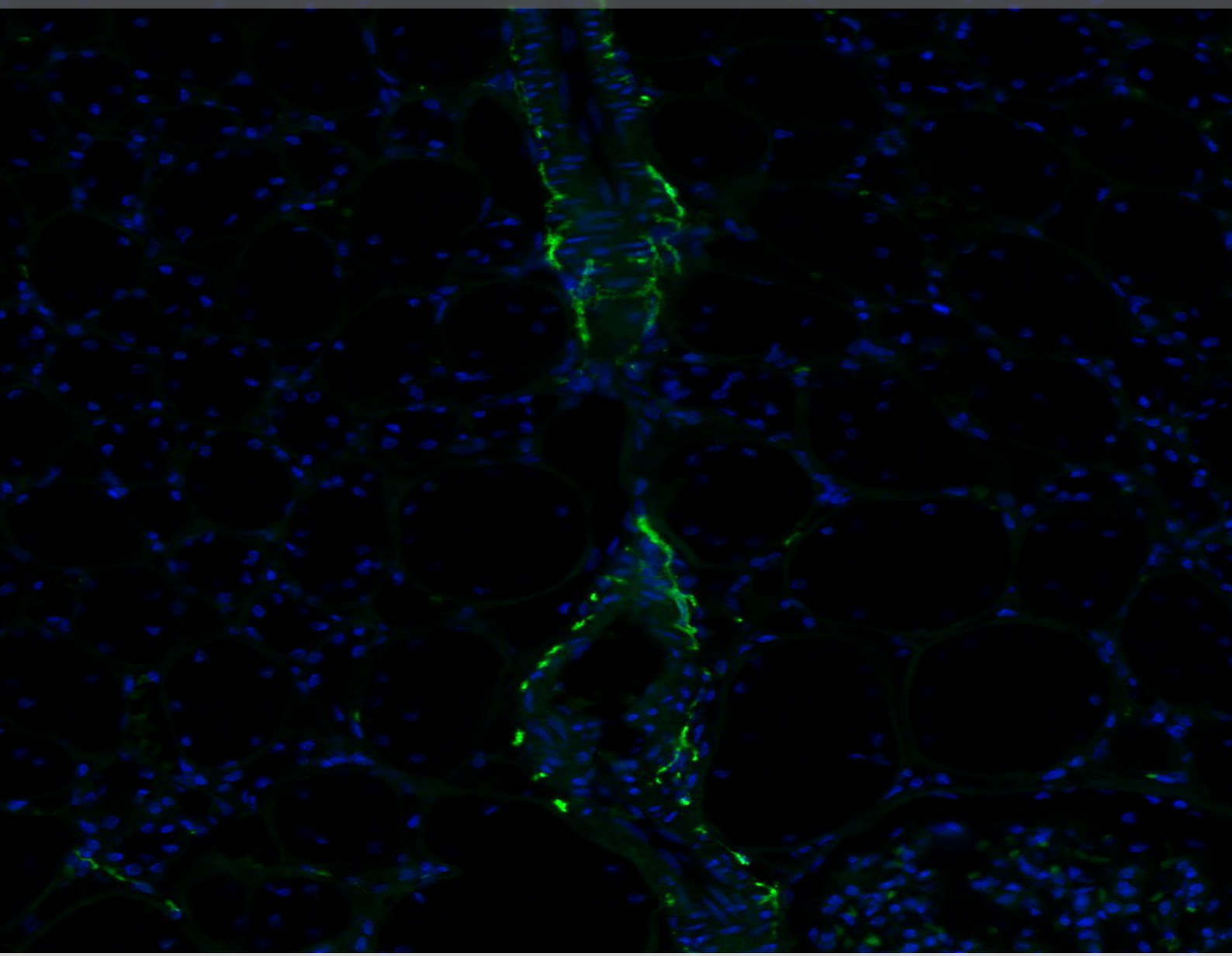
FUNCTION OF RENAL SYMPATHETIC NERVES

EDITED BY: Yutang Wang, Kyungjoon Lim and Kate M. Denton
PUBLISHED IN: Frontiers in Physiology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88945-295-8
DOI 10.3389/978-2-88945-295-8

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

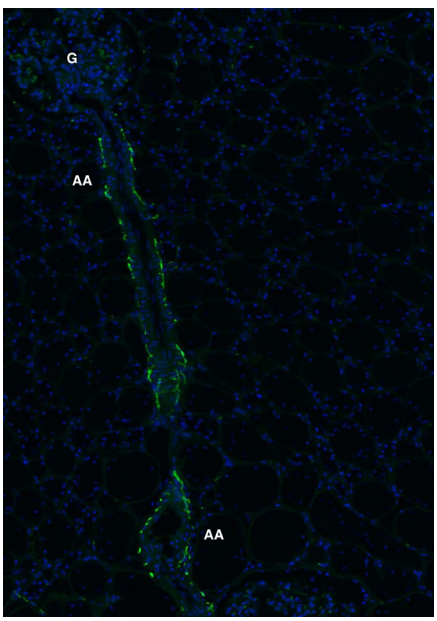
Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: **researchtopics@frontiersin.org**

FUNCTION OF RENAL SYMPATHETIC NERVES

Topic Editors:
Yutang Wang, Federation University Australia, Australia
Kyungjoon Lim, La Trobe University, Australia
Kate M. Denton, Monash University, Australia



Efferent nerve distribution in the renal cortex in an adult sheep as shown by tyrosine hydroxylase staining (green). Cell nuclei are stained with 4',6-diamidino-2-phenylindole (DAPI, blue). Depicted are two glomeruli (G) and their associated afferent arterioles (AA). Scale bar, 100 um.

Image credit: Reetu Singh

Sympathetic overactivity is associated with the development of hypertension. Renal denervation (RDN) prevents or delays hypertension in a variety of animal models, which laid the groundwork for the introduction of RDN as a clinical therapy in humans. In 2007, a novel, minimally invasive RDN ablation catheter was first trialled in hypertensive patients, with a 93% success rate of lowering blood pressure for at least three years post-RDN. However, a large scale, sham-controlled clinical trial (Symplicity HTN -3) failed to show reductions in BP greater than sham.

The aim of this research topic was to evaluate the efficacy and safety of RDN, to explore the contribution of both afferent and efferent renal nerve activity to hypertension and non-hypertension disorders, and to stimulate future research to better understand the function of the renal nerves and the effects of RDN by highlighting gaps in knowledge.

Citation: Wang, Y., Lim, K., Denton, K. M., eds (2017). Function of Renal Sympathetic Nerves. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-295-8

Table of Contents

Chapter 4: Long-term safety concerns following renal denervation

frontiers in Physiology

EDITORIAL
published: 28 August 2017
doi: 10.3389/fphys.2017.00642

Editorial: Function of Renal Sympathetic Nerves

Yutang Wang[1]*, *Kyungjoon Lim*[2] and *Kate M. Denton*[3]*

[1] School of Applied and Biomedical Science, Federation University Australia, Ballarat, VIC, Australia, [2] Neurophysiology, Department of Physiology, Anatomy and Microbiology, La Trobe University, Melbourne, VIC, Australia, [3] Cardiovascular Disease Program, Department of Physiology, School of Biomedical Sciences, Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

Keywords: atherosclerosis, heart failure, hypertension, kidney disease, renal denervation, renal sympathetic nerves

Editorial on the Research Topic

Function of Renal Sympathetic Nerves

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity worldwide. The incidence of CVD is increasing in association with the growing prevalence of hypertension, diabetes, and obesity. This is occurring despite established effective therapies for hypertension. Thus, new methods for risk reduction are still needed (Cutler et al., 2008). The aim of this research topic was to evaluate the efficacy and safety of renal denervation (RDN), to explore the contribution of both afferent and efferent renal nerve activity to hypertension and non-hypertension disorders, and to stimulate future research to better understand the function of the renal nerves and the effects of RDN by highlighting gaps in knowledge.

OPEN ACCESS

Edited and reviewed by:
Carolyn J. Barrett,
University of Auckland, New Zealand

***Correspondence:**
Yutang Wang
Yutang.wang@federation.edu.au
Kate M. Denton
kate.denton@monash.edu

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *05 July 2017*
Accepted: *15 August 2017*
Published: *28 August 2017*

Citation:
Wang Y, Lim K and Denton KM (2017) Editorial: Function of Renal Sympathetic Nerves. Front. Physiol. 8:642. doi: 10.3389/fphys.2017.00642

EVALUATION OF CATHETER-BASED RENAL DENERVATION FOR THE TREATMENT OF HYPERTENSION

Strong evidence that sympathetic overactivity is associated with the development of hypertension, combined with the demonstration that RDN prevents or delays hypertension in a variety of animal models, laid the groundwork for the introduction of RDN as a clinical therapy in humans (DiBona and Esler, 2010). In 2007, a novel, minimally invasive RDN ablation catheter was first trialed in hypertensive patients, with a 93% success rate of lowering blood pressure (BP) for at least 3 years post-RDN (Krum et al., 2014). These studies were met with acclaim (Lakhanpal and Domanski, 2012). However, a large scale, sham-controlled clinical trial (Symplicity HTN-3) failed to show reductions in BP greater than sham (Bhatt et al., 2014). It is now clear that poor trial design contributed to this outcome; only 19 of the 340 patients received at least the recommended 4+ ablations per artery and in these patients the falls in BP were greatest (Kandzari et al., 2015). In addition, the report by the Global SYMPLICITY Registry of 1,000 consecutively enrolled patients not only confirmed the safety of RDN but also suggested that RDN lowers office and ambulatory BP at 6 months post-procedure (Bohm et al., 2015). In the current volume, Fadl Elmula et al. summarizes the results of recent clinical RDN trials and rightly raised concerns regarding the interpretation of this data and called for caution in the implementation of this procedure. The authors point out that these trials did not confirm the marked BP lowering effect observed by initial RDN studies (Krum et al., 2009; Esler et al., 2010). In addition, they suggested that the BP lowering effect of RDN may be limited to discrete patient populations, and that it would be

worthwhile searching for potential predictors of true responders to RDN. Briasoulis and Bakris reviewed the role of renal sympathetic nerves in hypertension and clinical applications of RDN in resistant hypertension. In light of the results from the Symplicity HTN-3 trial, the authors suggested that future randomized trials should be performed in experienced centers, using newer catheters, and in carefully selected compliant patients with true resistant hypertension.

EFFECTS OF RENAL DENERVATION BEYOND BLOOD PRESSURE CONTROL

It is noteworthy that RDN has also been shown to be effective in the treatment of other conditions coexisting with resistant hypertension. Thorp and Schlaich contributed a comprehensive review on RDN for hypertension and beyond. The non-hypertensive disorders that may also be affected by RDN include chronic kidney disease, chronic heart failure, left ventricular hypertrophy, arrhythmias, and metabolic diseases. Evidence suggests that clinical benefits beyond BP reduction could be gained following RDN in patients with these disorders. However, randomized controlled trials are needed before the application of RDN becomes routine in clinical practice for these patient cohorts. Moreover, these intriguing findings suggest that there is still a lot we don't understand about the function of the renal nerves.

Heart Failure

The kidney has a rich afferent sensory and efferent sympathetic innervation. It is now apparent that afferent renal nerve activity can reflexly modulate sympathetic outflow, not just to the kidney, but also to other organs, which may be one mechanism whereby RDN might improve outcomes in heart failure. Ramchandra and Barrett reviewed the mechanisms that contribute to enhanced renal sympathetic nerve activity (SNA) during heart failure, with an emphasis on afferent reflexes and central mechanisms. Booth et al. also contributed a review on this topic, focusing on critically assessing recent preclinical and clinical evidence supporting RDN as a potential treatment for heart failure. It was concluded that a reflex reduction in cardiac SNA in heart failure may be one beneficial effect of RDN. Booth et al. also explored the effect of RDN on the renal afferents, and provided evidence for both the destruction and then significant regrowth of afferent renal nerves. However, what effect ablating the afferent nerves play in mediating the responses to RDN is still largely unknown. Finally, Schiller et al. (2015) reviewed the contribution of renal nerves to the pathogenesis of chronic heart failure, providing an overview of clinical RDN studies to date. This culminated in a call for more research to determine how RDN may be employed as a safe and effective treatment in chronic heart failure.

Kidney Disease

Strong evidence demonstrates that renal SNA is elevated in both acute and chronic kidney disease. Although less robust, evidence also implicates increased afferent sensory nerve activity in kidney disease. In a primary study, Salman et al. demonstrated using telemetry-based recordings in rats with chronic kidney disease (Lewis Polycystic Kidney rats) that renal SNA directly correlated with BP. This study confirmed the notion that elevated renal SNA is likely a key contributor to hypertensive kidney disease. In an acute study, Goulding et al. examined the contribution of the renal nerves to baroreflex control of renal SNA and excretory function in cisplatin-induced renal failure in rats. The authors found that impaired renal function was associated with blunting of baroreflex gain and increased renal SNA, and that RDN restored these abnormalities. The results of this study suggested that dysregulation of renal SNA plays an important role in renal failure and that RDN may provide benefits for patients with kidney disease. In combination, this evidence led to the suggestion that kidney disease may also be amenable to RDN and Sanders and Blankestijn contributed a review of the clinical evidence arguing that RDN may have a place in the management of chronic kidney disease. RDN has been trialed in models of chronic kidney disease and been shown to be effective in lowering BP, though concerns were raised regarding the ability to adequately mount a response to hemorrhage (Singh et al., 2017).

LONG-TERM SAFETY CONCERNS FOLLOWING RDN

The introduction of catheter-based RDN initially moved forward at a rapid pace and though this has slowed, interest still remains strong. In part, this reduced momentum stems from concerns surrounding the lack of understanding of the long-term impact of RDN. Wang pointed out that although RDN is generally regarded as a safe procedure, some reports with small sample size have shown that the incidence of renal artery stenosis after RDN could be up to 18.2%; though this may depend on operator experience and the type of catheter employed. However, the safety of RDN needs to be continuously monitored.

In another study, Wang et al. examined the possible side effects of RDN in apolipoprotein E-deficient mice with hypertension induced by angiotensin II infusion. It was found that RDN increased atherosclerosis in the aortic arch despite a reduction in systolic BP in the mice. In contrast, RDN has been reported to decrease atherosclerosis in normotensive apolipoprotein E-deficient mice fed a high fat diet (Wang et al., 2015). Therefore, whether RDN promotes atherosclerosis in patients with resistant hypertension requires investigation in follow-up studies. In fact, the consequences of RDN in all patient populations in which this procedure is being trialed requires longer term follow-up, given evidence that the renal nerves regrow over time (Booth et al., 2015).

CONCLUSION

Thus, the articles contained in this collection, contribute to our understanding of the function of the renal nerves, the effects of RDN as a treatment for hypertension and non-hypertensive disorders, and the side effects of RDN. However, much remains to be learned in terms of intra-procedural verification of the

completeness of RDN, patient selection and long-term safety of RDN.

AUTHOR CONTRIBUTIONS

YW, KL, and KD conceived the content. YW drafted the manuscript. KL and KD revised the manuscript.

ACKNOWLEDGMENTS

YW is supported by a grant from the National Health and Medical Research Council (1062671). KL is supported by a grant from La Trobe University (Understanding Disease RFA). KD is supported by an NHMRC Research Fellowship (1041844).

REFERENCES

Bhatt, D. L., Kandzari, D. E., O'Neill, W. W., D'Agostino, R., Flack, J. M., Katzen, B. T., et al. (2014). A controlled trial of renal denervation for resistant hypertension. *N. Engl. J. Med.* 370, 1393–1401. doi: 10.1056/NEJMoa1402670

Bohm, M., Mahfoud, F., Ukena, C., Hoppe, U. C., Narkiewicz, K., Negoita, M., et al. (2015). First report of the Global SYMPLICITY Registry on the effect of renal artery denervation in patients with uncontrolled hypertension. *Hypertension* 65, 766–774. doi: 10.1161/HYPERTENSIONAHA.114.05010

Booth, L. C., Nishi, E. E., Yao, S. T., Ramchandra, R., Lambert, G. W., Schlaich, M. P., et al. (2015). Reinnervation of renal afferent and efferent nerves at 5.5 and 11 months after catheter-based radiofrequency renal denervation in sheep. *Hypertension* 65, 393–400. doi: 10.1161/HYPERTENSIONAHA.114.04176

Cutler, J. A., Sorlie, P. D., Wolz, M., Thom, T., Fields, L. E., and Roccella, E. J. (2008). Trends in hypertension prevalence, awareness, treatment, and control rates in United States adults between 1988-1994 and 1999-2004. *Hypertension* 52, 818–827. doi: 10.1161/HYPERTENSIONAHA.108.113357

DiBona, G. F., and Esler, M. (2010). Translational medicine: the antihypertensive effect of renal denervation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 298, R245–253. doi: 10.1152/ajpregu.00647.2009

Esler, M. D., Krum, H., Sobotka, P. A., Schlaich, M. P., Schmieder, R. E., and Bohm, M. (2010). Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 376, 1903–1909. doi: 10.1016/S0140-6736(10)62039-9

Kandzari, D. E., Bhatt, D. L., Brar, S., Devireddy, C. M., Esler, M., Fahy, M., et al. (2015). Predictors of blood pressure response in the SYMPLICITY HTN-3 trial. *Eur. Heart J.* 36, 219–227. doi: 10.1093/eurheartj/ehu441

Krum, H., Schlaich, M. P., Sobotka, P. A., Bohm, M., Mahfoud, F., Rocha-Singh, K., et al. (2014). Percutaneous renal denervation in patients with treatment-resistant hypertension: final 3-year report of the Symplicity HTN-1 study. *Lancet* 383, 622–629. doi: 10.1016/S0140-6736(13)62192-3

Krum, H., Schlaich, M., Whitbourn, R., Sobotka, P. A., Sadowski, J., Bartus, K., et al. (2009). Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 373, 1275–1281. doi: 10.1016/S0140-6736(09)60566-3

Lakhanpal, J. R., and Domanski, M. J. (2012). Hypothesis: catheter-based renal sympathetic denervation should be the initial therapy for essential hypertension. *Med. Hypotheses* 78, 482–484 doi: 10.1016/j.mehy.2012.01.011

Schiller, A. M., Pellegrino, P. R., and Zucker, I. H. (2015). The renal nerves in chronic heart failure: efferent and afferent mechanisms. *Front. Physiol.* 6:224. doi: 10.3389/fphys.2015.00224

Singh, R. R., Sajeesh, V., Booth, L. C., McArdle, Z., May, C. N., Head, G. A., et al. (2017). Catheter-based renal denervation exacerbates blood pressure fall during hemorrhage. *J. Am. Coll. Cardiol.* 69, 951–964. doi: 10.1016/j.jacc.2016.12.014

Wang, H., Wang, J., Guo, C., Luo, W., Kleiman, K., and Eitzman, D. T. (2015). Renal denervation attenuates progression of atherosclerosis in apolipoprotein E-deficient mice independent of blood pressure lowering. *Hypertension* 65, 758–765. doi: 10.1161/HYPERTENSIONAHA.114.04648

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in PHYSIOLOGY

MINI REVIEW ARTICLE
published: 09 February 2015
doi: 10.3389/fphys.2015.00009

Renal sympathetic denervation after Symplicity HTN-3 and therapeutic drug monitoring in severe hypertension

Fadl Elmula M. Fadl Elmula[1,2], Anne C. Larstorp[1,2], Sverre E. Kjeldsen[1,2]*, Alexandre Persu[3,4], Yu Jin[5] and Jan A. Staessen[5,6]

[1] Departments of Cardiology and Internal Medicine, Oslo University Hospital, Ullevaal, Norway
[2] Faculty of Medicine, University of Oslo, Oslo, Norway
[3] Pole of Cardiovascular Research, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium
[4] Division of Cardiology, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium
[5] Studies Coordinating Centre, Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Leuven, Belgium
[6] VitaK Development and Research, Maastricht University, Maastricht, Netherlands

Edited by:
Kate Denton, Monash University, Australia

Reviewed by:
Jacqueline Kathleen Phillips, Macquarie University, Australia
Kenju Miki, Nara Women's University, Japan
Massimo Volpe, University of Rome Sapienza, Italy

***Correspondence:**
Sverre E. Kjeldsen, Oslo University Hospital, Ullevaal, University of Oslo, PO Box 4950, Nydalen, 0424 Oslo, Norway
e-mail: s.e.kjeldsen@medisin.uio.no

Renal sympathetic denervation (RDN) has been and is still proposed as a new treatment modality in patients with apparently treatment resistant hypertension (TRH), a condition defined as persistent blood pressure elevation despite prescription of at least 3 antihypertensive drugs including a diuretic. However, the large fall in blood pressure after RDN reported in the first randomized study, Symplicity HTN-2 and multiple observational studies has not been confirmed in five subsequent prospective randomized studies and may be largely explained by non-specific effects such as improvement of drug adherence in initially poorly adherent patients (the Hawthorne effect), placebo effect and regression to the mean. The overall blood-pressure lowering effect of RDN seems rather limited and the characteristics of true responders are largely unknown. Accordingly, RDN is not ready for clinical practice. In most patients with apparently TRH, drug monitoring and improvement of drug adherence may prove more effective and cost-beneficial to achieve blood pressure control. In the meantime, research should aim at identifying characteristics of those patients with truly TRH who may respond to RDN.

Keywords: hypertension, antihypertensive drugs, renal denervation, drug monitoring, treatment resistance

INTRODUCTION

Renal sympathetic denervation (RDN) has been and is still proposed as a new treatment modality in patients with apparently treatment resistant hypertension (TRH), a condition defined as persistent blood pressure elevation despite prescription of at least 3 antihypertensive drugs including a diuretic (Krum et al., 2009, 2014; Esler et al., 2010). However, with the recent publication of the Symplicity HTN-3 study in the U.S. (Bhatt et al., 2014) it is questioned whether RDN at all lowers blood pressure (Demaria, 2014). During 2014, a total of 5 prospective and randomized studies of RDN showing modest or no effect on blood pressure in patients with TRH have been published or presented. Other recent studies have shown that patients with TRH have surprisingly low drug adherence. The aim of this paper is to review all prospective and randomized studies of RDN in TRH and, and to review the issue of poor drug adherence and suggest therapeutic drug monitoring (TDM) as a cost-effective modality to control blood pressure and improve prognosis in this subset of hypertensive patients who are at risk and difficult-to-treat.

Abbreviations: ABPM, ambulatory blood pressure monitoring; RDN, renal sympathetic denervation; TRH, treatment resistant hypertension; TDM, therapeutic drug monitoring.

THE RISE AND FALL OF RENAL DENERVATION IN TREATMENT RESISTANT HYPERTENSION

The initial enthusiasm followed by the setback of RDN can probably be summarized by a handful of explanations: (1) The role of the sympathetic system in the pathophysiology of hypertension is substantiated by a wealth of experimental and clinical arguments (Julius and Esler, 1975; Eide et al., 1979; Kjeldsen et al., 1981). On this background, enthusiasm surged when an intervention in this system seemed to drastically lower blood pressure. (2) Market-driven industry interests significantly influenced the medical community. (3) Subsequently, pitfalls in the treatment of apparent TRH patients, which are simple but well-known for decades, were suddenly forgotten, including well described phenomena such as the placebo effect, poor drug adherence (Gifford, 1988; Klein, 1988; Ceral et al., 2011) and the Hawthorne effect (Mangione-Smith et al., 2002). Regression to the mean could also be involved which means that abnormal BP values tend to change toward normalization without an underlying biological explanation.

The first and for a long time the only prospective randomized clinical trial in this field, the Symplicity HTN-2 study (Esler et al., 2010), was monitored by Ardian (Medtronic) who collected and processed the data. Usually, when such a task is given to industry, all measures are taken to secure confidence and

trials are double-blinded (Julius et al., 2004). However, in this case, everything was open, making the trial particularly vulnerable to patient and physician related biases (Howard et al., 2013). In a recent editorial (Shun-Shin et al., 2014), the authors wrote that "measurement of a noisy variable by unblinded optimistic staff is a known recipe for calamitous exaggeration." It is also unfortunate that selection of patients enrolled in Symplicity HTN-2 and evaluation of efficacy were based on office rather than ambulatory blood pressure measurements (ABPM), which is state-of-the art (O'Brien et al., 2013), particularly in resistant hypertension (Persu et al., 2014d). ABPM reduces observer bias and measurement error, minimizes the white-coat effect and has greater reproducibility, and therefore provides a better estimate of a patient's usual blood pressure and cardiovascular prognosis (Kikuya et al., 2007; Salles et al., 2008). Notwithstanding the well-known, major contribution of poor drug adherence to apparently resistant hypertension (Gifford, 1988; Klein, 1988; Ceral et al., 2011), drug adherence was not monitored, either at baseline or during follow-up. This made the study vulnerable to the Hawthorne effect, i.e., patients changing behavior—in this case starting taking their drugs as prescribed -, in response to the intervention and massive attention devoted to them. The lack of blood pressure decrease in the control group also raises concerns. One would indeed suspect that patients in the control group had not taken their medications properly, in order to keep their blood pressure at a higher level that made them eligible for cross-over to RDN group (Azizi et al., 2012; Persu et al., 2012). Finally, placebo effect and regression to the mean must also be taken into account. Noteworthy, the placebo effect is small by using ABPM (Staessen et al., 1996; O'Brien et al., 2013); however, ABPM remains as sensitive to the Hawthorne effect as office blood pressure.

THE ROLE OF INDUSTRY IN PROMOTING RENAL DENERVATION

Despite the major limitations and potential biases of Symplicity HTN-2, RDN was adopted in hundreds of centers worldwide. Medtronic Inc® (Minneapolis, Minnesota) paid $800 million to purchase Ardian® (Mountain View, California), the company that had developed the technology (Demaria, 2014), and more than 10 companies developed their own RDN systems, five of which obtained the CE mark (Conformité Européenne, European Conformity). CE marking means that the product is assessed before being placed on the market and meets EU safety, health and environmental protection requirements. However, CE marking is unrelated to medical indication at variance with the USA where FDA approves a medical device only when it has been tested and proved effective for a certain medical condition. The procedure was quickly reimbursed in Germany, and later on in Switzerland, Sweden and the Netherlands. While RDN remained an investigational procedure in the U.S., at least 8000 (Lüscher and Mahfoud, 2014), possibly 15,000–20,000 procedures were performed in Europe and in the rest of the world in less than 4 years, most of them using the Ardian -Medtronic® catheter. It may be hypothesized that the massive incomes, generated by selling the Symplicity catheter to enthusiastic Europeans paid for the Symplicity HTN-3 study (Bhatt et al., 2014), required by the FDA before approval of RDN in the U.S. In Symplicity HTN-3, blinding of patients through the use of a sham procedure and wider use of ABPM balanced and limited the differential impact of the Hawthorne, white coat, placebo and regression to the mean effects in both arms, disclosing to the world the true size of blood pressure decrease attributable to RDN, at least in patients meeting the Symplicity criteria; it was less than 2 mmHg systolic based on ABPM.

For all aforementioned reasons, and in view of the complexity and multifactorial character of hypertension, the failure of RDN to normalize or substantially reduce blood pressure in all patients with apparently TRH was a reasonable working hypothesis for us, even before the Medtronic announcement that Symplicity HTN-3 had failed to meet its primary endpoint (http://www.tctmd.com/show.aspx?id=123265). We (Fadl Elmula et al., 2013; Persu et al., 2013a,b) and others (Azizi et al., 2012; Howard et al., 2013) had predicted that the true effect of RDN might have been overestimated and may considerably shrink in properly designed studies (Howard et al., 2013), and that "one size may not fit all" (Persu et al., 2012). In particular, in preliminary analysis of the European Network COordinating research on Renal Denervation (ENCOReD) network (Persu et al., 2014a) we were struck by the imbalance between the 17.6 mmHg decreases in office blood pressure, vs. only 5.9 mmHg for 24-h ambulatory blood pressure.

FINDING PATIENTS WITH TRUE TREATMENT-RESISTANT HYPERTENSION FOR RESEARCH

When we set out to investigate the effects of RDN in one of the centers with the longest experience in conducting randomized clinical trials in Europe (Helgeland, 1980), we had thus clearly in mind the limitations of previous studies. We needed a simple and practical way to deal with pitfalls in the recruitment of patients with resistant hypertension into a study protocol: Patients had to qualify for the RDN protocols by having elevated daytime ABPM after witnessed intake of their prescribed blood pressure medication (Fadl Elmula et al., 2013). Meanwhile, a leading hypertension center in Germany (Brinkmann et al., 2012) published a well-documented series of patients whose blood pressure remained unchanged after RDN. We were thus not surprised when we found no change in either office or ABPM following RDN, first in an open series of six patients (Fadl Elmula et al., 2013), later followed by a randomized study (Fadl Elmula et al., 2014). Patients who were randomly assigned to further improvement of drug treatment guided by non-invasive hemodynamic monitoring had normalized blood pressures (**Figures 1**, **2**). In contrast, patients exposed to RDN experienced only a small and probably partly placebo-induced fall in office and ABPM. The decreases averaged 20 mmHg more for office and 9 mmHg more for ambulatory systolic blood pressure in the hemodynamically guided drug treatment group ($n = 10$) compared to the RDN group ($n = 9$). Because of sustained elevation of AMBP in the RDN treated patients at 6 months of follow-up, we stopped randomization for ethical reason according to a pre-specified decision (Fadl Elmula et al., 2013).

THE PITFALLS WITH RENAL DENERVATION IN TREATMENT RESISTANT HYPERTENSION

In the absence of solid evidence of efficacy, how can we explain the uncontrolled deployment of RDN in Europe and worldwide (with the notable exception of the U.S. where RDN remained

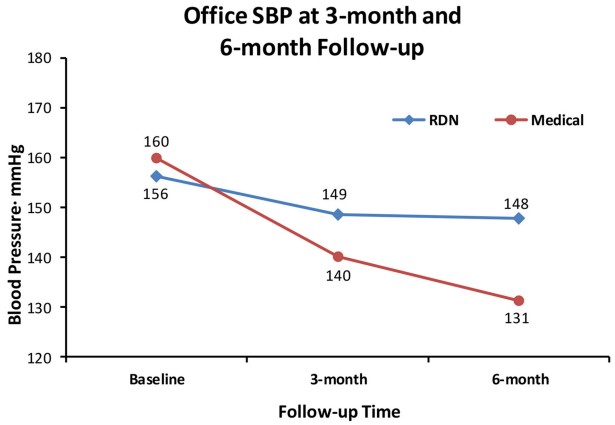


FIGURE 1 | Shows the effect of RDN on office systolic blood pressure (SBP) at 3-month and 6-months of follow-up, compared to drug treatment adjustment guided by non-invasive hemodynamic measurements. Differences were statistically significant (Fadl Elmula et al., 2014), favoring drug treatment adjustment, which is the recommended method to gain blood pressure control in patients with so-called treatment-resistant hypertension (Gifford, 1988).

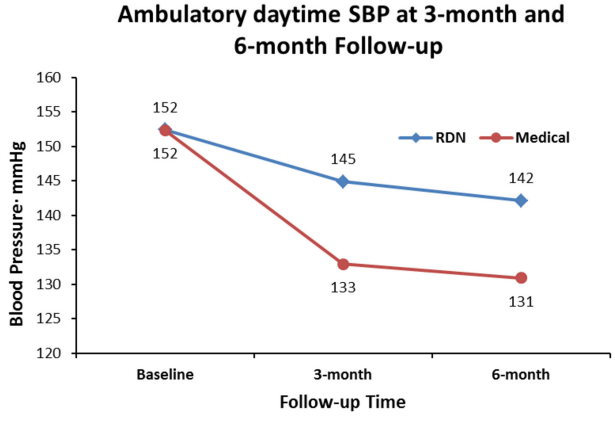


FIGURE 2 | Shows the effect of RDN on ambulatory daytime systolic blood pressure (SBP) at 3-month and 6-months of follow-up, compared to drug treatment adjustment guided by non-invasive hemodynamic measurements. Differences were statistically significant (Fadl Elmula et al., 2014), favoring drug treatment adjustment, which is the recommended method to gain blood pressure control in patients with so-called treatment resistant hypertension (Gifford, 1988).

an investigational procedure)? Of course, publications of the Symplicity studies and multiple observational studies, and enthusiastic editorials and reviews in top-ranking journals (Mahfoud et al., 2013; Ott et al., 2013) had a substantial impact, and the lack of strict rules for introduction of device-based therapies in Europe facilitated the large-scale implementation of the technique. However, this phenomenon would have remained limited without the huge promotion by device-producing industry. Probably industry has never launched such a strong campaign to market a new technology before. A multitude of national and international advisory boards organized educational meetings, developed a website (www.poweroverpressure.com) and produced guidelines, and corresponding author of this current review contributed to these. Medical journals were swamped by reviews and meta-analyses showing the powerful blood pressure lowering effects as recorded in observational studies and in the single available randomized study, Symplicity HTN-2. Comments pointing out the defects and inconsistencies in such meta-analysis encountered great delay in getting published (Jin et al., 2014). Many never questioned *whether* RDN should be implemented, but *when* it should start in an institution. By all means, the purpose was to disseminate the enthusiasm for RDN from the technically-oriented invasive radiologists and cardiologists who usually had little interest or experience in the treatment of hypertension to the "hypertension establishment." The European Society of Hypertension issued specific guidelines (Schmieder et al., 2012, 2013), but maintained reservations that more data was needed, and eventually it had to be proven that RDN would lower morbidity and mortality before being generally accepted in the treatment of true or apparent TRH.

In the aftermath of Symplicity HTN-3, it has been suggested that the lack of demonstrated efficacy of RDN in Symplicity HTN-3 may be due to lack of statistical power or even to chance (Lüscher and Mahfoud, 2014) or that the trial was well conceived but not rigorously executed (Esler, 2014; Schmieder, 2014; White et al., 2014; Lobo et al., 2015). In particular, a fraction of African American participants increased their antihypertensive medication, contrary to protocol, which masked a potential BP lowering effect of RDN in contrast to other participants. In addition, legitimate concerns were raised as to whether the denervation procedure was sub-optimal in many cases due to insufficient delivery of appropriate energy in the renal arteries as a consequence of the inexperience of the investigators. However, this criticism is all *post-hoc*, and the Symplicity HTN-3 findings are after all in line with the other RCTs published and presented in 2014 (Azizi et al., 2014; Desch et al., 2014; Fadl Elmula et al., 2014; Rosa et al., 2015). Furthermore, the Symplicity HTN-3 results are diluted by non-scientific comparisons with the Medtronic® registry (Pathak et al., 2014) which is hampered by all the weaknesses touched upon in this review, and even more as it is a pure industry-ran activity. Finally, while RDN will not become available in the U.S., and ongoing research in Asia was stopped, industry continues to make their catheters available for clinical use and promotes the technique in Europe.

COULD THERE BE RESPONDERS TO RENAL DENERVATION IN HYPERTENSION?

Does the failure of Symplicity HTN-3 mean the end of RDN? Not necessarily. Indeed, it has been shown in cohorts recruited from the third (The effect of progressive sympathectomy on blood pressure, Bradford Cannon, 1931) until the fifth decade of the last century (Smithwick and Thompson, 1953; Longland and Gibb, 1954) that abdominal sympathectomy associated to splanchnicectomy is effective in the treatment of severe hypertension. Accordingly, research should go on to find the minority of patients who are true responders to RDN, and identify predictors of effective RDN. The European Network COordinating research

on Renal Denervation (ENCOReD) is set up to include thousands of patients in randomized protocols, observational studies and registries independent of industry. Some early results (Persu et al., 2014a,b) from this joint effort have already been published and suggest that it may be worthwhile searching for potential predictors of response to RDN. When 2 prospective and randomized studies that have been published (Rosa et al., 2015) and reported (Azizi et al., 2014), plus Symplicity Flex, another sham-controlled study very recently reported (Desch et al., 2014) are added to the 3 published studies (Esler et al., 2010; Bhatt et al., 2014; Fadl Elmula et al., 2014) the overall picture shows that RDN is equal to drugs in lowering BP. However, individual data suggests that there may be cause for optimism that some truly responding patients may be identified.

Still, before going ahead, we have to draw the lessons of the RDN story. We must make sure that RDN is beneficial and does no harm. Many patients have probably undergone unneeded procedures. By a careful estimate, 20 000 renal arteries have been exposed to ablation in people with hypertension and an increasing number of cases of renal artery stenosis after RDN are being reported (Persu et al., 2014c). It remains to be seen whether the negative news that RDN is not for most people will reach Time Magazine (Oz, 2012) and Der Spiegel (Blech, 2013), or whether the old lessons (Bramley et al., 2006) remain for clinicians who treat people with hypertension in daily life.

THERAPEUTIC DRUG MONITORING IN RESISTANT HYPERTENSION

In view of the major contribution of poor drug adherence to apparent TRH, therapeutic drug monitoring (TDM) maybe a useful tool for detecting and reducing non-adherence, leading to substantial blood pressure (BP) improvement in this subset of hypertensive patients. (Chung et al., 2014) have assessed cost-effectiveness of TDM using a Markov model based on German data and life statistics to evaluate life-years, quality-adjusted life-years (QALYs), costs, and incremental cost-effectiveness ratios in TRH patients receiving either TDM optimized therapy or standard best medical therapy. Efficacy of TDM was modeled by reducing risk of hypertension-related morbidity and mortality. The authors showed that TDM is a cost-effective health care intervention in patients diagnosed with TRH, and that this finding is valid for a wide range of patients, irrespective of age and sex (Chung et al., 2014).

Poor drug adherence in apparent TRH is a serious issue that has drawn the attention of experienced clinicians for many years (Gifford, 1988; Klein, 1988). Recently, in a study of 84 patients taking on average 5 antihypertensive drugs it was shown by measurements that no drug was detectable in the blood in 34.5% of the patients, and 65.5% of the patients fulfilled the criteria of non-adherence (Ceral et al., 2011). Other investigators have provided similar results (Jung et al., 2013; Strauch et al., 2013; Tomaszewski et al., 2014). Beyond the clinical challenge of convincing people with severe hypertension to take their antihypertensive medication in order to control their high blood pressure and improve their prognosis, changes in drug adherence over time may have major, unpredictable effects on the results of clinical trials including patients with apparent TRH. People may change their behavior when given special attention in research (the Hawthorne-effect). This may introduce important biases, as patients with assumed TRH but with poor drug adherence, may start taking their drugs when exposed to additional intervention. We postulate that much of the recent controversy with RDN can be explained in this way (Esler et al., 2010; Bhatt et al., 2014).

Clinical assessment of non-adherence in routine practice is challenging (Burnier et al., 2003). Drug adherence is usually investigated using written patient's diary or somewhat more sophisticated by electronic pill boxes, or blood and urine measurements of prescribed drugs. Measurements of drugs can provide interesting information, but are not often used in practical clinical work especially in primary care, and the cost has been prohibitive until recently. Neither patient's diary nor electronic pill boxes are perfectly reliable to ensure drug intake. The only methods that 100% ensures true drug intake is witnessed drug intake, an approach that may yield quite interesting results in patients with TRH (Fadl Elmula et al., 2013, 2014). However, while witnessed intake of drugs may identify adherent patients for immediate inclusion into a study, this method is not particularly practical in the long-run for the follow-up in clinical practice or research.

In the long run, TDM in body fluids may thus prove the best tool for evaluation and improvement of adherence to drug therapy (Brinker et al., 2014). This approach allows an objective surveillance of patient adherence by repeatedly measuring concentrations of antihypertensive drugs in blood and urine. Moreover, when non-adherent patients were confronted with their low or undetectable drug levels and were provided additional counseling to overcome barriers of adherence, blood pressure control improved considerably without intensification of therapy (Brinker et al., 2014). While several studies as pointed out above focused on the objective exclusion or confirmation of non-adherence, this recent study (Brinker et al., 2014) utilized the information gained from TDM measurements for therapeutic purposes. The TDM results were discussed with the non-adherent patients to explore barriers to adherence and counseling was provided to overcome these barriers. During follow-up, SBP was reduced by 46 $\pm$ 10 mmHg in non-adherent compared to 12 $\pm$ 17 mmHg in adherent patients, without intensification of the antihypertensive therapy (Brinker et al., 2014).

TDM identifies and helps to resolve the key problem in many—possibly the majority—of patients with apparent TRH—that is poor adherence to prescribed drug regimen. As previously shown, the cost-effectiveness of this approach is supported by a solid rationale (Chung et al., 2014) and should not be compared to similar analyses of controversial device intervention (Geisler et al., 2012; Dorenkamp et al., 2013; Gladwell et al., 2014) in apparent TRH patients. So far, such analyses were indeed based on Symplicity HTN-2, an unblinded study largely open to the Hawthorne and placebo effects, whose results could not be replicated in any of five randomized trials published or presented in 2014.

REFERENCES

Azizi, M., Monge, M., Pereira, H., and Sapoval, M. (2014). The French DENERHTN Trial: renal denervation + standardized antihypertensive treatment

vs. standardized antihypertensive treatment alone in patients with resistant hypertension. *J. Hypertens.* 32(e-Suppl. 1), e89.

Azizi, M., Steichen, O., Frank, M., Bobrie, G., Plouin, P. F., and Sapoval, M. (2012). Catheter-based radiofrequency renal-nerve ablation in patients with resistant hypertension. *Eur. J. Vasc. Endovasc. Surg.* 43, 293–299. doi: 10.1016/j.ejvs.2011.11.022

Bhatt, D. L., Kandzari, D. E., O'Neill, W. W., D'Agostino, R., Flack, J. M., and Katzen, B. T. (2014). SYMPLICITY HTN-3 Investigators. A controlled trial of renal denervation for resistant hypertension. *N. Engl. J. Med.* 370, 1393–1401. doi: 10.1056/NEJMoa1402670

Blech, J. (2013). Vergebens verbrutzelt. Ein Eingriff an den Nierennerven gilt als neues Wundermittel gegen Bluthochdruck. Dabei ist nicht bewiesen, dass die Methode den Patienten wirklich nutzt. *Der. Spiegel No*. 28, 102–103.

Bradford Cannon, W. (1931). *The Effect of Progressive Sympathectomy on Blood Pressure*. Available online at: http://www.ncbi.nlm.nih.gov/pubmed/2204236

Bramley, T. J., Gerbino, P. P., Nightengale, B. S., and Frech-Tamas, F. (2006). Relationship of blood pressure control to adherence with antihypertensive monotherapy in 13 managed care organizations. *J. Manag. Care Pharm.* 12, 239–245.

Brinker, S., Pandey, A., Ayers, C., Price, A., Raheja, P., and Arbique, D. (2014). Therapeutic drug monitoring facilitates blood pressure control in resistant hypertension. *J. Am. Coll. Cardiol.* 63, 834–835. doi: 10.1016/j.jacc.2013.10.067

Brinkmann, J., Heusser, K., Schmidt, B. M., Menne, J., Klein, G., and Bauersachs, J. (2012). Catheter-based renal nerve ablation and centrally generated sympathetic activity in difficult-to-control hypertensive patients: prospective case series. *Hypertension* 60, 1485–1490. doi: 10.1161/HYPERTENSIONAHA.112.201186

Burnier, M., Santschi, V., Favrat, B., and Brunner, H. R. (2003). Monitoring compliance in resistant hypertension: an important step in patient management. *J. Hypertens Suppl.* 21, S37–S42. doi: 10.1097/00004872-200305002-00007

Ceral, J., Habrdova, V., Vorisek, V., Bima, M., Pelouch, R., and Solar, M. (2011). Difficult-to-control arterial hypertension or uncooperative patients? The assessment of serum antihypertensive drug levels to differentiate non-responsiveness from non-adherence to recommended therapy. *Hypertens Res.* 34, 87–90. doi: 10.1038/hr.2010.183

Chung, O., Vongpatanasin, W., Bonaventura, K., Lotan, Y., Sohns, C., Haverkamp, W., et al. (2014). Potential cost-effectiveness of therapeutic drug monitoring in patients with resistant hypertension. *J. Hypertens.* 32, 2411–2421. doi: 10.1097/HJH.0000000000000346

Demaria, A. N. (2014). Reflections on renal denervation. *J. Am. Coll. Cardiol.* 63, 1452–1453. doi: 10.1016/j.jacc.2014.03.001

Desch, S., Okon, T., Heinemann, T., Kulle, K., Röhnert, K., Sonnenabend, M., et al. (2014). "A randomized sham-controlled trial of renal sympathetic denervation in mild resistant hypertension," in *Transcatheter Cardiovascular Therapeutics Meeting*, (Washington, DC). Available online at: http://www.medpagetoday.com/MeetingCoverage/TCT/47688

Dorenkamp, M., Bonaventura, K., Leber, A. W., Boldt, J., Sohns, C., and Boldt, L. H. (2013). Potential lifetime cost-effectiveness of catheter-based renal sympathetic denervation in patients with resistant hypertension. *Eur. Heart J.* 34, 451–461. doi: 10.1093/eurheartj/ehs355

Eide, I., Kolloch, R., DeQuattro, V., Miano, L., Dugger, R., and Van der Meulen, J. (1979). Raised cerebrospinal fluid norepinephrine in some patients with primary hypertension. *Hypertension* 1, 255–260. doi: 10.1161/01.HYP.1.3.255

Esler, M. (2014). Illusions of thruth in the Symplicity HTN-3 Trial: generic design strengths but neuroscience failing. *J. Am. Soc. Hypertens* 8, 593–598. doi: 10.1016/j.jash.2014.06.001

Esler, M. D., Krum, H., Sobotka, P. A., Schlaich, M. P., Schmieder, R. E., and Bohm, M. (2010). Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 376, 1903–1909. doi: 10.1016/S0140-6736(10)62039-9

Fadl Elmula, F. E. M., Hoffmann, P., Fossum, E., Brekke, M., Gjønnæss, E., and Hjørnholm, U. (2013). Renal sympathetic denervation in patients with treatment-resistant hypertension after witnessed intake of medication before qualifying ambulatory blood pressure. *Hypertension* 62, 526–532. doi: 10.1161/HYPERTENSIONAHA.113.01452

Fadl Elmula, F. E. M., Hoffman, P., Larstorp, A. C., Fossum, E., Brekke, M., and Kjeldsen, S. E. (2014). Adjusted drug treatment is superior to sympathetic renal denervation in patients with true treatment resistant hypertension. *Hypertension* 63, 691–699. doi: 10.1161/HYPERTENSIONAHA.114.03246

Geisler, B. P., Egan, B. M., Cohen, J. T., Garner, A. M., Akehurst, R. L., and Esler, M. D. (2012). Cost-effectiveness and clinical effectiveness of catheter-based renal denervation for resistant hypertension. *J. Am. Coll. Cardiol.* 60, 1271–1217. doi: 10.1016/j.jacc.2012.07.029

Gifford, R. W. (1988). An algorithm for the management of resistant hypertension. *Hypertension* 11, I-171–175. doi: 10.1161/01.HYP.11.3_Pt_2.II101

Gladwell, D., Henry, T., Cook, M., and Akehurst, R. (2014). Cost effectiveness of renal denervation therapy for the treatment of resistant hypertension in the UK. *Appl. Health Econ. Health Policy* 12, 611–622. doi: 10.1007/s40258-014-0116-7

Helgeland, A. (1980). Treatment of mild hypertension: a five year controlled drug trial. The Oslo study. *Am. J. Med.* 69, 725–732. doi: 10.1016/0002-9343(80)90438-6

Howard, J. P., Nowbar, A. N., and Francis, D. P. (2013). Size of blood pressure reduction from renal denervation: insights from meta-analysis of antihypertensive drug trials of 4,121 patients with focus on trial design: the CONVERGE report. *Heart* 99, 1579–1587. doi: 10.1136/heartjnl-2013-304238

Jin, Y., Persu, A., and Staessen, J. A. (2014). Letter by Jin et al. regarding article, "ambulatory blood pressure changes after renal sympathetic denervation in patients with resistant hypertension". *Circulation* 129, e499. doi: 10.1161/CIRCULATIONAHA.113.005121

Julius, S., and Esler, M. (1975). Autonomic nervous cardiovascular regulation in borderline hypertension. *Am. J. Cardiol.* 36, 685–696. doi: 10.1016/0002-9149(75)90170-8

Julius, S., Kjeldsen, S. E., Weber, M., Brunner, H., Ekman, S., and Hansson, L. (2004). Cardiac events, stroke and mortality in high-risk hypertensives treated with valsartan or amlodipine: main outcomes of the value trial. *Lancet* 363, 2022–2031. doi: 10.1016/S0140-6736(04)16451-9

Jung, O., Gechter, J. L., Wunder, C., Paulke, A., Bartel, C., and Geiger, H. (2013). Resistant hypertension? Assessment of adherence by toxicological urine analysis. *J. Hypertens* 31, 766–774. doi: 10.1097/HJH.0b013e32835e2286

Kikuya, M., Hansen, T. W., Thijs, L., Björklund-Bodegård, K., Kuznetsova, T., and Ohkubo, T. (2007). For the IDACO investigators. Diagnostic thresholds for ambulatory blood pressure monitoring based on 10-year cardiovascular risk. *Blood Press Monit.* 12, 393–395. doi: 10.1097/MBP.0b013e3282f2b53d

Kjeldsen, S. E., Flaaten, B., Eide, I., Helgeland, A., and Leren, P. (1981). Increased peripheral release of noradrenaline and uptake of adrenaline in essential hypertension? *Clin. Sci. (Lond).* 61(Suppl. 7), 215s–217s.

Klein, L. E. (1988). Compliance and blood pressure control. *Hypertension* 11, I-161–164. doi: 10.1161/01.HYP.11.3_Pt_2.II61

Krum, H., Schlaich, M. P., Böhm, M., Mahfoud, F., Rocha-Singh, K., and Katholi, R. (2014). Percutaneous renal denervation in patients with treatment-resistant hypertension: final 3-year report of the Symplicity HTN-1 study. *Lancet* 383, 622–629. doi: 10.1016/S0140-6736(13)62192-3

Krum, H., Schlaich, M., Whitbourn, R., Sobotka, P. A., Sadowski, J., and Bartus, K. (2009). Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 373, 1275–1281. doi: 10.1016/S0140-6736(09)60566-3

Lobo, M. D., de Belder, M. A., Cleveland, T., Collier, D., Dasgupta, I., Deanfield, J., et al. (2015). Joint UK societies' 2014 consensus statement on renal denervation for resistant hypertension. *Heart* 101, 10–16. doi: 10.1136/heartjnl-2014-307029

Longland, C. J., and Gibb, W. E. (1954). Sympathectomy in the treatment of benign and malignant hypertension; A review of 76 patients. *Br. J. Surg.* 41, 382–392. doi: 10.1002/bjs.18004116814

Lüscher, T. F., and Mahfoud, F. (2014). Renal nerve ablation after SYMPLICITY HTN-3: confused at the higher level? *Eur. Heart J.* 35, 1706–1711. doi: 10.1093/eurheartj/ehu195

Mahfoud, F., Ukena, C., Schmieder, R. E., Cremers, B., Rump, L. C., and Vonend, O. (2013). Ambulatory blood pressure changes after renal sympathetic denervation in patients with resistant hypertension. *Circulation* 128, 132–140. doi: 10.1161/CIRCULATIONAHA.112.000949

Mangione-Smith, R., Elliott, M. N., McDonald, L., and McGlynn, E. A. (2002). An observational study of antibiotic prescribing behavior and the Hawthorne effect. *Health Serv. Res.* 37, 1603–1623. doi: 10.1111/1475-6773.10482

O'Brien, E., Parati, G., Stergiou, G., Asmar, R., Beilin, L., and Bilo, G. (2013). European society of hypertension position paper on ambulatory blood pressure monitoring. *J. Hypertens* 31, 1731–1768. doi: 10.1097/HJH.0000000000000221

Ott, C., Mahfoud, F., Schmid, A., Ditting, T., Sobotka, P. A., and Veelken, R. (2013). Renal denervation in moderate treatment-resistant hypertension. *J. Am. Coll. Cardiol.* 62, 1880–1886. doi: 10.1016/j.jacc.2013.06.023

Oz, M. (2012). Pressure relief. This year brings a breakthrough procedure to fight hypertension. *Time Mag.* 179, 40.

Pathak, A., Ewen, S., Fajadet, J., Honton, B., Mahfoud, F., and Marco, J. (2014). From SYMPLICITY HTN-3 to the renal denervation global registry: where do we stand and where should we go? *Eur. Intervent.* 10, 21–23. doi: 10.4244/EIJV10I1A4

Persu, A., Azizi, M., Burnier, M., and Staessen, J. A. (2013a). Residual effect of renal denervation in patients with truly resistant hypertension. *Hypertension* 62, 450–452. doi: 10.1161/HYPERTENSIONAHA.113.01632

Persu, A., Jin, Y., Azizi, M., Baelen, M., Völz, S., and Elvan, A. (2014a). On behalf of the European Network Coordinating research on REnal denervation (ENCOReD). Blood pressure changes after renal denervation at 10 European expert centers. *J. Hum. Hypertens* 28, 150–156. doi: 10.1038/jhh.2013.88

Persu, A., Jin, Y., Baelen, M., Vink, E., Verloop, W. L., and Schmidt, B. (2014b). European Network Coordinating research on REnal Denervation (ENCOReD) Consortium. Eligibility for renal denervation: experience at 11 European expert centers. *Hypertension* 63, 1319–1325. doi: 10.1161/HYPERTENSIONAHA.114.03194

Persu, A., Jin, Y., Fadl ElMula, M. F. E., Jacobs, L., Renkin, J., and Kjeldsen, S. E. (2014c). Renal denervation after symplicity HTN-3: an update. *Curr. Hypertens Rep.* 16, 460. doi: 10.1007/s11906-014-0460-x

Persu, A., O'Brien, E., and Verdecchia, P. (2014d). Use of ambulatory blood pressure measurement in the definition of resistant hypertension: a review of the evidence. *Hypertens. Res.* 37, 967–972. doi: 10.1038/hr.2014.83

Persu, A., Renkin, J., Asayama, K., O'Brien, E., and Staessen, J. A. (2013b). Renal denervation in treatment-resistant hypertension: the need for restraint and more and better evidence. *Expert Rev. Cardiovasc Ther.* 11, 739–749. doi: 10.1586/erc.13.52

Persu, A., Renkin, J., Thijs, L., and Staessen, J. A. (2012). Renal denervation: ultima ratio or standard in treatment-resistant hypertension. *Hypertension* 60, 596–606. doi: 10.1161/HYPERTENSIONAHA.112.195263

Rosa, J., Widimský, P., Tousek, P., Petrák, O., Curila, K., and Waldauf, P. (2015). Randomized comparison of renal denervation versus intensified pharmacotherapy including spironolactone in true-resistant hypertension. Six months results from the Prague-15 Study. *Hypertension* 65, 407–413. doi: 10.1161/HYPERTENSIONAHA.114.04019

Salles, G. F., Cardoso, C. R., and Muxfeldt, E. S. (2008). Prognostic influence of office and ambulatory blood pressures in resistant hypertension. *Arch. Intern. Med.* 168, 2340–2346. doi: 10.1001/archinte.168.21.2340

Schmieder, R. E. (2014). How should data from SYMPLICITY HTN-3 be interpreted? *Nat. Rev. Cardiol.* 11, 375–376. doi: 10.1038/nrcardio.2014.70

Schmieder, R. E., Redon, J., Grassi, G., Kjeldsen, S. E., Mancia, G., and Narkiewicz, K. (2012). ESH position paper: renal denervation—an interventional therapy of resistant hypertension. *J. Hypertens* 30, 837–841. doi: 10.1097/HJH.0b013e328352ce78

Schmieder, R. E., Redon, J., Grassi, G., Kjeldsen, S. E., Mancia, G., and Narkiewicz, K. (2013). Updated ESH position paper on interventional therapy of resistant hypertension. *Eur. Intervent.* 9, R58–R66. doi: 10.4244/EIJV9SRA11

Shun-Shin, M. J., Howard, J. P., and Francis, D. P. (2014). Removing the hype from hypertension. Symplicity HTN-3 illustrates the importance of randomisation and blinding for exciting new treatments. *BMJ* 348:g1937. doi: 10.1136/bmj.g1937

Smithwick, R. H., and Thompson, J. E. (1953). Splanchnicectomy for essential hypertension, results in 1,266 cases. *JAMA* 152, 1501–1504. doi: 10.1001/jama.1953.03690160001001

Staessen, J. A., Thijs, L., Bieniaszewski, L., O'Brien, E. T., Palatini, P., and Davidson, C. (1996). Ambulatory monitoring uncorrected for placebo overestimates long-term antihypertensive action. Systolic Hypertension in Europe (SYST-EUR) Trial Investigators. *Hypertension* 27, 414–420. doi: 10.1161/01.HYP.27.3.414

Strauch, B., Petrák, O., Zelinka, T., Rosa, J., Somlóová, Z., and Indra, T. (2013). Precise assessment of noncompliance with the antihypertensive therapy in patients with resistant hypertension using toxicological serum analysis. *J. Hypertens* 31, 2455–2461. doi: 10.1097/HJH.0b013e3283652c61

Tomaszewski, M., White, C., Patel, P., Masca, N., Damani, R., and Hepworth, J. (2014). High rates of non-adherence to antihypertensive treatment revealed by high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) urine analysis. *Heart* 100, 855–861. doi: 10.1136/heartjnl-2013-305063

White, W. B., Turner, J. R., Sica, D. A., Bisognano, J. D., Calhoun, D. A., Townsend, R. R., et al. (2014). Detection, evaluation, and treatment of severe and resistant hypertension: Proceedings from an American Society of Hypertension Interactive Forum held in Bethesda, MD, USA, October 10th 2013. *J. Am. Soc. Hypertens* 8, 743–757. doi: 10.1016/j.jash.2014.06.005

Conflict of Interest Statement: Sverre E. Kjeldsen, Fadl Elmula M. Fadl Elmula and Anne C. Larstorp have been supported by grant from Hemo Sapiens; and Sverre E. Kjeldsen by unrestricted grants from AZ and Pronova. Sverre E. Kjeldsen has received lecture and consultancy honoraria from AZ, Bayer, Medtronic, MSD, Novartis, Serodus, and Takeda, and royalty payments from Gyldendal (publisher). Fadl Elmula M. Fadl Elmula has received speaker honorarium from Medtronic and Hemo Sapiens and Anne C. Larstorp from Hemo Sapiens and Merck et Co. Alexandre Persu declares no conflict of interest.

Received: 21 October 2014; accepted: 08 January 2015; published online: 09 February 2015.

Citation: Fadl Elmula FE, Larstorp AC, Kjeldsen SE, Persu A, Jin Y and Staessen JA (2015) Renal sympathetic denervation after Symplicity HTN-3 and therapeutic drug monitoring in severe hypertension. Front. Physiol. ***6****:9. doi: 10.3389/fphys.2015.00009 This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology.*

frontiers in Physiology

MINI REVIEW
published: 25 March 2015
doi: 10.3389/fphys.2015.00075

A clinician's perspective of the role of renal sympathetic nerves in hypertension

*Alexandros Briasoulis and George L. Bakris**

American Society of Hypertension Comprehensive Hypertension Center, Department of Medicine, The University of Chicago Medicine, Chicago, IL, USA

The renal sympathetic nerves have significant contribution to the control of different aspects of kidney function. Early animal studies of renal denervation in a large number of different models of hypertension showed that that RDN improved BP control. Recently, data from prospective cohorts and randomized studies showed that renal denervation therapy (RDN) is a safe procedure but is associated with only modest reduction of ambulatory blood pressure (BP) in patients on intensive medical therapy. The main goal of this article is to review the results of preclinical and clinical studies on the contribution of the renal sympathetic nervous system to hypertension and the therapeutic applications of catheter-based renal denervation.

Keywords: resistant hypertension, renal denervation therapy

OPEN ACCESS

Edited by:
Kate Denton,
Monash University, Australia

Reviewed by:
Gregory Fink,
Michigan State University, USA
Olaf Grisk,
University of Greifswald, Germany
Sebastian Kelle,
German Heart Institute Berlin, Germany

****Correspondence:***
George L. Bakris,
American Society of Hypertension Comprehensive Hypertension Center, Department of Medicine, The University of Chicago Medicine, 5841 S. Maryland Ave. MC 1027, Chicago, IL 60637, USA
gbakris@gmail.com

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *28 January 2015*
Accepted: *23 February 2015*
Published: *25 March 2015*

Citation:
Briasoulis A and Bakris GL (2015) A clinician's perspective of the role of renal sympathetic nerves in hypertension. Front. Physiol. 6:75. doi: 10.3389/fphys.2015.00075

Introduction

Sympathetic activation participates in the development of the hypertension, by promoting the initial blood pressure elevation in the early clinical stages of the disease, and maintaining the blood pressure elevation (Johns et al., 2011). The adrenergic overdrive triggers not only elevations in blood pressure but contributes over time to end-organ damage and metabolic abnormalities detected in hypertensive patients.

Four decades ago Muller and Barajas (1972) reported the anatomical basis for a direct action of the sympathetic nervous system on renal tubular function by showing that norepinephrine-containing renal sympathetic nerve terminals are in direct contact with the basal membrane of all renal tubular segments, suggesting that renal sympathetic nerve activity can regulate renal tubular transport function. Indeed, the efferent and afferent renal nerves convey sensory stimuli between the sympathetic system and the kidney, thus providing a control system for the regulation of renal function (DiBona and Kopp, 1997). Under normal conditions, the renal sympathetic nerves regulate sodium homeostasis and participate in the arterial blood pressure (BP) control. In hypertensives, pathological alterations of this system contribute to abnormalities in sodium reabsorption and poorly controlled BP.

Despite the success of drug therapy in treating HTN and reducing associated adverse cardiovascular effects, the percentage of patients achieving adequate BP control worldwide remains low. According to the National Health and Nutrition Examination Survey (NHANES) dataset (Egan et al., 2011; Persell, 2011) the prevalence of resistant hypertension (RH) was 8.9% in hypertensives and 12.8% in treated hypertensives. In randomized controlled trials one third of patients did not achieve BP targets despite receiving $\geq$3 antihypertensive agents (Cushman et al., 2002; Gupta et al., 2011). In a recent report of data from the Kaiser Permanente health care systems, the incidence of RH was 1.9% within 1.5

years (Daugherty et al., 2012) and was related to increased cardiovascular and renal events.

The complex renal sympathetic system recently became of interest as renal sympathetic denervation treatment (RDN) was introduced into the treatment of patients with RH. Early uncontrolled cohort studies of patients with RH confirmed the safety of the procedure and reported substantial office BP reduction (Krum et al., 2009, 2013; Symplicity HTN-2 Investigators, 2010; Esler et al., 2014). However, evidence derived from the first randomized controlled trial Simplicity HTN-3 (Bhatt et al., 2014), failed to meet its primary efficacy end point, suggesting that RDN does not significantly lower office or ambulatory BP compared to medical therapy. The goal of this article is to review the role of the sympathetic renal nervous system in hypertension, in light of the recently reported results of RDN in patients with RH.

Anatomy and Physiology of the Renal Sympathetic Nerves and Implications in Blood Pressure Control

The neural pathways for the sympathetic innervation of the kidney originate from the intermediolateral column of the spinal cord. Preganglionic fibers connect to ganglia along the sympathetic chain, and the splachnic ganglia including the paravertebral aortorenal ganglia (DiBona and Kopp, 1997). Within the ganglia, the preganglionic fibers connect with postganglionic neurons that then project to the kidney. Sympathetic outflow to the kidney is controlled by neural projections from brain nuclei such as the rostral ventrolateral medulla (RVLM), to intermediolateral column region of the spinal cord. Afferent sensory information arising from the renal sympathetic system travels to the nucleus tractus solitarius (NTS) where the central integration begins and as a result pathways are activated that track to the caudal ventrolateral medulla and RVLM.

The sympathetic nerves pass from the aortorenal ganglia and come in proximity with the renal artery to enter the kidney at the hilus after which they divide into smaller bundles in parallel with the divisions of the arterial circulation. As the nerves traverse deeper into the kidney, they begin to divide further and to form a network of fibers that penetrate throughout the cortex, juxtamedullary regions, and to a lesser extent in the medulla (Fazan et al., 2002). Each renal nerve bundle contains approximately 900 fibers. The vast majority of postganglionic sympathetic nerve fibers entering the kidney are unmyelinated with variable diameters (Sato et al., 2006). In human and animal models the maximal mean number of nerves was observed in the proximal and middle segments of the renal artery, whereas the least average number of nerves was seen in the distal segment. The circumferential distribution was greatest in the ventral and least in the dorsal regions (Tellez et al., 2013; Sakakura et al., 2014). In the main renal artery, distribution of the distance of nerves from the renal arterial lumen varied considerably, from <1 mm to >10 mm; however, the 75th percentile of the distance was 4.28 mm. Interestingly, 20% of hypertensive patients have additional small accessory renal arteries which also have sympathetic nerves (Sakakura et al., 2014).

The primary neurotransmitter released by the renal sympathetic nerves is norepinephrine. Stimulation of the renal sympathetic nerves increases norepinephrine production that results in increased sodium reabsorption by the renal tubular epithelial cells, contraction of smooth muscle cells (Esler et al., 2003), and renin release by the granular cells of the juxtaglomerular apparatus (Kopp et al., 1980).

Afferent nerve fibers are also found intrarenally in close vicinity to efferent sympathetic nerve fibers mainly projecting from the renal pelvis to the first neuron in the dorsal root ganglion (Stella and Zanchetti, 1991). Afferent nerves are less abundant compared to efferent and their proportion is not different between the proximal, middle, and distal segments. The peripheral axons of afferent renal sensory nerves may release substance P and calcitonin gene-related peptide as primary sensory neurotransmitters (Kopp et al., 2001). They exert an inhibitory effect on both ipsilateral and contralateral efferent renal sympathetic nerve activity. Inhibitory renorenal reflexes regulate of arterial pressure and sodium balance in normotensive healthy individuals leading to decreased afferent renal sympathetic nerve activity (Kopp et al., 2009). In various pathological conditions, activation of the afferent renal sensory nerves and the inhibitory renorenal reflexes are impaired. In these conditions, the excitatory renorenal reflexes will contribute to increased sodium retention and arterial pressure (Kopp et al., 2009).

Under normal quiet and unstressed conditions the level of renal sympathetic nerve activity does not affect renal blood flow. However, in states of anxiety and tension or in pathophysiological states, renal sympathetic nerve activity is sufficiently elevated so as to increase renal vascular resistance and decrease renal blood flow (Yoshimoto et al., 2004).

The activation of the renal sympathetic fibers has several effects. Firstly, efferent renal sympathetic activation decreases renal blood flow and glomerular filtration rate via contraction of the preglomerular smooth muscle cells. Secondly, it stimulates the release of norepinephrine from renal sympathetic nerves' terminals leading to direct activation of the postsynaptic alpha-1 adrenoceptors located on the renal tubular epithelial cells and the activation of beta-1 adrenoceptors on juxtaglomerular granular cells (Pettinger et al., 1985). The activation of beta1-adrenoreceptors on juxtaglomerular granular cells increases renin secretion rate, the stimulation of alpha1b-adrenoreceptors on renal tubular epithelial cells increases renal tubular sodium reabsorption, and the stimulation of alpha1a-adrenoreceptors on the renal arterial resistance vessels decreases renal blood flow (Pettinger et al., 1985; Plato, 2001).

The importance of increased renal sympathetic nerve activity in the development of hypertension was supported by the finding that renal denervation in a large number of different experimental animal models of hypertension either prevented, delayed the onset, or reduced the magnitude of the hypertension (Bonjour et al., 1969; Hesse and Johns, 1984; Kompanowska-Jezierska et al., 2001; Yoshimoto et al., 2004; Schlaich et al., 2009; Salman et al., 2010). Bonjour et al., showed that renal denervation in anesthetized dogs (Bonjour et al., 1969), increased in urinary flow rate and sodium excretion while neither renal blood flow nor glomerular filtration rate changed. They concluded that

this increased output of water and sodium was due to the withdrawal of a direct action of the renal sympathetic nerves acting on renal tubules. Different groups showed that renal denervation decreases sodium and water reabsorption in all tubular segments including the proximal tubules, the loop of Henle and the distal convoluted tubule (Bello-Reuss et al., 1975, 1977). Additionally, renal denervation blunts the ability of the kidney to increase renin secretion in response to normal renin releasing stimuli (Johns, 1985).

