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Wei, L, Syed Mortadza, SA, Yan, J et al. (11 more authors) (2018) ATP-activated P2X7 receptor in the pathophysiology of mood disorders and as an emerging target for the development of novel antidepressant therapeutics. Neuroscience and Biobehavioral Reviews, 87. pp. 192-205. ISSN 0149-7634

https://doi.org/10.1016/j.neubiorev.2018.02.005

ATP-activated P2X7 receptor in the pathophysiology of mood disorders and as an emerging target for the development of novel antidepressant therapeutics

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Abstract

Mood disorders are a group of psychiatric conditions that represent leading global disease burdens. Increasing evidence from clinical and preclinical studies supports that innate immune system dysfunction plays an important part in the pathophysiology of mood disorders. P2X7 receptor, belonging to the ligand-gated ion channel P2X subfamily of purinergic P2 receptors for extracellular ATP, is highly expressed in immune cells including microglia in the central nervous system (CNS) and has a vital role in mediating innate immune response. The P2X7 receptor is also important in neuron-glia signalling in the CNS. The gene encoding human P2X7 receptor is located in a locus of susceptibility to mood disorders. In this review, we will discuss the recent progress in understanding the role of the P2X7 receptor in the pathogenesis and development of mood disorders and in discovering CNS-penetrable P2X7 antagonists for potential uses in *in vivo* imaging to monitor brain inflammation and antidepressant therapeutics.

Keywords: mood disorders; innate immune system dysfunction; P2X7 receptor; brain imaging; antidepressant therapeutics

1. Introduction

Mood disorders comprise a group of psychiatric diseases that exhibit a high phenotypic complexity but are all characterized by pervasive changes in thoughts, emotions and behaviours and impairments in social and cognitive functions. Among them, depression or major depressive disorder (MDD) (also known as unipolar depressive disorder), bipolar disorder (BPD) and anxiety disorder represent the three main types. Mood disorders are associated with elevated risk of early death due to suicide, and are comorbid with a wide range of conditions, such as arthritis, asthma, cardiovascular diseases, cancer, chronic pain, chronic respiratory disorders, diabetes, and neurodegenerative diseases including Alzheimer's disease and Parkinson's disease (Anisman et al., 2008; Kessler and Bromet, 2013; Walker et al., 2014). MDD, BPD and anxiety disorder together account for more than 60% of disability-adjusted life years due to mental and substance use disorders worldwide (Whiteford and Baxter, 2013). The World Health Organization has projected MDD to be the first leading cause of global disease burdens by 2030 (WHO, 2008). Analysis of epidemiological data from several surveys suggests that MDD is a commonly occurring and seriously incapacitating condition with high lifetime prevalence and persistence across nations and cultures (Kessler and Bromet, 2013; Walker et al., 2014). Since the serendipitous discovery of the first antidepressant drugs more than fifty years ago, substantial progress has been made in developing new antidepressant

treatments with primary mechanisms of action on the monoaminergic neurotransmission system and, in more recent years, on the glutamatergic and other neurotransmission systems (Di Giovanni and De Deurwaerdere, 2016; Duman and Voleti, 2012; Papakostas and Ionescu, 2015). Nonetheless, there are still considerable unmet clinical needs. Development of more effective therapeutics for mood disorders has been challenging because of the elusive aetiology. An increasing number of preclinical and clinical studies have been devoted to deciphering the mechanisms leading to mood disorders at the molecular, cellular, tissue and system levels. As discussed below, accumulating evidence supports a crucial role of innate immune system dysfunction and brain inflammation in the pathogenesis and development of mood disorders.

It is well-known that ATP is present in every living cell as the energy source for a diversity of cell functions. It has been firmly established that ATP is released from many cell types under physiological and pathological conditions and serves as an extracellular signalling molecule via acting on two structurally and functionally distinct subfamilies of P2 purinergic receptors on the cell surface, namely, the ligand-gated ion channel P2X receptors and G-protein-coupled P2Y receptors (Burnstock and Verkhratsky, 2009; North, 2002; Ralevic and Burnstock, 1998; von Kugelgen and Harden, 2011). ATP when released in large quantity, as known to occur at the site of tissue inflammation or damage, initiates innate immune response by activating the P2X7 receptor present on immune cells, including microglia in the central nervous system (CNS). A large body of evidence has been collected to support a critical role for the P2X7 receptor not only in the normal immune response but also in the pathogenesis of numerous inflammatory diseases (Jiang, 2009, 2012; Jiang et al., 2013), including those mentioned above that show comorbidity with mood disorders. The gene encoding human P2X7 receptor, *P2RX7*, is located on chromosome 12q24.31 (North, 2002), a locus known to confer the susceptibility to MDD and BPD (Morissette et al., 1999; Shink et al., 2005). The *P2RX7* gene contains a large number of single nucleotide polymorphisms (SNPs) including a dozen of non-synonymous SNPs (NS-SNPs) that alter the receptor function. Studies support association of such genetic variations with a predisposition to mood disorders. Preclinical studies by combining rodent behaviour models with genetic and pharmacological interventions show the P2X7 receptor to be crucial in stress-induced depressive-like and anxiety-like behaviours.

In this review, we will introduce the innate immune system dysfunction or inflammation hypothesis of mood disorders and describe the distinctive properties of P2X7 receptor and its role in such biological processes. We will discuss the studies that support the role of the P2X7 receptor in the pathophysiology of mood disorders. We will also highlight the

recent progress in discovering CNS-penetrable P2X7 antagonists and exploring their uses as imaging agents to monitor brain inflammation and as antidepressants.

