



P2X₇ Receptors in Neurodegeneration: Potential Therapeutic Applications From Basic to Clinical Approaches

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Purinergic receptors play important roles in central nervous system (CNS), where the bulk of these receptors are implicated in neuroinflammatory responses and regulation of cellular function of neurons, microglial and astrocytes. Within the P2X receptor family, P2X₇ receptor is generally known for its inactivity in normal conditions and activation by moderately high concentrations (> 100 μM) of extracellular adenosine 5'-triphosphate (ATP) released from injured cells as a result of brain injury or pathological conditions. Activation of P2X₇R contributes to the activation and proliferation of microglia and directly contribute to neurodegeneration by provoking microglia-mediated neuronal death, glutamate-mediated excitotoxicity, and NLRP3 inflammasome activation that results in initiation, maturity and release of the pro-inflammatory cytokines and generation of reactive oxygen and nitrogen species. These components of the inflammatory response play important roles in many neural pathologies and neurodegeneration disorders. In CNS, expression of P2X₇R on microglia, astrocytes, and oligodendrocytes are upregulated under neuroinflammatory conditions. Several *in vivo* studies have demonstrated beneficial effects of the P2X₇ receptor antagonists in animal model systems of neurodegenerative diseases. A number of specific and selective P2X₇ receptor antagonists have been developed, but only few of them have shown efficient brain permeability. Finding potent and selective P2X₇ receptor inhibitors which are also CNS penetrable and display acceptable pharmacokinetics (PK) has presented challenges for both academic researchers and pharmaceutical companies. In this review, we discuss the role of P2X₇ receptor function in neurodegenerative diseases, the pharmacological inhibition of the receptor, and PET radiopharmaceuticals which permit non-invasive monitoring of the P2X₇ receptor contribution to neuroinflammation associated with neurodegeneration.

Keywords: purinergic signaling, neurodegeneration, neuroinflammation, neuropsychiatric disease, P2X₇R

INTRODUCTION

Purinergic receptors or purinoceptors are plasma membrane proteins found in almost all mammalian tissues including the central nervous system (CNS) (Tozaki-Saitoh et al., 2011). These receptors participate in the synaptic processes via communications between neuron-glia and glia with other glia cell types (i.e., astrocytes, oligodendrocytes, and microglia) (Tozaki-Saitoh et al., 2011). Based on their endogenous ligands, the purinergic receptors are classified into P1 and P2 categories (Burnstock, 2008). P1 or adenosine receptors are a family of G protein-coupled receptors (GPCR) with four subtypes: A₁, A_{2A}, A_{2B}, and A₃. P2 receptors are further divided into the two structurally and functionally unique families of receptors that mediate intracellular signaling evoked by extracellular ATP. The ligand-gated ion channel P2X receptors with seven subtypes: P2X_{1–7} and the G protein-coupled metabotropic P2Y receptors with eight subtypes: P2Y_{1,2,4,6,11,12,13,14} (Ralevic and Burnstock, 1998; Burnstock, 2018). Several members of the purinergic receptors, specifically play major roles in CNS disorders including: Alzheimer's disease (AD), Parkinson disease (PD), Huntington's disease (HD), Frontotemporal dementia (FD), Amyotrophic Lateral Sclerosis (ALS), Multiple Sclerosis (MS), Traumatic Brain Injury (TBI), stroke, cerebral ischemia, epilepsy, psychiatric diseases, sleep disorder, and neuropathic pain (Burnstock, 2008, 2016; Tozaki-Saitoh et al., 2011; Beamer et al., 2016).

Depending on the receptor subtype, adenosine and the P2Y receptors are coupled to Gq/Gi/Gs proteins (Puchalowicz et al., 2015). Gq proteins activation prompt a signaling cascade through phospholipase C/inositol-1,4,5-triphosphate (PLC/IP3), a process that promotes the release of Ca²⁺ from the endoplasmic reticulum into the cytoplasm. Activation of the Gs/Gi proteins effect stimulation or inhibition of adenylate cyclase that subsequently modifies the production of cyclic AMP (cAMP), while the non-selective P2X ion channel receptors facilitate cellular exchange of cations like Ca²⁺, Mg²⁺, Na⁺, and K⁺ (Surprenant and North, 2009; Puchalowicz et al., 2015).

The energy source for neurons and glial cells, Adenosine 5'-triphosphate (ATP), also acts as an extracellular purinergic signaling that controls communication between brain cells (Burnstock, 2006a). The steady concentration of cytosolic ATP ranges between 5 to 10 mM, but very low nM in the extracellular space (Bhattacharya and Biber, 2016). However, under pathological conditions, and during CNS insults, high concentration of ATP is released from the damaged cells to the extracellular space as a danger signal and alters calcium signaling through modulation of purinergic receptors, and in turn results in neuroinflammatory responses, excitotoxicity, and apoptosis, a cascade of events that eventually damages the neurons (Yegutkin, 2008; Roszek and Czarnecka, 2015).

High level of extracellular ATP signals microglia to undergo chemotaxis to the site of injury in order to remove cell debris from these sites (Inoue, 2008). Microglial activation (Domercq et al., 2013) result in upregulation of P2X₄ and P2X₇ (Di Virgilio et al., 2017) and downregulation of P2Y₁₂ receptors expression (Haynes et al., 2006). The fine balance between expressions of

these three receptors dictates the destiny of microglia (Vazquez-Villoldo et al., 2014). A relatively high expression levels of P2X₄ and P2X₇ receptors are indicator of pro-inflammatory M1 phenotype microglia activation (Burnstock, 2007). Additionally, release of the large quantity of ATP [hundreds of micromolar (μM)], activates P2Y₁ receptor that enables movement of ramified microglia to the damage site, while P2Y₆ receptor, a normally expressed receptor on the activated microglia, initiates phagocytosis process (Burnstock, 2008; Boue-Grabot and Pankratov, 2017). Moreover, conversion of extracellular ATP to adenosine takes place by ectonucleotidases CD39 and CD73 that are present in microglia (Braun et al., 2000) and in turn activates adenosine receptors (Fredholm et al., 2001; Choi et al., 2015). Both adenosine and ATP are essential modulators of neuroinflammatory responses, excitotoxicity, oxidative stress and cell death, especially via A_{2A} and P2X₇ receptors activity, respectively (Cunha, 2016; Faas et al., 2017; He et al., 2017; Vuorimaa et al., 2017).

The ligand-gated ion channel P2X receptors have seven subunits that vary in length ranging from 377 amino acid residues in the P2X₆ receptor to 595 residues in the P2X₇ receptor (North, 2002). P2X₇R is mostly coexpressed with P2X₄R, and the two receptors are proven not to form heteromeric assemblies and function only as homomers (Guo et al., 2007). However, P2X₇R homotrimers were able to co-immunoprecipitate with P2X₄ and as such, there is an evidence of a structural and functional interaction between P2X₄ and P2X₇ receptors (Boumechache et al., 2009). Other P2X receptor subunits form both homomeric or heteromeric receptors such as: P2X_{1/2}, P2X_{1/4}, P2X_{1/5}, P2X_{2/3}, P2X_{2/5}, P2X_{2/6} and P2X_{4/6} receptors (Jiang et al., 2013). P2X receptors are widely distributed in neuronal and non-neuronal cells, and participate in many physiological and pathophysiological processes (Caseley et al., 2014). Countless *in vitro* and *in vivo* studies have shown changes in these receptors' expression under pain sensation, inflammation and nerve transmission conditions (Jacobson and Muller, 2016). In the CNS, P2X receptors, especially P2X₄ and P2X₇ contribute to modulation of neuron-glia communication, inflammation, and apoptosis (Burnstock, 2006b; Surprenant and North, 2009). With the exception of P2X₇R, other P2XR subtypes are typically activated at low micromolar (μM) or high nanomolar (nM) concentrations of ATP. Several publications have extensively discussed the distribution, pharmacological properties, physiological and pathophysiological functions of the P2X receptors (Surprenant and North, 2009; Jiang, 2012; Jiang et al., 2013; North and Jarvis, 2013).

P2X₇ Receptors

P2X₇ receptors are members of the P2X family of trimeric ligand-gated cation channel receptors encoded by the P2RX₇ gene and share the least homology (35–40%) with other P2XRs (North, 2002; Sperlagh et al., 2006). P2X₇R has the largest monomeric subunit in the P2X family (North, 2002). Each subunit has a short intracellular amino, long carboxyl termini that seems to be essential for most of the receptor activities and two hydrophobic transmembrane domains that are separated by a long glycosylated extracellular ATP-binding

domain (Nicke, 2008; Jiang et al., 2013). The C-terminus also plays a role in positioning of the receptor in membrane microdomains (Murrell-Lagnado, 2017) and/or signaling complexes (Kim et al., 2001; Kopp et al., 2019). Functional P2X₇ receptor forms a homo-trimeric structure (Jiang et al., 2013), and hold important physiological functions that distinguish it from the other receptor in its family (North, 2002; Sperlagh et al., 2006).