Also, the activation of the renal afferent nerves contributes directly to systemic hypertension by modulating central sympathetic nervous system activity and promoting vasopressin and oxytocin release from the neuro-hypophysis (Echtenkamp and Dandridge, 1989). Patients early in the course of essential hypertension often have been demonstrated to have increased efferent sympathetic activity to the kidneys (Katholi, 1983). On the other hand, patients with essential hypertension with chronic kidney disease have been found to have increased centrally mediated sympathetic activity, possibly mediated by increased afferent renal sensory nerve activity (Hausberg et al., 2002).

Based on results from animal models of hypertension, denervation of efferent nerves can reduce renin release and sodium retention, improve renal blood flow, and facilitate blood pressure control (Holmer et al., 1994) while the denervation of afferent sensory nerves could attenuate the kidneys' contribution to centrally mediated sympathetic nervous system activity (Katholi et al., 1983, 1984).

Renal Denervation Therapy for Patients with Treatment Resistant Hypertension

Surgical renal denervation has been studied in humans for the treatment of resistant hypertension and shown effective for reducing sympathetic outflow to the kidneys, and renin release, without adversely affecting other functions of the kidney such as glomerular filtration rate (GFR) and RBF. However, these surgical approaches were frequently complicated by severe orthostatic hypotension, and urinary incontinence impotence (Smithwick and Thompson, 1953).

Renal sympathetic denervation treatment (RDT) using a radiofrequency ablation catheter presents several significant advantages over surgical approaches targeting the renal sympathetic nerves. It is a localized procedure, it is minimally invasive, it has no systematic side effects, and its procedural and recovery times are very short. The Symplicity Renal Denervation System and newer multielectrode catheters comprise of endovascular energy delivery catheters and an automated radiofrequency generator. Once in place within the renal artery, the tip of the catheter is placed against the arterial wall in several places where it delivers radiofrequency energy to the surrounding sympathetic nerves according to a proprietary, computer-controlled algorithm. Typical procedure starts distally in the renal artery with the catheter being withdrawn by pulling and rotating the tip, and it involves at least 4 focal treatments with a distance of $\geq$5 mm between each site (Krum et al., 2009; Symplicity HTN-2 Investigators, 2010). Renal sympathetic nerves are more abundant in the superior area of the arterial ostium. Recent studies have shown that in the proximal segments of renal artery these nerved are localized >5 mm from the lumen, a distance which may be beyond the ablation depth of currently used catheters which is approximately 3–4 mm (Tzafriri et al., 2014).

Early Clinical Studies of RDN

The Symplicity HTN-1 study (Krum et al., 2009) assessed safety of RDN. Short-term repeat angiography and 6-month magnetic resonance angiography, available for 34 patients, revealed no residual luminal irregularities at any treatment site. The effectiveness of RDN was confirmed by renal norepinephrine (NE) spillover. This assay confirmed a significant mean post-treatment reduction in renal norepinephrine spillover of 47% in 10 randomly selected patients. In this cohort study, RDN lowered office systolic blood pressure by 27 mm Hg at 12 months, and 85% of the patients responded to therapy with a reduction of systolic blood pressure exceeding 10 mm Hg (Krum et al., 2009). Importantly, six of the 45 patients who underwent catheter-based renal denervation had office systolic blood pressure reductions of less than 10 mm Hg and were non-responders.

At 36 months office BP was reduced by an average of 32/14 mmHg in 88 patients with complete data with 6 non-responders only (Krum et al., 2013). Although, striking and sustained BP reductions were seen, ambulatory blood pressure monitoring was not used during follow-up and allowed medication adjustments during this period. After three years of follow-up of Symplicity HTN-1 there was still no indication that the number of antihypertensive medications could be reduced by RDN (Krum et al., 2013).

A recent larger prospective uncontrolled study specifically examined the BP response to RDN as measured by ambulatory BP monitoring (Mahfoud et al., 2013). In 346 subjects who underwent RDN following the Symplicity HTN-2 protocol were followed for up to 12 months, there was a significant reduction in 24-h systolic BP (−12 mm Hg) and diastolic BP (−7 mm Hg) at 12 months which was much smaller than the reported office SBP and DBP reduction. Both these studies had important limitations. Apart from being nonrandomized and uncontrolled, a high rate of subjects were lost to follow-up. In the study by Mahfoud et al (Kopp et al., 1980) the significant discrepancy between office BP and ambulatory BP reduction may have been due to large bias in office BP measurements.

The Symplicity HTN-2 multicenter, prospective, randomized trial (Symplicity HTN-2 Investigators, 2010) assessed the safety and change in office BP in 106 patients with RH. The inclusion criteria were the same with the first proof-of-concept study. Office BP was reduced by 32/12 mm Hg in the renal denervation group, but did not differ from baseline in the control group. Similar differences in home BP were seen between the two groups were observed. Also, RDN reduced BP during exercise without compromising chronotropic competence in patients with resistant hypertension (Ukena et al., 2011). There were no serious complications related to the device or procedure. The results of the 3-year follow-up analysis reported a pronounced sustained office SBP and DBP reduction with approximately 15% nonresponders and not substantial reduction in mean number

of medications (Esler et al., 2014). Despite the limited follow-up time, number of patients and lack of ambulatory BP this study showed a significant reduction in office BP can be safely achieved with catheter-based RDN in patients with resistant hypertension.

Symplicity HTN-3 and Recent Studies (Table 1)

The Symplicity HTN-3 (Bhatt et al., 2014) randomized 535 patients with resistant essential hypertension and an estimated glomerular filtration rate above 45 mL/min/1.73 m^2 to undergo renal denervation with previous treatment or to maintain previous treatment alone. At 6 months, the decrease in office and ambulatory systolic BP in the RDN group was a mean of 14.13 and 7 mm Hg respectively compared with a fall of 11.74 and 5 mm Hg in the control group. Neither of these differences in BP met the prespecified criteria for statistically significant superiority. Interestingly, the 7 mm Hg decrease in ambulatory systolic BP after RDN was similar to reduction in 24-h ambulatory systolic BP seen in the 12 patients in the Symplicity HTN-1 study (Krum et al., 2013) but less pronounced compared to the 11 mmHg difference seen in 20 patients from Symplicity HTN-2 (Symplicity HTN-2 Investigators, 2010). In the pre-specified subgroup analysis, office SBP was significantly reduced by RDN in the non-African American patients and those younger than age 60, but this was not translated into meaningful difference in ambulatory BP measurements (Bakris et al., 2014).

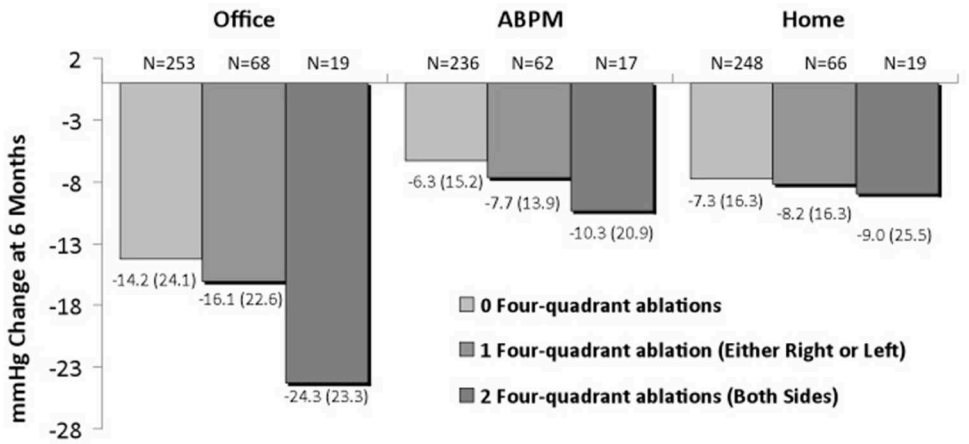
The major discrepancies between Symplicity HTN-3 and previous studies may be in part attributed to baseline population differences and selection bias. Symplicity HTN-3 included more obese patients, of African-American decent, at higher cardiovascular risk, treated with diuretics and aldosterone antagonists more frequently compared to Symplicity HTN-2. The Symplicity HTN-3 investigators may have been less experienced than the Symplicity HTN-1 and 2 investigators. Additionally, the selection of patients only based on elevated office BP in Symplicity HTN-1 and HTN-2 may have resulted in selection bias due to lack of standardization and substantial variability of BP affected by the regression to the mean phenomenon.

A more recent European cohort study of 109 patients with RH and a prospective uncontrolled trial of a new multielectrode catheter confirmed the disparities between office and ambulatory measurements and showed modest reduction of ambulatory BP (Worthley et al., 2013; Persu et al., 2014). In contrast to the disappointing reports of Symplicity HTN-3 and subsequent small studies, the recently reported Global SYMPLICITY Registry (Böhm et al., 2014) of 1000 consecutively enrolled patients not only confirmed the safety of RDN but also suggested that RDN lowers office and ambulatory BPs at 6 months. It is also noteworthy to mention that beyond BP reduction, RDN has also been shown to be effective in the treatment of other conditions coexisting with resistant hypertension such as impaired glucose tolerance (Mahfoud et al., 2011; Witkowski et al., 2011), obstructive sleep apnea severity (Witkowski et al., 2011), and left ventricular hypertrophy (Brandt et al., 2012). Brandt et al examined the effect of RDN on diastolic function and LVH in patients with resistant hypertension. Besides reduction of systolic and diastolic blood pressure at 1 and 6 months, similar to

TABLE 1 | Prospective randomized controlled trials of renal denervation therapy in resistant Hypertension.

Studies	Sample size (RDN/Controls)	Age	Number of antihypertensives	Catheter type	Method of BP measurement	ABPM difference from baseline (mmHg)	Office SBP/DBP difference from baseline (mmHg)	Follow-up (months)	Country
Symplicity-HTN 3, Bhatt et al., 2014	364/171	57.9 ± 10.4	5.1 ± 1.4	Symplicity	Office BP ABPM	RDN: −7/−4 Control:−6/−3	RDN: −14/−7 Control: −12/−5	6	USA
Rosa et al., 2014	52/54	56 ± 12	5.1 ± 1.2	Symplicity	Office BP ABPM	RDN: −9/−5 Control:−9/−4	RDN: −12/−7 Control: −14/−7	6	Czech Republic
Fadl Elmula et al., 2014	9/10	57 ± 10.9	5.1 ± 1.6	Symplicity	Office BP ABPM	RDN: −10/−7 Control:−19/−11	RDN: −12/−2 Control: −28/−11	6	Norway
Symplicity HTN-2 Investigators, 2010; Esler et al., 2014	52/54	58 ± 12	5.2 ± 1.5	Symplicity	Office BP ABPM	RDN: −11/−7 Control: −3/−1	RDN: −32/−12 Control: 1/0	6	Australia, Europe
Ukena et al., 2011	37/9	59 ± 9.4	5.9 ± 1.4	Symplicity	Office BP	-	RDN:−31/−9 Control:0/1	3	Germany
Pokushalov et al., 2012	13/14	56.5 ± 9	3.8 ± 0.4	Navistar/ThermoCool	Office BP	-	RDN:−15/−7 Control: −10/−2	12	Russia, USA

BP, Blood Pressure; ABPM, Ambulatory Blood Pressure Monitoring; HTN, Hypertension; RDN, Renal Denervation Therapy.



Baseline SBP Measurements (mm Hg)			
0 four-quadrant tx*	179.6	158.7	168.5
1 Four-quadrant tx	178.8	161.2	171.3
2 four-quadrant tx	186.9	159.9	170.4

*1 superior, 1 inferior and 2 anterior/ posterior; tx, treatment

FIGURE 1 | Systolic blood pressure change at symplicity HTN-3 study at 6 months according to ablation pattern.

the effect observed in the Symplicity-2 HTN trial, RDN significantly reduced LVH and improved E/E′ prime ratio and isovolumetric relaxation time as well as systolic LV function (Brandt et al., 2012). Notably, in 5 non-responders LV mass index was significantly decreased while in 4 non-responders the diastolic function was significantly improved, indicating BP-independent effects of RD on LVH and diastolic dysfunction. More recently, Mahfoud et al demonstrated a decrease in LV mass index, as assessed by using cardiac Magnetic Resonce, in both responders and non-responders undergoing RDN (Mahfoud et al., 2014). The beneficial effects of RDN on LV mass independently of BP reduction were confirmed by Doltra et al in 23 patients undergoing RDN who exhibited reduction of LV mass not exclusively due to a reversion of myocyte hypertrophy but also to reduction of interstitial myocardial fibrosis (Doltra et al., 2014). It's presumed that these beneficial effects are linked to actions of RDN on renin-angiotensin-aldosterone axis and sympathic nervous system activity as discussed above.

However, the favorable metabolic effects of RDN were not confirmed by Symplicity HTN-3, which did not show any significant between-group difference in the change in glycated hemoglobin levels in the RDN group or in the subgroup of patients with diabetes (Bhatt et al., 2014).

Based on the findings of the first cohorts and randomized trials a number of concerns arise regarding the utility of RDN on patients with RH: (i) A limited number of patients with RH are candidates for the procedure due to presence of secondary form of HTN, CKD, normal home BP measurement or unsuitable anatomy. (ii) A significant portion of patients (15–30%) will have less than 10/5 mmHg BP reduction with RDT due to procedural-related limitations, operator experience and number of treatment delivered. In a subgroup analysis of Symplicity HTN-3 higher number of ablations (10–13) and also ablations in all for quadrants of the arterial wall cross sections (**Figure 1**) were associated with significant ambulatory BP reduction compared to the sham control group (Kandzari et al., 2015). (iii) Non-adherence to antihypertensive regimens affects more than 50% of patients with difficult to control hypertension (Jung et al., 2013). In Symplicity-HTN 3, appropriate combination and dosage of antihypertensive regimens, improved patient compliance and assessment with home and ambulatory BP led to substantial BP reduction in the control group which was greater compared to previous RDN trials. The importance of medication adherence and structured adjustment of antihypertensive medications was also shown in the recently published Oslo RDN trial (Fadl Elmula et al., 2014), which stopped early in view of the dramatic superiority of adjusted drug treatment and witnessed medication intake compared to RDN at 6 months of follow-up. (iv) Finally, RDN may not be suitable for all subgroups of patients regardless of the degree of sympathetic activity. In SYMPLICITY HTN-3 subgroup analysis revealed that African American control patients demonstrated an unusually greater decrease in systolic blood pressure compared with non-African American controls and a blunted response to RDN compared to non-African Americans. The marked reduction in blood pressure in the sham control group could be related to a change in medical adherence, type

of therapy or degree of sympathetic activation (Kandzari et al., 2015).

Conclusion

The renal sympathetic nerves have significant contribution to the control of different aspects of kidney function. Early animal studies of renal denervation in a large number of different models of hypertension showed that that RDN either prevented, delayed the onset, or reduced the magnitude of the hypertension. Additionally, the preclinical and clinical studies reviewed above, have provided comprehensive insight into the mechanisms that account for the BP lowering during suppression of renal sympathetic outflow and propose an alternative approach to improve BP control in patients with resistant hypertension. Future randomized trials should be performed in experienced centers using newer catheters and better designed techniques in carefully selected compliant patients on appropriate antihypertensive drug combinations in whom all other measures have failed.

References

Bakris, G. L., Townsend, R. R., Liu, M., Cohen, S. A., D'Agostino, R., Flack, J. M., et al. (2014). SYMPLICITY HTN-3 investigators. Impact of renal denervation on 24-hour ambulatory blood pressure: results from SYMPLICITY HTN-3. *J. Am. Coll. Cardiol.* 64, 1071–1078. doi: 10.1016/j.jacc.2014.05.012

Bello-Reuss, E., Colindres, R. E., Pastoriza-Munoz, E., Mueller, R. A., and Gottschalk, C. W. (1975). Effects of acute unilateral renal denervation in the rat. *J. Clin. Invest.* 56, 208–217. doi: 10.1172/JCI108069

Bello-Reuss, E., Pastoriza-Munoz, E., and Colindres, R. E. (1977). Acute unilateral renal denervation in rats with extracellular volume expansion. *Am. J. Physiol. Renal. Physiol.* 232, F26–F32.

Bhatt, D. L., Kandzari, D. E., O'Neill, W. W., D'Agostino, R., Flack, J. M., Katzen, B. T., et al. (2014). A controlled trial of renal denervation for resistant hypertension. *N. Engl. J. Med.* 370, 1393–1401. doi: 10.1056/NEJMoa1402670

Böhm, M., Mahfoud, F., Ukena, C., Sinhal, A., Chew, D. P., Meredith, I. T., et al. (2014). *Effect of Renal Denervation in a Real World Population of Patients with Uncontrolled Hypertension - The Global SYMPLICITY Registry*. Washington, DC: Late Breaking Clinical Trials.

Bonjour, J. P., Churchill, P. C., and Malvin, R. L. (1969). Change of tubular reabsorption of sodium and water after renal denervation in the dog. *J. Physiol.* 204, 571–582. doi: 10.1113/jphysiol.1969.sp008932

Brandt, M. C., Mahfoud, F., and Reda, S. (2012). Renal sympathetic denervation reduces left ventricular hypertrophy and improves cardiac function in patients with resistant hypertension. *J. Am. Coll. Cardiol.* 59, 901–909. doi: 10.1016/j.jacc.2011.11.034

Cushman, W. C., Ford, C. E., Cutler, J. A., Margolis, K. L., Davis, B. R., and Grimm, R. H. (2002). For the ALLHAT Collaborative Research Group Success and predictors of blood pressure control in diverse North American settings: the Antihypertensive and lipid-lowering and treatment to prevent heart attack trial (ALLHAT). *J. Clin. Hypertens.* 4, 393–404. doi: 10.1111/j.1524-6175.2002.02045.x

Daugherty, S. L., Powers, J. D., Magid, D. J., Tavel, H. M., Masoudi, F. A., Margolis, K. L., et al. (2012). Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation* 125, 1635–1642. doi: 10.1161/CIRCULATIONAHA.111.068064

DiBona, G. F., and Kopp, U. C. (1997). Neural control of renal function. *Physiol. Rev.* 77, 75–197.

Doltra, A., Messroghli, D., Stawowy, P., Hassel, J. H., Gebker, R., Leppänen, O., et al. (2014). Potential reduction of interstitial myocardial fibrosis with renal denervation. *J. Am. Heart Assoc.* 3:e001556. doi: 10.1161/JAHA.114.001353

Echtenkamp, S. T., and Dandridge, P. F. (1989). Sympathetic nerve stimulation on renal function in the primate. *Am. J. Physiol.* 257, F204–F209.

Egan, B. M., Zhao, Y., Axon, R. N., Brzezinski, W. A., and Ferdinand, K. C. (2011). Uncontrolled and apparent treatment resistant hypertension in the United States, 1988 to 2008. *Circulation* 124, 1046–1058. doi: 10.1161/CIRCULATIONAHA.111.030189

Esler, M. D., Böhm, M., Sievert, H., Rump, C. L., Schmieder, R. E., Krum, H., et al. (2014). Catheter-based renal denervation for treatment of patients with treatment-resistant hypertension: 36 month results from the SYMPLICITY HTN-2 randomized clinical trial. *Eur. Heart J.* 35, 1752–1759. doi: 10.1093/eurheartj/ehu209

Esler, M., Lambert, G., Brunner-La Rocca, H. P., Vaddadi, G., and Kaye, D. (2003). Sympathetic nerve activity and neurotransmitter release in humans: translation from pathophysiology into clinical practice. *Acta Physiol. Scand.* 177, 275–284. doi: 10.1046/j.1365-201X.2003.01089.x

Fadl Elmula, F. E., Hoffmann, P., Larstorp, A. C., Paulke, A., Bartel, C., Geiger, H., et al. (2014). Adjusted drug treatment is superior to renal sympathetic denervation in patients with true treatment-resistant hypertension. *Hypertension* 63, 991–999. doi: 10.1161/HYPERTENSIONAHA.114.03246

Fazan, V. P., Ma, X., Chapleau, M. W., and Barreira, A. (2002). A. Qualitative and quantitative morphology of renal nerves in C57BL/6J mice. *Anat. Rec.* 268, 399–404. doi: 10.1002/ar.10174

Gupta, A. K., Nasothimiou, E. G., Chane, C. L., Sever, P. S., Dahlöf, B., Poulter, N. R., et al. (2011). Baseline predictors of resistant hypertension in the Anglo-Scandinavian Cardiac Outcome Trial (ASCOT): a risk score to identify those at high-risk. *J. Hypertens.* 29, 2004–2013. doi: 10.1097/HJH.0b013e32834a8a42

Hausberg, M., Kosch, M., Harmelinck, P., Barenbrock, M., Hohage, H., Kisters, K., et al. (2002). Sympathetic nerve activity in end-stage renal disease. *Circulation* 106, 1974–1979. doi: 10.1161/01.CIR.0000034043.16664.96

Hesse, I. F., and Johns, E. J. (1984). The effect of graded renal nerve stimulation on renal function in the anaesthetized rabbit. *Comp. Biochem. Physiol. A* 79, 409–414. doi: 10.1016/0300-9629(84)90536-X

Holmer, S., Rinne, B., Eckardt, K. U., Le Hir, M., Schricker, K., Kaissling, B., et al. (1994). Role of renal nerves for the expression of renin in adult rat kidney. *Am. J. Physiol.* 266, F738–F745.

Johns, E. J. (1985). Role of the renal nerves in modulating renin release during pressure reduction at the feline kidney. *Clin Sci (Lond.)* 69, 185–195.

Johns, E. J., Kopp, U. C., and DiBona, G. F. (2011). Neural control of renal function. *Compr. Physiol.* 1, 731–767. doi: 10.1002/cphy.c100043

Jung, O., Gechter, J. L., Wunder, C., Paulke, A., Bartel, C., Geiger, H., et al. (2013). Resistant hypertension? Assessment of adherence by toxicological urine analysis. *J. Hypertens.* 31, 766–774. doi: 10.1097/HJH.0b013e32835e2286

Kandzari, D., Bhatt, D., Brar, S., Devireddy, C. M., Esler, M., Fahy, M., et al. (2015). Predictors of blood pressure response in the SYMPLICITY HTN-3 trial. *Eur. Heart J.* 36, 219–227. doi: 10.1093/eurheartj/ehu441

Katholi, R. E. (1983). Renal nerves in the pathogenesis of hypertension in experimental animals and humans. *Am. J. Physiol.* 245, F1–F14.

Katholi, R. E., Hageman, G. R., Whitlow, P. L., and Woods, W. T. (1983). Hemodynamic and afferent renal nerve responses to intrarenal adenosine in the dog. *Hypertension* 5(Suppl. I), I-149–I-154. doi: 10.1161/01.HYP.5.2_Pt_2.I149

Katholi, R. E., Whitlow, P. L., Hageman, G. R., and Woods, W. T. (1984). Intrarenal adenosine produces hypertension by activating the sympathetic nervous system via the renal nerves in the dog. *J. Hypertens.* 2, 349–359. doi: 10.1097/00004872-198402040-00005

Kompanowska-Jezierska, E.,Walkowska, A., Johns, E. J., and Sadowski, J. (2001). Early effects of renal denervation in the anaesthetised rat: natriuresis and increased cortical blood flow. *J. Physiol.* 531, 527–534. doi: 10.1111/j.1469-7793.2001.0527i.x

Kopp, U., Aurell, M., Nilsson, I. M., and Ablad, B. (1980). The role of beta-1-adrenoceptors in the renin release response to graded renal sympathetic nerve stimulation. *Pflugers Arch.* 387, 107–113. doi: 10.1007/BF00584260

Kopp, U. C., Cicha, M. Z., Smith, L. A., and Hokfelt, T. (2001). Nitric oxide modulates renal sensory nerve fibers by mechanisms related to substance P receptor activation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 281, R279–R290.

Kopp, U. C., Grisk, O., Cicha, M. Z., Smith, A., Steinbach, A., Schluter, T., et al. (2009). Dietary sodium modulates the interaction between efferent renal sympathetic nerve activity and afferent renal nerve activity: Role of endothelin. *Am. J. Physiol.* 297, R337–R351. doi: 10.1152/ajpregu.91029.2008

Krum, H., Schlaich, M. P., Böhm, M., Böhm, M., Mahfoud, F., Rocha-Singh, K., et al. (2013). Percutaneous renal denervation in patients with treatment-resistant hypertension: final 3-year report of the Symplicity HTN-1 study. *Lancet* 383, 583–584. doi: 10.1016/S0140-6736(13)62192-3

Krum, H., Schlaich, M., Whitbourn, R., Sobotka, P. A., Sadowski, J., Bartus, K., et al. (2009). Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 373, 1275–1281. doi: 10.1016/S0140-6736(09)60566-3

Mahfoud, F., Schlaich, M., Kindermann, I., Ukena, C., Cremers, B., Brandt, M. C., et al. (2011). Effect of renal sympathetic denervation on glucose metabolism in patients with resistant hypertension: a pilot study. *Circulation* 123, 1940–1946. doi: 10.1161/CIRCULATIONAHA.110.991869

Mahfoud, F., Ukena, C., Schmieder, R. E., Cremers, B., Rump, L. C., Vonend, O., et al. (2013). Ambulatory blood pressure changes after renal sympathetic denervation in patients with resistant hypertension. *Circulation*. 128, 132–140. doi: 10.1161/CIRCULATIONAHA.112.000949

Mahfoud, F., Urban, D., Teller, D., Linz, D., Stawowy, P., Hassel, J. H., et al. (2014). Effect of renal denervation on left ventricular mass and function in patients with resistant hypertension: data from a multi-centre cardiovascular magnetic resonance imaging trial. *Eur. Heart J.* 35, 2224–2231. doi: 10.1093/eurheartj/ehu093

Muller, J., and Barajas, L. (1972). Electron microscopic and histochemical evidence for a tubular innervation in the renal cortex of the monkey. *J. Ultrastruct. Res.* 41, 533–549. doi: 10.1016/S0022-5320(72)90054-8

Persell, S. D. (2011). Prevalence of resistant hypertension in the United States, 2003-2008. *Hypertension* 57, 1076–1080. doi: 10.1161/HYPERTENSIONAHA.111.170308

Persu, A., Jin, Y., Azizi, M., Baelen, M., Völz, S., Elvan, A., et al. (2014). Blood pressure changes after renal denervation at 10 European expert centers. *J. Hum. Hypertens.* 28, 150–156. doi: 10.1038/jhh.2013.88

Pettinger, W. A., Smyth, D. D., and Umemura, S. (1985). Renal alpha 2-adrenoceptors, their locations and effects on sodium excretion. *J. Cardiovasc. Pharmacol.* 7, S24–S27. doi: 10.1097/00005344-198500078-00004

Plato, C. F. (2001). Alpha-2 and beta-adrenergic receptors mediate NE's biphasic effects on rat thick ascending limb chloride flux. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 281, R979–R986.

Pokushalov, E., Romanov, A., Corbucci, G., Artyomenko, S., Baranova, V., Turov, A., et al. (2012). A randomized comparison of pulmonary vein isolation with versus without concomitant renal artery denervation in patients with refractory symptomatic atrial fibrillation and resistant hypertension. *J. Am. Coll. Cardiol.* 60, 1163–1170. doi: 10.1016/j.jacc.2012.05.036

Rosa, J., Widimsky, P., Tousek, P., Petrák, O., Čurila, K., Waldauf, P., et al. (2014). Randomized comparison of renal denervation versus intensified pharmacotherapy including spironolactone in true-resistant hypertension: six-month results from the Prague-15 study. *Hypertension* 65, 407–413. doi: 10.1161/HYPERTENSIONAHA.114.04019

Sakakura, K., Ladich, E., Cheng, Q., Otsuka, F., Yahagi, K., Fowler, D. R., et al. (2014). Anatomic assessment of sympathetic peri-arterial renal nerves in man. *J. Am. Coll. Cardiol.* 64, 635–643. doi: 10.1016/j.jacc.2014.03.059

Salman, I. M., Sattar, M. A., Abdullah, N. A., Ameer, O. Z., Hussain, F. B., Hye Khan, M. A., et al. (2010). Renal functional and hemodynamic changes following unilateral renal denervation in Sprague-Dawley rats. *Indian J. Med. Res.* 131, 76–82.

Sato, K. L., do Carmo, J. M., and Fazan, V. P. (2006). Ultrastructural anatomy of the renal nerves in rats. *Brain Res.* 1119, 94–100. doi: 10.1016/j.brainres.2006.08.044

Schlaich, M. P., Sobotka, P. A., Krum, H., Lambert, E., and Esler, M. D. (2009). Renal sympathetic-nerve ablation for uncontrolled hypertension. *N. Engl. J. Med.* 361, 932–934. doi: 10.1056/NEJMc0904179

Smithwick, R. H., and Thompson, J. E. (1953). Splanchnicectomy for essential hypertension; results in 1,266 cases. *J. Am. Med. Assoc.* 152, 1501–1504. doi: 10.1001/jama.1953.03690160001001

Stella, A., and Zanchetti, A. (1991). Functional role of renal afferents. *Physiol. Rev.* 71, 659–682.

Symplicity HTN-2 Investigators. (2010). Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomized controlled trial. *Lancet* 376, 1903–1909. doi: 10.1016/S0140-6736(10)62039-9

Tellez, A., Rousselle, S., Palmieri, T., Rate, W. R. IV, Wicks, J., Degrange, A., et al. (2013). Renal artery nerve distribution and density in the porcine model: biologic implications for the development of radiofrequency ablation therapies. *Transl. Res.* 162, 381–389. doi: 10.1016/j.trsl.2013.07.002

Tzafriri, A. R., Mahfoud, F., Keating, J. H., Markham, P. M., Spognardi, A., Wong, G., et al. (2014). Innervation patterns may limit response to endovascular renal denervation. *J. Am. Coll. Cardiol.* 64, 1079–1087. doi: 10.1016/j.jacc.2014.07.937

Ukena, C., Mahfoud, F., Kindermann, I., Barth, C., Lenski, M., Kindermann, M., et al. (2011). Cardiorespiratory response to exercise after renal sympathetic denervation in patients with resistant hypertension. *J. Am. Coll. Cardiol.* 58, 1176–1182. doi: 10.1016/j.jacc.2011.05.036

Witkowski, A., Prejbisz, A., Florczak, E., Kądziela, J., Śliwiński, P., Bieleñ, P., et al. (2011). Effects of renal sympathetic denervation on blood pressure, sleep apnea course, and glycemic control in patients with resistant hypertension and sleep apnea. *Hypertension* 58, 559–565. doi: 10.1161/HYPERTENSIONAHA.111.173799

Worthley, S. G., Tsioufis, C. P., Worthley, M. I., Sinhal, A., Chew, D. P., Meredith, I. T., et al. (2013). Safety and efficacy of a multi-electrode renal sympathetic denervation system in resistant hypertension: the EnligHTN I trial. *Eur. Heart J.* 34, 2132–2140. doi: 10.1093/eurheartj/eht197

Yoshimoto, M., Sakagami, T., Nagura, S., and Miki, K. (2004). Relationship between renal sympathetic nerve activity and renal blood flow during natural behavior in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 286, R881–R887. doi: 10.1152/ajpregu.00105.2002

Conflict of Interest Statement: Professor Bakris is a consultant for Medtronic and Kona and served as co-principal investigator of SYMPLICITY HTN-3. The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in PHYSIOLOGY

GENERAL COMMENTARY
published: 19 December 2014
doi: 10.3389/fphys.2014.00505

Is isolated systolic hypertension an indication for renal denervation?

Yutang Wang*

School of Applied and Biomedical Sciences, Federation University Australia, Mount Helen, VIC, Australia
**Correspondence: yutangwang000@gmail.com*

Edited by:
Carolyn J. Barrett, University of Auckland, New Zealand

Reviewed by:
Rohit Ramchandra, Florey Neuroscience Institute, Australia
Mark Knuepfer, Saint Louis University School of Medicine, USA

Keywords: blood pressure, clinical trials, isolated systolic hypertension, renal artery stenosis, renal denervation

A commentary on

Reduced effect of percutaneous renal denervation on blood pressure in patients with isolated systolic hypertension
by Ewen, S., Ukena, C., Linz, D., Kindermann, I., Cremers, B., Laufs, U., et al. (2014). Hypertension. doi: 10.1161/HYPERTENSIONAHA.114.04336

Ewen et al. recently reported in the journal *Hypertension* that they investigated, for the first time, the effect of renal denervation on blood pressure in 63 patients with isolated systolic hypertension (Ewen et al., 2014). The authors concluded that renal denervation reduced office and ambulatory blood pressure in patients with isolated systolic hypertension (Ewen et al., 2014). However, this conclusion may not be drawn, as renal denervation may not decrease ambulatory blood pressure in these patients. The potential risk of renal denervation may overweigh its benefit in patients with isolated systolic hypertension. Therefore, adjusted drug treatment may be recommended to these patients before renal denervation.

Ambulatory blood pressure monitoring is the gold standard to diagnose true hypertension and removes the white coat effect (Hermida et al., 2013). Ambulatory blood pressure is superior to office blood pressure in predicting cardiovascular events (Staessen et al., 1999) and mortality (Dolan et al., 2005). The 24-h ambulatory systolic blood pressure in these 63 patients in Ewen et al.'s report decreased by 8 ± 8 and 7 ± 8 mm Hg at 6 and 12 months respectively after renal denervation. However, this study lacked a control group as the authors pointed out as a limitation. It has been reported that the sham procedure reduced 24-h ambulatory systolic blood pressure by 5 ± 15 mm Hg at 6 months (Bhatt et al., 2014). Therefore, compared with the sham procedure, renal denervation may not decrease ambulatory blood pressure in those patients with isolated systolic hypertension.

Consequently, the risk posed to patients with isolated systolic hypertension by renal denervation may overweigh the minimal benefit of renal denervation via lowering blood pressure. For example, renal artery stenosis after renal denervation is of concern. The renal artery stenosis rate in the Symplicity HTN trials ($N = 45$, 106, and 535 for the Symplicity HTN-1, HTN-2, and HTN-3 trials, respectively) ranges from 0.3 to 2.2% (Krum et al., 2009; Esler et al., 2010; Bhatt et al., 2014). However, more and more studies with a smaller sample size (Worthley et al., 2013; Versaci et al., 2014) and case reports (Kaltenbach et al., 2012; Vonend et al., 2012; Aguila et al., 2014; Bacaksiz et al., 2014; Chandra et al., 2014; Pucci et al., 2014) showed relatively higher rates of development or progression of renal artery stenosis after renal denervation. Ewen et al. did not observe any hemodynamically significant renal artery stenosis in these 63 patients with isolated systolic hypertension within 12 months (Ewen et al., 2014). However, ultrasonography, which was used by the authors, has limitations in detecting renal artery stenosis (Zhang et al., 2009; Lao et al., 2011).

Renal denervation is regarded as a last resort for patients with resistant hypertension (Persu et al., 2012). It is reported that about 9% of adults with hypertension suffer from resistant hypertension (Persell, 2011), which is often defined as elevated blood pressure despite treatment with at least 3 antihypertensive agents including a diuretic at maximal tolerated or highest recommended doses (Bohm et al., 2014). The prevalence of resistant hypertension is likely overestimated due to drug non-adherence. For example, blood pressure in 20 of 65 patients with resistant hypertension was normalized after witnessed intake of antihypertensive drugs (Fadl Elmula et al., 2014). Resistant hypertension has been classified as "true" resistant hypertension if blood pressure is still elevated after witnessed intake of antihypertensive drugs (Fadl Elmula et al., 2014). Blood pressure in some patients with "true" resistant hypertension could be controlled by adjusted drug treatment. For example, Fadl Elmula et al. reported that adjusted drug treatment significantly decreased ambulatory systolic blood pressure from 152 ± 12 mm Hg at baseline to 133 ± 11 mm Hg at 6 months in 10 patients with "true" resistant hypertension (Fadl Elmula et al., 2014). In addition, adjusted drug treatment lowered ambulatory systolic blood pressure to below 135 mm Hg in 7 of these 10 patients with "true" resistant hypertension (Fadl Elmula et al., 2014). Therefore, patients with isolated systolic hypertension may be offered with adjusted drug treatment before being offered with renal denervation.

In summary, renal denervation may not decrease ambulatory blood pressure in patients with isolated systolic hypertension. Adjusted drug treatment may be recommended to these patients before renal denervation, as the risk might overweigh the benefit of renal denervation in these patients.

ACKNOWLEDGMENT

Yutang Wang is supported by the National Health and Medical Research Council (1062671).

REFERENCES

Aguila, F. J., Mediavilla Garcia, J. D., Navarro, E. M., Vargas Hitos, J. A., and Fernandez-Torres, C. (2014). Bilateral renal artery stenosis after renal denervation. *Hypertension* 63, e126–e127. doi: 10.1161/HYPERTENSIONAHA.113.03065

Bacaksiz, A., Uyarel, H., Jafarov, P., and Kucukbuzcu, S. (2014). Iatrogenic renal artery stenosis after renal sympathetic denervation. *Int. J. Cardiol.* 172, e389–e390. doi: 10.1016/j.ijcard.2013.12.270

Bhatt, D. L., Kandzari, D. E., O'Neill, W. W., D'Agostino, R., Flack, J. M., Katzen, B. T., et al. (2014). A controlled trial of renal denervation for resistant hypertension. *N. Engl. J. Med.* 370, 1393–1401. doi: 10.1056/NEJMoa1402670

Bohm, M., Linz, D., Ukena, C., Esler, M., and Mahfoud, F. (2014). Renal denervation for the treatment of cardiovascular high risk-hypertension or beyond? *Circ. Res.* 115, 400–409. doi: 10.1161/CIRCRESAHA.115.302522

Chandra, A. P., Marron, C. D., Puckridge, P., and Spark, J. I. (2014). Severe bilateral renal artery stenosis after transluminal radiofrequency ablation of renal sympathetic nerve plexus. *J. Vasc. Surg.* doi: 10.1016/j.jvs.2013.11.005. [Epub ahead of print].

Dolan, E., Stanton, A., Thijs, L., Hinedi, K., Atkins, N., McClory, S., et al. (2005). Superiority of ambulatory over clinic blood pressure measurement in predicting mortality: the Dublin outcome study. *Hypertension* 46, 156–161. doi: 10.1161/01.HYP.0000170138.56903.7a

Esler, M. D., Krum, H., Sobotka, P. A., Schlaich, M. P., Schmieder, R. E., and Bohm, M. (2010). Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 376, 1903–1909. doi: 10.1016/S0140-6736(10)62039-9

Ewen, S., Ukena, C., Linz, D., Kindermann, I., Cremers, B., Laufs, U., et al. (2014). Reduced effect of percutaneous renal denervation on blood pressure in patients with isolated systolic hypertension. *Hypertension.* doi: 10.1161/HYPERTENSIONAHA.114.04336. [Epub ahead of print].

Fadl Elmula, F. E., Hoffmann, P., Larstorp, A. C., Fossum, E., Brekke, M., Kjeldsen, S. E., et al. (2014). Adjusted drug treatment is superior to renal sympathetic denervation in patients with true treatment-resistant hypertension. *Hypertension* 63, 991–999. doi: 10.1161/HYPERTENSIONAHA.114.03246

Hermida, R. C., Smolensky, M. H., Ayala, D. E., and Portaluppi, F. (2013). 2013 ambulatory blood pressure monitoring recommendations for the diagnosis of adult hypertension, assessment of cardiovascular and other hypertension-associated risk, and attainment of therapeutic goals. *Chronobiol. Int.* 30, 355–410. doi: 10.3109/07420528.2013.750490

Kaltenbach, B., Id, D., Franke, J. C., Sievert, H., Hennersdorf, M., Maier, J., et al. (2012). Renal artery stenosis after renal sympathetic denervation. *J. Am. Coll. Cardiol.* 60, 2694–2695. doi: 10.1016/j.jacc.2012.09.027

Krum, H., Schlaich, M., Whitbourn, R., Sobotka, P. A., Sadowski, J., Bartus, K., et al. (2009). Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 373, 1275–1281. doi: 10.1016/S0140-6736(09)60566-3

Lao, D., Parasher, P. S., Cho, K. C., and Yeghiazarians, Y. (2011). Atherosclerotic renal artery stenosis–diagnosis and treatment. *Mayo Clin. Proc.* 86, 649–657. doi: 10.4065/mcp.2011.0181

Persell, S. D. (2011). Prevalence of resistant hypertension in the United States, 2003-2008. *Hypertension* 57, 1076–1080. doi: 10.1161/HYPERTENSIONAHA.111.170308

Persu, A., Renkin, J., Thijs, L., and Staessen, J. A. (2012). Renal denervation: ultima ratio or standard in treatment-resistant hypertension. *Hypertension* 60, 596–606. doi: 10.1161/HYPERTENSIONAHA.112.195263

Pucci, G., Battista, F., Lazzari, L., Dominici, M., Boschetti, E., and Schillaci, G. (2014). Progression of renal artery stenosis after renal denervation. *Circ. J.* 78, 767–768. doi: 10.1253/circj.CJ-13-0997

Staessen, J. A., Thijs, L., Fagard, R., O'Brien, E. T., Clement, D., de Leeuw, P. W., et al. (1999). Predicting cardiovascular risk using conventional vs ambulatory blood pressure in older patients with systolic hypertension. *JAMA* 282, 539–546.

Versaci, F., Trivisonno, A., Olivieri, C., Caranci, F., Brunese, L., and Prati, F. (2014). Late renal artery stenosis after renal denervation: is it the tip of the iceberg? *Int. J. Cardiol.* 172, e507–e508. doi: 10.1016/j.ijcard.2014.01.018

Vonend, O., Antoch, G., Rump, L. C., and Blondin, D. (2012). Secondary rise in blood pressure after renal denervation. *Lancet* 380, 778. doi: 10.1016/S0140-6736(12)61145-3

Worthley, S. G., Tsioufis, C. P., Worthley, M. I., Sinhal, A., Chew, D. P., Meredith, I. T., et al. (2013). Safety and efficacy of a multi-electrode renal sympathetic denervation system in resistant hypertension: the EnligHTN I trial. *Eur. Heart J.* 34, 2132–2140. doi: 10.1093/eurheartj/eht197

Zhang, H. L., Sos, T. A., Winchester, P. A., Gao, J., and Prince, M. R. (2009). Renal artery stenosis: imaging options, pitfalls, and concerns. *Prog. Cardiovasc. Dis.* 52, 209–219. doi: 10.1016/j.pcad.2009.10.003

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 October 2014; accepted: 03 December 2014; published online: 19 December 2014.

*Citation: Wang Y (2014) Is isolated systolic hypertension an indication for renal denervation? Front. Physiol. **5**:505. doi: 10.3389/fphys.2014.00505*

This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology.

frontiers in Physiology

REVIEW
published: 08 July 2015
doi: 10.3389/fphys.2015.00193

Device-based approaches for renal nerve ablation for hypertension and beyond

Alicia A. Thorp[1,2] *and Markus P. Schlaich*[1,3,4,5]*

[1] Neurovascular Hypertension and Kidney Disease Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia, [2] School of Public Health and Preventive Medicine, Monash University, Melbourne, VIC, Australia, [3] Department of Cardiovascular Medicine, Alfred Hospital, Melbourne, VIC, Australia, [4] Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, VIC, Australia, [5] Royal Perth Hospital Unit, School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia

OPEN ACCESS

Edited by:
Yutang Wang, Federation University Australia, Australia

Reviewed by:
Yutang Wang, Federation University Australia, Australia
Maarten Koeners, University Medical Centre Utrecht, Netherlands
Luciana A. Campos, Universidade Camilo Castelo Branco, Brazil

***Correspondence:**
Markus P. Schlaich, Royal Perth Hospital Unit, School of Medicine and Pharmacology, The University of Western Australia, Level 3, MRF Building, Rear 50 Murray St., Perth, WA 6000, Australia
markus.schlaich@uwa.edu.au

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *28 April 2015*
Accepted: *21 June 2015*
Published: *08 July 2015*

Citation:
Thorp AA and Schlaich MP (2015) Device-based approaches for renal nerve ablation for hypertension and beyond. Front. Physiol. 6:193. doi: 10.3389/fphys.2015.00193

Animal and human studies have demonstrated that chronic activation of renal sympathetic nerves is critical in the pathogenesis and perpetuation of treatment-resistant hypertension. Bilateral renal denervation has emerged as a safe and effective, non-pharmacological treatment for resistant hypertension that involves the selective ablation of efferent and afferent renal nerves to lower blood pressure. However, the most recent and largest randomized controlled trial failed to confirm the primacy of renal denervation over a sham procedure, prompting widespread re-evaluation of the therapy's efficacy. Disrupting renal afferent sympathetic signaling to the hypothalamus with renal denervation lowers central sympathetic tone, which has the potential to confer additional clinical benefits beyond blood pressure control. Specifically, there has been substantial interest in the use of renal denervation as either a primary or adjunct therapy in pathological conditions characterized by central sympathetic overactivity such as renal disease, heart failure and metabolic-associated disorders. Recent findings from pre-clinical and proof-of-concept studies appear promising with renal denervation shown to confer cardiovascular and metabolic benefits, largely independent of changes in blood pressure. This review explores the pathological rationale for targeting sympathetic renal nerves for blood pressure control. Latest developments in renal nerve ablation modalities designed to improve procedural success are discussed along with prospective findings on the efficacy of renal denervation to lower blood pressure in treatment-resistant hypertensive patients. Preliminary evidence in support of renal denervation as a possible therapeutic option in disease states characterized by central sympathetic overactivity is also presented.

Keywords: sympathetic overactivity, renal denervation, blood pressure, resistant hypertension, cardiovascular disease, renal nerve activity

Introduction

Treatment-resistant hypertension (rHTN) is a clinically important condition that is associated with increased cardiovascular morbidity and mortality risk (Daugherty et al., 2012; Irvin et al., 2014). Defined by the failure to achieve target blood pressure (BP) despite taking ≥3 antihypertensive medications at optimal doses, rHTN is estimated to affect 8−10% of all hypertensive adults

(Persell, 2011; De La Sierra et al., 2011). Poor adherence to pharmacotherapy is ubiquitous in this patient cohort hindering efforts for timely and consistent BP management (Jung et al., 2013), which has caused some clinicians to question whether rHTN really exists or is a case of difficult-to-treat hypertension. Despite the controversy, there is compelling evidence that chronic sympathetic outflow to and from the kidneys is involved in the pathophysiology of rHTN (Esler et al., 1989; Schlaich et al., 2004; Smith et al., 2004).

The emergence of catheter-based renal denervation (RDN)- a minimally invasive procedure used to ablate renal sympathetic nerves- has marked a paradigm shift in the therapeutic management of rHTN. The long-term safety and efficacy of RDN to control BP has principally been evidenced by open-label studies. In the last 12-months, several rigorously designed, randomized controlled trials have brought into question the efficacy of RDN to treat rHTN. Specifically, data from the Symplicity HTN-3 study failed to show the primacy of RDN in lowering BP compared to a sham-procedure in rHTN patients (Bhatt et al., 2014).

In addition to rHTN, sympathetic overactivity is a cardinal feature of several pathological conditions including renal disease, heart failure, left-ventricular hypertrophy, insulin resistance, and sleep apnea. The ability of RDN to alter renal afferent signaling and reduce whole body sympathetic nerve activity in these disease states is currently being explored in a number of preclinical and clinical studies with promising results.

This review will we focus on the importance of renal sympathetic nerves in the pathophysiology of rHTN, technological advancements in ablation modalities for RDN and latest prospective clinical findings. Novel therapeutic applications for RDN beyond BP control will also be discussed along with several critical issues that must be addressed for research going forward.

Renal Nerves: An Important Target for Blood Pressure Control

The kidneys are connected to the autonomic nervous system via a dense, neuronal network of post-ganglionic sympathetic nerve fibers located within the adventitia of the renal artery (Dibona, 2005). Renal efferent motor fibers innervate all parts of the renal vasculature, tubules, and juxtaglomerular apparatus (Barajas, 1978); while afferent sensory nerves are located principally in the renal pelvic wall and serve to connect the kidneys with autonomic centers in the central nervous system (Kopp, 1992).

Animal (Dibona and Kopp, 1997) and human (Esler, 2000) studies suggest chronic activation of renal sympathetic nerves is important in the pathogenesis and perpetuation of essential hypertension as well as other pathological conditions including heart failure, chronic kidney disease, and diabetes (see review Malpas, 2010). As highlighted in **Figure 1**, sustained efferent sympathetic outflow to the kidneys (via peripheral and central sensory inputs) elevates BP by altering renal vascular resistance (Kirchheim et al., 1989), stimulating renin release from juxaglomerular granular cells (Zanchetti, 1977) and increasing tubular sodium and water reabsorption (Bell-Reuss et al., 1976). Excitatory reflexes originating from afferent renal nerves in the kidney can also contribute to development of hypertension, particularly rHTN (Hering et al., 2014). Under normal physiological conditions, changes in hydrostatic pressures or chemical composition of the renal environment activate sensory afferent renal nerves to stimulate a centrally-mediated decrease in efferent renal sympathetic outflow via an inhibitory feedback mechanism, known as the renorenal reflex response.

Pathological activation of renal sensory afferent nerve fibers through renal ischemia, injury or elevated adenosine concentrations (Esler, 2010), can alter the activity of central integrative neuronal circuits involved in cardiovascular homeostasis and shift the renorenal reflex response from inhibitory to excitatory. The consequence is an increase in central efferent sympathetic outflow to the kidneys and to other highly innervated organs (such as the heart and vasculature) initiating the development and/or maintenance of hypertension and other pathological conditions.

The application of radiofrequency energy to ablate renal nerves in the adventitia can reduce efferent renal sympathetic activity [as evidenced by a reduction in noradrenaline spillover in the kidney (Krum et al., 2009)] to promote urinary sodium excretion and BP reduction; while the ablation of afferent renal sympathetic nerves lowers BP via the inhibition of central sympathetic outflow (Schlaich et al., 2009a).

Changing Face of Renal Denervation: Latest Developments in Device-based Nerve Ablation

Concomitant with the refinement of radiofrequency-based catheter ablation systems, there has been an emergence of novel treatment modalities that use highly differentiated approaches (i.e., ultrasonic nerve, pharmacological, and cryoablation) to ablate renal sympathetic nerves. These next-generation RDN systems may potentially offer several clinical advantages to radiofrequency-based modalities namely the ability to deliver energy circumferentially and penetrate deeper into the adventitia to optimize neural damage, while preserving artery integrity.

Intravascular and fully non-invasive ultrasound nerve ablation modalities have undergone extensive clinical investigation in the last 12–18 months (refer **Table 1**). Intravascular ultrasound ablation modalities use transducer-based catheters to deliver high-intensity, non-focused ultrasonic emissions (rapid mechanical oscillations) to renal nerves at a depth range of 0.5–10 mm. The latest iteration of the ReCor Percutaneous Renal Denervation System (the RADIANCE™ catheter) uses a novel radial access approach to deliver ultrasonic waves to the renal adventitia, promoting minimal invasiveness to the patient. The therapeutic intra-vascular ultrasound (TIVUS™) catheter system (Cardiosonic Ltd, Tel Aviv, Israel) similarly delivers ultrasonic waves to the renal adventitia but in the absence of endoluminal surface contact, preserving the

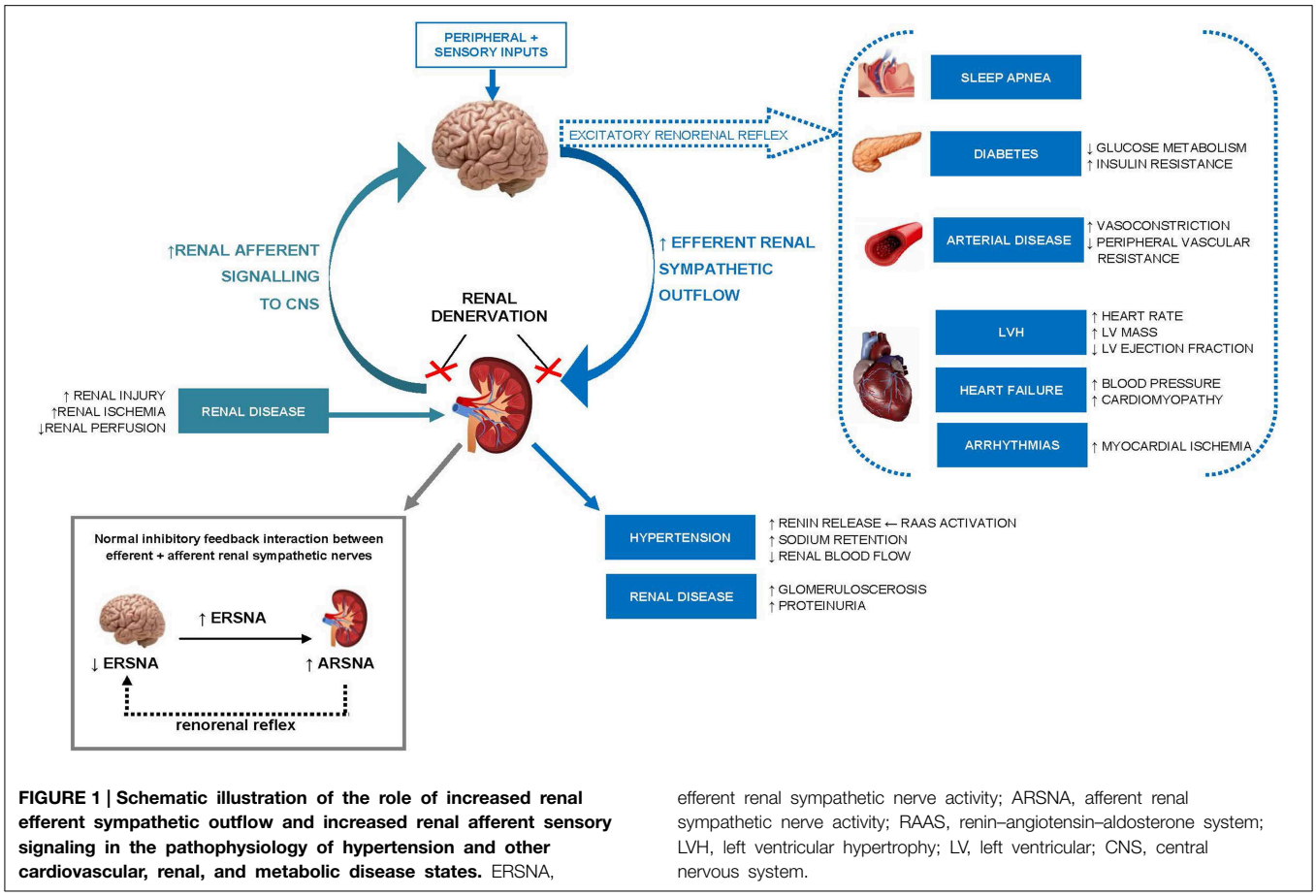


FIGURE 1 | Schematic illustration of the role of increased renal efferent sympathetic outflow and increased renal afferent sensory signaling in the pathophysiology of hypertension and other cardiovascular, renal, and metabolic disease states. ERSNA, efferent renal sympathetic nerve activity; ARSNA, afferent renal sympathetic nerve activity; RAAS, renin–angiotensin–aldosterone system; LVH, left ventricular hypertrophy; LV, left ventricular; CNS, central nervous system.

integrity of the artery. Results from first-in-man studies highlight the efficacy and safety of these latest modalities to reduce BP short-term (Jonas et al., 2014; Shetty et al., 2014; Daemen and Van Mieghem, 2015) with prospective, multi-center, clinical studies currently underway.

The Surround Sound Hypertension Therapy System (Kona Medical, Washington, USA) is the only fully external ultrasound-based RDN system being investigated as an adjunct therapy to medication in rHTN patients. Circumferential nerve ablation is achieved using externally focused, low-frequency ultrasonic waves delivered to the renal adventitia via an ultrasound imaging transducer. Initial data from a series of small clinical trials (WAVE I and WAVE II) found that 81% of patients (total $n = 41$ across the two studies) experienced clinically significant reductions in office SBP ($\geq$10 mmHg) at 6-months follow-up with no serious adverse events reported (Neuzil et al., 2014; Ormiston et al., 2014). Primary outcomes from a multi-center randomized, sham-controlled, double-blinded trial involving 132 subjects (WAVE IV) are expected to be released later this year. The findings will be of interest as only the second, sham-controlled clinical trial in rHTN patients and first to include a treatment arm of patients who have failed radiofrequency RDN modalities (Daemen and Van Mieghem, 2015).

Clinical Findings Update: Where is the Evidence-base on Renal Denervation?

In the past 12-months there have been 5 prospective, randomized controlled trials that have reported either a modest or no effect of RDN on BP reduction in patients with rHTN (Bhatt et al., 2014; Fadl Elmula et al., 2014; Azizi et al., 2015; Desch et al., 2015; Rosa et al., 2015). Of most relevance is the Symplicity HTN-3 study, the largest and most rigorously designed trial to date, which failed to meet its primary efficacy endpoint (mean 6-month change in office SBP) (Bhatt et al., 2014). Previously, the only prospective trial to compare the BP lowering effects of RDN to usual care had been the open-label Symplicity HTN-2 Study (Esler et al., 2010), which reported a significant office BP reduction of −32/−12 mmHg at 6-months following RDN. The Symplicity HTN-2 study is recognized as having several limitations, the most notable being the use of office BP over 24-h ambulatory BP monitoring to assess the efficacy of RDN and the absence of a blinded control drug adherence monitoring in the study.

As the first randomized, double-blinded, sham-controlled trial, the Symplicity HTN-3 study was expected to provide the definitive statement on the superiority of RDN in the treatment of severe rHTN (Bhatt et al., 2014). A total of 535

TABLE 1 | Overview of emerging intravascular and non-invasive ultrasound modalities for circumferential renal nerve ablation.

Product name	RDN modality	Description/feature	CE mark	Clinical trial data	Ongoing/planned trials
ReCor RADIANCE catheter system	Intravascular ultrasound nerve ablation	Cylindrical catheter advanced into renal artery via radial access. 3 unfocused, ultrasonic emissions delivered bilaterally.	Yes	First-in-man study ($n = 2$) At 3-mo follow-up mean decline in office BP (−40/−29 mmHg) and 24-h ambulatory BP (−11/−8 mmHg).	Prospective, single-arm, open-label study (REALISE Trial; $n = 20$)- ongoing not recruiting patients.
Cardiosonic TIVIS catheter system	Intravascular ultrasound nerve ablation	Catheter delivers high-frequency, high-intensity directional ultrasound emissions. Radiopaque tip positions catheter using fluoroscopic guidance. No endoluminal surface contact is required.	Yes	First-in-man study (TIVUS I; $n = 18$ with rHTN). Prospective, non-randomized, single-arm, open label study. Mean decline in office BP at 1-mo (−28/−10 mmHg; $n = 18$) and 3-mo follow-up (−25/−10 mmHg; $n = 16$).	Prospective, multicenter, non-randomized, single-arm, open-label clinical study using system next generation Multidirectional Catheter TIVUS™ (TIVUS II). Study will include treatment arm for patients who have failed radio-frequency RDN. To date 6 patients have been enrolled in TIVUS™ II – ongoing and recruiting patients.
Sound Interventions SOUND 360 catheter system	Intravascular ultrasound nerve ablation	Cylindrical transducer encased in a non-cylindrical, non-occlusive balloon delivers therapeutic ultrasound at specific dosimetry. 2 unfocused ultrasonic emissions delivered bilaterally.	No	First-in-man study (SOUND-ITV; $n = 10$ with rHTN). Mean decline in office BP (−25.6/−12.5 mmHg) and 24-h ambulatory BP (−23.1/−11.9 mmHg) at 3-mo follow-up. 3 patients developed groin hematomas not requiring intervention.	Nil
Kona Medical Surround Sound Hypertension Therapy system	Non-invasive ultrasound nerve ablation	Externally focused, low frequency ultrasonic emissions delivered bilaterally to the renal adventitia.	No	First-in-man study (WAVE I; $n = 24$) At 6-mo follow-up, mean −27 mmHg decline in office SBP. WAVE II trial: $n = 13$ with rHTN. At 6-week follow-up ($n = 8$), mean −18 mmHg decline in office SBP, no decline in office DBP. No serious adverse events. WAVE III trial- completed, results not yet disclosed.	Multi-center, randomized, sham-controlled, double-blind trial (WAVE IV; target $n = 132$). Study will include treatment arm for patients who have failed alternate forms of RDN-ongoing and recruiting patients.

rHTN, resistant hypertension.

patients were assigned in a 2:1 ratio to receive either the RDN or sham-procedure. Treatment rHTN was confirmed at baseline using 24-h ambulatory BP monitoring following 2-weeks of stable, maximally tolerated doses of ≥3 antihypertensive medications of complementary classes (including a diuretic). The 6-month follow-up data revealed significant office BP reductions in both treatment groups (RDN: −14.1/6.8 mmHg vs. SHAM: −11.7/4.8 mmHg; both $p < 0.001$). However, between-treatment differences in office BP reduction at 6-months were not significant (−2.4/2.0 mmHg; $P = 0.26$). Concomitantly, no superior treatment effect of RDN over the sham-procedure for mean change in 24-h ($p = 0.98$), daytime ($p = 0.52$), or nighttime ($p = 0.06$) ambulatory SBP was observed (Bakris et al., 2014).

Clearly, the failure of Symplicity HTN-3 to show a clear-cut superiority of RDN over the sham-procedure in lowering BP (modest ∼2 mmHg SBP reduction only) was disappointing, but contemplated by some (Howard et al., 2013). Evidence from

a recent meta-analysis that combined office and ambulatory BP data from 10 European centers predicted similar, modest 6-month BP reductions following RDN and large variability in patient's BP responsiveness (Persu et al., 2014). A number caveats with the Symplicity HTN-3 study design have since been identified that may well account for some of the neutral findings. Specifically, despite the inclusion of a sham-procedure, the majority of interventional cardiologists were inexperienced in performing the procedure (one third performed one procedure only), only 6% of patients received bilateral circumferential ablation (as per protocol) with energy preferentially applied to the proximal portion of the renal artery, renal and total body noradrenaline spillover testing (measure of renal efferent activity and central sympathetic drive via renal afferent pathway respectively) was not performed, and patient's medication regime prior to testing at baseline and 6-months was not stable.

In the aftermath of the Symplicity HTN-3 study announcement, 3 rigorously designed, prospective, open-label randomized controlled trials using the same single-electrode Symplicity radiofrequency catheter system (Medtronic Inc, Minnesota, USA) have been published (Fadl Elmula et al., 2014; Azizi et al., 2015; Rosa et al., 2015). As highlighted in **Table 2**, two of the studies showed intensive pharmacotherapy to be equally effective (Fadl Elmula et al., 2014; Rosa et al., 2015) to RDN in lowering BP in patients with true rHTN, highlighting the ability of RDN to lower BP at least to the extent of additional pharmacologic treatment. The third study (DENER-HTN) comparing RDN in combination with standardized, stepped-care antihypertensive treatment (SSAHT) observed a modest, albeit significant reduction in 6-month daytime SBP [adjusted mean difference of −5.9 mmHg (95%CI: −11.3, −0.5); $p = 0.03$] compared to SSAHT alone (Azizi et al., 2015).

Despite their smaller cohort sizes, a major strength of these latest studies was the careful selection of patients with true treatment rHTN. Patients were only recruited if they had elevated ambulatory daytime systolic BP after witnessed intake (Fadl Elmula et al., 2014) or quantitative plasma/urine levels (Azizi et al., 2015; Rosa et al., 2015) confirmation of prescribed antihypertensive medications. The absence of a sham-procedure in favor of standardized pharmacological intervention in the studies was also compensated by the use of ambulatory BP monitoring to assess the efficacy of RDN (which is preferential to the use of office BP) in combination with drug adherence monitoring. Such measures help to minimize the potential over-inflation of treatment-effects caused by white coat hypertension or the Hawthorn effect, which is a criticism of the Symplicity HTN-3 study.

Preliminary data from a recent study using the Symplicity-FLEX catheter is somewhat more encouraging for RDN (Desch et al., 2015). The smaller study ($n = 71$) designed to emulate the Symplicity HTN-3 in patients with mild refractory hypertension (defined as daytime systolic BP of 135−149 mmHg and/or diastolic BP of 90−94 mmHg on ≥3 antihypertensive medications) found that in the per-protocol analyses, those who underwent RDN ($n = 29$) experienced a significant reduction in mean 24-h and daytime systolic BP at 6-months follow-up compared to patients treated with a sham-procedure ($n = 34$). On average, RDN reduced 24-h and daytime systolic BP by −4.8 mmHg (mean ± SD: −8.3 ± 8.9 mmHg vs. −3.5 ± 9.5 mmHg; $p = 0.04$) and −6.2 mmHg (mean ± SD: −9.9 ± 9.0 mmHg vs. −3.7 ± 9.9 mmHg; $p = 0.01$), respectively. In the intention-to-treat analyses (primary outcome) the significant treatment-effects observed in the per-protocol analyses group were attenuated ($p = 0.15$). The inclusion of patients who should a priori not have been eligible for the study has been suggested as a possible explanation (Desch et al., 2015).

Beyond Blood Pressure Control: Other Therapeutic Uses of Renal Denervation

The localized effect of disrupting renal afferent signaling suggests RDN may offer distinct clinical benefits beyond BP control in pathological conditions characterized by central sympathetic overactivity. Specifically, there has been intense interest in the application of RDN in patients with renal disease, heart failure, and metabolic disorders (Mahfoud et al., 2011).

Chronic Kidney Disease/Dialysis

Excessive sympathetic nerve activity is a hallmark of both chronic kidney disease (CKD) and end-stage renal disease (ESRD). In CKD patients, augmented sympathetic tone is present in the early clinical stages of the disease and increases with disease progression (Grassi et al., 2011). Animal (Campese et al., 1995) and human (Converse et al., 1992; Grassi et al., 2011) studies have identified afferent signaling from diseased kidneys as playing a critical role in the progression of renal function decline. Indeed, in hypertensive patients, increased muscle sympathetic nerve activity is strongly associated with a decline in estimated glomerular filtration rate (eGFR) (Grassi et al., 2011). Bilateral nephrectomy is also shown to normalize elevated muscle sympathetic nerve activity in non-dialysis ESRD patients (Converse et al., 1992).

Despite the known importance of BP control for optimal renal function (Bakris et al., 2000) and mounting evidence that RDN does not induce structural or functional renal damage in hypertensive patients (Mahfoud et al., 2012; Dorr et al., 2013), international consensus states the procedure remain limited to patients with preserved renal indices. Hering et al. first reported on the efficacy and short-term safety of RDN in 15 moderate-to-severe CKD (mean creatinine-based eGFR 31.2 mL/min/1.73 m^2) patients with rHTN (Hering et al., 2012). The study showed RDN safely reduced peripheral arterial stiffness and office BP while preserving renal blood flow, electrolytes and eGFR at 6-months follow-up. Pilot data from 24 predominantly stage 2 CKD patients (mean eGRF 64.4 mL/min/1.73 m^2) with treatment rHTN (Kiuchi et al., 2013) showed a similar beneficial effect on 24-h ambulatory BP (mean reduction from baseline −19/−7 mmHg; $p < 0.001$) that was accompanied by short-term improvements in renal function. Compared to baseline, patients reported significantly higher eGFR and reduced urine albumin: creatinine ratio and serum creatinine (all $P < 0.001$) at 6-months follow-up. The conflicting short-term effects on eGRF reported

TABLE 2 | Randomised controlled trials using renal nerve ablation in the treatment of resistant hypertension in the last 12-months.

