P2X<sub>7</sub> 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, P2X<sub>7</sub> 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 P2X<sub>7</sub>R 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 P2X<sub>7</sub>R on microglia, astrocytes, and oligodendrocytes are upregulated under neuroinflammatory conditions. Several in vivo studies have demonstrated beneficial effects of the P2X<sub>7</sub> receptor antagonists in animal model systems of neurodegenerative diseases. A number of specific and selective P2X<sub>7</sub> receptor antagonists have been developed, but only few of them have shown efficient brain permeability. Finding potent and selective P2X<sub>7</sub> 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 P2X<sub>7</sub> receptor function in neurodegenerative diseases, the pharmacological inhibition of the receptor, and PET radiopharmaceuticals which permit non-invasive monitoring of the P2X<sub>7</sub> 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, A2A, A2B, 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: P2X1−7 and the G protein-coupled metabotropic P2Y receptors with eight subtypes: P2Y1,2,4,6,11,12,13,14 (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 Scleroses (MS), Traumatic Brain Injury (TBI), stroke, cerebral ischemia, epilepsy, psychiatric diseases, sleep disorder, and neutropathic 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 Ca\textsuperscript{2+} from the endoplasmic reticulum into the cytoplasm. Activation of the Gs/Gi proteins effect stimulation or inhibition of adenylyl cyclase that subsequently modifies the production of cyclic AMP (cAMP), while the non-selective P2X ion channel receptors facilitate cellular exchange of cations like Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, Na\textsuperscript{+}, and K\textsuperscript{+} (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 P2Y2 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 A2A and P2X7 receptors activity, respectively (Cunha, 2016; Faas et al., 2017; He et al., 2017; Vuorima 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). P2X7-R 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, P2X7-R 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 homomic or heteromic receptors such as: P2X1/2, P2X1/4, P2X1/5, P2X2/3, P2X2/5, P2X2/6 and P2X4/6 receptors (Jiang et al., 2013). P2X receptors are widely distributed in neuronal and non-neuronal cells, and participate in many physiological 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 P2X7-R, other P2XRs 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). P2X7-R 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 micro-domains (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 glumatamergic 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-1β 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 TNFα, production of reactive oxygen species (ROS), and in particular oxygen superoxides, which stimulate the NFκB 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 ≥ 100 µM) (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 P2X7 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 baring 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 P2X7-R 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-1β secretion (Stokes et al., 2010), while only the H521Q has been described as neutral (Wiley et al., 2011). By contrast, the loss-of-function 1568N 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).
P2X<sub>7</sub> 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, P2X<sub>7</sub> receptor activation does occur, resulting in dye uptake, IL-1β 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 P2X<sub>7</sub> receptors share 77–85% sequence homology (Surprent et al., 1996; Bartlett et al., 2014), which results in variations in the receptor affinities for ATP (Table 1).

P2X<sub>7</sub> 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 P2X<sub>7</sub>-R activation on microglia (Sperlagh et al., 2006; Burnstock et al., 2011; Sperlagh and Illes, 2014) (Figure 1). ATP-dependent P2X<sub>7</sub>-Rs 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. P2X<sub>7</sub>-Rs driven microglial activation has been implicated in neuroinflammation and neurodegeneration (Monif et al., 2010; Illes et al., 2017). Therefore, P2X<sub>7</sub> 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).

P2X<sub>7</sub> Receptor in Alzheimer's Disease

The appearance of plaques that consist of extracellular β-amyloid (Aβ) peptide surrounded by reactive microglial is a major hallmark of AD (Sanz et al., 2009). Aβ peptide, produced via cleavage of the amyloid precursor protein (APP) by β- and γ-secretases has shown to trigger increases in Ca<sup>2+</sup>, ATP and IL-1β, induce plasma membrane permeabilization and consecutively damage neurons (Delarasse et al., 2011; Martin et al., 2019) (Figure 1). P2X<sub>7</sub> 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 Aβ injection (Parvathenani et al., 2003; Mclarnon et al., 2006). Additionally, P2X<sub>7</sub> receptor deficient microglia was not activated with Aβ (Sanz et al., 2009); P2X<sub>7</sub> receptor silencing enhanced microglia phagocytosis and clearance of Aβ (Ni et al., 2013); and receptor deficiency reduced Aβ plaque, improve synaptic plasticity and resulted in reduction of cognitive deficits in AD mice model (Chen et al., 2014). The P2X<sub>7</sub>-R antagonist BBG has shown to decrease Aβ 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 P2X<sub>7</sub>-R antagonist) via reduction of GSK3β 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 P2X<sub>7</sub>-R did not influence IL-1β release or non-amyloidogenic fragment sAPPα level (Martin et al., 2019). Furthermore, in vitro studies has indicated that stimulation P2X<sub>7</sub>-R for a short time (~30 minutes) increased α-secretase activity (Delarasse et al., 2011). Therefore, the involvement of P2X<sub>7</sub>-R in AD is still somewhat unclear and requires more investigation.