2. The inflammation hypothesis of mood disorders

The innate immune system is equipped with a repertoire of pattern recognition receptors that stereotypically detect a large assortment of pathogen-associated molecules, for example, lipopolysaccharide (LPS) from bacterial infection, or danger-associated molecules, such as ATP. Activation of pattern recognition receptors induces a multitude of intrinsic signalling pathways leading to generation of cytokines, chemokines and other pro-inflammatory mediators (Franchi et al., 2009a; Guo et al., 2015; Martinon et al., 2009). Interleukin (IL)-1β is the master cytokine in innate immune response as it induces generation of other important pro-inflammatory cytokines, such as IL-6 and tumour necrosis factor (TNF)-α. Assembly and activation of a multi-protein complex, designated the NLRP3 inflammasome, composed of NLRP3 (nucleotide-binding, leucine-rich repeat, pyrin domain containing 3), ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) and pro-caspase-1. The NLRP3 inflammasome converts pro-caspase-1 to caspase-1, which in turn cleaves the biologically inactive IL-1β precursor to generate IL-1β.

Studies over the past decades have collected substantial evidence to support the importance of alterations in the innate immune system function in the pathophysiology of mood disorders (Bauer et al., 2014; Bhattacharya et al., 2016; Bhattacharya and Drevets, 2017; Brown et al., 2017; Dantzer et al., 2008; Iwata et al., 2013; Jones and Thomsen, 2013; Kaufmann et al., 2017; Maes et al., 2012; Raison et al., 2006; Slavich and Irwin, 2014; Wohleb et al., 2016; Yirmiya et al., 1999). For example, there were significant increases in the expression of NLRP3 and caspase-1 in blood cells from MDD patients (Alcocer-Gomez et al., 2014). Consistently, the levels of IL-1β, IL-6 and TNF-α were higher in blood plasma and monocytes from MDD and BPD patients than healthy subjects (Alcocer-Gomez et al., 2014; Jones and Thomsen, 2013). An elevation in the IL-1β level in the cerebrospinal fluid (CSF) was also observed in BPD patients compared to control subjects (Soderlund et al., 2011). It is also noted that the IL-1β level was well correlated with the severity of depression (Alcocer-Gomez et al., 2014) or significantly higher in BPD patients having a recent manic episode (Soderlund et al., 2011). Similar changes in the innate immune system function have been documented by recent studies in rodents exhibiting depressive-like and anxiety-like behaviours induced by psychological stress. The expression of NLRP3 and ASC, and/or activation of the NLRP3 inflammasome and caspase-1 in hippocampus and prefrontal cortex, the two major

brain regions implicated in mood disorders, were increased in rats subjected to chronic unpredictable or mild stress (CUS/CMS), a widely used model of MDD (Iwata et al., 2016; Yue et al., 2017) or in female mice due to estrogen deficiency induced by ovariectomy (Xu et al., 2016). Thus, an increase in the IL-1β level in the CSF and prefrontal cortex was also detected in mice and rats following exposure to CUS (Cao et al., 2013; Iwata et al., 2016; Pan et al., 2014). Similarly, there was an increase in the IL-1β level in ovariectomized female mice (Xu et al., 2016). An elevated level of TNF-α in hippocampus was also detected in rats exposed to immobilization stress (Iwata et al., 2016). Moreover, the TNF-α level was increased with a noticeable delay relative to the IL-1β level, consistent with the idea that generation of TNF-α depends on generation of IL-1β (Iwata et al., 2016). In further support of causative association of mood disorders with innate immune system dysfunctions, studies have documented anti-inflammatory effects of antidepressant medications in MDD patients (Alcocer-Gomez et al., 2014; Hannestad et al., 2011; Hwang et al., 2008) and CUS-exposed mice (Tan et al., 2017). Recent meta-analyses of the currently available data suggest that antidepressant treatments result in a significant reduction in the levels of IL-1β, IL-6 or TNF-α in MDD and BPD patients (Goldsmith et al., 2016; Kohler et al., 2017). Conversely, studies show antidepressant phenotypes of anti-inflammatory interventions in BPD and MDD patients and rodent models of mood disorders (Abbasi et al., 2012; Lu et al., 2014; Nery et al., 2008; Xu et al., 2016; Zhang et al., 2015). For example, a randomized double-blind placebo-controlled study in 40 MDD patients showed that treatment with celecoxib, an anti-inflammatory drug, reduced the serum IL-6 level and Hamilton Depression rating score and that the serum IL-6 levels and rating scores were significantly correlated (Abbasi et al., 2012). In mice, genetic deletion of the NLRP3 expression or pharmacological inhibition of the NLRP3 inflammasome activation conferred anti-depressive and anti-anxiety phenotypes (Iwata et al., 2016). Treatment with VX-765, a caspase-1 inhibitor, mitigated depressive-like and anxiety-like behaviours as well as reversing the elevated IL-1β level in hippocampus in CMS-exposed mice (Zhang et al., 2015) or ovariectomized mice (Xu et al., 2016). Moreover, as mentioned above, there is increasing evidence to show mood disorders to be comorbid with numerous diseases related to peripheral inflammation and inflammation in the CNS (Anisman et al., 2008; Kessler and Bromet, 2013; Walker et al., 2014). Meta-analysis of comorbidity of depression and rheumatoid arthritis suggests several-fold greater tendency towards depression in the subgroup of patients who were more severely affected with rheumatoid arthritis (Godha et al., 2010). In summary, both clinical and preclinical studies support a crucial role of innate immune system dysfunction, particularly IL-1β, IL-6 and TNF-α, in the pathophysiology of mood disorders.