P2X₇ Receptor Expression and Activation in the CNS

P2X₇ receptors are expressed in a number of cell types in the mammalian system including the peripheral and central nervous systems cells (Burnstock, 2008). In the CNS, the highest concentrations of the receptor is expressed on microglia (Weisman et al., 2012; Bhattacharya and Biber, 2016), astrocytes (Ballerini et al., 1996), and oligodendrocytes (Matute et al., 2007) as well as glutamatergic pyramidal neurons of the hippocampus (Metzger et al., 2017). While, P2X₇R expression on neurons is controversial (Illes et al., 2017), it has been detected on some populations of the spinal cord, cerebellum, hypothalamus, and substantia nigra neurons (Bartlett et al., 2014). Activation of P2X₇ receptor on microglia subsequently prompts activation of the NLRP3 inflammasome, which induces the release of pro-inflammatory cytokines IL-1 β and IL-18, the key mediators of chronic inflammation (Beamer et al., 2016), chronic pain (Bartlett et al., 2014; Beamer et al., 2016; He et al., 2017), neuroinflammation (Monif et al., 2010; Bhattacharya et al., 2013; He et al., 2017), and inflammatory cell death (Leeson et al., 2019). Additionally, activation of P2X₇R on microglia induces the release of TNF α , production of reactive oxygen species (ROS), and in particular oxygen superoxides, which stimulate the NF κ B signaling, release of more pro-inflammatory and pro-apoptotic genes, causing cell-death of surrounding neurons (Parvathenani et al., 2003). Differently from other P2Xs, the P2X₇ receptor is generally known for its inactivity in normal conditions and activation under toxic effects of high extracellular ATP and therefore, is known as a toxic or death-inducing receptor (Di Virgilio, 2020). P2X₇R is only activated after injury, infection, in tumor microenvironments, or in conditions that cause increase in extracellular ATP concentration (in the millimolar range of EC₅₀ \geq 100 μ M) (Surprenant et al., 1996; Donnelly-Roberts et al., 2009; Bianchi et al., 2014; Fiebich et al., 2014). P2X₇ receptor is also activated when ectonucleotidases, which degrade ATP and other nucleotides, are downregulated (Di Virgilio et al., 2009; Bartlett et al., 2014). Short exposure of the receptor to ATP results in the channel opening to small cations, including Ca²⁺, Mg²⁺, Na⁺, and K⁺ (Surprenant et al., 1996; Bartlett et al., 2014), while sustained exposure of the receptor to ATP leads to formation of larger pores that allow for the uptake of large organic ions of up to 900 Da (Volonte et al., 2012), and has shown to result in inflammation, cytotoxicity (Di Virgilio et al., 2001; Liang and Schwiebert, 2005) and cell death (Surprenant et al., 1996; Illes et al., 2017). Additionally, activation of P2X₇R for a longer duration allows for recruitment of pannexin pores (Volonte et al., 2012; Sun et al., 2013; Idzko et al., 2014) allowing the

release of even larger amounts of ATP and leading to activation of caspases (Franke et al., 2012), and has been shown to lead to neuro-pathology and cell death (Bartlett et al., 2014). ATP removal from the receptor within 10–15 min of the addition, have resulted in resealing of the plasma membrane and recovery of normal cell functions (Di Virgilio et al., 2018). The major site for neuronal P2X₇ receptor expression appears to be at presynaptic terminals (Miras-Portugal et al., 2003), resulting in this receptor's participation in the release and regulation of neurotransmitters such as GABA and glutamate (Sperlagh et al., 2002).

P2X₇ Receptor Polymorphism

The P2RX genes encode for the human P2X receptors, mainly the human P2X₇ receptor that is highly polymorphic and contains a large set of single nucleotide polymorphisms (SNPs). Genetic association studies suggest that non-synonymous SNPs (NS-SNPs) in the P2RX genes are important genetic factors in susceptibility of individuals to various diseases (Jiang et al., 2013). The disease-associated NS-SNPs have provided novel insights into disease mechanisms associated with these receptors (Sorge et al., 2012). For instance, ATP-induced influx observed in the T357S polymorphism results in a partial loss of function in human monocytes, lymphocytes, and macrophages, and impaired mycobacterial killing (Shemon et al., 2006; Miller et al., 2011). Similarly, the Q460R polymorphism has been associated with major depressive and bipolar disorders (Barden et al., 2006; Lucae et al., 2006), while ATP-induced ethidium uptake assays in HEK293 cells bearing this SNP showed a reduction in pore formation (Stokes et al., 2010), subsequent reduced Ca²⁺ flux, and diminished channel currents associated with impaired cellular signaling. Moreover, in the P2X₇R knock-in mouse model, which harbors the Q460R polymorphism, if co-expressed with the non-polymorphic variants, results in reductions in sleep quality compared with controls (Aprile-Garcia et al., 2016; Metzger et al., 2017). Similarly, the E496A SNP, has also been associated with cancer metastasis and shown to result in impaired ATP-induced ethidium uptake, Ba²⁺ permeation, and induction of apoptosis in human B-lymphocytes (Gu et al., 2001; Ghiringhelli et al., 2009). Moreover, the human A348T SNP has been shown to induce pore formation and IL-1 β secretion (Stokes et al., 2010), while only the H521Q has been described as neutral (Wiley et al., 2011). By contrast, the loss-of-function I568N polymorphism has been reported to prevent cell surface expression and cytosolic receptor trafficking (Wiley et al., 2003). Importantly, the P451L SNP results in loss-of-function, and is found in many common murine strains (i.e., AKR/J, C3H/HeJ, C57BL/6, C57BL/10, CBA/J, DBA/1, DBA/2, FVB/NJ, and NZO/HILtJ), but is not observed in rats, humans, or wild derived mouse strains (i.e., CAST/EiJ, WSB/EiJ and PWK/PhJ) (Yang et al., 2021). This SNP impairs ATP-induced cation fluxes, pore formation, externalization, and apoptosis (Schwarz et al., 2012; Rissiek et al., 2015), and has been associated with reduced pain sensitivity and inflammation in these model systems which harbor the mutation (Sorge et al., 2012).

P2X₇ Receptor in Different Species

Significant species differences in receptor pharmacology exists in mouse, rat, and human resulting in altered affinity of these receptors for their native ligand (Donnelly-Roberts et al., 2009). Despite this, P2X₇ receptor activation does occur, resulting in dye uptake, IL-1 β release, and initiation of apoptosis (i.e., phosphatidylserine-flip) present in all isoforms (Kopp et al., 2019). In general human, Rhesus macaque, dog, rat, and mouse P2X₇ receptors share 77–85% sequence homology (Surprenant et al., 1996; Bartlett et al., 2014), which results in variations in the receptor affinities for ATP (Table 1).

P2X₇ Receptor in Neurodegeneration

Neurodegenerative diseases (ND) are a cluster of disorders caused by either hereditary or sporadic conditions and characterized by progressive dysfunction of the nervous system that leads to inflammation, gliosis and degeneration of neurons in the brain and/or the spinal cord (Kanellopoulos and Delarasse, 2019). In addition to aging which is the main contributor to the neurodegenerative diseases, abnormal protein aggregation in the brain cells represents a common hallmark in these pathologies as seen in both Alzheimer's disease (AD) and Parkinson's disease (PD), the two most common neurodegenerative disorders. Neuroinflammation, the harmful shared characteristic of neuronal degeneration is the consequence of P2X₇R activation on microglia (Sperlagh et al., 2006; Burnstock et al., 2011; Sperlagh and Illes, 2014) (Figure 1). ATP-dependent P2X₇Rs activation induce necrosis by encouraging membrane pores opening that subsequently promote loss of intracellular proteins and promote activation of the caspase pathway, bringing apoptosis to glial cells. P2X₇Rs driven microglial activation has been implicated in neuroinflammation and neurodegeneration (Monif et al., 2010; Illes et al., 2017). Therefore, P2X₇ receptor modulation has proven to be a promising option for treatment of neurodegenerative diseases and antagonists of this receptor have shown to slow deposition of the amyloid plaque and progression of AD disease in animal model systems (Diaz-Hernandez et al., 2012; Rodrigues et al., 2015).