P2X<sub>7</sub> Receptor in Parkinson's Disease

P2X<sub>7</sub>-induced microglia activation has been detected in PD pathology (Carmo et al., 2014). α-Synuclein binding to, and activating of the P2X<sub>7</sub> 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). P2X<sub>7</sub>-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 P2X<sub>7</sub> receptor antagonists (Marcellino et al., 2010; Carmo et al., 2014; Ferrazoli et al., 2017; Kumar et al., 2017), suggesting P2X<sub>7</sub> receptor play a pro-inflammatory role in microglia activation in PD (Kumar et al., 2017). Additionally, P2X<sub>7</sub> 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 P2X<sub>7</sub>-R 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 P2X<sub>7</sub>-R did not induce neuro-protection in the MPTP mouse model of Parkinson's disease (Hracsiko et al., 2011). More research is needed to further clarify the relationship between P2X<sub>7</sub>-R activation and PD.

P2X<sub>7</sub> 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 P2X<sub>7</sub>- 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 P2X<sub>7</sub>-R 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).
| P2X<sub>7</sub> Receptor Antagonists | K<sub>i</sub> (nM) | IC<sub>50</sub> (nM) | Application in CNS indications | References |
|-----------------------------------|----------------|----------------|--------------------------------|------------|
| A-438079                          | 7.1 ± 0.08 (h) | 6.0 ± 0.02 (h) | 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; Hracsiko 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; 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                          | 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 [³H]A-740003 was used in in vitro 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 |
| A-804598                          | 8.0 ± 0.04 (h) | 7.7 ± 0.13 (h) | 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 [³H]A-804598 radioligand to study recombinant rat receptors expressed in 1321N1 cells. PET radioligand [¹⁸F]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; Fabbrizio et al., 2017; Ly et al., 2020; Freire et al., 2019; Ribeiro et al., 2019; Zarrinmayeh and Territo, 2020 |
| GSK1482160                        | 2.63 (h) | 8.5 (h) | Brain penetrable. Entered phase I for treating inflammatory pain in arthritis, but failed to proceed further. PET radioligand [¹¹C]GSK1482160 showed high affinity to brain P2X<sub>7</sub>R. PET radioligand [¹⁸F]UR-1601 showed similar affinity and selectivity for P2X<sub>7</sub>R as [¹¹C]GSK1482160. | Abdi et al., 2010; Ali et al., 2013; Territo et al., 2017; Glaxosmithkline, 2009; Zarrinmayeh and Territo, 2020; Gao et al., 2018 |
| SMW139                            | 32 (h) | | Entered clinical trial for evaluation of neuroinflammation in MS patients. PET radioligand [¹¹C]SMW139 was developed to study in vivo marker of neuroinflammation in multiple sclerosis. | Janssen et al., 2018; Hospital, 2019; Hagens et al., 2020; Zarrinmayeh and Territo, 2020 |

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

[^18]F: Brain-penetrant (brain/plasma = 1).[^11]C: Brain penetrable (Brain/Palms = 1).[^3]H: Brain permeable (Brain/Palms = 1).[^8]F: Brain penetrable with brain/plasma ratio (Brain/Palms = 1).[^3]H: Brain penetrable.[^11]C: Brain-penetrable with brain/plasma ratio (Brain/Palms = 1).[^3]H: Brain penetrable.[^11]C: Brain-penetrable with brain/plasma ratio (Brain/Palms = 1).[^3]H: Brain penetrable.[^11]C: Brain-penetrable with brain/plasma ratio (Brain/Palms = 1).[^3]H: Brain penetrable.
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 P2X7 receptor 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).