3. P2X7 receptor in the pathophysiology of mood disorders

3.1 The properties of P2X7 receptor

The P2X7 receptor, as introduced above, belongs to the ligand-gated ion channel P2X receptor subfamily that has a structure distinct from other classical ligand-gated ion channels, such as ionotropic glutamate receptors and cysteine-loop receptors (Browne et al., 2010; Khakh and North, 2006, 2012). The P2X receptor is trimeric, with each subunit composed of intracellular N- and C-termini, and two α-helical transmembrane segments connected by a large extracellular domain. As shown in the atomic structures of the zebrafish P2X4 (Hattori and Gouaux, 2012; Kawate et al., 2009), human P2X3 (Mansoor et al., 2016) and panda P2X7 receptors (Karasawa and Kawate, 2016), the second transmembrane segments intertwine each other to form a central, aqueous and cation-permeating pathway that opens upon ATP binding at the subunit interfaces in the extracellular part of the receptor (Browne et al., 2010; Hattori and Gouaux, 2012). The P2X7 receptor has exceptional structural and functional properties (Jiang et al., 2013). All mammalian P2X7 subunits are 595 amino acid residues in length, with an exception of the guinea-pig P2X7 subunit that is one residue shorter due to loss of Asp77 (according to the human P2X7 subunit numbering used from this point onward), and their C-terminus is about 70-220 residues longer than that of the other mammalian P2X subunits. The P2X7 receptor is activated by sub-millimolar concentrations of ATP and thus approximately 10- to 100-fold less sensitive to ATP than the other P2X receptors. BzATP (2',3'-O-(4-benzoyl-benzoyl)-ATP), a synthetic ATP analogue, is widely used in studies as it is more potent than ATP at the P2X7 receptor. Upon brief stimulation, the P2X7 receptor functions as a ligand-gated ion channel that is restricted to permeate small cations such as K^+, Na^+ and Ca^{2+}. Of interest, on the contrary to all other P2X receptors, the P2X7 receptor does not show desensitization but a facilitation process that increases its functional activity during agonist exposure (Roger et al., 2010a; Roger et al., 2008). Moreover, prolonged or repeated activation of the P2X7 receptor increases the membrane permeability to molecules of up to 900 Daltons. This functionality is commonly referred to as large pore formation and measured often using a fluorescent dye uptake assay. The mechanisms underlying P2X7 receptor-dependent large pore formation still remain enigmatic (Wei et al., 2016). The large pore formation appears to be influenced by many different parts of the receptor, including the unique C-terminal tail as evidenced by the finding that change from proline at position 451 to leucine (P451L), due to NS-SNP in the mouse P2X7 receptor gene, prevents the large pore formation without affecting

the small ion-permeable channel function (Sorge et al., 2012). There is evidence to show that the P2X7 receptor channel does allow passage of large molecules (Browne and North, 2013). In one proposed mechanism for large pore formation, the P2X7 receptor channel can undergo progressive dilatation of its permeation pathway (Alves et al., 2014; North, 2002; Pelegrin, 2011). However, a recent study suggests no change in the size of the P2X7 receptor channel (Pippel et al., 2017). Another recent study shows that the purified panda P2X7 receptor, upon reconstitution into liposomes, forms a lipid-composition dependent dye-permeable pore that is independent of its unique C-terminal tail but facilitated by palmitoylated cysteine residues near the pore-lining helix (Karasawa et al., 2017). Nonetheless, recent studies have discovered compounds that preferentially inhibit the large pore formation without effect on the ion channel function (Caseley et al., 2016; Fowler et al., 2014). Furthermore, there is evidence to indicate an important role of the P2X7 receptor, particularly its large pore formation, in inflammatory diseases such as chronic pain (Sorge et al., 2012) and aged-related macular degeneration (Fowler et al., 2014). Finally, the conspicuous or unique property of the P2X7 receptor among the P2X receptors is that its sustained activation leads to cell death, rendering the P2X7 receptor to be long known as the cytolytic receptor (Surprenant et al., 1996).

3.2 Role of the P2X7 receptor in the innate immune and CNS functions

The P2X7 receptor is highly expressed in immune cells, particularly those engaged in innate immune response such as macrophage, monocytes and microglia. A number of studies using transgenic knockout mice and/or selective antagonists provide unequivocal evidence to support an essential role for the P2X7 receptor in ATP-induced activation of the NLRP3 inflammasome and generation of IL-1β (Giuliani et al., 2017; Shieh et al., 2014; Solle et al., 2001; Stokes et al., 2010; Stokes et al., 2006). Consistently, ATP-induced generation of IL-1β is impaired by loss-of-function NS-SNP mutation such as E496A (Ali et al., 2013; Sluyter et al., 2004) or enhanced by gain-of-function NS-SNP mutation like A348T in the human P2X7 receptor (Stokes et al., 2010). The relationship between P2X7 receptor activation and generation of IL-6 or TNF-α remains less well-defined and is further complicated by the finding that TNF-α can enhance ATP-induced activation of the NLRP3 inflammasome and caspase-1 (Franchi et al., 2009b). Nonetheless, genetic and pharmacological studies provide evidence to suggest that the P2X7 receptor is critical in the signalling mechanisms for microglial generation of IL-6 and TNF-α (Iwata et al., 2016; Lu et al., 2017; Masuch et al., 2016; Shieh et al., 2014). The loss-of-function E496A mutation in the human P2X7 receptor

also attenuates the generation of TNF-α (Denlinger et al., 2005). There is a large volume of evidence that shows an important role the P2X7 receptor plays in the normal innate immune response and in the development of brain inflammation leading to neurodegenerative and neuropsychiatric diseases, as well as in a diversity of diseases due to peripheral inflammation (Bartlett et al., 2014; Bhattacharya and Biber, 2016; Di Virgilio et al., 2017; Eltzschig et al., 2012; Idzko et al., 2014; Ortiz et al., 2015; Platania et al., 2017).

In the CNS, ATP can be released as a neurotransmitter from presynaptic neurons and also as a gliotransmitter from glial cells, particularly from astrocytes, and thus it is a key mediator in the communications between neurons and glial cells (Abbracchio et al., 2009; Del Puerto et al., 2013; Fields and Stevens, 2000; Khakh and North, 2012). Alterations in ATP-induced purinergic signalling in neuron-glia communications have been strongly implicated in mood disorders and other pathophysiological conditions leading to behavioural and cognitive dysfunctions (Bhattacharya and Biber, 2016; Illes and Verkhratsky, 2016; Jarvis, 2010; Krugel, 2016; Sperlagh and Illes, 2014; Tsuda and Inoue, 2016). While its neuronal expression still remains controversial, the P2X7 receptor is well-documented to locate on astrocyte and oligodendrocyte as well as microglia and play an important role in neuron-glia signalling (Illes et al., 2017; Miras-Portugal et al., 2017). Specifically, it is widely recognised that the P2X7 receptor on glial cells, on one hand, responds to ATP released from presynaptic neurons and, on the other hand, induces glial cells to release ATP and other gliotransmitters that reciprocally modulates synaptic function. As discussed below, emerging evidence shows that psychological stress induces depressive-like and anxiety-like behaviours in rodents via stimulating ATP release from astrocytes and activation of the P2X7 receptor on microglia to generate IL-1β, IL-6 and TNF-α in hippocampus and prefrontal cortex.