P2X₇ Receptor in Alzheimer's Disease

The appearance of plaques that consist of extracellular β -amyloid (A β) peptide surrounded by reactive microglial is a major hallmark of AD (Sanz et al., 2009). A β peptide, produced via cleavage of the amyloid precursor protein (APP) by β - and γ -secretases has shown to trigger increases in Ca²⁺, ATP and IL-1 β , induce plasma membrane permeabilization and consecutively damage neurons (Delarasse et al., 2011; Martin et al., 2019) (Figure 1). P2X₇ receptor has shown to be upregulated on activated microglia in the hippocampus of Tg2576 transgenic mice models of AD, and in rat's brain following intra-hippocampal A β injection (Parvathani et al., 2003; Mclarnon et al., 2006). Additionally, P2X₇ receptor deficient microglia was not activated with A β (Sanz et al., 2009); P2X₇ receptor silencing enhanced microglia phagocytosis and clearance of A β (Ni et al., 2013); and receptor deficiency reduced A β plaque, improve synaptic plasticity and resulted in reduction of cognitive

deficits in AD mice model (Chen et al., 2014). The P2X₇R antagonist BBG has shown to decrease A β plaque buildup in hippocampal neurons and improve cognition in J20 mice model of AD (Chen et al., 2014). This effect has been due to the boost in α -secretase activity by BBG (a P2X₇R antagonist) via reduction of GSK3 β activity (Diaz-Hernandez et al., 2012), an APP phosphorylating enzyme (Mclarnon et al., 2006; Ryu and Mclarnon, 2008). However, in AD mice, the absence of P2X₇R did not influence IL-1 β release or non-amyloidogenic fragment sAPP α level (Martin et al., 2019). Furthermore, *in vitro* studies has indicated that stimulation P2X₇R for a short time (~30 minutes) increased α -secretase activity (Delarasse et al., 2011). Therefore, the involvement of P2X₇R in AD is still somewhat unclear and requires more investigation.

P2X₇ Receptor in Parkinson's Disease

P2X₇-induced microglia activation has been detected in PD pathology (Carmo et al., 2014). α -Synuclein binding to, and activating of the P2X₇ receptor on microglia (Jiang et al., 2015; Wilkaniec et al., 2017) have been detected in the brains of patients with PD (Durrenberger et al., 2012; Jiang et al., 2015). P2X₇ receptor has also been shown to participate in the nigrostriatal degeneration in rat model of PD. Motor and memory deficit induced by 6-hydroxydopamine (6-OHDA) animal model of PD was ameliorated by administration of selective P2X₇ receptor antagonists (Marcellino et al., 2010; Carmo et al., 2014; Ferrazoli et al., 2017; Kumar et al., 2017), suggesting P2X₇ receptor play a pro-inflammatory role in microglia activation in PD (Kumar et al., 2017). Additionally, P2X₇ receptor antagonist BBG significantly prevented, and in some cases reversed loss of dopaminergic neurons in the 6-OHDA model (Carmo et al., 2014; Ferrazoli et al., 2017). However, A-438079, another P2X₇R antagonist maintained striatal dopamine; but, did not prevent the loss of dopaminergic cells in the 6-OHDA model (Marcellino et al., 2010). Furthermore, inhibition and/or genetic deletion of P2X₇R did not induce neuro-protection in the MPTP mouse model of Parkinson's disease (Hracsco et al., 2011). More research is needed to further clarify the relationship between P2X₇R activation and PD.

P2X₇ Receptor in Multiple Sclerosis

Multiple sclerosis (MS) is an inherited degenerative disease, which results in focal inflammatory lesions in both white and gray matter (Hagens et al., 2020). MS is caused by immune cell infiltration, loss of oligodendrocytes, axonal damage, demyelination, and neuronal death. Increased level of P2X₇ receptor expression has been detected on microglia (Yiangou et al., 2006), astrocytes (Narcisse et al., 2005), and oligodendrocytes (Matute et al., 2007) of the post-mortem multiple sclerosis patients (Narcisse et al., 2005; Grygorowicz et al., 2010; Burnstock, 2015; Sadovnick et al., 2017). In the acute phase of the disease, elevated expression of P2X₇R have been observed in neurons, astrocytes and oligodendrocytes, causing the release the pro-inflammatory cytokines that has shown to contribute to the progressive inflammation, degeneration and cells death in Experimental Autoimmune Encephalomyelitis (EAE) model of MS (Grygorowicz et al., 2016) (Figure 1).

TABLE 1 | P2X₇ Receptor Antagonist and Radioligands and their application in CNS indications.

P2X ₇ Receptor Antagonists P2X ₇ Receptor Radioligands	K _i (nM)	IC ₅₀ (nM)	Application in CNS indications	References
A-438079	7.1 ± 0.08 (h) 6.7 ± 0.1 (r)	6.0 ± 0.02 (h) 5.9 ± 0.2 (r) 5.5 ± 0.2 (m)	Studied in PD, epilepsy, depression, anxiety, and bipolar disorders. Maintained striatal dopamine; but did not prevent the loss of dopaminergic cells in the 6-OHDA model. Reduced noxious and innocuous evoked activity of different classes of spinal neurons in neuropathic rat model. Suppressed seizures and exhibited neuroprotective effects in immature rats. Reduced induced status epileptic seizure. Exhibited antidepressant effects in chronic unpredictable mild stress (CUMS) mice model of depression.	Nelson et al., 2006 Donnelly-Roberts et al., 2009 Bhattacharya et al., 2013 Marcellino et al., 2010 Hracsco et al., 2011 Mesuret et al., 2014 Henshall et al., 2013 Engel et al., 2016 Yue et al., 2017 Bhattacharya et al., 2018 Park and Kim, 2017 Ribeiro et al., 2019
A-839977		20 - 150 (h, r, m)	Produced antinociception in animal models of inflammatory pain. Reduced thermal hyperalgesia in rats. Produced antihyperalgesia in the model of inflammatory pain in mice. Did not induce antihyperalgesic effects in IL-1 knockout mice.	Florjancic et al., 2008 Honore et al., 2009 Friedle et al., 2010
A-740003 [³ H]A-740003 [¹¹ C]A-740003		40 (h) 20 (r)	Claimed to be brain-permeable, persisting in brain tissues at least for 1 h after administration. But has already been shown to not enter the brain. Reduced neuropathic pain in rat. Radioligand [³ H]A-740003 was used in <i>in vitro</i> study in post mortem brain sections of MS patients and rat brain sections of a rat model of EAE model of MS.	Honore et al., 2006 Janssen et al., 2014 Beaino et al., 2017 Zarrinmayeh and Territo, 2020
A-804598 [³ H]A-804598 [¹⁸ F]EFB	8.0 ± 0.04 (h) 8.8 ± 0.06 (r)	7.7 ± 0.13 (h) 6.8 ± 0.17 (r) 7.0 ± 0.06 (m)	Prevented stress-induced depressive-like and anxiety-like behaviors in mice and rats. Induced antidepressant-like effects in FST mice model of depression. Failed to reverse behavioral changes caused by foot shocks in rat. Decreased hepatic inflammation in mice fed a high fat diet and ethanol. Reduced inflammatory markers in hippocampus without altering many neurotransmitters. Converted to [³ H]A-804598 radioligand to study recombinant rat receptors expressed in 1321N1 cells. PET radioligand [¹⁸ F]EFB, a fluorinated analog of A-804598, showed limited yet quantifiable brain penetration.	Donnelly-Roberts et al., 2009 Bhattacharya et al., 2013 Karasawa and Kawate, 2016 Fabrizio et al., 2017 Ly et al., 2020 Ruiz-Ruiz et al., 2020 Freire et al., 2019 Ribeiro et al., 2019 Zarrinmayeh and Territo, 2020
GSK1482160 [¹¹ C]GSK1482160 [¹⁸ F]IUR-1601	2.63 (h)	8.5 (h) 6.5 (r)	Brain penetrable. Entered phase I for treating inflammatory pain in arthritis, but failed to proceed further. PET radioligand [¹¹ C]GSK1482160 showed high affinity to brain P2X ₇ R. PET radioligand [¹⁸ F]IUR-1601 showed similar affinity and selectivity for P2X ₇ R as [¹¹ C]GSK1482160.	Abdi et al., 2010 Ali et al., 2013 Territo et al., 2017 Glaxosmithkline, 2009 Zarrinmayeh and Territo, 2020 Gao et al., 2018
SMW139 [¹¹ C]SMW139	32 (h)		Entered clinical trial for evaluation of neuroinflammation in MS patients. PET radioligand [¹¹ C]SMW139 was developed to study <i>in vivo</i> marker of neuroinflammation in multiple sclerosis.	Janssen et al., 2018 Hospital, 2019 Hagens et al., 2020 Zarrinmayeh and Territo, 2020