**P2X<sub>7</sub> 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 P2X<sub>7</sub> 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 P2X<sub>7</sub>-R has been found upregulated, and in some case showed altered splicing, in the brain of HD subjects.

**P2X<sub>7</sub> 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 P2X<sub>R</sub>-Rs in pathogenesis (Volonte et al., 2016). Increased expression of P2X<sub>R</sub>-Rs 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<sup>G93A</sup>) mouse model of ALS, and application of the P2X<sub>R</sub> 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 P2X<sub>R</sub> antagonists such as A804598 and JNJ-42253432 for a week (Amhaoul et al., 2016). Another P2X<sub>R</sub> antagonist A-438079 suppressed seizures and exhibited neuroprotective effects in immature rats (Mesuret et al., 2014). P2X<sub>R</sub>-R 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 P2X<sub>R</sub>-R 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 P2X<sub>R</sub> receptor antagonists in treating epilepsy, including drug-resistant epilepsy (Beamer et al., 2016; Cieslak et al., 2017; Rodriguez-Alvarez et al., 2017). Furthermore, P2X<sub>R</sub>-R have been recognized as targets for treatment of hypoxic/ischemic encephalopathy (Beamer et al., 2017). Recent anticonvulsant studies using P2X<sub>R</sub>-R antagonists, which include BBG, AFC-5128, JNJ-47965567, and tanshinone IIA sulfonate (traditional Chinese herbal medicine, TIHAS) in animal models have illustrated the potential of these agents to modulate seizures. Remarkably in the pentyleneetrazol-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 TIHAS were observed, whereas the P2X<sub>R</sub>-R 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, P2X<sub>R</sub>- receptors have three distinct functions depending on the situations of the extracellular environment. P2X<sub>R</sub>-R initiation cell death in the presence of elevated extracellular ATP, while mediates calcium signal transduction in response to ATP that regulates proliferation and differentiation. Finally, P2X<sub>R</sub>-R activation promotes phagocytosis in the absence of extracellular ATP (Zheng et al., 2017).

**P2X<sub>7</sub> 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, 2016; 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). P2X<sub>7</sub> 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 P2X<sub>7</sub>-R antagonists (Jimenez-Pacheco et al., 2016). BBG treatment of rats with spontaneous recurrent seizures helped treatment by reducing the P2X<sub>7</sub> receptor expression (Fischer et al., 2016; Song et al., 2019). Additionally, significant reduction in severe convulsive seizures was observed after treatment with P2X<sub>7</sub>-R antagonist JNJ-42253432 for a week (Amhaoul et al., 2016). Another P2X<sub>R</sub>-R antagonist A-438079 suppressed seizures and exhibited neuroprotective effects in immature rats (Mesuret et al., 2014). P2X<sub>R</sub>-R 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 P2X<sub>R</sub>-R 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 P2X<sub>R</sub> receptor antagonists in treating epilepsy, including drug-resistant epilepsy (Beamer et al., 2016; Cieslak et al., 2017; Rodriguez-Alvarez et al., 2017). Furthermore, P2X<sub>R</sub>-R have been recognized as targets for treatment of hypoxic/ischemic encephalopathy (Beamer et al., 2017). Recent anticonvulsant studies using P2X<sub>R</sub>-R antagonists, which include BBG, AFC-5128, JNJ-47965567, and tanshinone IIA sulfonate (traditional Chinese herbal medicine, TIHAS) in animal models have illustrated the potential of these agents to modulate seizures. Remarkably in the pentyleneetrazol-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 TIHAS were observed, whereas the P2X<sub>R</sub>-R 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, P2X<sub>R</sub>- receptors have three distinct functions depending on the situations of the extracellular environment. P2X<sub>R</sub>-R initiation cell death in the presence of elevated extracellular ATP, while mediates calcium signal transduction in response to ATP that regulates proliferation and differentiation. Finally, P2X<sub>R</sub>-R activation promotes phagocytosis in the absence of extracellular ATP (Zheng et al., 2017).