3.3 P2X7 receptor in mood disorders: genetic association studies

The human *P2RX7* gene, as introduced above, is situated in a locus of susceptibility to mood disorders and is prolific of SNPs. A number studies have observed significant association of NS-SNPs with a vulnerability to mood disorders as well as other clinical conditions, including multiple sclerosis (370T>V, 489C>T, 835G>A and 946G>A), chronic pain (489C>T and 835G>A), severe sepsis (474G>A and 1405>G) and cardiovascular disease (1513A>C) (Caseley et al., 2014; Gu et al., 2015). A study analysing a cohort comprising 1,000 German Caucasian MDD patients and 1029 control subjects firstly reported association of 1405A>G with MDD (Lucae et al., 2006). Association of this NS-SNP with BPD was also described in a study published in the same year that examined 231 Canadian BPD patients and 214 control

subjects (Barden et al., 2006), and in a later study of 604 BPD patients and 560 control subjects with UK and Irish background (McQuillin et al., 2009). Analysis of combined data from these different three cohorts revealed stronger association of 1405A>G with mood disorders (McQuillin et al., 2009). There was also association of 1405A>G with mood disorders in a cohort of 171 Hungarian MDD or BPD patients and 178 control subjects (Hejjas et al., 2009). A study of 178 BPD patients and 272 MDD patients from three independent clinical cohorts and 1322 control subjects in Finland suggested that 489C>T and 1405A>G significantly increase the risk to familial mood disorder (Soronen et al., 2011). However, neither of these two NS-SNPs was found to associate with MDD or remission after treatment in a later study of the pooled data from two Finland cohorts, consisting of 119 patients with treatment-resistant MDD and receiving electroconvulsive therapy in the first cohort and 99 depressive outpatients treated with selective serotonin reuptake inhibitors in the second cohort (Viikki et al., 2011). Meta-analysis of 6,962 mood disorder patients and 9,262 control subjects collected in 13 different studies only noticed significant association of mood disorders with 1405A>G in family-based cohorts (Feng et al., 2014). An early study examining 218 Hungarian diabetic patients also supported association of 1405A>G with depressive symptoms, with patients with the G-allele scoring higher on the hospital anxiety and depression scale (Nagy et al., 2008). Analysis of 315 Hungarian patients (195 with MDD and 120 with BPD) and 373 control subjects showed association of 1405A>G with the depression score in the group of BPD patients, based on the hospital anxiety and depression scale (Halmai et al., 2013). However, significant association with 1405A>G was not observed in other studies, for example, that examined a UK cohort of 687 BPD patients and 1036 patients with unipolar recurrent major depression and 1204 control subjects (Green et al., 2009) or a cohort of 1445 BD patients and 2006 control subjects from four Central and Eastern European countries (Ferreira et al., 2008; Grigoroiu-Serbanescu et al., 2009). Genome-wide analysis also failed to support a role for the *P2RX7* in BPD (Ferreira et al., 2008; Psychiatric, 2011; Sklar et al., 2008). A study of 179 Caucasian patients with anxiety disorder and syndromal panic attacks and 462 control subjects indicated a trend of association between 1068G>A with anxiety disorder (Erhardt et al., 2007). In summary, many, but not all, genetic studies support association of variations in the *P2RX7* gene due to NS-SNPs with a predisposition to mood disorders.

Electrophysiological studies have shown that many NS-SNP mutations alter the human P2X7 receptor function, either leading to loss-of-function (e.g., V76A, G150R, R270H, R307Q and E496A, resulting from 370T>V, 474G>A, 835G>A, 946G>A and 1513A>C, respectively) or gain-of-function (e.g., H155Y and A348T, due to 489C>T and 1068G>A, respectively) in

terms of the ion channel function and large pore formation (Bradley et al., 2011; Caseley et al., 2014; Gu et al., 2015; Roger et al., 2010b; Stokes et al., 2010; Sun et al., 2010). In addition, as mentioned above, the human P2X7 receptor bearing the gain-of-function A348T mutation increases generation of IL-1β (Stokes et al., 2010), whereas the human P2X7 receptor containing the loss-of-function E496A mutation reduces generation of IL-1β (Ali et al., 2013; Sluyter et al., 2004) and TNF-α (Denlinger et al., 2005), consistent with critical dependence of generation of these proinflammatory cytokines on the P2X7 receptor function. Intriguingly, the mutant human P2X7 receptor carrying the Q460R mutation, arising from 1405A>G that as discussed above is most relevant to mood disorders, was found to be functionally similar to the wild-type (WT) receptor. A recent study has however revealed impaired interactions between the WT and Q460R mutant subunits when they were co-expressed that reduce the function of the P2X7 receptor made of WT and mutant subunits (Aprile-Garcia et al., 2016). Consistently, As has reported in a more recent study, primary hippocampal cells from homozygous humanized mice expressing the WT human P2X7 receptor or the G560R mutant human P2X7 receptor showed similar Ca^{2+} responses to BzATP, but BzATP-induced Ca^{2+} response was significantly smaller in cells from heterozygous humanized mice co-expressing the WT and Q460R mutant human P2X7 subunits (Metzger et al., 2017). The same study has further uncovered that such WT/Q460R P2X7 receptor heterozygosity reduced the sleep quality of the heterozygous humanized mice although there was no significant increase in depressive-like and anxiety-like behaviours as examined using behavioural tests, including the open field test (OFT), elevated plus maze (EPM), forced swim test (FST), and chronic social defeat stress.