(Continued)

TABLE 1 | Continued

P2X ₇ Receptor Antagonists P2X ₇ Receptor Radioligands	K _i (nM)	IC ₅₀ (nM)	Application in CNS indications	References
JNJ-42253432	7.9 ± 0.08 (h) 9.1 ± 0.07 (r)	7.7 ± 0.07 (h) 7.8 ± 0.1 (r) 7.1 ± 0.2 (m)	Brain permeable (Brain/Plasma = 1). Significantly reduced severe convulsive seizures after one-week treatment.	Lord et al., 2014 Amhaoul et al., 2016
JNJ-47965567	7.9 ± 0.07 (h) 8.7 ± 0.07 (r)	8.3 ± 0.08 (h) 7.2 ± 0.08 (r) 7.5 ± 0.1 (m)	Brain penetrable. Blocked the Bz-ATP induced IL-1β release. Attenuated amphetamine-induced hyperactivity. Efficacious in rat model of neuropathic pain. Reduced temporal lobe epilepsy. Produced long-lasting delay in kindling development. Chronic administration to SOD mice model of ALS modified disease progression in female animals, but had no effect in male animals.	Bhattacharya et al., 2013 Jimenez-Pacheco et al., 2016 Ruiz-Ruiz et al., 2020 Fabrizio et al., 2017 Ly et al., 2020
JNJ-55308942	8.12 ± 0.08 (h) 8.5 ± 0.04 (r)	7.87 ± 0.2 (h) 7.81 ± 0.2 (r) 7.55 ± 0.5 (m) 7.96 ± 0.1 (mk) 7.72 ± 0.06 (d)	Brain-penetrant (brain/plasma = 1) Engaged brain targets, modulated microglial activation and reduced IL-1β release. Efficacious in models of anhedonia in rodents. Entered phase I clinical trial and is currently in clinical development to study P2X ₇ R occupancy in the brain. In clinical trial to assess the safety, tolerability, and pharmacokinetics in healthy participants after administration of single and multiple oral doses.	Ali et al., 2013 Letavic et al., 2017 Bhattacharya, 2018 Chrovian et al., 2018 Letavic et al., 2017 Bhattacharya, 2018 Nv, 2017b Watch, 2021
JNJ-54175446	8.3 ± 0.1 (h) 8.3 ± 0.05 (r)	8.46 ± 0.36 (h) 8.81 ± 0.24 (r)	Brain-penetrant. Entered phase I clinical trial to study safety, tolerability, and pharmacodynamics in participants with major depressive disorder.	Letavic et al., 2017 Kolb et al., 2019 Bhattacharya, 2018 Cctu-Core, 2019
JNJ-64413739 [¹⁸ F]JNJ-64413739	15.9 (h) 2.7 (rat cortex)	1.0 ± 0.2 (h) 2.0 ± 0.6 (r)	Potent, selective and brain permeable. PET radioligand [¹⁸ F]JNJ-64413739 was used to study P2X ₇ R in human brain, and can be used for testing target engagement of other brain permeable P2X ₇ antagonists.	Kolb et al., 2019 Koole et al., 2019 Nv, 2017a Zarrinmayeh and Territo, 2020
JNJ-54232334 [³ H] JNJ-54232334	7.8 ± 0.05 (h) 9.3 ± 0.1 (r)	9.5 ± 0.02 (h) 7.5 ± 0.02 (r)	Higher levels of P2X ₇ <i>ex vivo</i> occupancy were measured using [³ H] JNJ-54232334 due to less non-specific binding. Radioligand [³ H] JNJ-54232334 showed improved properties over [³ H] A-804598.	Lord et al., 2015 Rudolph et al., 2015 Zarrinmayeh and Territo, 2020
JNJ-54140515	7.7 (h) 8.9 ± 0.01 (r)	7.7 ± 2.6 (h) 8 ± 2.9 (r)	Readily crossed the blood-brain barrier. Studied brain P2X ₇ R occupancy. Has shown a 10-fold increase in brain penetration over JNJ-54232334.	Lord et al., 2015 Bhattacharya and Jones, 2018 Hempel et al., 2013 Rudolph et al., 2015
JNJ-54173717 [¹¹ C]JNJ-54173717	1.6 ± 0.1 nM rat cortex	4.2 ± 0.01 (h) 7.6 ± 0.01 (r)	Brain-penetrable. Studied in models of depression, epilepsy and PD. PET radioligand [¹¹ C]JNJ-54173717 used for studying the brain P2X ₇ R functions in both rats and nonhuman primates. [¹¹ C]JNJ54173717 was also studied in healthy volunteers and PD patients in human.	Ory et al., 2016 Rudolph et al., 2015 Savall et al., 2015 Van Weehaeghe et al., 2019. Zarrinmayeh and Territo, 2020
JNJ-54166060	7 (h) 8 (r)	4 (h) 115 (r) 72 (m)	Brain-penetrable with brain/plasma ratio (~3). Bioavailable P2X ₇ R antagonist with moderate clearance. Exhibited dose dependent occupancy in the rat brain with an ED ₅₀ = 2.3 mg/kg.	Swanson et al., 2016

P2X₇R, P2X₇ receptor; h, human; r, rat; m, mouse; d, dog; mk, monkey; PD, Parkinson's disease; 6-OHDA, 6-hydroxydopamine; CUMS, chronic unpredictable mild stress; PET, positron emission tomography; Bz-ATP, 3'-O-(4-benzoylbenzoyl)-adenosine 5'-triphosphate; SOD, superoxide dismutase; ALS, Amyotrophic Lateral Sclerosis.