Study ID	Symplicity HNT-3 (Bhatt and Bakris, 2014)	PRAGUE-15 study (Rosa et al., 2015)	DENER-HTN trial (Azizi et al., 2015)	Symplicity FLEX study (Desch et al., 2015)	OSLO RDN study (Fadl Elmula et al., 2014)
Study design	Prospective, single-blind multi-center RCT with 2:1 treatment (RDN v SHAM) randomization	Prospective, open-label, multi-center RCT with 1:1 treatment randomization	Prospective, single-blind, multi-center RCT with 1:1 treatment randomization	Prospective, open-label RCT with 1:1 treatment randomization	Prospective, open-label RCT with 1:1 treatment randomization
Control	Sham-procedure (renal angiography)	Intensive pharmacotherapy with spironolactone	Standardized stepped-care antihypertensive treatment (SSAHT)	Sham-procedure (renal angiography)	Antihypertensive drug adjustment
Patient Cohort	RDN: $n = 364$ Mean age: 58 ± 10 years Number of BP medications: 5.1 ± 1.4 SHAM: n=171 Mean age: 56 ± 11 years Number of BP medications: 5.2 ± 1.4	RDN: $n = 52$ Mean age: 56 ± 12 years Mean office SBP:159 ± 19 mmHg Number of BP medications: 5.1 ± 1.2 PHAR: n=54 Mean age: 59 ± 9 years Mean office SBP:155 ± 17 mmHg Number of BP medications: 5.4 ± 1.2	RDN + SSAHT: $n = 53$ Mean age: 55 ± 11 years Mean office SBP:159 ± 23 mmHg SSAHT: n=53 Mean age: 55 ± 10 years Mean office SBP:156 ± 22 mmHg	RDN: $n = 35$ Mean age: 65 ± 8 years Mean daytime SBP:144 ± 5 mmHg Number of BP medications: 4.4 ± 1.3 SHAM: n=36 Mean age: 57 ± 9 years Mean daytime SBP:143 ± 5 mmHg Number of BP medications: 4.3 ± 1.3	RDN: $n = 9$ Mean age: 57 ± 11 years Mean office SBP:156 ± 13 mmHg Number of BP medications: 5.1 ± 1.6 PHAR: n=10 Mean age: 63 ± 5 years Mean office SBP:160 ± 12 mmHg Number of BP medications: 5.0 ± 1.2
RDN Modality	Symplicity single-electrode radiofrequency catheter system (Medtronic Inc.)	Symplicity single-electrode radiofrequency catheter system (Medtronic Inc.)	Symplicity single-electrode radiofrequency catheter system (Medtronic Inc.)	Symplicity FLEX multi-electrode radiofrequency catheter system (Medtronic Inc.)	Symplicity single-electrode radiofrequency catheter system (Medtronic Inc.)
Drug Adherence	Patient diary	Plasma drug concentration	8-item Morisky Medication Adherence Scale	Patient diary	Witnessed intake
Primary Outcome	Change in 6-mo office SBP	Changes in 6-mo 24-h ambulatory BP	Change in 6-mo daytime ambulatory SBP	Change in 6-mo 24-h ambulatory SBP (intention-to-treat)	Change in 6-mo office SBP
Secondary Outcomes	Change in 6-mo 24-h ambulatory SBP	1-year spironolactone (PHAR) or medical (RDN) effects	Change in 6-mo ambulatory, home and office BP measures; eGFR; incidence of adverse events;	Change in 6-mo 24-h ambulatory DBP and mean ambulatory BP (intention-to-treat); change in 24-h ambulatory SBP (per-protocol)	Change in 3-mo and 6-mo daytime ambulatory BP
Results	Mean change in 6-mo office SBP RDN: −14.13 ± 23.93 mmHg SHAM: −11.74 ± 25.94 mmHg (both $P < 0.001$) No treatment differences for change in 6-mo office SBP ($p = 0.26$) or 24-h ambulatory SBP ($p = 0.98$)	Mean change in 6-mo 24-h ambulatory SBP: RDN: −8.6 mmHg (−11.8, −5.3) PHAR: −8.1 mmHg (−12.7, −3.4) (both $P = 0.001$) No treatment differences for change in 6-mo 24-h, daytime or night-time ambulatory BP (all $P > 0.36$)	Mean change in 6-mo daytime ambulatory SBP: RDN + SSAHT: −15.8 mmHg (−19.7, −11.9) SSAHT: −9.9 mmHg (−13.6, −6.2) Significant treatment difference for change in 6-mo daytime SBP ($p = 0.03$) in favor of RDN group	Mean change in 6-mo 24-h ambulatory SBP: RDN: −7.0 mmHg (−10.8, −3.2) SHAM: −3.5 mmHg (−6.7, −0.2) No treatment difference for change in 6-mo 24-h ambulatory SBP ($p = 0.15$) Per-protocol: significant treatment difference for change in 6-mo 24-h ambulatory SBP ($p = 0.042$) in favor of RDN group	Office SBP at baseline and 6-mo: RDN: 156 ± 13 vs. 148 ± 7 mmHg ($p = 0.42$) SHAM: 160 ± 14 vs. 132 ± 10 mmHg ($P < 0.0005$) Significant treatment differences for change in 6-mo office SBP ($p = 0.002$) and office DBP ($p = 0.004$) in favor of PHAR group

(Continued)

TABLE 2 | Continued

Study ID	Symplicity HNT-3 (Bhatt and Bakris, 2014)	PRAGUE-15 study (Rosa et al., 2015)	DENER-HTN trial (Azizi et al., 2015)	Symplicity FLEX study (Desch et al., 2015)	OSLO RDN study (Fadl Elmula et al., 2014)
Conclusion	BP lowering effects of RDN is comparable to a sham-operation rHTN in patients with.	BP lowering effects of RDN is comparable to intensive pharmacotherapy in patients with true rHTN.	BP lowering effects of RDN + SSAHT is superior to SSAHT alone in patients with rHTN.	BP lowering effects of RDN is comparable to a sham-operation in patients with rHTN.	BP lowering effects of intensified pharmacological therapy is superior to RDN in patients with true rHTN.

Data presented as mean ± SD or mean [95%CI]. RCT, randomized controlled trial; RDN, renal denervation; SHAM = invasive sham-procedure; PHAR, pharmacological treatment; rHTN, resistant hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; mo, month.

between these two aforementioned pilot studies, suggests RDN may afford the greatest clinical benefit to patients in the early stages of CKD who have not yet undergone extensive vascular remodeling.

Two recent prospective longitudinal studies have reported sustained benefits in renal function parameters following RDN (Kiuchi et al., 2014; Ott et al., 2015). Kiuchi et al. observed of 27 CKD patients with rHTN that underwent RDN, those ($n = 22$) who achieved BP control (defined as office SBP <140 mmHg) at 12-months follow-up also reported a significantly higher eGRF (mean ± SD difference 18.54 ± 8.15 ml/min/1.73 m^2; $p = 0.03$). Furthermore, improvements in office diastolic BP, serum creatinine and ACR were only observed at 12-months follow-up in patients who achieved BP control ($P < 0.05$ for all) (Kiuchi et al., 2014).

In a separate multi-center observational study (Ott et al., 2015), RDN was prospectively shown to reduce mean 24-h ambulatory BP (mean ± SD; 9 ± 14/4 ± 7 mmHg; $P < 0.02$) and improve mean eGFR by 1.5 ± 10 ml/min/1.73 m^2 ($p = 0.009$) after 12-months in 27 rHTN patients with moderate-to-severe CKD. The efficacy of RDN to preserve renal function was evidenced by retrospective analyses showing patients experienced an average decline in eGFR of −4.8 ± 3.8 ml/min/1.73 m^2 per year prior to the RDN procedure. Interestingly, the magnitude of change in 24-h ambulatory systolic BP at 12-months was not shown to predict change in eGFR. This contrasts with Kiuchi et al.'s study (Kiuchi et al., 2014) and suggests RDN may attenuate renal function decline via mechanisms unrelated to BP. Importantly, neither procedural complications nor evidence of acute kidney injury following RDN was reported in patients.

Central sympathetic activity present in ESRD patients is driven principally by afferent renal nerve signaling from the diseased native kidneys (Converse et al., 1992). To date, the use of RDN to reduce increased cardiovascular mortality in ESRD patients with renal hypertension has been explored in a limited number of clinical studies (Schlaich et al., 2009b; Spinelli et al., 2014). In a proof-of-concept study of 9 patients with ESRD and hypertension, RDN resulted in sustained reductions in office SBP of −18, −16, and −28 mmHg at 3−, 6−, and 12-months, respectively (Schlaich et al., 2009b). Two patients ($n = 2$) who underwent additional measures of sympathetic activity at 3-months, also displayed reductions in muscle sympathetic nerve activity, and renal and whole body noradrenaline release. With respect to safety, only 2 patients developed perioperative femoral pseudo-aneurysms that were resolved without further sequelae. Findings from the pilot trial are supported by an elegant case series involving four patients (age range: 22–65 years) with ESRD and difficult anatomy (renal arteries < 4 mm) (Spinelli et al., 2014), which reported a mean reduction in 24-h ambulatory BP of −36/−16 mmHg at 12-months follow-up. With the exception of notches detected on the final angiogram, no other procedure related complications were reported. Larger, randomized controlled clinical trials are planned or currently ongoing to substantiate the seminal findings that RDN is a safe and efficacious therapeutic approach to both lower BP and regulate sympathetic activity in patients with impaired kidney function.

Chronic Heart Failure

Chronic heart failure (CHF) patients often exhibit renal dysfunction with augmented sympathetic tone (Hasking et al., 1986). Renal and cardiac noradrenaline spillover is a stronger predictor of mortality (Hasking et al., 1986; Petersson et al., 2005) than whole-body noradrenaline spillover in CHF patients suggesting chronic renal afferent nerve signaling is involved in the maintenance and progression of the pathological state.

Two small-scale trials assessing the potential benefits of RDN have been undertaken in CHF patients (Davies et al., 2013). The REACH pilot study evaluated the safety of RDN in 7 normotensive patients with CHF (class III–IV) and mean ejection fraction of 45% (Davies et al., 2013). At 6-months, all patients showed an improvement in their functional capacity (as assessed by a 6-min walk test) and overall quality of life. Importantly, no procedural complications or symptomatic adverse effects were reported and renal hemodynamics and function were also preserved.

In a larger observational study (OLOMOUC 1 Study) involving 51 patients with advanced CHF (mean ejection fraction 25%), RDN was associated with improved ventricular systolic function compared to standardized drug therapy (beta-blockers, ACE inhibitors or ARBs and diuretics) (Taborsky et al., 2012). Of the 26 patients randomized to RDN, left ventricular ejection fraction improved by an average of 6% at 12-months follow-up (mean ± SD: 25 ± 12% vs. 31 ± 14%; $p < 0.01$). No change was reported in the 25 patients randomized to standardized drug therapy ($p = 0.36$). Patients in the RDN group also reported significant reductions in heart rate and NT-pro brain natriuretic peptide levels and twice as fewer hospitalisations (31 vs. 72%; $p < 0.001$) during follow-up. Both treatments were shown to preserved patients renal function (as measured by eGFR; $p = 0.55$).

The stabilizing effect on RDN on heart failure progression is currently being investigated in several larger, randomized

controlled trials. Studies in diastolic heart failure patients are also ongoing. Specifically, the DIASTOLE study will use magnetic resonance imaging to assess the efficacy of RDN to improve diastolic functional parameters in a multicenter-randomized controlled trial of 60 heart failure patients with preserved ejection fraction (Verloop et al., 2013).

Left Ventricular Hypertrophy

Left ventricular hypertrophy (LVH) is an indicator of end-organ damage in arterial hypertension and is associated with increased cardiovascular morbidity and mortality risk, independent of BP (Ruilope and Schmieder, 2008; Bombelli et al., 2009). Chronic sympathetic nerve activity is shown to mediate hypertension-induced cardiac remodeling (Perlini et al., 2005). In hypertensive adults, cardiac noradrenaline spillover is positively related to LV mass index, suggesting a direct relationship between increased cardiac sympathetic activity in the development of LVH (Schlaich et al., 2003).

Two echocardiographic pilot studies have reported beneficial effects of RDN on measures of cardiac function and structure (Brandt et al., 2012; Schirmer et al., 2014). In 46 patients with rHTN, RDN was shown to significantly reduce LV mass (by 17%) and interventricular septum thickness (by −1.6 mm) at 6-months follow-up (both $p < 0.001$). Improvements in LV ejection fraction, LV end-systolic volume, diastolic LV filling pressures and isovolumic relation time were also observed (all $p < 0.006$). With the exception of LV mass and left atrial size, which was higher at 6-months follow-up compared to RDN patients, no other significant changes were observed in the control patients ($n = 18$). The effect of RDN on LV mass regression and diastolic function, while most prominent in patients who displayed the greatest reduction in systolic BP, was not exclusively associated with 6-month BP changes, suggesting RDN exerts effects on cardiac remodeling independent of BP. Schirmer et al. similarly reported in 66 overweight patients with rHTN a reduction in LV mass, improved diastolic function and increased vascular compliance at 6-months following RDN (all $P < 0.001$) (Schirmer et al., 2014). Compared to baseline, reductions in office BP (mean reduction −22/−10 mmHg) and heart rate (mean reduction −7 bpm) were also observed (both $p < 0.001$). With the exception of vascular compliance, which was directly correlated with BP reduction ($r^2 = 0.29, p < 0.001$), the degree of LV mass regression or diastolic improvement reported post-RDN was not dependent on the magnitude of reduction in BP or heart rate.

A recent prospective multi-center, blinded study using cardiac magnetic resonance imaging (a more reliable measure of cardiac function and morphology), confirms the aforementioned echocardiographic findings that RDN reduces LV mass regression and improves LV ejection fraction largely independent of the significant changes in BP (Mahfoud et al., 2014). Compared to 17 patients who received medical treatment only, 55 patients with rHTN treated with RDN reported a significant 7.1% reduction in LV mass (mean ± SD: 46.3 ± 13.6 vs. 43.0 ± 12.6 g/m$^{1.7}$; $P < 0.001$) and 3.4% improvement in ejection fraction (mean ± SD: 55.7 ± 11.1 vs. 57.6 ± 9.3%; $P = 0.048$) at 6-months follow-up. Importantly, LV mass was reduced in those patients who did not show significant clinical reductions in systolic BP at 6-months (defined as systolic BP reduction < 10 mmHg). In a sub-group of patients ($n = 19$) with markedly reduced systolic LV ejection fraction (defined as <50%) at baseline, the effect of RDN was even more pronounced resulting in a 7.3% improvement in LV ejection fraction; $P < 0.001$) at 6-months. Left ventricular wall stress, defined as a function of chamber size and configuration, thickness of the ventricular wall and intraventricular pressure was also significantly reduced following RDN at 6-months ($p = 0.03$). No changes in any cardiac parameters were reported in the control group.

Given the current findings support RDN having a prognostic benefit on LVH regression in resistant hypertensive patients who are at heighten risk for heart failure, larger studies analyzing clinical outcomes are warranted.

Arrhythmias

Preliminary evidence suggests that RDN may have a salutary effect in the management of recurrent arrhythmias in heart failure patients, particularly those with atrial fibrillation (Pokushalov et al., 2012) and ventricular tachycardia (Ukena et al., 2012; Hoffmann et al., 2013), via a reduction in BP and central sympathetic cardiac stimulation.

The potential antiarrhythmic efficacy of RDN was first described in a normotensive porcine model (Linz et al., 2013a) and canine with pacing-induced heart failure model (Zhao et al., 2013). First-in-man experience comes from a small prospective study of 27 patients with refractory symptomatic atrial fibrillation and rHTN (Pokushalov et al., 2012). Compared to patients who underwent usual treatment for atrial fibrillation pulmonary vein isolation (PVI) ($n = 14$), patients who underwent a combined therapy of PVI and RDN ($n = 13$) exhibited a two-fold reduction in the occurrence of atrial fibrillation episodes (defined as <30 s of atrial fibrillation during 9-months follow-up). Patients on combined therapy also demonstrated a significant and sustained BP reduction of −25/−10 mmHg and reduction in LV mass of approximately 10% at follow up. Findings from a two-study meta-analysis suggest the salutary effects of RDN when used as an adjunct therapy to PVI on atrial fibrillation reoccurrence is even more pronounced in patients with severe rHTN (Pokushalov et al., 2014). A recent study by McLellan et al. suggests electrical remodeling, specifically an increase in global and atrial conduction velocity, may in conjunction with structural changes promote a reduction in atrial fibrillation reoccurrence following RDN (McLellan et al., 2015).

Ventricular tachyarrhthmias (VTA) are associated with a high, irregular heart rate (>100 bpm) and significant risk for sudden death. Elevated cardiac sympathetic nerve activity has been linked to the pathogenesis of VTAs (Meredith et al., 1991) with structural changes in myocardial tissue (i.e., myocardial hypertrophy and heart failure) caused by elevations in BP thought to predispose patients to VTA (Anderson, 1984; Bryant et al., 1999).

In a porcine model of acute coronary myocardial infarction, RDN was shown to significantly reduce the incidence of ventricular arrhythmia compared to a sham procedure (86 vs. 17%; $p = 0.03$) (Linz et al., 2013b). The efficacy of RDN

to suppress ventricular tachycardia in adults has only been explored in a few case studies involving patients with ventricular electrical storm (Ukena et al., 2012; Hoffmann et al., 2013). Ukena et al. reported the first-in-man experience in 2 patients suffering from asymptomatic CHF (non-obstructive hypertrophic and dilated cardiomyopathy, NYHA III) and treatment resistance ventricular electrical storm (Ukena et al., 2012). In both patients, RDN was shown to eliminate the occurrence of ventricular tachyarrhythmic episodes at 6-months without altering BP. In a 63 year old male with recurrent ventricular electrical storm in the setting of acute myocardial infarction, the use of RDN in combination with ventricular tachycardia catheter ablation (standard therapy) was shown to eliminate both ventricular tachycardia and ventricular fibrillation episodes at 23, 100, and 160 days follow-up (Hoffmann et al., 2013). The patient also experienced normalization of their BP, which warranted a reduction in their antihypertensive medication.

While the observations reported in these case studies are promising for RDN as an adjunctive therapy for patients with serious cardiac electrical instability they underscore the need for future randomized controlled trials.

Metabolic Diseases

Accumulating data from animal and human studies suggest chronic sympathoexcitaion plays a pivotal role in the etiology and complications of metabolic conditions. Even in the absence of hypertension, elevated urinary noradrenaline levels, increased efferent muscle sympathetic nerve activity, and elevated rates of plasma noradrenaline spillover, are present in patients with obesity, insulin resistance and the metabolic syndrome (Lee et al., 2001; Grassi et al., 2005; Straznicky et al., 2008; Schlaich et al., 2015).

Retrospective sub-cohort analyses of 37 rHTN patients who underwent RDN in the Symplicity HTN-1 Study, showed improvements in office BP (−32/−12 mmHg; $P < 0.001$) were accompanied by significant reductions in fasting plasma glucose, insulin, C-peptide and 2-h post load glucose levels at 3-months follow-up (all $p > 0.04$) (Mahfoud et al., 2011). Insulin sensitivity as measured by the HOMA-IR and IS_{QUICKI} index measures was also improved by −62 and 13%, respectively, at 3-months follow-up (both $P = 0.001$). Importantly, these beneficial metabolic alterations were preserved in patients diagnosed with type 2 diabetes at baseline ($n = 20$). No BP or metabolic improvements were reported in the control group ($n = 13$) who continued their usual medication regime.

These impressive findings contrast with the recently published Denervation of the Renal Artery in Metabolic Syndrome (DREAMs) study that prospectively reported no effect of RDN on measures of insulin sensitivity after 12-months in 29 patients with metabolic syndrome on ≤1 antihypertensive and/or diabetic medication (Verloop et al., 2015). Of note, whole-body sympathetic activity, as measured by microneurography ($n = 10$), and heart rate variability ($n = 26$) did not change at 12-months post-RDN despite a modest reduction in 24-h ambulatory BP (mean change from baseline $-6 \pm 12/-5 \pm 7$ mmHg; $p < 0.02$).

Improved glucose metabolism following RDN has been reported in patients with polycystic ovary syndrome (Schlaich et al., 2011) and obstructive sleep apnea (Witkowski et al., 2011), two conditions that are characterized by multiple metabolic disturbances. In 2 obese women with polycystic ovary syndrome, RDN was shown to lower fasting plasma glucose, improve insulin sensitivity (assessed by euglycaemic hyperinsulinemic clamp) and reduce both muscle sympathetic nerve activity and whole-body noradrenaline spillover at 3-months follow-up (Schlaich et al., 2011). Importantly, these metabolic effects were shown to occur in the absence of any changes in body weight. In 10 patients with obstructive sleep apnea, RDN was associated with improved 2-h glucose levels (median: 7.0 vs. 6.4 mmol/L; $p = 0.05$) during an oral glucose tolerance test and reduced HbA1c levels (median: 6.1% vs. 5.6%; $p < 0.05$) at 6-months follow-up (Witkowski et al., 2011). Improvements in office BP (−34/−13 mmHg: $p < 0.01$) and severity of obstructive sleep apnoea for 80% of patients was also observed. Overall, the evidence suggests acute improvements in insulin resistance and glycemic control ensue from RDN. Understanding the longer term effects of RDN in patients with metabolic disease is expected to be a focus for further investigation.

Future of Renal Denervation: Where to from Here?

Despite the recent disappointment of Symplicity HTN-3, support remains for the efficacy of RDN in the real world setting (Bohm et al., 2015). Recent analyses from Medtronic's Global SYMPLICITY Registry showed 998 patients who underwent RDN experienced a significant lowering in office and ambulatory systolic BP by an average of −19.8 and −9.2 mmHg, respectively, after 6-months. For patients with a baseline systolic BP ≥ 160 mmHg, the BP reduction following RDN was even more pronounced. The registry also confirmed the well-established short-term safety profile of RDN with only six procedure-related events reported (<1% of the cohort) during the 6-months. Collectively, the results lend support to RDN being a safe and viable therapeutic option in patients with severe rHTN when traditional pharmacotherapy has failed.

In 2014, the Joint UK societies' consensus statement on RDN for rHTN called for a temporary moratorium on RDN in routine clinical practice following the Symplicity HTN-3 announcement but was hesitant to abandon the therapy entirely, citing the need for further research (Lobo et al., 2015). In this respect, Symplicity HTN-3 has been invaluable in helping guide the design and execution of future clinical studies. The finding that patients who received ablations in all four quadrants of the renal artery were more likely to experience significant reductions in BP in Symplicity HTN-3 (Bhatt et al., 2014) coupled with contemporary anatomical insights into the distribution of renal artery nerves (Sakakura et al., 2014) demonstrates certain ablation patterns are more efficacious for lowing BP than others. Indeed, there is now a preferential shift toward the use of modalities that circumferentially ablate nerves toward the distal portion of both renal arteries. Patient selection is also critical with meticulous identification of true treatment-rHTN using ambulatory BP monitoring during a period of stable medication (>8 weeks) confirmed by drug adherence testing necessary to

eliminate patients without "neurogenic" hypertension and those who simply are non-compliant with their BP medication.

At present, there is no intraprocedural marker to confirm whether RDN has been successfully achieved. Instead, physicians have naively relied on changes in BP to define procedural success. Noradrenaline spillover testing has been performed in small subgroups of patients to quantify whole-body (Krum et al., 2009) and renal sympathetic nerve activity (Esler, 2014a) prior to and 30-days post RDN. While a reduction in renal noradrenaline spillover correlates with a reduction in BP, the response can be highly variable between individual patients (Esler, 2014b). Furthermore, the validated test is not suitable for use in large-scale trials.

Identification of a novel biomarker of renal nerve injury that can be measured in either urine or plasma immediately following RDN would be the ne plus ultra to inform interventionists of procedural success. Recent animal data suggests intraluminal electrical stimulation of renal arteries pre- and post-RDN may provide valuable insight in the acute efficacy of the procedure. Indeed, Chinushi et al. reported an increase in BP, serum catecholamines and heart rate variability immediately following high-frequency electrical stimulation of renal arteries in a hypertensive canine model, which was attenuated following RDN (Chinushi et al., 2013). Adenosine infusion into the renal artery, which under normal conditions potentiates a rise in BP, has also been suggested as an immediate measure of renal afferent nerve ablation success following RDN (Esler, 2014a).

Conclusion

Latest clinical trial data suggests RDN is a safe treatment option in patients with true rHTN that is most efficacious when used as an adjunct therapy to intensive pharmacotherapy. It is hoped the development of next generation ablation modalities that enable circumferential ablation coupled with more stringent screening of true treatment rHTN will help improve the clinical efficacy of RDN. Encouraging data from a number of pre-clinical studies highlights that clinical benefits beyond BP reduction can be gained following RDN in patients with renal disease, heart failure, arrhythmias and metabolic-related disease. However, caution is warranted not to over-interpret the findings of these small studies with larger, randomized controlled trials needed before the application of RDN becomes routine in clinical practice for these patient cohorts.

Author Contributions

AT and MS contributed to the conception and interpretation of data in the review; AT prepared the manuscript, tables, and figures; MS reviewed and edited the manuscript; AT and MS approved the final version of the manuscript and are accountable for all aspects of the review presented.

Funding

MS is supported by a Senior Research Fellowship from the National Health and Medical Research Council (NHMRC) of Australia. AT is supported by an NHMRC of Australia Grant. This research was funded in part by the grants from the National Health and Research Council of Australia (NHMRC) and the Victorian Government's Operational Infrastructure Support Program.

References

Anderson, K. P. (1984). Sudden death, hypertension, and hypertrophy. *J. Cardiovasc. Pharmacol.* 6(Suppl. 3), S498–S503. doi: 10.1097/00005344-198400063-00009

Azizi, M., Sapoval, M., Gosse, P., Monge, M., Bobrie, G., Delsart, P., et al. (2015). Optimum and stepped care standardised antihypertensive treatment with or without renal denervation for resistant hypertension (DENERHTN): a multicentre, open-label, randomised controlled trial. *Lancet* 385, 1957–1965. doi: 10.1016/S0140-6736(14)61942-5

Bakris, G. L., Townsend, R. R., Liu, M., Cohen, S. A., D'Agostino, R., Flack, J. M., et al. (2014). Impact of renal denervation on 24-hour ambulatory blood pressure: results from SYMPLICITY HTN-3. *J. Am. Coll. Cardiol.* 64, 1071–1078. doi: 10.1016/j.jacc.2014.05.012

Bakris, G. L., Williams, M., Dworkin, L., Elliott, W. J., Epstein, M., Toto, R., et al. (2000). Preserving renal function in adults with hypertension and diabetes: a consensus approach. National kidney foundation hypertension and diabetes executive committees working group. *Am. J. Kidney Dis.* 36, 646–661. doi: 10.1053/ajkd.2000.16225

Barajas, L. (1978). Innervation of the renal cortex. *Fed. Proc.* 37, 1192–1201.

Bell-Reuss, E., Trevino, D. L., and Gottschalk, C. W. (1976). Effect of renal sympathetic nerve stimulation on proximal water and sodium reabsorption. *J. Clin. Invest.* 57, 1104–1107. doi: 10.1172/JCI108355

Bhatt, D. L., and Bakris, G. L. (2014). Renal denervation for resistant hypertension. *N. Engl. J. Med.* 371, 184. doi: 10.1056/NEJMoa1402670

Bhatt, D. L., Kandzari, D. E., O'Neill, W. W., D'Agostino, R., Flack, J. M., Katzen, B. T., et al. (2014). A controlled trial of renal denervation for resistant hypertension. *N. Engl. J. Med.* 370, 1393–1401. doi: 10.1056/NEJMoa1402670

Bohm, M., Mahfoud, F., Ukena, C., Hoppe, U. C., Narkiewicz, K., Negoita, M., et al. (2015). First report of the global SYMPLICITY registry on the effect of renal artery denervation in patients with uncontrolled hypertension. *Hypertension* 65, 766–774. doi: 10.1161/HYPERTENSIONAHA.114.05010

Bombelli, M., Facchetti, R., Carugo, S., Madotto, F., Arenare, F., Quarti-Trevano, F., et al. (2009). Left ventricular hypertrophy increases cardiovascular risk independently of in-office and out-of-office blood pressure values. *J. Hypertens.* 27, 2458–2464. doi: 10.1097/HJH.0b013e328330b845

Brandt, M. C., Mahfoud, F., Reda, S., Schirmer, S. H., Erdmann, E., Bohm, M., et al. (2012). Renal sympathetic denervation reduces left ventricular hypertrophy and improves cardiac function in patients with resistant hypertension. *J. Am. Coll. Cardiol.* 59, 901–909. doi: 10.1016/j.jacc.2011.11.034

Bryant, S. M., Shipsey, S. J., and Hart, G. (1999). Normal regional distribution of membrane current density in rat left ventricle is altered in catecholamine-induced hypertrophy. *Cardiovasc. Res.* 42, 391–401. doi: 10.1016/S0008-6363(99)00033-4

Campese, V. M., Kogosov, E., and Koss, M. (1995). Renal afferent denervation prevents the progression of renal disease in the renal ablation model of chronic

renal failure in the rat. *Am. J. Kidney Dis.* 26, 861–865. doi: 10.1016/0272-6386(95)90456-5

Chinushi, M., Izumi, D., Iijima, K., Suzuki, K., Furushima, H., Saitoh, O., et al. (2013). Blood pressure and autonomic responses to electrical stimulation of the renal arterial nerves before and after ablation of the renal artery. *Hypertension* 61, 450–456. doi: 10.1161/HYPERTENSIONAHA.111.00095

Converse, R. L. Jr., Jacobsen, T. N., Toto, R. D., Jost, C. M., Cosentino, F., Fouad-Tarazi, F., et al. (1992). Sympathetic overactivity in patients with chronic renal failure. *N. Engl. J. Med.* 327, 1912–1918. doi: 10.1056/NEJM199212313272704

Daemen, J., and Van Mieghem, N. (2015). First-in-man radial access renal denervation with the ReCor Radiance catheter. *EuroIntervention* 10, 1209–1212. doi: 10.4244/EIJY14M12_03

Daugherty, S. L., Powers, J. D., Magid, D. J., Tavel, H. M., Masoudi, F. A., Margolis, K. L., et al. (2012). Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation* 125, 1635–1642. doi: 10.1161/CIRCULATIONAHA.111.068064

Davies, J. E., Manisty, C. H., Petraco, R., Barron, A. J., Unsworth, B., Mayet, J., et al. (2013). First-in-man safety evaluation of renal denervation for chronic systolic heart failure: primary outcome from REACH-Pilot study. *Int. J. Cardiol.* 162, 189–192. doi: 10.1016/j.ijcard.2012.09.019

De La Sierra, A., Segura, J., Banegas, J. R., Gorostidi, M., De La Cruz, J. J., Armario, P., et al. (2011). Clinical features of 8295 patients with resistant hypertension classified on the basis of ambulatory blood pressure monitoring. *Hypertension* 57, 898–902. doi: 10.1161/HYPERTENSIONAHA.110.168948

Desch, S., Okon, T., Heinemann, D., Kulle, K., Rohnert, K., Sonnabend, M., et al. (2015). Randomized sham-controlled trial of renal sympathetic denervation in mild resistant hypertension. *Hypertension* 65, 1202–1208. doi: 10.1161/HYPERTENSIONAHA.115.05283

Dibona, G. F. (2005). Physiology in perspective: the wisdom of the body. Neural control of the kidney. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R633–R641. doi: 10.1152/ajpregu.00258.2005

Dibona, G. F., and Kopp, U. C. (1997). Neural control of renal function. *Physiol. Rev.* 77, 75–197.

Dorr, O., Liebetrau, C., Mollmann, H., Achenbach, S., Sedding, D., Szardien, S., et al. (2013). Renal sympathetic denervation does not aggravate functional or structural renal damage. *J. Am. Coll. Cardiol.* 61, 479–480. doi: 10.1016/j.jacc.2012.09.051

Esler, M. (2000). The sympathetic system and hypertension. *Am. J. Hypertens.* 13, 99S–105S. doi: 10.1016/S0895-7061(00)00225-9

Esler, M. (2010). The 2009 Carl ludwig lecture: pathophysiology of the human sympathetic nervous system in cardiovascular diseases: the transition from mechanisms to medical management. *J. Appl. Physiol.* 108, 227–237. doi: 10.1152/japplphysiol.00832.2009

Esler, M. (2014a). Illusions of truths in the symplicity HTN-3 trial: generic design strengths but neuroscience failings. *J. Am. Soc. Hypertens.* 8, 593–598. doi: 10.1016/j.jash.2014.06.001

Esler, M. (2014b). "Louise F. Bishop Lecture, Treament of drug-resistant hypertension with renal denervation: rationale, results and recent challenges," in *American College of Cardiology 63rd Annual Scientific Session 29 March 2014* (Washington, DC).

Esler, M. D., Krum, H., Sobotka, P. A., Schlaich, M. P., Schmieder, R. E., and Bohm, M. (2010). Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 376, 1903–1909. doi: 10.1016/S0140-6736(10)62039-9

Esler, M., Lambert, G., and Jennings, G. (1989). Regional norepinephrine turnover in human hypertension. *Clin. Exp. Hypertens. A* 11(Suppl. 1), 75–89. doi: 10.3109/10641968909045414

Fadl Elmula, F. E., Hoffmann, P., Larstorp, A. C., Fossum, E., Brekke, M., Kjeldsen, S. E., et al. (2014). Adjusted drug treatment is superior to renal sympathetic denervation in patients with true treatment-resistant hypertension. *Hypertension* 63, 991–999. doi: 10.1161/HYPERTENSIONAHA.114.03246

Grassi, G., Dell'oro, R., Quarti-Trevano, F., Scopelliti, F., Seravalle, G., Paleari, F., et al. (2005). Neuroadrenergic and reflex abnormalities in patients with metabolic syndrome. *Diabetologia* 48, 1359–1365. doi: 10.1007/s00125-005-1798-z

Grassi, G., Quarti-Trevano, F., Seravalle, G., Arenare, F., Volpe, M., Furiani, S., et al. (2011). Early sympathetic activation in the initial clinical stages of chronic renal failure. *Hypertension* 57, 846–851. doi: 10.1161/HYPERTENSIONAHA.110.164780

Hasking, G. J., Esler, M. D., Jennings, G. L., Burton, D., Johns, J. A., and Korner, P. I. (1986). Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. *Circulation* 73, 615–621. doi: 10.1161/01.CIR.73.4.615

Hering, D., Mahfoud, F., Walton, A. S., Krum, H., Lambert, G. W., Lambert, E. A., et al. (2012). Renal denervation in moderate to severe CKD. *J. Am. Soc. Nephrol.* 23, 1250–1257. doi: 10.1681/ASN.2011111062

Hering, D., Marusic, P., Walton, A. S., Lambert, E. A., Krum, H., Narkiewicz, K., et al. (2014). Sustained sympathetic and blood pressure reduction 1 year after renal denervation in patients with resistant hypertension. *Hypertension* 64, 118–124. doi: 10.1161/HYPERTENSIONAHA.113.03098

Hoffmann, B. A., Steven, D., Willems, S., and Sydow, K. (2013). Renal sympathetic denervation as an adjunct to catheter ablation for the treatment of ventricular electrical storm in the setting of acute myocardial infarction. *J. Cardiovasc. Electrophysiol.* 24, 1175–1178. doi: 10.1111/jce.12282

Howard, J. P., Nowbar, A. N., and Francis, D. P. (2013). Size of blood pressure reduction from renal denervation: insights from meta-analysis of antihypertensive drug trials of 4,121 patients with focus on trial design: the CONVERGE report. *Heart* 99, 1579–1587. doi: 10.1136/heartjnl-2013-304238

Irvin, M. R., Booth, J. N. III, Shimbo, D., Lackland, D. T., Oparil, S., Howard, G., et al. (2014). Apparent treatment-resistant hypertension and risk for stroke, coronary heart disease, and all-cause mortality. *J. Am. Soc. Hypertens.* 8, 405–413. doi: 10.1016/j.jash.2014.03.003

Jonas, M., Rapoport, J., Gendelman, G., Diehm, N., and Geaorge, J. (2014). "In-stent renal denervation using the TIVUS (trademark) system: worldwide first cases," in *EuroPCR 2014* (Paris).

Jung, O., Gechter, J. L., Wunder, C., Paulke, A., Bartel, C., Geiger, H., et al. (2013). Resistant hypertension? Assessment of adherence by toxicological urine analysis. *J. Hypertens.* 31, 766–774. doi: 10.1097/HJH.0b013e32835e2286

Kirchheim, H., Ehmke, H., and Persson, P. (1989). Sympathetic modulation of renal hemodynamics, renin release and sodium excretion. *Klin. Wochenschr.* 67, 858–864. doi: 10.1007/BF01717340

Kiuchi, M. G., Chen, S., Andrea, B. R., Kiuchi, T., Carreira, M. A., Graciano, M. L., et al. (2014). Renal sympathetic denervation in patients with hypertension and chronic kidney disease: does improvement in renal function follow blood pressure control? *J. Clin. Hypertens. (Greenwich).* 16, 794–800. doi: 10.1111/jch.12415

Kiuchi, M. G., Maia, G. L., De Queiroz Carreira, M. A., Kiuchi, T., Chen, S., Andrea, B. R., et al. (2013). Effects of renal denervation with a standard irrigated cardiac ablation catheter on blood pressure and renal function in patients with chronic kidney disease and resistant hypertension. *Eur. Heart J.* 34, 2114–2121. doi: 10.1093/eurheartj/eht200

Kopp, U. C. (1992). Renorenal reflexes: interaction between efferent and afferent renal nerve activity. *Can. J. Physiol. Pharmacol.* 70, 750–758. doi: 10.1139/y92-099

Krum, H., Schlaich, M., Whitbourn, R., Sobotka, P. A., Sadowski, J., Bartus, K., et al. (2009). Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 373, 1275–1281. doi: 10.1016/S0140-6736(09)60566-3

Lee, Z. S., Critchley, J. A., Tomlinson, B., Young, R. P., Thomas, G. N., Cockram, C. S., et al. (2001). Urinary epinephrine and norepinephrine interrelations with obesity, insulin, and the metabolic syndrome in Hong Kong Chinese. *Metabolism* 50, 135–143. doi: 10.1053/meta.2001.19502

Linz, D., Mahfoud, F., Schotten, U., Ukena, C., Neuberger, H. R., Wirth, K., et al. (2013a). Effects of electrical stimulation of carotid baroreflex and renal denervation on atrial electrophysiology. *J. Cardiovasc. Electrophysiol.* 24, 1028–1033. doi: 10.1111/jce.12171

Linz, D., Wirth, K., Ukena, C., Mahfoud, F., Poss, J., Linz, B., et al. (2013b). Renal denervation suppresses ventricular arrhythmias during acute ventricular ischemia in pigs. *Heart Rhythm* 10, 1525–1530. doi: 10.1016/j.hrthm.2013.07.015

Lobo, M. D., De Belder, M. A., Cleveland, T., Collier, D., Dasgupta, I., Deanfield, J., et al. (2015). Joint UK societies' 2014 consensus statement on renal denervation for resistant hypertension. *Heart* 101, 10–16. doi: 10.1136/heartjnl-2014-307029

Mahfoud, F., Cremers, B., Janker, J., Link, B., Vonend, O., Ukena, C., et al. (2012). Renal hemodynamics and renal function after catheter-based renal sympathetic denervation in patients with resistant hypertension. *Hypertension* 60, 419–424. doi: 10.1161/HYPERTENSIONAHA.112.193870

Mahfoud, F., Schlaich, M., Kindermann, I., Ukena, C., Cremers, B., Brandt, M. C., et al. (2011). Effect of renal sympathetic denervation on glucose metabolism in patients with resistant hypertension: a pilot study. *Circulation* 123, 1940–1946. doi: 10.1161/CIRCULATIONAHA.110.991869

Mahfoud, F., Urban, D., Teller, D., Linz, D., Stawowy, P., Hassel, J. H., et al. (2014). Effect of renal denervation on left ventricular mass and function in patients with resistant hypertension: data from a multi-centre cardiovascular magnetic resonance imaging trial. *Eur. Heart J.* 35, 2224b–2231b. doi: 10.1093/eurheartj/ehu093

Malpas, S. C. (2010). Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. *Physiol. Rev.* 90, 513–557. doi: 10.1152/physrev.00007.2009

McLellan, A. J., Schlaich, M. P., Taylor, A. J., Prabhu, S., Hering, D., Hammond, L., et al. (2015). Reverse cardiac remodeling after renal denervation: atrial electrophysiologic and structural changes associated with blood pressure lowering. *Heart Rhythm* 12, 982–990. doi: 10.1016/j.hrthm.2015.01.039

Meredith, I. T., Broughton, A., Jennings, G. L., and Esler, M. D. (1991). Evidence of a selective increase in cardiac sympathetic activity in patients with sustained ventricular arrhythmias. *N. Engl. J. Med.* 325, 618–624. doi: 10.1056/NEJM199108293250905

Neuzil, P., Whitbourn, R., Starek, Z., Esler, M., Brinton, T. J., and Gertner, M. (2014). "Optimized external focused ultrasound for renal sympathetic denervation- wave II trial," in *EuroPCR 2014* (Paris).

Ormiston, J. A., Anderson, T., Brinton, T. J., Dawood, O., Gertner, M., Kay, P., et al. (2014). "Non-invasive renal denervation using externallt delivered focused ultrasound: early experience using doppler based imaging tracking and targeting for treatment," in *EuroPCR 2014* (Paris).

Ott, C., Mahfoud, F., Schmid, A., Toennes, S. W., Ewen, S., Ditting, T., et al. (2015). Renal denervation preserves renal function in patients with chronic kidney disease and resistant hypertension. *J. Hypertens.* 33, 1261–1266. doi: 10.1097/HJH.0000000000000556

Perlini, S., Palladini, G., Ferrero, I., Tozzi, R., Fallarini, S., Facoetti, A., et al. (2005). Sympathectomy or doxazosin, but not propranolol, blunt myocardial interstitial fibrosis in pressure-overload hypertrophy. *Hypertension* 46, 1213–1218. doi: 10.1161/01.HYP.0000185689.65045.4c

Persell, S. D. (2011). Prevalence of resistant hypertension in the United States, 2003–2008. *Hypertension* 57, 1076–1080. doi: 10.1161/HYPERTENSIONAHA.111.170308

Persu, A., Jin, Y., Azizi, M., Baelen, M., Volz, S., Elvan, A., et al. (2014). Blood pressure changes after renal denervation at 10 European expert centers. *J. Hum. Hypertens.* 28, 150–156. doi: 10.1038/jhh.2013.88

Petersson, M., Friberg, P., Eisenhofer, G., Lambert, G., and Rundqvist, B. (2005). Long-term outcome in relation to renal sympathetic activity in patients with chronic heart failure. *Eur. Heart J.* 26, 906–913. doi: 10.1093/eurheartj/ehi184

Pokushalov, E., Romanov, A., Corbucci, G., Artyomenko, S., Baranova, V., Turov, A., et al. (2012). A randomized comparison of pulmonary vein isolation with versus without concomitant renal artery denervation in patients with refractory symptomatic atrial fibrillation and resistant hypertension. *J. Am. Coll. Cardiol.* 60, 1163–1170. doi: 10.1016/j.jacc.2012.05.036

Pokushalov, E., Romanov, A., Katritsis, D. G., Artyomenko, S., Bayramova, S., Losik, D., et al. (2014). Renal denervation for improving outcomes of catheter ablation in patients with atrial fibrillation and hypertension: early experience. *Heart Rhythm* 11, 1131–1138. doi: 10.1016/j.hrthm.2014.03.055

Rosa, J., Widimsky, P., Tousek, P., Petrak, O., Curila, K., Waldauf, P., et al. (2015). Randomized comparison of renal denervation versus intensified pharmacotherapy including spironolactone in true-resistant hypertension: six-month results from the Prague-15 study. *Hypertension* 65, 407–413. doi: 10.1161/HYPERTENSIONAHA.114.04019

Ruilope, L. M., and Schmieder, R. E. (2008). Left ventricular hypertrophy and clinical outcomes in hypertensive patients. *Am. J. Hypertens.* 21, 500–508. doi: 10.1038/ajh.2008.16

Sakakura, K., Ladich, E., Cheng, Q., Otsuka, F., Yahagi, K., Fowler, D. R., et al. (2014). Anatomic assessment of sympathetic peri-arterial renal nerves in man. *J. Am. Coll. Cardiol.* 64, 635–643. doi: 10.1016/j.jacc.2014.03.059

Schirmer, S. H., Sayed, M. M., Reil, J. C., Ukena, C., Linz, D., Kindermann, M., et al. (2014). Improvements in left ventricular hypertrophy and diastolic function following renal denervation: effects beyond blood pressure and heart rate reduction. *J. Am. Coll. Cardiol.* 63, 1916–1923. doi: 10.1016/j.jacc.2013.10.073

Schlaich, M. P., Kaye, D. M., Lambert, E., Sommerville, M., Socratous, F., and Esler, M. D. (2003). Relation between cardiac sympathetic activity and hypertensive left ventricular hypertrophy. *Circulation* 108, 560–565. doi: 10.1161/01.CIR.0000081775.72651.B6

Schlaich, M. P., Lambert, E., Kaye, D. M., Krozowski, Z., Campbell, D. J., Lambert, G., et al. (2004). Sympathetic augmentation in hypertension: role of nerve firing, norepinephrine reuptake, and Angiotensin neuromodulation. *Hypertension* 43, 169–175. doi: 10.1161/01.HYP.0000103160.35395.9E

Schlaich, M. P., Sobotka, P. A., Krum, H., Lambert, E., and Esler, M. D. (2009a). Renal sympathetic-nerve ablation for uncontrolled hypertension. *N. Engl. J. Med.* 361, 932–934. doi: 10.1056/NEJMc0904179

Schlaich, M. P., Socratous, F., Hennebry, S., Eikelis, N., Lambert, E. A., Straznicky, N., et al. (2009b). Sympathetic activation in chronic renal failure. *J. Am. Soc. Nephrol.* 20, 933–939. doi: 10.1681/ASN.2008040402

Schlaich, M. P., Straznicky, N., Grima, M., Ika-Sari, C., Dawood, T., Mahfoud, F., et al. (2011). Renal denervation: a potential new treatment modality for polycystic ovary syndrome? *J. Hypertens.* 29, 991–996. doi: 10.1097/HJH.0b013e328344db3a

Schlaich, M., Straznicky, N., Lambert, E., and Lambert, G. (2015). Metabolic syndrome: a sympathetic disease? *Lancet Diabetes Endocrinol.* 3, 148–157. doi: 10.1016/S2213-8587(14)70033-6

Shetty, S. V., Blessing, E., Roenschein, U., Scheinert, D., Diehm, N., and Jonas, M. (2014). "Renal denervation using the novel therapeutic intra-vascular ultrasound (TIVUSTM) catheter system- Preliminary report of first-in-man safety and performance study," in *EuroPCR 2014* (Paris).

Smith, P. A., Graham, L. N., Mackintosh, A. F., Stoker, J. B., and Mary, D. A. (2004). Relationship between central sympathetic activity and stages of human hypertension. *Am. J. Hypertens.* 17, 217–222. doi: 10.1016/j.amjhyper.2003.10.010

Spinelli, A., Da Ros, V., Morosetti, D., Onofrio, S. D., Rovella, V., Di Daniele, N., et al. (2014). Technical aspects of renal denervation in end-stage renal disease patients with challenging anatomy. *Diagn. Interv. Radiol.* 20, 267–270. doi: 10.5152/dir.2013.13408

Straznicky, N. E., Eikelis, N., Lambert, E. A., and Esler, M. D. (2008). Mediators of sympathetic activation in metabolic syndrome obesity. *Curr. Hypertens. Rep.* 10, 440–447. doi: 10.1007/s11906-008-0083-1

Taborsky, M., Lazarova, M. L., and Vaclavik, J. (2012). The effect of renal denervation in patients with advanced heart failure. *Eur. Heart J.* 33 (Abstract Supplement), 517.

Ukena, C., Bauer, A., Mahfoud, F., Schreieck, J., Neuberger, H. R., Eick, C., et al. (2012). Renal sympathetic denervation for treatment of electrical storm: first-in-man experience. *Clin. Res. Cardiol.* 101, 63–67. doi: 10.1007/s00392-011-0365-5

Verloop, W. L., Beeftink, M. M., Nap, A., Bots, M. L., Velthuis, B. K., Appelman, Y. E., et al. (2013). Renal denervation in heart failure with normal left ventricular ejection fraction. Rationale and design of the DIASTOLE (DenervatIon of the renAl Sympathetic nerves in hearT failure with nOrmal Lv Ejection fraction) trial. *Eur. J. Heart Fail.* 15, 1429–1437. doi: 10.1093/eurjhf/hft119

Verloop, W. L., Spiering, W., Vink, E. E., Beeftink, M. M., Blankestijn, P. J., Doevendans, P. A., et al. (2015). Denervation of the Renal Arteries in Metabolic Syndrome: The DREAMS-Study. *Hypertension* 65, 751–757. doi: 10.1161/HYPERTENSIONAHA.114.04798

Witkowski, A., Prejbisz, A., Florczak, E., Kadziela, J., Sliwinski, P., Bielen, P., et al. (2011). Effects of renal sympathetic denervation on blood pressure, sleep apnea course, and glycemic control in patients with

resistant hypertension and sleep apnea. *Hypertension* 58, 559–565. doi: 10.1161/HYPERTENSIONAHA.111.173799

Zanchetti, A. S. (1977). Neural regulation of renin release: experimental evidence and clinical implications in arterial hypertension. *Circulation* 56, 691–698. doi: 10.1161/01.CIR.56.5.691

Zhao, Q., Yu, S., Huang, H., Tang, Y., Xiao, J., Dai, Z., et al. (2013). Effects of renal sympathetic denervation on the development of atrial fibrillation substrates in dogs with pacing-induced heart failure. *Int. J. Cardiol.* 168, 1672–1673. doi: 10.1016/j.ijcard.2013.03.091

Conflict of Interest Statement: MS is an investigator in studies sponsored by Medtronic and has received honoraria and lecture fees. AT has no conflicts of interest to disclose.

frontiers in Physiology

REVIEW
published: 25 August 2015
doi: 10.3389/fphys.2015.00238

Regulation of the renal sympathetic nerves in heart failure

Rohit Ramchandra and Carolyn J. Barrett*

Department of Physiology, The University of Auckland, Auckland, New Zealand

Heart failure (HF) is a serious debilitating condition with poor survival rates and an increasing level of prevalence. HF is associated with an increase in renal norepinephrine (NE) spillover, which is an independent predictor of mortality in HF patients. The excessive sympatho-excitation that is a hallmark of HF has long-term effects that contribute to disease progression. An increase in directly recorded renal sympathetic nerve activity (RSNA) has also been recorded in animal models of HF. This review will focus on the mechanisms controlling sympathetic nerve activity (SNA) to the kidney during normal conditions and alterations in these mechanisms during HF. In particular the roles of afferent reflexes and central mechanisms will be discussed.

Keywords: heart failure, renal sympathetic nerve activity, central control

OPEN ACCESS

Edited by:
Kate Denton,
Monash University, Australia

Reviewed by:
Kenju Miki,
Nara Women's University, Japan
Luciana A. Campos,
University Camilo Castelo Branco, Brazil

****Correspondence:***
Rohit Ramchandra,
Department of Physiology,
The University of Auckland,
Building 503, 85 Park Road,
Auckland 1142, New Zealand
r.ramchandra@auckland.ac.nz

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *26 June 2015*
Accepted: *07 August 2015*
Published: *25 August 2015*

Citation:
Ramchandra R and Barrett CJ (2015) Regulation of the renal sympathetic nerves in heart failure. Front. Physiol. 6:238. doi: 10.3389/fphys.2015.00238

Introduction

Heart failure (HF) is a complex syndrome, arising secondary to a wide range of cardiac structural and functional abnormalities, with the manifestations being shortness of breath, fatigue, exercise intolerance, and oedema. Elevated sympathetic drive is well recognized to play a key role in the pathophysiology of HF, with increased sympathetic nerve activity (SNA) to the kidneys resulting in renal vasoconstriction, increased renal sodium retention and increased renin release, and consequently elevated angiotensin II and aldosterone levels (DiBona and Kopp, 1997). In turn, angiotensin II is known to drive adverse cardiac remodeling and deterioration in cardiac function via a number of different pathways, including inflammatory pathways and fibrosis (Zhao et al., 2014). While initially activation of renal sympathetic nerve activity (RSNA) may assist in maintaining cardiac output, in the long-term neurohumoral activation drives the fluid overload associated with HF, significantly impacting both morbidity and mortality.

The significance of the sympathetic activation in HF is highlighted by the successful use of ACE inhibitors and β-blockers in the clinical management of the syndrome (Yancy et al., 2013). β-Blockers are well established to reduce the rate of mortality (Esler and Kaye, 1998), however, even when the majority of patients are prescribed β-blockers, increased renal norepinephrine (NE) spillover is directly linked to mortality (Petersson et al., 2005). Kidney function is an important determinant of outcomes during HF, with glomerular filtration rate, being an independent predictor of mortality in HF (Hillege et al., 2006). Understanding the mechanisms regulating renal function, and the changes that occur during the development of HF is thus crucial in understanding the pathophysiology of HF. In this review we will review the evidence for an increase in RSNA in HF and focus on three key areas potentially involved in mediating the changes: the arterial baroreceptors, the peripheral chemoreceptors, and the central regulation of RSNA within the paraventricular nucleus of the hypothalamus. A more in depth discussion of additional afferent signals such as from cardiopulmonary volume receptors and the afferent kidney reflex in control of RSNA in HF is presented by Booth et al. in this same special edition.

Resting Levels of Renal Sympathetic Nerve Activity in HF

Early studies in patients with severe HF have indicated that the spillover of NE from the kidney is increased (Hasking et al., 1986; Aggarwal et al., 2003). **Figure 1** illustrates the progressive increase in spillover of NE from the kidney observed in clinical studies. As the figure indicates, as the progression of HF reaches ejection fractions of below 30%, there is a significant increase in renal spillover of NE. Rundqvist et al. (1997) indicate that at ejection fractions of 29 ± 7%, the renal spillover of NE is not significantly different to a normal matched cohort. However, once ejection fraction decreases below 25%, there is a significant increase in renal spillover of NE. Interestingly at ejection fractions of 29%, a three-fold increase in cardiac spillover of NE is observed indicating that the increase in cardiac spillover of NE occurs earlier than that in the kidney (Rundqvist et al., 1997).

An increase in spillover of NE from the kidney may be driven by changes in directly recorded SNA or may be secondary to changes in reuptake of NE at the synapse. Various animal models have been employed to study the role of the renal sympathetic nerves in HF. These include the myocardial infarction (MI) induced HF model in the rat and pacing induced HF in the rabbit, sheep, and the dog. The majority of studies have indicated an increase in directly recorded RSNA during HF with ejection fractions of 36–45%. The MI induced model of HF in rodents results in a significant increase in baseline resting levels of RSNA (DiBona et al., 1996, 1997). Similar to this, the pacing induced model of HF in rabbits is also associated with an increase in baseline levels of sympathetic drive to the kidney (Sun et al., 1999a; Liu et al., 2000). The finding of a significant increase in RSNA is not universal in all animal models of HF. Previous studies have found that baseline levels of RSNA are not increased in doxorubicin induced HF (Rossi et al., 2008) or in pacing induced HF in sheep (Ramchandra et al., 2009a). Given the progressive increase in renal spillover of NE as HF worsens, it is possible that these findings merely reflect models of HF which are not severe enough.

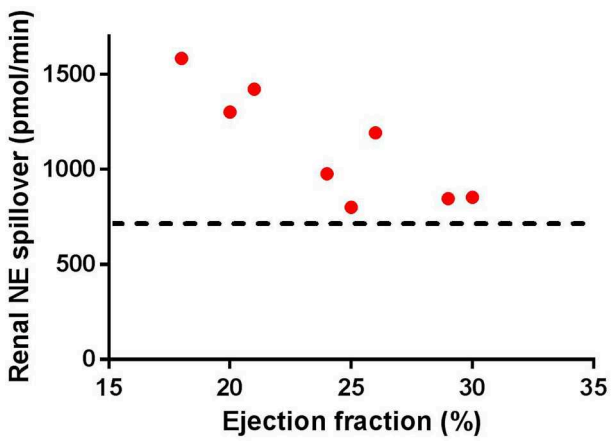


FIGURE 1 | Renal norepinephrine (NE) spillover increases as the ejection fraction decreases in separate groups of heart failure patients (individual numbers taken from Rundqvist et al., 1997; Brunner-La Rocca et al., 2001; Al-Hesayen and Parker, 2004, 2008; Petersson et al., 2005). Note the straight line indicates the average baseline renal NE spillover in normal age-matched controls (taken from Rundqvist et al., 1997; Petersson et al., 2005).

Clinical Relevance

It is interesting that animal models of HF that have indicated an increase in RSNA show increases at ejection fractions of 50% while clinically at the same stage of HF, there is no increase in renal spillover of NE. Considering this discrepancy, it is important to note that the majority of HF patients are on medications in particular ACE inhibitors and angiotensin II receptor blockers. Given that angiotensin II has been shown to facilitate release of NE from sympathetic nerve endings (Johnson et al., 1974; Burgdorf et al., 2003), it is tempting to speculate that any effects of RSNA in moderate stages of HF may be attenuated by these drugs although direct evidence of this is currently lacking. In addition, the re-uptake of NE at the synapse in the kidney is lower compared to the heart and this has meant that the role of reuptake mechanisms in modulating spillover of NE in the kidney has not been adequately explored.

The Role of Afferent Inputs in Regulating RSNA

The regulation of RSNA is dependent on the integration of many afferent inputs, including the cardiopulmonary receptors, cardiac chemoreceptor afferents, and feedback from muscle receptors (**Figure 2**). In HF the general consensus is that there is a blunting of the inhibitory reflexes, including the arterial and cardiopulmonary baroreceptors and an increase in the sensitivity of the excitatory reflexes, including the peripheral chemoreceptors, cardiac chemosensory afferent reflexes, and muscle metaboreceptors (as reviewed by Floras, 2009; Zucker et al., 2012). Recently focus has been drawn to the role of the arterial baroreceptor reflex and the peripheral chemoreceptors, due to the potential to modulate these reflexes clinically. We will focus on these two afferent reflexes.

Arterial Baroreceptor Reflex

Perhaps the most well recognized modulator of SNA is the arterial baroreceptor reflex. With receptors located primarily in the carotid sinus and aortic arch any increase in stretch of the arterial wall results in an increase in firing of afferent baroreceptor nerve activity. Stimulation of the arterial baroreceptors results in inhibition of SNA, playing an important role in buffering rapid changes in arterial pressure (Lohmeier and Iliescu, 2015). While initial studies suggested that the arterial baroreflex plays a minor role in chronic regulation, more recent studies indicate arterial baroreceptor reflexes are capable of regulating arterial pressure over much longer periods (Thrasher, 2005; Lohmeier et al., 2009). Sustained baroreflex-mediated inhibition of RSNA is observed in response to increases in arterial pressure induced by pressor doses of angiotensin II in both rats

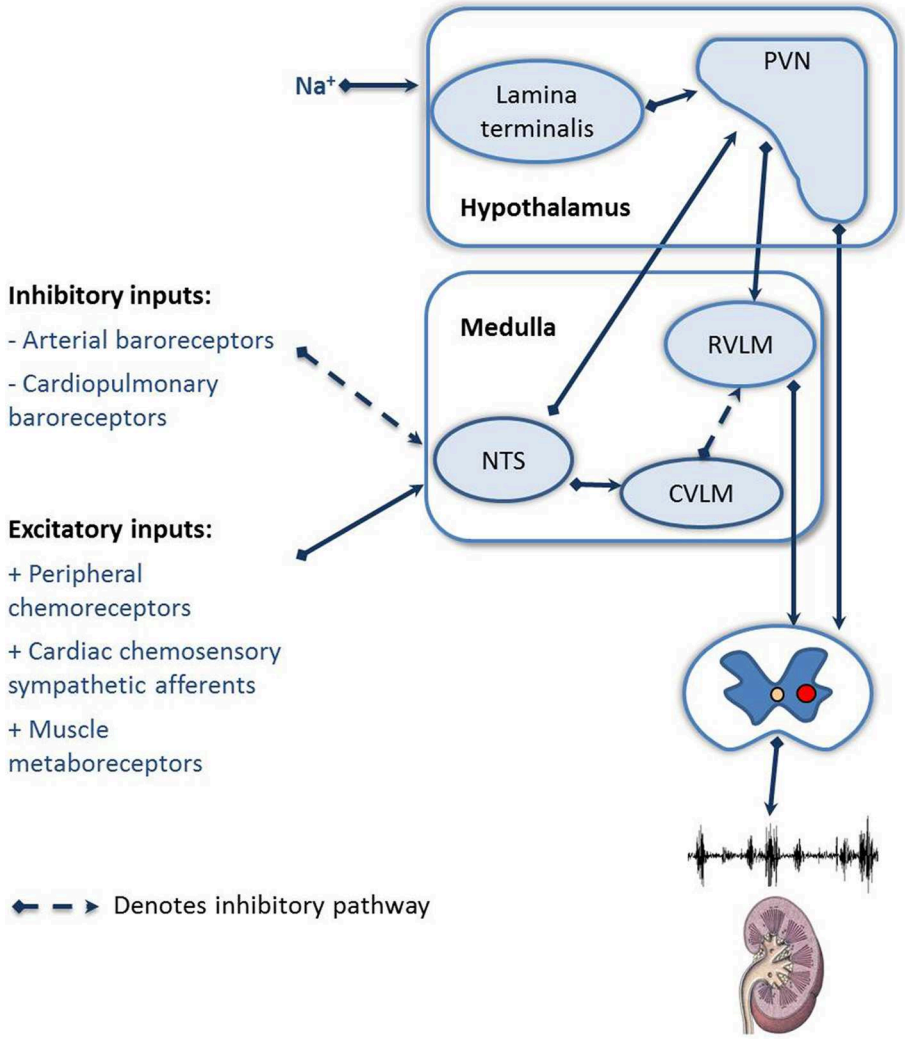


FIGURE 2 | Schematic showing the afferent reflexes which modulate renal sympathetic nerve activity during heart failure. NTS, Nucleus of the solitary tract; RVLM, rostral ventral lateral medulla; CVLM, caudal ventral lateral medulla; PVN, paraventricular nucleus of the hypothalamus.

and rabbit models (Barrett et al., 2003, 2005; Yoshimoto et al., 2010), suggesting that the baroreflex is capable of influencing RSNA chronically. Whether the baroreflex plays a role in driving the changes in RSNA observed during HF remains controversial. Most reports have suggested that in HF arterial baroreflex control of RSNA is blunted, with a reduced sensitivity (DiBona et al., 1995; Murakami et al., 1997) and often also a reduced range of control (DiBona and Sawin, 1994; Liu et al., 1996; Zhang et al., 1999). As a consequence it is often argued that blunting of the arterial baroreflex may play a permissive role in HF, allowing the excitatory reflexes to predominate, resulting in an increase in RSNA (DiBona and Sawin, 1995). However, there is growing evidence that the arterial baroreflex regulation of RSNA in HF is more complicated than first presented. Recent research suggests that the degree of HF, female sex, and methods of normalizing RSNA can all influence the interpretation of data (Pinkham et al., 2015). Pinkham et al. showed that a blunting of arterial baroreflex regulation of RSNA was positively associated with deterioration in left ventricular function in males and ovariectomized females, but not ovary intact females, and only when the data was normalized (Pinkham et al., 2015). These findings fit with those of Ramchandra et al. (2009a) who have demonstrated that arterial baroreflex control of RSNA is unaltered in conscious female sheep with mild pacing induced HF. It should also be noted that elevations in plasma NE levels are still observed with the development of HF in dogs with baroreceptor denervation (Brändle et al., 1996). Thus, while the majority of studies suggest that at least in males with severe HF there may be an impaired ability of the baroreflex to inhibit RSNA, it is difficult to suggest that the arterial baroreceptor reflex is the primary driver for the increase in RSNA associated with HF. Furthermore, clinical studies suggest that whilst baroreflex control of heart rate (HR) is

impaired in HF, there is little evidence to suggest that impairment of the baroreceptor reflex control of muscle SNA plays a role in driving the increase in muscle SNA (Floras, 2009).

Peripheral Chemoreflex

The carotid body (CB) is the most perfused organ per gram weight in the body [2000 ml min^{-1} (100 mg tissue)$^{-1}$]. It receives its blood supply via an arterial branch arising from the internal or external carotid artery, which ensures that the CB responds acutely to changes in arterial oxygen levels and other contents (e.g., CO_2, acidotic pH, hypoglycaemia, and hypoperfusion). Stimulation of the CB drives an increase in systemic sympathetic tone through direct signaling to the nucleus tractus solitarii and rostral ventrolateral medulla (Marshall, 1994). The CB chemoreflex not only serves as a protective mechanism during hypoxia (Prabhakar, 2013), but also contributes eupnoeic drive to breathing and control of blood flow during exercise (Dempsey, 2012). Activation of the CB by hypoxia or chemical stimuli increases respiratory drive and SNA to various vascular beds (Xing and Pilowsky, 2010), including the heart (Kollai et al., 1978).

More recently the role of the CB chemoreflex has been implicated in mediating the increased RSNA during HF (Li et al., 2005, 2006; Schultz and Li, 2007; Ding et al., 2011) and hypertension (McBryde et al., 2013; Paton et al., 2013; Moraes et al., 2015). In a rabbit model of pacing-induced HF, Schultz and colleagues have documented that maladaptive CB activity not only contributes to the enhanced RSNA response to hypoxia (Ding et al., 2011), but also provides a tonic excitatory influence to increase RSNA (Sun et al., 1999b). This enhanced responsiveness appears to be mediated at least in part by the CB chemoreflex, and depends on reduced CB blood flow, increased angiotensin II, and down-regulation of nitric oxide (NO) (Li et al., 2005, 2006; Ding et al., 2011). Importantly, inhalation of 100% oxygen decreased RSNA levels in pacing-induced HF animals to a greater extent than in normal animals (Sun et al., 1999b), suggesting a tonic excitatory input from this reflex. Interestingly, acute inhibition of the CB also reduces cardiac SNA (Xing et al., 2014) and consistent with this, denervation of the CB has led to an improvement in heart function (Del Rio et al., 2013) further strengthening the case for targeting the CB in HF.

Clinical Relevance

In clinical studies, the issue is less clear with some reports finding that breathing 100% oxygen does not decrease muscle SNA in HF (van de Borne et al., 1996), whereas others have found reduced SNA suggesting a role for tonic chemoreflex activation (Despas et al., 2009; Franchitto et al., 2010). This discrepancy is probably due to the variety of methods used to assess peripheral chemoreflex sensitivity, and the patients selected for the studies. The discrepancy could also reflect differential control of SNA to the muscle and the kidney. Additionally and more importantly, only 40% of patients with HF indicate an augmented response to the chemoreflex (Chua et al., 1997). This was classified as an increase in ventilation rate during hypoxia induced using transient inhalations of 100% nitrogen. We can conclude that there is clearly a large percentage of the HF population where any putative increases in RSNA are driven by other mechanisms. Why some patients are sensitive to the chemoreflex but others not remains unclear. This may be related to anemia induced by HF since one previous study has shown that deactivation of chemoreceptors reduces muscle SNA in chronic HF patients with anemia but does not change levels of muscle SNA in chronic HF patients with no anemia (Franchitto et al., 2010). These subtleties of HF have not been adequately reproduced in animal models of HF and remain an important area where more basic research is required.

Central Regulators of RSNA

We will now shift focus from the afferent reflexes to central brain regions which integrate the input of these reflexes. In this context, sympathetic drive to various organs arises from sympathetic premotor neurons in the central nervous system. These include the "traditional" sympathetic premotor regions such as the rostral ventral lateral medulla, the rostral ventral medial medulla, the medullary raphe, the paraventricular nucleus of the hypothalamus (PVN), and the A5 noradrenergic cell group (Strack et al., 1989; Schramm et al., 1993) as well as recent additions including the locus coeruleus, lateral hypothalamus, and periaqueductal gray (Cano et al., 2004), Barrington's nucleus (Cano et al., 2000) and subcoeruleus nucleus (Cano et al., 2004). When it comes to ascertaining the central brain regions involved in regulating sympathetic drive to the kidney the majority of studies have focused on the PVN due to the important role this region plays in blood volume regulation. Anatomically the PVN includes vasopressin-producing magnocellular neurons and parvocellular neurons which project to both the spinal preganglionic sympathetic neurons and premotor sympathetic neurons in the brain stem (Shafton et al., 1998). Moreover, plasma and CSF [Na^+] are monitored via sodium/osmo receptors in the lamina terminalis (McKinley et al., 2003) and studies have indicated an important pathway from the lamina terminalis to the PVN in mediating changes in RSNA (May et al., 2000; Shi et al., 2007).