3.5 P2X7 receptor in mood disorders: rodent behaviour studies

A number of recent studies have investigated, using genetic or pharmacological interventions, the role of the P2X7 receptor in stress-induced depressive-like, manic-like and anxiety-like behaviours in rodents. Basso et al were first to examine the P2X7-knockout (P2X7-KO) mice (Basso et al., 2009), generated by Pfizer (Solle et al., 2001). While resulting in no effect on the spontaneous locomotor activity over a period of 90 min in the OFT, P2X7-KO significantly alleviated depressive-like behaviours as evidenced by shortened immobility time in both the FST and tail suspension test (TST) (Basso et al., 2009). In addition, the P2X7-KO mice were better responsive to treatment with a low dose of imipramine, an antidepressant drug. However, there was no difference in exploration-linked anxiety-like behaviours between the WT and P2X7-KO mice in the EPM test and novelty suppressed feeding (NSF) test. A subsequent and independent study has confirmed these findings and, in addition, documented

a manic mood-stabilizing phenotype of P2X7-KO in the amphetamine-induced hyperactivity (AH) test as a behaviour model of the manic pole of BPD (Csolle et al., 2013a). Such anti-depressive-like and anti-manic phenotypes in the TST and AH test were also observed in the WT mice following 7-day treatment with brilliant blue G (BBG), a P2X7 antagonist, via intraperitoneal administration (Csolle et al., 2013a). Furthermore, the study found no reduction in the depressive-like and manic-like behaviours in chimeras lacking the P2X7 expression in haematopoietic cells, suggesting that genetic deletion of the P2X7 receptor on non-haematopoietic cells may give rise to the antidepressant phenotypes (Csolle et al., 2013a). Another study examined the same strain of P2X7-KO mice during repeated behaviour testing over three consecutive days, and showed in the FST that the depressive-like behaviours of the P2X7-KO mice were similar on the first day but significantly reduced on the second and third days compared to the WT mice (Boucher et al., 2011). This study also examined anxiety-like behaviours in the light dark emergence (LDE) test as well as the EPM test. In the LDE test, there was no difference in the anxiety-like behaviours between the WT and P2X7-KO mice but a reduction in the locomotor activity in the P2X7-KO mice during 10 min recording. Intriguingly, the EPM test revealed exacerbated anxiety-like behaviours in the P2X7-KO mice (Boucher et al., 2011), a finding that is noticeably different from that reported by the two above-mentioned studies (Basso et al., 2009; Csolle et al., 2013a). A recent study has further explored the role of the P2X7 receptor in the development of manic-like behaviours induced by acute and chronic treatments of amphetamine (Gubert et al., 2016). Both acute and chronic amphetamine treatments induced a remarkable increase in the locomotor activity or hyperactivity, which was almost completely abolished by P2X7-KO. The manic-like behaviours in mice induced by chronic amphetamine treatments were also prevented by intracerebroventricular microinjection of BBG or another P2X7 antagonist, A-438079 (Gubert et al., 2016). Similarly, AH induced by chronic treatment with amphetamine was attenuated in rats treated with JNJ-47965567 and JNJ-42253432, two potent P2X7 antagonists, via subcutaneous injection (Bhattacharya et al., 2013; Lord et al., 2014). LPS-induced depressive-like behaviours in the TST and anhedonia in the sucrose preference test (SPT) were also attenuated in the P2X7-KO mice, or in the WT mice after intraperitoneal administration of BBG or another P2X7 antagonist, AZ-10606120 (Csolle et al., 2013b). Another two recent studies have examined the role of the P2X7 receptor in the development of depressive-like and anxiety-like behaviours in rats and mice subjected to CUS (Iwata et al., 2016; Yue et al., 2017). Both anhedonia as shown in the SPT and anxiety-like behaviours in the NSF and EPM tests in CUS-exposed rats were reversed by intraperitoneal administration of A-804598, a P2X7

antagonist (Iwata et al., 2016). The anti-depressant and anti-anxiety phenotypes of P2X7-KO were also observed in the OFT, FST and EPM test in CUS-exposed mice (Yue et al., 2017). Development of the depressive-like behaviours in the OPT and FST in rats induced by CUS or microinjection in hippocampus of ATP or BzATP was prevented by treatment with BBG or A-438079 (Yue et al., 2017). Finally, the WT mice that developed learned helplessness behaviours after exposure to inescapable footshocks showed an increase in the latency and the number of escape failures to subsequent escapable footshocks and, in striking contrast, the P2X7-KO mice exhibited strong resilience to repeated stressful stimuli (Otrokocsi et al., 2017). It is not straightforward to translate the findings from rodents to humans and, nonetheless, these studies provide strong evidence to support the notion that the P2X7 receptor has an important role in the pathophysiology of mood disorders.

3.6 The P2X7 receptor mechanisms leading to mood disorders

Bennett proposed ten years ago a regenerative loop hypothesis centring on neuron-glia communications at the glutamatergic synapse in hippocampus in an attempt to provide a testable theory to the emerging role of the P2X7 receptor in the pathogenesis of mood disorders (Bennett, 2007). According to this hypothesis, the quad-partite synapse comprises presynaptic and post-synaptic neurons in interactions with astrocyte and microglia in the close vicinity. Neuron-derived glutamate induces release of ATP from both astrocytes and microglia, and subsequently ATP is released from astrocytes in an autocrine fashion and acts on the presynaptic P2X7 receptor to stimulate release of more glutamate, thereby sustaining the levels of ATP and glutamate in the synapse to remodel the synaptic transmission. In addition, ATP activates the P2X7 receptor on microglia to induce generation of IL-1β and TNF-α and glutamate induces release of TNF-α from microglia. IL-1β negatively and TNF-α positively regulate expression and function of the post-synaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors, which are well-known for their key role in cognitive functions. Therefore, the P2X7 receptor is central to this theory in modulating the synaptic and neuronal network functions via directly modulating presynaptic release of glutamate and indirectly regulating post-synaptic expression of the AMPA receptors. As introduced above, it still remains controversial regarding expression of the P2X7 receptor in CNS neurons. Such a hypothesis may be amenable to further elaborations or amendments but does help us to evolve a better understanding of the P2X7 receptor mechanisms in the pathophysiology of mood disorders.