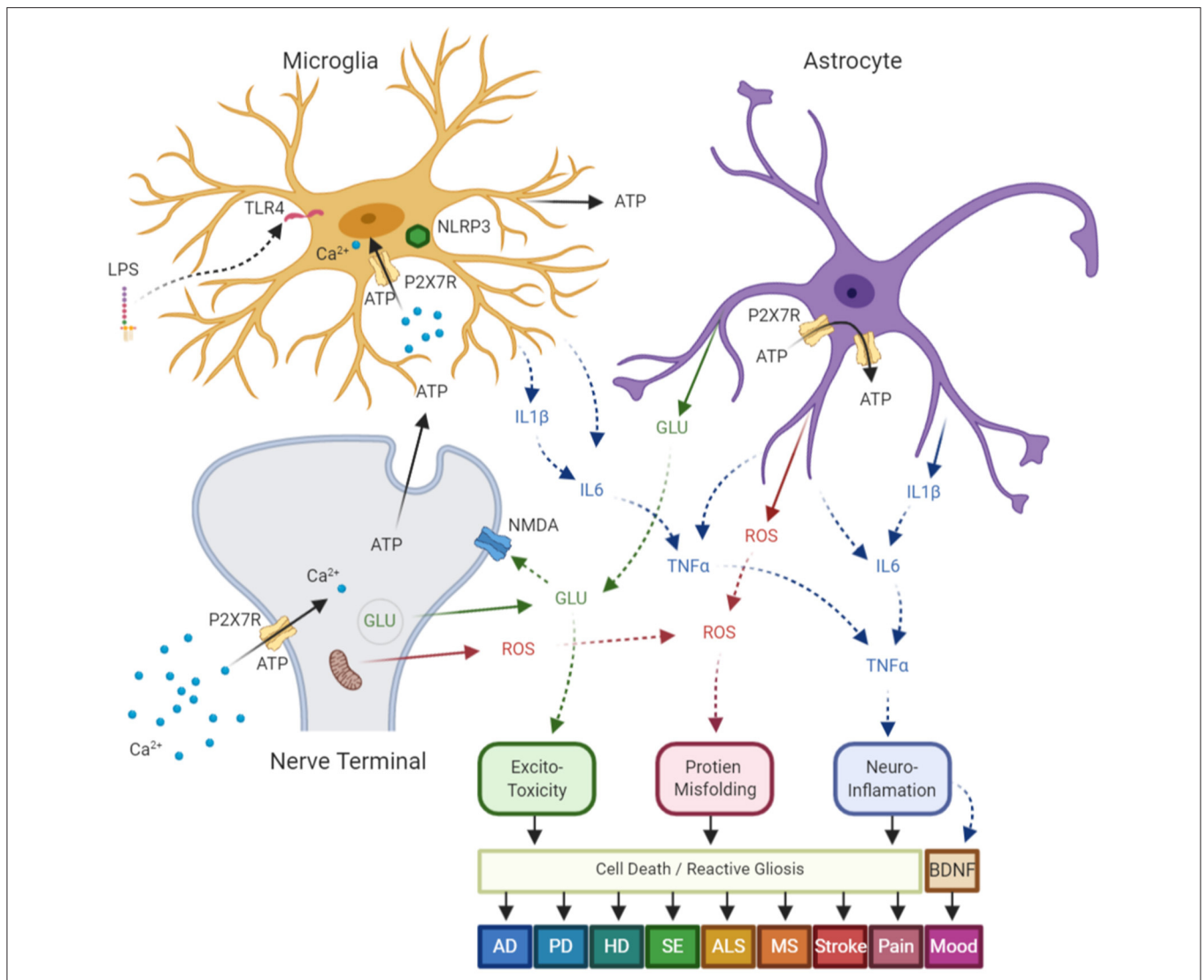


FIGURE 1 | Diagram of common neurologic diseases mediated via P2X₇ receptors (P2X₇R)- in central nervous system (CNS). P2X₇R are expressed on a number of CNS cells, which include: nerve terminals, astrocytes, and microglia. These receptors are upregulated in response to stress signals such as mechanical injury, bacterial or chemical toxins, and hypoxia/ischemia, and lead to a self-amplified release of ATP, which results in further activation of P2X₇R on neighboring cells. Following ATP dependent Ca²⁺ influx through the receptor ion channel complex, P2X₇R activation results in: (1) releases glutamate from nerve terminals and astrocytes by both exo and endocytotic mechanisms, which results in excite-toxicity; (2) synthesis and post-translational modification of pro-interleukin-1β (pro-IL-1β) which leads to mature IL-1β and ultimate release via the NLRP3 inflammasome. This activation then leads to other cytokine release and activation leading to neuroinflammation; (3) enhance reactive oxygen and nitrogen species which results in neuronal damage and protein misfolding; which in turn (4) leads to cell death and reactive astrogliosis; and (5) the downregulation of brain-derived neurotrophic factor (BDNF) and alterations in neuronal plasticity. The aforementioned mechanisms have been shown independently, or in concert, to contribute to disease pathology in Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), status epilepticus (SE), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), stroke, pain, and mood disorders. ATP, Adenosine Triphosphate; GLU, glutamate; ROS, reactive oxygen species. Figure adapted from Sperlagh and Illes (2014) with modification, and generated using the online software at BioRender.com.

Remarkably, P2X₇R expression is down-regulated on peripheral monocytes of MS patients during the acute phase of the disease (Amadio et al., 2017). This behavior of the receptor has created a gap in understanding of the receptor function in MS and the treatment of the disease. Several cohort studies have acknowledged an association of loss-of-function, minor allele frequency P2X₇ receptor SNP, rs28360457, coding for R307G with protection against MS and a gain-of-function

haplotype rs208294, coding for H155T, which increases risk of MS (Oyanguren-Desez et al., 2011; Gu et al., 2015). Additionally, in a case-control study, elevated frequency of a gain-of-function SNP, rs17525809 coding for A76V in MS patients was observed (Oyanguren-Desez et al., 2011), suggesting that P2X₇R variants may play a significant role in the pathogenesis of MS disease. Moreover, studies have reported that P2X₇ receptor antagonist decreased astrogliosis, abridged demyelination, and

improved neurological symptoms in an EAE rat model of MS (Grygorowicz et al., 2016). Some of these antagonists, including BBG, have shown to improve motor deficits in EAE by decreasing demyelination that consequently improves axonal conduction (Bartlett et al., 2017).

P2X₇ Receptor in Huntington's Disease

Huntington's disease (HD) is an inherited neurodegenerative disorder caused by progressive degeneration of nerve cells in the cortex and striatum of the brain (Oliveira-Giacomelli et al., 2018). Patients with HD experience progressive cognitive decline in addition to motor and psychological dysfunction. Brains of Tet/HD94 and R6/1 mice, which are genetic models of HD, have shown increased mRNA and protein levels of P2X₇ receptor (Diaz-Hernandez et al., 2009), and BBG has shown to prevent loss of body weight and motor coordination deterioration in the R6/1 mouse model of HD (Diaz-Hernandez et al., 2009). Importantly, recent work by Olla et al. (2020) has shown that P2X₇R has been found upregulated, and in some case showed altered splicing, in the brain of HD subjects.

P2X₇ Receptor in Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by decline and loss of motor neurons (i.e., both upper and lower) (Oliveira-Giacomelli et al., 2018). Inflammation and autophagy play critical roles in the pathogenesis of ALS, while several studies have implicated the role of P2X₇Rs in pathogenesis (Volonte et al., 2016). Increased expression of P2X₇Rs has been detected in microglia (D'ambrosi et al., 2009; Rudnick et al., 2017) or astrocytes (Gandelman et al., 2010; Apolloni et al., 2014) isolated from superoxide dismutase 1 (SOD1^{G93A}) mouse model of ALS, and application of the P2X₇R antagonist BBG improved spinal cord pathology and ameliorated the disease in these mice (Apolloni et al., 2014; Bartlett et al., 2017). Additionally, more potent and selective P2X₇R antagonists such as A804598 and JNJ-47965567 have provided some beneficial effects in ALS mouse models (Fabrizio et al., 2017; Ly et al., 2020; Ruiz-Ruiz et al., 2020). Furthermore, ATP-induced activation of P2X₇R has shown to activate kinase ERK1/2 and NOX2 in microglia of SOD1^{G93A} mice (Apolloni et al., 2013). Nevertheless, P2X₇R down-regulation has also been detected in peripheral circulating monocytes of ALS patients (Liu et al., 2016), which contrasts the up-regulation of the receptor in spinal cord and nervous tissues of post-mortem ALS patients post-mortem (Yiangou et al., 2006). Thus far, studies of the P2X₇R function in ALS has not been conclusive and it seems that this receptor plays a dual role in ALS (Volonte et al., 2020).

P2X₇ Receptor in Epilepsy

Epilepsy can occur from an insult to the brain (stroke and head trauma such as in TBI), repeated episodes of status epilepticus or genetic malfunction (Rees, 2010; Pitkanen and Lukasiuk, 2011). In adults, the most common form of acquired epilepsy is temporal lobe epilepsy (TLE) which is characterized by a pattern of reactive gliosis and selective neuronal loss, a phenomenon

that is also called hippocampal sclerosis (Chang and Lowenstein, 2003). P2X₇ receptor activation and upregulation has been associated with TLE and inhibition of receptor activity with antagonist JNJ-47965567 has shown to reduce TLE both during and past the time of drug presence (Jimenez-Pacheco et al., 2016). Hippocampal sections of these treated mice displayed reduction of activated microglia and astrocytes, suggesting anticonvulsant and anti-epilepsy property of P2X₇R antagonists (Jimenez-Pacheco et al., 2016). BBG treatment of rats with spontaneous recurrent seizures helped treatment by reducing the P2X₇ receptor expression (Fischer et al., 2016; Song et al., 2019). Additionally, significant reduction in severe convulsive seizures was observed after treatment with P2X₇R antagonist JNJ-42253432 for a week (Amhaoul et al., 2016). Another P2X₇R antagonist A-438079 suppressed seizures and exhibited neuroprotective effects in immature rats (Mesuret et al., 2014). P2X₇R antagonists BBG and A-438079 have also been shown to reduce status epileptic seizure caused by unilateral injection of kainic acid into the mice amygdala in rodent model of temporal lobe epilepsy (Henshall et al., 2013; Engel et al., 2016). Additionally, several lines of evidence confirms increase of P2X₇R in hippocampus of patients with pharmaco-resistant temporal lobe epilepsy and in hippocampal subfields of mice that experienced status epilepticus (Jimenez-Pacheco et al., 2016). These studies support the role of P2X₇ receptor antagonists in treating epilepsy, including drug-resistant epilepsy (Beamer et al., 2016; Cieslak et al., 2017; Rodriguez-Alvarez et al., 2017). Furthermore, P2X₇R have been recognized as targets for treatment of hypoxic/ischemic encephalopathy (Beamer et al., 2017). Recent anticonvulsant studies using P2X₇R antagonists, which include BBG, AFC-5128, JNJ-47965567, and tanshinone IIA sulfonate (traditional Chinese herbal medicine, TIIAS) in animal models have illustrated the potential of these agents to modulate seizures. Remarkably in the pentylenetetrazol-kindling (PTZ-kindling) and maximal electroshock seizure (MES) models threshold test, none of the compounds showed anticonvulsant effects when given by itself; however, when given in combination with carbamazepine, AFC-5128 and JNJ-47965567 increased the threshold in the MES test (Fischer et al., 2016). Similarly, in the PTZ-kindling rat model anti-epileptogenic activities for BBG and TIIAS were observed, whereas the P2X₇R inhibitors AFC-5128 and JNJ-47965567 showed long-lasting delay in kindling development, while results in fully kindled animal showed reductions in seizure stage (Fischer et al., 2016). In the case of epilepsy, P2X₇ receptors have three distinct functions depending on the situations of the extracellular environment. P2X₇R initiates cell death in the presence of elevated extracellular ATP, while mediates calcium signal transduction in response to ATP that regulates proliferation and differentiation. Finally, P2X₇R activation promotes phagocytosis in the absence of extracellular ATP (Zheng et al., 2017).

