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Urothelial TRPV1: TRPV1-reporter mice, a way to clarify the debate?

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A commentary on

Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells
by Cavanaugh, D. J., Chesler, A. T., Jackson, A. C., Sigal, Y. M., Yamanaka, H., Grant, R., O'Donnell, D., Nicoll, R. A., Shah, N. M., Julius, D., and Basbaum, A. I. (2011). J. Neurosci. 31, 5067–5077.

The urothelium is a complex and dynamic epithelial layer with a sensory role contributing to mechano- and chemosensation in the bladder. This primary source of sensory inputs modulates micturition via an urothelial–sensory fibers crosstalk. The communication is mediated by ATP and NO release from epithelial cells. Therefore, a great interest has emerged in the urothelial molecular sensors during the last decade. Efforts were mainly focused on the transient receptor potential (TRP) channels. The TRP channels operate as polymodal cellular sensors involved in the fine tuning of many physiological processes. Based on sequence homology, the 28 mammalian members are divided into 6 families. TRPV1 and TRPV4 are the two most described members expressed in the urothelium. TRPV4 is a mechanosensitive channel involved in normal micturition (Gevaert et al., 2007) and has been highlighted as a putative pharmacological target to treat overactive bladder symptoms (Everaerts et al., 2010b). However, the extent to which functional TRPV1 channels are expressed in the bladder is debated.

Experimental studies using TRPV1$^{-/-}$ mice suggested that TRPV1 might function as a mechanosensor in the bladder influencing the micturition threshold under general anesthesia (Birder et al., 2002). They also described urothelial ATP release upon capsaicin (the most potent TRPV1 agonist) application. The activation of TRPV1 by capsaicin induced intracellular calcium rise and inward cation current in both rat and human cultured urothelial cells (Charrua et al., 2009; Kullmann et al., 2009). The expression of TRPV1 in urothelium has been reported using diverse methods including quantitative PCR, immunohistochemistry, and western blot in different species (Lazzeri et al., 2004; Charrua et al., 2009; Heng et al., 2011). Recently, it has been shown that TRPV1 expression is not different from bladder dome and trigone in human biopsies at mRNA level (Sánchez Freire et al., 2011). In urothelial carcinoma, TRPV1 mRNA and protein levels were decreased (Lazzeri et al., 2005; Kalogris et al., 2010). The oral administration of a TRPV1 antagonist counteracted the bladder hyperactivity and the related hyperalgesia in cystitis animal model (Charrua et al., 2009). Altogether, these studies tend to demonstrate the functional expression of TRPV1 in urothelial cells and its implication in micturition in both physiological and pathological contexts.

However, another school of thought exists. From that point of view, TRPV1 is expressed in small diameter bladder afferent fibers running through the urothelium but not by the epithelial cells themselves (Yamada et al., 2009; Yu et al., 2011). This expression is decreased following intradetrusor injections of botulinum toxin in patients (Apostolidis et al., 2005). Intravesical capsaicin and resiniferatoxin dissolved in high ethanol concentrations (5–30%) were able to suppress neurogenic detrusor overactivity in patients, but the relative role of the vanilloids and the ethanol have never been clarified (Ost et al., 2003). The specificity of TRPV1 antibodies have been questioned and appropriate controls (i.e., knock out animals) are not always used (Everaerts et al., 2009). All these studies question the TRPV1 expression in the urothelium. Moreover, a discrepancy also exists between the functional data. Indeed, two independent groups did not record TRPV1 positive signals (intracellular calcium rise or current) in cultured urothelial cells from guinea pig and mice (Xu et al., 2009; Everaerts et al., 2010*a*). The authors also doubted that Kullmann et al. (2009) recorded rat TRPV1 current considering that the current–voltage is linear whereas rat TRPV1 typical current is outwardly rectifying (Xu et al., 2005). However, TRPV1 gating properties are responsible for the rectification as TRPV1 single channel recordings are linear. In one hand, it is described that the stimulus strength can linearize the TRPV1 current–voltage relationship; therefore high concentration of capsaicin may modify the biophysical signature of the current. In the other hand, unknown adaptor proteins and/or subunits may exist in the urothelium; and this would be fascinating! In part, the lack of consensus reflects the limitations of traditional approaches to determine gene expression, including variable sensitivity, poor signal-to-noise, and lack of specificity. These divergent data need to be understood and explained to settle this important controversy for basic and clinical urology to determine whether TRPV1-based drugs could treat urothelial pathologies.

Undoubtedly, researchers need new tools to assess this question. It might have been published in Journal of Neuroscience! In this study, the authors proposed to solve the mystery of TRPV1 expression in brain using a new genetic tool. They designed a TRPV1-reporter mouse using the insertion of two reporter genes after an IRES sequence. This genetic system allows the expression of a nuclear LacZ and the placental alkaline phosphatase (PLAP) with the putative TRPV1 expression pattern without disturbing TRPV1 function (Cavanaugh et al., 2011). These authors did not reveal any TRPV1 expression in bladder cDNA by

PCR, but surprisingly they did not use their own reporter mice to confirm this result! They also created TRPV1 Cre mice that may help to distinguish differential expression and function among the urothelium layers via imaging and functional experiments. Nevertheless, scientists should bear in mind that gene expression under IRES sequence control is often inferior to the expression level of the upstream gene. Therefore, it could be possible that the reporter gene is not expressed or not detectable in case of low TRPV1 expression. However, these new transgenic mice might not be the panacea but they might surely help.

These tools are now available, let us use them!

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