Pertinent to such regenerative loop theory, stress-induced increase in the synaptic level of glutamate and ATP in hippocampus, particularly the role of P2X7 receptor in ATP-induced glutamate release, has gained supports by studies using rodent models. A recent study has shown an increase in the levels of both ATP and glutamate in hippocampus and prefrontal cortex in rats subjected to immobilization stress (Iwata et al., 2016). A more recent study has also documented an increase in the level of ATP in hippocampus in rats after exposed to CUS for 2-3 weeks (Yue et al., 2017). There is evidence that glutamate can induce ATP release from astrocytes (Iwata et al., 2016). Conversely, ATP elicited glutamate release from mouse hippocampal slices that was largely prevented by P2X7-KO (Csolle et al., 2013b) and, while the cell type releasing glutamate was not established, astrocytes remain a strong candidate. Thus accumulating evidence supports the notion that the P2X7 receptor mediates ATP-induced release of glutamate which in return stimulates ATP release from astrocytes, resulting in elevated synaptic levels of ATP and glutamate in hippocampus and prefrontal cortex (Fig. 1).

Studies using rodent models also provide strong evidence to support a critical role of the P2X7 receptor in mediating stress-induced innate immune system dysfunction, particularly generation of IL-1β, IL-6 and TNF-α. As has been recently shown, immobilization stress stimulated an increase in the NLRP3 inflammasome activation and the levels of IL-1β and TNF-α in hippocampus in rats, which were almost completely prevented by treatment with A-804598 (Iwata et al., 2016). Similarly, there was an increase in the levels of IL-1β and TNF-α in hippocampus of mice receiving chronic treatment with amphetamine and BzATP in the model of mania, which was ablated by BBG or A-438079 (Gubert et al., 2016). Another recent study has also demonstrated that LPS-induced elevation in the levels of IL-1β, IL-6 and TNF-α in serum and hippocampus as well as LPS-induced depressive-like behaviours was remarkably reduced by blocking the P2X7 receptor (Zhang et al., 2016). As discussed above, there was no reduction in the depressive-like and manic-like behaviours in chimeras lacking the P2X7 expression on non-haematopoietic cells (Csolle et al., 2013a), suggesting a critical role for the P2X7 receptor on immune cells such as microglia. Taken together, these recent studies provide compelling evidence to support an important role for the P2X7 receptor in mediating stress-induced innate immune system dysfunction, particularly IL-1β, IL-6 and TNF-α, which is consistent with the inflammation hypothesis of mood disorders (Fig. 1).

Deficiency in neuron-microglia signalling contributes in the development of psychiatric diseases by impairing functional brain connectivity (Zhan et al., 2014). Furthermore, there is evidence to show that the expression of synapse-related genes in prefrontal cortex is attenuated in MDD patients and recapitulation of such changes in mice resulted in loss of dendritic spines

and dendrites and led to depressive behaviours (Kang et al., 2012). These findings support an important role of neuron-glia signalling in altering synaptic and neuronal network functions in the pathology of depression. Consistent with the P2X7 receptor as a key player in neuron-glia commination is the finding from whole genome microarray analysis that P2X7-KO induced widespread up or down-regulation of genes that are crucial for synaptic function (Csolle et al., 2013a), including an elevated expression of the NR2B subunit of the NMDA-type glutamate receptors in hippocampus (Csolle et al., 2013b). As shown by examining the expression of c-Fos, a marker of neuronal activity, P2X7-KO prevented stress-induced neuronal activity in the dentate gyrus (DG) of hippocampus and in amygdala in mice (Boucher et al., 2011). A recent study also shows a reduction in the level of synaptopodin expression in hippocampus and in the density of spine synapses in the molecular layer of DG that is associated with inescapable footshock-induced learned helplessness behaviours in mice, which were abolished by P2X7-KO (Otrokocsi et al., 2017). Brain-derived neurotrophic factor (BDNF) is thought to play an important role in the synaptic plasticity and neurogenesis and be critically involved in depression (Berton and Nestler, 2006; Duman and Voleti, 2012; Groves, 2007). Studies also examined the role of P2X7 receptor in regulating the BDNF expression. P2X7-KO or intraperitoneal injection of BBG significantly increased, whereas injection of BzATP reduced, the BDNF expression level in mouse hippocampus (Csolle et al., 2013b). BzATP-induced reduction in the BDNF level was prevented by treatment with CNQX, a non-N-methyl-D-aspartate (NMDA)-type glutamate receptor antagonist, TCN-201, a NR1/NR2A-containing NMDA-type glutamate receptor selective antagonist, or RO-256981, a NR2A-containing NMDA-type glutamate receptor antagonist, suggesting engagement of both NMDA-type and non-NMDA-type glutamate receptors (Csolle et al., 2013b). A recent study has shown that the BDNF level was attenuated in prefrontal cortex in mice following chronic amphetamine treatment, which however was insensitive to treatment with either BzATP or A-438079 (Gubert et al., 2016). Finally, recent studies show using bromodeoxyuridine-labelling that cell proliferation in the DG of hippocampus in mice, including the subventricular zone of the granular cell layer, was enhanced by P2X7-KO or treatment with A-804598, supporting a role for the P2X7 receptor in down-regulating neurogenesis (Csolle et al., 2013b; Iwata et al., 2016). Such a regulation has been proposed to be mediated by IL-1β via activation of the IL-1 receptor and NF-κB signalling pathway (Iwata et al., 2016; Koo and Duman, 2008). In summary, studies have gathered substantial evidence to support an important role for stress-induced activation of the P2X7 receptor in changes in the synaptic, neuron-glial and neuronal network function that

give arise to behaviour and cognitive impairments associated with mood disorders (Fig. 1). Further investigations are required to provide a better understanding of the signalling mechanisms underlying these changes.