Role of the PVN in Mediating Increased RSNA during HF

Previous studies that have examined the role of the PVN in the baseline control of RSNA in normal animals have found variable effects. Microinjection of muscimol, a GABA receptor agonist into the PVN, results in increases (Badoer et al., 2002), decreases (Zhang et al., 2002; Akine et al., 2003), or no change in resting levels of RSNA (Ng et al., 2004; Stocker et al., 2004, 2005; Ramchandra et al., 2013). These different RSNA responses are likely to be due to the effects of anesthesia in previous studies, as anesthesia can significantly modify the responses to stimulation of the PVN (Kannan et al., 1989). These data suggest that in conscious animals, there is little contribution by the PVN to baseline levels of RSNA, although we cannot categorically rule out a tonic inhibitory role of the PVN in mediating baseline levels of RSNA (Zhang et al., 2002; Ramchandra et al., 2013).

In contrast to normal animals, the PVN appears to play an important role in mediating the high resting levels of RSNA observed in animal models of HF. Activation of the PVN in HF is indicated by the increased expression of *c-fos* as well as *Fos* related antigens in neurons in the PVN (Vahid-Ansari and Leenen, 1998; Patel et al., 2000). More importantly, studies in a rat model of HF indicate that activation of the PVN contributes to the increased levels of RSNA (Li and Patel, 2003; Zheng et al., 2009). Microinjection of muscimol into the PVN of rats with HF results in diminished depressor responses in MAP, HR, and RSNA (Patel, 2000; Zhang et al., 2002; Wang et al., 2009) suggesting a reduction in the GABAergic inhibitory input to the PVN may mediate the increase in baseline levels of RSNA (Carillo et al., 2012).

In terms of neuromodulators involved, studies have focused on the role of NO within the PVN. Reduced synthesis of NO, due to down regulation of the neuronal isoform of nitric oxide synthase (NOS), has been suggested as a cause of the centrally mediated sympathoexcitation in HF. In conscious normal rats, unilateral PVN microinjections of the NO donor, sodium nitroprusside (SNP) (0.5–1.0 M, about 13–26 μg), decreased MAP (Martins-Pinge et al., 2012). This is also associated with a decrease in RSNA in anesthetized rats (Zhang et al., 1997) indicating a sympathoinhibitory action of NO within the PVN. Although the inhibition of RSNA in these studies is likely a direct central effect of SNP, as the renal inhibition occurred in the presence of a fall in blood pressure, it is important to note that the intravenous dose of SNP required to produce large falls in blood pressure in rats is 5–10 μg. This raises the possibility that changes observed after SNP infusions or microinjections may result from supraphysiological increases in NO levels. In this regard, studies with inhibition of NOS are clearer to interpret. Acute inhibition of central NOS with the nonselective inhibitor N^{ω}-nitro-l-arginine methyl ester (l-NAME), microinjected in the PVN increased baseline levels of RSNA (Zhang et al., 1997; Zhang and Patel, 1998). These findings suggest that NO within the PVN regulates baseline levels of RSNA in rodents however it is important to note that in conscious sheep and rabbits, this is not the case (Ng et al., 2004; Ramchandra et al., 2014).

Previous studies have indicated that neuronal NOS (nNOS) levels are decreased in HF, in particular, in neurons of the PVN (Patel et al., 1996a; Zhang et al., 1998; Ramchandra et al., 2014). The functional importance of this as a cause of the increased RSNA in HF is shown by the beneficial effects of nNOS gene transfer into the PVN (Zheng et al., 2011). Administration of a recombinant adenovirus that overexpressed nNOS resulted in an attenuated increase in HR and RSNA due to microinjection of N-methylD-aspartic acid into the PVN (Zheng et al., 2011). These studies indicate that during HF the PVN is at least partly responsible for the increase in baseline levels of RSNA.

Studies have also examined the role of the PVN in modulating the reflex regulation of RSNA. The primary reflex studied in this regard has been the cardiopulmonary afferent reflex. In essence, an increase in circulating blood volume using infusion of plasma or plasma expanders results in an increase in renal blood flow that is mediated by a decrease in SNA to the kidney. In this context, the PVN plays a critical role in mediating this inhibition of RSNA in a variety of species (Badoer et al., 2002; Li et al., 2003; Ramchandra et al., 2013). For example, microinjection of muscimol into the PVN caused attenuation of the inhibition of RSNA during volume expansion in conscious rabbits (Ng et al., 2004) and sheep (Ramchandra et al., 2013). Furthermore, lesions of the PVN in anesthetized rats attenuated the inhibition of RSNA and renal vasodilatation following volume expansion (Lovick et al., 1993; Haselton et al., 1994). Together these data indicate that in the normal state volume expansion stimulates a reflex that increases the level of GABAergic inhibitory input into the PVN and inhibits RSNA. In contrast, volume expansion during HF fails to activate central pathways (Akama et al., 1998) and results in an attenuated decrease in RSNA (Dibner-Dunlap and Thames, 1992; Patel et al., 1996b; Ramchandra et al., 2009b). Interestingly, this attenuated inhibition of RSNA during volume expansion is restored post exercise training (Pliquett et al., 2003; Zheng et al., 2006) probably mediated by an up-regulation of nNOS positive neurons within the PVN (Zheng et al., 2005).

It must be mentioned that in anesthetized animals, *dis*inhibition of the PVN attenuates the RSNA inhibition in contrast to conscious animals where inhibition of the PVN attenuated the RSNA inhibition. One important unanswered question is how inhibition of the PVN leads to an inhibition of RSNA. In this context, there are numerous inhibitory interneurons in the PVN. When muscimol is microinjected into the PVN, it is still unclear what subsets of neurons are being inhibited and whether inhibition of inhibitory interneurons occurs. This makes it hard to decipher neuronal networks within the PVN. Irrespective of these limitations, the PVN plays an important role in reflex regulation of RSNA and appears to be important in mediating the impaired reflex regulation of RSNA during HF.

Conclusions

Understanding the role of RSNA in HF is difficult in humans where measurement is indirect at best. When considering findings from animal studies we must be mindful that the degree and model of HF, species and sex of the animals and presence of anesthesia all add complexity to the interpretation. Evidence does suggest that deterioration in cardiac function in HF is closely associated with elevations in RSNA, although it would appear that increased NE spillover only becomes significant once the ejection fraction is reduced below 30%. The exact mechanisms driving the increase in RSNA remain controversial, in part reflecting the diverse nature of the origins of HF. While any changes in sensitivity of the reflex regulation of SNA may simply be secondary to the increase in RSNA, there is emerging evidence that, at least in some instances, the peripheral chemoreceptors may be involved in driving the increase in RSNA. While in severe HF, there may be an impaired ability of the baroreflex to inhibit RSNA in males, it is difficult to suggest that the arterial baroreceptor reflex is the primary driver for the increase in RSNA. Additionally HF is characterized by impaired regulation of body water content. The PVN is highlighted as one central area which is associated with the impairment of the regulation

of blood volume in HF, with an impaired ability of the PVN to inhibit RSNA in response to the increased blood volume observed in HF. Understanding the drivers for the increased RSNA and the variability that occurs between individuals is key to the successful management of HF.

Acknowledgments

Part of this work was supported by a National Health and Medical Research Council of Australia Project Grant and the University of Auckland Faculty Research Development Fund.

References

Aggarwal, A., Esler, M. D., Morris, M. J., Lambert, G., and Kaye, D. M. (2003). Regional sympathetic effects of low-dose clonidine in heart failure. *Hypertension* 41, 553–557. doi: 10.1161/01.HYP.0000055779.93635.A2

Akama, H., McGrath, B. P., and Badoer, E. (1998). Volume expansion fails to normally activate neural pathways in the brain of conscious rabbits with heart failure. *J. Auton. Nerv. Syst.* 73, 54–62. doi: 10.1016/S0165-1838(98)00124-6

Akine, A., Montanaro, M., and Allen, A. M. (2003). Hypothalamic paraventricular nucleus inhibition decreases renal sympathetic nerve activity in hypertensive and normotensive rats. *Auton. Neurosci.* 108, 17–21. doi: 10.1016/j.autneu.2003.08.009

Al-Hesayen, A., and Parker, J. D. (2004). Impaired baroreceptor control of renal sympathetic activity in human chronic heart failure. *Circulation* 109, 2862–2865. doi: 10.1161/01.CIR.0000129313.73750.57

Al-Hesayen, A., and Parker, J. D. (2008). The effects of dobutamine on renal sympathetic activity in human heart failure. *J. Cardiovasc. Pharmacol.* 51, 434–436. doi: 10.1097/FJC.0b013e3181684026

Badoer, E., Ng, C. W., and De Matteo, R. (2002). Tonic sympathoinhibition arising from the hypothalamic PVN in the conscious rabbit. *Brain Res.* 947, 17–24. doi: 10.1016/S0006-8993(02)02901-3

Barrett, C. J., Guild, S. J., Ramchandra, R., and Malpas, S. C. (2005). Baroreceptor denervation prevents sympathoinhibition during angiotensin II-induced hypertension. *Hypertension* 46, 168–172. doi: 10.1161/01.HYP.0000168047.09637.d4

Barrett, C. J., Ramchandra, R., Guild, S. J., Lala, A., Budgett, D. M., and Malpas, S. C. (2003). What sets the long-term level of renal sympathetic nerve activity: a role for angiotensin II and baroreflexes? *Circ. Res.* 92, 1330–1336. doi: 10.1161/01.RES.0000078346.60663.A0

Brändle, M., Wang, W., and Zucker, I. H. (1996). Hemodynamic correlates of baroreflex impairment of heart rate in experimental canine heart failure. *Basic Res. Cardiol.* 91, 147–154. doi: 10.1007/BF00799687

Brunner-La Rocca, H. P., Kaye, D. M., Woods, R. L., Hastings, J., and Esler, M. D. (2001). Effects of intravenous brain natriuretic peptide on regional sympathetic activity in patients with chronic heart failure as compared with healthy control subjects. *J. Am. Coll. Cardiol.* 37, 1221–1227. doi: 10.1016/S0735-1097(01)01172-X

Burgdorf, C., Richardt, D., Kurz, T., and Richardt, G. (2003). Presynaptic regulation of norepinephrine release in a model of nonfailing hypertrophied myocardium. *J. Cardiovasc. Pharmacol.* 41, 813–816. doi: 10.1097/00005344-200305000-00020

Cano, G., Card, J. P., Rinaman, L., and Sved, A. F. (2000). Connections of Barrington's nucleus to the sympathetic nervous system in rats. *J. Auton. Nerv. Syst.* 79, 117–128. doi: 10.1016/S0165-1838(99)00101-0

Cano, G., Card, J. P., and Sved, A. F. (2004). Dual viral transneuronal tracing of central autonomic circuits involved in the innervation of the two kidneys in rat. *J. Comp. Neurol.* 471, 462–481. doi: 10.1002/cne.20040

Carillo, B. A., Oliveira-Sales, E. B., Andersen, M., Tufik, S., Hipolide, D., Santos, A. A., et al. (2012). Changes in GABAergic inputs in the paraventricular nucleus maintain sympathetic vasomotor tone in chronic heart failure. *Auton. Neurosci.* 171, 41–48. doi: 10.1016/j.autneu.2012.10.005

Chua, T. P., Ponikowski, P., Webb-Peploe, K., Harrington, D., Anker, S. D., Piepoli, M., et al. (1997). Clinical characteristics of chronic heart failure patients with an augmented peripheral chemoreflex. *Eur. Heart J.* 18, 480–486. doi: 10.1093/oxfordjournals.eurheartj.a015269

Del Rio, R., Marcus, N. J., and Schultz, H. D. (2013). Carotid chemoreceptor ablation improves survival in heart failure: rescuing autonomic control of cardiorespiratory function. *J. Am. Coll. Cardiol.* 62, 2422–2430. doi: 10.1016/j.jacc.2013.07.079

Dempsey, J. A. (2012). New perspectives concerning feedback influences on cardiorespiratory control during rhythmic exercise and on exercise performance. *J. Physiol.* 590(Pt 17), 4129–4144. doi: 10.1113/jphysiol.2012.233908

Despas, F., Detis, N., Dumonteil, N., Labrunee, M., Bellon, B., Franchitto, N., et al. (2009). Excessive sympathetic activation in heart failure with chronic renal failure: role of chemoreflex activation. *J. Hypertens.* 27, 1849–1854. doi: 10.1097/HJH.0b013e32832e8d0f

Dibner-Dunlap, M. E., and Thames, M. D. (1992). Control of sympathetic nerve activity by vagal mechanoreflexes is blunted in heart failure. *Circulation* 86, 1929–1934. doi: 10.1161/01.CIR.86.6.1929

DiBona, G. F., Jones, S. Y., and Brooks, V. L. (1995). ANG II receptor blockade and arterial baroreflex regulation of renal nerve activity in cardiac failure. *Am. J. Physiol.* 269(5 Pt 2), R1189–R1196.

DiBona, G. F., Jones, S. Y., and Sawin, L. L. (1997). Reflex influences on renal nerve activity characteristics in nephrosis and heart failure. *J. Am. Soc. Nephrol.* 8, 1232–1239.

DiBona, G. F., and Kopp, U. C. (1997). Neural control of renal function. *Physiol. Rev.* 77, 75–197.

DiBona, G. F., and Sawin, L. L. (1994). Reflex regulation of renal nerve activity in cardiac failure. *Am. J. Physiol.* 266(1 Pt 2), R27–R39.

DiBona, G. F., and Sawin, L. L. (1995). Increased renal nerve activity in cardiac failure: arterial vs. cardiac baroreflex impairment. *Am. J. Physiol.* 268(1 Pt 2), R112-R116.

DiBona, G. F., Sawin, L. L., and Jones, S. Y. (1996). Characteristics of renal sympathetic nerve activity in sodium-retaining disorders. *Am. J. Physiol.* 271(1 Pt 2), R295–R302.

Ding, Y., Li, Y. L., and Schultz, H. D. (2011). Role of blood flow in carotid body chemoreflex function in heart failure. *J. Physiol.* 589(Pt 1), 245–258. doi: 10.1113/jphysiol.2010.200584

Esler, M., and Kaye, D. (1998). Increased sympathetic nervous system activity and its therapeutic reduction in arterial hypertension, portal hypertension and heart failure. *J. Auton. Nerv. Syst.* 72, 210–219. doi: 10.1016/S0165-1838(98)00107-6

Floras, J. S. (2009). Sympathetic nervous system activation in human heart failure: clinical implications of an updated model. *J. Am. Coll. Cardiol.* 54, 375–385. doi: 10.1016/j.jacc.2009.03.061

Franchitto, N., Despas, F., Labrunée, M., Roncalli, J., Boveda, S., Galinier, M., et al. (2010). Tonic chemoreflex activation contributes to increased sympathetic nerve activity in heart failure-related anemia. *Hypertension* 55, 1012–1017. doi: 10.1161/HYPERTENSIONAHA.109.146779

Haselton, J. R., Goering, J., and Patel, K. P. (1994). Parvocellular neurons of the paraventricular nucleus are involved in the reduction in renal nerve discharge during isotonic volume expansion. *J. Auton. Nerv. Syst.* 50, 1–11. doi: 10.1016/0165-1838(94)90117-1

Hasking, G. J., Esler, M. D., Jennings, G. L., Burton, D., Johns, J. A., and Korner, P. I. (1986). Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. *Circulation* 73, 615–621. doi: 10.1161/01.CIR.73.4.615

Hillege, H. L., Nitsch, D., Pfeffer, M. A., Swedberg, K., McMurray, J. J., Yusuf, S., et al. (2006). Renal function as a predictor of outcome in a broad spectrum of patients with heart failure. *Circulation* 113, 671–678. doi: 10.1161/CIRCULATIONAHA.105.580506

Johnson, E. M. Jr., Marshall, G. R., and Needleman, P. (1974). Modification of responses to sympathetic nerve stimulation by the renin-angiotensin system in rats. *Br. J. Pharmacol.* 51, 541–547. doi: 10.1111/j.1476-5381.1974.tb09672.x

Kannan, H., Hayashida, Y., and Yamashita, H. (1989). Increase in sympathetic outflow by paraventricular nucleus stimulation in awake rats. *Am. J. Physiol.* 256(6 Pt 2), R1325–R1330.

Kollai, M., Koizumi, K., and Brooks, C. M. (1978). Nature of differential sympathetic discharges in chemoreceptor reflexes. *Proc. Natl. Acad. Sci. U.S.A.* 75, 5239–5243. doi: 10.1073/pnas.75.10.5239

Li, Y. F., Mayhan, W. G., and Patel, K. P. (2003). Role of the paraventricular nucleus in renal excretory responses to acute volume expansion: role of nitric oxide. *Am. J. Physiol. Heart Circ. Physiol.* 285, H1738–H1746. doi: 10.1152/ajpheart.00727.2002

Li, Y. F., and Patel, K. P. (2003). Paraventricular nucleus of the hypothalamus and elevated sympathetic activity in heart failure: the altered inhibitory mechanisms. *Acta Physiol. Scand.* 177, 17–26. doi: 10.1046/j.1365-201X.2003.01043.x

Li, Y. L., Li, Y. F., Liu, D., Cornish, K. G., Patel, K. P., Zucker, I. H., et al. (2005). Gene transfer of neuronal nitric oxide synthase to carotid body reverses enhanced chemoreceptor function in heart failure rabbits. *Circ. Res.* 97, 260–267. doi: 10.1161/01.RES.0000175722.21555.55

Li, Y. L., Xia, X. H., Zheng, H., Gao, L., Li, Y. F., Liu, D., et al. (2006). Angiotensin II enhances carotid body chemoreflex control of sympathetic outflow in chronic heart failure rabbits. *Cardiovasc. Res.* 71, 129–138. doi: 10.1016/j.cardiores.2006.03.017

Liu, J. L., Irvine, S., Reid, I. A., Patel, K. P., and Zucker, I. H. (2000). Chronic exercise reduces sympathetic nerve activity in rabbits with pacing-induced heart failure: a role for angiotensin II. *Circulation* 102, 1854–1862. doi: 10.1161/01.CIR.102.15.1854

Liu, J. L., Murakami, H., and Zucker, I. H. (1996). Effects of NO on baroreflex control of heart rate and renal nerve activity in conscious rabbits. *Am. J. Physiol.* 270(6 Pt 2), R1361–R1370.

Lohmeier, T. E., Hildebrandt, D. A., Dwyer, T. M., Iliescu, R., Irwin, E. D., Cates, A. W., et al. (2009). Prolonged activation of the baroreflex decreases arterial pressure even during chronic adrenergic blockade. *Hypertension* 53, 833–838. doi: 10.1161/HYPERTENSIONAHA.109.128884

Lohmeier, T. E., and Iliescu, R. (2015). The baroreflex as a long-term controller of arterial pressure. *Physiology* 30, 148–158. doi: 10.1152/physiol.00035.2014

Lovick, T. A., Malpas, S., and Mahony, M. T. (1993). Renal vasodilatation in response to acute volume load is attenuated following lesions of parvocellular neurones in the paraventricular nucleus in rats. *J. Auton. Nerv. Syst.* 43, 247–255. doi: 10.1016/0165-1838(93)90331-N

Marshall, J. M. (1994). Peripheral chemoreceptors and cardiovascular regulation. *Physiol. Rev.* 74, 543–594.

Martins-Pinge, M. C., Mueller, P. J., Foley, C. M., Heesch, C. M., and Hasser, E. M. (2012). Regulation of arterial pressure by the paraventricular nucleus in conscious rats: interactions among glutamate, GABA, and nitric oxide. *Front. Physiol.* 3:490. doi: 10.3389/fphys.2012.00490

May, C. N., McAllen, R. M., and McKinley, M. J. (2000). Renal nerve inhibition by central NaCl and ANG II is abolished by lesions of the lamina terminalis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 279, R1827–R1833.

McBryde, F. D., Abdala, A. P., Hendy, E. B., Pijacka, W., Marvar, P., Moraes, D. J., et al. (2013). The carotid body as a putative therapeutic target for the treatment of neurogenic hypertension. *Nat. Commun.* 4, 2395. doi: 10.1038/ncomms3395

McKinley, M. J., McAllen, R. M., Davern, P., Giles, M. E., Penschow, J., Sunn, N., et al. (2003). The sensory circumventricular organs of the mammalian brain. *Adv. Anat. Embryol. Cell Biol.* 172, III–XII, 1–122, back cover. doi: 10.1007/978-3-642-55532-9

Moraes, D. J., Machado, B. H., and Paton, J. F. (2015). Carotid body over activity induces respiratory neurone channelopathy contributing to neurogenic hypertension. *J. Physiol.* 593, 3055–3063. doi: 10.1113/JP270423

Murakami, H., Liu, J. L., and Zucker, I. H. (1997). Angiotensin II blockade [corrected] enhances baroreflex control of sympathetic outflow in heart failure. *Hypertension* 29, 564–569. doi: 10.1161/01.HYP.29.2.564

Ng, C. W., De Matteo, R., and Badoer, E. (2004). Effect of muscimol and L-NAME in the PVN on the RSNA response to volume expansion in conscious rabbits. *Am. J. Physiol. Renal Physiol.* 287, F739–F746. doi: 10.1152/ajprenal.00431.2003

Patel, K. P. (2000). Role of paraventricular nucleus in mediating sympathetic outflow in heart failure. *Heart Fail. Rev.* 5, 73–86. doi: 10.1023/A:1009850224802

Patel, K. P., Zhang, K., Kenney, M. J., Weiss, M., and Mayhan, W. G. (2000). Neuronal expression of Fos protein in the hypothalamus of rats with heart failure. *Brain Res.* 865, 27–34. doi: 10.1016/S0006-8993(00)02186-7

Patel, K. P., Zhang, K., Zucker, I. H., and Krukoff, T. L. (1996a). Decreased gene expression of neuronal nitric oxide synthase in hypothalamus and brainstem of rats in heart failure. *Brain Res.* 734, 109–115. doi: 10.1016/S0006-8993(96)00620-8

Patel, K. P., Zhang, P. L., and Carmines, P. K. (1996b). Neural influences on renal responses to acute volume expansion in rats with heart failure. *Am. J. Physiol.* 271(4 Pt 2), H1441–H1448.

Paton, J. F., Sobotka, P. A., Fudim, M., Engelman, Z. J., Hart, E. C., McBryde, F. D., et al. (2013). The carotid body as a therapeutic target for the treatment of sympathetically mediated diseases. *Hypertension* 61, 5–13. doi: 10.1161/HYPERTENSIONAHA.111.00064

Petersson, M., Friberg, P., Eisenhofer, G., Lambert, G., and Rundqvist, B. (2005). Long-term outcome in relation to renal sympathetic activity in patients with chronic heart failure. *Eur. Heart J.* 26, 906–913. doi: 10.1093/eurheartj/ehi184

Pinkham, M. I., Whalley, G., Guild, S. J., Malpas, S. C., and Barrett, C. J. (2015). Arterial baroreceptor reflex control of renal sympathetic nerve activity following chronic myocardial infarction in male, female and ovariectomized female rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 309, R169-R178. doi: 10.1152/ajpregu.00026.2015

Pliquett, R. U., Cornish, K. G., Patel, K. P., Schultz, H. D., Peuler, J. D., and Zucker, I. H. (2003). Amelioration of depressed cardiopulmonary reflex control of sympathetic nerve activity by short-term exercise training in male rabbits with heart failure. *J. Appl. Physiol.* 95, 1883–1888. doi: 10.1152/japplphysiol.00486.2003

Prabhakar, N. R. (2013). Sensing hypoxia: physiology, genetics and epigenetics. *J. Physiol.* 591(Pt 9), 2245–2257. doi: 10.1113/jphysiol.2012.247759

Ramchandra, R., Hood, S. G., Denton, D. A., Woods, R. L., McKinley, M. J., McAllen, R. M., et al. (2009a). Basis for the preferential activation of cardiac sympathetic nerve activity in heart failure. *Proc. Natl. Acad. Sci. U.S.A.* 106, 924–928. doi: 10.1073/pnas.0811929106

Ramchandra, R., Hood, S. G., Frithiof, R., and May, C. N. (2009b). Discharge properties of cardiac and renal sympathetic nerves and their impaired responses to changes in blood volume in heart failure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297, R665–R674. doi: 10.1152/ajpregu.00191.2009

Ramchandra, R., Hood, S. G., Frithiof, R., McKinley, M. J., and May, C. N. (2013). The role of the paraventricular nucleus of the hypothalamus in the regulation of cardiac and renal sympathetic nerve activity in conscious normal and heart failure sheep. *J. Physiol.* 591(Pt 1), 93–107. doi: 10.1113/jphysiol.2012.236059

Ramchandra, R., Hood, S. G., and May, C. N. (2014). Central exogenous nitric oxide decreases cardiac sympathetic drive and improves baroreflex control of heart rate in ovine heart failure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 307, R271–R280. doi: 10.1152/ajpregu.00057.2014

Rossi, N. F., Maliszewska-Scislo, M., and Chen, H. (2008). Central endothelin: effects on vasopressin and the arterial baroreflex in doxorubicin heart failure rats. *Can. J. Physiol. Pharmacol.* 86, 343–352. doi: 10.1139/Y08-027

Rundqvist, B., Elam, M., Bergmann-Sverrisdottir, Y., Eisenhofer, G., and Friberg, P. (1997). Increased cardiac adrenergic drive precedes generalized sympathetic activation in human heart failure. *Circulation* 95, 169–175. doi: 10.1161/01.CIR.95.1.169

Schramm, L. P., Strack, A. M., Platt, K. B., and Loewy, A. D. (1993). Peripheral and central pathways regulating the kidney: a study using pseudorabies virus. *Brain Res.* 616, 251–262. doi: 10.1016/0006-8993(93)90216-A

Schultz, H. D., and Li, Y. L. (2007). Carotid body function in heart failure. *Respir. Physiol. Neurobiol.* 157, 171–185. doi: 10.1016/j.resp.2007.02.011

Shafton, A. D., Ryan, A., and Badoer, E. (1998). Neurons in the hypothalamic paraventricular nucleus send collaterals to the spinal cord and to the rostral ventrolateral medulla in the rat. *Brain Res.* 801, 239–243. doi: 10.1016/S0006-8993(98)00587-3

Shi, P., Stocker, S. D., and Toney, G. M. (2007). Organum vasculosum laminae terminalis contributes to increased sympathetic nerve activity induced by central hyperosmolality. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 293, R2279–R2289. doi: 10.1152/ajpregu.00160.2007

Stocker, S. D., Hunwick, K. J., and Toney, G. M. (2005). Hypothalamic paraventricular nucleus differentially supports lumbar and renal sympathetic

outflow in water-deprived rats. *J. Physiol.* 563(Pt 1), 249–263. doi: 10.1113/jphysiol.2004.076661

Stocker, S. D., Keith, K. J., and Toney, G. M. (2004). Acute inhibition of the hypothalamic paraventricular nucleus decreases renal sympathetic nerve activity and arterial blood pressure in water-deprived rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 286, R719–R725. doi: 10.1152/ajpregu.00494.2003

Strack, A. M., Sawyer, W. B., Hughes, J. H., Platt, K. B., and Loewy, A. D. (1989). A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. *Brain Res.* 491, 156–162. doi: 10.1016/0006-8993(89)90098-X

Sun, S. Y., Wang, W., Zucker, I. H., and Schultz, H. D. (1999a). Enhanced activity of carotid body chemoreceptors in rabbits with heart failure: role of nitric oxide. *J. Appl. Physiol.* 86, 1273–1282.

Sun, S. Y., Wang, W., Zucker, I. H., and Schultz, H. D. (1999b). Enhanced peripheral chemoreflex function in conscious rabbits with pacing-induced heart failure. *J. Appl. Physiol.* 86, 1264–1272.

Thrasher, T. N. (2005). Effects of chronic baroreceptor unloading on blood pressure in the dog. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288, R863–R871. doi: 10.1152/ajpregu.00489.2004

Vahid-Ansari, F., and Leenen, F. H. (1998). Pattern of neuronal activation in rats with CHF after myocardial infarction. *Am. J. Physiol.* 275(6 Pt 2), H2140–H2146.

van de Borne, P., Oren, R., Anderson, E. A., Mark, A. L., and Somers, V. K. (1996). Tonic chemoreflex activation does not contribute to elevated muscle sympathetic nerve activity in heart failure. *Circulation* 94, 1325–1328. doi: 10.1161/01.CIR.94.6.1325

Wang, R. J., Zeng, Q. H., Wang, W. Z., and Wang, W. (2009). GABA(A) and GABA(B) receptor-mediated inhibition of sympathetic outflow in the paraventricular nucleus is blunted in chronic heart failure. *Clin. Exp. Pharmacol. Physiol.* 36, 516–522. doi: 10.1111/j.1440-1681.2008.05101.x

Xing, D. T., May, C. N., Booth, L. C., and Ramchandra, R. (2014). Tonic arterial chemoreceptor activity contributes to cardiac sympathetic activation in mild ovine heart failure. *Exp. Physiol.* 99, 1031–1041. doi: 10.1113/expphysiol.2014.079491

Xing, T., and Pilowsky, P. M. (2010). Acute intermittent hypoxia in rat *in vivo* elicits a robust increase in tonic sympathetic nerve activity that is independent of respiratory drive. *J. Physiol.* 588(Pt 16), 3075–3088. doi: 10.1113/jphysiol.2010.190454

Yancy, C. W., Jessup, M., Bozkurt, B., Butler, J., Casey, D. E. Jr., Drazner, M. H., et al. (2013). 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. *J. Am. Coll. Cardiol.* 62, e147–e239. doi: 10.1161/cir.0b013e31829e8807

Yoshimoto, M., Miki, K., Fink, G. D., King, A., and Osborn, J. W. (2010). Chronic angiotensin II infusion causes differential responses in regional sympathetic nerve activity in rats. *Hypertension* 55, 644–651. doi: 10.1161/HYPERTENSIONAHA.109.145110

Zhang, K., Li, Y. F., and Patel, K. P. (2002). Reduced endogenous GABA-mediated inhibition in the PVN on renal nerve discharge in rats with heart failure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 282, R1006–R1015. doi: 10.1152/ajpregu.00241.2001

Zhang, K., Mayhan, W. G., and Patel, K. P. (1997). Nitric oxide within the paraventricular nucleus mediates changes in renal sympathetic nerve activity. *Am. J. Physiol.* 273(3 Pt 2), R864–R872.

Zhang, K., and Patel, K. P. (1998). Effect of nitric oxide within the paraventricular nucleus on renal sympathetic nerve discharge: role of GABA. *Am. J. Physiol.* 275(3 Pt 2), R728–R734.

Zhang, K., Zucker, I. H., and Patel, K. P. (1998). Altered number of diaphorase (NOS) positive neurons in the hypothalamus of rats with heart failure. *Brain Res.* 786, 219–225. doi: 10.1016/S0006-8993(97)01449-2

Zhang, W., Huang, B. S., and Leenen, F. H. (1999). Brain renin-angiotensin system and sympathetic hyperactivity in rats after myocardial infarction. *Am. J. Physiol.* 276(5 Pt 2), H1608–H1615.

Zhao, Z., Wang, H., Jessup, J. A., Lindsey, S. H., Chappell, M. C., and Groban, L. (2014). Role of estrogen in diastolic dysfunction. *Am. J. Physiol. Heart Circ. Physiol.* 306, H628–H640. doi: 10.1152/ajpheart.00859.2013

Zheng, H., Li, Y. F., Cornish, K. G., Zucker, I. H., and Patel, K. P. (2005). Exercise training improves endogenous nitric oxide mechanisms within the paraventricular nucleus in rats with heart failure. *Am. J. Physiol. Heart Circ. Physiol.* 288, H2332–H2341. doi: 10.1152/ajpheart.00473.2004

Zheng, H., Li, Y. F., Wang, W., and Patel, K. P. (2009). Enhanced angiotensin-mediated excitation of renal sympathetic nerve activity within the paraventricular nucleus of anesthetized rats with heart failure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297, R1364–R1374. doi: 10.1152/ajpregu.00149.2009

Zheng, H., Li, Y. F., Zucker, I. H., and Patel, K. P. (2006). Exercise training improves renal excretory responses to acute volume expansion in rats with heart failure. *Am. J. Physiol. Renal Physiol.* 291, F1148–F1156. doi: 10.1152/ajprenal.00501.2005

Zheng, H., Liu, X., Li, Y., Sharma, N. M., and Patel, K. P. (2011). Gene transfer of neuronal nitric oxide synthase to the paraventricular nucleus reduces the enhanced glutamatergic tone in rats with chronic heart failure. *Hypertension* 58, 966–973. doi: 10.1161/HYPERTENSIONAHA.111.176222

Zucker, I. H., Patel, K. P., and Schultz, H. D. (2012). Neurohumoral stimulation. *Heart Fail. Clin.* 8, 87–99. doi: 10.1016/j.hfc.2011.08.007

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

MINI REVIEW
published: 01 October 2015
doi: 10.3389/fphys.2015.00270

The role of the renal afferent and efferent nerve fibers in heart failure

*Lindsea C. Booth *, Clive N. May and Song T. Yao*

Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, VIC, Australia

Renal nerves contain afferent, sensory and efferent, sympathetic nerve fibers. In heart failure (HF) there is an increase in renal sympathetic nerve activity (RSNA), which can lead to renal vasoconstriction, increased renin release and sodium retention. These changes are thought to contribute to renal dysfunction, which is predictive of poor outcome in patients with HF. In contrast, the role of the renal afferent nerves remains largely unexplored in HF. This is somewhat surprising as there are multiple triggers in HF that have the potential to increase afferent nerve activity, including increased venous pressure and reduced kidney perfusion. Some of the few studies investigating renal afferents in HF have suggested that at least the sympatho-inhibitory reno-renal reflex is blunted. In experimentally induced HF, renal denervation, both surgical and catheter-based, has been associated with some improvements in renal and cardiac function. It remains unknown whether the effects are due to removal of the efferent renal nerve fibers or afferent renal nerve fibers, or a combination of both. Here, we review the effects of HF on renal efferent and afferent nerve function and critically assess the latest evidence supporting renal denervation as a potential treatment in HF.

Keywords: radiofrequency-ablation, renal denervation, arterial baroreflex, renal sympathetic nerve activity, renal afferent

OPEN ACCESS

Edited by:
Kate Denton,
Monash University, Australia

Reviewed by:
Olaf Grisk,
University of Greifswald, Germany
Sebastian Kelle,
German Heart Institute Berlin, Germany

****Correspondence:***
Lindsea C. Booth,
Florey Institute of Neuroscience and Mental Health, University of Melbourne, 30 Royal Parade (corner Genetics Lane), Parkville, Melbourne, VIC 3010, Australia
lindsea.booth@florey.edu.au

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *25 June 2015*
Accepted: *14 September 2015*
Published: *01 October 2015*

Citation:
Booth LC, May CN and Yao ST (2015) The role of the renal afferent and efferent nerve fibers in heart failure. Front. Physiol. 6:270. doi: 10.3389/fphys.2015.00270

Patients with heart failure (HF) have a poor prognosis, with a 5-year mortality rate of 75% (Levy et al., 2002). In HF, the reduced cardiac output and inadequate perfusion of organs triggers a complex set of compensatory mechanisms, including activation of the sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS) (Weiss et al., 2003). The increased renal sympathetic nerve activity (RSNA) leads to increased release of renin, renal vasoconstriction (RVR), reduced renal blood flow (RBF), and renal sodium and water retention, with renal dysfunction being predictive of poor outcome (Goldberg et al., 2005; Petersson et al., 2005; Jose et al., 2006; Aspromonte et al., 2011). Although multiple therapies have been developed for the treatment of HF, these have only partially reduced the disease burden. As such, new treatments and novel approaches for tackling the disease are desperately needed.

Recently, catheter-based radiofrequency ablation of the renal nerves has been used as a treatment for drug-resistant hypertension and it has been proposed as a treatment for HF. The beneficial effects of renal denervation (RDN) are thought to depend on destruction of both the efferent, sympathetic and the afferent, sensory renal nerve fibers. This review will focus on the effects of the renal efferent and afferent nerve fibers in HF. We will also review the latest evidence supporting catheter-based RDN as a treatment in HF.

Increased Sympathetic Nerve Activity in Heart Failure

There are differential increases in sympathetic activity to individual organs in HF, as shown by measurement of regional noradrenaline spillover in HF patients (Hasking et al., 1986). It has been

shown in HF patients and animal models of HF that the greatest increase in SNA is to the heart, with a smaller increase to the kidneys (Hasking et al., 1986; Ramchandra et al., 2009a). Importantly, these increases in SNA to the heart and kidneys are predictive of poor outcome (Kaye et al., 1995; Petersson et al., 2005). Relatively large increases in RSNA have been reported in rats 4 weeks after myocardial infarction, with burst incidence increased from 35 to 47% (DiBona et al., 1988; Feng et al., 1994), and RSNA was increased from 30 to 60% of maximum in rabbits with pacing-induced HF (Liu et al., 2000, 2001). However, such large increases in RSNA are not always seen in the early stages of HF. For example, in sheep paced into HF (ejection fractions: 35–40%), cardiac SNA (CSNA) was increased three-fold, whereas a modest increase in RSNA was only observed when activity was expressed as bursts per minute, mostly driven by an increase in heart rate (HR) (Ramchandra et al., 2009b). Similarly in patients, renal noradrenaline spillover is not increased in mild HF (ejection fraction: 29%) but is significantly increased in severe HF (ejection fraction: 18%) (Rundqvist et al., 1997).

Causes of Increased Renal Efferent Sympathetic Nerve Activity in HF

Increased sympathetic drive to the kidneys in HF causes renal vasoconstriction, RAAS activation and sodium and water retention, leading to increases in blood volume and BP. Although this may initially be beneficial in improving perfusion, with deteriorating heart function, the enhanced sympathetic drive puts extra load on an already stressed cardiovascular system. This leads to a vicious cycle of increasingly high levels of sympathetic drive and a progressively deteriorating cardiac system (**Figure 1**). The mechanisms underlying the specific increase in sympathetic drive to the heart and kidneys in HF remain incompletely understood.

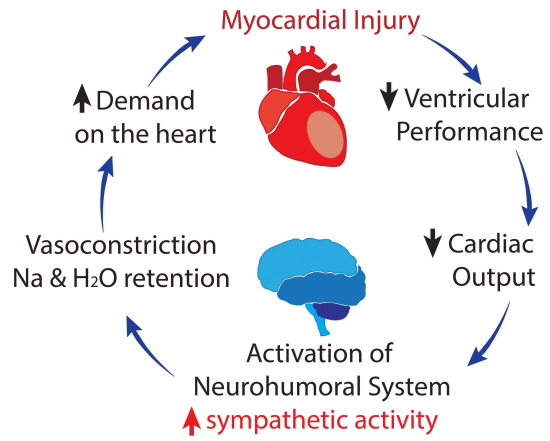


FIGURE 1 | Vicious cycle of heart failure; where activation of the sympathetic and renin-angiotensin-aldosterone systems contributes to a progressive deterioration in cardiac function.

Blunted Arterial Baroreflex Control in HF

There is extensive evidence indicating that altered control by inhibitory and excitatory reflexes contributes to the sympathoexcitation in HF. The arterial baroreflex is the main inhibitory reflex controlling SNA and desensitization of this reflex could contribute to increased SNA levels. Desensitized arterial baroreflex control has been shown for muscle SNA in HF patients (Leimbach et al., 1986; Grassi et al., 1995), and for RSNA in rabbits (Liu et al., 2000), dogs (Wang et al., 1991), and rats (Feng et al., 1994; DiBona and Sawin, 1995) with experimentally-induced HF. Impaired baroreflex control of SNA has, however, not been shown in all studies. For example, preserved arterial baroreflex control of muscle SNA has been reported in patients with HF (Dibner-Dunlap et al., 1996) and it has been argued that even in patients with advanced HF, the baroreflex control of muscle SNA is not desensitized (Floras, 2001). In anesthetized dogs with pacing-induced HF, baroreflex control of RSNA was preserved although baroreflex control of HR was desensitized (Dibner-Dunlap and Thames, 1989). Similarly, in ovine pacing-induced HF, the baroreflex control of RSNA and CSNA were unchanged; however, there was impaired baroreflex control of HR (Watson et al., 2007; Ramchandra et al., 2009a).

Attenuated Cardiopulmonary Reflex Inhibition of SNA in HF

The increase in blood volume and thus cardiac pressures that occur in HF would be expected to stimulate the cardiopulmonary reflex and inhibit SNA. There is extensive evidence that in HF the sensitivity of this inhibitory reflex is reduced. In rats, the reflex decrease in RSNA in response to acute volume expansion is reduced (DiBona et al., 1988). Similarly, we demonstrated that inhibition of RSNA, as well as CSNA, by volume expansion in normal sheep was largely abolished in sheep with HF (Ramchandra et al., 2009b). These findings indicate that the cardiopulmonary mechanoreceptor reflex is largely ineffective in HF, allowing SNA to remain elevated in the face of expanded blood volume.

Exaggerated Responses to Chemoreceptor Stimulation in HF

There is also evidence that the sympathoexcitatory chemoreflex is sensitized in patients with HF and that this is strongly associated with severity of the disease and poor outcome (Chua et al., 1997; Ponikowski et al., 2001). In support of these findings, in rabbits with pacing-induced HF, deactivation of the carotid chemoreflex with hyperoxia or cryoablation of the carotid bodies decreased RSNA (Sun et al., 1999; Marcus et al., 2014). Similarly, in ovine pacing-induced HF, deactivation of the carotid chemoreflex with hyperoxia decreased CSNA (Xing et al., 2014). Although, as described above, RSNA burst rate is not significantly elevated in this ovine HF model, hyperoxia decreased RSNA, as expressed as bursts/minute due to a decrease in HR (Xing et al., 2014). There is evidence that increased angiotensin II (AngII) levels, acting on angiotensin type-1 (AT-1) receptors in the carotid body, contributes to the sensitization of the chemoreflex in HF (Li et al., 2006).

Central Mechanisms Stimulating SNA in HF

A more in-depth discussion of the central control of RSNA in HF is presented by Ramchandra et al. in this same special edition. As such we will only touch upon this briefly.

There is extensive evidence that the central angiotensinergic system plays a critical role in stimulating the increased SNA in HF. Blockade of central AT-1 receptors with losartan reduced the elevated RSNA in rats with HF induced by myocardial infarction (DiBona et al., 1995; Zhang et al., 1999) and reduced the high level of CSNA in ovine HF (Ramchandra et al., 2012). In addition, there are increased levels of AT1 receptors in a number of central autonomic areas, including the subfornical organ, paraventricular nucleus of the hypothalamus (PVN), nucleus of the solitary tract (NTS), and rostral ventrolateral medulla (RVLM) (Yoshimura et al., 2000; Gao et al., 2008). In particular, there is strong evidence that the PVN plays an important role in setting the increased levels of RSNA in HF (Patel, 2000), although the same is not true for CSNA (Ramchandra et al., 2013). In addition to AngII, a number of other factors within the PVN are likely to contribute to the changes in RSNA, including impaired nitric oxide function (Reddy et al., 2007), increased cytokine levels and oxidative stress (Guggilam et al., 2007; Kang et al., 2010).

Renal Afferent Nerve Fibers in HF

Compared with the widely studied renal efferents, there have been few studies of the renal afferent nerve fibers in HF. Renal afferent nerve activity is influenced by two main classes of receptors; mechanoreceptors and chemoreceptors. Mechanoreceptors are found within the renal parenchyma and in the wall of the renal pelvis (Niijima, 1975). These respond to increases in intra-renal pressure (Ueda et al., 1967) and can be stimulated experimentally by renal vein occlusion/compression in rats (Ueda et al., 1967), cats (Astrom and Crafoord, 1968), and dogs (Kostreva et al., 1981) and physical compression of the hilus of the kidney (Ueda et al., 1967; Astrom and Crafoord, 1968). Stimulation of renal mechanoreceptors with increases in renal venous pressure has been shown to lead to an increase in ipsilateral renal *afferent* activity and decreases in ipsilateral and contralateral *efferent* RSNA (Ueda et al., 1967; Kopp et al., 1985). Mirroring the decrease in contralateral efferent RSNA, mechanoreceptor activation generally results in decreased contralateral RVR (Kostreva et al., 1981). RVR on the ipsilateral side, however, has been reported to increase in direct response to increased renal venous pressure via non-neural mechanisms (Dilley et al., 1983; Kopp et al., 1985). Activation of renal mechanoreceptors has also been shown to affect renal function, with an increase in contralateral urine flow and contra- and ispilateral increases in sodium excretion (Kopp et al., 1985), although some studies have shown no change in ipsilateral sodium excretion and instead showed a decrease in potassium excretion (Dilley et al., 1983).

In addition to effects on the kidney, renal mechanoreceptor activation has been shown to inhibit SNA from the ansa subclavia and decrease right ventricular contractility and blood pressure (BP) (Kostreva et al., 1981). However, other studies have found no change in HR, BP or RBF with increases in intrarenal pressure (Kopp et al., 1984, 1985). A decrease in renal perfusion by balloon inflation in the aorta for 2 min (which is likely to activate chemo- and inhibit mechanoreceptors) caused an increase in hindlimb vascular resistance in anesthetized rabbits (Rankin et al., 1992). The decreased renal perfusion is thought to elicit hypoxic-driven release of local mediators, such prostaglandin E2, bradykinin, and adenosine, which stimulate renal afferents leading to neutrally-mediated increases in hindlimb vascular resistance (Ashton et al., 1994). The main responses to renal mechanoreceptor activation are abolished by spinal cord transection at T6, indicating that the mechanoreceptor reno-renal reflex is dependent on central integration (Francisco et al., 1980; Kopp et al., 1985).

The second class of renal sensory receptors are the chemoreceptors: R1 and R2 receptors, which are activated by the chemical environment of intrarenal tissue and renal pelvis, respectively (Recordati et al., 1978, 1980). R1 receptors are activated by renal ischaemia, stimulated experimentally by prolonged arterial and venous occlusion and systemic asphyxia (Recordati et al., 1978). R1 activation, induced by renal artery occlusion, is associated with an increase in ipsilateral efferent RSNA, which persists after spinal cord transection at T6 in rats (Recordati et al., 1982). R2 receptors are activated experimentally by backflow of concentrated urine (Rogenes, 1982), hypertonic NaCl, and hypotonic KCl (Recordati et al., 1980). Activation of R2 chemoreceptors results in an increase in both ipsilateral and contralateral efferent RSNA, which is more pronounced if backflow of urine is bilateral, and is variably accompanied by small increases in BP and HR (Recordati et al., 1982; Rogenes, 1982). Like the response of R1 receptors, the R2 response remains after spinal cord transection at T6 (Recordati et al., 1982) and is enhanced by transection at C3 (Rogenes, 1982); therefore, a reflex integrated at a spinal level.

Renal afferent nerve fibers are mainly unmyelinated (primarily C- fibers) with a small population of faster conducting, A-delta, myelinated fibers (Knuepfer and Schramm, 1987). Studies in rats indicate that the renal afferent nerve fibers project from the kidney to the ipsilateral dorsal root ganglia, between T6 and L2 (Donovan et al., 1983; Knuepfer and Schramm, 1987), with the peak number at T12–13. By stimulating myelinated renal afferent fibers, investigators have shown that there are direct projections from the kidney to the most medial segment of the nucleus gracilis and the caudal half of the NTS (Simon and Schramm, 1984) and fluorescent tracer studies between the kidneys and posterior medulla show that monosynaptic connections make up approximately 8% of renal afferents (Wyss and Donovan, 1984). In addition to these brainstem regions, in cats, electrical stimulation of the renal afferents effects activity of medullary neurons in the lateral tegmental field, paramedical reticular nucleus and dorsal vagal complex, and hypothalamic neurons in the lateral preoptic area, lateral hypothalamic area, and PVN (Calaresu and Ciriello, 1981). Additionally, the ventral medulla has been shown to receive input from renal afferents in the cat (Vizzard et al., 1992). Indeed, Xu et al. (2015) have recently shown that there is a neural connection from the RVLM to the PVN that is activated by stimulation of renal afferents. Importantly for the role of the renal afferents in HF, the same

authors have previously shown that RVLM projecting PVN neurons are more active in rats with chronic HF (Xu et al., 2012). As the RVLM plays a crucial role in the regulation of SNA, this may be a pathway by which renal afferent activation in HF influences sympathetic tone; however, this remains to be confirmed. Electrical stimulation of renal afferent nerve fibers has also been studied using Fos (a marker of neuronal activation) immunohistochemistry (see Solano-Flores et al., 1997).

Potential Factors Driving the Changes in Renal Afferent Activity in HF

There are very few studies that have examined the role of the renal afferent nerve fibers in HF. HF is associated with a number of symptoms which would be expected to stimulate renal afferent activity, such increased venous pressure and decreased RBF. Kopp et al. showed that the inhibitory mechanoreceptor reno-renal reflex is blunted in HF, due to high circulating AngII (Kopp et al., 2003) and activation of endothelin A receptors (Kopp et al., 2010). Blunting of the inhibitory reno-renal reflex may be a mechanism by which sodium is retained and efferent sympathetic drive to non-renal vascular beds is stimulated in HF. It is unknown whether the excitatory renal-chemoreflex is enhanced in HF, potentially in parallel with the enhanced arterial chemoreflex.

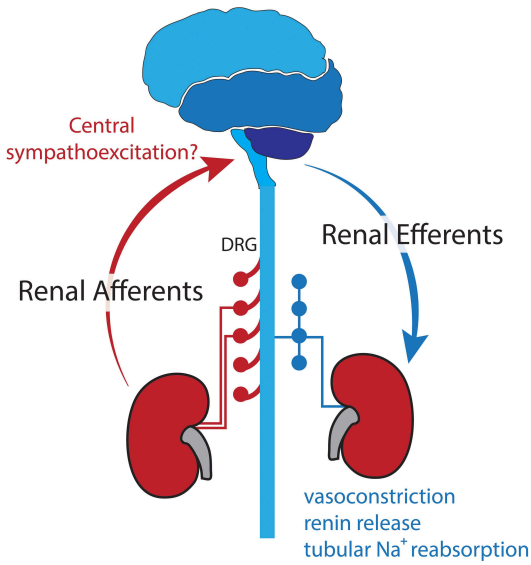


FIGURE 2 | Sympathetic efferent and sensory afferent renal nerves postulated to be interrupted with RDN.

Ablation of the Renal Nerve Fibers in Heart Failure: Evidence For Potential Benefit Following Catheter-based RDN

Discussed above are some of the potential factors stimulating SNA in HF and the effects of the renal sympathetic and sensory nerves. The critical question is whether removing the effect of these nerves is beneficial in HF. The development of catheter-based renal nerve ablation has led to increasing interest in RDN as a treatment for hypertension and HF. Although not without controversy (Bhatt et al., 2014), RDN has been shown to be effective in lowering BP in patients with drug-resistant hypertension (Krum et al., 2009; Esler et al., 2010). Recently, the First Report of the Global SYMPLICITY Registry showed a significant reduction in 24 h ambulatory BP after RDN in nearly 1000 patients (Böhm et al., 2015), supporting the population effect of catheter-RDN.

The BP lowering effects of RDN are *postulated* to be due to destruction of both renal efferent and afferent nerve fibers (**Figure 2**). As outlined previously, efferent renal nerves play a major role in stimulating renin release, renal vasoconstriction, and sodium retention (DiBona and Kopp, 1997), thus removal of these nerves decreases BP. It has also been suggested that in hypertension, increased afferent renal nerve activity causes a reflex increase in sympathetic outflow and worsening hypertension (Katholi and Woods, 1987; Campese et al., 1995) and there is evidence that ablation of the afferent nerve fibers reduced muscle SNA (Schlaich et al., 2009) and plasma noradrenaline (Ezzahti et al., 2014). These effects of both efferent and afferent RDN are likely to be beneficial in HF.

Successful destruction of the renal nerves depends heavily on the ablation sites in relation to the renal nerves. It has recently been highlighted that although the number of renal nerves is higher in proximal regions of the renal artery, the renal nerves are closest to the renal artery in distal regions in humans (Sakakura et al., 2014), pigs (Tellez et al., 2013), and sheep (Booth et al., 2015a). Therefore, starting ablations as close as possible to the kidney may be the most effective method of ablating the renal nerves. We have previously shown a ∼80% reduction in renal noradrenaline levels with six ablations started as close as anatomically possible to the kidney in sheep (Booth et al., 2015b).

Clinical Studies of RDN in HF

While it is intuitive to use RDN in hypertensive patients to reduce BP, this is less so in HF where BP is reduced in the majority of cases. However, the ability of RDN to reduce RSNA and thus the increased renal vasoconstriction, renin release, and sodium retention is likely to have beneficial effects. Indeed, a safety trial in HF patients showed that there were no significant reductions in BP following RDN in the seven systolic HF patients and, importantly, RDN was associated with an increase in 6-min walk distance 6 months after RDN (Davies et al., 2013). Importantly, in a pilot study RDN was shown to reduce ventricular tachyarrhythmias in two patients with chronic HF (Ukena et al., 2012). Further, RDN trials in hypertensive patients with cardiomyopathy have shown that 6 months after RDN patients had reduced left ventricular mass (Doltra et al., 2014; Mahfoud et al., 2014) and increased EF (Mahfoud et al., 2014). Larger clinical trials of renal denervation in HF are ongoing (Verloop et al., 2013).

Effect of RDN on Renal Function in Experimental HF

As previously described, RSNA is increased in severe HF and this has detrimental actions suggesting that RDN would be

beneficial. Indeed, bilateral surgical RDN attenuated the sodium retention following myocardial infarction in rats (DiBona and Sawin, 1991; Souza et al., 2004) and in dogs with HF (Villarreal et al., 1994). Studies in rats, 3–4 weeks after myocardial infarction (LVEDP ∼ 18 mmHg), showed impaired water and sodium excretion following an acute salt load, a finding reversed by prior RDN (DiBona et al., 1988). Increased sodium reabsorption in HF is likely to be at least partially driven by increased expression of the Na-K-2Cl cotransporter in the thick ascending loop of Henle, which has been shown in HF rats and was reduced following RDN (Torp et al., 2012). In addition to altered sodium handling, large myocardial infarcts have been associated with increased RVR, decreased renal plasma flow and an inability to increase glomerular filtration rate after volume loading (Hostetter et al., 1983). As mentioned above, reduced RBF and renal dysfunction are predictive of poor outcome in HF patients (Goldberg et al., 2005; Petersson et al., 2005; Jose et al., 2006). In rabbits paced into HF, unilateral RDN prevented the reduction in RBF, increase in RVR and upregulation of AT-1 receptor expression in renal cortical blood vessels otherwise seen with HF (Clayton et al., 2011). Together these studies indicate that RDN improves renal function in experimental models of HF, probably mainly by efferent denervation.

Effect of RDN on Cardiac Function in Experimental HF

Surgical RDN has been shown to reduce left ventricular filling pressure and improve function following myocardial infarction in rats (Nozawa et al., 2002; Hu et al., 2014a); while, catheter-based RDN, prior to pacing-induced HF, has been shown to reduce the incidence of atrial and ventricular fibrillation and left ventricular filling pressure in dogs (Zhao et al., 2013; Guo et al., 2014). In contrast, in rabbits with pacing–induced HF, unilateral surgical RDN did not improve cardiac function but reduced the sensitivity of the HR baroreflex and decreased plasma noradrenaline levels (Schiller et al., 2013). The majority of studies investigating RDN in HF have assessed the effects before or at the induction of HF. One of the few studies investigating RDN in established HF showed improved cardiac and renal function in rats when surgical denervation was performed 1 and 4 weeks post-myocardial infarction (Hu et al., 2014b). In addition, a recent study investigating the effects of surgical RDN in rats with cardiac dysfunction secondary to chronic pressure overload showed that RDN reduced myocardial fibrosis, increased cardiac β-adrenergic receptor expression and decreased cardiac AT-1 receptor levels (Li et al., 2015). In pacing-induced ovine HF, the high resting level of CSNA was not reduced shortly after catheter-based RDN, but the baroreflex-mediated increase in CSNA in response to the fall in BP was inhibited following the procedure (Booth et al., 2015c). This lack of a reflex increase in CSNA resulted from a leftward shift of the CSNA arterial baroreflex curve (Booth et al., 2015c). These findings indicate that RDN can have beneficial cardiac effects in experimental HF, but further studies are required to determine the mechanisms involved. In addition, the extent to which any effects of RDN in HF depend on ablation of the afferent versus efferent nerve fibers remains, at present, unknown. This could be addressed using methods of selective denervation; such as destruction of renal afferent fibers with capsaicin (Foss et al., 2015) or destruction of renal efferent fibers with 6-hydroxydopamine (LeNoble et al., 1985).

Conclusions

The renal nerves are made up of afferent sensory and efferent sympathetic nerve fibers. Although the activities of both types of nerve fibers are postulated to increase in HF, the role of the sympathetic nerves have been much more widely investigated. In HF there is an increase in sympathetic outflow, especially to the heart and kidneys, which is associated with poor outcome. In experimentally induced HF, RDN, both surgical and catheter-based, has been associated with some improvements in renal and cardiac function. In contrast, the role of renal afferents remains largely unexplored in HF, although there are multiple triggers that could potentially increase afferent nerve activity. Some of the few studies investigating this have suggested that at least the inhibitory reno-renal reflex is blunted in HF. This may be one mechanism stimulating efferent sympathetic drive in HF, which leads to renal vasoconstriction, renin release, and sodium retention. Although the evidence outlined above indicates the beneficial effects of removing the renal nerves in HF, it remains unknown whether the effects are due to removal of the efferent, sympathetic renal nerves or sensory, afferent renal nerves, or a combination of both.

Sources of Funding

This work was supported by National Health and Medical Research Council of Australia (NHMRC) and the Victorian Government's Operational Infrastructure Support Program. LB is supported by a NHMRC Early Career Fellowship and CM was supported by a NHMRC Research Fellowship.

Acknowledgments

The authors thank Dr. Rohit Ramchandra for helpful discussions.

References

Ashton, N., Clarke, C. G., Eddy, D. E., and Swift, F. V. (1994). Mechanisms involved in the activation of ischemically sensitive, afferent renal nerve mediated reflex increases in hind-limb vascular resistance in the anesthetized rabbit. *Can. J. Physiol. Pharmacol.* 72, 637–643. doi: 10.1139/y94-090

Aspromonte, N., Cruz, D. N., Valle, R., and Ronco, C. (2011). Management and monitoring of haemodynamic complications in acute heart failure. *Heart Fail. Rev.* 16, 575–581. doi: 10.1007/s10741-011-9229-3

Aström, A., and Crafoord, J. (1968). Afferent and efferent activity in the renal nerves of cats. *Acta Physiol. Scand.* 74, 69–78. doi: 10.1111/j.1365-201X.1968.tb10901.x

Bhatt, D. L., Kandzari, D. E., O'Neill, W. W., D'Agostino, R., Flack, J. M., Katzen, B. T., et al. (2014). A controlled trial of renal denervation for resistant hypertension. *N. Engl. J. Med.* 370, 1393–1401. doi: 10.1056/NEJMoa1402670

Böhm, M., Mahfoud, F., Ukena, C., Hoppe, U. C., Narkiewicz, K., Negoita, M., et al. (2015). First report of the global SYMPLICITY registry on the effect of renal artery denervation in patients with uncontrolled hypertension. *Hypertension* 65, 766–774. doi: 10.1161/HYPERTENSIONAHA.114.05010

Booth, L. C., Nishi, E. E., Yao, S. T., Ramchandra, R., Lambert, G. W., Schlaich, M. P., et al. (2015a). Reinnervation following catheter-based radio-frequency renal denervation. *Exp. Physiol.* 100, 485–490. doi: 10.1113/expphysiol.2014.079871

Booth, L. C., Nishi, E. E., Yao, S. T., Ramchandra, R., Lambert, G. W., Schlaich, M. P., et al. (2015b). Reinnervation of renal afferent and efferent nerves at 5.5 and 11 months after catheter-based radiofrequency renal denervation in sheep. *Hypertension* 65, 393–400. doi: 10.1161/HYPERTENSIONAHA.114.04176

Booth, L. C., Schlaich, M. P., Nishi, E. E., Yao, S. T., Xu, J., Ramchandra, R., et al. (2015c). Short-term effects of catheter-based renal denervation on cardiac sympathetic drive and cardiac baroreflex function in heart failure. *Int. J. Cardiol.* 190, 220–226. doi: 10.1016/j.ijcard.2015.03.440

Calaresu, F. R., and Ciriello, J. (1981). Renal afferent nerves affect discharge rate of medullary and hypothalamic single units in the cat. *J. Auton. Nerv. Syst.* 3, 311–320. doi: 10.1016/0165-1838(81)90072-2

Campese, V. M., Kogosov, E., and Koss, M. (1995). Renal afferent denervation prevents the progression of renal disease in the renal ablation model of chronic renal failure in the rat. *Am. J. Kidney Dis.* 26, 861–865. doi: 10.1016/0272-6386(95)90456-5

Chua, T. P., Ponikowski, P., Webb-Peploe, K., Harrington, D., Anker, S. D., Piepoli, M., et al. (1997). Clinical characteristics of chronic heart failure patients with an augmented peripheral chemoreflex. *Eur. Heart J.* 18, 480–486. doi: 10.1093/oxfordjournals.eurheartj.a015269

Clayton, S. C., Haack, K. K., and Zucker, I. H. (2011). Renal denervation modulates angiotensin receptor expression in the renal cortex of rabbits with chronic heart failure. *Am. J. Physiol. Renal Physiol.* 300, F31–F39. doi: 10.1152/ajprenal.00088.2010

Davies, J. E., Manisty, C. H., Petraco, R., Barron, A. J., Unsworth, B., Mayet, J., et al. (2013). First-in-man safety evaluation of renal denervation for chronic systolic heart failure: primary outcome from REACH-Pilot study. *Int. J. Cardiol.* 162, 189–192. doi: 10.1016/j.ijcard.2012.09.019

Dibner-Dunlap, M. E., Smith, M. L., Kinugawa, T., and Thames, M. D. (1996). Enalaprilat augments arterial and cardiopulmonary baroreflex control of sympathetic nerve activity in patients with heart failure. *J. Am. Coll. Cardiol.* 27, 358–364. doi: 10.1016/0735-1097(95)00484-X

Dibner-Dunlap, M. E., and Thames, M. D. (1989). Baroreflex control of renal sympathetic nerve activity is preserved in heart failure despite reduced arterial baroreceptor sensitivity. *Circ. Res.* 65, 1526–1535. doi: 10.1161/01.RES.65.6.1526

DiBona, G. F., Herman, P. J., and Sawin, L. L. (1988). Neural control of renal function in edema-forming states. *Am. J. Physiol.* 254(6 Pt 2), R1017–R1024.

DiBona, G. F., Jones, S. Y., and Brooks, V. L. (1995). ANG II receptor blockade and arterial baroreflex regulation of renal nerve activity in cardiac failure. *Am. J. Physiol.* 269(5 Pt 2), R1189–R1196.

DiBona, G. F., and Kopp, U. C. (1997). Neural control of renal function. *Physiol. Rev.* 77, 75–197.

DiBona, G. F., and Sawin, L. L. (1991). Role of renal nerves in sodium retention of cirrhosis and congestive heart failure. *Am. J. Physiol.* 260(2 Pt 2), R298–R305.

DiBona, G. F., and Sawin, L. L. (1995). Increased renal nerve activity in cardiac failure: arterial vs. cardiac baroreflex impairment. *Am. J. Physiol.* 268(1 Pt 2), R112-R116.

Dilley, J. R., Corradi, A., and Arendshorst, W. J. (1983). Glomerular ultrafiltration dynamics during increased renal venous pressure. *Am. J. Physiol.* 244, F650–F658.

Doltra, A., Messroghli, D., Stawowy, P., Hassel, J. H., Gebker, R., Leppänen, O., et al. (2014). Potential reduction of interstitial myocardial fibrosis with renal denervation. *J. Am. Heart Assoc.* 3:e001353. doi: 10.1161/jaha.114.001353

Donovan, M. K., Wyss, J. M., and Winternitz, S. R. (1983). Localization of renal sensory neurons using the fluorescent dye technique. *Brain Res.* 259, 119–122. doi: 10.1016/0006-8993(83)91072-7

Esler, M. D., Krum, H., Sobotka, P. A., Schlaich, M. P., Schmieder, R. E., and Böhm, M. (2010). Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 376, 1903–1909. doi: 10.1016/S0140-6736(10)62039-9

Ezzahti, M., Moelker, A., Friesema, E. C., van der Linde, N. A., Krestin, G. P., and van den Meiracker, A. H. (2014). Blood pressure and neurohormonal responses to renal nerve ablation in treatment-resistant hypertension. *J. Hypertens.* 32, 135–141. doi: 10.1097/HJH.0b013e3283658ef7

Feng, Q. P., Carlsson, S., Thoren, P., and Hedner, T. (1994). Characteristics of renal sympathetic nerve activity in experimental congestive heart failure in the rat. *Acta Physiol. Scand.* 150, 259–266. doi: 10.1111/j.1748-1716.1994.tb09685.x

Floras, J. S. (2001). Arterial baroreceptor and cardiopulmonary reflex control of sympathetic outflow in human heart failure. *Ann. N.Y. Acad. Sci.* 940, 500–513. doi: 10.1111/j.1749-6632.2001.tb03701.x

Foss, J. D., Wainford, R. D., Engeland, W. C., Fink, G. D., and Osborn, J. W. (2015). A novel method of selective ablation of afferent renal nerves by periaxonal application of capsaicin. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 308, R112–R122. doi: 10.1152/ajpregu.00427.2014

Francisco, L. L., Hoversten, L. G., and DiBona, G. F. (1980). Renal nerves in the compensatory adaptation to ureteral occlusion. *Am. J. Physiol.* 238, F229–F234.

Gao, L., Wang, W. Z., Wang, W., and Zucker, I. H. (2008). Imbalance of angiotensin type 1 receptor and angiotensin II type 2 receptor in the rostral ventrolateral medulla: potential mechanism for sympathetic overactivity in heart failure. *Hypertension* 52, 708–714. doi: 10.1161/HYPERTENSIONAHA.108.116228

Goldberg, A., Hammerman, H., Petcherski, S., Zdorovyak, A., Yalonetsky, S., Kapeliovich, M., et al. (2005). Inhospital and 1-year mortality of patients who develop worsening renal function following acute ST-elevation myocardial infarction. *Am. Heart J.* 150, 330–337. doi: 10.1016/j.ahj.2004.09.055

Grassi, G., Seravalle, G., Cattaneo, B. M., Lanfranchi, A., Vailati, S., Giannattasio, C., et al. (1995). Sympathetic activation and loss of reflex sympathetic control in mild congestive heart failure. *Circulation* 92, 3206–3211. doi: 10.1161/01.CIR.92.11.3206

Guggilam, A., Haque, M., Kerut, E. K., McIlwain, E., Lucchesi, P., Seghal, I., et al. (2007). TNF-alpha blockade decreases oxidative stress in the paraventricular nucleus and attenuates sympathoexcitation in heart failure rats. *Am. J. Physiol. Heart Circ. Physiol.* 293, H599–H609. doi: 10.1152/ajpheart.00286.2007

Guo, Z., Zhao, Q., Deng, H., Tang, Y., Wang, X., Dai, Z., et al. (2014). Renal sympathetic denervation attenuates the ventricular substrate and electrophysiological remodeling in dogs with pacing-induced heart failure. *Int. J. Cardiol.* 175, 185–186. doi: 10.1016/j.ijcard.2014.04.189

Hasking, G. J., Esler, M. D., Jennings, G. L., Burton, D., Johns, J. A., and Korner, P. I. (1986). Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. *Circulation* 73, 615–621. doi: 10.1161/01.CIR.73.4.615

Hostetter, T. H., Pfeffer, J. M., Pfeffer, M. A., Dworkin, L. D., Braunwald, E., and Brenner, B. M. (1983). Cardiorenal hemodynamics and sodium excretion in rats with myocardial infarction. *Am. J. Physiol.* 245, H98–H103.