P2X₇ Receptor in Ischemia, Stroke and Trauma

Ischemic stroke, one of the major type of strokes, results from oxygen and glucose deprivation that cause cell death (Hempel

et al., 2013; Yoshida et al., 2015). Neuroinflammation and over expression of the P2X₇ receptor has been detected in this ischemic stroke (**Figure 1**) and the neuroprotective effects of P2X₇R suppression has been proven to be successful in this type of stroke (Melani et al., 2006; Eyo et al., 2013). The ATP degrading enzyme apyrase and P2X₇ receptor antagonists have shown to relieve the damage initiated by ischemia and improve action potential recovery (Domercq et al., 2010). In hemorrhagic stroke, where a sudden rupture of cerebral blood vessels and cell death promote quick release and accumulation of large quantity of ATP, P2X₇R inhibition has been promising in prevention of acute neuroinflammation and cell death (Chen et al., 2013a,b). Striatal P2X₇R has also shown to intensify neuroinflammation and brain damage in intracerebral hemorrhage (ICH) possibly via activation of NLRP3 inflammasome and release of IL-1 β /IL-18. The P2X₇R antagonist BBG treatment following ICH has shown to downregulate the release of these proinflammatory cytokines (Feng et al., 2015). Inhibition of P2X₇Rs improved global cerebral ischemia/reperfusion injury. This positive effect is evidenced by increase in survival rate, reduction of neuronal death in the hippocampal CA1 region, and improvement in learning memory (Chu et al., 2012; Yu et al., 2013). P2X₇Rs are also involved in cerebral neurological damage and edema after traumatic brain injury. Application of P2X₇R antagonist BBG has resulted in decreased expression of Glial fibrillary acidic protein (GFAP) and reduction of aquaporin-4, which is an astrocytic water channel that promotes cellular edema (Kimble et al., 2012; Leeson et al., 2019).

P2X₇ Receptor in Depression

P2X₇ receptor activation by ATP followed by NLRP3 induced IL-1 β release that results in neuroinflammation are major contributors of neuropsychiatric disorders, especially depression (Adinolfi et al., 2018; Bhattacharya and Jones, 2018; Franklin et al., 2018; Li and Barres, 2018; Liu et al., 2018). An anti-inflammatory Chinese medicine, Chrysophanol has shown an anti-depressant effects by mediation of the P2X₇R/NF κ B signaling pathway (Zhang et al., 2016) supporting the role of P2X₇ receptor participation in depression. Additionally, the P2X₇R knock out mice did not show signs of depression in forced swim and tail suspension tests (Basso et al., 2009; Leeson et al., 2019), consistent with this hypothesis. Some brain penetrable P2X₇R antagonists such as BBG and A-438079 have exhibited antidepressant effects in chronic unpredictable mild stress (CUMS) mice model of depression by inhibiting the activation of P2X₇/NLRP3/IL-1 β pathway (Yue et al., 2017; Bhattacharya, 2018). Furthermore, stress is known to prompt production of excessive glutamate that is proven to stimulate large ATP release from astrocytes, activating P2X₇R and subsequently increasing IL-1 β level in the brain (Faloia et al., 2012).

P2X₇ RECEPTOR LIGANDS

Agonists

Two major agonist of the P2X₇ receptor are ATP and BzATP, which is 10–30 fold more potent than ATP (Surprenant et al., 1996; Beigi et al., 2003), but also activates P2X₁ and P2X₃

receptors (De Marchi et al., 2016). Activation of P2X₇R by ATP induces neuroinflammation that results in pathogenesis of many diseases of CNS, suggesting significant potential for P2X₇ receptor antagonists to combat the diseases of neuroinflammatory origin (Mehta et al., 2014). Therefore, there has been extensive effort to develop several potent and selective P2X₇R antagonists.

Antagonists

There are two non-selective ATP derivative antagonists TNP-ATP and periodate-oxidized ATP (oATP) with high μ mol potencies (Beigi et al., 2003; Di Virgilio, 2003; De Marchi et al., 2016). The major group of P2X₇R antagonists are the non-ATP based compounds. Depending on their interaction with the receptors, some are orthostatic antagonists that bind competitively to the ATP binding pocket, while majority are allosteric antagonists that bind to other locations than the ATP-binding site, and reduce ATP binding affinity to the receptor (De Marchi et al., 2016).

First Generation Antagonists

The first generation of non-ATP antagonists were mostly designed for *in vitro* study of the receptor and included compounds such as: Reactive Blue 2 (Bartlett et al., 2014), Suramin (Leff et al., 1990), Brilliant Blue G (BBG) (Jiang et al., 2000), the irreversible PPADS (Jiang et al., 2000), and KN-62 (Gever et al., 2006; Bartlett et al., 2014). Among this list, KN-62 (Bartlett et al., 2014) and the non-specific BBG exhibited brain permeability property (Wang et al., 2004, 2017; Donnelly-Roberts et al., 2009; Peng et al., 2009; Jo and Bean, 2011; Carmo et al., 2014). BBG is currently the most widely used P2X₇R antagonist in research (Peng et al., 2009). BBG has also shown to block pannexin-1 (Bin Dayel et al., 2019). Additional primary antagonists include Chelerythrine and other benzophenanthridine alkaloids (Shemon et al., 2004), CAY10593 which is a synthetic phospholipase D blocker (Pupovac et al., 2013), Ca²⁺ and Mg²⁺ cations (Jiang, 2009). Some of the aforementioned antagonist exhibited poor stability and less desirable pharmacokinetics properties, preventing their use for *in vivo* studies of the receptor (Jiang, 2012).

Second Generation Antagonists

The second generation of the P2X₇R antagonists were developed aiming for higher potency, selectivity, *in vivo* stability and possible equal potency at different species. Among this list are the tetrazole-based compounds A-438079 and A-839977 (**Table 1**). Antagonist A-438079 [$K_i = 7.1 \pm 0.08$ (h) and $K_i = 6.7 \pm 0.1$ (r); $IC_{50} = 6.0 \pm 0.02$ (h), $IC_{50} = 5.9 \pm 0.2$ nM (r), and $IC_{50} = 5.5 \pm 0.2$ nM (m)] (Nelson et al., 2006; Donnelly-Roberts et al., 2009; Bhattacharya et al., 2013) has been studied in PD (Marcellino et al., 2010) and while maintained striatal dopamine, it did not prevent the loss of dopaminergic cells in the 6-OHDA model of PD (Marcellino et al., 2010). It suppressed seizures and exhibited neuroprotective effects in immature rats (Mesuret et al., 2014). A-438079, also exhibited antidepressant effects in chronic unpredictable mild stress (CUMS) mice model of depression (Yue et al., 2017; Bhattacharya, 2018). Another tetrazole based

antagonist A-839977 [IC₅₀ = 20–150 nM (h, r, m)] reduced thermal hyperalgesia in rats and produced antihyperalgesia in the CFA model of inflammatory pain in mice (Florjancic et al., 2008; Honore et al., 2009; Friedle et al., 2010). However, A-839977 did not induce any antihyperalgesic effects in IL-1 knockout mice (Honore et al., 2009).