4. Development of CNS-penetrable P2X7 antagonists

The recognition of the P2X7 receptor to be critical in the pathogenesis of inflammatory diseases sparked tremendous interest in the P2X7 receptor as a drug target (Arulkumaran et al., 2011; Bartlett et al., 2014; Baudelet et al., 2015; Bhattacharya and Biber, 2016; Chrovian et al., 2014; Guile et al., 2009; Jacobson and Muller, 2016; Lambertucci et al., 2015; Mehta et al., 2014; North and Jarvis, 2013; Park and Kim, 2017; Rech et al., 2016). A huge amount of medicinal chemistry efforts, largely driven by the pharmaceutical industry, have been devoted over the past nearly twenty years, leading to the discovery of numerous structurally novel compounds as potent, selective, competitive or non-competitive P2X7 antagonists. Much of the early attention has been drawn to such P2X7 antagonists as therapeutics treating diseases related to peripheral inflammation, resulting in advancement of AZD-9056 from AstraZeneca and CE-224,535 from Pfizer into clinical trials for rheumatoid arthritis (Keystone et al., 2012; Stock et al., 2012) and Crohn's disease (Eser et al., 2015). With increasing appreciation of the role of the P2X7 receptor in brain inflammation leading to neurodegenerative and psychiatric diseases, the interest in developing P2X7 antagonists that can penetrate the blood-brain barrier (BBB) is fast growing.

One of the challenges in developing therapeutics for brain diseases is the lack of tractable central biomarkers to monitor the progression of disease. Positron emission tomography (PET) using ligands for the translocator protein, a 18 kDa protein in the outer mitochondrial membrane of steroid-synthesizing cells including those in the CNS, has been attractive in monitoring microglia activation and brain inflammation, with potential use for diagnosis of neurodegenerative and neuropsychiatric diseases as well as in drug discovery (Dupont et al., 2017; Rupprecht et al., 2010). The translocator protein as a PET microglial biomarker however has various limitations such as polymorphism of TSPO, reference region for quantification and cellular specificity, leading to an increasing interest in developing PET tracers targeting other proteins including the P2X7 receptor (Tronel et al., 2017). A P2X7 PET tracer can be useful as a biomarker for brain inflammation and provide additional information such as the location and expression of the P2X7 receptor, which should facilitate a better understanding of the disease mechanism. A CNS-penetrable P2X7 PET tracer would also be beneficial for therapeutic purpose. During the drug development, the PET tracer would be able

to evaluate drug penetration, occupancy and metabolism and, thereafter, could be used as a companion diagnostic to improve patient stratification and determine responders and non-responders to therapy but also to follow up treatment efficacy.

Several reviews have extensively covered the medicinal chemistry efforts in the development of P2X7 antagonists (Arulkumaran et al., 2011; Bartlett et al., 2014; Baudelet et al., 2015; Bhattacharya and Biber, 2016; Chrovian et al., 2014; Guile et al., 2009; Jacobson and Muller, 2016; Lambertucci et al., 2015; Park and Kim, 2017; Rech et al., 2016). It may be worth mentioning that a number of compounds that are potent at the rodent P2X7 receptor do not present the same activity for the human P2X7 receptor. The recent breakthroughs in the structural biology of P2X receptors are expected to accelerate the progress of discovering new P2X7 antagonists (Ahmadi and Shahlaei, 2015; Caseley et al., 2016; Fantoni et al., 2017) and provide the molecular mechanism of antagonism (Barniol-Xicota et al., 2017; Caseley et al., 2015; Karasawa and Kawate, 2016). Below we will highlight the CNS-penetrable P2X7 antagonists, focusing on the studies that explores their potential uses as a PET tracer and/or an antidepressant.

4.1. Abbott compounds

A-740003, A-438079 and A-804598 were discovered by Abbott as P2X7 antagonists (Tab.1). A-740003 was reported to inhibit the human and rat P2X7 receptors with IC_{50} values of 44 nM and 18 nM in a Ca^{2+} influx assay or 93 nM and 100 nM in a dye uptake assay, respectively (Honore et al., 2006). This compound is penetrable to BBB with a brain to plasma (B/P) ratio of 0.11 following intraperitoneal administration (Honore et al., 2006). ^{11}C-A-740003 was synthesized as a PET tracer, but the very low in vivo brain accumulation in healthy rats (<0.05% of injected dose/g) renders this compound not appropriate for imaging (Janssen et al., 2014). A-438079 has IC_{50} values of 126 nM and 316 nM at the human and rat P2X7 receptors, respectively (Nelson et al., 2006) in a Ca^{2+} influx assay. It is worth mentioning that the potency of this compound seems much lower in a subsequent study also using a Ca^{2+} influx assay, with IC_{50} values of 1 μM, 1 μM, 1.3 μM, 3.2 μM and 4 μM at the human, monkey, rat, mouse and dog P2X7 receptors, respectively (Bhattacharya et al., 2013). A-438079 shows good BBB penetration with a B/P ratio of 0.36 following intraperitoneal administration (McGaraughty et al., 2007). As discussed above, A-438079 was used in recent studies via intracerebroventricular or hippocampal microinjection to show a role of the P2X7 receptor in amphetamine-induced manic-like behaviours and CUS-induced depressive-like and anxiety-

like behaviours in rats (Gubert et al., 2016; Yue et al., 2017). A-804598 shows IC_{50} values of 11 nM, 9.9 nM and 8.9 nM at the human, rat, and mouse P2X7 receptors, respectively, in the initial study (Donnelly-Roberts et al., 2009) or 20 nM, 40 nM, 158 nM, 100 nM and 32 nM at the human, monkey, rat, mouse and dog P2X7 receptors, respectively, in a subsequent study (Iwata et al., 2013), both using a Ca^{2+} influx assay. A-804598 has been shown to penetrate BBB to reach the brain upon intraperitoneal injection and, as discussed above, prevent stress-induced activation of the NLRP3 inflammasome and generation of IL-1β and TNF-α in hippocampus and prefrontal cortex as well as depressive-like and anxiety-like behaviours in rats (Iwata et al., 2016).