Hu, J., Li, Y., Cheng, W., Yang, Z., Wang, F., Lv, P., et al. (2014a). A comparison of the efficacy of surgical renal denervation and pharmacologic therapies in post-myocardial infarction heart failure. *PLoS ONE* 9:e96996. doi: 10.1371/journal.pone.0096996

Hu, J., Yan, Y., Zhou, Q., Ji, M., Niu, C., Hou, Y., et al. (2014b). Effects of renal denervation on the development of post-myocardial infarction heart failure and cardiac autonomic nervous system in rats. *Int. J. Cardiol.* 172, e414–e416. doi: 10.1016/j.ijcard.2013.12.254

Jose, P., Skali, H., Anavekar, N., Tomson, C., Krumholz, H. M., Rouleau, J. L., et al. (2006). Increase in creatinine and cardiovascular risk in patients with systolic dysfunction after myocardial infarction. *J. Am. Soc. Nephrol.* 17, 2886–2891. doi: 10.1681/ASN.2006010063

Kang, Y. M., Wang, Y., Yang, L. M., Elks, C., Cardinale, J., Yu, X. J., et al. (2010). TNF-alpha in hypothalamic paraventricular nucleus contributes to sympathoexcitation in heart failure by modulating AT1 receptor and neurotransmitters. *Tohoku J. Exp. Med.* 222, 251–263. doi: 10.1620/tjem.222.251

Katholi, R. E., and Woods, W. T. (1987). Afferent renal nerves and hypertension. *Clin. Exp. Hypertens. A* 9(Suppl. 1), 211–226. doi: 10.3109/10641968709160175

Kaye, D. M., Lefkovits, J., Jennings, G. L., Bergin, P., Broughton, A., and Esler, M. D. (1995). Adverse consequences of high sympathetic nervous activity in the

failing human heart. *J. Am. Coll. Cardiol.* 26, 1257–1263. doi: 10.1016/0735-1097(95)00332-0

Knuepfer, M. M., and Schramm, L. P. (1987). The conduction velocities and spinal projections of single renal afferent fibers in the rat. *Brain Res.* 435, 167–173. doi: 10.1016/0006-8993(87)91598-8

Kopp, U. C., Cicha, M. Z., and Jones, S. Y. (2010). Activation of endothelin A receptors contributes to impaired responsiveness of renal mechanosensory nerves in congestive heart failure. *Can. J. Physiol. Pharmacol.* 88, 622–629. doi: 10.1139/Y10-035

Kopp, U. C., Cicha, M. Z., and Smith, L. A. (2003). Impaired responsiveness of renal mechanosensory nerves in heart failure: role of endogenous angiotensin. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284, R116–R124. doi: 10.1152/ajpregu.00336.2002

Kopp, U. C., Olson, L. A., and DiBona, G. F. (1984). Renorenal reflex responses to mechano- and chemoreceptor stimulation in the dog and rat. *Am. J. Physiol.* 246(1 Pt 2), F67–F77.

Kopp, U. C., Smith, L. A., and DiBona, G. F. (1985). Renorenal reflexes: neural components of ipsilateral and contralateral renal responses. *Am. J. Physiol.* 249(4 Pt 2), F507–F517.

Kostreva, D. R., Seagard, J. L., Castaner, A., and Kampine, J. P. (1981). Reflex effects of renal afferents on the heart and kidney. *Am. J. Physiol.* 241, R286–R292.

Krum, H., Schlaich, M., Whitbourn, R., Sobotka, P. A., Sadowski, J., Bartus, K., et al. (2009). Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 373, 1275–1281. doi: 10.1016/S0140-6736(09)60566-3

Leimbach, W. N. Jr., Wallin, B. G., Victor, R. G., Aylward, P. E., Sundlof, G., and Mark, A. L. (1986). Direct evidence from intraneural recordings for increased central sympathetic outflow in patients with heart failure. *Circulation* 73, 913–919. doi: 10.1161/01.CIR.73.5.913

LeNoble, L. M., Lappe, R. W., Brody, M. J., Struyker Boudier, H. A., and Smits, J. F. (1985). Selective efferent chemical sympathectomy of rat kidneys. *Am. J. Physiol.* 249(4 Pt 2), R496–R501.

Levy, D., Kenchaiah, S., Larson, M. G., Benjamin, E. J., Kupka, M. J., Ho, K. K., et al. (2002). Long-term trends in the incidence of and survival with heart failure. *N. Engl. J. Med.* 347, 1397–1402. doi: 10.1056/NEJMoa020265

Li, Y. L., Xia, X. H., Zheng, H., Gao, L., Li, Y. F., Liu, D., et al. (2006). Angiotensin II enhances carotid body chemoreflex control of sympathetic outflow in chronic heart failure rabbits. *Cardiovasc. Res.* 71, 129–138. doi: 10.1016/j.cardiores.2006.03.017

Li, Z. Z., Jiang, H., Chen, D., Liu, Q., Geng, J., Guo, J. Q., et al. (2015). Renal sympathetic denervation improves cardiac dysfunction in rats with chronic pressure overload. *Physiol. Res.* [Epub ahead of print].

Liu, J. L., Irvine, S., Reid, I. A., Patel, K. P., and Zucker, I. H. (2000). Chronic exercise reduces sympathetic nerve activity in rabbits with pacing-induced heart failure: a role for angiotensin II. *Circulation* 102, 1854–1862. doi: 10.1161/01.cir.102.15.1854

Liu, J. L., Pliquett, R. U., Brewer, E., Cornish, K. G., Shen, Y. T., and Zucker, I. H. (2001). Chronic endothelin-1 blockade reduces sympathetic nerve activity in rabbits with heart failure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 280, R1906–R1913.

Mahfoud, F., Urban, D., Teller, D., Linz, D., Stawowy, P., Hassel, J., et al. (2014). Effect of renal denervation on left ventricular mass and function in patients with resistant hypertension: data from a multi-centre cardiovascular magnetic resonance imaging trial. *Eur. Heart J.* 35, 2224b–2231b. doi: 10.1093/eurheartj/ehu093

Marcus, N. J., Del Rio, R., Schultz, E. P., Xia, X. H., and Schultz, H. D. (2014). Carotid body denervation improves autonomic and cardiac function and attenuates disordered breathing in congestive heart failure. *J. Physiol.* 592(Pt 2), 391–408. doi: 10.1113/jphysiol.2013.266221

Niijima, A. (1975). Observation on the localization of mechanoreceptors in the kidney and afferent nerve fibres in the renal nerves in the rabbit. *J. Physiol.* 245, 81–90. doi: 10.1113/jphysiol.1975.sp010836

Nozawa, T., Igawa, A., Fujii, N., Kato, B., Yoshida, N., Asanoi, H., et al. (2002). Effects of long-term renal sympathetic denervation on heart failure after myocardial infarction in rats. *Heart Vessels* 16, 51–56. doi: 10.1007/s380-002-8317-8

Patel, K. P. (2000). Role of paraventricular nucleus in mediating sympathetic outflow in heart failure. *Heart Fail. Rev.* 5, 73–86. doi: 10.1023/A:1009850224802

Petersson, M., Friberg, P., Eisenhofer, G., Lambert, G., and Rundqvist, B. (2005). Long-term outcome in relation to renal sympathetic activity in patients with chronic heart failure. *Eur. Heart J.* 26, 906–913. doi: 10.1093/eurheartj/ehi184

Ponikowski, P., Chua, T. P., Anker, S. D., Francis, D. P., Doehner, W., Banasiak, W., et al. (2001). Peripheral chemoreceptor hypersensitivity: an ominous sign in patients with chronic heart failure. *Circulation* 104, 544–549. doi: 10.1161/hc3101.093699

Ramchandra, R., Hood, S. G., Denton, D. A., Woods, R. L., McKinley, M. J., McAllen, R. M., et al. (2009a). Basis for the preferential activation of cardiac sympathetic nerve activity in heart failure. *Proc. Natl. Acad. Sci. U.S.A.* 106, 924–928. doi: 10.1073/pnas.0811929106

Ramchandra, R., Hood, S. G., Frithiof, R., and May, C. N. (2009b). Discharge properties of cardiac and renal sympathetic nerves and their impaired responses to changes in blood volume in heart failure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297, R665–R674. doi: 10.1152/ajpregu.00191.2009

Ramchandra, R., Hood, S. G., Frithiof, R., McKinley, M. J., and May, C. N. (2013). The role of the paraventricular nucleus of the hypothalamus in the regulation of cardiac and renal sympathetic nerve activity in conscious normal and heart failure sheep. *J. Physiol.* 591(Pt 1), 93–107. doi: 10.1113/jphysiol.2012.236059

Ramchandra, R., Hood, S. G., Watson, A. M., Allen, A. M., and May, C. N. (2012). Central angiotensin type 1 receptor blockade decreases cardiac but not renal sympathetic nerve activity in heart failure. *Hypertension* 59, 634–641. doi: 10.1161/HYPERTENSIONAHA.111.181131

Rankin, A. J., Ashton, N., and Swift, F. V. (1992). The reflex effect of changes in renal perfusion on hindlimb vascular resistance in anaesthetized rabbits. *Pflugers Arch.* 421, 585–590. doi: 10.1007/BF00375055

Recordati, G., Genovesi, S., and Cerati, D. (1982). Renorenal reflexes in the rat elicited upon stimulation of renal chemoreceptors. *J. Auton. Nerv. Syst.* 6, 127–142. doi: 10.1016/0165-1838(82)90046-7

Recordati, G. M., Moss, N. G., Genovesi, S., and Rogenes, P. R. (1980). Renal receptors in the rat sensitive to chemical alterations of their environment. *Circ. Res.* 46, 395–405. doi: 10.1161/01.RES.46.3.395

Recordati, G. M., Moss, N. G., and Waselkov, L. (1978). Renal chemoreceptors in the rat. *Circ. Res.* 43, 534–543. doi: 10.1161/01.RES.43.4.534

Reddy, M. K., Schultz, H. D., Zheng, H., and Patel, K. P. (2007). Altered nitric oxide mechanism within the paraventricular nucleus contributes to the augmented carotid body chemoreflex in heart failure. *Am. J. Physiol. Heart Circ. Physiol.* 292, H149–H157. doi: 10.1152/ajpheart.00117.2006

Rogenes, P. R. (1982). Single-unit and multiunit analyses of renorenal reflexes elicited by stimulation of renal chemoreceptors in the rat. *J. Auton. Nerv. Syst.* 6, 143–156. doi: 10.1016/0165-1838(82)90047-9

Rundqvist, B., Elam, M., Bergmann-Sverrisdottir, Y., Eisenhofer, G., and Friberg, P. (1997). Increased cardiac adrenergic drive precedes generalized sympathetic activation in human heart failure. *Circulation* 95, 169–175. doi: 10.1161/01.CIR.95.1.169

Sakakura, K., Ladich, E., Cheng, Q., Otsuka, F., Yahagi, K., Fowler, D. R., et al. (2014). Anatomic assessment of sympathetic peri-arterial renal nerves in man. *J. Am. Coll. Cardiol.* 64, 635–643. doi: 10.1016/j.jacc.2014.03.059

Schiller, A. M., Haack, K. K., Pellegrino, P. R., Curry, P. L., and Zucker, I. H. (2013). Unilateral renal denervation improves autonomic balance in conscious rabbits with chronic heart failure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 305, R886–R892. doi: 10.1152/ajpregu.00269.2013

Schlaich, M. P., Sobotka, P. A., Krum, H., Lambert, E., and Esler, M. D. (2009). Renal sympathetic-nerve ablation for uncontrolled hypertension. *N. Engl. J. Med.* 361, 932–934. doi: 10.1056/NEJMc0904179

Simon, O. R., and Schramm, L. P. (1984). The spinal course and medullary termination of myelinated renal afferents in the rat. *Brain Res.* 290, 239–247. doi: 10.1016/0006-8993(84)90941-7

Solano-Flores, L. P., Rosas-Arellano, M. P., and Ciriello, J. (1997). Fos induction in central structures after afferent renal nerve stimulation. *Brain Res.* 753, 102–119. doi: 10.1016/S0006-8993(96)01497-7

Souza, D. R., Mill, J. G., and Cabral, A. M. (2004). Chronic experimental myocardial infarction produces antinatriuresis by a renal nerve-dependent mechanism. *Braz. J. Med. Biol. Res.* 37, 285–293. doi: 10.1590/S0100-879X2004000200017

Sun, S. Y., Wang, W., Zucker, I. H., and Schultz, H. D. (1999). Enhanced peripheral chemoreflex function in conscious rabbits with pacing-induced heart failure. *J. Appl. Physiol.(1985)* 86, 1264–1272.

Tellez, A., Rousselle, S., Palmieri, T., Rate, W. R. IV, Wicks, J., Degrange, A., et al. (2013). Renal artery nerve distribution and density in the porcine model: biologic implications for the development of radiofrequency ablation therapies. *Transl. Res.* 162, 381–389. doi: 10.1016/j.trsl.2013.07.002

Torp, M., Brond, L., Nielsen, J. B., Nielsen, S., Christensen, S., and Jonassen, T. E. (2012). Effects of renal denervation on the NKCC2 cotransporter in the thick ascending limb of the loop of Henle in rats with congestive heart failure. *Acta Physiol. (Oxf)* 204, 451–459. doi: 10.1111/j.1748-1716.2011.02351.x

Ueda, H., Uchida, Y., and Kamisaka, K. (1967). Mechanism of the reflex depressor effect by the kidney in dog. *Jpn. Heart J.* 8, 597–606. doi: 10.1536/ihj.8.597

Ukena, C., Bauer, A., Mahfoud, F., Schreieck, J., Neuberger, H. R., Eick, C., et al. (2012). Renal sympathetic denervation for treatment of electrical storm: first-in-man experience. *Clin. Res. Cardiol.* 101, 63–67. doi: 10.1007/s00392-011-0365-5

Verloop, W. L., Beeftink, M. M., Nap, A., Bots, M. L., Velthuis, B. K., Appelman, Y. E. et al. (2013). Renal denervation in heart failure with normal left ventricular ejection fraction. Rationale and design of the DIASTOLE (DenervatIon of the renAl Sympathetic nerves in hearT failure with nOrmal Lv Ejection fraction) trial. *Eur. J. Heart Fail.* 15, 1429–1437. doi: 10.1093/eurjhf/hft119

Villarreal, D., Freeman, R. H., Johnson, R. A., and Simmons, J. C. (1994). Effects of renal denervation on postprandial sodium excretion in experimental heart failure. *Am. J. Physiol.* 266(5 Pt 2), R1599–R1604.

Vizzard, M. A., Standish, A., and Ammons, W. S. (1992). Renal afferent input to the ventrolateral medulla of the cat. *Am. J. Physiol.* 263(2 Pt 2), R412–R422.

Wang, W., Chen, J. S., and Zucker, I. H. (1991). Carotid sinus baroreceptor reflex in dogs with experimental heart failure. *Circ. Res.* 68, 1294–1301. doi: 10.1161/01.RES.68.5.1294

Watson, A. M., Hood, S. G., Ramchandra, R., McAllen, R. M., and May, C. N. (2007). Increased cardiac sympathetic nerve activity in heart failure is not due to desensitization of the arterial baroreflex. *Am. J. Physiol. Heart Circ. Physiol.* 293, H798–H804. doi: 10.1152/ajpheart.00147.2007

Weiss, M., Kenney, M., Musch, T., and Patel, K. (2003). Modifications to central neural circuitry during heart failure. *Acta Physiol. Scand.* 177, 57–67. doi: 10.1046/j.1365-201X.2003.01047.x

Wyss, J. M., and Donovan, M. K. (1984). A direct projection from the kidney to the brainstem. *Brain Res.* 298, 130–134. doi: 10.1016/0006-8993(84)91154-5

Xing, D. T., May, C. N., Booth, L. C., and Ramchandra, R. (2014). Tonic arterial chemoreceptor activity contributes to cardiac sympathetic activation in mild ovine heart failure. *Exp. Physiol.* 99, 1031–1041. doi: 10.1113/expphysiol.2014.079491

Xu, B., Zheng, H., Liu, X., and Patel, K. P. (2015). Activation of afferent renal nerves modulates RVLM projecting PVN neurons. *Am. J. Physiol. Heart Circ. Physiol.* 308, H1103–H1111. doi: 10.1152/ajpheart.00862.2014

Xu, B., Zheng, H., and Patel, K. P. (2012). Enhanced activation of RVLM-projecting PVN neurons in rats with chronic heart failure. *Am. J. Physiol. Heart Circ. Physiol.* 302, H1700–H1711. doi: 10.1152/ajpheart.00722.2011

Yoshimura, R., Sato, T., Kawada, T., Shishido, T., Inagaki, M., Miyano, H., et al. (2000). Increased brain angiotensin receptor in rats with chronic high-output heart failure. *J. Card. Fail.* 6, 66–72. doi: 10.1016/S1071-9164(00)00013-0

Zhang, W., Huang, B. S., and Leenen, F. H. (1999). Brain renin-angiotensin system and sympathetic hyperactivity in rats after myocardial infarction. *Am. J. Physiol.* 276(5 Pt 2), H1608–H1615.

Zhao, Q., Yu, S., Huang, H., Tang, Y., Xiao, J., Dai, Z., et al. (2013). Effects of renal sympathetic denervation on the development of atrial fibrillation substrates in dogs with pacing-induced heart failure. *Int. J. Cardiol.* 168, 1672–1673. doi: 10.1016/j.ijcard.2013.03.091

Conflict of Interest Statement: Clive N. May has received honoraria and travel support for presentations from Medtronic. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

MINI REVIEW
published: 07 August 2015
doi: 10.3389/fphys.2015.00224

The renal nerves in chronic heart failure: efferent and afferent mechanisms

Alicia M. Schiller, Peter R. Pellegrino and Irving H. Zucker*

Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE, USA

The function of the renal nerves has been an area of scientific and medical interest for many years. The recent advent of a minimally invasive catheter-based method of renal denervation has renewed excitement in understanding the afferent and efferent actions of the renal nerves in multiple diseases. While hypertension has been the focus of much this work, less attention has been given to the role of the renal nerves in the development of chronic heart failure (CHF). Recent studies from our laboratory and those of others implicate an essential role for the renal nerves in the development and progression of CHF. Using a rabbit tachycardia model of CHF and surgical unilateral renal denervation, we provide evidence for both renal efferent and afferent mechanisms in the pathogenesis of CHF. Renal denervation prevented the decrease in renal blood flow observed in CHF while also preventing increases in Angiotensin-II receptor protein in the microvasculature of the renal cortex. Renal denervation in CHF also reduced physiological markers of autonomic dysfunction including an improvement in arterial baroreflex function, heart rate variability, and decreased resting cardiac sympathetic tone. Taken together, the renal sympathetic nerves are necessary in the pathogenesis of CHF via both efferent and afferent mechanisms. Additional investigation is warranted to fully understand the role of these nerves and their role as a therapeutic target in CHF.

Keywords: heart failure, afferent pathways, efferent pathways, renal sympathetic nerves, renal denervation

OPEN ACCESS

Edited by:
Kyungjoon Lim, Baker IDI Heart and Diabetes Institute, Australia

Reviewed by:
Kazuhiro Nakamura, Nagoya University, Japan
Anna M. D. Watson, Baker IDI Heart and Diabetes Institute, Australia

****Correspondence:***
Alicia M. Schiller, Cellular and Integrative Physiology, University of Nebraska Medical Center, 985850 Nebraska Medical Center, Omaha, NE 69198-5850, USA
alicia.schiller.phd@gmail.com

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *16 June 2015*
Accepted: *24 July 2015*
Published: *07 August 2015*

Citation:
Schiller AM, Pellegrino PR and Zucker IH (2015) The renal nerves in chronic heart failure: efferent and afferent mechanisms. Front. Physiol. 6:224. doi: 10.3389/fphys.2015.00224

Introduction

Chronic heart failure (CHF) is a diverse clinical syndrome in which impairments of ventricular filling or emptying compromise the ability of the heart to match cardiac output to metabolic demand. This activates multiple maladaptive mechanisms such as inflammation, oxidative stress, the renin-angiotensin system (RAS), and the sympathetic nervous system (SNS), which over time contribute to disease progression (Felder et al., 2003; Tsutsui et al., 2011; Gullestad et al., 2012).

Demographics of CHF

In the United States, approximately six million adults are living with CHF, a number that is expected to increase to over eight million in the next 15 years (Heidenreich et al., 2013). These patients suffer from dyspnea, fatigue, exercise intolerance, and edema, which degrade their quality of life and lead to frequent and costly hospitalizations. The American financial burden of CHF in 2012 was approximately $31 billion and is projected to balloon to nearly $70 billion by 2030 (Heidenreich et al., 2013). The prognosis of CHF is poor, with a 5-year mortality of

approximately 50% (Roger et al., 2004), emphasizing the need for new therapeutic strategies.

Cardiorenal Interactions in CHF

Cardiac and renal dysfunction are inextricably intertwined in CHF, evidenced by the fact that over 80% of patients have renal insufficiency (McAlister et al., 2004). The hemodynamic disturbances of CHF lead to decreased renal blood flow with progressive proteinuria, diminished glomerular filtration rate, and renal fibrosis. Clinical studies have delineated an important relationship between markers of systemic venous congestion and renal function that is independent of other common prognostic markers (Mullens et al., 2008, 2009). Moreover, therapies that improve cardiac function, like cardiac resynchronization and left ventricular assist devices (LVAD), also improve renal function in CHF patients (Boerrigter et al., 2008; Sandner et al., 2009).

Several pieces of evidence implicate the kidney in the pathogenesis of CHF. Hypertension precedes 75% of incident CHF cases (Lloyd-Jones et al., 2002), and higher urinary albumin-to-creatinine ratios (Velagaleti et al., 2010) and hematocrit levels (Coglianese et al., 2012) are significant risk factors for the development of CHF. In established CHF, decreased glomerular filtration and increased renal sodium retention worsens the volume load on the already failing heart. In fact, renal dysfunction is a stronger predictor of mortality than New York Heart Association (NYHA) Functional Class and left ventricular ejection fraction (LVEF) in CHF patients (Hillege et al., 2000). The renal contribution to heart failure is further highlighted by a study of CHF patients on dialysis in whom renal transplantation improved LVEF from 32% pre-transplant to 52% 12 months post-transplant, normalizing cardiac function in nearly 70% of the cohort (Wali et al., 2005).

Despite the importance of hemodynamic dysregulation in CHF, the notion of heart failure as a purely hemodynamic disorder with purely hemodynamic cardiorenal interactions has been discredited (Bongartz et al., 2005; Bock and Gottlieb, 2010). Maladaptive mechanisms, including inflammation, oxidative stress, RAS activation, and sympatho-excitation, also drive morbidity and mortality in CHF patients.

The Sympathetic Nervous System in Chronic Heart Failure

Sympatho-excitation is a major component of the pathological relationship between the kidney and heart in CHF. The arterial baroreflex, chemoreflex, cardiac sympathetic afferent reflex, exercise pressor reflex, and cardiopulmonary reflexes modulate sympathetic outflow, and all of these reflexes are aberrant in CHF (Zucker et al., 1995). Sympatho-excitation activates the RAS and the immune system which increase sympatho-excitation in a deleterious feed forward fashion (Testa et al., 1996; Tsutsui et al., 2011; Sousa-Pinto et al., 2014). CHF is characterized by global sympatho-excitation, with cardiac and renal sympathetic outflows being particularly increased (Hasking et al., 1986), thus further implicating the SNS as an important mediator of deleterious cardiorenal interactions. Clinical evidence has demonstrated correlations between cardiac and renal sympathetic efferent activity and mortality in CHF (Brunner-La Rocca et al., 2001; Ogita, 2001; Petersson et al., 2005). A new minimally invasive therapeutic strategy targeting the renal sympathetic innervation has renewed interest in the role of the afferent and efferent renal nerves in health and disease.

Rabbit Tachycardia Model as a Model for Human CHF

Recent studies from our laboratory investigated the role of the renal nerves in CHF in the rabbit rapid ventricular pacing model (Clayton et al., 2011; Schiller et al., 2013). The animals were chronically instrumented with ventricular pacing leads, arterial pressure radiotelemetries, and renal flow probes (**Figure 1A**). The kidney instrumented with a flow probe either underwent surgical denervation (DNV) or remained innervated (INV). CHF was induced over several weeks and validated by echocardiography. This model exhibits increases in renal sympathetic nerve activity (RSNA), plasma angiotensin II, and plasma norepinephrine (Mousa et al., 2008; Schiller et al., 2013), recapitulating the pathophysiology of human CHF. All studies were performed in the conscious state in rabbits that were well acclimated to the experimental procedures and environment.

Efferent Renal Sympathetic Nerve Activity in CHF

The kidney receives rich sympathetic efferent innervation of many structures (Barajas et al., 1992). These renal sympathetic efferents mediate renal vasoconstriction, sodium reabsorption, and renin release.

Several studies indicate that the efferent renal nerves are essential to the renal hypoperfusion in CHF. **Figure 1B** shows the effect of renal denervation on renal blood flow (RBF) in our rabbit model of CHF. CHF significantly reduces RBF to INV but not DNV kidneys by increasing renal vascular resistance. These data support the necessity of the renal nerves in the development of renal hypoperfusion in CHF. Taken with the observation that acute renal denervation of anesthetized rats significantly increases RBF in CHF but not control rats (Kon et al., 1985; DiBona and Sawin, 2004), it seems the renal nerves exert a tonic vasoconstrictive action in CHF. Importantly, Kon et al. also found that acute renal denervation increases glomerular filtration rate while reducing glomerular capillary pressure in CHF, providing evidence that the elevated efferent RSNA in CHF impairs renal function.

Several studies have examined the contribution of the renal nerves to volume dysregulation in CHF. Renal denervation does not affect sodium excretion or balance in CHF animals unless they are challenged, for example by sodium restriction (DiBona and Sawin, 1991) or volume or salt loading (DiBona et al., 1988; Villarreal et al., 1994; Souza et al., 2004). Regardless of the challenge, denervated animals with CHF excrete more sodium than their innervated counterparts. A recent rodent study

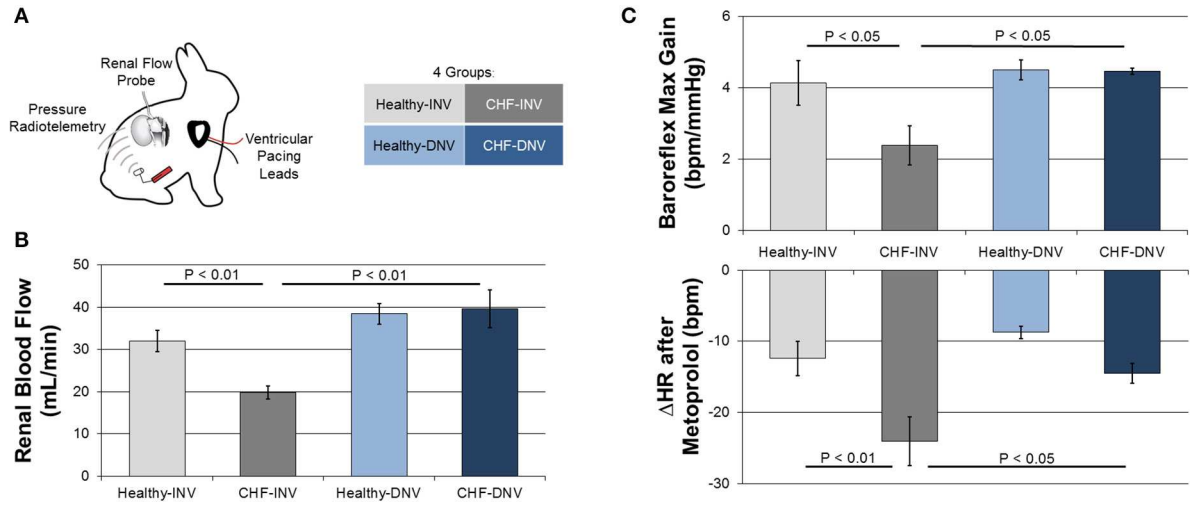


FIGURE 1 | Efferent and afferent signaling in the development of experimental CHF. Rabbits were chronically instrumented with ventricular pacing leads, arterial pressure radiotelemeters, and renal flow probes, and underwent surgical denervation or a sham procedure **(A)**. Induction of CHF by ventricular pacing reduced blood flow in innervated (INV) rabbits but not in denervated (DNV) rabbits **(B)**. Modified from Clayton et al. (2011). Induction of CHF also reduced baroreflex gain and potentiated the heart rate response to metoprolol in INV but not DNV rabbits **(C)**. Modified from Schiller et al. (2013).

suggests that this is because the increased efferent RSNA in CHF increases Na^+-K^+-$2Cl^-$ co-transporter expression in the thick ascending limb (Torp et al., 2012).

One study has shown that renal denervation attenuates but does not normalize the increased plasma renin activity in experimental CHF (Witty et al., 1972), indicating that the renal nerves are partly responsible for the maladaptive activation of the systemic RAS. In summary, efferent RSNA may contribute to the renal hypoperfusion, volume dysregulation, and RAS activation in CHF.

Afferent Renal Sympathetic Nerve Activity in CHF

The kidney is heavily innervated with sensory nerves that transmit information from the renal chemo- and mechano-sensitive receptors that monitor parameters including composition of the interstitial fluid and hydrostatic pressure changes (Nijima, 1971; Uchida et al., 1971; Recordati et al., 1981). Activation of the renal afferent nerves modulates hypothalamic activity, pain sensation, and sympathetic outflow to multiple organs, including the heart and kidney (Stella and Zanchetti, 1991; Xu et al., 2015). While much of the attention on therapeutic renal denervation has focused on the role of efferent renal nerves in cardiovascular disease, renal denervation was first used clinically to eliminate renal afferent signaling to alleviate kidney pain (Oldham, 1950). Evidence of the importance of renal afferent signaling in hypertensive patients has brought more focus to this limb of the renal nerves in other cardiovascular diseases (Hering et al., 2013).

Given the ability of the afferent renal nerves to modulate central reflexes and sympathetic outflow, we investigated the effects of renal denervation on common markers of autonomic dysfunction in the rabbit pacing model of CHF (Schiller et al., 2013). Rabbits were administered an intravenous bolus of metoprolol, which results in a decrease in heart rate that is proportional to the resting cardiac sympathetic tone. The response to metoprolol was greater for CHF-INV rabbits than healthy rabbits, consistent with elevated cardiac sympathetic tone in CHF, while CHF-DNV rabbits had a significantly attenuated metoprolol response compared to CHF-INV rabbits (**Figure 1C**). This indicates that the renal nerves play a role in the development of the cardiac sympatho-excitation in CHF, most likely due to increased renal afferent feedback.

In the same rabbits, cardiac baroreflex control was assessed by infusion of vasoactive drugs. CHF-INV rabbits exhibited decreased baroreflex gain, which was prevented in CHF-DNV rabbits (**Figure 1C**). These data indicate that renal afferent signaling is important in the development of baroreflex dysfunction in CHF. Additionally, CHF-DNV rabbits had improved heart rate variability compared to CHF-INV rabbits, further implicating the renal nerves in the development of cardiac autonomic dysfunction in CHF. Cardiac sympathetic tone, and baroreflex sensitivity, and heart rate variability are prognostic for risk of sudden cardiac death in CHF. Another study in anesthetized pigs found that renal denervation decreased ventricular arrhythmias in response to acute ventricular ischemia (Linz et al., 2013), bolstering the idea that renal afferent signaling may be pro-arrhythmogenic in CHF. Cumulatively, these data suggest that the renal sympathetic afferents play a role in the development of autonomic dysfunction and possibly sudden cardiac death in CHF.

A recent study employed catheter-based renal denervation after the development of CHF in sheep (Booth et al., 2015b). Despite convincing evidence of complete functional afferent and efferent renal denervation as well as a drop in blood pressure,

renal denervation did not decrease cardiac sympathetic tone or improve cardiac baroreflex gain in CHF sheep. The findings of this study contrast from our own data, but many possible explanations for these differences exist. First, the experiments are performed the day after denervation whereas ours were performed several weeks after denervation, and the short-term effects of nerve ablation may be markedly different from the long-term effects as time is required for neural circuit reorganization and renal and systemic molecular changes. Second, the ovine CHF model does not exhibit increased RSNA, and renal afferent signaling may not be important for autonomic dysfunction in this model. Along these lines, model-specific contributions of the renal afferents to hypertension has been recently demonstrated (Foss et al., 2015). Finally, because renal denervation was performed after induction of CHF, this study raises the very real possibility that renal afferent nerves are necessary for the development of autonomic dysfunction in CHF but do not maintain the autonomic dysfunction in established CHF. The reconciliation of this study with our own has important bearing on the viability of the renal nerves as a therapeutic target in CHF.

Renal Sympathetic Nerves and Neurohumoral Activation in CHF

Evidence from our studies also indicates that the renal nerves play an important role in the neurohumoral activation of CHF mediated by both efferent and afferent mechanisms. CHF-INV rabbits had increased plasma norepinephrine which was normalized in CHF-DNV rabbits (Schiller et al., 2013). Furthermore, our laboratory has shown that renal denervation prevents deleterious molecular changes in the renal cortical microvasculature, which may be influenced by both local and circulating factors. Specifically, CHF-INV rabbits exhibited increased expression of the vasoconstrictive, profibrotic Angiotensin-II Type 1 Receptor (AT_1R) compared to healthy rabbits (Clayton et al., 2011) while CHF-DNV rabbits did not show an increase in AT_1R protein. Of note, this model, like human CHF, exhibits increases in circulating Angiotensin-II, which can, in turn, upregulate the deleterious AT_1R in a feed-forward manner (Zucker et al., 2001). Whether these changes are a local phenomenon or if similar effects can be seen throughout the vasculature of denervated animals awaits further investigation.

Renal Sympathetic Nerves and Cardiac Function in CHF

In our rapid ventricular pacing model of CHF, the degree of heart failure is verified by echocardiography, and rabbits are defined to be in CHF after their LVEF falls below a threshold. Thus, cardiac function is a controlled parameter, and our studies are unable to answer questions about the role of the renal nerves in the development of cardiac dysfunction. However, in a rat myocardial infarction CHF model, chronic renal denervation decreased left ventricular end-diastolic diameter and increased left ventricular fractional shortening (Nozawa et al., 2002), indicating that the renal nerves play a role in the development of cardiac dysfunction in this model suggesting that they are important in the feed-forward pathophysiology of CHF.

The Renal Nerves as a Therapeutic Target in CHF

Taken together, the above studies implicate both the afferent and efferent renal nerves in the development of the pathophysiology of CHF. Despite importance of the renal nerves in the development of CHF, several open questions remain about their potential as a therapeutic target. Specifically, the efficacy and longevity of catheter-based renal denervation and the role of the renal nerves in established CHF are points of further discussion.

Catheter-based Renal Denervation

In most pre-clinical studies, renal denervation is performed surgically by stripping the neural tissue from the renal artery (Clayton et al., 2011; Schiller et al., 2013). The result is essentially complete denervation, evidenced by nearly 100% reductions in renal cortical norepinephrine levels in the rabbit, dog, and rat (Lohmeier et al., 2007; Clayton et al., 2011; Schiller et al., 2013; Linz et al., 2015). This contrasts from the partial denervation achieved by bilateral radiofrequency ablation with a catheter which is performed in patients. The only clinical study that has directly addressed the efficacy of catheter-based renal denervation was performed on 10 patients who underwent renal denervation with the Medtronic Symplicity™ catheter in whom renal norepinephrine spillover was assessed before and 15–30 days after denervation (Krum et al., 2009). After denervation, renal norepinephrine spillover was reduced by an average of 47%, but this response was highly variable between subjects. Much better results have been achieved in both sheep and pigs (Booth et al., 2015b), but the fact that the renal nerves do not form a network around the main renal artery in man may render such complete denervation impossible (Oldham, 1950). The partial efficacy of catheter-based techniques in patients contrasts from surgical denervation, which consistently and completely eliminates renal norepinephrine in all species. As it stands, it appears that partial denervation is the best that can be achieved by catheter-based renal ablation in humans, but this may be sufficient for a therapeutic benefit, as evidenced by our studies of unilateral denervation.

In addition to concerns about the completeness of catheter-based renal denervation, the duration of denervation in patients remains unknown. The longevity of afferent and efferent denervation after both surgical and catheter-based denervation of animals is only a few months (Mulder et al., 2013; Booth et al., 2015a). In renal transplant patients, histochemical studies have shown abundant allograft innervation at 8 months post-transplant (Gazdar and Dammin, 1970), but this innervation may not be functional even years after transplantation (Hansen et al., 1994). The time course of both molecular and functional renal reinnervation after catheter-based denervation remains to be investigated and will certainly impact the therapeutic potential of this technique.

The Renal Nerves in Established CHF

A very important point that our studies were not designed to address is whether or not the renal nerves remain a therapeutic target in established CHF. We performed surgical renal denervation prior to ventricular pacing, and thus the findings in our studies represent the cumulative contribution of the renal nerves through the development of CHF. This does not necessarily mean that the renal nerves actively drive these same processes in established CHF, a distinction which is emphasized by studies in other disease models in which renal denervation is preventative but not therapeutic (Stella and Zanchetti, 1991; Kim and Padanilam, 2013) and has been suggested by the aforementioned short-term study in sheep with CHF (Booth et al., 2015b). Whether or not renal denervation performed after the development of CHF can slow or reverse the renal hypoperfusion, autonomic imbalance, and neurohumoral activation in CHF remains an open question.

Clinical Studies of Renal Denervation in CHF

The excitement surrounding the Medtronic Symplicity renal nerve ablation catheter in the wake of promising Phase 1 and 2 clinical trials in hypertension gave way to despair after the Phase 3 SYMPLICITY HTN-3 failed to achieve its efficacy endpoints (Bhatt et al., 2014). The reasons for the disparities between the earlier trials and SYMPLICITY HTN-3 have been discussed at length by many other authors (Esler, 2014; Kandzari et al., 2015), and include completeness of denervation, patient selection, the placebo effect, and in-trial medication regimen changes (Bhatt and Bakris, 2014). Recently, DENERHTN, which was not sham-controlled but was carried out at select hypertension centers by experienced interventionalists in patients on a carefully controlled medication regimen, showed that the addition of renal denervation to a standard medication regimen significantly decreased 24-h ambulatory systolic blood pressure (Azizi et al., 2015). Despite questions about its efficacy, catheter-based denervation is generally safe. HTN-3 showed that denervation was no worse than renal angiography in terms of renovascular complications, renal function, all-cause mortality, and cardiovascular events, while two patients in DENERHTN experienced lumbar pain and one developed a groin hematoma.

Presently, ClinicalTrials.gov lists 18 studies for renal denervation in CHF. Only one, a small pilot safety study in seven CHF patients, has been completed (Davies et al., 2013). These patients did not have hypotensive complications or deterioration of renal function after renal denervation with the Medtronic Symplicity™ catheter. All patients felt better after denervation and improved their 6-min walk distance despite no change in echocardiographic parameters. Many of the other studies are aimed at particular CHF subgroups, including heart failure with preserved ejection fraction, CHF with renal impairment, and CHF secondary to Chagas disease. Also notable is that some of these trials were withdrawn or terminated following the efficacy shortcomings of SYMPLICITY HTN-3.

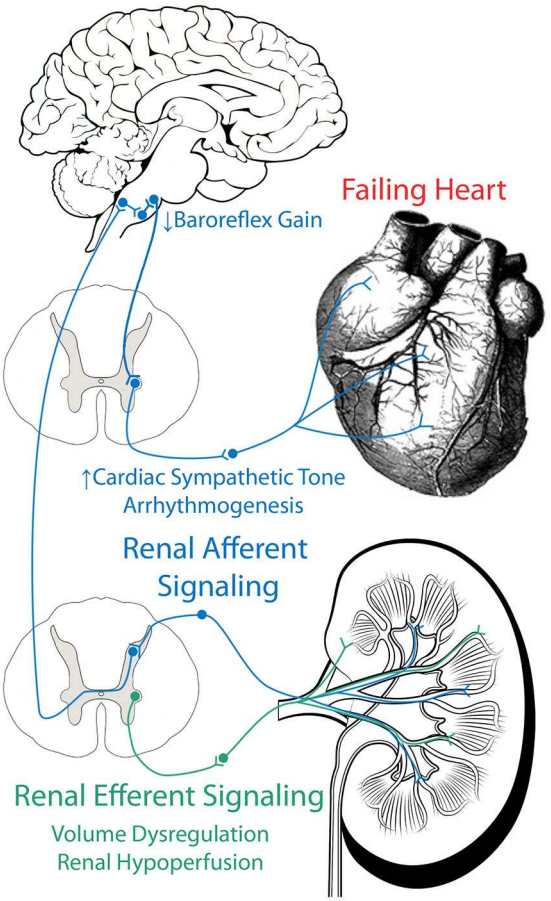


FIGURE 2 | Role of efferent and afferent renal nerves in CHF. Efferent renal signaling decreases renal blood flow and contributes to volume dysregulation. Afferent renal signaling contributes to cardiac sympatho-excitation, baroreflex dysfunction, and arrhythmogenesis.

Conclusion

We have reviewed evidence which implicates both the efferent and afferent renal sympathetic nerves in the development several of the pathophysiological hallmarks of CHF, including renal hypoperfusion, volume dysregulation, cardiac sympatho-excitation, baroreflex dysfunction, arrhythmogenesis, and neurohumoral activation (**Figure 2**). More research, both basic and clinical, is necessary to determine if and exactly how renal denervation can be employed as an effective therapeutic strategy for CHF patients.

Funding

This work was supported by a National Institutes of Health National Heart, Lung, and Blood Institute (NIH NHLBI) Grant P01 HL62222. AS was supported by an American Heart Association pre-doctoral fellowship (13PRE14700045); PP was supported by a NIH NHLBI F30 pre-doctoral fellowship (1F30HL118974-01A1).

References

Azizi, M., Sapoval, M., Gosse, P., Monge, M., Bobrie, G., Delsart, P., et al. (2015). Optimum and stepped care standardised antihypertensive treatment with or without renal denervation for resistant hypertension (DENERHTN): a multicentre, open-label, randomised controlled trial. *Lancet* 385, 1957–1965. doi: 10.1016/S0140-6736(14)61942-5

Barajas, L., Liu, L., and Powers, K. (1992). Anatomy of the renal innervation: intrarenal aspects and ganglia of origin. *Can. J. Physiol. Pharmacol.* 70, 735–749. doi: 10.1139/y92-098

Bhatt, D. L., and Bakris, G. L. (2014). Renal denervation for resistant hypertension. *N. Engl. J. Med.* 371, 184. doi: 10.1056/NEJMoa1402670

Bhatt, D. L., Kandzari, D. E., O'Neill, W. W., D'Agostino, R., Flack, J. M., Katzen, B. T., et al. (2014). A controlled trial of renal denervation for resistant hypertension. *N. Engl. J. Med.* 370, 1393–1401. doi: 10.1056/NEJMoa1402670

Bock, J. S., and Gottlieb, S. S. (2010). Cardiorenal syndrome: new perspectives. *Circulation* 121, 2592–2600. doi: 10.1161/CIRCULATIONAHA.109.886473

Boerrigter, G., Costello-Boerrigter, L. C., Abraham, W. T., Sutton, M. G., Heublein, D. M., Kruger, K. M., et al. (2008). Cardiac resynchronization therapy improves renal function in human heart failure with reduced glomerular filtration rate. *J. Card. Fail.* 14, 539–546. doi: 10.1016/j.cardfail.2008.03.009

Bongartz, L. G., Cramer, M. J., Doevendans, P. A., Joles, J. A., and Braam, B. (2005). The severe cardiorenal syndrome: 'Guyton Revisited.' *Eur. Heart J.* 26, 11–17. doi: 10.1093/eurheartj/ehi020

Booth, L. C., Nishi, E. E., Yao, S. T., Ramchandra, R., Lambert, G. W., Schlaich, M. P., et al. (2015a). Reinnervation of renal afferent and efferent nerves at 5.5 and 11 Months after catheter-based radiofrequency renal denervation in sheep. *Hypertension* 65, 393–400. doi: 10.1161/HYPERTENSIONAHA.114.04176

Booth, L. C., Schlaich, M. P., Nishi, E. E., Yao, S. T., Xu, J., Ramchandra, R., et al. (2015b). Short-term effects of catheter-based renal denervation on cardiac sympathetic drive and cardiac baroreflex function in heart failure. *Int. J. Cardiol.* 190, 220–226. doi: 10.1016/j.ijcard.2015.03.440

Brunner-La Rocca, H. P., Esler, M. D., Jennings, G. L., and Kaye, D. M. (2001). Effect of cardiac sympathetic nervous activity on mode of death in congestive heart failure. *Eur. Heart J.* 22, 1136–1143. doi: 10.1053/euhj.2000.2407

Clayton, S. C., Haack, K. K., and Zucker, I. H. (2011). Renal denervation modulates angiotensin receptor expression in the renal cortex of rabbits with chronic heart failure. *Am. J. Physiol. Renal Physiol.* 300, F31–F39. doi: 10.1152/ajprenal.00088.2010

Coglianese, E. E, Qureshi, M. M., Vasan, R. S., Wang, T. J., and Moore, L. L. (2012). Usefulness of the blood hematocrit level to predict development of heart failure in a community. *Am. J. Cardiol.* 109, 241–245. doi: 10.1016/j.amjcard.2011.08.037

Davies, J. E., Manisty, C. H., Petraco, R., Barron, A. J., Unsworth, B., Mayet, J., et al. (2013). First-in-man safety evaluation of renal denervation for chronic systolic heart failure: primary outcome from REACH-Pilot study. *Int. J. Cardiol.* 162, 189–192. doi: 10.1016/j.ijcard.2012.09.019

DiBona, G. F., Herman, P. J., and Sawin, L. L. (1988). Neural control of renal function in edema-forming states. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 254, R1017–R1024.

DiBona, G. F., and Sawin, L. L. (1991). Role of renal nerves in sodium retention of cirrhosis and congestive heart failure. *Am. J. Physiol.* 260(2 Pt 2), R298–R305.

DiBona, G. F., and Sawin, L. L. (2004). Effect of renal denervation on dynamic autoregulation of renal blood flow. *Am. J. Physiol. Renal Physiol.* 286, F1209–F1218. doi: 10.1152/ajprenal.00010.2004

Esler, M. (2014). Illusions of truths in the symplicity HTN-3 trial: generic design strengths but neuroscience failings. *JASH* 8, 593–598. doi: 10.1016/j.jash.2014.06.001

Felder, R. B., Francis, J., Zhang, Z.-H., Wei, S.-G., Weiss, R. M., and Johnson, A. K. (2003). Heart failure and the brain: new perspectives. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284, R259–R276. doi: 10.1152/ajpregu.00317.2002

Foss, J. D., Wainford, R. D., Engeland, W. C., Fink, G. D., and Osborn, J. W. (2015). A novel method of selective ablation of afferent renal nerves by periaxonal application of capsaicin. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 308, R112–R122. doi: 10.1152/ajpregu.00427.2014

Gazdar, A. F., and Dammin, G. J. (1970). Neural degeneration and regeneration in human renal transplants. *N. Engl. J. Med.* 283, 222–224. doi: 10.1056/NEJM197007302830502

Gullestad, L., Ueland, T., Vinge, L. E., Finsen, A., Yndestad, A., and Aukrust, P. (2012). Inflammatory cytokines in heart failure: mediators and markers. *Cardiology* 122, 23–35. doi: 10.1159/000338166

Hansen, J. M., Abildgaard, U., Fogh-Andersen, N., Kanstrup, I.-L., Bratholm, P., Plum, I., et al. (1994). The transplanted human kidney does not achieve functional reinnervation. *Clin. Sci.* 87, 13–20.

Hasking, G. J., Esler, M. D., Jennings, G. L., Burton, D., Johns, J. A., and Korner, P. I. (1986). Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. *Circulation* 73, 615–621. doi: 10.1161/01.CIR.73.4.615

Heidenreich, P. A., Albert, N. M., Allen, L. A., Bluemke, D. A., Butler, J., Fonarow, G. C., et al. (2013). Forecasting the impact of heart failure in the united states: a policy statement from the american heart association. *Circ. Heart Fail.* 6, 606–619. doi: 10.1161/HHF.0b013e318291329a

Hering, D., Lambert, E. A., Marusic, P., Walton, A. S., Krum, H., Lambert, G. W., et al. (2013). Substantial reduction in single sympathetic nerve firing after renal denervation in patients with resistant hypertension. *Hypertension* 61, 457–464. doi: 10.1161/HYPERTENSIONAHA.111.00194

Hillege, H. L., Girbes, A. R. J., de Kam, P. J., Boomsma, F., de Zeeuw, D., Charlesworth, A., et al. (2000). Renal function, neurohormonal activation, and survival in patients with chronic heart failure. *Circulation* 102, 203–210. doi: 10.1161/01.CIR.102.2.203

Kandzari, D. E, Bhatt, D. L., Brar, S., Devireddy, C. M., Esler, M., Fahy, M., et al. (2015). Predictors of blood pressure response in the SYMPLICITY HTN-3 Trial. *Eur. Heart J.* 36, 219–227. doi: 10.1093/eurheartj/ehu441

Kim, J., and Padanilam, B. J. (2013). Renal nerves drive interstitial fibrogenesis in obstructive nephropathy. *JASN* 24, 229–242. doi: 10.1681/ASN.2012070678

Kon, V., Yared, A., and Ichikawa, I. (1985). Role of renal sympathetic nerves in mediating hypoperfusion of renal cortical microcirculation in experimental congestive heart failure and acute extracellular fluid volume depletion. *J. Clin. Invest.* 76, 1913–1920. doi: 10.1172/JCI112187

Krum, H., Schlaich, M., Whitbourn, R., Sobotka, P. A., Sadowski, J., Bartus, K., et al. (2009). Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 373, 1275–1281. doi: 10.1016/S0140-6736(09)60566-3

Linz, D., Hohl, M., Schütze, J., Mahfoud, F., Speer, T., Linz, B., et al. (2015). Progression of kidney injury and cardiac remodeling in obese spontaneously hypertensive rats: the role of renal sympathetic innervation. *Am. J. Hypertens.* 28, 256–265. doi: 10.1093/ajh/hpu123

Linz, D., Wirth, K., Ukena, C., Mahfoud, F., Pöss, J., Linz, B., et al. (2013). Renal denervation suppresses ventricular arrhythmias during acute ventricular ischemia in pigs. *Heart Rhythm* 10, 1525–1530. doi: 10.1016/j.hrthm.2013.07.015

Lloyd-Jones, D. M., Larson, M. G., Leip, E. P., Beiser, A., D'Agostino, R. B., Kannel, W. B., et al. (2002). Lifetime risk for developing congestive heart failure: the framingham heart study. *Circulation* 106, 3068–3072. doi: 10.1161/01.CIR.0000039105.49749.6F

Lohmeier, T. E., Hildebrandt, D. A., Dwyer, T. M., Barrett, A. M., Irwin, E. D., Rossing, M. A., et al. (2007). Renal denervation does not abolish sustained baroreflex-mediated reductions in arterial pressure. *Hypertension* 49, 373–379. doi: 10.1161/01.HYP.0000253507.56499.bb

McAlister, F. A., Ezekowitz, J., Tonelli, M., and Armstrong, P. W. (2004). Renal insufficiency and heart failure: prognostic and therapeutic implications from a prospective cohort study. *Circulation* 109, 1004–1009. doi: 10.1161/01.CIR.0000116764.53225.A9

Mousa, T. M., Liu, D., Cornish, K. G., and Zucker, I. H. (2008). Exercise training enhances baroreflex sensitivity by an angiotensin II-Dependent mechanism in chronic heart failure. *J. Appl. Physiol. (Bethesda, Md. 1985)* 104, 616–624. doi: 10.1152/japplphysiol.00601.2007

Mulder, J., Hökfelt, T., Knuepfer, M. M., and Kopp, U. C. (2013). Renal sensory and sympathetic nerves reinnervate the kidney in a similar time-dependent fashion after renal denervation in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 304, R675–R682. doi: 10.1152/ajpregu.00599.2012

Mullens, W., Abrahams, Z., Francis, G. S., Sokos, G., Taylor, D. O., Starling, R. C., et al. (2009). Importance of venous congestion for worsening of renal function in advanced decompensated heart failure. *J. Am. Coll. Cardiol.* 53, 589–596. doi: 10.1016/j.jacc.2008.05.068

Mullens, W., Abrahams, Z., Skouri, H. N., Francis, G. S., Taylor, D. O., Starling, R. C., et al. (2008). Elevated intra-abdominal pressure in acute decompensated heart failure: a potential contributor to worsening renal function? *J. Am. Coll. Cardiol.* 51, 300–306. doi: 10.1016/j.jacc.2007.09.043

Nijima, A. (1971). Afferent discharges from arterial mechanoreceptors in the kidney of the rabbit. *J. Physiol.* 219, 477–485. doi: 10.1113/jphysiol.1971.sp009673

Nozawa, T., Igawa, A., Fujii, N., Kato, B., Yoshida, N., Asanoi, H., et al. (2002). Effects of long-term renal sympathetic denervation on heart failure after myocardial infarction in rats. *Heart Vessels* 16, 51–56. doi: 10.1007/s380-002-8317-8

Ogita, H. (2001). Prognostic significance of cardiac 123I metaiodobenzylguanidine imaging for mortality and morbidity in patients with chronic heart failure: a prospective study. *Heart* 86, 656–660. doi: 10.1136/heart.86.6.656

Oldham, J. B. (1950). Denervation of the kidney. *Ann. R. Coll. Surg. Engl.* 7, 222–245.

Petersson, M., Friberg, P., Eisenhofer, G., Lambert, G., and Rundqvist, B. (2005). Long-term outcome in relation to renal sympathetic activity in patients with chronic heart failure. *Eur. Heart J.* 26, 906–913. doi: 10.1093/eurheartj/ehi184

Recordati, G., Moss, N. G., Genovesi, S., and Rogenes, P. (1981). Renal chemoreceptors. *J. Auton. Nerv. Syst.* 3, 237–251. doi: 10.1016/0165-1838(81)90066-7

Roger, V. L., Weston, S. A., Redfield, M. M., Hellermann-Homan, J. P., Killian, J., Yawn, B. P., et al. (2004). Trends in heart failure incidence and survival in a community-based population. *JAMA* 292, 344–350. doi: 10.1001/jama.292.3.344

Sandner, S. E., Zimpfer, D., Zrunek, P., Rajek, A., Schima, H., Dunkler, D., et al. (2009). Renal function and outcome after continuous flow left ventricular assist device implantation. *Ann. Thorac. Surg.* 87, 1072–1078. doi: 10.1016/j.athoracsur.2009.01.022

Schiller, A. M., Haack, K. K., Pellegrino, P. R., Curry, P. L., and Zucker, I. H. (2013). Unilateral renal denervation improves autonomic balance in conscious rabbits with chronic heart failure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 305, R886–R892. doi: 10.1152/ajpregu.00269.2013

Sousa-Pinto, B., Ferreira-Pinto, M. J., Santos, M., and Leite-Moreira, A. F. (2014). Central nervous system circuits modified in heart failure: pathophysiology and therapeutic implications. *Heart Fail. Rev.* 19, 759–779. doi: 10.1007/s10741-014-9427-x

Souza, D. R. B., Mill, J. G., and Cabral, A. M. (2004). Chronic experimental myocardial infarction produces antinatriuresis by a renal nerve-dependent mechanism. *Braz. J. Med. Biol. Res.* 37, 285–293. doi: 10.1590/S0100-879X2004000200017

Stella, A., and Zanchetti, A. (1991). Functional role of renal afferents. *Physiol. Rev.* 71, 659–682.

Testa, M., Yeh, M., Lee, P., Fanelli, R., Loperfido, F., Berman, J. W., et al. (1996). Circulating levels of cytokines and their endogenous modulators in patients with mild to severe congestive heart failure due to coronary artery disease or hypertension. *J. Am. Coll. Cardiol.* 28, 964–971. doi: 10.1016/S0735-1097(96)00268-9

Torp, M., Brønd, L., Nielsen, J. B., Nielsen, S., Christensen, S., and Jonassen, T. E. N. (2012). Effects of renal denervation on the NKCC2 cotransporter in the thick ascending limb of the loop of henle in rats with congestive heart failure. *Acta Physiol. (Oxf).* 204, 451–459. doi: 10.1111/j.1748-1716.2011.02351.x

Tsutsui, H., Kinugawa, S., and Matsushima, S. (2011). Oxidative stress and heart failure. *AJP-Heart Circ. Physiol.* 301, 2181–2190. doi: 10.1152/ajpheart.00554.2011

Uchida, Y., Kamisaka, K., and Ueda, H. (1971). Two types of renal mechanoreceptors. *Jpn. Heart J.* 12, 233–241. doi: 10.1536/ihj.12.233

Velagaleti, R. S., Gona, P., Larson, M. G., Wang, T. J., Levy, D., Benjamin, E. J., et al. (2010). Multimarker approach for the prediction of heart failure incidence in the community. *Circulation* 122, 1700–1706. doi: 10.1161/CIRCULATIONAHA.109.929661

Villarreal, D., Freeman, R. H., Johnson, R. A., and Simmons, J. C. (1994). Effects of renal denervation on postprandial sodium excretion in experimental heart failure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 266, R1599–R1604.

Wali, R. K., Wang, G. S., Gottlieb, S. S., Bellumkonda, L., Hansalia, R., Ramos, E., et al. (2005). Effect of kidney transplantation on left ventricular systolic dysfunction and congestive heart failure in patients with end-stage renal disease. *J. Am. Coll. Cardiol.* 45, 1051–1060. doi: 10.1016/j.jacc.2004.11.061

Witty, R. T., Davis, J. O., Shade, R. E., Johnson, J. A., and Prewitt, R. L. (1972). Mechanisms regulating renin release in dogs with thoracic caval constriction. *Circ. Res.* 31, 339–347. doi: 10.1161/01.RES.31.3.339

Xu, B., Zheng, H., Liu, X., and Patel, K. P. (2015). Activation of afferent renal nerves modulates RVLM projecting PVN neurons. *Am. J. Physiol. Heart Circ. Physiol.* 308, H1103–H1111. doi: 10.1152/ajpheart.00862.2014

Zucker, I. H., Wang, W., Brändle, M., Schultz, H. D., and Patel, K. P. (1995). Neural regulation of sympathetic nerve activity in heart failure. *Prog. Cardiovasc. Dis.* 37, 397–414. doi: 10.1016/S0033-0620(05)80020-9

Zucker, I. H., Wang, W., Pliquett, R. U., Liu, J. L., and Patel, K. P. (2001). The regulation of sympathetic outflow in heart failure. The roles of Angiotensin II, nitric oxide, and exercise training. *Ann. N. Y. Acad. Sci.* 940, 431–443. doi: 10.1111/j.1749-6632.2001.tb03696.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 04 August 2015
doi: 10.3389/fphys.2015.00218

Direct conscious telemetry recordings demonstrate increased renal sympathetic nerve activity in rats with chronic kidney disease

Ibrahim M. Salman[1], *Divya Sarma Kandukuri*[1], *Joanne L. Harrison*[2], *Cara M. Hildreth*[1] *and Jacqueline K. Phillips*[1]*

[1] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia, [2] School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA, Australia

OPEN ACCESS

Edited by:
Kate Denton,
Monash University, Australia

Reviewed by:
Rohit Ramchandra,
University of Auckland, New Zealand
Joseph Francis,
Louisiana State University, USA

***Correspondence:**
Jacqueline K. Phillips,
Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, 2 Technology Place, Sydney, NSW 2109, Australia
jacqueline.phillips@mq.edu.au

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: 29 May 2015
Accepted: 20 July 2015
Published: 04 August 2015

Citation:
Salman IM, Sarma Kandukuri D, Harrison JL, Hildreth CM and Phillips JK (2015) Direct conscious telemetry recordings demonstrate increased renal sympathetic nerve activity in rats with chronic kidney disease. Front. Physiol. 6:218. doi: 10.3389/fphys.2015.00218

Chronic kidney disease (CKD) is associated with sympathetic hyperactivity and impaired blood pressure control reflex responses, yet direct evidence demonstrating these features of autonomic dysfunction in conscious animals is still lacking. Here we measured renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP) using telemetry-based recordings in a rat model of CKD, the Lewis Polycystic Kidney (LPK) rat, and assessed responses to chemoreflex activation and acute stress. Male LPK and Lewis control animals (total $n = 16$) were instrumented for telemetric recording of RSNA and MAP. At 12–13 weeks-of-age, resting RSNA and MAP, sympathetic and haemodynamic responses to both peripheral (hypoxia: 10% O_2) and central chemoreflex (hypercapnia: 7% CO_2) activation and acute stress (open-field exposure), were measured. As indicators of renal function, urinary protein (U_{Pro}) and creatinine (U_{Cr}) levels were assessed. LPK rats had higher resting RSNA (1.2 ± 0.1 vs. $0.6 \pm 0.1\,\mu V$, $p < 0.05$) and MAP (151 ± 8 vs. 97 ± 2 mmHg, $p < 0.05$) compared to Lewis. MAP was negatively correlated with U_{Cr} ($r = -0.80$, $p = 0.002$) and positively correlated with RSNA ($r = 0.66$, $p = 0.014$), with multiple linear regression modeling indicating the strongest correlation was with U_{cr}. RSNA and MAP responses to activation of the central chemoreflex and open-field stress were reduced in the LPK relative to the Lewis (all $p < 0.05$). This is the first description of dual conscious telemetry recording of RSNA and MAP in a genetic rodent model of CKD. Elevated RSNA is likely a key contributor to the marked hypertension in this model, while attenuated RSNA and MAP responses to central chemoreflex activation and acute stress in the LPK indicate possible deficits in the neural processing of autonomic outflows evoked by these sympathoexcitatory pathways.

Keywords: renal sympathetic nerve activity, blood pressure, chemoreflex, open-field stress, hypertension, polycystic kidney disease, telemetry

Introduction

Sympathetic nervous system (SNS) hyperactivity is synonymous with chronic kidney disease (CKD), contributing to hypertension, renal disease progression, and consequent cardiovascular morbidity and mortality (Penne et al., 2009; Grassi et al., 2011). Plasma catecholamine levels (Zoccali et al., 2002; Grassi et al., 2011) and renal noradrenaline spillover (Schlaich et al., 2013) are increased, and direct measurements of muscle sympathetic nerve activity (SNA) reveal elevated tonic levels (Neumann et al., 2007; Grassi et al., 2011; Schlaich et al., 2013). However, despite the various approaches to quantify sympathetic activity in humans and experimental animals, each has inherent limitations. Measurement of plasma catecholamine levels can reveal global activity but not the discrete contributions of organ-specific sympathetic nerve beds and noradrenaline spillover studies, while organ specific, must take into consideration not only SNA but also altered neurotransmitter uptake as an underlying mechanism. Elevated levels of muscle SNA features in many cardiovascular diseases and as a tool has provided an incredibly valuable snapshot into SNA at both the multiunit and single unit recording level in humans (Lambert et al., 2008) and has been used to assess for example the impact of renal denervation on muscle SNA in essential hypertension (Hering et al., 2013), however muscle SNA cannot provide mechanistic data as to what pattern of SNA is influencing visceral organs such as the kidney (Grassi et al., 2015) and it does not provide a long-term monitoring approach. Direct recordings of SNA to specific organ beds can be acquired from acute recordings in unconscious animals (Yao et al., 2015), however these may be unavoidably confounded by the effect of anesthetic on both SNA and blood pressure. Urethane anesthesia, for example, has been shown to both promote and inhibit SNA (Shimokawa et al., 1998; Wang et al., 2014) and altered baseline levels of blood pressure due to anesthetic will alter SNA through baroreflex mechanisms. Many of the reflex autonomic responses tested through pharmacological manipulation of blood pressure may also be influenced by anesthesia. For example responses to the ganglionic blocker hexamethonium have been shown to have different responses on sympathetic control of blood pressure in anesthetized vs. conscious animals, in both normotensive and hypertensive models (Biancardi et al., 2007).

Knowing the baseline level of SNA to a specific target organ can be of critical importance. For example, increased sympathetic outflow to the kidney may have a greater role in the long-term control of blood pressure in CKD, given the role of renal SNA (RSNA) in not only altering blood flow, but also regulating renin secretion and salt and water reabsorption (Johns et al., 2011). This is supported by recent evidence showing that renal denervation, using a catheter-based approach that disrupts renal sympathetic nerves in the adventitia of the renal arteries, can mitigate sympathetic hyperactivity in CKD patients, contributing to not only reductions in blood pressure but also improving renal haemodynamics, enhancing glomerular filtration rate, and reducing albuminuria (Hering et al., 2012; Kiuchi et al., 2013; Schlaich et al., 2013). Our current direct knowledge of RSNA and autonomic reflex control in CKD is still limited however.

The Lewis Polycystic Kidney (LPK) rat is a model of nephronophthisis, a form of autosomal recessive polycystic kidney disease arising from a spontaneous mutation in the *Nek8* gene (McCooke et al., 2012). We have previously demonstrated both indirect and direct evidence for sympathetic overactivity in the LPK. Indirectly, hypotensive responses to ganglionic blockade (Phillips et al., 2007; Ameer et al., 2014), bradycardic responses to β_1-adrenoceptor blockade (Harrison et al., 2010) and low-frequency power of systolic blood pressure variability (Harrison et al., 2010; Hildreth et al., 2013b) are all increased in the LPK. Directly, we have recently shown that RSNA in anesthetized animals display elevated absolute baseline tonic levels (Salman et al., 2014, 2015; Yao et al., 2015). Given the potential effect of anesthesia on SNA, which may be influenced to a greater degree in the disease state (Hildreth et al., 2013a), a key objective of the present study was to record RSNA in conscious unrestrained animals using telemetry, examining the hypothesis that a sustained elevation in RSNA is a key pathological feature of CKD. We also sought to identify if altered sympathetic activity in the conscious LPK is associated with deficits in regulatory pathways known to impact autonomic neuroregulation of the cardiovascular system including the chemoreceptor reflex and behavioral stress. Tonic activation of excitatory chemoreceptor afferents has been proposed as a driver of elevated SNA in CKD patients (Hering et al., 2007; Despas et al., 2009) and we have recently shown under anesthetized conditions that the LPK animals have impaired peripheral and central chemoreflex responses (Yao et al., 2015). Accordingly, our second aim was to assess RSNA and blood pressure responses to central (hypercapnia) and peripheral (hypoxia) chemoreceptor stimulation in the conscious LPK. Another potential contributor to sympathoexcitation in CKD is altered reactivity to stress, with exaggerated sympathetic responses to acute stress reported in other hypertensive models (DiBona et al., 1995; Head and Burke, 2004; D'Angelo et al., 2006). In CKD however, results are variable, with reports of unchanged (Agarwal et al., 1991) or exaggerated (Seliger et al., 2008) haemodynamic response to mental stressors documented. Therefore, our third aim was to assess sympathetic and blood pressure reactivity to acute stress in the conscious LPK.

Materials and Methods

Animals

Telemetry probes were implanted in male Lewis ($n = 13$) and LPK ($n = 9$) rats. From the control group, 9 provided successful recordings of both SNA and blood pressure, while from the LPK group, 7 were successful recordings. Those animals that did not provide dual recordings for the duration of the study were removed from the study (final $n = 16$). Animals were kept under a 12-h light/dark cycle and received a standard pellet diet and water *ad libitum*. All studies were approved by the Animal Ethics Committee of Macquarie University and carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th Edition, 2013).

Telemetry Probe Implantation

At 10–11 weeks of age, rats underwent surgery to implant a telemeter equipped with nerve recording electrodes and arterial catheter (Model TR46SP/56SP,Telemetry Research/Millar, Auckland, New Zealand). Animals were anesthetized with isoflurane (5% in 100% O_2) for induction and 1–3% for maintenance. Pre-operative pain relief (carprofen 2.5 mg/kg s.c., Norbrook Laboratories, VIC, Australia) and antibiotic (cephazolin 55 mg/kg i.m., Hospira Pty Ltd, VIC, Australia) were administered. An abdominal and dorsal skin incision was made and the nerve recording electrode subcutaneously tunneled from the abdominal through to dorsal incision site. The left kidney was exposed retroperitoneally and a 2-mm portion of the renal nerve, coursing between the abdominal aorta and left renal artery, isolated. The recording electrode was anchored to the aorta and renal artery using non-absorbable 7/0 prolene sutures and the renal nerve gently placed on the electrode and embedded in a silicone elastomer (Kwik-sil®, World Precision Instruments, FL, USA). The ground electrode was sutured to the flank muscles exterior to the dorsal incision site and the dorsal incision closed. The blood pressure catheter was placed by one of two methods depending upon catheter type. Either, the peritoneal cavity was exposed and the catheter (Millar type/TR56SP) was inserted into the abdominal aorta, such that the tip of the catheter was distal to the renal artery. The catheter was secured in place using a plastic mesh (Telemetry Research) and cyanoacrylate cement (Histoacryl®, B Braun, NSW, Australia), and lidocaine (1%, Pfizer, NSW, Australia) applied to induce vasodilation and improve hind limb blood flow. Alternatively, the blood pressure catheter (fluid-filled type/TR46SP) was placed into the femoral artery with the probe body placed within the peritoneal cavity as we have described previously (Hildreth et al., 2013b). Supplemental fluids were provided (saline, 6 ml/kg i.p.) and the peritoneal cavity closed. All skin incisions were closed using wound closure clips. Following cessation of anesthesia, post-operative pain relief was provided [buprenorphine (Temgesic®, Reckitt Benckiser, NSW, Australia, 50 μg/kg s.c.). Rats were allowed to recover for at least 1 week in order to re-establish circadian rhythms (Hildreth et al., 2013b). Pain relief (carprofen, 2.5 mg/kg, s.c. and/or buprenorphine, 50 μg/kg s.c.) and supplemental fluid therapy (up to 60 ml/kg/day 0.9% saline and/or 5% glucose s.c.) were administered as required.