**P2X<sub>7</sub> 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 overexpression 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-1β/IL-18. The P2X7-R antagonist BBG treatment following ICH has shown to downregulate the release of these proinflammatory cytokines (Feng et al., 2015). Inhibition of P2X7-Rs 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). P2X7-Rs are also involved in cerebral neurological damage and edema after traumatic brain injury. Application of P2X7-R 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 (Kimbler et al., 2012; Leeson et al., 2019).

**P2X7 Receptor in Depression**

P2X7 receptor activation by ATP followed by NLRP3 induced IL-1β 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 P2X7-R/NFkB signaling pathway (Zhang et al., 2016) supporting the role of P2X7 receptor participation in depression. Additionally, the P2X7-R 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 P2X7-R 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-1β 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 P2X7-R and subsequently increasing IL-1β 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 P2X7-R 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 P2X7-R antagonists.

**Antagonists**

There are two non-selective ATP derivative antagonists TNP-ATP and peridate-oxidized ATP (oATP) with high μmol potencies (Beigi et al., 2003; Di Virgilio, 2003; De Marchi et al., 2016). The major group of P2X7-R 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 P2X7-R 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 benzopanethanthdine 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 P2X7-R 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 [Kᵢ = 7.1 ± 0.08 (h) and Kᵢ = 6.7 ± 0.1 (r); IC₅₀ = 6.0 ± 0.02 (h), IC₅₀ = 5.9 ± 0.2 nM (r), and IC₅₀ = 5.5 ± 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 [IC$_{50} = 20–150$ 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 [IC$_{50} = 40$ nM (h) and IC$_{50} = 20$ nM at (r)] dose-dependently reduces neuropathic pain in rat (Honore et al., 2006). This antagonist was converted to $[^{13}$C]A-740003 PET radioligand and while has been shown not to enter the brain (Janssen et al., 2014), its tritiated analog $[^{3}\text{H}]$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 EAEd model of MS (Beaino et al., 2017). Antagonist A-804598 ($K_i = 8.0 \pm 0.4$ nM (h), $K_i = 8.8 \pm 0.06$ nM (r); IC$_{50} = 7.7 \pm 0.13$ nM (h), IC$_{50} = 6.8 \pm 0.17$ nM (r), IC$_{50} = 7.0 \pm 0.06$ nM (m)) (Donnelly-Roberts et al., 2009; Bhattacharya et al., 2013; Karasawa and Kawate, 2016) was used to study the functional role of P2X$_7$R 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 ($[^{3}\text{H}]$A-804598) was also prepared and utilized as a P2X$_7$R 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 $[^{18}\text{F}]$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., 2010; AZ-10606120 (Michel et al., 2007); GW791343 (Felix et al., 2012); GSK314181A (Poulos 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 $[^{11}\text{C}]$GSK1482160 and showed high affinity ($K_d = 1.15 \pm 0.12$ nM) in targeting P2X$_7$R (Territo et al., 2017). Recent work from our lab has also developed an F-18 PET radioligand $[^{18}\text{F}]$JUR-1601 that shows similar affinity and selectivity ($K_i = 4.31$ nM (h), IC$_{50} = 7.86$ nM (h)) for P2X$_7$R as $[^{11}\text{C}]$GSK1482160 (Gao et al., 2018). Another benzamide, compound, SMW139 has also been converted to PET radioligand $[^{11}\text{C}]$SMW139 to study the P2X$_7$R receptor expression on pro-inflammatory microglia (Janssen et al., 2018). $[^{11}\text{C}]$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 P2X$_7$R antagonists have specifically been designed to penetrate CNS and enable evaluation of the P2X$_7$R 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 P2X$_7$R antagonist [$K_i = 7.9 \pm 0.08$ (h) and $K_i = 9.1 \pm 0.07$ nM (r); IC$_{50} = 7.7 \pm 0.07$ nM (h), IC$_{50} = 7.8 \pm 0.1$ nM (r) and IC$_{50} = 7.1 \pm 0.2$ 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-1$\beta$ 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 [$K_i = 7.9 \pm 0.07$ nM (h) and $K_i = 8.7 \pm 0.07$ nM (r); IC$_{50} = 8.3 \pm 0.08$ nM (h), IC$_{50} = 7.2 \pm 0.08$ nM (r), and IC$_{50} = 7.5 \pm 0.1$ nM (m)]. It exhibited target engagement in rat brain (EC$_{50} = 78 \pm 19$ ng/ml in P2X$_7$R autoradiography) and functionally blocked the Bz-ATP-induced IL-1$\beta$ 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 P2X$_7$R 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 P2X$_7$R antagonist with high potency [$K_i = 8.12 \pm 0.08$ nM (h) and 8.5 $\pm 0.04$ nM (r)] (Ali et al., 2013; Letavic et al., 2017; Bhattacharya et al., 2018; Chrovin 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 P2X$_7$R [IC$_{50} = 7.87 \pm 0.2$ nM (h), IC$_{50} = 7.81 \pm 0.2$ nM (r), IC$_{50} = 7.55 \pm 0.5$ nM (m), IC$_{50} = 7.96 \pm 0.1$ nM (mm), and IC$_{50} = 7.72 \pm 0.06$ (d)] (Letavic et al., 2017;
Nv, 2017b; Bhattacharya et al., 2018). JNJ-55308942 has shown to engage brain targets, modulate microglial activation, reduce IL-1β release and has been efficacious in models of anhedonia in rodents (Letavic et al., 2017; Bhattacharya et al., 2018; Chrovián 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-triazole based P2X7-R antagonist is the highly potent and brain penetrant JNJ-54173717. Its high affinity JNJ-54173717 [K_i = 3.0 ± 0.1 nM (h) and K_i = 3.0 ± 0.5 nM (r)]; IC_50 = 8.46 ± 0.36 (h), and IC_50 = 8.81 ± 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 ± 0.2 nM (h) and IC_50 = 2.0 ± 0.6 nM (r)] (Kolb et al., 2019; Koole et al., 2019). The F-18 PET radioligand of JNJ-64413739, [^{18}F]JNJ-64413739 was developed to study brain P2X7 function (Zarrinmeyeh and Territo, 2020). Micro-dosing in mice showed a 38% lower compound binding in P2X7-R knock out compared to wild type mice receptor and the uptake of [^{18}F]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 [^{18}F]JNJ-64413739 in healthy human volunteer also showed the tracer to be an appropriate PET ligand for quantification of P2X7-R expression in the human brain (Table 1) (Nv, 2017a; Kolb et al., 2019; Koole et al., 2019).