Another class of antagonists in the second generation group are the cyanoguanidine based compounds A-740003 and A-804598 (Table 1). Cyanoguanidine A-740003 [IC₅₀ = 40 nM (h) and IC₅₀ = 20 nM (r)] dose-dependently reduces neuropathic pain in rat (Honore et al., 2006). This antagonist was converted to [¹¹C]A-740003 PET radioligand and while has been shown not to enter the brain (Janssen et al., 2014), its tritiated analog [³H]A-740003 was used in an *in vitro* study in post mortem brain sections of MS patients and rat brain sections of a rat model of EAE model of MS (Beaino et al., 2017). Antagonist A-804598 [K_i = 8.0 ± 0.04 nM (h), K_i = 8.8 ± 0.06 nM (r); IC₅₀ = 7.7 ± 0.13 nM (h), IC₅₀ = 6.8 ± 0.17 nM (r), IC₅₀ = 7.0 ± 0.06 nM (m)] (Donnelly-Roberts et al., 2009; Bhattacharya et al., 2013; Karasawa and Kawate, 2016) was used to study the functional role of P2X₇R in inflammatory response in the liver and brain of the C57BL/6J mice fed a high fat diet and those with chronic ethanol consumption. It showed reduction of inflammatory markers in hippocampus without altering many neurotransmitters and decrease in hepatic inflammation but not steatosis (Freire et al., 2019). A-804598 has also shown to induce antidepressant-like effects in the FST mice model of depression (Ribeiro et al., 2019), but failed to reverse behavioral changes caused by foot shocks in rat (Catanzaro et al., 2014). Tritiated A-804598 ([³H]A-804598) was also prepared and utilized as a P2X₇R radioligand to study recombinant rat receptors expressed in 1321N1 cells (Donnelly-Roberts et al., 2009). Fluorinated analog of A-804598 was also converted to F-18 PET radioligand [¹⁸F]EFB that showed limited yet quantifiable brain penetration (Zarrinmayeh and Territo, 2020).

Other second-generation antagonists included: AZD9056 (Keystone et al., 2012); AZ-11645373 (Stokes et al., 2006; Syed and Kennedy, 2012; Mehta et al., 2014); AZ-10606120 (Michel et al., 2007); GW791343 (Felix et al., 2012); GSK314181A (Broom et al., 2008); GSK1482160 (Ali et al., 2013); CE-224,535 (Stock et al., 2012); AFC-5128 (Fischer et al., 2016); SMW139 (Hansen et al., 2018); and EVT-401 (Zhu et al., 2017). Among the list of antagonists, AZD9056 (Keystone et al., 2012) and CE-224,535 (Stock et al., 2012) entered clinical trials in patients with rheumatoid arthritis and while passed acceptable safety and tolerability hurdles, they failed in phase II efficacy (Stokes et al., 2006; Keystone et al., 2012). EVT-401 also entered phase I clinical trial in patients with rheumatoid arthritis, but did not advance further (Zhu et al., 2017; Evotec, 2020). GSK1482160 entered phase I clinical trials for treating inflammatory pain in arthritis, but failed to proceed beyond phase I (Glaxosmithkline, 2009). GSK1482160 was also converted to C-11 PET radioligand [¹¹C]GSK1482160 and showed high affinity (K_d = 1.15 ± 0.12 nM) in targeting P2X₇R (Territo et al., 2017). Recent work from our lab has also developed an F-18 PET radioligand [¹⁸F]IUR-1601 that shows similar affinity and selectivity [K_i = 4.31 nM (h), IC₅₀ = 7.86 nM (h)] for P2X₇R as [¹¹C]GSK1482160

(Gao et al., 2018). Another benzamide, compound, SMW139 has also been converted to PET radioligand [¹¹C]SMW139 to study the P2X₇R receptor expression on pro-inflammatory microglia (Janssen et al., 2018). [¹¹C]SMW139 proceeded to first in-man study to evaluate its potential in identifying *in vivo* neuroinflammation in MS patients (Hospital, 2019; Hagens et al., 2020).

New Generation Antagonists

The newer generation of the P2X₇R antagonists have specifically been designed to penetrate CNS and enable evaluation of the P2X₇R functions in the CNS disorders including neuroinflammation (Bhattacharya, 2018). This collection of highly potent and selective antagonists has been clustered into four groups based on their chemical scaffolds. They are presented in Table 1 and are briefly mentioned herein:

Group 1

The phenylpiperazine based compounds JNJ-42253432 and JNJ-47965567. JNJ-42253432 is a potent P2X₇R antagonist [K_i = 7.9 nM ± 0.08 (h) and K_i = 9.1 ± 0.07 nM (r); IC₅₀ = 7.7 ± 0.07 nM (h), IC₅₀ = 7.8 ± 0.1 nM (r) and IC₅₀ = 7.1 ± 0.2 nM (m)] that penetrates into the CNS (Brain/Plasma = 1) (Lord et al., 2014). With its excellent pharmacokinetic and pharmacodynamic properties, JNJ-42253432 has shown to block the Bz-ATP-induced release of IL-1β in a concentration-dependent manner (Lord et al., 2014). Significant reduction in severe convulsive seizures was also detected after one-week treatment with JNJ-42253432 (Amhaoul et al., 2016). Another centrally permeable phenylpiperazine based antagonist is JNJ-47965567 that has shown high potency [K_i = 7.9 ± 0.07 nM (h) and K_i = 8.7 ± 0.07 nM (r); IC₅₀ = 8.3 ± 0.08 nM (h), IC₅₀ = 7.2 ± 0.08 nM (r), and IC₅₀ = 7.5 ± 0.1 nM (m)]. It exhibited target engagement in rat brain (EC₅₀ = 78 ± 19 ng/ml in P2X₇R autoradiography) and functionally blocked the Bz-ATP induced IL-1β release (Bhattacharya et al., 2013). JNJ-47965567 reduced amphetamine-induced hyperactivity and showed substantial efficacy in neuropathic rat model of pain (Bhattacharya et al., 2013). JNJ-47965567 significantly reduced temporal lobe epilepsy characterized by a pattern of selective neuronal loss and reactive gliosis (Jimenez-Pacheco et al., 2016). Chronic administration of JNJ-47965567 (4X/week) to SOD mice model of ALS modified disease progression in female animals, but had no effect in male animals, suggesting partial effect of P2X₇R in progression of ALS (Ruiz-Ruiz et al., 2020).

Group 2

The 1,2,3-triazolo based antagonists include JNJ-55308942, JNJ-54175446, and JNJ-64413739. JNJ-55308942 is a P2X₇R antagonist with high potency [K_i = 8.12 ± 0.08 nM (h) and 8.5 ± 0.04 nM (r)] (Ali et al., 2013; Letavic et al., 2017; Bhattacharya et al., 2018; Chrovian et al., 2018). JNJ-55308942 is a brain-penetrant antagonist (brain/plasma = 1) that has shown a prominent pharmacology at recombinant human, rat, mouse, macaque, and dog P2X₇R [IC₅₀ = 7.87 ± 0.2 nM (h), IC₅₀ = 7.81 ± 0.2 nM (r), IC₅₀ = 7.55 ± 0.5 nM (m), IC₅₀ = 7.96 ± 0.1 nM (mm), and IC₅₀ = 7.72 ± 0.06 (d)] (Letavic et al., 2017;

Nv, 2017b; Bhattacharya et al., 2018). JNJ-55308942 has shown to engage brain targets, modulates microglial activation, reduce IL-1 β release and has been efficacious in models of anhedonia in rodents (Letavic et al., 2017; Bhattacharya et al., 2018; Chrovian et al., 2018). JNJ-55308942 entered phase I of clinical trial in 2017 to assess the safety, tolerability, and pharmacokinetics in healthy participants after administration of single and multiple oral doses (Nv, 2017a; Watch, 2021). Another 1,2,3-triazolo based P2X₇R antagonist is the highly potent and brain penetrant JNJ-54175446 [$K_i = 8.3 \pm 0.1$ nM (h) and $K_i = 8.3 \pm 0.05$ nM (r), $IC_{50} = 8.46 \pm 0.36$ (h), and $IC_{50} = 8.81 \pm 0.24$ (r)] with dose-dependent occupancy ($ED_{50} = 0.46$ mg/kg, corresponding to plasma $EC_{50} = 105$ ng/ml) (Kolb et al., 2019). JNJ-54175446 has also entered phase I of clinical trial to study its antidepressant activity (Bhattacharya, 2018; Cctu-Core, 2019). Third 1,2,3-triazole based antagonist is the selective and potent JNJ-64413739 [$K_i = 15.9$ nM (h) and $K_i = 2.7$ nM at rat cortex; $IC_{50} = 1.0 \pm 0.2$ nM (h) and $IC_{50} = 2.0 \pm 0.6$ nM (r)] (Kolb et al., 2019; Koole et al., 2019). The F-18 PET radioligand of JNJ-64413739, [¹⁸F]JNJ-64413739 was developed to study brain P2X₇ function (Zarrinmayeh and Territo, 2020). Micro-dosing in mice showed a 38% lower compound binding in P2X₇R knock out compared to wild type mice receptor and the uptake of [¹⁸F]JNJ-64413739 was reduced by JNJ-54175446 in a dose-related manner in a monkey PET study (Nv, 2017a; Kolb et al., 2019; Koole et al., 2019). Study of [¹⁸F]JNJ-64413739 in healthy human volunteer also showed the tracer to be an appropriate PET ligand for quantification of P2X₇R expression in the human brain (Table 1) (Nv, 2017a; Kolb et al., 2019; Koole et al., 2019).