Experimental Protocol

All protocols were carried out when animals were aged between 12 and 13 weeks over a ∼7-day period, allowing a minimum 1 week post-surgical recovery period for reestablishment of circadian rhythms, adequate wound healing and increase in body weight and food and water intake. Animals were individually housed and RSNA and blood pressure data acquired using a receiver (TR162 or TR180: Telemetry Research/Millar) interfaced with a CED Micro 1401 data acquisition system (Cambridge Electronic Designs Ltd, Cambridge, UK). A battery charging pad (TR802/TR180 Telemetry Research/Millar) was placed under each individual animal cage. The blood pressure signal was sampled at a minimum of 500 Hz and RSNA at 2 kHz and continuously displayed on Spike 2 software (v7, CED Ltd., Cambridge, UK). The original RSNA signal was amplified, filtered between 50 and 2000 Hz, full-wave rectified and integrated (1 s smoothing constant). All experiments were conducted between 9:00 a.m. and 5:00 p.m. Each rat underwent no more than one study per day. Resting data was collected at the beginning of the experimental period. Chemoreflex and stress response experiments (see below) were then performed in random order. Ganglionic blockade was the last protocol undertaken. Note not all animals contributed to the data set for each protocol.

Resting Data

Blood pressure and RSNA were recorded for 5 min every 15 min from 9:00 a.m. to 5:00 p.m. over two consecutive days. No other intervention was undertaken on these days.

Chemoreceptor Reflex

Animals were placed in a custom-made plexiglass chamber to which they were previously acclimatized. The chamber was initially filled with medical grade air [21% O_2 balance N_2 (BOC Ltd, NSW, Australia),0.5–1 L/min] and O_2 and CO_2 levels continuously monitored (CapStar-100 CO_2 analyser®, CWE Inc., Ardmore, PA, USA and Gas analyser®, ADInstruments Pty Ltd, NSW, Australia). Once the animal was resting quietly, as indicated by stable measurements of mean arterial pressure (MAP) and RSNA, a 5-min baseline recording was obtained. Following this, the chamber was flushed with either a hypoxic (10% O_2 balance N_2, BOC Ltd,) or hypercapnic (7% CO_2 balance O_2, BOC Ltd,) gas mixture to activate the peripheral and central chemoreceptors, respectively. Approximately 15 s were required to reach the target concentration of O_2 (10%) and CO_2 (7%) within the chamber. Reducing inspired O_2 from 21 to ∼10% in conscious rats has been shown previously to effectively reduce PaO_2, but not $PaCO_2$, while increasing inspired CO_2 from 0 to ∼7% results in an increase in $PaCO_2$without a change in PaO_2(Pepelko and Dixon, 1975). Animals were exposed to these gas mixtures for no more than 5 min, following which the chamber was flushed with medical grade air. Following a 10–20 min recovery period, another 5 min baseline recording was obtained and the chamber was filled with the alternate gaseous mixture and MAP and RSNA recorded for another period of 5 min. The order of gas exposure was randomized and at the end of the experiment, the rat was returned to its home cage.

Open-field Stress

Baseline levels of MAP and RSNA were recorded for 10 min while the animal was in its home cage. The animal was then gently transferred into a brightly lit open field (∼90 cm diameter circular chamber with 40 cm wall) and the MAP and RSNA recorded for 40 min. The animal was then returned to its home cage.

Ganglionic Blockade

Baseline MAP and RSNA was recorded for 5 min while the animal was in its home cage. The ganglionic blocker, hexamethonium (20 mg/kg s.c., Sigma Aldrich, NSW, Australia) was then administered and MAP and RSNA recorded for at least

30 min. The peak response to hexamethonium however was seen in under 10 min in both strains.

Indicators of Renal Function

Animals were individually held in metabolic cages (Tecniplast, NSW, Australia) for 24 h to collect urine samples and determine 24 h urine production and water consumption. Urine was then centrifuged at 3000 rpm for 5 min and stored at −20°C until further assayed for urinary protein (U_{Pro}) and creatinine (U_{Cr}) using an IDEXX VetLab analyser (IDEXX Laboratories Pty Ltd., NSW, Australia).

Euthanasia

After all protocols had been undertaken, rats were euthanased with an overdose of 60 mg/kg sodium pentobarbital i.p. (Lethabarb Euthanasia®, Virbac Pty Ltd, NSW, Australia) and death levels of RSNA recorded.

Data Analysis

All data was analyzed offline using Spike 2 software and GraphPad Prism (GraphPad Prism software v6 Inc., La Jolla, CA, USA). Background nerve activity acquired following ganglionic blockade was subtracted from all RSNA recordings. The level of nerve activity following ganglionic blockade was compared against that obtained following euthanasia and the quality of nerve activity verified by assessing the pulse modulation of RSNA (**Figure 1**) as described previously (Guild et al., 2012; Stocker and Muntzel, 2013).

Resting Data

From the MAP and RSNA recording taken between 9:00 a.m. to 5:00 p.m., a 3-h period, where no signal dropout was observed was identified for each animal. Care was taken to ensure that the time of data analysis was not biased toward morning vs. afternoon in the Lewis vs. LPK, and thus the 3-h periods were randomly distributed. Blood pressure and RSNA was averaged over each 3-h period over the two consecutive days to create one estimate for each variable per animal.

Chemoreflex Data

To temporally capture changes in the chemoreflex response, blood pressure, and nerve responses were averaged into 1-min bins. The level of RSNA, determined during the 1 min period immediately prior to exposure to either hypoxic or hypercapnic gas mixture was set as 100%. Maximum changes in RSNA (μV) relative to the 1 min baseline were also measured. Changes in MAP were expressed relative to the level of MAP 1 min prior to chemoreflex activation.

Open-field Stress

The response to exposure to the open field chamber was evident in less than 10 min after initial exposure and consequently, only the first 10 min of data acquired was analyzed. To temporally capture changes during the open-field exposure, blood pressure, and nerve responses were averaged into 2-min bins. The averaged level of MAP and RSNA determined during the 2 min period immediately prior to exposure to the open-field was set as 100% and changes then expressed relative these baseline levels.

Ganglionic Blockade

Maximum MAP and RSNA responses to hexamethonium were measured relative to a 5-min baseline of these variables.

Correlation of MAP with Indicators of Renal Function and RSNA

In order to determine if there was any relationship between MAP and the indicators renal function [U_{Pro}, U_{Cr} and urinary protein: creatinine ratio (UPC)] and/or RSNA, and the relative strength of the relationships, multiple linear regression modeling was undertaken, using the IBM Statistical Package for the Social Sciences (SPSS; v22, IL, USA). MAP was set as the dependent variable and U_{Pro}, U_{Cr}, UPC, and RSNA as the independent variables, using a stepwise selection method of entry and pairwise exclusion of missing values. Correlation analysis [Pearson correlation coefficient (r) and significance (1-tailed)] and results of the full model [adjusted R^2 value, being the relative predictive powers of the model adjusted for degrees of freedom), significance and β-standardized regression coefficient (β) for the entered independent variables] are provided. n = Number of data pairs.

Statistical Analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism (GraphPad Prism software v6 Inc.). A Brown-Forsythe test was used to determine if there were any differences in the variance, and if so, the data was log-transformed before statistical analysis. Baseline levels of MAP and RSNA, renal function, maximum RSNA (μV) responses to chemoreflex activation and open-field exposure, responses to ganglionic blockade and stability of RSNA were analyzed between strains using a two-tailed Student's *t*-test. The effect of chemoreflex activation or stress on the level of MAP and RSNA was identified within each strain using a repeated measures One-Way ANOVA. Any strain differences in the response to chemoreflex activation or stress were identified using a Two-Way ANOVA. Significance was defined as $p \leq 0.05$.

Results

Phenotypic Characteristics of Lewis and LPK Rats

Daily water intake and urine output, U_{Pro}, UPC, MAP, and resting absolute RSNA were significantly higher in the LPK rats vs. Lewis controls (**Table 1**). U_{Cr} excretion levels were significantly lower in the LPK compared with Lewis (**Table 1**). MAP was significantly negatively correlated with U_{cr}(**Figure 2A**) and positively correlated with RSNA (**Figure 2B**). In the multiple linear regression model however, U_{cr} was the only significant predictor variable (n = 11 data pairs, adjusted R^2 = 0.595, β coefficient = −0.797, p = 0.003), indicating a stronger relationship between MAP and U_{cr} than MAP and RSNA. U_{Pro} and UPC were not significantly correlated with MAP and were both excluded from the final model.

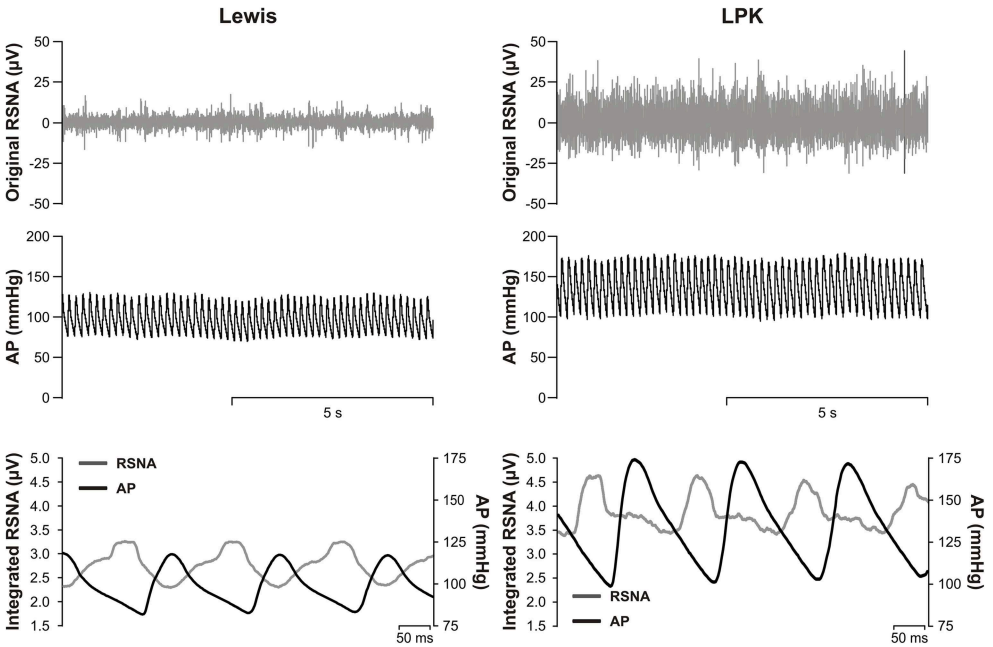


FIGURE 1 | Top, representative raw data traces of original renal sympathetic nerve activity (RSNA) and arterial pressure (AP) from conscious Lewis (left) and Lewis Polycystic Kidney (LPK, right) rats. Bottom, pulse modulation of RSNA using pulse triggered averages of AP and integrated RSNA (solid lines) from the same Lewis (left) and LPK (right) animals. Note the inverse phasic relationship between AP and RSNA in the pulse-triggered averages, typical of pulse modulation of SNA.

TABLE 1 | Phenotypic characteristics of Lewis and LPK rats.

$Parameter_n$	$Lewis_8$	LPK_6	*p*-value
Body weight (g)	332 ± 8	254 ± 8	< 0.0001
Water intake (ml/24 h)	25 ± 1	50 ± 3	< 0.0001
Urine output (ml/24 h)	11 ± 1	51 ± 3	< 0.0001
U_{Pro} (g/L)	0.05 ± 0.01	0.89 ± 0.41	< 0.0001
U_{Cr} (g/L)	1.2 ± 0.2	0.2 ± 0.1	0.0006
UPC	0.06 ± 0.02	5.9 ± 3.2	< 0.0001
MAP (mmHg)	97 ± 2	151 ± 8	< 0.0001
RSNA (μV)	0.6 ± 0.1	1.2 ± 0.1	0.0190

LPK, Lewis Polycystic Kidney; U_{Pro}, urinary total protein; U_{Cr}, urinary creatinine; UPC, urinary protein-to-creatinine ratio; RSNA, renal sympathetic nerve activity and MAP, mean arterial pressure. Results are expressed as mean ± SEM. In the Lewis, UPC was not calculated in 3/9 animals due to lack of detectable protein levels in the urine. Accordingly, only 6 Lewis animals were used to compare mean values of UPC. p-values determined using a two-tailed Student's t-test. (n) values denoted in subscript and represent the minimum number in each group.

Responses to Chemoreceptor Reflex Activation

Peripheral Chemoreflex Activation

Exposure to hypoxia (**Figure 3**) did not significantly change MAP in either the LPK or Lewis (both $p \geq 0.05$). A trend for an overall increase in RSNA in response to hypoxia was evident, most notably in the Lewis animals (**Figure 3B**), however, Two-Way ANOVA indicated no significant effect for either strain ($p = 0.29$) or time ($p = 0.15$) nor was there an interaction between strain and time ($p = 0.13$, $n = 9$).

Central Chemoreflex Activation

Exposure to hypercapnia produced an increase in MAP ($p = 0.005$) alongside a trend toward increasing RSNA ($p = 0.06$) in the Lewis (**Figure 4A**). In the LPK, no change in MAP ($p = 0.10$) or RSNA ($p = 0.68$) was observed (**Figure 4A**). Consequently, both the pressor and sympathoexcitatory response to exposure to hypercapnia was greater in the Lewis compared with the LPK (both $p < 0.05$, **Figure 4B**).

Responses to Acute Open-field Stress

Exposure to the open-field environment produced an increase in both MAP ($p < 0.001$) and RSNA ($p = 0.001$) in the Lewis (**Figure 5A**). In the LPK, only an increase in RSNA ($p = 0.02$) was observed (**Figure 5A**). Consequently, both the pressor ($p = 0.003$) and sympathoexcitatory ($p < 0.001$) response to exposure to the open-field environment was greater in the Lewis compared with LPK (**Figure 5B**).

Responses to Ganglionic Blockade

Administration of hexamethonium produced a fall in MAP and RSNA in both the Lewis [RSNA: 2.1 ± 0.1 vs. 1.5 ± 0.2 μV ($n = 8$); MAP: 105 ± 3 vs. 49 ± 4 mmHg ($n = 7$), baseline vs. hexamethonium; all $p < 0.05$] and LPK [RSNA: 3.0 ± 0.2 vs. 1.6 ± 0.2 μV ($n = 4$), MAP: 157 ± 5 vs. 56 ± 6 mmHg ($n = 4$), baseline vs. hexamethonium; all $p < 0.05$]. The absolute magnitude of the fall was significantly greater in the LPK compared to Lewis for MAP (−1.5 ± 8 vs. -56 ± 3 mmHg) and RSNA (−1.3 ± 0.3 vs. −0.6 ± 0.1 μV) (respectively, both $p < 0.05$). The level of RSNA recorded following administration

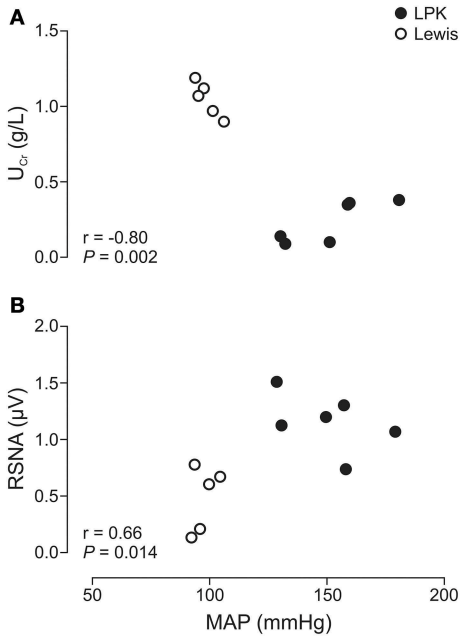


FIGURE 2 | Correlations of mean arterial pressure (MAP) vs. (A) urinary creatinine (U_{Cr}) and (B) renal sympathetic nerve activity (RSNA), in Lewis and Lewis Polycystic Kidney (LPK) rats derived from multiple linear regression ($n = 11$). Pearson correlation coefficient (r) and significance are provided for each relationship within the figure panel.

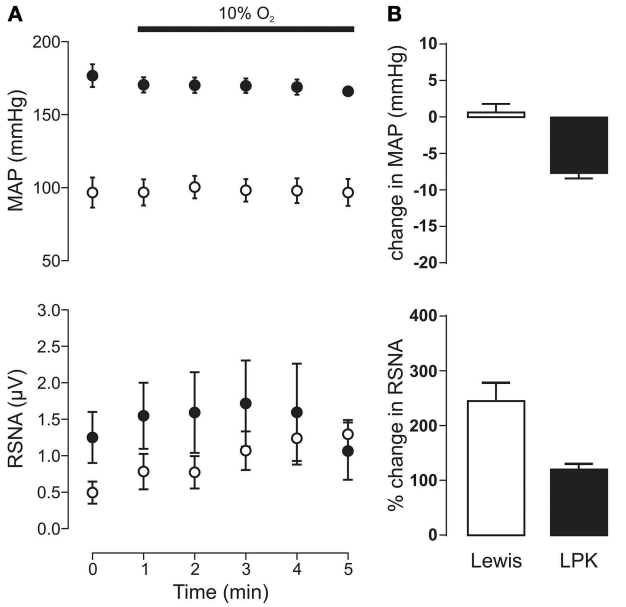


FIGURE 3 | Mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) responses to peripheral chemoreflex activation in Lewis and Lewis Polycystic Kidney (LPK) rats. (A) Data points are 1-min averages of MAP and RSNA measured for 1 min before (time zero) and 5 min during a hypoxic challenge (10% O_2). Results are expressed as mean ± SEM. Minimum *n*/group = 4. **(B)** Average change in MAP and RSNA during the challenge. Results are expressed as mean ± SEM.

of hexamethonium was not different between Lewis and LPK and in both groups was comparable to that recorded following euthanasia [Lewis: 1.5 ± 0.2 ($n = 8$) vs. 1.3 ± 0.2 ($n = 9$) μV and LPK: 1.6 ± 0.2 ($n = 4$) vs. 1.3 ± 0.2 ($n = 7$) μV, hexamethonium vs. euthanasia; all $p > 0.05$].

Discussion

This is the first description of conscious concurrent telemetric recordings of RSNA and blood pressure in a genetic rodent model of CKD. The major novel findings of the present study are: (1) LPK rats have sustained elevated absolute RSNA under conscious conditions compared to Lewis control animals; (2) MAP is most strongly correlated with urinary creatinine as an indicator of renal function across both strains of rat, as well as showing a correlation with RSNA, (3) conscious LPK show reduced RSNA and MAP responses to central chemoreceptor reflex activation and acute stress, indicating possible deficits in the neural processing of autonomic outflows evoked by these typically sympathoexcitatory pathways. Together, this shows that in CKD, sympathetic overdrive, as assessed by direct conscious recording of RSNA, is an archetypal feature, likely contributing to the maintained hypertensive state, and there is altered reactivity to reflexogenic and stressful stimuli.

In our study, we used absolute microvolt measures to report baseline RSNA, and percentage change scores to assess autonomic reflex responses. The reporting of SNA is a methodological consideration, as it has been suggested that absolute levels of SNA in voltage units cannot be compared between animals, as SNA level is dependent upon conditions at the recording site, the number and size of the nerve fibers and the proximity of the active fibers to the electrode (Burke et al., 2011), which increases variability between different nerve recordings. Normalizing nerve activity data by setting baseline to 100% and reporting percentage change scores is proposed to avoid any bias that would therefore otherwise arise, and allow for significant differences to be detected in smaller groups of rats. This method, however, does not allow for comparison of baseline activity or maximal changes in absolute units (Burke et al., 2011). This has led some to suggest that reporting properties of nerve activity using both normalized responses and raw rectified voltage is extremely valuable (Huber and Schreihofer, 2010).

The use of telemetry to directly record SNA in conscious animals over a sustained period overcomes many of the limitations associated with other direct and indirect methods of determining SNA. It does however have inherent technical challenges. The surgical procedure and placement of the telemetry probe is limited by animal size, with >200 grams being the minimum bodyweight for probe implantation. This limits the age range of animals that can be studied, which can be confounded by disease if that is a factor that reduces an animal's body weight further. Another key challenge is the ability to determine sympathetic activity from background or artifact related noise (Stocker and Muntzel, 2013). In this study, we obtained successful blood pressure and RSNA recordings from 16 out of 22 (∼70%) of animals implanted with probes, with the

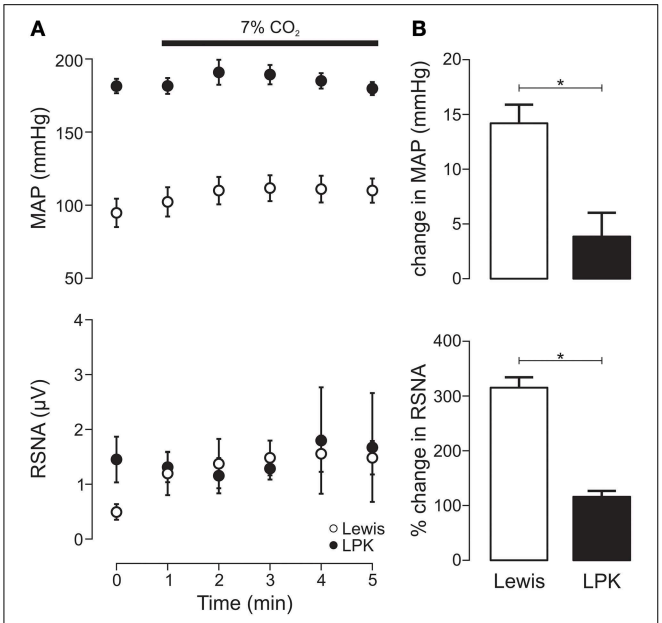


FIGURE 4 | Mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) responses to central chemoreflex activation in Lewis and Lewis Polycystic Kidney (LPK) rats. (A) Data points are 1-min averages of RSNA and MAP measured for 1 min before (time zero) and 5 min during a hypercapnic challenge (7% CO_2). **(B)** average change in MAP and RSNA during the challenge. Results are expressed as mean ± SEM. *$p < 0.05$. Minimum n/group = 4.

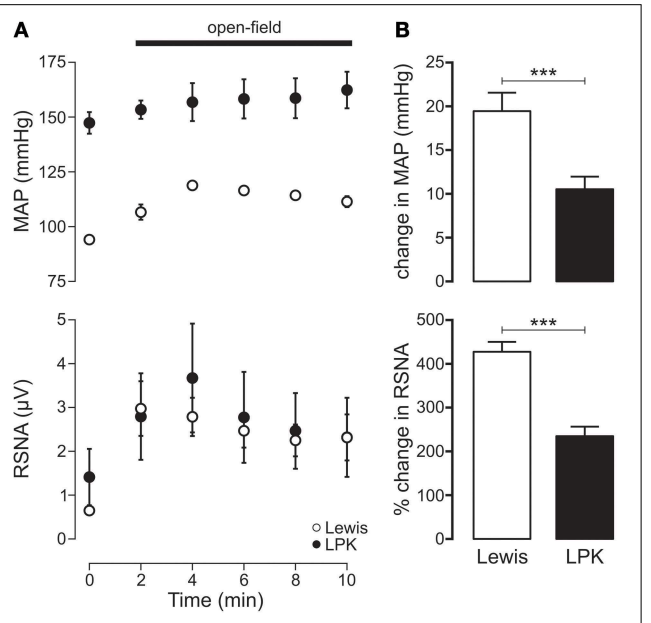


FIGURE 5 | (A) Mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) responses to acute open-field stress in Lewis and Lewis Polycystic Kidney (LPK) rats. Data points are 2-min averages of RSNA and MAP measured for 2 min before (time zero) and the first 10 min during acute stress exposure. **(B)** Average change in MAP and RSNA during the first 10 min of the acute stress exposure. Results are expressed as mean ± SEM. ***$p < 0.001$. Minimum n/group = 6.

validity of our recordings confirmed by the burst like activity evident within the recordings, strong coupling of the SNA to the cardiac cycle as evidenced by the pulse modulation of SNA, and the effective elimination of the response after ganglionic blockade with hexamethonium, indicative of the postganglionic nature of the signal (Stocker and Muntzel, 2013). Recordings for a prolonged period require reliable battery capacity and charging of the battery *in-vivo* can be problematic, however, this has recently been greatly facilitated by the use of individual recharging pads optimized to fit a standard rodent cage, thereby allowing for continual home cage charging operation. Other technical difficulties experienced included: signal dropout, which influenced the recording period over which we could obtain resting data; activity-induced signal noise; and the ease of data analysis, which for our study required the generation of custom-made scripts to analyse the data in our chosen software package.

Our work here confirms our previous studies demonstrating evidence of increased sympathetic activity in the LPK, as measured by other direct and indirect approaches (Phillips et al., 2007; Harrison et al., 2010; Hildreth et al., 2013b; Salman et al., 2014; Yao et al., 2015). This includes our confirmation of previous findings that the LPK display enhanced depressor responses to ganglionic blockade (Phillips et al., 2007; Ameer et al., 2014), another indicator of increased sympathetic vasomotor tone that as a methodology has been the subject of some debate (Moretti et al., 2009). Clinically, elevated SNA in CKD patients is of marked significance being associated with all-cause mortality and nonfatal cardiovascular events (Penne et al., 2009), left ventricular hypertrophy (Guízar-Mendoza et al., 2006) and vascular damage (Bruno et al., 2012). While not indicative of any causal relationship, our observation of blood pressure being strongly correlated with urinary creatinine, as an indicator of declining renal function, as well as having a positive association with RSNA could offer insight to a potential mechanism underlying the blood pressure lowering and renoprotective effects reported after renal denervation in CKD patients (Hering et al., 2012; Kiuchi et al., 2013; Schlaich et al., 2013) and animal models (Eriguchi et al., 2015).

Whether the RSNA measures that we have observed in the LPK model reflect SNA to other vascular beds remains unknown. We have recently shown that in the anesthetized preparation, simultaneous recordings of lumbar, renal, and splanchnic nerves in the LPK demonstrate higher baseline absolute levels of SNA (μV) when compared to Lewis controls (Yao et al., 2015). Future, studies in conscious animals are required to verify this. Current evidence suggests that overall activity of the SNS cannot be simply judged from the recording of a single sympathetic nerve bed (Knuepfer and Osborn, 2010). For example, in Dahl hypertensive rats, targeted ablation of the renal nerves and/or the splanchnic nerves independently influenced MAP, with the greatest decline in MAP seen in those rats that underwent both ablations (Foss et al., 2013), suggesting distinct roles for both the renal and splanchnic sympathetic outflows in driving hypertension in this model. In contrast, in an angiotensin II-high salt induced hypertension model, RSNA decreased over the study period, while lumbar SNA did not change (Yoshimoto et al.,

2010) and that SNA to either vascular bed was not critical in the pathogenesis of angiotensin II-salt hypertension.

We have previously demonstrated that elevated sympathetic drive in the LPK is associated with an inability to effectively restrain SNA by the baroreflex (Salman et al., 2014) and that peripheral and central chemoreflex responses are blunted in these animals (Yao et al., 2015). An objective of the present work was to assess if the LPK display comparable chemoreflex abnormalities in the conscious state and to test a psychological stressful stimulus, being another reflex response known to modulate SNA (van den Buuse et al., 2001). In contrast to our anesthetized study, activation of the peripheral chemoreflex pathway using hypoxia did not cause a change in SNA or MAP in either the Lewis or LPK animals. This may be because the exposure was not of sufficient time frame and/or in the conscious state the animals were able to respond with a reflex increase in ventilation. Additionally, and with specific reference to the LPK animals, the peripheral chemoreceptors may be already maximally activated. In human studies, in order to test if tonic activation of excitatory chemoreceptor afferents contributes to elevated SNA in patients with CKD, the effect of chemoreflex deactivation on SNA is instead tested, comparing the inhibitory effects of 100% oxygen with that of breathing room air on MSNA and blood pressure (Hering et al., 2007). We will require future experiments using alternative experimental paradigms to investigate the effect of stimulation of the peripheral chemoreflex on RSNA in the conscious state in our CKD model. In the CKD animals however, the central chemoreflex response demonstrated an attenuated sympathoexcitatory and pressor response to hypercapnia when compared to the Lewis control animals. This is directly comparable to our studies in the anesthetized animals (Yao et al., 2015). The mechanisms underlying these effects were not examined in this study, however it is possible that tonic activation of the central chemoreflex pathway in CKD may impair the ability of this reflex to drive further increases in SNA.

Like chemoreflex activation, altered cardiovascular responses to emotional stress have been implicated in the setting of high blood pressure (Ming et al., 2004) and are often evident in different models of hypertension (DiBona and Jones, 1995; Head and Burke, 2004; D'Angelo et al., 2006). The principal response to a stressful stimulus involves the activation of the SNS, release of catecholamines from the adrenal medulla and activation of the hypothalamic-pituitary-adrenocortical axis (Fontes et al., 2011), mechanisms which may become altered during disease. Comparable to our central chemoreflex response, we observed diminution of the sympathetic responses to acute stress in the LPK relative to Lewis, such that the acute rise in RSNA was attenuated and no change in blood pressure was noted. In CKD, previous studies have shown that sympathetic reactivity to mental stress was either unchanged (Agarwal et al., 1991) or exaggerated (Seliger et al., 2008). In these studies, however, sympathetic reactivity was inferred indirectly from assessing differences in the haemodynamic response to stress in test subjects. To our knowledge, this is the first report to show sympathetic responses to stress in CKD using direct recording of both SNA and blood pressure. It is possible that the attenuated sympathetic response and absence of a blood pressure response in the LPK may have been the result of an effective activation of the buffering capacity of the baroreflex; however we believe this is unlikely because we have shown previously that baroreflex control of RSNA and heart rate are markedly impaired in the LPK at this age (Salman et al., 2014). Alternatively, it may indicate RSNA is maximally increased in the LPK, such that it cannot be increased further, or that in the LPK there is an underlying upregulation of endogenous stress pathways (Armitage et al., 2012). Either way, when this response and/or pathway are activated by external factors, the net increase to maximal response is proportionally less than in the control animals.

In conclusion, direct conscious recording of SNA in undisturbed freely moving rats overcomes many of the limitations that other indirect or direct assessments of sympathetic activity are associated with. In a conscious rodent model of CKD, we describe for the first time direct telemetric recording of SNA to the kidney and provide evidence that high blood pressure is significantly correlated with increased RSNA and reduced renal function. The study further shows that the renal disease is associated with both reduced central chemoreflex and stress-induced regulation of RSNA and MAP, indicating that the ability of the CNS to regulate sympathetic outflow and blood pressure is compromised in CKD, therefore emphasizing the complexity of this pathological condition. Findings from the present study are relevant to better understanding the complex nature of this global clinical problem and novel therapies such as the targeting of sympathetic activity to specific organs to limit cardiovascular disease in CKD.

Author Contributions

IS Contributed to design of research, performed experiments; analyzed data; interpreted results of experiments; prepared figures; drafted manuscript; approved final version of manuscript. DK Assisted in the undertaking of experiments and approved final version of manuscript. JH Contributed to the design of research, performed preliminary experiments and approved final version of manuscript. CH Contributed to experimental design, analyzed data, interpreted results of experiments; edited and revised manuscript; prepared figures and approved final version of manuscript. JP–Conception and design of research; analyzed data; interpreted results of experiments, edited and drafted manuscript and responsible for submission of manuscript.

Acknowledgments

The authors acknowledge technical advice and assistance provided by Dr. Sarah-Jane Guild (University of Auckland), Dr. Mark Butlin and Ms. Christine Sutter (Macquarie University). The National Health and Medical Research Council of Australia project grants GNT1030297 and GNT1030301 supported this study. IS and DK were recipients of Macquarie University Research Scholarships while undertaking this research.

References

Agarwal, A., Anand, I. S., Sakhuja, V., and Chugh, K. S. (1991). Effect of dialysis and renal transplantation on autonomic dysfunction in chronic renal failure. *Kid. Int.* 40, 489–495. doi: 10.1038/ki.1991.236

Ameer, O. Z., Hildreth, C. M., and Phillips, J. K. (2014). Sympathetic overactivity prevails over the vascular amplifier phenomena in a chronic kidney disease rat model of hypertension. *Physiol. Rep.* 2, 11. doi: 10.14814/phy2.12205

Armitage, J. A., Burke, S. L., Prior, L. J., Barzel, B., Eikelis, N., Lim, K., et al. (2012). Rapid onset of renal sympathetic nerve activation in rabbits fed a high-fat diet. *Hypertension* 60, 163–171. doi: 10.1161/HYPERTENSIONAHA.111.190413

Biancardi, V. C., Bergamaschi, C. T., Lopes, O. U., and Campos, R. R. (2007). Sympathetic activation in rats with L-NAME-induced hypertension. *Braz. J. Med. Biol. Res.* 40, 401–408. doi: 10.1590/S0100-879X2006005000077

Bruno, R. M., Ghiadoni, L., Seravalle, G., Dell'oro, R., Taddei, S., and Grassi, G. (2012). Sympathetic regulation of vascular function in health and disease. *Front. Physiol.* 3:284. doi: 10.3389/fphys.2012.00284

Burke, S. L., Lambert, E., and Head, G. A. (2011). New approaches to quantifying sympathetic nerve activity. *Curr. Hypertens. Rep.* 13, 249–257. doi: 10.1007/s11906-011-0196-9

D'Angelo, G., Mintz, J. D., Tidwell, J. E., Schreihofer, A. M., Pollock, D. M., and Stepp, D. W. (2006). Exaggerated cardiovascular stress responses and impaired beta-adrenergic-mediated pressor recovery in obese Zucker rats. *Hypertension* 48, 1109–1115. doi: 10.1161/01.HYP.0000247306.53547.d4

Despas, F., Detis, N., Dumonteil, N., Labrunee, M., Bellon, B., Franchitto, N., et al. (2009). Excessive sympathetic activation in heart failure with chronic renal failure: role of chemoreflex activation. *J. Hypertens.* 27, 1849–1854. doi: 10.1097/HJH.0b013e32832e8d0f

DiBona, G. F., and Jones, S. Y. (1995). Analysis of renal sympathetic nerve responses to stress. *Hypertension* 25, 531–538. doi: 10.1161/01.HYP.25.4.531

DiBona, G. F., Jones, S. Y., and Brooks, V. L. (1995). ANG II receptor blockade and arterial baroreflex regulation of renal nerve activity in cardiac failure. *Am. J. Physiol.* 269, R1189–R1196.

Eriguchi, M., Tsuruya, K., Haruyama, N., Yamada, S., Tanaka, S., Suehiro, T., et al. (2015). Renal denervation has blood pressure-independent protective effects on kidney and heart in a rat model of chronic kidney disease. *Kid. Int.* 87, 116–127. doi: 10.1038/ki.2014.220

Fontes, M. A., Xavier, C. H., de Menezes, R. C., and Dimicco, J. A. (2011). The dorsomedial hypothalamus and the central pathways involved in the cardiovascular response to emotional stress. *Neuroscience* 184, 64–74. doi: 10.1016/j.neuroscience.2011.03.018

Foss, J. D., Fink, G. D., and Osborn, J. W. (2013). Reversal of genetic salt-sensitive hypertension by targeted sympathetic ablation. *Hypertension* 61, 806–811. doi: 10.1161/HYPERTENSIONAHA.111.00474

Grassi, G., Mark, A., and Esler, M. (2015). The sympathetic nervous system alterations in human hypertension. *Circ. Res.* 116, 976–990. doi: 10.1161/CIRCRESAHA.116.303604

Grassi, G., Quarti-Trevano, F., Seravalle, G., Arenare, F., Volpe, M., Furiani, S., et al. (2011). Early sympathetic activation in the initial clinical stages of chronic renal failure. *Hypertension* 57, 846–851. doi: 10.1161/HYPERTENSIONAHA.110.164780

Guild, S. J., McBryde, F. D., Malpas, S. C., and Barrett, C. J. (2012). High dietary salt and angiotensin II chronically increase renal sympathetic nerve activity: a direct telemetric study. *Hypertension* 59, 614–620. doi: 10.1161/HYPERTENSIONAHA.111.180885

Guízar-Mendoza, J. M., Amador-Licona, N., Lozada, E. E., Rodriguez, L., Gutiérrez-Navarro, M., Dubey-Ortega, L. A., et al. (2006). Left ventricular mass and heart sympathetic activity after renal transplantation in children and young adults. *Pediatr. Nephrol.* 21, 1413–1418. doi: 10.1007/s00467-006-0238-8

Harrison, J. L., Hildreth, C. M., Callahan, S. M., Goodchild, A. K., and Phillips, J. K. (2010). Cardiovascular autonomic dysfunction in a novel rodent model of polycystic kidney disease. *Auton. Neurosci.* 152, 60–66. doi: 10.1016/j.autneu.2009.09.019

Head, G. A., and Burke, S. L. (2004). Sympathetic responses to stress and rilmenidine in 2K1C rabbits: evidence of enhanced nonvascular effector mechanism. *Hypertension* 43, 636–642. doi: 10.1161/01.HYP.0000116301.02975.aa

Hering, D., Lambert, E. A., Marusic, P., Walton, A. S., Krum, H., Lambert, G. W., et al. (2013). Substantial reduction in single sympathetic nerve firing after renal denervation in patients with resistant hypertension. *Hypertension* 61, 457–464. doi: 10.1161/HYPERTENSIONAHA.111.00194

Hering, D., Mahfoud, F., Walton, A. S., Krum, H., Lambert, G. W., Lambert, E. A., et al. (2012). Renal denervation in moderate to severe CKD. *J. Am. Soc. Nephrol.* 23, 1250–1257. doi: 10.1681/asn.2011111062

Hering, D., Zdrojewski, Z., Krol, E., Kara, T., Kucharska, W., Somers, V. K., et al. (2007). Tonic chemoreflex activation contributes to the elevated muscle sympathetic nerve activity in patients with chronic renal failure. *J. Hypertens.* 25, 157–161. doi: 10.1097/HJH.0b013e3280102d92

Hildreth, C. M., Goodchild, A. K., and Phillips, J. K. (2013a). "Insight into autonomic nervous system control of heart rate in the rat using analysis of heart rate variability and baroreflex sensitivity," in *Stimulation and Inhibition of Neurons*, Vol, *78*, eds P. M. Pilowsky, M. M. J. Farnham and A. Y. Fong (New York, NY: Humana Press), 203–223.

Hildreth, C. M., Kandukuri, D. S., Goodchild, A. K., and Phillips, J. K. (2013b). Temporal development of baroreceptor dysfunction in a rodent model of chronic kidney disease. *Clin. Exp. Pharmacol. Physiol.* 40, 458–465. doi: 10.1111/1440-1681.12110

Huber, D. A., and Schreihofer, A. M. (2010). Attenuated baroreflex control of sympathetic nerve activity in obese Zucker rats by central mechanisms. *J. Physiol.* 588, 1515–1525. doi: 10.1113/jphysiol.2009.186387

Johns, E. J., Kopp, U. C., and DiBona, G. F. (2011). Neural control of renal function. *Compr. Physiol.* 1, 731–767. doi: 10.1002/cphy.c100043

Kiuchi, M. G., Maia, G. L., de Queiroz Carreira, M. A., Kiuchi, T., Chen, S., Andrea, B. R., et al. (2013). Effects of renal denervation with a standard irrigated cardiac ablation catheter on blood pressure and renal function in patients with chronic kidney disease and resistant hypertension. *Eur. Heart J.* 34, 2114–2121. doi: 10.1093/eurheartj/eht200

Knuepfer, M. M., and Osborn, J. W. (2010). Direct assessment of organ specific sympathetic nervous system activity in normal and cardiovascular disease states. *Exp. Physiol.* 95, 32–33. doi: 10.1113/expphysiol.2008.045492

Lambert, E., Dawood, T., Schlaich, M., Straznicky, N., Esler, M., and Lambert, G. (2008). Single-unit sympathetic discharge pattern in pathological conditions associated with elevated cardiovascular risk. *Clin. Exp. Pharmacol. Physiol.* 35, 503–507. doi: 10.1111/j.1440-1681.2008.04905.x

McCooke, J., Appels, R., Barrero, R., Ding, A., Ozimek-Kulik, J., Bellgard, M., et al. (2012). A novel mutation causing nephronophthisis in the Lewis polycystic kidney rat localises to a conserved RCC1 domain in Nek8. *BMC Genomics* 13:393. doi: 10.1186/1471-2164-13-393

Ming, E. E., Adler, G. K., Kessler, R. C., Fogg, L. F., Matthews, K. A., Herd, J. A., et al. (2004). Cardiovascular reactivity to work stress predicts subsequent onset of hypertension: the Air Traffic Controller Health Change Study. *Psychosom. Med.* 66, 459-465. doi: 10.1097/01.psy.0000132872.71870.6d

Moretti, J. L., Burke, S. L., Evans, R. G., Lambert, G. W., and Head, G. A. (2009). Enhanced responses to ganglion blockade do not reflect sympathetic nervous system contribution to angiotensin II-induced hypertension. *J. Hypertens.* 27, 1838–1848. doi: 10.1097/HJH.0b013e32832dd0d8

Neumann, J., Ligtenberg, G., Klein, I. H., Boer, P., Oey, P. L., Koomans, H. A., et al. (2007). Sympathetic hyperactivity in hypertensive chronic kidney disease patients is reduced during standard treatment. *Hypertension* 49, 506–510. doi: 10.1161/01.HYP.0000256530.39695.a3

Penne, E. L., Neumann, J., Klein, I. H., Oey, P. L., Bots, M. L., and Blankestijn, P. J. (2009). Sympathetic hyperactivity and clinical outcome in chronic kidney disease patients during standard treatment. *J. Nephrol.* 22, 208–215.

Pepelko, W. E., and Dixon, G. A. (1975). Arterial blood gases in conscious rats exposed to hypoxia, hypercapnia, or both. *J. Appl. Physiol.* 38, 581–587.

Phillips, J. K., Hopwood, D., Loxley, R. A., Ghatora, K., Coombes, J. D., Tan, Y. S., et al. (2007). Temporal relationship between renal cyst development, hypertension and cardiac hypertrophy in a new rat model of autosomal recessive polycystic kidney disease. *Kid. Blood Press. Res.* 30, 129–144. doi: 10.1159/000101828

Salman, I. M., Hildreth, C. M., Ameer, O. Z., and Phillips, J. K. (2014). Differential contribution of afferent and central pathways to the development

of baroreflex dysfunction in chronic kidney disease. *Hypertension* 63, 804–810. doi: 10.1161/HYPERTENSIONAHA.113.02110

Salman, I. M., Phillips, J. K., Ameer, O. Z., and Hildreth, C. M. (2015). Abnormal central control underlies impaired baroreflex control of heart rate and sympathetic nerve activity in female Lewis Polycystic Kidney rats. *J. Hypertens.* 33, 1418–1428. doi: 10.1097/hjh.0000000000000572

Schlaich, M. P., Bart, B., Hering, D., Walton, A., Marusic, P., Mahfoud, F., et al. (2013). Feasibility of catheter-based renal nerve ablation and effects on sympathetic nerve activity and blood pressure in patients with end-stage renal disease. *Int. J. Cardiol.* 168, 2214–2220. doi: 10.1016/j.ijcard.2013.01.218

Seliger, S. L., Katzel, L. I., Fink, J. C., Weir, M. R., and Waldstein, S. R. (2008). Renal function and cardiovascular response to mental stress. *Am. J. Nephrol.* 28, 304–310. doi: 10.1159/000111386

Shimokawa, A., Kunitake, T., Takasaki, M., and Kannan, H. (1998). Differential effects of anesthetics on sympathetic nerve activity and arterial baroreceptor reflex in chronically instrumented rats. *J. Auton. Nerv. Syst.* 72, 46–54. doi: 10.1016/S0165-1838(98)00084-8

Stocker, S. D., and Muntzel, M. S. (2013). Recording sympathetic nerve activity chronically in rats: surgery techniques, assessment of nerve activity, and quantification. *Am. J. Physiol. Heart Circ. Physiol.* 305, H1407–H1416. doi: 10.1152/ajpheart.00173.2013

van den Buuse, M., Van Acker, S. A., Fluttert, M., and De Kloet, E. R. (2001). Blood pressure, heart rate, and behavioral responses to psychological "novelty" stress in freely moving rats. *Psychophysiology* 38, 490–499. doi: 10.1111/1469-8986.3830490

Wang, G. F., Mao, X. J., and Chen, Z. J. (2014). Urethane suppresses renal sympathetic nerve activity in Wistar rats. *Eur. Rev. Med. Pharmacol. Sci.* 18, 1454–1457.

Yao, Y., Hildreth, C. M., Farnham, M. M., Saha, M., Sun, Q. J., Pilowsky, P. M., et al. (2015). The effect of losartan on differential reflex control of sympathetic nerve activity in chronic kidney disease. *J. Hypertens.* 33, 1249–1260. doi: 10.1097/HJH.0000000000000535

Yoshimoto, M., Miki, K., Fink, G. D., King, A., and Osborn, J. W. (2010). Chronic angiotensin II infusion causes differential responses in regional sympathetic nerve activity in rats. *Hypertension* 55, 644–651. doi: 10.1161/HYPERTENSIONAHA.109.145110

Zoccali, C., Mallamaci, F., Tripepi, G., Parlongo, S., Cutrupi, S., Benedetto, F. A., et al. (2002). Norepinephrine and concentric hypertrophy in patients with end-stage renal disease. *Hypertension* 40, 41–46. doi: 10.1161/01.HYP.0000022063.50739.60

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 30 June 2015
doi: 10.3389/fphys.2015.00192

Neural regulation of the kidney function in rats with cisplatin induced renal failure

*Niamh E. Goulding and Edward J. Johns**

Renal Research Laboratory, Department of Physiology, University College Cork, Cork, Ireland

Aim: Chronic kidney disease (CKD) is often associated with a disturbed cardiovascular homeostasis. This investigation explored the role of the renal innervation in mediating deranged baroreflex control of renal sympathetic nerve activity (RSNA) and renal excretory function in cisplatin-induced renal failure.

Methods: Rats were either intact or bilaterally renally denervated 4 days prior to receiving cisplatin (5 mg/kg i.p.) and entered a chronic metabolic study for 8 days. At day 8, other groups of rats were prepared for acute measurement of RSNA or renal function with either intact or denervated kidneys.

Results: Following the cisplatin challenge, creatinine clearance was 50% lower while fractional sodium excretion and renal cortical and medullary TGF-β1 concentrations were 3–4 fold higher in both intact and renally denervated rats compared to control rats. In cisplatin-treated rats, the maximal gain of the high-pressure baroreflex curve was only 20% that of control rats, but following renal denervation not different from that of renally denervated control rats. Volume expansion reduced RSNA by 50% in control and in cisplatin-treated rats but only following bilateral renal denervation. The volume expansion mediated natriuresis/diuresis was absent in the cisplatin-treated rats but was normalized following renal denervation.

Conclusions: Cisplatin-induced renal injury impaired renal function and caused a sympatho-excitation with blunting of high and low pressure baroreflex regulation of RSNA, which was dependent on the renal innervation. It is suggested that in man with CKD there is a dysregulation of the neural control of the kidney mediated by its sensory innervation.

Keywords: renal innervation, baroreflexes, chronic kidney disease, inflammation, renal sodium excretion

OPEN ACCESS

Edited by:
Kate Denton,
Monash University, Australia

Reviewed by:
Kenju Miki,
Nara Women's University, Japan
Anna M. D. Watson,
Baker IDI Heart and Diabetes Institute, Australia
Jaap Joles,
University Medical Center Utrecht, Netherlands

****Correspondence:***
Edward J. Johns,
Department of Physiology, Western Gateway Building, Western Road, University College Cork, Cork, Ireland
e.j.johns@ucc.ie

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *27 March 2015*
Accepted: *19 June 2015*
Published: *30 June 2015*

Citation:
Goulding NE and Johns EJ (2015) Neural regulation of the kidney function in rats with cisplatin induced renal failure. Front. Physiol. 6:192. doi: 10.3389/fphys.2015.00192

Introduction

Chronic kidney disease (CKD) is often initiated as a consequence of structural deterioration within the renal vascular and tubular structures and impairs renal function which eventually impinges on cardiovascular homeostasis (Khawaja and Wilcox, 2011; Sobotka et al., 2011). Hypertension frequently develops in CKD patients, which not only causes further progressive damage to the kidneys, but is a major contributing factor to increased risk of cardiovascular events and mortality (Klag et al., 1996).

There is now compelling evidence that the sympathetic nervous system is over-activated in CKD. Plasma catecholamine levels have been shown to be increased in hemodialysis patients

(Henrich et al., 1977). Moreover, patients with end stage renal disease have elevated muscle sympathetic nerve activity (MSNA) and that removal of the diseased kidneys, at or following transplantation of a functional kidney, decreased blood pressure, peripheral resistance, and the bursting rate in MSNA to normal values (Converse et al., 1992; Hausberg et al., 2002). Moreover, it has been reported that ablation of the renal nerves in patients with CKD, whilst not impacting on renal function itself not only delayed the deterioration in kidney function but also resulted in a chronic reduction in blood pressure (Ott et al., 2015).

It is not clear how the development of CKD may cause a sympatho-excitation as the disease progresses. Activation of the renal sympathetic nerves, that is the efferent innervation, can influence cardiovascular homeostasis by impacting on the regulation of extracellular fluid volume and hence blood pressure. This is due to the direct actions of the sympathetic nerves on renal resistance vessels, to increase vascular resistance and at the nephrons to stimulate tubular sodium and water reabsorption (Johns et al., 2011). Increased renal sympathetic nerve activity (RSNA) will also enhance the release of renin and the generation of angiotensin II which itself is not only a vasoconstrictor, but will also act directly on the proximal tubule to stimulate fluid reabsorption, and indirectly by increasing aldosterone production which causes sodium reabsorption at the distal tubule.

The kidney itself contains sensory nerves which have an important physiological role in the neural control of kidney function and may contribute to the deranged autonomic control in CKD (Kopp, 2015). Sensory nerves present in the renal pelvis appear to be sensitive chemo- and mechano-receptors which upon activation cause a renal sympatho-inhibition and a renal nerve dependent natriuresis at the contra-lateral kidney (Dibona and Rios, 1980). This is termed an inhibitory reno-renal reflex as it is likely to be involved in ensuring that excretion of a sodium and water load is distributed equitably between the two kidneys. There is evidence of an excitatory reno-renal reflex (Ditting et al., 2012; Johns, 2014) which elicits a sympatho-excitation. Early evidence by Katholi et al. (1983) using intrarenal adenosine administration in the dog and Smits and Brody (1984) and more recently Barry and Johns (2015) using intra-renal bradykinin infusion in the rat demonstrated acute increases in blood pressure and RSNA which was blocked in animals subjected to a bilateral renal denervation.

The recent debate and apparently conflicting findings on the role of the renal innervation in pathophysiological states in man, such as resistant hypertension, CKD and diabetes (Bhatt et al., 2014; Esler, 2014; Krum et al., 2014), has created uncertainty especially as the underlying physiological mechanisms are unclear. The hypothesis explored in this investigation was that injury to the kidney, which may induce inflammatory responses, would cause an activation of the renal sensory innervation leading to a renal sympatho-excitation and a dysregulation of baroreflexes and an inability to excrete a saline volume load which was mediated by the renal innervation. The model chosen was the cisplatin induced renal failure rat model in which the high and low pressure baroreflex regulation was tested by increasing and decreasing blood pressure and administering an acute saline volume load, respectively.

Methods

Male Wistar rats, weighing 250–300 g were housed under a 12 h light/dark regime at 20 ± 3°C and 35% humidity. All procedures were approved by the Animal Experimentation Ethical Committee at National University Ireland, Cork and were performed in accordance with the European Community Directive 86/609/EC. All rats were maintained on a normal diet and tap water *ad libitum*.

Cisplatin Induced Renal Failure

Groups of rats were injected with either 5 mg/kg cisplatin (10 mg/ml, Hospira, Illinois, USA) in a volume of 6 ml/kg to induce renal injury, or the equivalent volume of saline (0.9% sodium chloride) intraperitoneally in the control groups of rats (Khan et al., 2007; Salman et al., 2011). Metabolic studies were performed over the subsequent 8 day period and acute studies were undertaken on day 8, post cisplatin administration.

Metabolic Studies

Rats were housed in metabolic cages for 8 days. Urine flow, sodium excretion, and water intake were measured over 24 h periods. Day 1 measurements represented basal values and were obtained prior to administration of either vehicle or cisplatin.

Bilateral renal denervation: Rats were anaesthetized (2–3% isoflurane in O_2). Sequentially, left and right kidneys were exposed retroperitoneally, each renal artery was identified, stripped of its adventitia and then coated with a solution of 10% phenol in absolute ethanol for 1 min and then rinsed with saline. In control rats, the renal arteries were only exposed, but not manipulated further. The muscles and skin were sutured and an analgesic (carprofen 5 mg/kg, Pfizer Inc, USA) given subcutaneously. The animals were allowed 4 days to recover before cisplatin administration and entering the metabolic study.

Food was given *ad libitum* over the 8-day period. Urine samples and tail vein blood samples (0.4 ml) were taken on Days 1 and 8. The blood samples were centrifuged, the plasma was removed and frozen at −20°C. Day 1 and 8 samples of urine were also frozen at −20°C. The plasma and urine sodium concentrations were measured using Flame photometry (Corning, Halstead, Essex, UK); plasma and urine creatinine levels were measured (Quanticom Creatinine Assay DICT 500, Bioassay Systems, Hayward CA, USA) to allow estimation of the clearance of creatinine. Noradrenaline levels in plasma and urine were measured using an ELISA (LDN, Nordhorn, Germany). Noradrenaline excretion rates most likely reflect both filtration from plasma as well as renal production of the catecholamine and will be influenced by the ongoing level of glomerular filtration rate. In order to take account of the low filtration rate in the RF rats, noradrenaline excretion was calculated per unit filtrate (creatinine clearance) to attain a more direct measure of noradrenaline production. On day 8, the animals were killed with an overdose of anaesthetic (5 ml i.p. chloralose-urethane), the kidneys were excised, decapsulated, the cortices and medullae

homogenized, and the supernatent frozen at −80°C for the later estimation of TGF-β1 (RnD Systems, Minneapolis, USA).

Acute Studies

General Surgical Preparation: Rats were anaesthetized using 1 ml i.p. of chloralose-urethane (16.5 and 250 mg/ml, respectively) and maintained under anaesthesia with further doses of 0.05 ml every 30 min. Rats were prepared for renal nerve recordings or renal functional measurements from the left kidney as described previously (Zhang et al., 1997; Huang and Johns, 1998). Briefly, cannulae were placed in the right femoral artery for measurement of mean arterial pressure (MAP) and right femoral vein for administration of sustaining saline and drugs. MAP and heart rate (Henrich et al.) were recorded using a pressure transducer (Spectromed, Oxnard, CA, USA) and an amplifier (Grass Instruments, Quincy, MA, USA). The renal nerves of the left kidney were dissected free and sealed onto multi-stranded stainless steel wire recording electrodes (Medwire Mt. Vernon, NY, USA) using dental glue (Klasse4Dental, Augsburg, Germany). Following surgical preparation, the rats were allowed to recover for 2 h before the experimental protocol began.

RSNA is the efferent nerve traffic passing from the spinal cord to the kidney whereas renal afferent, or sensory, nerve activity represents that arising within the kidney and passing to the CNS. In the present study, afferent nerve activity was not recorded as it was filtered out by the low and high pass filters in the amplifier. RSNA was verified by audio recognition and was amplified using an optically isolated amplifier (Grayden Electronics, Birmingham, UK) having a gain of 100 thousand with high and low pass filters set at 100 and 1000 Hz, respectively. MAP, HR, and RSNA were recorded using LabVIEW software (National Instruments, Austin, TX, USA), for offline analysis. At the end of the experiment the rats were killed with an overdose of anaesthetic and 20 min later, background RSNA was determined and this value taken from all other RSNA measurements. RSNA values for all rats were normalized to 100% at baseline levels.

In the acute studies, where left RSNA was recorded, bilateral renal denervation comprised exposing the right kidney and denervating it as described above. Thereafter, the nerves of the left kidney were mechanically occluded between the electrode and the kidney, just before application of the dental glue, to allow recording of RSNA only.

Low Pressure Baroreceptor Challenge: This comprised an acute saline volume expansion where an i.v. infusion of saline was administered at a rate of 0.25% of body weight per minute for 30 min. The MAP, HR, and RSNA responses to volume expansion were recorded continuously and averaged over 5 min periods for the duration of the 30 min protocol.

High Pressure Baroreceptor Challenge: High pressure baroreflex regulation of RSNA was evaluated using i.v. doses of phenylephrine and nitroprusside (10 μg in 0.2 ml of saline each) to increase and decrease MAP respectively, by approximately 50–60 mmHg. Each drug was infused at a rate of 0.05 ml per 10 s over a 40 s period. The order in which each drug was infused first was randomized throughout each experiment. A 30 min recovery period was allowed after the infusion of each drug, to enable RSNA to return to baseline value.

Baroreflex gain curves were generated by plotting the relationship between RSNA and MAP from offline-stored data. The voltage level of RSNA recorded in each animal is very much dependent on technical conditions, anatomical display, ease of dissecton and fatty tissue surrounding the nerve bundle and this creates large variability in the signal recorded. In order to decrease this variability, RSNA was normalized by taking the basal level recorded immediately prior to the administration of the vasopressor or vasodepressor agent as 100%. The resultant sigmoidal relationship was analyzed by means of the following 4 parameter logistic regression equation (Kent et al., 1972), using the software MATLAB (The Mathworks Inc, Cambridge, UK):

$$RSNA/HR = A4 + A1\left\{1 + \exp\left[A2 * (MAP - A3)\right]\right\}$$

where, A1 is the response range of HR and RSNA, A2 is the slope of the curve, A3 is the pressure at the midrange of the curve and A4 is the minimum response of HR and RSNA. A1–A4 values were determined for each rat and a mean value for A1–A4 calculated for each group.

The gain of the baroreflex control of HR and RSNA was calculated from the first derivative of the logistic equations as follows:

$$Gain = -A1.A2.exp\left(A2\left(MAP - A3\right)\right)/(1 + exp\left(A2\left(MAP - A3\right)\right))^2$$

The maximum slope or gain (Gmax) was calculated at the midpoint (A3) from the 1st derivative of the logistic function:

$$G_{max} = -A1 * A2/4$$

Renal Functional Protocol: Rats were anaesthetized and prepared as described above but following exposure of the left kidney, the ureter was cannulated for urine collection.

Following surgical preparation, the rat was allowed to recover for 2 h before the experimental protocol began. The protocol (**Figure 1**) involved 10 clearance periods (C): two 20 min clearances to determine baseline values, followed by six 5 min collections during the saline volume expansion and then a further two 20 min clearances commencing 30 min after the end of the saline infusion. Plasma samples were collected into heparinized syringes from the arterial line at the four time points (P1–P4). The protocol of urine collections and plasma sampling are shown in **Figure 1**.

The metabolic study, acute RSNA and renal functional studies comprised the following groups of animals:

Control rats ($n = 12$) received a saline i.p. (6 ml/kg body weight)
RF rats ($n = 12$) were given 5 mg/kg of cisplatin i.p.
Bilaterally renally denervated (DNX) rats, control ($n = 8$)
Bilaterally renally denervated (DNX) RF rats ($n = 8$).

Statistics

All statistical analyses were performed using Graphpad Prism Version 6.0c. Results are presented as means ± standard error of the mean (SEM). For all comparisons, statistical significance

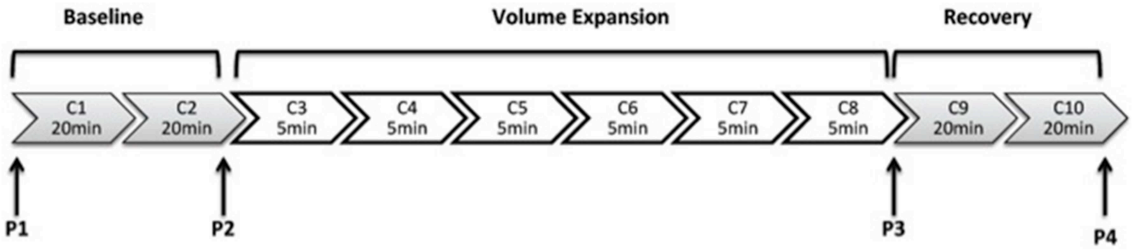


FIGURE 1 | This illustrates the protocol for urine collections for clearance (C) measurements. C1, C2, C9, and C10 are of 20 min duration and C3–C8 are of 5 min duration.

was taken at $P < 0.05$. When multiple groups were compared, a Two-Way analysis of variance (ANOVA) with Bonferonni's corrections for multiple comparisons were used, followed by Tukey's *post hoc* test. Paired and unpaired, two-tailed student's *t*-tests were employed when comparing two groups.

Results

Metabolic Study

Table 1 shows that in the control rats, the fractional excretion of sodium (FENa), noradrenaline excretion and urine flow rate did not change from day 1 to 8. By contrast, following cisplatin administration in the RF rats, FENa and noradrenaline excretion were some three-fold higher on day 8 (both $P < 0.05$), although urine flow rate remained at the same level. Cisplatin given to the renally denervated rats (RF DNX) had no effect on FENa, noradrenaline excretion or urine flow rate at day 8 as the values were not significantly different from those recorded on day 1.

Creatinine clearance (**Figure 2**) remained at a stable unchanged value on day 8 in the control rats given vehicle on day 1. However, in the rats with an intact innervation but given cisplatin (RF) and those subject to prior renal denervation (RF DNX), there were significant reductions in creatinine clearance by day 8 of over 50% ($P < 0.001$). The day 1 value for creatinine clearance was significantly ($P < 0.001$) higher in the RF DNX compared to the control rats. There were no significant differences in absolute sodium excretion (UNaV) between any groups (**Table 1** and **Figure 2**).

TGF-β1

Renal cortical and medullary concentrations of TGF-β1 of kidneys taken from rats at day 8 were significantly ($P < 0.0001$) higher in the cisplatin treated than in control rats (**Figure 3**).

Noradrenaline

Figure 4 shows that noradrenaline excretion rates did not change significantly in control rats from day 1 to 8. However, noradrenaline excretion increased significantly ($P < 0.0001$) from baseline levels at day 1 by approximately four fold by day 8 in the RF group of rats, whereas in the RF DNX group, noradrenaline excretion at day 8 was not significantly different from that measured at day 1.

TABLE 1 | Metabolic data in all experimental groups of renal failure (RF), with (DNX) or without (INN) renal denervation, and control rats.

	Group	Day 1	Day 8
Body Weight (g)	Control INN	272 ± 8	286 ± 7
	RF INN	268 ± 6	248 ± 12
	RF DNX	271 ± 5	281 ± 7*#
UNaV ($\mu Mh^{-1}kg^{-1}$)	Control INN	157 ± 5	164 ± 7
	RF INN	157 ± 4	154 ± 6
	RF DNX	155 ± 5	159 ± 5
FE_{Na}(%)	Control INN	0.9 ± 0.11	0.8 ± 0.10
	RF INN	0.7 ± 0.02#	2.5 ± 0.36*#
	RF DNX	1.12 ± 0.21	4.86 ± 0.22
NER ($ngmL^{-1}h^{-1}$)	Control INN	0.037 ± 0.006	0.024 ± 0.005
	RF INN	0.053 ± 0.005	0.189 ± 0.024*#
	RF DNX	0.045 ± 0.010	0.063 ± 0.013
GFR ($mLmin^{-1}kg^{-1}$)	Control INN	2.3 ± 0.2	2.0 ± 0.1
	RF INN	2.7 ± 0.3#	1.1 ± 0.2*#
	RF DNX	4.0 ± 0.2*	1.4 ± 0.5
UV ($mLh^{-1}kg^{-1}$)	Control INN	1.0 ± 0.1	0.5 ± 0.1
	RF INN	1.6 ± 0.3	1.8 ± 0.5*
	RF DNX	0.5 ± 0.1	0.6 ± 0.2

*Statistical analysis was performed using Two-Way ANOVA followed by Tukey's post hoc test. Data presented as mean SEM. (Control INN n = 12, RF and RF DNX, n = 8 per group). UNaV, absolute sodium excretion; FE_{Na}, fractional excretion of sodium; NER, noradrenaline excretion rate; GFR, glomerular filtration rate (creatinine clearance); UV, urine flow rate. *P < 0.05 vs. control INN, #P < 0.05 vs. RF DNX.*

Acute Studies

High Pressure Baroreflex challenge: It can be seen from **Table 2** that baseline values of HR and MAP were not different in all groups of rats. Basal integrated RSNA was significantly ($P < 0.005$) higher in the RF group when compared with all other groups. In the RF animals, both the range (A1) and the slope (A2) of the gain curve were significantly lower and mid-point blood pressure (A3) higher ($P < 0.05$, <0.01, and <0.05, respectively) compared to those of the control rats although and the minimum point to which RSNA could be driven (A4) were no different. Renal denervation of the control rats had little effect on the

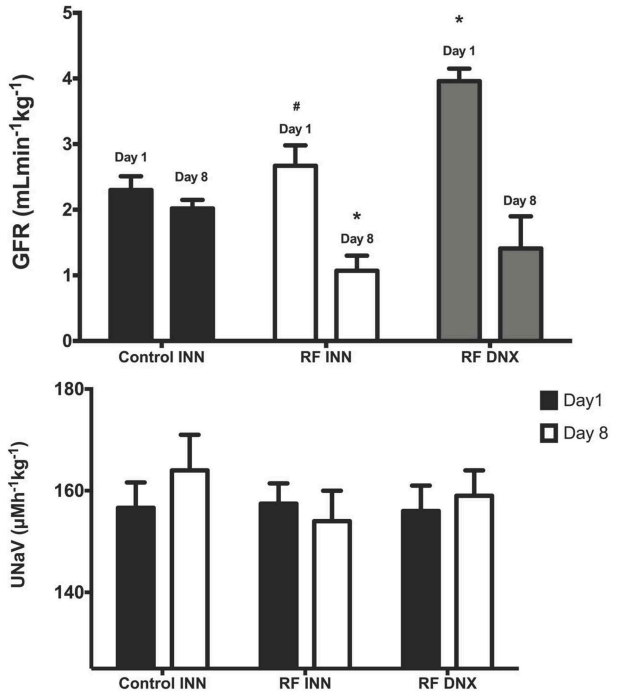


FIGURE 2 | Creatinine clearance (glomerular filtration rate, GFR), upper graph (A) and sodium excretion (Lower graph, B) for innervated (INN) control, renal failure (RF INN) and renally denervated RF (RF DNX) rats on day 1 and 8 of the metabolic study. Statistical analysis was performed using Two-Way ANOVA followed by Tukey's *post-hoc* test. Data presented as mean SEM. ($n = 12$ for all groups). *$P < 0.05$ vs. Control INN; #$P < 0.05$ vs. RF DNX. *P*-value for interaction <0.005.