### Group 3
The 1,2,4-triazole based P2X7-R antagonists are three close analogs JNJ-54232334, JNJ-54140515, and JNJ-54173717. The high affinity JNJ-54232334 [K_i = 7.8 ± 0.05 nM (h) and K_i = 9.3 ± 0.1 nM (r)]; IC_50 = 9.5 ± 0.02 nM (h), and IC_50 = 7.5 ± 0.02 nM (r)] was tritiated to produce [^{3}H]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 [^{3}H]JNJ-54232334 in rat brain sections was enhanced compared to that of the [^{3}H] 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 ± 0.01 nM (r); IC_50 = 7.7 ± 2.6 nM (h) and IC_50 = 8.0 ± 2.9 nM (r)], readily crossed the blood-brain barrier and facilitated the high level of brain P2X7-R 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 P2X7-R antagonists at human and rat receptor [K_i = 1.6 ± 0.1 nM in rat cortex; IC_50 = 4.2 ± 0.01 nM (h) and IC_50 = 7.6 ± 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 Weeegahe et al., 2019). JNJ-54173717 was also converted to PET radioligand [^{11}C]JNJ-54173717 for studying the brain P2X7-R 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). [^{11}C]JNJ54173717 was studies in healthy volunteers and PD patients in human and showed selectivity for P2X7-R (Van Weeegahe et al., 2019).

### Group 4
The imidazolopyridin JNJ-54166060 is another P2X7-R 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 P2X7-R in neurodegenerative disorders, there are still unanswered questions that could add clarity to our understanding of the role of P2X7-R in CNS disease progression. For example, in patients with MS, P2X7-R 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, P2X7-R 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 (Yangou et al., 2006). The fact that P2X7-R 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 P2X7-R 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 bares the P451L SNP, as such it is unclear if these mice show the full complement of signaling associated with the P2X7-R 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 P2X7-R in C57BL/6) 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 P2X7-R 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-glial 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-1β 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-1β from stimulated cells. Since P2X7 receptor is integral in the processing and release of many inflammatory mediators (IL-1β, NFκB, TNFα 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 P2X7-R 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 P2X7-R 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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