Group 3

The 1,2,4-triazolo based P2X₇R antagonists are three close analogs JNJ-54232334, JNJ-54140515, and JNJ-54173717. The high affinity JNJ-54232334 [$K_i = 7.8 \pm 0.05$ nM (h) and $K_i = 9.3 \pm 0.1$ nM (r); $IC_{50} = 9.5 \pm 0.02$ nM (h), and $IC_{50} = 7.5 \pm 0.02$ nM (r)] was tritiated to produce [³H]JNJ-54232334 that reached saturable binding, and equilibrium dissociation rate constant (K_d) of 4.9 ± 1.3 nM (Lord et al., 2015; Rudolph et al., 2015). The specific binding of [³H]JNJ-54232334 in rat brain sections was enhanced compared to that of the [³H] A-804598 as a result of low non-specific binding (Lord et al., 2015). JNJ-54140515, an analog of JNJ-54232334 with comparable *in vitro* pharmacology [$K_i = 7.7$ nM (h) and $K_i = 8.9 \pm 0.01$ nM (r); $IC_{50} = 7.7 \pm 2.6$ nM (h) and $IC_{50} = 8.0 \pm 2.9$ nM (r)], readily crossed the blood-brain barrier and facilitated the high level of brain P2X₇R occupancy (Lord et al., 2015; Bhattacharya and Jones, 2018). While similar in potency, JNJ-54140515 has shown a 10-fold increase in brain penetration over JNJ-54232334 (Hempel et al., 2013; Lord et al., 2015; Rudolph et al., 2015). JNJ-54173717 is another high affinity P2X₇R antagonists at human and rat receptor [$K_i = 1.6 \pm 0.1$ nM in rat cortex; $IC_{50} = 4.2 \pm 0.01$ nM (h) and $IC_{50} = 7.6 \pm 0.01$ nM (r)]. JNJ-54173717 has good drug-like properties and high P2X₇ receptor occupancy in rat subsequent oral administration (Ory et al., 2016). JNJ-54173717 has been studied in models of depression, epilepsy and PD (Rudolph et al., 2015; Van Weehaeghe et al., 2019). JNJ-54173717 was also converted to PET radioligand [¹¹C]JNJ-54173717 for

studying the brain P2X₇R functions in both rats and nonhuman primates (Ory et al., 2016). This tracer crossed the blood-brain barrier, and was cleared from plasma via hepatobiliary pathways in rat bio-distribution study (Rudolph et al., 2015; Savall et al., 2015; Ory et al., 2016). [¹¹C]JNJ54173717 was studied in healthy volunteers and PD patients in human and showed selectivity for P2X₇R (Van Weehaeghe et al., 2019).

Group 4

The imidazolopyridin JNJ-54166060 is another P2X₇R antagonist with high potency [$K_i = 7$ nM (h) and $K_i = 8$ nM (r); $IC_{50} = 4$ nM (h), $IC_{50} = 115$ nM (r), and $IC_{50} = 72$ nM (m)] that has shown great oral bioavailability and low-moderate clearance in preclinical animal models. It has brain penetrable property, exhibiting a significant brain/plasma ratio (~ 3) with $ED_{50} = 2.3$ mg/kg when dosed orally (Swanson et al., 2016).

Controversies, Research Gaps, and Potential Developments

While numerous research studies have been conducted to unravel the function of P2X₇R in neurodegenerative disorders, there are still unanswered questions that could add clarity to our understanding of the role of P2X₇R in CNS disease progression. For example, in patients with MS, P2X₇R expression is down-regulated on peripheral monocytes during the acute phase of the disease (Amadio et al., 2017), while it appears to be up regulated on myeloid derived cells in the CNS. Similarly, P2X₇R down-regulation has also been detected in peripheral circulating monocytes of ALS patients (Liu et al., 2016), while this contrasts the up-regulation of the receptor in spinal cord and nervous tissues of post-mortem ALS patients (Yiangou et al., 2006). The fact that P2X₇R shows opposite expression patterns in peripheral vs. central compartments, and in some cases shows a temporal expression pattern which is also tissue specific, suggest that additional work is needed to better understand the interdependency of these compartments on disease progression. Another aspect of P2X₇R expression is related to the genetic context in which the receptor is being expressed. As noted above, many of the mouse models of AD, PD, HD, ALS, MS, TBI, stroke, and depression have been conducted in mice which bears the P451L SNP, as such it is unclear if these mice show the full complement of signaling associated with the P2X₇R system, since the polymorphism results in reduction in sensitivity to ATP by several orders of magnitude. Based on work from our laboratory (Territo et al., 2017), we showed that activation of P2X₇R in C57BL6/J mice required 5–10 fold higher levels of lipopolysaccharide than have been reported for mice which do not carry this polymorphism. Provided this, we believe that both face and construct validity are required in model systems to ensure that they faithfully replicate the type of signaling observed in human cell lines and clinical studies (see Table 1). Therefore, to better understand the role P2X₇R in neurodegenerative diseases, we believe it is imperative that one asks the right question, at the right time, and using the right model systems to maximize our understanding so that these results can be used to help inform and guide clinical trials.

CONCLUDING REMARKS

Purinergic receptors (i.e., purinoceptors) are plasma membrane proteins that play key physiological roles in mammalian central nervous system (CNS), and regulate neurotransmission, neuromodulation, and intra and inter-glia network communication. Purinoceptors are found in a variety of cells in the CNS that include microglia, astrocytes and oligodendrocytes. Astrocytes express many types of purinergic receptors, and release adenosine triphosphate (ATP) as an intercellular signaling molecule allowing communication of these cells with microglia, neurons, oligodendrocytes and the vascular walls of capillaries. In the CNS, purinergic receptor signaling in oligodendrocyte cells helps them in their development and for myelination, while in microglia purinergic receptors are known to function as immunocompetent. In all of these cases, ATP and other nucleotides work as danger signals by activating microglia in pathophysiological conditions. Importantly, dysregulations of purinoceptors have been associated with major CNS disorders including neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson disease (PD), Amyotrophic lateral sclerosis (ALS), brain trauma, ischemia, epilepsy, and chronic pain associated with neuroinflammation, as well as neuropsychiatric diseases, including depression, anxiety, and schizophrenia. Importantly, the P2X₇ receptor has been involved in all of the aforementioned diseases, and in many cases influenced by mutations that increase (or in some cases decrease) function, thus altering one's susceptibility for developing the disease. Moreover, P2X₇ receptors have been shown to activate the NLRP3 inflammasome and the release of pro-inflammatory cytokines, which drives neuroinflammation, and recent work suggests that inhibition of these receptors may serve as a viable drug target.

The role of P2X₇ receptor in the production and release of active pro-inflammatory cytokine IL-1 β has inspired major efforts to develop antagonists. A number of research groups

have disclosed potent P2X₇ receptor antagonists, which have been shown to attenuate the release of IL-1 β from stimulated cells. Since P2X₇ receptor is integral in the processing and release of many inflammatory mediators (IL-1 β , NF κ B, TNF α etc.), the inhibition of this receptor may provide therapeutic benefit in diseases including cancer, tuberculosis, diabetes, asthma, and all of the neurodegenerative diseases. While, full understanding of the P2X₇R localization and function in the brain is currently incomplete, development of high potency and selective antagonists that cross the BBB have helped our understanding of the receptor in multiple rodent models of peripheral inflammatory diseases, neuroinflammatory disorders, and cancer. Although these data have begun to fill this knowledge gap, it is important to recognize that many of the rodent models are based on base-strains that retain SNPs, and therefore show dampened or reduced sensitivity to ATP and thus reduced signaling. Despite this, several antagonists have been studied in depression, anxiety, bipolar disorders, and PD. In some cases, clinical trials have been conducted to evaluate inflammatory pathologies such as rheumatoid arthritis, Crohn's, and basal cell carcinoma. The latest generation of antagonists have been designed to specifically penetrate brain and evaluate brain disorders. Several antagonists from this group have entered clinical trials for evaluation of the P2X₇R participation in CNS disorders. In the future, the development of P2X₇ receptor antagonist that are potent, selective, and have acceptable pharmacokinetic and pharmacodynamic readouts will be needed to advance the field.

AUTHOR CONTRIBUTIONS

PT and HZ contributed equally to the review article. Each author wrote various sections and provided extensive edits to the final document. PT generated the graphical abstract using online tools found at BioRender. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: 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.

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