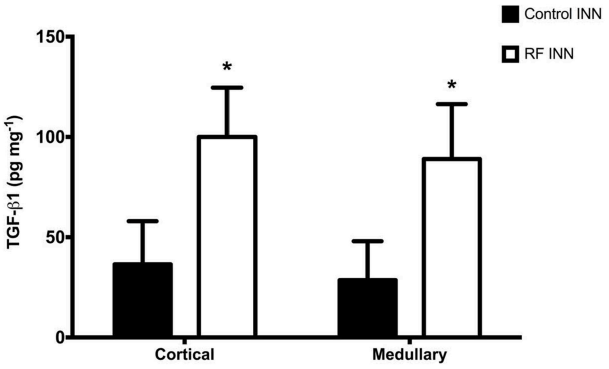


FIGURE 3 | This presents the renal cortical and medullary concentrations of TGF-β1 in control and RF groups of rats. *$P < 0.001$ vs. Control INN.

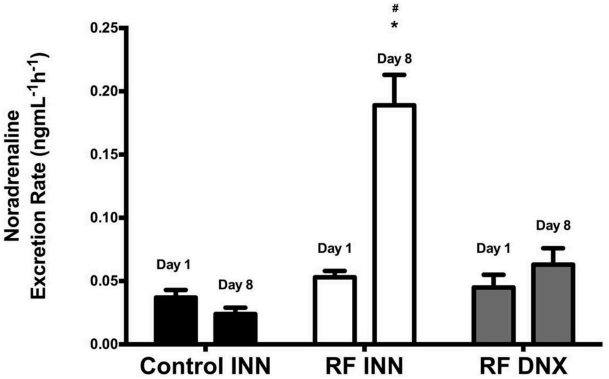


FIGURE 4 | This provides the fractional noradrenaline excretion rates (ng/mlGFR/h) for innervated control, innervated cisplatin treated (RF INN) and renally denervated renal failure (RF DNX) rats. Statistical analysis was performed using Two-Way ANOVA followed by Tukey's *post hoc* test. Data presented as mean SEM. ($n = 12$ for all groups). *$P < 0.05$ vs. Control INN #$P < 0.05$ vs. RF DNX. *P*-value for interaction <0.0001.

values of A1, A2, or A3, although A4 was reduced. However, following renal denervation in the RF rats the values of A1, A2, A3, and A4 could not be distinguished statistically from those of the control DNX rats. These findings are illustrated as full baroreceptor gain curves in **Figure 5**. **Figure 6** presents the maximal gain of the RSNA baroreflex curve for all groups and it was significantly ($P < 0.05$) lower in the RF compared to the control rats. Furthermore, renal denervation of the RF rats (RF DNX) resulted in maximal gain values for RSNA which were no different from either the control or control DNX groups of rats.

Low pressure baroreflex challenge: The infusion of the acute saline load (**Figure 7**) significantly decreased RSNA by approximately 50% after 30 min in both the intact control rats and those subjected to renal denervation (both $P < 0.05$). By contrast, in the RF rats, the acute saline load did not change RSNA, which was a response significantly ($P < 0.002$) different from that obtained in the control rats. However, after prior renal denervation of the RF rats, the 30 min of saline infusion decreased RSNA to the same degree as that obtained in the control and control DNX groups of rats, and to a significantly ($P < 0.001$) greater degree than that obtained in the intact RF group (**Figure 7**).

The excretory responses of all groups of rats control and renal failure rats over the course of the volume expansion are presented

TABLE 2 | Baseline mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA) and baroreflex gain curve parameters.

	Control INN	RF INN	Control DNX	RF DNX
Baseline HR (beats min^{-1})	346 ± 27	347 ± 13	358 ± 18	361 ± 20
Baseline MAP (mmHg)	94 ± 7	83 ± 4	72 ± 5	78 ± 3
Baseline RSNA (mVs^{-1})	7 ± 3	29 ± 6*#	4 ± 1	2 ± 0.3
A1 (% RSNA)	98 ± 19	43 ± 15*	103 ± 8	98 ± 15
A2 (% RSNA $mmHg^{-1}$)	0.14 ± 0.01	0.07 ± 0.02*#	0.11 ± 0.03	0.14 ± 0.02
A3 (mmHg)	94 ± 17	138 ± 17*#	88 ± 8	104 ± 6
A4 (% RSNA)	68 ± 23	68 ± 9#	31 ± 2	37 ± 7

*A1, the range of the curve; A2, the slope or sensitivity of the curve; A3, the mid-point blood pressure; and A4, the minimum point to which the RSNA could be driven in all experimental groups. Renal failure (RF) and control rats, with (DNX) or without (INN) renal denervation. Statistical analysis was performed using Two-Way ANOVA followed by Tukey's post hoc test. Data presented as mean SEM. (n = 12 for control and n = 8 for RF groups). *P < 0.05 vs. control INN; *P < 0.005 vs. control #P < 0.05 vs. RF DNX.*

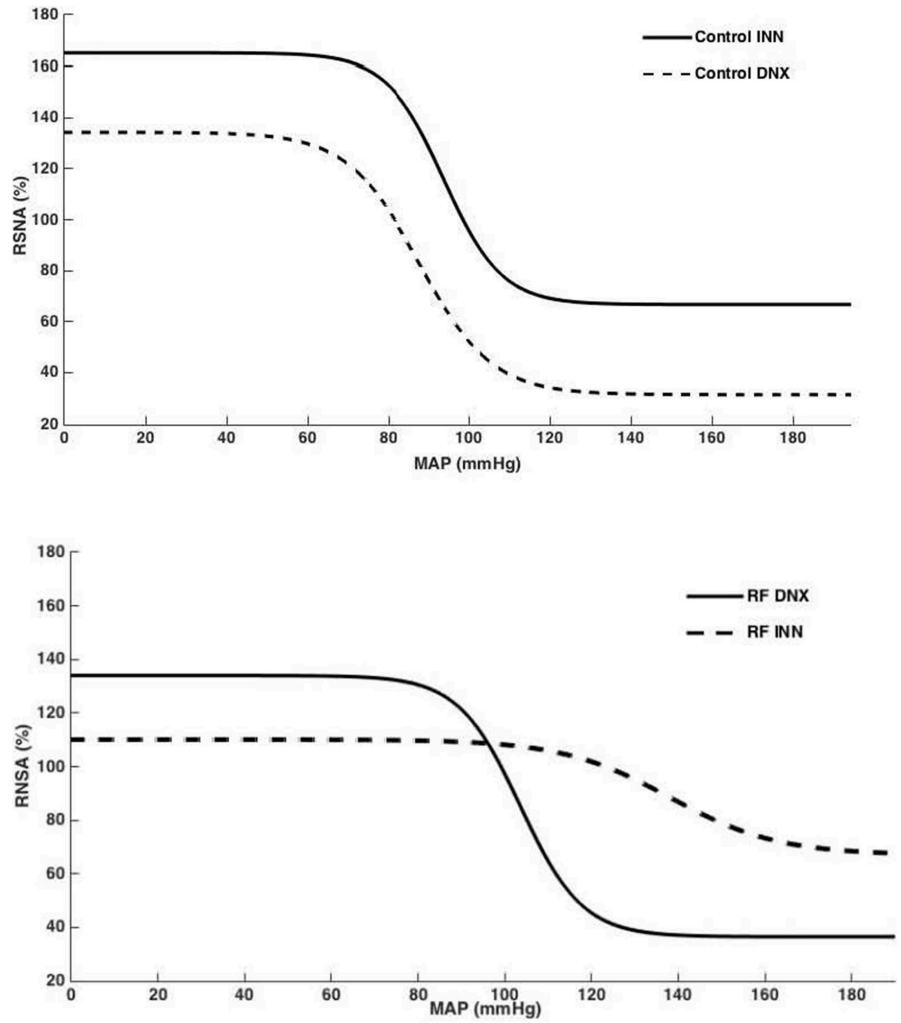


FIGURE 5 | Baroreflex curves for RSNA (%), generated from mean A1–A4 values for control innervated (control INN) and denervated (control DNX) and renal failure (RF) innervated (RF INN) and denervated (RF DNX) groups.

in **Figure 8**. The acute volume load significantly increased UV and UNaV from 81.1 ± 2.0 to 1941.3 ± 67.5 μl/min/kg and 0.22 ± 0.01 to 25.24 ± 6.66 μmol/ml/kg, respectively in the control group and to a significantly greater extent in the control rats subjected to renal denervation, from 78.3 ± 19.1 to 2665.5 ± 598.5 μl/min/kg and 0.23 ± 0.01 to 34.65 ± 5.97 μmol/ml/kg, respectively ($P < 0.01$). The magnitude of increase in UV was significantly ($P < 0.001$) larger in the control compared to the RF group. The magnitudes of increase in UV and UNaV in response to the saline volume expansion were significantly blunted in the RF rats (both $P < 0.001$) but not in the RF DNX rats in which the increases in UV and UNaV were similar to those of the control rats.

Discussion

The role of the renal sensory innervation in the regulation of autonomic and renal function remains enigmatic. There has recently been renewed interest in the contribution of the renal innervation in specific patient groups; those with resistant hypertension (Krum et al., 2014), chronic renal disease (Ott et al., 2015) and diabetes and obesity (Mahfoud et al., 2011). However, the apparent failure of the Symplicity HTN3 trial (Bhatt et al., 2014) has called into question the contribution of the renal nerves in these patho-physiological states and indicates that further study is necessary.

The present investigation set out to explore how renal failure following injury to the kidney with cisplatin impacted on the neural regulation of kidney function. There were two novel findings. Firstly, that the cisplatin induced renal injury was associated with a sympatho-excitation, as shown by the increase in fractional noradrenaline excretion, and that it was an effect dependent on an intact renal innervation. Secondly, that the deranged high and low pressure baroreflex regulation of RSNA following the renal injury with cisplatin resulted in a blunted ability to excrete a sodium load and, moreover, was dependent

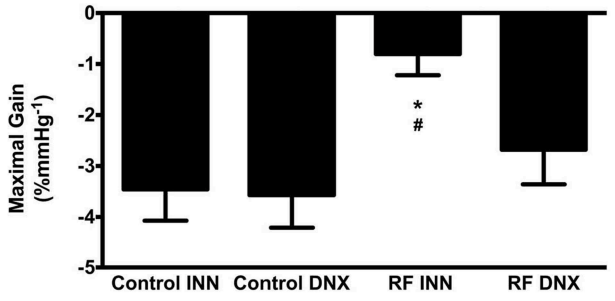


FIGURE 6 | This illustrates the maximal gain of the RSNA baroreflex curve for intact control (Control INN), control renally denervated rats (control DNX), cisplatin treated renal failure (RF) and renally denervated renal failure (RF DNX) groups of rats. Statistical analysis was performed using Two-Way ANOVA followed by Tukey's *post hoc* test. Data presented as mean SEM. ($n = 12$ for all groups). *$P < 0.05$ vs. Control INN; #$P < 0.05$ vs. RF DNX.

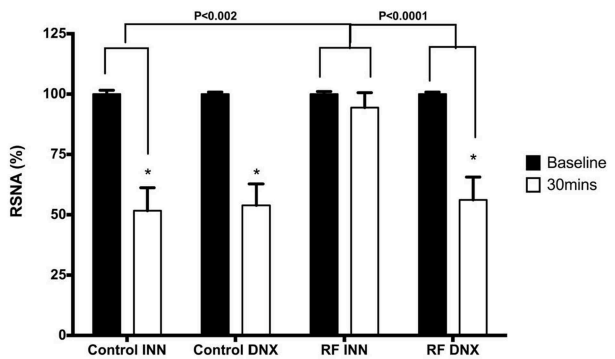


FIGURE 7 | This compares the changes in renal sympathetic nerve activity (RSNA) in response to the saline volume expansion at baseline (over the 5 min prior to the start of infusion) and over the last 5 min of the infusion (30 min) in intact control (Control INN), control renally denervated rats (control DNX), intact cisplatin treated renal failure (RF) and renally denervated renal failure (RF DNX) groups of rats. Statistical analysis was performed using Two-Way ANOVA followed by Tukey's *post hoc* test. Data presented as mean SEM. ($n = 12$ for all groups). *$P < 0.001$ against baseline. P-value for interaction <0.005.

on the renal innervation as the natriuretic and diuretic responses were normalized following removal of the renal innervation. These findings reveal that the injured and failing kidney seems to elicit an activation of the renal sensory innervation which blunts sympathetic control preventing the dynamic regulation of renal excretory function necessary for the regulation of extracellular fluid volume and hence the chronic level at which blood pressure is set.

The administration of a single dose of cisplain caused a marked reduction in creatinine clearance indicative of decreased glomerular filtration comparable to that reported earlier using this model (Khan et al., 2007). This likely reflects damage caused by cisplatin to the proximal epithelial cells, which slough off and generate hyaline casts that in turn block the tubules decreasing filtration. Indeed, injury to the kidney is supported by the observation that concentrations of TGF-β1, a biomarker of renal damage and fibrosis (Bottinger, 2007), was increased to high levels in both the renal cortex and medulla by day 8. One of the consequences of cisplatin induced renal injury will be an activation of the renin-angiotensin-aldosterone system. Any increase in angiotensin II production will contribute to the deterioration in renal function in two ways, directly by causing constriction of the renal resistance vessels, and indirectly via a facilitation of noradrenaline release from the varicosities of the postganglionic sympathetic fibers at the neuroeffector junctions. Although the cisplatin injury decreased creatinine clearance, absolute sodium excretion was maintained at levels comparable to those of the control rats, but this most likely reflects an adjustment of fluid handling along the later portions of the nephrons. The end result was that by day 8, fractional sodium excretion, was markedly elevated in the cisplatin treated rats, which indicated that there was decreased reabsorption of fluid along the nephron as a greater proportion of the filtered load was excreted. It was clear that in the renal failure group subjected to the renal denervation, following the cisplatin challenge there was a similarly elevated fractional sodium excretion by day 8 suggesting that the renal sympathetic innervation was playing a minor role in the regulation of basal fluid excretion under these conditions.

A primary aim of the investigation was to determine whether in the cisplatin model of renal injury there was an excitation of the sympathetic nervous system. To this end, the urinary excretion of noradrenaline was evaluated. Fractional noradrenaline excretion was evaluated even though this was a less reliable indicator of sympathetic nerve activity in the kidney than measurement of noradrenaline content, but it did have the advantage of allowing repeated measures in the same animals. The first novel finding was that fractional noradrenaline excretion was markedly elevated by day 8, indicative of an increase in sympathetic activity. It is likely that there are two sources of noradrenaline in the kidney, that filtered from the plasma, and that released at the neuroeffector junctions of the renal sympathetic innervation. Clearly, there is a reduced filtered load of both fluid and noradrenaline in the injured kidney but if this was taken into account, by calculating fractional noradrenaline excretion, then it became evident that that there was a large increase in noradrenaline excreted by the kidney. This conclusion was supported by the observation that in the animals subjected to the bilateral renal denervation, there was no change in fractional noradrenaline excretion on day 8, compared with baseline, following the cisplatin injection even though there was a comparable reduction in glomerular filtration rate. A limitation of the present study was that a group of control rats subjected to renal denervation were not studied but there was a low probability that any major responses in renal function would have occurred over the 8 day period of study. Interestingly, the level of RSNA recorded from the multifiber nerve recordings in the acute studies was higher in the renal failure model compared with the control rats. However, such a direct comparison of this nature is not valid because there are technical challenges which impact on the ability to get consistent nerve recordings between animals because of differing anatomical displays, the ability to clear fatty tissue from the nerve bundle and the number of nerve

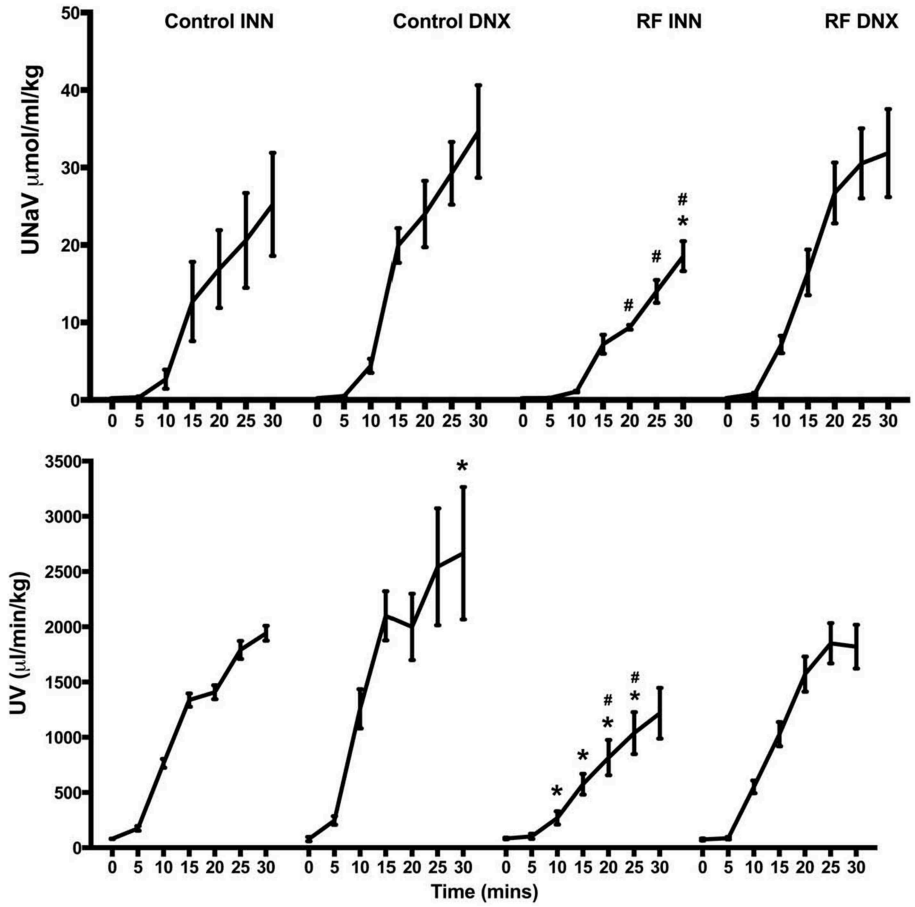


FIGURE 8 | This presents the values of urine flow (UV) and sodium excretion (UNaV) for each of the 5 min periods during the 30 min of saline volume expansion in control, control renally denervated rats (control DNX), cisplatin treated renal failure (RF) and renally denervated renal failure (RF DNX) groups of rats. Statistical analysis was performed using Two-Way ANOVA followed by Tukey's *post hoc* test. Data presented as mean SEM. ($n = 12$ for all groups). *$P < 0.01$ vs. control #$P < 0.05$ vs. RF DNX. P-value for interaction is <0.001.

bundles that can be placed on the electrodes. Together, these findings imply that there is a sympatho-excitation following the cisplatin induced renal failure but perhaps more importantly, they suggest that it is the kidney which is the source of the sensory information passing into the central nervous system. Moreover, these observations closely reflect the reports in chronic renal disease in man where an elevated level of sympathetic activity has been reported (Converse et al., 1992; Hausberg et al., 2002).

The second major objective of the investigation was to determine how the cisplatin induced renal injury disrupted the baroreflex regulation of RSNA and renal nerve dependent excretory function. It was evident that there were marked alterations in the baroreflex gain curves in the renal failure rats. Perhaps surprising was the elevation in mid-point blood pressure (A3) in renal failure at a time when basal blood pressure was similar to that of the control rats. The reasons are unclear but one possibility is that basal blood pressure is influenced by the level of anaesthesia whereas the baroreflex gives a better reflection of the overall regulatory mechanisms that determine the level at which blood pressure is set. The maximal gain of the high pressure baroreflex was very much depressed in the renal failure rats demonstrating that autonomic control was blunted. Importantly, bilateral renal denervation restored the maximal gain of the baroreflex to normal values indicating that a neural signal was originating from the kidneys under these circumstances. These findings support an earlier report in renal failure in rats (Khan et al., 2014) evaluating slightly different characteristics of the baroreflex curves but nevertheless also demonstrate major dysfunction in baroreflex regulation in renal failure. Nonetheless, injury to the kidney elicits an inappropriate neural signal which, within the CNS, blunts normal high pressure baroreflex regulation of at least one major organ, the kidney, and could seriously impair cardiovascular homeostasis.

Challenging the cardiopulmonary reflex using an acute saline volume expansion caused a prompt renal sympatho-inhibition, which was a response absent in the renal failure animals but that could be restored by prior renal denervation. These observations support those of Khan et al. (2014) and reinforce the concept that inappropriate sensory information arising from the injured kidneys impairs the normal operation of the cardiopulmonary

reflex. The second important novel observation was that the ability to increase sodium and water excretion in response to the volume expansion was very much attenuated but could be restored if the influence of the renal nerves was removed. Two interesting points arise from this observation. Firstly, that part of the inability to excrete the saline load in the renal failure rats could be due to the increased RSNA which, via the direct action of the nerves on proximal tubular fluid reabsorption, would cause a relative fluid retention. Secondly, the restoration of the excretory responses in the renal failure rats following renal denervation was compatible with an inappropriate sensory signal arising from the injured kidneys which was both causing an elevated RSNA as well as blunting the normal renal sympatho-inhibitory response to a volume expansion.

This investigation set out to examine how injury to the kidney, induced by cisplatin, caused a derangement of the reflex regulation of RSNA and the neural regulation of kidney excretory function. There is good evidence that in experimental models and man CKD is associated with a sympatho-excitation that may be due to the intra-renal generation of inflammatory mediators (Campese and Kogosov, 1995; Campese et al., 2011; Koeners et al., 2014). It was apparent in the present study that cisplatin induced renal failure was associated with an increased noradrenaline excretion consistent with a sympatho-excitation. There was also a marked attenuation of both the high and low pressure baroreflex regulation of RSNA and in terms of function, prevented the volume expansion mediated natriuresis and diuresis. Derangement of these reflexes means that the dynamic handling of sodium and water during normal everyday activity is lost which will seriously impact on cardiovascular homeostasis. Importantly, these dysfunctions appear dependent on the renal innervation as they are normalized when the kidneys are denervated. The question arises as to how an inappropriate sensory signal is generated within the kidneys under these conditions. In this renal failure model, an inflammatory response takes place as expressed by the increase in TGF-β1 concentrations within the kidney. One significant pro-inflammatory mediator within the kidney is bradykinin which is a key mediator of increased sensory nerve activity (Kopp, 2015) and recently it has been reported that intra-renal bradykinin infusion can increase RSNA, but not if the infused kidney is denervated (Barry and Johns, 2015). It may well be that an inflammatory response induced by renal injury is responsible for the deranged neural control of the kidney as renal disease develops.

Acknowledgments

This work was funded by the Health Research Board grant number: RP/2008/53

References

Barry, E. F., and Johns, E. J. (2015). Intrarenal bradykinin elicits reno-renal reflex sympatho-excitation and renal nerve-dependent fluid retention. *Acta Physiol. (Oxf).* 213, 731–739. doi: 10.1111/apha.12420

Bhatt, D. L., Kandzari, D. E., O'neill, W. W., D'agostino, R., Flack, J. M., Katzen, B. T., et al. (2014). A controlled trial of renal denervation for resistant hypertension. *N. Engl. J. Med.* 370, 1393–1401. doi: 10.1056/NEJMoa1402670

Bottinger, E. P. (2007). TGF-beta in renal injury and disease. *Semin. Nephrol.* 27, 309–320. doi: 10.1016/j.semnephrol.2007.02.009

Campese, V. M., and Kogosov, E. (1995). Renal afferent denervation prevents hypertension in rats with chronic renal failure. *Hypertension* 25, 878–882. doi: 10.1161/01.HYP.25.4.878

Campese, V. M., Ku, E., and Park, J. (2011). Sympathetic renal innervation and resistant hypertension. *Int. J. Hypertens.* 2011:814354. doi: 10.4061/2011/814354

Converse, R. L. Jr., Jacobsen, T. N., Toto, R. D., Jost, C. M., Cosentino, F., Fouad-Tarazi, F., et al. (1992). Sympathetic overactivity in patients with chronic renal failure. *N. Engl. J. Med.* 327, 1912–1918. doi: 10.1056/NEJM199212313272704

Dibona, G. F., and Rios, L. L. (1980). Renal nerves in compensatory renal response to contralateral renal denervation. *Am. J. Physiol.* 238, F26–F30.

Ditting, T., Freisinger, W., Siegel, K., Fiedler, C., Small, L., Neuhuber, W., et al. (2012). Tonic postganglionic sympathetic inhibition induced by afferent renal nerves? *Hypertension* 59, 467–476. doi: 10.1161/HYPERTENSIONAHA.111.185538

Esler, M. (2014). Illusions of truths in the Symplicity HTN-3 trial: generic design strengths but neuroscience failings. *J. Am. Soc. Hypertens.* 8, 593–598. doi: 10.1016/j.jash.2014.06.001

Hausberg, M., Kosch, M., Harmelink, P., Barenbrock, M., Hohage, H., Kisters, K., et al. (2002). Sympathetic nerve activity in end-stage renal disease. *Circulation* 106, 1974–1979. doi: 10.1161/01.CIR.0000034043.16664.96

Henrich, W. L., Katz, F. H., Molinoff, P. B., and Schrier, R. W. (1977). Competitive effects of hypokalemia and volume depletion on plasma renin activity, aldosterone and catecholamine concentrations in hemodialysis patients. *Kidney Int.* 12, 279–284. doi: 10.1038/ki.1977.112

Huang, C., and Johns, E. J. (1998). Role of ANG II in mediating somatosensory-induced renal nerve-dependent antinatriuresis in the rat. *Am. J. Physiol.* 275, R194–R202.

Johns, E. J. (2014). The neural regulation of the kidney in hypertension and renal failure. *Exp. Physiol.* 99, 289–294. doi: 10.1113/expphysiol.2013.072686

Johns, E. J., Kopp, U. C., and Dibona, G. F. (2011). Neural control of renal function. *Compr. Physiol.* 1, 731–767. doi: 10.1002/cphy.c100043

Katholi, R. E., Hageman, G. R., Whitlow, P. L., and Woods, W. T. (1983). Hemodynamic and afferent renal nerve responses to intrarenal adenosine in the dog. *Hypertension* 5, I149–I154. doi: 10.1161/01.HYP.5.2_Pt_2.I149

Kent, B. B., Drane, J. W., Blumenstein, B., and Manning, J. W. (1972). A mathematical model to assess changes in the baroreceptor reflex. *Cardiology* 57, 295–310. doi: 10.1159/000169528

Khan, A. H., Sattar, M. A., Abdullah, N. A., and Johns, E. J. (2007). Influence of cisplatin-induced renal failure on the alpha(1)-adrenoceptor subtype causing vasoconstriction in the kidney of the rat. *Eur. J. Pharmacol.* 569, 110–118. doi: 10.1016/j.ejphar.2007.04.063

Khan, S. A., Sattar, M. A., Rathore, H. A., Abdulla, M. H., Ud Din Ahmad, F., Ahmad, A., et al. (2014). Renal denervation restores the baroreflex control of renal sympathetic nerve activity and heart rate in Wistar-Kyoto rats with cisplatin-induced renal failure. *Acta Physiol. (Oxf).* 210, 690–700. doi: 10.1111/apha.12237

Khawaja, Z., and Wilcox, C. S. (2011). Role of the kidneys in resistant hypertension. *Int. J. Hypertens.* 2011:143471. doi: 10.4061/2011/143471

Klag, M. J., Whelton, P. K., Randall, B. L., Neaton, J. D., Brancati, F. L., Ford, C. E., et al. (1996). Blood pressure and end-stage renal disease in men. *N. Engl. J. Med.* 334, 13–18. doi: 10.1056/NEJM199601043340103

Koeners, M. P., Vink, E. E., Kuijper, A., Gadellaa, N., Rosenberger, C., Mathia, S., et al. (2014). Stabilization of hypoxia inducible factor-1alpha ameliorates acute renal neurogenic hypertension. *J. Hypertens.* 32, 587–597. doi: 10.1097/HJH.0000000000000060

Kopp, U. C. (2015). Role of renal sensory nerves in physiological and pathophysiological conditions. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 308, R79–R95. doi: 10.1152/ajpregu.00351.2014

Krum, H., Schlaich, M. P., Sobotka, P. A., Bohm, M., Mahfoud, F., Rocha-Singh, K., et al. (2014). Percutaneous renal denervation in patients with treatment-resistant hypertension: final 3-year report of the Symplicity HTN-1 study. *Lancet* 383, 622–629. doi: 10.1016/S0140-6736(13)62192-3

Mahfoud, F., Schlaich, M., Kindermann, I., Ukena, C., Cremers, B., Brandt, M. C., et al. (2011). Effect of renal sympathetic denervation on glucose metabolism in patients with resistant hypertension: a pilot study. *Circulation* 123, 1940–1946. doi: 10.1161/CIRCULATIONAHA.110.991869

Ott, C., Mahfoud, F., Schmid, A., Toennes, S. W., Ewen, S., Ditting, T., et al. (2015). Renal denervation preserves renal function in patients with chronic kidney disease and resistant hypertension. *J Hypertens.* 33, 1261–1266. doi: 10.1097/HJH.0000000000000556

Salman, I. M., Ameer, O. Z., Sattar, M. A., Abdullah, N. A., Yam, M. F., Najim, H. S., et al. (2011). Characterization of renal hemodynamic and structural alterations in rat models of renal impairment: role of renal sympathoexcitation. *J. Nephrol.* 24, 68–77. doi: 10.5301/JN.2010.6

Smits, J. F., and Brody, M. J. (1984). Activation of afferent renal nerves by intrarenal bradykinin in conscious rats. *Am. J. Physiol.* 247, R1003–R1008.

Sobotka, P. A., Mahfoud, F., Schlaich, M. P., Hoppe, U. C., Bohm, M., and Krum, H. (2011). Sympatho-renal axis in chronic disease. *Clin. Res. Cardiol.* 100, 1049–1057. doi: 10.1007/s00392-011-0335-y

Zhang, T., Huang, C., and Johns, E. J. (1997). Neural regulation of kidney function by the somatosensory system in normotensive and hypertensive rats. *Am. J. Physiol.* 273, R1749–R1757.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 08 June 2016
doi: 10.3389/fphys.2016.00220

Chronic Kidney Disease As a Potential Indication for Renal Denervation

Margreet F. Sanders and Peter J. Blankestijn *

Department of Nephrology and Hypertension, University Medical Centre Utrecht, Utrecht, Netherlands

Renal denervation is being used as a blood pressure lowering therapy for patients with apparent treatment resistant hypertension. However, this population does not represent a distinct disease condition in which benefit is predictable. In fact, the wide range in effectiveness of renal denervation could be a consequence of this heterogeneous pathogenesis of hypertension. Since renal denervation aims at disrupting sympathetic nerves surrounding the renal arteries, it seems obvious to focus on patients with increased afferent and/or efferent renal sympathetic nerve activity. In this review will be argued, from both a pathophysiological and a clinical point of view, that chronic kidney disease is particularly suited to renal denervation.

Keywords: renal denervation, chronic kidney disease, hypertension, sympathetic nervous system, renin-angiotensin-aldosterone-system

OPEN ACCESS

Edited by:
Kyungjoon Lim,
Baker IDI Heart and Diabetes Institute, Australia

Reviewed by:
Jacqueline Kathleen Phillips,
Macquarie University, Australia
Yusuke Sata,
Baker IDI Heart and Diabetes Institute, Australia

***Correspondence:**
Peter J. Blankestijn
p.j.blankestijn@umcutrecht.nl

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *11 November 2015*
Accepted: *26 May 2016*
Published: *08 June 2016*

Citation:
Sanders MF and Blankestijn PJ (2016) Chronic Kidney Disease As a Potential Indication for Renal Denervation. Front. Physiol. 7:220. doi: 10.3389/fphys.2016.00220

INTRODUCTION

Renal denervation (RDN) is an invasive procedure in which a catheter is percutaneously introduced into the renal arteries. By applying radiofrequency energy against the blood vessel wall, nerve fibers surrounding the artery are damaged (Steigerwald et al., 2012). Sympathetic nerves fibers are the specific target (Schlaich et al., 2009a). The procedure is non-selective, meaning in this context that both afferent and efferent pathways are affected (Booth et al., 2015). So far, RDN is mostly applied to apparent treatment resistant hypertensive patients (aTRH). Resistant means uncontrolled blood pressure despite the use of $\geq$3 optimally dosed blood pressure lowering drugs, including a diuretic, or treatment with $\geq$4 blood pressure lowering drugs (Rossignol et al., 2015). When applying RDN, the assumption is that renal sympathetic nerves are too active and that this activity is the main contributor to hypertension in these patients. This assumption might actually not be true for all hypertensive patients. RDN studies so far have all shown an exceptionally large range in blood pressure lowering effect (Bhatt et al., 2014; Azizi et al., 2015). Especially the first sham-controlled trial, Symplicity HTN-3, did not show superiority of RDN to a sham procedure in lowering blood pressure and raised many questions (Bhatt et al., 2014). Secondary analyses and subsequent studies indicated that possibly in many cases the denervation was incomplete, because of too few or inappropriate location of ablation points (Kandzari et al., 2015). There was also uncertainty about medication change during the studies. These and possibly other unidentified factors may have substantially affected the outcome of the study. Furthermore, there is increasing debate on which patient categories should be offered this therapy. The major disadvantage of the aTRH patient as candidate for RDN is that this type of patients does not represent a distinct disease condition. In fact the opposite is true, with RDN being applied to very mixed groups of patients (Verloop et al., 2013; Persu et al., 2014a). So, failure to prove RDN efficacy might partly be explained by incomplete

nerve ablation and partly by inaccurate patient selection. Therefore, we believe that RDN research should be focused on more distinct patient groups, in whom it is theoretically likely that they suffer from (highly) activated renal sympathetic nerves. In this review it will be argued that hypertensive patients with chronic kidney disease (CKD) could be such a target population, and indeed there is now evidence available to suggest that CKD patients may benefit from RDN (Hering et al., 2012; Kiuchi et al., 2013; Schlaich et al., 2013; Ott et al., 2015).

RENAL DENERVATION IN PATIENTS WITH CKD AND HYPERTENSION

RDN is performed from within the renal arteries and aims at disrupting renal sympathetic nerve traffic. It is well known that (renal) sympathetic nerve activity is increased in CKD patients and that the prognostic consequences of this may be significant (Zoccali et al., 2002). Therefore it seems logical to investigate CKD as a specific indication for RDN. The arguments to support this will be discussed from both a clinical and a pathophysiological stance.

Unmet Need in CKD from a Clinical Point of View

Hypertension is highly prevalent in CKD patients and is both a cause and a consequence of chronic kidney failure (defined as kidney damage and/or impaired kidney function for at least 3 months, with health implications) (Rossignol et al., 2015). More than 20% of individuals with CKD suffer from hypertension, increasing to over 80% in patients with stage 4 kidney disease (Mahmoodi et al., 2012). In a recent meta-analysis the additional mortality and end-stage kidney disease (ESKD) risk of hypertension in CKD patients was investigated. They showed that the risk of (cardiovascular) mortality and ESKD was not much different in hypertensive CKD patients compared to CKD patients without hypertension (Mahmoodi et al., 2012). The hazard ratio remained the same after a sensitivity analysis in which the definition "hypertension" also included blood pressure lowering drug use. This suggests that the risk of cardiovascular mortality and ESKD is partially blood pressure independent. Several other studies have shown that in CKD patients with inadequately controlled blood pressure, outcome is particularly poor (de Nicola et al., 2011; Daugherty et al., 2012; de Beus et al., 2015; Thomas et al., 2016). Prevalence of aTRH in hypertensive CKD patients ranges from 23 to 42%, increasing with severity of kidney disease (Muntner et al., 2010; Mahmoodi et al., 2012; Tanner et al., 2013; de Beus et al., 2015). Such patients have a poorer prognosis despite extensive pharmacological treatment. Both the degree of albuminuria and reduction of GFR are associated with outcome and with the presence of aTRH (Sarnak et al., 2003; Foster et al., 2007; Tanner et al., 2013). Although often accompanied by comorbidities, such as diabetes and hypertension, CKD as such is an independent risk factor for cardiovascular disease and mortality (Mahmoodi et al., 2012; Matsushita et al., 2015). Close interaction between the heart and kidney is often called cardio-renal syndrome. Secondary cardiac involvement in CKD has been defined as chronic renocardiac syndrome type 4 (Ronco et al., 2008). Across all stages of reduced GFR, primary CKD can lead to decreased cardiac function, cardiac hypertrophy and to higher risk of cardiovascular events (Ronco et al., 2008; Hatamizadeh et al., 2013). Furthermore, as is addressed in the next paragraph, increased sympathetic nerve activity is also an independent predictor of morbidity and mortality in patients with ESKD (Zoccali et al., 2002). It seems clear that given the fact that aTRH is so common in CKD and related to poor outcome, there is a clinically relevant yet unmet need to improve the quality of treatment. This "call for action" was emphasized in a recent review (Rossignol et al., 2015). In the next section will be discussed how RDN might be an effective treatment for these high risk patients.

Rationale from a Pathophysiological Point of View

The prominent role of the sympathetic nervous system (SNS) in CKD and hypertension has extensively been discussed by us and others (Schlaich et al., 2009b; Vink and Blankestijn, 2012; Grassi et al., 2015; Sata and Schlaich, 2016). We will briefly summarize this. In many studies sympathetic activity is measured by quantifying muscle sympathetic nerve activity (MSNA), which is the centrally originated sympathetic outflow toward the resistance vasculature. Organ specific sympathetic activity can be assessed by regional spillover techniques, including that to the kidneys (Grassi et al., 2015). Regardless of the underlying cause of disease, sympathetic nerve activity is increased in most patients with CKD (Blankestijn, 2004). Even in early stages of the disease, this overactivity is already present. It increases in parallel with the progression of kidney function impairment (Grassi et al., 2011). Importantly, markers of sympathetic activity, such as MSNA, noradrenaline and neuropeptide Y are on average elevated in CKD patients, but show considerable variation (Zoccali et al., 2002, 2003; Blankestijn, 2004). In 1992, Converse et al. compared sympathetic activity of haemodialysis patients with normal subjects and also with haemodialysis patients who underwent bilateral nephrectomy (Converse et al., 1992). MSNA appeared to be increased in haemodialysis patients and hypertensive patients with chronic kidney failure who are not yet on dialysis (Converse et al., 1992; Ligtenberg et al., 1999; Klein et al., 2003; Neumann et al., 2007). After bilateral nephrectomy in haemodialysis patients, MSNA decreased to normal levels, whereas no change in MSNA was observed after unilateral donor nephrectomy (Converse et al., 1992; Klein et al., 2003). So, removing a healthy kidney does not affect MSNA (despite GFR reduction), whereas removing diseased kidneys will lower MSNA. This finding suggests that the sympathetic overactivity is not explained by a reduced kidney function *per se* or by the uremic state, but that it is generated within the diseased kidneys (Hausberg et al., 2002; Klein et al., 2003). There is much evidence that kidney ischemia is the primary cause for sympathetic nerve activation. Inducing ischemia by acute renal artery stenosis in conscious rats results

in both neurogenic and humoral responses (activation of the renin-angiotensin-aldosterone system, RAAS) and consequently in hypertension (Navar et al., 1998). In humans, MSNA was significantly reduced after angioplasty of renal artery stenosis (Miyajima et al., 1991). In hypertensive patients with polycystic kidney disease, a condition characterized by regional hypoxia, sympathetic nerve activity is increased, although kidney function was not impaired (Klein et al., 2001; Bernhardt et al., 2007). Sympathetic nerve stimulation from the brain to the kidney and vice versa seems to be a vicious cycle: interruption of the renal afferent nerves by dorsal rhizotomy (a definitive method for afferent RDN) prevented hypertension, secretion of noradrenaline from the posterior hypothalamic nuclei and prevented the progression of renal disease in rats (Campese et al., 1995). As earlier explained, catheter-based RDN is non-selective and thus targets both afferent and efferent renal sympathetic pathways (Booth et al., 2015; Sata and Schlaich, 2016).

INTERACTION BETWEEN SYMPATHETIC NERVES AND RENIN-ANGIOTENSIN-ALDOSTERONE-SYSTEM

As has long been recognized, the SNS and the RAAS are often simultaneously upregulated. For instance, after angioplasty in patients with renal artery stenosis, activity of both SNS and RAAS decrease. This is also illustrated by Klein et al: in patients with chronic kidney failure, both MSNA and the level of plasma renin activity were higher compared to controls and reacted parallel to each other along with changes in extracellular volume status (Klein et al., 2003). There is strong evidence that these systems also interact in their contribution to hypertension and progression of kidney failure. In the renal ablation rat model (renal mass reduction), kidney function can be preserved by ACE inhibition (ACEi), angiotensin-II receptor blocker (ARB), or by alpha- or beta blockade (Joles and Koomans, 2004). Treating hypertensive, chronic kidney failure patients with ACEi or ARB, lowers blood pressure and MSNA (Ligtenberg et al., 1999; Klein et al., 2003). Furthermore, intravenous administration of angiotensin-II to healthy individuals results in an increase in MSNA, independently of baroreceptor reflexes (Matsukawa et al., 1991). It is thought that the stimulated RAAS (by angiotensin-II) leads to hypertension in several different ways: directly by arteriolar constriction, via baroreceptor reflexes, via sympathetic nerve terminals and ganglia, via the central nervous system and via the kidneys by influencing salt and water handling (Reid, 1992). The effects on the SNS seem to play a significant role: RDN in rats attenuates hypertension that is caused by chronic angiotensin-II infusion (Hendel and Collister, 2006). Similar, Eriguchi et al. found that in rats with cardiorenal syndrome, induced by chronic L-NAME administration (causing NO depletion), bilateral RDN decreased local RAAS activity (Eriguchi et al., 2015). It is clear that interaction between the two systems is bidirectional. This could be due to bidirectional cause-effect relations, a common origin, or both.

LOWERING SYMPATHETIC NERVE ACTIVITY

As stated earlier, MSNA represents the sympathetic outflow toward the resistance vasculature. It is thought to be involved in the pathogenesis of hypertension. Indeed, in an earlier study we found a positive relationship between MSNA levels and blood pressure (Siddiqi et al., 2009). In order to appreciate the effect of RDN on the SNS, it is important to know to what extent the currently often used drugs lower sympathetic activity in disease conditions characterized by sympathetic hyperactivity. It seems logical to aim at normalization. Insufficient reduction, i.e. not normalization, could suggest an unmet need. Basically, there are at least two types of sympathetic activity quantified by MSNA: a control level and an overactivated state. The control activity is generated by the central nervous system, influenced by baroreceptors and is present in every individual, even in bilaterally nephrectomized subjects (Blankestijn and Ritz, 2011). As explained, overactivity can be generated in the diseased kidney. In patients with sympathetic overactivity, the aim of therapy is to reduce this activity, back to levels comparable to normal subjects. We were the first to show that an ACE-inhibitor reduces MSNA in CKD patients (Ligtenberg et al., 1999; Neumann et al., 2007). However, MSNA did not normalize during treatment with normal dosages of ACEi or ARB (**Figure 1**) (Klein et al., 2003). Insufficient suppression has been confirmed in multiple clinical trials studying hypertensive CKD patients (Neumann et al., 2004, 2007; Siddiqi et al., 2011). When moxonidine (a centrally acting sympatholytic agent) was added to the treatment, both blood pressure and MSNA further decreased (Neumann et al., 2004). Monotherapy of certain oral beta-blocking agents leads to a reduction in MSNA in hypertensive and in chronic heart failure patients (Wallin et al., 1984; de Matos et al., 2004). However, like the MSNA

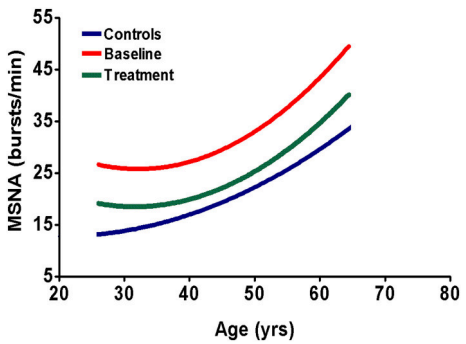


FIGURE 1 | Schematic representation of sympathetic nerve activity in CKD patients and normal subjects. The blue line represents muscle sympathetic nerve activity (MSNA) in healthy subjects, the red line represents MSNA in untreated CKD patients and the green line represents MSNA in CKD patients, when treated chronically with a RAAS-inhibitor (Ligtenberg et al., 1999; Klein et al., 2003; Neumann et al., 2004, 2007; Siddiqi et al., 2011). Sympathetic activity increases with age, irrespective of treatment. The figure shows that chronic treatment with RAAS-inhibitors does not result in full normalization of MSNA. This indicates the need for additional sympatholytic therapy.

lowering effect of RAAS inhibitors, sympathetic activity does not reach control levels: MSNA can be further decreased for example by regular exercise (Fraga et al., 2007). Carvedilol (a beta-blocker) added to standard therapy (including at least one RAAS inhibitor) in hemodialysis patients with chronic heart failure reduced morbidity and mortality, which could be due to sympatholytic actions (Azevedo et al., 2001; Cice et al., 2003). One could argue that there might be a dose-effect relation: increasing the dosage, to double or three times the normal dosage of RAAS inhibition or beta-blocking agents, would possibly further reduce MSNA. There is not much known about the dose-related sympathetic lowering effects of drugs. It is also unclear whether MSNA can completely normalize when only targeting the SNS, pharmacologically or otherwise. Since components of the RAAS and SNS are often simultaneously released, it seems reasonable to believe that a combination therapy of RAAS- and beta-blockade is essential when both systems are expected to be overactivated. Changes in activity of SNS after RDN, e.g. measured by MSNA, have not been consistently shown (Hart et al., 2013; Hering et al., 2013; Vink et al., 2014a). Evidence of parallel activation of RAAS and SNS indicate that variables of RAAS might also be indicative of an RDN effect, a hypothesis that is already supported by some early results by our group (Vink et al., 2014b). To conclude, in view of the pathophysiology, there is rationale to target both RAAS and SNS in order to adequately lower sympathetic overactivity. It is worth addressing the hypothesis that the combination of RAAS inhibitors and RDN is more effective than either intervention alone.

EFFECTS OF RDN IN CKD PATIENTS AND IN EXPERIMENTAL STUDIES

Is there any information on the effects of RDN in CKD? In RDN research, CKD patients were originally excluded from participation, mainly because of lack of safety data. Data now available seem to suggest that the procedure is safe, including in more advanced CKD, but only few studies investigated the effects of RDN specifically in a CKD population (Hering et al., 2012; Kiuchi et al., 2013, 2016; Schlaich et al., 2013; Ott et al., 2015). Longer follow-up reports describe a slight decrease in creatinine clearance, which could have many explanations, for instance, change in medication use or natural progression due to existing comorbidities (Krum et al., 2014; Tsioufis et al., 2015). In the CKD population, available studies showed a significant blood pressure reduction up to 2 years after the procedure, although most studies were limited by a small sample size, lack of control group and the uncertainty of medication adherence (Hering et al., 2012; Kiuchi et al., 2013; Schlaich et al., 2013; Ott et al., 2015; Kiuchi et al., 2016). No worsening of kidney function was reported. In fact, in some studies the opposite was found: Ott and colleagues observed beneficial effects on kidney function. In this pilot study in CKD patients with hypertension, decline of creatinine clearance slowed down after RDN (Ott et al., 2015). These potentially beneficial effects were also demonstrated in experimental studies. For example, in the study by Eriguchi et al. it became clear that both hydralazine (a direct vasodilating agent) and bilateral RDN lowered the L-NAME induced hypertension. However, the denervated rats showed protective effects, associated with local RAAS inhibition, that were blood pressure independent (Eriguchi et al., 2015). Interestingly, the results of this experimental study might be in line with the previously cited meta-analysis, showing that the morbidity and mortality risks in CKD patients are partially blood pressure independent (Siddiqi et al., 2011). So these early studies indicate that RDN in (advanced) CKD is safe. Based on limited evidence, RDN might even be renoprotective, an effect that could be blood pressure-independent.

PERSPECTIVES

There are several aspects of the topic of this review that have not yet been touched upon here and seem appropriate to briefly discuss. First of all, we need to focus on how to improve the selection for RDN *within* the CKD population. Clinical studies investigating baseline differences between responders and non-responders to RDN did not consistently produce useful pretreatment predictors (Kaiser et al., 2014; Persu et al., 2014b; Vink et al., 2014b; Kandzari et al., 2015). In a recent review, potential strategies for selecting CKD patients for RDN are described in more detail (de Beus et al., 2014). These strategies are more based on the pathophysiology and could therefore be a potential focus of further research. Another important issue is the recent knowledge of the ability of renal sympathetic nerves to re-innervate after RDN. Although long-term follow-up studies, up to three years after RDN, showed a persistent blood pressure lowering effect, preclinical research demonstrated both functionally and anatomically that re-innervation occurred within 12 months after the procedure (Mulder et al., 2013; Esler et al., 2014; Krum et al., 2014; Booth et al., 2015). It should be investigated whether this is likely to happen in humans as well. Despite of the uncertainties of the procedure, the concept of a single intervention with prolonged effect is very attractive and most likely cost effective, especially when the price of RDN devices decreases. A so far unexplored collateral idea is the potential environmental benefit of RDN. There is emerging awareness that metabolites of pharmaceutical drugs inevitably pollute the environment (Valcarcel et al., 2013). The consequences are poorly understood but potentially disastrous (Cok et al., 2011). RDN as a one-time non-pharmaceutical treatment may be more environmentally friendly. Although the rationale behind RDN in CKD patients is strong, there is reason to be cautious. As the use of contrast imaging is part of the intervention, there has been some concern about contrast induced nephropathy in CKD patients. Most nephrologists believe that this complication is rare provided that the patient is sufficiently hydrated. It is important to realize that RDN is an elective procedure. There is sufficient time to adequately prepare the patient for the procedure and the risk seems minimal. There is concern on possible detrimental effects of the procedure on renal artery anatomy. There are some reports on renal artery abnormalities, such as stenosis, observed during follow-up (Templin et al., 2013; Persu et al., 2014c). Most of these studies

lack a control group, so it is uncertain whether the stenosis was indeed caused by the intervention or a feature of the natural disease course in these high risk patients.

SUMMARY AND CONCLUSIONS

We are only at the beginning of correctly positioning RDN in the field. Up to now, RDN is mainly applied to patients with *apparent* TRH. It is now clear that this non-specific indication is not supported by knowledge on the pathophysiology of a specific condition. Nephrologists and hypertension specialists tend to think that *true* TRH is a very rare condition. In fact, aTRH seems to be a very heterogeneous population, including non-adherence, white coat hypertension, insufficiently dosed patients, high salt intake and undiagnosed secondary forms. Admittedly, aTRH is rather common, and should perhaps be redefined as "poorly controlled blood pressure for whatever reason." Therefore, we need to change our way of thinking and use a more logical, possibly blood-pressure independent, approach for patient selection, i.e., based on the knowledge of the clinic and the pathophysiology. In CKD patients there is a clear unmet need, because aTRH is so often present, persistent and associated with poor prognosis. Furthermore, there is rather convincing evidence that the kidneys are involved in the pathogenesis and that the renal sympathetic nerves play a role. The bottom line is: in which patients will the addition of sympatholytic therapy such as RDN to existing standard therapy, that in most cases includes a RAAS inhibitor, reduce sympathetic activity. CKD is one of the disease conditions in which there is already some evidence that the addition of sympatholytic therapy to RAAS inhibition may improve prognosis. Therefore, it seems appealing to focus on this disease condition.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

Renal denervation related research at the Department of Nephrology and Hypertension at the University Medical Centre Utrecht is supported by grants from The Netherlands Organization for Health Research and Development (ZonMw), the Dutch Kidney Foundation and an unrestricted grant from Medtronic (Sympathy).

REFERENCES

Azevedo, E. R., Kubo, T., Mak, S., Al-Hesayen, A., Schofield, A., Allan, R., et al. (2001). Nonselective versus selective beta-adrenergic receptor blockade in congestive heart failure: differential effects on sympathetic activity. *Circulation* 104, 2194–2199. doi: 10.1161/hc4301.098282

Azizi, M., Sapoval, M., Gosse, P., Monge, M., Bobrie, G., Delsart, P., et al. (2015). Optimum and stepped care standardised antihypertensive treatment with or without renal denervation for resistant hypertension (DENERHTN): a multicentre, open-label, randomised controlled trial. *Lancet* 385, 1957–1965. doi: 10.1016/S0140-6736(14)61942-5

Bernhardt, W. M., Wiesener, M. S., Weidemann, A., Schmitt, R., Weichert, W., Lechler, P., et al. (2007). Involvement of hypoxia-inducible transcription factors in polycystic kidney disease. *Am. J. Pathol.* 170, 830–842. doi: 10.2353/ajpath.2007.060455

Bhatt, D. L., Kandzari, D. E., O'Neill, W. W., D'Agostino, R., Flack, J. M., Katzen, B. T., et al. (2014). A controlled trial of renal denervation for resistant hypertension. *N. Engl. J. Med.* 370, 1393–1401. doi: 10.1056/NEJMoa1402670

Blankestijn, P. J. (2004). Sympathetic hyperactivity in chronic kidney disease. *Nephrol. Dial. Transplant* 19, 1354–1357. doi: 10.1093/ndt/gfh242

Blankestijn, P. J., and Ritz, E. (2011). Renal denervation: potential impact on hypertension in kidney disease? *Nephrol. Dial. Transplant* 26, 2732–2734. doi: 10.1093/ndt/gfr190

Booth, L. C., Nishi, E. E., Yao, S. T., Ramchandra, R., Lambert, G. W., Schlaich, M. P., et al. (2015). Reinnervation of renal afferent and efferent nerves at 5.5 and 11 months after catheter-based radiofrequency renal denervation in sheep. *Hypertension* 65, 393–400. doi: 10.1161/HYPERTENSIONAHA.114.04176

Campese, V. M., Kogosov, E., and Koss, M. (1995). Renal afferent denervation prevents the progression of renal disease in the renal ablation model of chronic renal failure in the rat. *Am. J. Kidney Dis.* 26, 861–865.

Cice, G., Ferrara, L., D'Andrea, A., D'Isa, S., Di, B. A., Cittadini, A., et al. (2003). Carvedilol increases two-year survivalin dialysis patients with dilated cardiomyopathy: a prospective, placebo-controlled trial. *J. Am. Coll. Cardiol.* 41, 1438–1444. doi: 10.1016/S0735-1097(03)00241-9

Cok, I., Ulutas, O. K., Okusluk, O., Durmaz, E., and Demir, N. (2011). Evaluation of DNA damage in common carp (*Cyprinus carpio* L.) by comet assay for determination of possible pollution in Lake Mogan (Ankara). *Scientific World Journal* 11, 1455–1461. doi: 10.1100/tsw.2011.140

Converse, R. L. Jr., Jacobsen, T. N., Toto, R. D., Jost, C. M., Cosentino, F., Fouad-Tarazi, F., et al. (1992). Sympathetic overactivity in patients with chronic renal failure. *N. Engl. J. Med.* 327, 1912–1918.

Daugherty, S. L., Powers, J. D., Magid, D. J., Tavel, H. M., Masoudi, F. A., Margolis, K. L., et al. (2012). Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation* 125, 1635–1642. doi: 10.1161/CIRCULATIONAHA.111.068064

de Beus, E., Bots, M. L., van Zuilen, A. D., Wetzels, J. F., and Blankestijn, P., J (2015). Prevalence of apparent therapy-resistant hypertension and its effect on outcome in patients with chronic kidney disease. *Hypertension* 66, 998–1005. doi: 10.1161/HYPERTENSIONAHA.115.05694

de Beus, E., de Jager, R. L., Joles, J. A., Grassi, G., and Blankestijn, P. J. (2014). Sympathetic activation secondary to chronic kidney disease: therapeutic target for renal denervation? *J. Hypertens.* 32, 1751–1761. doi: 10.1097/HJH.0000000000000210

de Matos, L. D., Gardenghi, G., Rondon, M. U., Soufen, H. N., Tirone, A. P., Barretto, A. C., et al. (2004). Impact of 6 months of therapy with carvedilol on muscle sympathetic nerve activity in heart failure patients. *J. Card. Fail.* 10, 496–502. doi: 10.1016/j.cardfail.2004.03.006

de Nicola, L., Borrelli, S., Gabbai, F. B., Chiodini, P., Zamboli, P., Iodice, C., et al. (2011). Burden of resistant hypertension in hypertensive patients with non-dialysis chronic kidney disease. *Kidney Blood Press. Res.* 34, 58–67. doi: 10.1159/000322923

Eriguchi, M., Tsuruya, K., Haruyama, N., Yamada, S., Tanaka, S., Suehiro, T., et al. (2015). Renal denervation has blood pressure-independent protective effects on kidney and heart in a rat model of chronic kidney disease. *Kidney Int.* 87, 116–127. doi: 10.1038/ki.2014.220

Esler, M. D., Bohm, M., Sievert, H., Rump, C. L., Schmieder, R. E., Krum, H., et al. (2014). Catheter-based renal denervation for treatment of patients with treatment-resistant hypertension: 36 month results from the SYMPLICITY HTN-2 randomized clinical trial. *Eur. Heart J.* 35, 1752–1759. doi: 10.1093/eurheartj/ehu209

Foster, M. C., Hwang, S. J., Larson, M. G., Parikh, N. I., Meigs, J. B., Vasan, R. S., et al. (2007). Cross-classification of microalbuminuria and reduced

glomerular filtration rate: associations between cardiovascular disease risk factors and clinical outcomes. *Arch. Intern. Med.* 167, 1386–1392. doi: 10.1001/archinte.167.13.1386

Fraga, R., Franco, F. G., Roveda, F., de Matos, L. N., Braga, A. M., Rondon, M. U., et al. (2007). Exercise training reduces sympathetic nerve activity in heart failure patients treated with carvedilol. *Eur. J. Heart Fail.* 9, 630–636. doi: 10.1016/j.ejheart.2007.03.003

Grassi, G., Mark, A., and Esler, M. (2015). The sympathetic nervous system alterations in human hypertension. *Circ. Res.* 116, 976–990. doi: 10.1161/CIRCRESAHA.116.303604

Grassi, G., Quarti-Trevano, F., Seravalle, G., Arenare, F., Volpe, M., Furiani, S., et al. (2011). Early sympathetic activation in the initial clinical stages of chronic renal failure. *Hypertension* 57, 846–851. doi: 10.1161/HYPERTENSIONAHA.110.164780

Hart, E. C., McBryde, F. D., Burchell, A. E., Ratcliffe, L. E., Stewart, L. Q., Baumbach, A., et al. (2013). Translational examination of changes in baroreflex function after renal denervation in hypertensive rats and humans. *Hypertension* 62, 533–541. doi: 10.1161/HYPERTENSIONAHA.113.01261

Hatamizadeh, P., Fonarow, G. C., Budoff, M. J., Darabian, S., Kovesdy, C. P., and Kalantar-Zadeh, K. (2013). Cardiorenal syndrome: pathophysiology and potential targets for clinical management. *Nat. Rev. Nephrol.* 9, 99–111. doi: 10.1038/nrneph.2012.279

Hausberg, M., Kosch, M., Harmelink, P., Barenbrock, M., Hohage, H., Kisters, K., et al. (2002). Sympathetic nerve activity in end-stage renal disease. *Circulation* 106, 1974–1979. doi: 10.1161/01.CIR.0000034043.16664.96

Hendel, M. D., and Collister, J. P. (2006). Renal denervation attenuates long-term hypertensive effects of Angiotensin ii in the rat. *Clin. Exp. Pharmacol. Physiol.* 33, 1225–1230. doi: 10.1111/j.1440-1681.2006.04514.x

Hering, D., Lambert, E. A., Marusic, P., Walton, A. S., Krum, H., Lambert, G. W., et al. (2013). Substantial reduction in single sympathetic nerve firing after renal denervation in patients with resistant hypertension. *Hypertension* 61, 457–464. doi: 10.1161/HYPERTENSIONAHA.111.00194

Hering, D., Mahfoud, F., Walton, A. S., Krum, H., Lambert, G. W., Lambert, E. A., et al. (2012). Renal denervation in moderate to severe CKD. *J. Am. Soc. Nephrol.* 23, 1250–1257. doi: 10.1681/ASN.2011111062

Joles, J. A., and Koomans, H. A. (2004). Causes and consequences of increased sympathetic activity in renal disease. *Hypertension* 43, 699–706. doi: 10.1161/01.HYP.0000121881.77212.b1

Kaiser, L., Beister, T., Wiese, A., von Wedel, J., Meincke, F., Kreidel, F., et al. (2014). Results of the ALSTER BP real-world registry on renal denervation employing the Symplicity system. *EuroIntervention* 10, 157–165. doi: 10.4244/EIJV10I1A24

Kandzari, D. E., Bhatt, D. L., Brar, S., Devireddy, C. M., Esler, M., Fahy, M., et al. (2015). Predictors of blood pressure response in the SYMPLICITY HTN-3 trial. *Eur. Heart J.* 36, 219–227. doi: 10.1093/eurheartj/ehu441

Kiuchi, M. G., Graciano, M. L., Carreira, M. A., Kiuchi, T., Chen, S., and Lugon, J. R. (2016). Long-term effects of renal sympathetic denervation on hypertensive patients with mild to moderate chronic kidney disease. *J. Clin. Hypertens. (Greenwich).* 18, 190–196. doi: 10.1111/jch.12724

Kiuchi, M. G., Maia, G. L., de Queiroz Carreira, M. A., Kiuchi, T., Chen, S., Andrea, B. R., et al. (2013). Effects of renal denervation with a standard irrigated cardiac ablation catheter on blood pressure and renal function in patients with chronic kidney disease and resistant hypertension. *Eur. Heart J.* 34, 2114–2121. doi: 10.1093/eurheartj/eht200

Klein, I. H., Ligtenberg, G., Neumann, J., Oey, P. L., Koomans, H. A., and Blankestijn, P. J. (2003). Sympathetic nerve activity is inappropriately increased in chronic renal disease. *J. Am. Soc. Nephrol.* 14, 3239–3244. doi: 10.1097/01.ASN.0000098687.01005.A5

Klein, I. H., Ligtenberg, G., Oey, P. L., Koomans, H. A., and Blankestijn, P. J. (2001). Sympathetic activity is increased in polycystic kidney disease and is associated with hypertension. *J. Am. Soc. Nephrol.* 12, 2427–2433.

Krum, H., Schlaich, M. P., Bohm, M., Mahfoud, F., Rocha-Singh, K., Katholi, R., et al. (2014). Percutaneous renal denervation in patients with treatment-resistant hypertension: final 3-year report of the Symplicity HTN-1 study. *Lancet* 383, 622–629. doi: 10.1016/S0140-6736(13)62192-3

Ligtenberg, G., Blankestijn, P. J., Oey, P. L., Klein, I. H., Jkhorst-Oei, L. T., Boomsma, F., et al. (1999). Reduction of sympathetic hyperactivity by enalapril in patients with chronic renal failure. *N. Engl. J. Med.* 340, 1321–1328.

Mahmoodi, B. K., Matsushita, K., Woodward, M., Blankestijn, P. J., Cirillo, M., Ohkubo, T., et al. (2012). Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without hypertension: a meta-analysis. *Lancet* 380, 1649–1661. doi: 10.1016/S0140-6736(12)61272-0

Matsukawa, T., Gotoh, E., Minamisawa, K., Kihara, M., Ueda, S., Shionoiri, H., et al. (1991). Effects of intravenous infusions of angiotensin II on muscle sympathetic nerve activity in humans. *Am. J. Physiol.* 261, R690–R696.

Matsushita, K., Coresh, J., Sang, Y., Chalmers, J., Fox, C., Guallar, E., et al. (2015). Estimated glomerular filtration rate and albuminuria for prediction of cardiovascular outcomes: a collaborative meta-analysis of individual participant data. *Lancet Diabetes Endocrinol.* 3, 514–525. doi: 10.1016/S2213-8587(15)00040-6

Miyajima, E., Yamada, Y., Yoshida, Y., Matsukawa, T., Shionoiri, H., Tochikubo, O., et al. (1991). Muscle sympathetic nerve activity in renovascular hypertension and primary aldosteronism. *Hypertension* 17, 1057–1062.

Mulder, J., Hokfelt, T., Knuepfer, M. M., and Kopp, U. C. (2013). Renal sensory and sympathetic nerves reinnervate the kidney in a similar time-dependent fashion after renal denervation in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 304, R675–R682. doi: 10.1152/ajpregu.00599.2012

Muntner, P., Judd, S. E., Krousel-Wood, M., McClellan, W. M., and Safford, M. M. (2010). Low medication adherence and hypertension control among adults with CKD: data from the REGARDS (Reasons for Geographic and Racial Differences in Stroke) Study. *Am. J. Kidney Dis.* 56, 447–457. doi: 10.1053/j.ajkd.2010.02.348

Navar, L. G., Zou, L., Von Thun, A., Tarng, W. C., Imig, J. D., and Mitchell, K. D. (1998). Unraveling the mystery of goldblatt hypertension. *News Physiol. Sci.* 13, 170–176.

Neumann, J., Ligtenberg, G., Klein, I. H., Boer, P., Oey, P. L., Koomans, H. A., et al. (2007). Sympathetic hyperactivity in hypertensive chronic kidney disease patients is reduced during standard treatment. *Hypertension* 49, 506–510. doi: 10.1161/01.HYP.0000256530.39695.a3

Neumann, J., Ligtenberg, G., Oey, L., Koomans, H. A., and Blankestijn, P. J. (2004). Moxonidine normalizes sympathetic hyperactivity in patients with eprosartan-treated chronic renal failure. *J. Am. Soc. Nephrol.* 15, 2902–2907. doi: 10.1097/01.ASN.0000143471.10750.8C

Ott, C., Mahfoud, F., Schmid, A., Toennes, S. W., Ewen, S., Ditting, T., et al. (2015). Renal denervation preserves renal function in patients with chronic kidney disease and resistant hypertension. *J. Hypertens.* 33, 1261–1266. doi: 10.1097/hjh.0000000000000556

Persu, A., Azizi, M., Jin, Y., Volz, S., Rosa, J., Fadl Elmula, F. E., et al. (2014b). Hyperresponders vs. nonresponder patients after renal denervation: do they differ? *J. Hypertens.* 32, 2422–2427. doi: 10.1097/HJH.0000000000000347

Persu, A., Jin, Y., Baelen, M., Vink, E., Verloop, W. L., Schmidt, B., et al. (2014a). Eligibility for renal denervation: experience at 11 European expert centers. *Hypertension* 63, 1319–1325. doi: 10.1161/HYPERTENSIONAHA.114.03194

Persu, A., Sapoval, M., Azizi, M., Monge, M., Danse, E., Hammer, F., et al. (2014c). Renal artery stenosis following renal denervation: a matter of concern. *J. Hypertens.* 32, 2101–2105. doi: 10.1097/HJH.0000000000000323

Reid, I. A. (1992). Interactions between ANG II, sympathetic nervous system, and baroreceptor reflexes in regulation of blood pressure. *Am. J. Physiol.* 262, E763–E778.

Ronco, C., Haapio, M., House, A. A., Anavekar, N., and Bellomo, R. (2008). Cardiorenal syndrome. *J. Am. Coll. Cardiol.* 52, 1527–1539. doi: 10.1016/j.jacc.2008.07.051

Rossignol, P., Massy, Z. A., Azizi, M., Bakris, G., Ritz, E., Covic, A., et al (2015). The double challenge of resistant hypertension and chronic kidney disease. *Lancet* 386, 1588–1598. doi: 10.1016/S0140-6736(15)00418-3

Sarnak, M. J., Levey, A. S., Schoolwerth, A. C., Coresh, J., Culleton, B., Hamm, L. L., et al. (2003). Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American heart association councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. *Circulation* 108, 2154–2169. doi: 10.1161/01.CIR.0000095676.90936.80

Sata, Y., and Schlaich, M. P. (2016). The potential role of catheter-based renal sympathetic denervation in chronic and end-stage kidney disease. *J. Cardiovasc. Pharmacol. Ther.* 21, 344–352. doi: 10.1177/1074248415624156

Schlaich, M. P., Bart, B., Hering, D., Walton, A., Marusic, P., Mahfoud, F., et al. (2013). Feasibility of catheter-based renal nerve ablation and effects on sympathetic nerve activity and blood pressure in patients with end-stage renal disease. *Int. J. Cardiol.* 168, 2214–2220. doi: 10.1016/j.ijcard.2013.01.218

Schlaich, M. P., Sobotka, P. A., Krum, H., Lambert, E., and Esler, M. D. (2009a). Renal sympathetic-nerve ablation for uncontrolled hypertension. *N. Engl. J. Med.* 361, 932–934. doi: 10.1056/NEJMc0904179

Schlaich, M. P., Socratous, F., Hennebry, S., Eikelis, N., Lambert, E. A., Straznicky, N., et al. (2009b). Sympathetic activation in chronic renal failure. *J. Am. Soc. Nephrol.* 20, 933–939. doi: 10.1681/ASN.2008040402

Siddiqi, L., Joles, J. A., Grassi, G., and Blankestijn, P. J. (2009). Is kidney ischemia the central mechanism in parallel activation of the renin and sympathetic system? *J. Hypertens.* 27, 1341–1349. doi: 10.1097/HJH.0b013e32832b521b

Siddiqi, L., Oey, P. L., and Blankestijn, P. J. (2011). Aliskiren reduces sympathetic nerve activity and blood pressure in chronic kidney disease patients. *Nephrol. Dial. Transplant* 26, 2930–2934. doi: 10.1093/ndt/gfq857

Steigerwald, K., Titova, A., Malle, C., Kennerknecht, E., Jilek, C., Hausleiter, J., et al. (2012). Morphological assessment of renal arteries after radiofrequency catheter-based sympathetic denervation in a porcine model. *J. Hypertens.* 30, 2230–2239. doi: 10.1097/HJH.0b013e32835821e5

Tanner, R. M., Calhoun, D. A., Bell, E. K., Bowling, C. B., Gutierrez, O. M., Irvin, M. R., et al. (2013). Prevalence of apparent treatment-resistant hypertension among individuals with CKD. *Clin. J. Am. Soc. Nephrol.* 8, 1583–1590. doi: 10.2215/CJN.00550113

Templin, C., Jaguszewski, M., Ghadri, J. R., Sudano, I., Gaehwiler, R., Hellermann, J. P., et al. (2013). Vascular lesions induced by renal nerve ablation as assessed by optical coherence tomography: pre- and post-procedural comparison with the Simplicity catheter system and the EnligHTN multi-electrode renal denervation catheter. *Eur. Heart J.* 34, 2141-8, 2148b. doi: 10.1093/eurheartj/eht141

Thomas, G., Xie, D., Chen, H. Y., Anderson, A. H., Appel, L. J., Bodana, S., et al. (2016). Prevalence and prognostic significance of apparent treatment resistant hypertension in chronic kidney disease: report from the chronic renal insufficiency cohort study. *Hypertension* 67, 387–396. doi: 10.1161/HYPERTENSIONAHA.115.06487

Tsioufis, C. P., Papademetriou, V., Dimitriadis, K. S., Kasiakogias, A., Tsiachris, D., Worthley, M. I., et al. (2015). Catheter-based renal denervation for resistant hypertension: twenty-four month results of the EnligHTN I first-in-human study using a multi-electrode ablation system. *Int. J. Cardiol.* 201, 345–350. doi: 10.1016/j.ijcard.2015.08.069

Valcarcel, Y., Alonso, S. G., Rodriguez-Gil, J. L., Castano, A., Montero, J. C., Criado-Alvarez, J. J., et al. (2013). Seasonal variation of pharmaceutically active compounds in surface (Tagus River) and tap water (Central Spain). *Environ. Sci. Pollut. Res. Int.* 20, 1396–1412. doi: 10.1007/s11356-012-1099-2

Verloop, W. L., Vink, E. E., Voskuil, M., Vonken, E. J., Rookmaaker, M. B., Bots, M. L., et al. (2013). Eligibility for percutaneous renal denervation: the importance of a systematic screening. *J. Hypertens.* 31, 1662–1668. doi: 10.1097/hjh.0b013e328362152e

Vink, E. E., and Blankestijn, P. J. (2012). Evidence and consequences of the central role of the kidneys in the pathophysiology of sympathetic hyperactivity. *Front. Physiol.* 3:29. doi: 10.3389/fphys.2012.00029

Vink, E. E., Verloop, W. L., Bost, R. B., Voskuil, M., Spiering, W., Vonken, E. J., et al. (2014b). The blood pressure-lowering effect of renal denervation is inversely related to kidney function. *J. Hypertens.* 32, 2045–2053. doi: 10.1097/HJH.0000000000000282

Vink, E. E., Verloop, W. L., Siddiqi, L., van Schelven, L. J., Liam, O. P., and Blankestijn, P. J. (2014a). The effect of percutaneous renal denervation on muscle sympathetic nerve activity in hypertensive patients. *Int. J. Cardiol.* 176, 8–12. doi: 10.1016/j.ijcard.2014.06.021

Wallin, B. G., Sundlof, G., Stromgren, E., and Aberg, H. (1984). Sympathetic outflow to muscles during treatment of hypertension with metoprolol. *Hypertension* 6, 557–562.

Zoccali, C., Mallamaci, F., Parlongo, S., Cutrupi, S., Benedetto, F. A., Tripepi, G., et al. (2002). Plasma norepinephrine predicts survival and incident cardiovascular events in patients with end-stage renal disease. *Circulation* 105, 1354–1359. doi: 10.1161/hc1102.105261

Zoccali, C., Mallamaci, F., Tripepi, G., Benedetto, F. A., Parlongo, S., Cutrupi, S., et al. (2003). Prospective study of neuropeptide y as an adverse cardiovascular risk factor in end-stage renal disease. *J. Am. Soc. Nephrol.* 14, 2611–2617. doi: 10.1097/01.ASN.0000089026.28617.33

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in PHYSIOLOGY

GENERAL COMMENTARY
published: 15 August 2014
doi: 10.3389/fphys.2014.00311

What is the true incidence of renal artery stenosis after sympathetic denervation?

Yutang Wang[1,2]*

[1] Queensland Research Centre for Peripheral Vascular Disease, School of Medicine and Dentistry, James Cook University, Townsville, QLD, Australia
[2] School of Health Sciences, Federation University Australia, Mount Helen, VIC, Australia
*Correspondence: yutangwang000@gmail.com

Edited by:
Clive May, University of Melbourne, Australia

Reviewed by:
Francesco Versaci, Cardiology Dept. Cardarelli Hospital - Campobasso - Italy, Italy

Keywords: clinical trials, renal artery stenosis, renal denervation, resistant hypertension, side effect

Renal denervation (RDN), a recently developed therapy for resistant hypertension, is generally regarded as a safe procedure (Krum et al., 2009; Esler et al., 2010; Bhatt et al., 2014). The Symplicity HTN trials reported that the rate of renal artery stenosis after RDN was low. For example, the Symplicity HTN-1 trial showed that 1 of 45 (2.2%) denervated patients developed a non-obstructive renal artery stenosis in an untreated area at 6 months after RDN (Krum et al., 2009). This low rate of renal artery stenosis after RDN was confirmed by the 6-month report of the Symplicity HTN-2 trial ($N = 106$) (Esler et al., 2010) and the recently published 6-month report of the Symplicity HTN-3 trial ($N = 535$) (Bhatt et al., 2014), which was 1.9 and 0.3%, respectively. In these Symplicity HTN trials, renal artery stenosis occurred at a low rate (0.3–2.2%) and was not reported to cause further complications.

However, some studies reported that renal artery stenosis occurred at a higher rate. For example, the EnligHTN I study (Worthley et al., 2013) reported that 2 of 46 (4.3%) patients showed progression of a pre-existing renal artery stenosis; a study from 10 European expert centers (Persu et al., 2014) reported that 3 of 109 (2.8%) patients showed progression of a non-significant (<30%) renal artery stenosis; and Versaci et al. (2014a) reported that 2 of 11 (18.2%) patients developed severe renal artery stenosis. It is worthwhile to point out that the sample size of 11 in Versaci et al.'s report is relatively small (Versaci et al., 2014a).

In 2012, Vonend et al. reported that a patient developed a 75% stenosis near the ostium of the right renal artery, which caused recurrent hypertension (Vonend et al., 2012). Subsequently, another 3 case reports reported that renal artery stenosis after RDN caused recurrent hypertension (Kaltenbach et al., 2012; Aguila et al., 2014; Pucci et al., 2014).

The causal role of RDN in promoting renal artery stenosis is currently speculative. However, given that (1) renal artery stenosis causes recurrent hypertension in denervated patients (Kaltenbach et al., 2012; Vonend et al., 2012; Aguila et al., 2014; Pucci et al., 2014) and (2) treatment of renal artery stenosis is not always safe and sometimes leads to death (Soriano-Perez et al., 2012), it is important to thoroughly investigate the effect of RDN on renal artery stenosis (Mahfoud and Kjeldsen, 2013; Wang, 2014a,b,c,d). To do this, the following three points need to be emphasized in future clinical trials on RDN:

(1) Long-term randomized trials are needed. Two major randomized trials on RDN, i.e., the Symplicity HTN-2 and HTN-3 trials (Esler et al., 2012; Kandzari et al., 2012), allowed patients in the randomized control group to receive RDN after completion of the 6-month study. This crossover design makes it difficult to investigate possible long-term side effects of RDN, e.g., promoting renal artery stenosis. Therefore, long-term randomized trials without a short-term crossover design are needed to investigate the effect of RDN on renal artery stenosis.
(2) Imaging methods monitoring renal artery stenosis need to be standardized. Symplicity HTN trials did not standardize renal artery imaging methods during follow ups. Ultrasonography, magnetic resonance angiography, and computerized tomographic angiography were used (Krum et al., 2009; Esler et al., 2010). The computerized tomographic angiography, the gold standard method to detect renal artery stenosis (Persu et al., 2012), was not the major imaging method in these trials. It is known that ultrasonography has limited visualization on renal artery stenosis because (1) the imaging is interfered by overlying adipose tissue and bowel gas (Zhang et al., 2009); and (2) the entire length of the renal artery or an accessory renal artery can be overlooked (Lao et al., 2011). Therefore, it is worthwhile to use computerized tomographic angiography as the standardized method during follow ups in future trials to investigate the effect of RDN on renal artery stenosis.
(3) It is likely that improved catheters for RDN using lower power radiofrequency over a shorter time will reduce the local tissue injury at the ablation site compared with that caused by the first generation RDN systems (Versaci et al., 2014b). However data regarding the vascular injury induced by these new devices are lacking.

ACKNOWLEDGMENT

Yutang Wang is supported by the National Health and Medical Research Council (1062671).

REFERENCES

Aguila, F. J., Mediavilla Garcia, J. D., Navarro, E. M., Vargas Hitos, J. A., and Fernandez-Torres, C. (2014). Bilateral renal artery stenosis after renal denervation. *Hypertension* 63, e126–e127. doi: 10.1161/HYPERTENSIONAHA.113.03065

Bhatt, D. L., Kandzari, D. E., O'Neill, W. W., D'Agostino, R., Flack, J. M., Katzen, B. T., et al. (2014). A controlled trial of renal denervation for resistant hypertension. *N. Engl. J. Med.* 370, 1393–1401. doi: 10.1056/NEJMoa1402670

Esler, M. D., Krum, H., Schlaich, M., Schmieder, R. E., Bohm, M., and Sobotka, P. A. (2012). Renal sympathetic denervation for treatment of drug-resistant hypertension: one-year results from the Symplicity HTN-2 randomized, controlled trial. *Circulation* 126, 2976–2982. doi: 10.1161/CIRCULATIONAHA.112.130880

Esler, M. D., Krum, H., Sobotka, P. A., Schlaich, M. P., Schmieder, R. E., and Bohm, M. (2010). Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 376, 1903–1909. doi: 10.1016/S0140-6736(10)62039-9

Kaltenbach, B., Id, D., Franke, J. C., Sievert, H., Hennersdorf, M., Maier, J., et al. (2012). Renal artery stenosis after renal sympathetic denervation. *J. Am. Coll. Cardiol.* 60, 2694–2695. doi: 10.1016/j.jacc.2012.09.027

Kandzari, D. E., Bhatt, D. L., Sobotka, P. A., O'Neill, W. W., Esler, M., Flack, J. M., et al. (2012). Catheter-based renal denervation for resistant hypertension: rationale and design of the SYMPLICITY HTN-3 Trial. *Clin. Cardiol.* 35, 528–535. doi: 10.1002/clc.22008

Krum, H., Schlaich, M., Whitbourn, R., Sobotka, P. A., Sadowski, J., Bartus, K., et al. (2009). Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 373, 1275–1281. doi: 10.1016/S0140-6736(09)60566-3

Lao, D., Parasher, P. S., Cho, K. C., and Yeghiazarians, Y. (2011). Atherosclerotic renal artery stenosis–diagnosis and treatment. *Mayo Clin. Proc.* 86, 649–657. doi: 10.4065/mcp.2011.0181.

Mahfoud, F., and Kjeldsen, S. E. (2013). Catheter-based renal denervation: a word of caution. *Eurointervention* 8, 997–998. doi: 10.4244/EIJV8I9A152

Persu, A., Jin, Y., Azizi, M., Baelen, M., Volz, S., Elvan, A., et al. (2014). Blood pressure changes after renal denervation at 10 European expert centers. *J. Hum. Hypertens.* 28, 150–156. doi: 10.1038/jhh.2013.88

Persu, A., Renkin, J., Thijs, L., and Staessen, J. A. (2012). Renal denervation: ultima ratio or standard in treatment-resistant hypertension. *Hypertension* 60, 596–606. doi: 10.1161/HYPERTENSIONAHA.112.195263

Pucci, G., Battista, F., Lazzari, L., Dominici, M., Boschetti, E., and Schillaci, G. (2014). Progression of renal artery stenosis after renal denervation. *Circ. J.* 78, 767–768. doi: 10.1253/circj.CJ-13-0997

Soriano-Perez, A. M., Baca-Morilla, Y., Galindo-De Blas, B., Bejar-Palma, M. P., Martin-Ortiz, M., and Bueno-Millan, M. P. (2012). Renal artery rupture during complicated recovery from angioplasty to treat renal stenosis. *Nefrologia* 32, 258–260. doi: 10.3265/Nefrologia.pre2011.Nov.11174

Versaci, F., Trivisonno, A., Olivieri, C., Caranci, F., Brunese, L., and Prati, F. (2014a). Late renal artery stenosis after renal denervation: is it the tip of the iceberg? *Int. J. Cardiol.* 172, e507–e508. doi: 10.1016/j.ijcard.2014.01.018

Versaci, F., Trivisonno, A., Olivieri, C., Caranci, F., Brunese, L., and Prati, F. (2014b). Vascular response after percutaneous sympathectomy: not all devices are equal. *Int. J. Cardiol.* 174, 406–407. doi: 10.1016/j.ijcard.2014.04.049

Vonend, O., Antoch, G., Rump, L. C., and Blondin, D. (2012). Secondary rise in blood pressure after renal denervation. *Lancet* 380, 778. doi: 10.1016/S0140-6736(12)61145-3

Wang, Y. (2014a). It may be not suitable to perform renal denervation in renal arteries with significant stenosis. *Int. J. Cardiol.* 174, 750. doi: 10.1016/j.ijcard.2014.04.087

Wang, Y. (2014b). Limitations in current clinical trials on renal denervation. *Int. J. Cardiol.* 174, 225. doi: 10.1016/j.ijcard.2014.04.054

Wang, Y. (2014c). Patients with renal artery stenosis may not be suitable for renal denervation. *Clin. Res. Cardiol.* 103, 585–586. doi: 10.1007/s00392-014-0700-8

Wang, Y. (2014d). Renal artery stenosis may be responsible for the gradual return of high blood pressure after renal denervation. *J. Clin. Hypertens. (Greenwich)* 16, 313. doi: 10.1111/jch.12265

Worthley, S. G., Tsioufis, C. P., Worthley, M. I., Sinhal, A., Chew, D. P., Meredith, I. T., et al. (2013). Safety and efficacy of a multi-electrode renal sympathetic denervation system in resistant hypertension: the EnligHTN I trial. *Eur. Heart J.* 34, 2132–2140. doi: 10.1093/eurheartj/eht197

Zhang, H. L., Sos, T. A., Winchester, P. A., Gao, J., and Prince, M. R. (2009). Renal artery stenosis: imaging options, pitfalls, and concerns. *Prog. Cardiovasc. Dis.* 52, 209–219. doi: 10.1016/j.pcad.2009.10.003

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 13 July 2014; paper pending published: 28 July 2014; accepted: 31 July 2014; published online: 15 August 2014.

*Citation: Wang Y (2014) What is the true incidence of renal artery stenosis after sympathetic denervation? Front. Physiol. **5**:311. doi: 10.3389/fphys.2014.00311*

This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology.

frontiers in Physiology

ORIGINAL RESEARCH
published: 13 April 2017
doi: 10.3389/fphys.2017.00215

Renal Denervation Promotes Atherosclerosis in Hypertensive Apolipoprotein E-Deficient Mice Infused with Angiotensin II

Yutang Wang[1,2], Tam N. Dinh[1,2], Alexander Nield[1], Smriti M. Krishna[2], Kate Denton[3] and Jonathan Golledge[2,4]*

[1] School of Applied and Biomedical Science, Federation University Australia, Ballarat, VIC, Australia, [2] The Vascular Biology Unit, Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University, Townsville, QLD, Australia, [3] Cardiovascular and Renal Physiology, Department of Physiology, Monash University, Clayton, VIC, Australia, [4] Department of Vascular and Endovascular Surgery, The Townsville Hospital, Townsville, QLD, Australia

Objective: To determine the effect of renal denervation (RDN) on the severity of atherosclerosis and aortic aneurysm in hypertensive mice.

Methods: Hypertension, atherosclerosis and aortic aneurysm were induced by subcutaneous infusion of angiotensin II (1 μg/kg/min) for 28 days in apolipoprotein E-deficient mice. RDN was conducted using combined surgical and local chemical denervation. The norepinephrine concentration in the kidney was measured by high-performance liquid chromatography. Blood pressure was measured by the tail-cuff method. Atherosclerosis was assessed by Sudan IV staining of the aortic arch. The aortic diameter was measured by the morphometric method. The mRNA expression of genes associated with atherosclerosis and aortic aneurysm were analyzed by quantitative PCR.

Results: RDN decreased the median norepinephrine content in the kidney by 93.4% ($n = 5$–7, $P = 0.003$) 5 days after the procedure, indicating that the RDN procedure was successful. RDN decreased systolic blood pressure in apolipoprotein E-deficient mice. Mice that had RDN had more severe aortic arch atherosclerosis (median percentage of Sudan IV positive area: 13.2% in control mice, $n = 12$, and 25.4% in mice having RDN, $n = 12$, $P = 0.028$). The severity of the atherosclerosis was negatively correlated with the renal norepinephrine content (spearman $r = -0.6557$, $P = 0.005$). RDN did not affect the size of aortic aneurysms formed or the incidence of aortic rupture in mice receiving angiotensin II. RDN significantly increased the aortic mRNA expression of matrix metalloproteinase-2 (MMP-2).

Conclusion: RDN promoted atherosclerosis in apolipoprotein E-deficient mice infused with angiotensin II associated with upregulation of MMP-2. The higher MMP-2 expression could be the results of the greater amount of atheroma in the RDN mice. The findings suggest further research is needed to assess potentially deleterious effects of RDN in patients.

Keywords: angiotensin II, aortic aneurysm, atherosclerosis, blood pressure, matrix metalloproteinase-2, renal denervation

OPEN ACCESS

Edited by:
Brian G. Drew,
Baker IDI Heart and Diabetes Institute, Australia

Reviewed by:
Olaf Grisk,
University of Greifswald, Germany
Mark Ian Munro Noble,
University of Aberdeen, UK

****Correspondence:***
Yutang Wang
yutang.wang@federation.edu.au

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *22 December 2016*
Accepted: *24 March 2017*
Published: *13 April 2017*

Citation:
Wang Y, Dinh TN, Nield A, Krishna SM, Denton K and Golledge J (2017) Renal Denervation Promotes Atherosclerosis in Hypertensive Apolipoprotein E-Deficient Mice Infused with Angiotensin II. Front. Physiol. 8:215. doi: 10.3389/fphys.2017.00215

INTRODUCTION

Renal denervation (RDN) is used in clinical practice to lower blood pressure in treatment-resistant hypertension (Krum et al., 2009; Esler et al., 2010; Worthley et al., 2013; Bhatt et al., 2014; Papademetriou et al., 2014) by inhibiting the sympathetic outflow from the brain (Schlaich et al., 2009). RDN is generally regarded as a safe procedure (Krum et al., 2009; Esler et al., 2010; Bhatt et al., 2014). However, some studies have suggested that RDN may cause renal artery stenosis in 5–18% of patients (Kaltenbach et al., 2013; Worthley et al., 2013; Papademetriou et al., 2014; Versaci et al., 2014).

A number of experimental studies suggest that both chemical and surgical sympathetic denervation promote atherosclerosis (Murphy et al., 1957; Snyder and Campbell, 1958; Kacem et al., 1997, 2006; Kacem and Sercombe, 2008; Hachani et al., 2010). A recent study reported that RDN inhibited atherosclerosis formation in normotensive apolipoprotein E-deficient ($ApoE^{-/-}$) mice fed a high-fat diet (Wang et al., 2015). The effects of RDN on atherosclerosis in mouse models that have hypertension has not however been investigated. This is important since RDN is performed in hypertensive patients. This study was designed to investigate whether RDN affects atherosclerosis severity in hypertensive $ApoE^{-/-}$ mice infused with angiotensin II. As angiotensin II infusion also induces aortic aneurysm we also assessed the effect of RDN on aortic aneurysm severity.

METHODS

Animals

Male $ApoE^{-/-}$ mice (3 months old) were purchased from the Animal Resources Centre, Perth, Australia. All experiments were conducted in a temperature-controlled animal house ($21 \pm 1°C$) under a 12:12-h light-dark cycle and mice were given standard chow and water *ad libitum*. All animal protocols conformed to the Guide for the Care and use of Laboratory Animals by the United States National Institutes of Health and the Australian Code of Practice for the Care and Use of Animals for Scientific Purpose (8th Edition, 2013). Institutional ethics approval was obtained from both James Cook University and Federation University Australia.

Experimental Protocol

A preliminary study was carried out to confirm the success of the RDN procedure. Five $ApoE^{-/-}$ mice underwent sham surgery and seven mice underwent bilateral RDN. Five days later, the mice were euthanized and the right kidney was collected and norepinephrine content determined.

For the main experiment, 18 $ApoE^{-/-}$ mice underwent bilateral RDN and 20 $ApoE^{-/-}$ mice underwent sham surgery 1 day after the baseline blood pressure was measured (**Figure 1**). Five days after RDN or sham surgery, blood pressure was measured and all the mice were then subjected to angiotensin II infusion at a dose of 1 μg/kg/min for the ensuing 28 days. Blood pressure was measured at Day 14 and Day 27 after the angiotensin II infusion commenced. The mice were euthanized at Day 28 (**Figure 1**) and the right kidney was collected for norepinephrine content measurement, and the aorta was isolated for morphometric analysis. The aortic arch was then used for Sudan IV staining and the thoracic aorta used for RNA extraction.

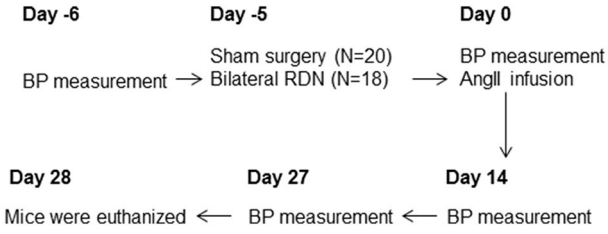


FIGURE 1 | A flowchart of the experiment. The baseline blood pressure was measured followed by RDN or sham surgery on the next day. Five days after RDN or sham surgery, blood pressure was measured and all the mice were then subjected to angiotensin II infusion. Blood pressure was then measured at Day 14 and Day 27 during the angiotensin II infusion. The mice were euthanized at Day 28. Eight mice from the sham surgery group and six mice from the RDN group died of aortic rupture during the 28-day angiotensin II infusion. AngII, angiotensin II; BP, blood pressure; RDN, renal denervation.

Renal Denervation

Bilateral RDN was carried out as previously described (O'Neill et al., 1991; Ye et al., 1997). In brief, after the renal arteries and veins were exposed, all visible nerves around the renal arteries were cut, and connective tissues passing next to and along the course of the renal arteries and veins were dissected and stripped off the adventitia under a dissecting microscope with a 4× magnification. Then the renal arteries and veins were painted with a solution of 10% phenol in 95% ethanol (O'Neill et al., 1991; Ye et al., 1997). After being washed with saline (0.9% sodium chloride), the abdominal cavity was closed. For the mice in the sham surgery group, the renal arteries were exposed as with the RDN procedure, but the renal nerves were kept intact.

Norepinephrine Measurement

The right kidney was homogenized in 1 mM ethylenediaminetetraacetic acid (EDTA) and 4 mM sodium metabisulfite. The norepinephrine content in the homogenate was measured by phase isocratic high-performance liquid chromatography (HPLC) (Wang et al., 1999) coupled with an electrochemical detector, with an Atlantis C18 column (5 μm particle size, 4.6 × 150 mm) and a mobile phase of 50 mM Na_2HPO_4, 27 μM EDTA, 0.6 mM sodium octane sulfonic acid, and 3.5% acetonitrile (pH 4.0). This method had a good reproducibility with an inter-assay coefficient of variation of 4.7% ($n = 10$).

Non-invasive Tail-cuff Blood Pressure Measurement

Blood pressure was measured using a computerized, non-invasive, tail-cuff system (Kent Scientific, USA) (Seto et al., 2013). Animals were habituated to the device before measuring blood pressure. Good reproducibility of this technique has been established previously (Seto et al., 2013).

Induction of Atherosclerosis and Aortic Aneurysm

Atherosclerosis and aortic aneurysm were induced by subcutaneous infusion of angiotensin II at a dose of 1 μg/kg/min for 28 days (Daugherty et al., 2000). Briefly, under general anesthesia using isoflurane, osmotic minipumps (Model 2004, ALZET, USA) were placed into the subcutaneous space along the dorsal midline to deliver 1 μg/kg/min of angiotensin (Sigma-Aldrich, Castle Hill, Australia) dissolved in distilled water over 28 days.

Quantification of Atherosclerotic Lesion Area

Atherosclerosis in the aortic arch was quantified by *en face* staining as described previously (Krishna et al., 2012). Briefly, the aortic arch was opened longitudinally and pinned down on a wax coated petri-dish. Tissue samples were transferred to a 70% ethanol solution and stained with 0.1% Sudan IV dissolved in equal parts of acetone and 70% ethanol for 10 min to identify areas of atherosclerosis. Sudan IV stained areas were quantified using Adobe Photoshop software (version CS5.1) and expressed as a percentage of the total aortic arch luminal surface area. We have previously established that these measurements can be repeated with good reproducibility (Golledge et al., 2010; Krishna et al., 2012).

Measurement of the Diameter of the Aortic Arch, Thoracic, and Suprarenal Aorta

After the 28-day infusion with angiotensin II, mice were euthanized and aortas were harvested from their origin at the left ventricle to the iliac bifurcation, placed beside a ruler, and digitally photographed. The maximum diameters of the aortic arch, thoracic aorta and the suprarenal aorta were determined using the Adobe Photoshop CS5.1 software (Rush et al., 2009). We have previously established that these measurements can be repeated with good intraobserver reproducibility (Rush et al., 2009).

Gene Expression Analysis

Six thoracic aortas were randomly selected from each group for RNA extraction. RNA was extracted using the TRI-reagent (Sigma-Aldrich, Castle Hill, Australia) in Eppendorf tubes according to the manufacturer's guidelines. The RNA yield of one sample from the sham surgery group was too low and not sufficient for the experiment, and thus this sample was not used for the subsequent cDNA synthesis and quantitative PCR. RNA was reverse transcribed to cDNA using the High Capacity Reverse Transcription Kit (Life Technologies). Gene expression was assessed by quantitative PCR using SYBR reagents. Primer sets are outlined in **Table 1**. The cycling conditions were as follows: a hold at 95°C for 2 min, followed by 40 cycles at 95°C for 15 s, 58°C for 20 s, and 72°C for 20 s. Relative gene expression was assessed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Gene expression analysis was represented using relative gene expression compared with the control gene eukaryotic translation elongation factor 2 (EEF2) (Kouadjo et al., 2007).

TABLE 1 | Primer sets.

Gene	Primer Sets	Tm (°C)	Product length
Adra2			
F	CAGCTCGCTGAACCCTGTTA	59.96	117
R	CACGATGCGTTTTCTGTCCC	60.04	
AT1A			
F	AGTTGGGAGGGACTGGATGA	59.88	149
R	GTTAAGTCCGGGAGAGCAGC	60.46	
AT1B			
F	GCAGGGAGTAACAGAGACCA	58.73	134
R	GTGAATTCAAAATGCACCCGT	57.97	
AT2			
F	TTTTAAGGAGTGCATGCGGGA	60.27	148
R	GGTAATGTTTCTGCTGGTGGC	59.8	
EEF2			
F	ACATGTCAGTCATCGCCCAT	59.46	166
R	GAGATGGCGGTGGATTTGATTG	59.97	
IL-6			
F	CGGCCTTCCCTACTTCACAA	59.68	149
R	GCCATTGCACAACTCTTTTCTCA	60.24	
iNOS			
F	CCTGCTTTGTGCGAAGTGTC	60.04	140
R	CCCTTTGTGCTGGGAGTCAT	59.96	
MCP-1			
F	CTTCTGGGCCTGCTGTTCA	59.93	127
R	CCAGCCTACTCATTGGGATCA	59.23	
MMP-2			
F	AACGGTCGGGAATACAGCAG	60.11	125
R	GTAAACAAGGCTTCATGGGGG	59.18	
MMP-9			
F	CAGCCGACTTTTGTGGTCTTC	59.74	87
R	ATAGCGGTACAAGTATGCCTCTG	59.99	
NF-κB			
F	GGCAGTGACGCGACGA	59.73	129
R	AAACAGATCGTCCATGGTCAGG	60.36	
TNF-α			
F	TAGCCCACGTCGTAGCAAAC	60.39	136
R	ACAAGGTACAACCCATCGGC	60.32	

Adra2, α2 adrenergic receptors; AT1A, type 1A angiotensin receptor; AT1B, type 1B angiotensin receptor; AT2, type 2 angiotensin receptor; EEF2, eukaryotic translation elongation factor 2; F, forward; IL-6, Interleukin-6; iNOS, inducible nitric oxide synthase; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; NF-κB, Nuclear factor-kappa B; R, reverse; Tm, melting temperature; TNF-α, tumor necrosis factor-alpha.

Plasma Cholesterol Measurements

Blood was collected by cardiac puncture at the time of mice sacrifice. The concentration of total cholesterol, low-density lipoprotein/very low-density lipoprotein (LDL/VLDL) cholesterol and high-density lipoprotein (HDL) cholesterol in the plasma were quantified using a commercial available kit (Abcam,

San Francisco, CA, USA; catalog number: ab65390) (Wang et al., 2014), according to the manufacturer's instructions.

Statistical Analyses

Continuous numbers were presented as a median and interquartile range (IQR). The difference between two groups was analyzed using Mann-Whitney *U*-test. Blood pressure was compared between mice that had RDN and controls during the experimental period using a linear mixed effect (LME) model using S Plus (software version 8.2). The difference in survival between the two groups was analyzed using log rank test. The correlation between atherosclerosis severity and renal norepinephrine content was assessed using the correlation analysis function of the GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA). Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Baseline Parameters

The baseline body weight, systolic, diastolic and mean blood pressure, and the heart rate were similar in the sham surgery and the RDN groups ($P > 0.05$, **Table 2**).

Success of the RDN Procedure

In a preliminary study, we performed RDN in seven mice and sham surgery in five mice. Five days later, mice were euthanized and norepinephrine content in the kidney was determined. RDN significantly decreased the median norepinephrine content in the kidney by 93.4% ($P = 0.003$, **Figure 2A**), which is similar to the previously reported ability of RDN to decrease norepinephrine content by 95% (Nakashima et al., 1996), suggesting that the RDN procedure was successful.

The norepinephrine in the kidney at the end of the main experiment (i.e., at the end of the 28-day angiotensin II infusion) was lower in the RDN group compared with sham surgery group ($P = 0.005$, **Figure 2B**). The median norepinephrine content in the RDN group was 71.7% of that in mice from the sham surgery group, suggesting a sustained reduction in renal innervation density.

The Effect of RDN on Blood Pressure

Baseline systolic, diastolic and mean blood pressure, and heart rate were not significantly different in mice prior to RDN and sham surgery (**Figure 3**). After RDN and sham surgery systolic blood pressure was significantly lower in mice that had RDN compared to controls ($P = 0.017$, **Figure 3**). Diastolic and mean blood pressure were not significantly different in mice having RDN and controls (**Figure 3**). RDN did not affect heart rate ($P > 0.05$, **Figure 3**).

The Effect of RDN on Atherosclerosis

After 28 days of angiotensin II infusion, aortic arch Sudan IV staining area was significantly greater in the RDN group compared to the sham surgery group ($P = 0.028$, **Figures 4A–C**). In addition, the norepinephrine content in the kidney at the end of the experiment was negatively correlated with the Sudan IV staining area (**Figure 4D**).

The Effect of RDN on Aortic Aneurysm Severity

During the angiotensin II infusion, six mice died in the RDN group and eight died in the sham surgery group due to aortic rupture. There was no difference in the survival rate between the two groups ($P > 0.05$, **Figure 5A**). Angiotensin II infusion induced aortic aneurysm formation in the suprarenal and thoracic aorta (Krishna et al., 2015). The maximum diameter of the aortic arch, thoracic aorta and suprarenal aorta were not significantly different in mice receiving RDN and sham surgery ($P > 0.05$, **Figures 5B–D**).

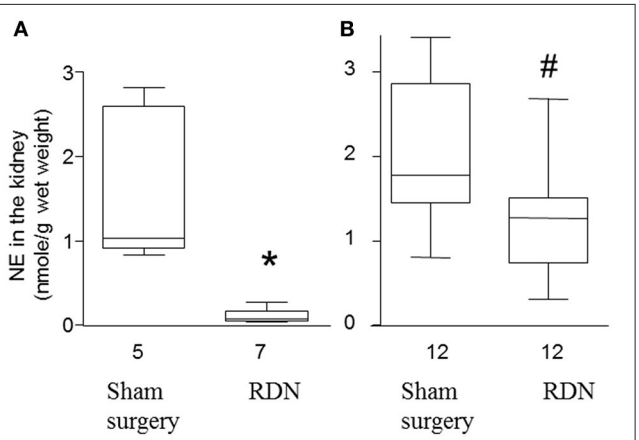


FIGURE 2 | Norepinephrine content in the kidney of apolipoprotein E-deficient mice. (A) Mice were euthanized 5 days after sham surgery or RDN and the norepinephrine (NE) content in the kidney was determined. **(B)** Mice were euthanized after 28 days angiotensin II infusion and then the NE content in the kidney was determined. $^{*}P = 0.003$, $^{\#}P = 0.005$, compared with sham surgery.

TABLE 2 | The baseline parameters of the mice.

Group (sample size)	Body weight (g)	SBP (mm Hg)	DBP (mm Hg)	MAP (mm Hg)	Heart rate (beats/min)
Sham surgery($N = 20$)	29.6 (28.6–31.0)	101.8 (96.5–103.5)	81.0 (77.5–85.1)	87.7 (83.5–90.8)	502 (437–613)
RDN ($N = 18$)	30.4 (29.2–31.1)	101.5 (96.5–106.0)	79.8 (74.4–84.3)	86.0 (83.0–90.7)	422 (364–564)

The baseline parameters were measured 1 day before the RDN or sham surgery procedures were conducted. Data were shown as a median and interquartile range (IQR). DBP, diastolic blood pressure; g, gram; MAP, mean arterial blood pressure; N, number; RDN, renal denervation; SBP, systolic blood pressure.

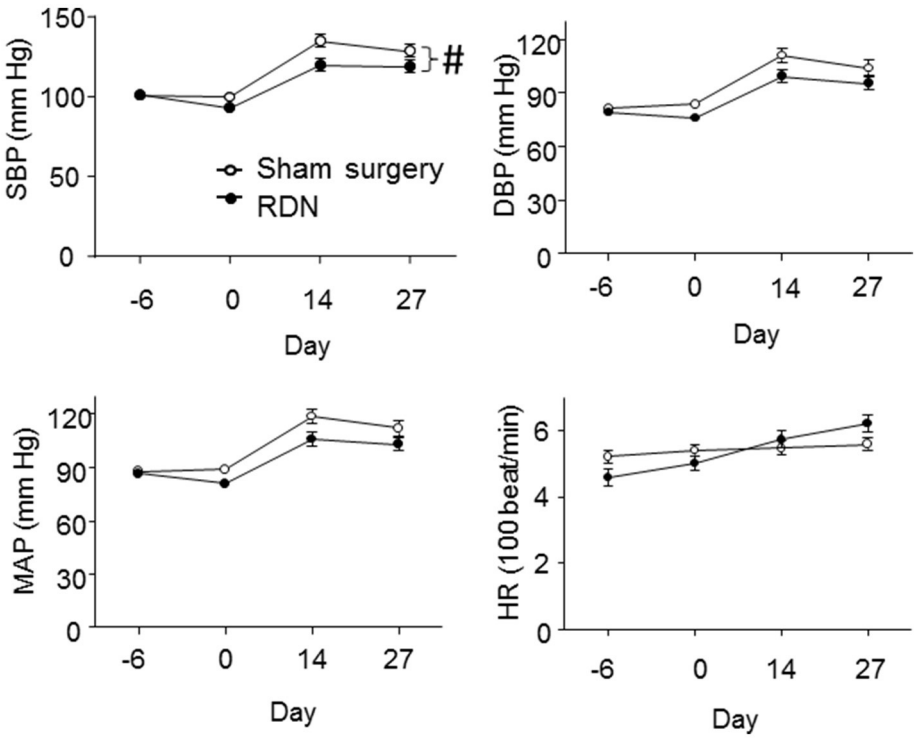


FIGURE 3 | Blood pressure and heart rate of the mice. Blood pressure and the heart rate were measured by the tail-cuff method at baseline (Day -6), 5 days after RDN or sham surgery (Day 0), 14 days (Day 14) and 27 days (Day 27) after starting the angiotensin II infusion. SBP (#$P = 0.017$), but not DBP ($P = 0.152$) and MAP ($P = 0.076$), was significantly lower in mice having RDN compared to controls, as analyzed by LME models. DBP, diastolic pressure; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure.

The Effect of RDN on the Aortic mRNA Expression of Some Atherosclerosis Associated Genes

mRNA expression of interleulin-6 (IL-6), inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein-1 (MCP-1), nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α) and matrix metalloproteinase-9 (MMP-9) within the thoracic aorta were similar in mice receiving RDN and sham surgery (**Table 3**). Mice receiving RDN had higher thoracic aortic mRNA expression of MMP-2 than mice receiving sham surgery (**Figure 6**).

The Effect of RDN on the Aortic mRNA Expression of Angiotensin Receptors and Adrenoceptors

mRNA expression of angiotensin receptors (both type 1 and 2) and α2 adrenoceptors in the thoracic aorta were similar in mice that received RDN and sham surgery (**Table 3**).

The Effect of RDN on Plasma Cholesterol Levels

RDN did not affect plasma levels of LDL/VLDL cholesterol, HDL cholesterol, or total cholesterol ($P > 0.05$, **Figure 7**).

DISCUSSION

This study focussed on assessing the effect of RDN on atherosclerosis severity in a mouse model. RDN was successful performed as evidenced by substantially lower norepinephrine content of the kidney 5 and 33 days after the procedure and lower systolic blood pressure in mice having RDN compared to controls. The main finding of the study was that the severity of aortic arch atherosclerosis, as assessed by Sudan IV staining area, was greater in mice that received RDN than controls. The severity of atherosclerosis was correlated with the extent of the RDN, as assessed by renal norepinephrine levels. Our results suggest that RDN did not affect aortic aneurysm severity or rupture.

A number of previous experimental studies have reported that sympathetic denervation promoted atherosclerosis at sites remote to the denervation procedure (Murphy et al., 1957; Snyder and Campbell, 1958; Kacem et al., 1997, 2006; Kacem and Sercombe, 2008; Hachani et al., 2010). Bilateral surgical lumbar sympathectomy has been reported to increase atherosclerosis severity in the thoracic and abdominal aorta, and iliac and femoral arteries in rabbits fed a high cholesterol diet (Murphy et al., 1957; Snyder and Campbell, 1958). Similarly, sympathetic denervation induced by intravenous administration of 6-hydroxydopamine was reported to increase atherosclerosis within the basilar and femoral arteries of rabbits fed a high cholesterol diet (Kacem et al., 2006; Kacem and Sercombe, 2008).

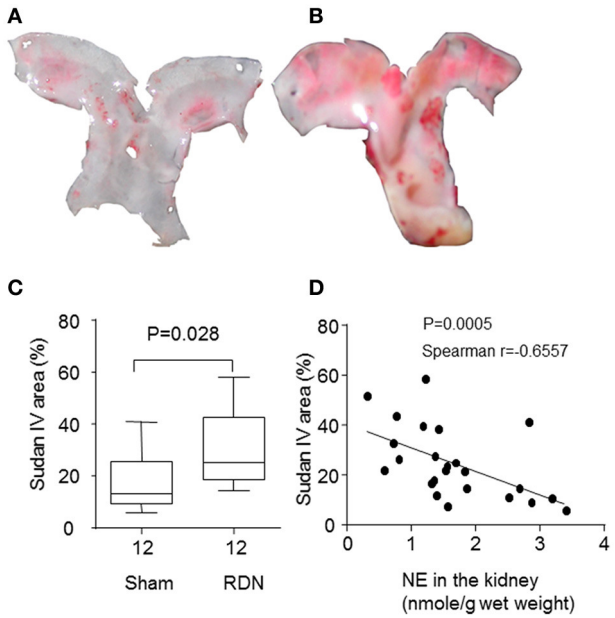


FIGURE 4 | RDN promoted more severe atherosclerosis in the apolipoprotein E-deficient mice infused with angiotensin II. Mice were euthanized after infusion with angiotensin II for 28 days, and the aortas were collected. The surface of the aortic arch was stained *en face* with Sudan IV. **(A,B)** were representative Sudan IV images of the aortic arch from sham surgery and RDN groups, respectively. The percentage of Sudan IV-positive area was calculated and expressed as a percentage of the total aortic arch luminal surface area **(C)**. **(D)** The correlation between the norepinephrine (NE) content in the kidney and atherosclerosis in the aortic arch.

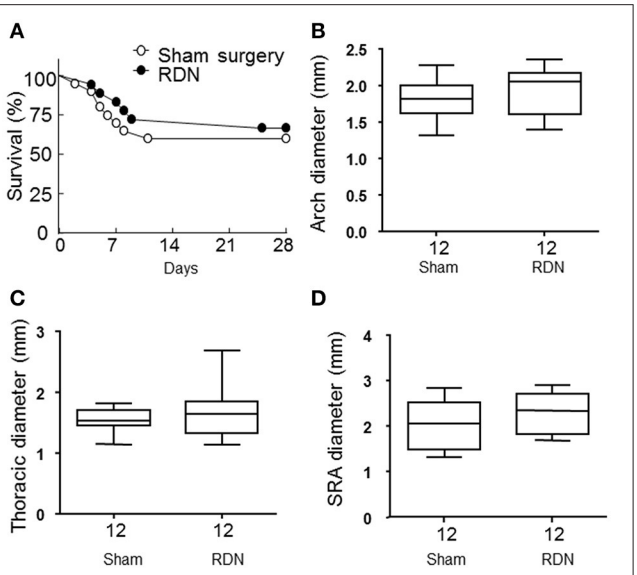


FIGURE 5 | RDN did not affect aortic rupture and the severity of the aortic aneurysm in the apolipoprotein E-deficient mice infused with angiotensin II. (A) Mice in the sham surgery and RDN groups were subcutaneously infused with angiotensin II for 28 days. The death of mice was recorded during this period and the survival curves were then constructed. $P = 0.299$ between the two survival curves using log rank test. **(B–D)** Mice were euthanized after infusion with angiotensin II for 28 days. The aorta was then dissected for aortic aneurysm assessment. The maximal aortic diameter in the aortic arch **(B)**, thoracic **(C)**, and suprarenal (SRA, **D**) aorta were measured.

TABLE 3 | The effect of RDN on mRNA expression of pro-atherosclerosis markers, angiotensin receptors, and α2 adrenergic receptors.

	Sham	RDN
AT1A	0.801 (0.735–1.364)	0.984 (0.574–1.295)
AT1B	0.834 (0.004–1.996)	0.039 (0.005–0.534)
AT2	0.803 (0.657–1.442)	1.669 (0.559–2.252)
Adra2	1.076 (0.784–1.178)	0.951 (0.546–1.293)
IL-6	1.011 (0.713–1.282)	0.501 (0.312–1.402)
iNOS	1.022 (0.741–1.0248)	1.311 (0.429–1.872)
MCP-1	0.633 (0.431–1.713)	0.433 (0.300–0.587)
NF-κB	0.951 (0.724–1.300)	0.890 (0.628–1.369)
MMP-9	0.969 (0.778–1.238)	1.472 (0.761–2.281)
TNF-α	0.863 (0.709–1.359)	0.992 (0.613–1.273)

Mice were euthanized after infusion with angiotensin II for 28 days, and the thoracic aortic was used for RNA extraction. mRNA expression was analyzed using quantitative PCR. The relative gene expression was normalized using EEF2 as a reference gene. Data were expressed as median and interquartile range. N = 5 for sham surgery, and N = 6 for RDN. P > 0.05 for all the genes tested in the table. Adra2, alpha-2 adrenergic receptor; AT1A, type 1A angiotensin receptor; AT1B, type 1B angiotensin receptor; AT2, type 2 angiotensin receptor; IL, interleukin; iNOS, inducible nitric oxide synthase; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; NF-κB, nuclear factor-kappa B; RDN, renal denervation; TNF-α, tumor necrosis factor-alpha.

Sympathetic denervation by subcutaneous administration of guanethidine was reported to promote intima thickening within the abdominal aorta of rats fed a high cholesterol diet (Hachani et al., 2010).

The mechanisms underlying the atherosclerosis-promoting effect of sympathetic denervation are not well understood. It has been reported that this effect of sympathetic denervation may be due to stimulating migration of adventitial fibroblasts to the media and the associated loss and dedifferentiation of smooth muscle cells (Kacem and Sercombe, 2008; Hachani et al., 2010). It has been reported that following sympathetic denervation that the aortic expression of immature smooth muscle cell markers, such as vimentin, increased but that the expression of the mature smooth muscle cell markers (α-smooth muscle actin, h-caldesmon) decreased (Hachani et al., 2010). However, the importance of these changes in smooth muscle cell phenotype on the development of atherosclerosis have not been established.

Our results are in contrast to the results of a recent report which used another atherosclerotic model, i.e., ApoE$^{-/-}$ mice fed a high-fat diet for 10 weeks (Wang et al., 2015). That study Wang et al. (2015) reported that RDN decreased atherosclerosis as assessed by oil-red-O staining within the aortic root and the aortic tree (including aortic arch, brachiocephalic artery, common carotid arteries and subclavian arteries). The discrepancy may be due to the disparate animal models used, i.e., high-fat diet vs. angiotensin II infusion in our study. The angiotensin II infusion model represents an advanced atherosclerosis model, as suggested by the large Sudan IV staining area reported in our study (Wang et al., 2015). In addition, the angiotensin II infusion model is a hypertensive model, whereas the high-fat diet model is a normotensive model.

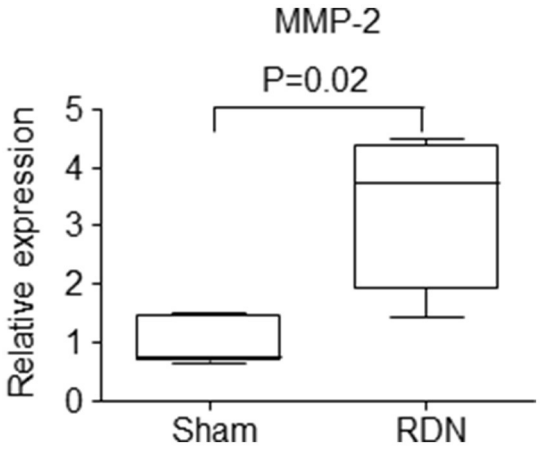


FIGURE 6 | The effect of RDN on the mRNA expression of MMP-2. Mice were euthanized after infusion with angiotensin II for 28 days, and the thoracic aortic was used for RNA extraction. mRNA expression was analyzed using quantitative PCR. The relative gene expression was normalized using EEF2 as a reference gene. $N = 5$ for sham surgery, and $N = 6$ for RDN. MMP, matrix metalloproteinase.

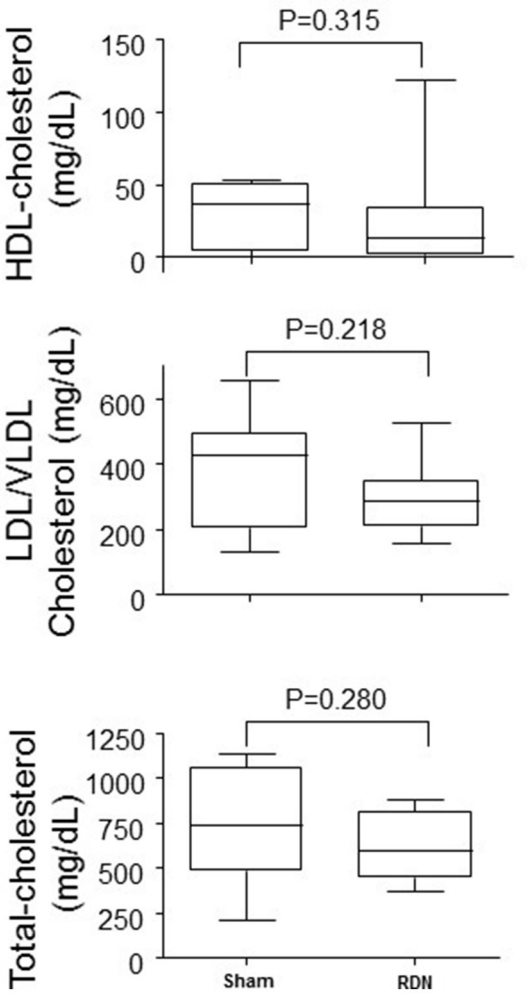


FIGURE 7 | Plasma lipid concentrations in mice undergoing RDN and controls. Plasma lipid concentrations were measured in blood collected at the completion of the 28-day angiotensin II infusion. $N = 10$ for each group. HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

The latter model is different from the clinical setting as RDN is performed in treatment-resistant hypertensive patients.

MMP-2 expression within the thoracic aorta was greater in mice receiving RDN than controls. MMP-2 plays an important role in degrading extracellular matrix and has been implicated in the initiation, development and eventual rupture of atherosclerotic plaques (Li et al., 1996; Nagase and Woessner, 1999; Johnson et al., 2006; Kuzuya et al., 2006). It has been reported that MMP-2 protein and activity levels are increased in human aortic atherosclerotic lesions compared with normal regions of the aorta (Li et al., 1996). The severity of atherosclerosis in MMP-2 and ApoE double gene knockout mice has been reported to be less than that in ApoE single gene knockout mice (Kuzuya et al., 2006). The activity of MMP-2 and other MMPs can be inhibited by their endogenous tissue inhibitors (TIMPs) (Nagase and Woessner, 1999). It has been reported that over-expression of TIMP-2 by adenovirus technology significantly reduces atherosclerotic formation in the brachiocephalic artery of $ApoE^{-/-}$ mice fed a high-fat diet (Johnson et al., 2006), and over-expression of TIMP-2 has also been reported to promote atherosclerotic plaque stability (Johnson et al., 2006). It is therefore possible that the upregulation of MMP-2 identified in mice receiving RDN may have promoted atherosclerosis within the aorta. It is also possible that the higher MMP-2 expression measured simply reflected the greater atherosclerosis in the mice receiving RDN although the fact other atherosclerosis-associated genes were not different supports a causative association. Further studies are needed to examine this theory. The expression of a range of other genes implicated in inflammation and matrix remodeling and plasma lipids were similar in mice receiving RDN and controls.

Other possible explanation for the greater atherosclerosis in mice receiving RDN include increased arterial pressure variability or functional changes within the media. These have not been investigated in the current study.

RDN has been reported to promote renal artery stenosis in humans. The Symplicity HTN studies (Krum et al., 2009; Esler et al., 2010; Bhatt et al., 2014) reported a renal artery stenosis rate of 0.3–2.2%, whereas other trials with smaller sample sizes have reported a higher rate of 2.8–18% (Kaltenbach et al., 2013; Worthley et al., 2013; Papademetriou et al., 2014; Persu et al., 2014; Versaci et al., 2014). The EnligHTN I trial (Worthley et al., 2013) reported that the progression of pre-existing renal artery stenosis was possibly related to the RDN procedure. Atherosclerosis is responsible for most primary renal artery stenoses but those developing after RDN may represent a local intimal hyperplasia response to the procedure rather than promotion of pre-existing atherosclerosis as identified in the mouse model studies here (Lao et al., 2011). Whether the renal artery stenoses reported in these trials are related to the

promotion of atherosclerosis within the distant site of the aortic arch found in the current mouse study is not clear.

Renal denervation has been shown to decrease blood pressure in a large number of clinical (Krum et al., 2009; Esler et al., 2010) and preclinical (Nishihara et al., 2016) studies. However, the recent blinded, sham-controlled, randomized Symplicity HTN-3 trial (Bhatt et al., 2014) and a number of non-randomized trials (Brinkmann et al., 2012; Vase et al., 2012; Fadl Elmula et al., 2013; Hart et al., 2013; Ezzahti et al., 2014) suggested that RDN did not decrease blood pressure, and this may be due to an ineffective RDN procedure (Mahfoud et al., 2015). Currently there are no well-defined ways to immediately tell whether RDN has been technically successful in patients (Mahfoud et al., 2015). RDN was successful in our experiment as indicated by a substantial decrease in the renal norepinephrine content, and this was associated with a decrease in systolic blood pressure. RDN in our study did not affect heart rate, which is consistent with other reports (Bhatt et al., 2014; Vink et al., 2014).

Limitations

This study has several limitations. First, the sample size of the study was small; second, this study employed only one animal model; third, blood pressure was measured by the tail-cuff method rather than the gold standard telemetry method; and fourth, the RDN procedure in mice is different from the clinical practice in which catheter-based methods are used. Finally the plasma concentrations of lipids varied substantially in different mice and the reasons for this are not clear.

Potential Clinical Implication

Based on our results and other experimental evidence (Murphy et al., 1957; Snyder and Campbell, 1958; Kacem et al., 1997, 2006; Kacem and Sercombe, 2008; Hachani et al., 2010), there is concern that RDN might promote more severe distant atherosclerosis severity. Clinical studies are needed to examine this possibility in patients undergoing RDN.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

This work was funded by grants from the National Health and Medical Research Council (1062671, 1079369, 1079193, 1063476, 1021416, and 1000967) and Queensland Government. JG is supported by a Practitioner Fellowship from the National Health and Medical Research Council (1117061).

REFERENCES

Bhatt, D. L., Kandzari, D. E., O'Neill, W. W., D'Agostino, R., Flack, J. M., Katzen, B. T., et al. (2014). A controlled trial of renal denervation for resistant hypertension. *N. Engl. J. Med.* 370, 1393–1401. doi: 10.1056/NEJMoa1402670

Brinkmann, J., Heusser, K., Schmidt, B. M., Menne, J., Klein, G., Bauersachs, J., et al. (2012). Catheter-based renal nerve ablation and centrally generated sympathetic activity in difficult-to-control hypertensive patients: prospective case series. *Hypertension* 60, 1485–1490. doi: 10.1161/HYPERTENSIONAHA.112.201186

Daugherty, A., Manning, M. W., and Cassis, L. A. (2000). Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J. Clin. Invest.* 105, 1605–1612. doi: 10.1172/JCI7818

Esler, M. D., Krum, H., Sobotka, P. A., Schlaich, M. P., Schmieder, R. E., and Bohm, M. (2010). Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 376, 1903–1909. doi: 10.1016/S0140-6736(10)62039-9

Ezzahti, M., Moelker, A., Friesema, E. C., van der Linde, N. A., Krestin, G. P., and van den Meiracker, A. H. (2014). Blood pressure and neurohormonal responses to renal nerve ablation in treatment-resistant hypertension. *J. Hypertens.* 32, 135–141. doi: 10.1097/HJH.0b013e3283658ef7

Fadl Elmula, F. E., Hoffmann, P., Fossum, E., Brekke, M., Gjonnaess, E., Hjornholm, U., et al. (2013). Renal sympathetic denervation in patients with treatment-resistant hypertension after witnessed intake of medication before qualifying ambulatory blood pressure. *Hypertension* 62, 526–532. doi: 10.1161/HYPERTENSIONAHA.113.01452

Golledge, J., Cullen, B., Moran, C., and Rush, C. (2010). Efficacy of simvastatin in reducing aortic dilatation in mouse models of abdominal aortic aneurysm. *Cardiovasc. Drugs Ther.* 24, 373–378. doi: 10.1007/s10557-010-6262-8

Hachani, R., Dab, H., Sakly, M., Vicaut, E., Callebert, J., Sercombe, R., et al. (2010). Influence of antagonist sensory and sympathetic nerves on smooth muscle cell differentiation in hypercholesterolemic rat. *Auton. Neurosci.* 155, 82–90. doi: 10.1016/j.autneu.2010.02.001

Hart, E. C., McBryde, F. D., Burchell, A. E., Ratcliffe, L. E., Stewart, L. Q., Baumbach, A., et al. (2013). Translational examination of changes in baroreflex function after renal denervation in hypertensive rats and humans. *Hypertension* 62, 533–541. doi: 10.1161/HYPERTENSIONAHA.113.01261

Johnson, J. L., Baker, A. H., Oka, K., Chan, L., Newby, A. C., Jackson, C. L., et al. (2006). Suppression of atherosclerotic plaque progression and instability by tissue inhibitor of metalloproteinase-2: involvement of macrophage migration and apoptosis. *Circulation* 113, 2435–2444. doi: 10.1161/CIRCULATIONAHA.106.613281

Kacem, K., Bonvento, G., and Seylaz, J. (1997). Effect of sympathectomy on the phenotype of smooth muscle cells of middle cerebral and ear arteries of hyperlipidaemic rabbits. *Histochem. J.* 29, 279–286. doi: 10.1023/A:1026418413313

Kacem, K., Sercombe, C., Hammami, M., Vicaut, E., and Sercombe, R. (2006). Sympathectomy causes aggravated lesions and dedifferentiation in large rabbit atherosclerotic arteries without involving nitric oxide. *J. Vasc. Res.* 43, 289–305. doi: 10.1159/000093010

Kacem, K., and Sercombe, R. (2008). Similar pathological effects of sympathectomy and hypercholesterolemia on arterial smooth muscle cells and fibroblasts. *Acta Histochem.* 110, 302–313. doi: 10.1016/j.acthis.2007.11.007

Kaltenbach, B., Franke, J., Bertog, S. C., Steinberg, D. H., Hofmann, I., and Sievert, H. (2013). Renal sympathetic denervation as second-line therapy in mild resistant hypertension: a pilot study. *Catheter. Cardiovasc. Interv.* 81, 335–339. doi: 10.1002/ccd.24557

Kouadjo, K. E., Nishida, Y., Cadrin-Girard, J. F., Yoshioka, M., and St-Amand, J. (2007). Housekeeping and tissue-specific genes in mouse tissues. *BMC Genomics* 8:127. doi: 10.1186/1471-2164-8-127

Krishna, S. M., Seto, S. W., Jose, R. J., Biros, E., Moran, C. S., Wang, Y., et al. (2015). A peptide antagonist of thrombospondin-1 promotes abdominal aortic aneurysm progression in the angiotensin II-infused apolipoprotein-E-deficient mouse. *Arterioscler. Thromb. Vasc. Biol.* 35, 389–398. doi: 10.1161/ATVBAHA.114.304732

Krishna, S. M., Seto, S. W., Moxon, J. V., Rush, C., Walker, P. J., Norman, P. E., et al. (2012). Fenofibrate increases high-density lipoprotein and sphingosine 1 phosphate concentrations limiting abdominal aortic aneurysm progression in a mouse model. *Am. J. Pathol.* 181, 706–718. doi: 10.1016/j.ajpath.2012.04.015

Krum, H., Schlaich, M., Whitbourn, R., Sobotka, P. A., Sadowski, J., Bartus, K., et al. (2009). Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 373, 1275–1281. doi: 10.1016/S0140-6736(09)60566-3

Kuzuya, M., Nakamura, K., Sasaki, T., Cheng, X. W., Itohara, S., and Iguchi, A. (2006). Effect of MMP-2 deficiency on atherosclerotic lesion formation in apoE-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 26, 1120–1125. doi: 10.1161/01.ATV.0000218496.60097.e0

Lao, D., Parasher, P. S., Cho, K. C., and Yeghiazarians, Y. (2011). Atherosclerotic renal artery stenosis–diagnosis and treatment. *Mayo Clin. Proc.* 86, 649–657. doi: 10.4065/mcp.2011.0181

Li, Z., Li, L., Zielke, H. R., Cheng, L., Xiao, R., Crow, M. T., et al. (1996). Increased expression of 72-kd type IV collagenase (MMP-2) in human aortic atherosclerotic lesions. *Am. J. Pathol.* 148, 121.

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262

Mahfoud, F., Bohm, M., Azizi, M., Pathak, A., Durand Zaleski, I., Ewen, S., et al. (2015). Proceedings from the European clinical consensus conference for renal denervation: considerations on future clinical trial design. *Eur. Heart J.* 36, 2219–2227. doi: 10.1093/eurheartj/ehv192

Murphy, T. O., Haglin, J. J., and Felder, D. A. (1957). The progression of experimental atherosclerosis after lumbar sympathectomy. *Surg. Forum* 7, 332–336.

Nagase, H., and Woessner, J. F. (1999). Matrix metalloproteinases. *J. Biol. Chem.* 274, 21491–21494. doi: 10.1074/jbc.274.31.21491

Nakashima, A., Matsuoka, H., Yasukawa, H., Kohno, K., Nishida, H., Nomura, G., et al. (1996). Renal denervation prevents intraglomerular platelet aggregation and glomerular injury induced by chronic inhibition of nitric oxide synthesis. *Nephron* 73, 34–40. doi: 10.1159/000188995

Nishihara, M., Takesue, K., and Hirooka, Y. (2016). Renal denervation enhances GABA-ergic input into the PVN leading to blood pressure lowering in chronic kidney disease. *Auton. Neurosci.* 204, 88–97. doi: 10.1016/j.autneu.2016.09.018

O'Neill, P. A., Wait, R. B., and Kahng, K. U. (1991). Role of renal sympathetic nerve activity in renal failure associated with obstructive jaundice in the rat. *Am. J. Surg.* 161, 662–667. doi: 10.1016/0002-9610(91)91251-D

Papademetriou, V., Tsioufis, C. P., Sinhal, A., Chew, D. P., Meredith, I. T., Malaiapan, Y., et al. (2014). Catheter-based renal denervation for resistant hypertension: 12-month results of the enlightn i first-in-human study using a multielectrode ablation system. *Hypertension* 64, 565–572. doi: 10.1161/HYPERTENSIONAHA.114.03605

Persu, A., Jin, Y., Azizi, M., Baelen, M., Volz, S., Elvan, A., et al. (2014). Blood pressure changes after renal denervation at 10 European expert centers. *J. Hum. Hypertens.* 28, 150–156. doi: 10.1038/jhh.2013.88

Rush, C., Nyara, M., Moxon, J. V., Trollope, A., Cullen, B., and Golledge, J. (2009). Whole genome expression analysis within the angiotensin II-apolipoprotein E deficient mouse model of abdominal aortic aneurysm. *BMC Genomics* 10:298. doi: 10.1186/1471-2164-10-298

Schlaich, M. P., Sobotka, P. A., Krum, H., Lambert, E., and Esler, M. D. (2009). Renal sympathetic-nerve ablation for uncontrolled hypertension. *N. Engl. J. Med.* 361, 932–934. doi: 10.1056/NEJMc0904179

Seto, S. W., Krishna, S. M., Yu, H., Liu, D., Khosla, S., and Golledge, J. (2013). Impaired acetylcholine-induced endothelium-dependent aortic relaxation by caveolin-1 in angiotensin II-infused apolipoprotein-E (ApoE−/−) knockout mice. *PLoS ONE* 8:e58481. doi: 10.1371/journal.pone.0058481

Snyder, D. D., and Campbell, G. S. (1958). Effect of aortic constriction on experimental atherosclerosis in rabbits. *Proc. Soc. Exp. Biol. Med.* 99, 563–564. doi: 10.3181/00379727-99-24420

Vase, H., Mathiassen, O. N., Kaltoft, A., Pedersen, E. B., Christensen, K. L., Buus, N. H., et al. (2012). Catheter-based renal denervation for treatment of resistant hypertension. *Dan. Med. J.* 59, A4439.

Versaci, F., Trivisonno, A., Olivieri, C., Caranci, F., Brunese, L., and Prati, F. (2014). Late renal artery stenosis after renal denervation: is it the tip of the iceberg? *Int. J. Cardiol.* 172, e507–e508. doi: 10.1016/j.ijcard.2014.01.018

Vink, E. E., Verloop, W. L., Siddiqi, L., van Schelven, L. J., Liam Oey, P., and Blankestijn, P. J. (2014). The effect of percutaneous renal denervation on muscle sympathetic nerve activity in hypertensive patients. *Int. J. Cardiol.* 176, 8–12. doi: 10.1016/j.ijcard.2014.06.021

Wang, H., Wang, J., Guo, C., Luo, W., Kleiman, K., and Eitzman, D. T. (2015). Renal denervation attenuates progression of atherosclerosis in apolipoprotein E-deficient mice independent of blood pressure lowering. *Hypertension* 65, 758–765. doi: 10.1161/HYPERTENSIONAHA.114.04648

Wang, Y., Fice, D. S., and Yeung, P. K. (1999). A simple high-performance liquid chromatography assay for simultaneous determination of plasma norepinephrine, epinephrine, dopamine and 3,4-dihydroxyphenyl acetic acid. *J. Pharm. Biomed. Anal.* 21, 519–525. doi: 10.1016/S0731-7085(99)00117-X

Wang, Y., Krishna, S. M., Moxon, J., Dinh, T. N., Jose, R. J., Yu, H., et al. (2014). Influence of apolipoprotein E, age and aortic site on calcium phosphate induced abdominal aortic aneurysm in mice. *Atherosclerosis* 235, 204–212. doi: 10.1016/j.atherosclerosis.2014.04.033

Worthley, S. G., Tsioufis, C. P., Worthley, M. I., Sinhal, A., Chew, D. P., Meredith, I. T., et al. (2013). Safety and efficacy of a multi-electrode renal sympathetic denervation system in resistant hypertension: the EnligHTN I trial. *Eur. Heart J.* 34, 2132–2140. doi: 10.1093/eurheartj/eht197

Ye, S., Ozgur, B., and Campese, V. M. (1997). Renal afferent impulses, the posterior hypothalamus, and hypertension in rats with chronic renal failure. *Kidney Int.* 51, 722–727. doi: 10.1038/ki.1997.103

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read, for greatest visibility

COLLABORATIVE PEER-REVIEW
Designed to be rigorous – yet also collaborative, fair and constructive

FAST PUBLICATION
Average 85 days from submission to publication (across all journals)

COPYRIGHT TO AUTHORS
No limit to article distribution and re-use

TRANSPARENT
Editors and reviewers acknowledged by name on published articles

SUPPORT
By our Swiss-based editorial team

IMPACT METRICS
Advanced metrics track your article's impact

GLOBAL SPREAD
5'100'000+ monthly article views and downloads

LOOP RESEARCH NETWORK
Our network increases readership for your article

Frontiers
EPFL Innovation Park, Building I • 1015 Lausanne • Switzerland
Tel +41 21 510 17 00 • Fax +41 21 510 17 01 • info@frontiersin.org
www.frontiersin.org

Find us on