4.2 AstraZeneca compound

AZ-10606120 (compound-17: (Michel et al., 2007)) (Tab.1) was reported as a P2X7 antagonist with IC_{50} values of 2.6 nM, 28 nM and 58 nM at the human, rat and dog P2X7 receptors, respectively, in an early study using a dye uptake assay (Roman et al., 2009). The IC_{50} values determined in a recent study using a Ca^{2+} influx assay are 1.3 nM, 4 nM, 2 μM, 630 nM and 50 nM at the human, monkey, rat, mouse and dog P2X7 receptors, respectively (Iwata et al., 2013). The BBB penetrability has not been examined specifically. However, it was shown that intraperitoneal administration of AZ-10606120 inhibited LPS-induced depressive-like behaviours in mice (Csolle et al., 2013a), suggesting that AZ-10606120 is BBB-penetrable.

4.3 GSK compounds

Several CNS-penetrable P2X7 antagonists have been developed by GSK, including GSK compound 16, GSK compound 18, GSK compound 21 and GSK1482160 (Tab.1). GSK compound 16, GSK compound 18, and GSK compound 21 inhibit the human P2X7 receptor with IC_{50} values of 8-10 nM and are much less potent at the rat P2X7 receptor with IC_{50} values of 80 nM, 398 nM and 63 nM in a dye uptake assay, and B/P ratios of 0.23, 0.33 and <0.1, respectively (Abberley et al., 2010; Beswick et al., 2010). GSK1482160 (GSK compound 31: (Abdi et al., 2010)) exhibits IC_{50} values of 3 nM and 316 nM at the human and rat P2X7 receptors in a dye uptake assay, respectively, and excellent BBB penetrability with a B/P ratio closing to 2.1 (Abdi et al., 2010). ^{11}C-GSK1482160 has been synthesized as a PET tracer (Gao et al., 2015) and characterized for its binding in LPS-treated and control mice (Territo et al., 2016). There was higher distribution of ^{11}C-GSK1482160 across all tissues including the brain

in LPS-induced inflamed mice. ^{11}C-GSK1482160 showed a significant increase in the total distribution volume in multiple cortical regions and hippocampus, which was blocked by GSK1482160 (Territo et al., 2016). Another recent study has also used ^{11}C-GSK1482160 as a PET imaging tracer to examine neuroinflammation in experimental autoimmune encephalomyelitis (EAE), a rat model of multiple sclerosis (Liu et al., 2017). ^{11}C-GSK1482160 exhibited high retention and homogeneous distribution in the brain and, furthermore, high uptake in lumbar spinal cord at the peak and remitting stages of EAE that was correlated with microglia activation and disease severity (Liu et al., 2017).

4.4 Janssen compounds

A large number of CNS-penetrable P2X7 antagonists has been described by Janssen. Examples include JNJ-47965567, JNJ-42253432, JNJ-54232334, JNJ-54140515, JNJ-54173717, JNJ compound 12d and compound 12f, JNJ compound 7u, JNJ compound 11d, JNJ compound 25 and compound 26, JNJ-54166060, JNJ compound 20 and compound 30, JNJ-54175446, JNJ-55308942 and JNJ-64413739 (Tab.1). Some of these JNJ compounds are strong candidates for the development of therapeutics for mood disorders and other CNS diseases as well as effective PET imaging tracers for monitoring disease progresses and investigating disease mechanisms.

JNJ-47965567 (compound 7: (Letavic et al., 2013)) was characterized in a Ca^{2+} influx assay with IC_{50} values of 5 nM, 2.5 nM, 63 nM, 32 nM and 3.2 nM inhibiting the human, monkey, rat, mouse and dog P2X7 receptors, respectively. JNJ-42253432 (compound 8: (Letavic et al., 2013)) has IC_{50} values of 20 nM, 16 nM and 79 nM at the human, rat and mouse P2X7 receptors, respectively, in a Ca^{2+} influx assay. Both JNJ-47965567 and JNJ-42253432 exhibit strong target engagement and brain penetration after subcutaneous administration with B/P ratios of 0.5-0.58 and ~1, respectively (Bhattacharya et al., 2013; Letavic et al., 2013). Administration of JNJ-47965567 blocked BzATP-induced IL-1β release in the brain *in vivo* (Bhattacharya et al., 2013). As discussed above, administration of JNJ-47965567 or JNJ-42253432 attenuated AH in rats induced by chronic treatment with amphetamine (Bhattacharya et al., 2013; Lord et al., 2014). A recent study, examining the role of P2X7 receptor-mediated neuroinflammation in the pathogenesis of temporal lobe epilepsy, has shown that 5-d treatment with systemic injection of JNJ-47965567 significantly reduced spontaneous epileptic seizures as well as microgliosis and astrogliosis in mice (Jimenez-Pacheco et al., 2016).

JNJ-54232334 and JNJ-54140515 are another two structurally related P2X7 antagonists that inhibit agonist-induced Ca^{2+} influx with IC_{50} values of 0.3 nM and 32 nM for JNJ-

54232334 at the human and rat P2X7 receptors, respectively, and 79 nM at the rat P2X7 receptor for JNJ-54140515 (Lord et al., 2015). A previous study using ^{3}H-JNJ-54232334 displayed homogenous binding of JNJ-54140515 in rat and mouse brains, which was prevented by A-740003 as well as P2X7-KO. As shown in *ex vivo* autoradiography, oral or subcutaneous dosing of JNJ-54140515 inhibited ^{3}H-JNJ-54232334 and ^{3}H-A-804598 binding in the rat brain, demonstrating the BBB penetration of JNJ-54140515 (Lord et al., 2015).

Another recent study has shown JNJ-54173717 as a CNS-penetrable P2X7 antagonist (Ory et al., 2016). JNJ-54173717 inhibits the human and rat P2X7 receptors in a Ca^{2+} influx assay with IC_{50} values of 4.2 nM and 7.6 nM, respectively. ^{11}C-JNJ-54173717 as a PET imaging agent has been evaluated in rat and rhesus monkey brains (Ory et al., 2016). In the rat brain, a high level of ^{11}C-JNJ-54173717 was observed in striatum expressing the recombinant human P2X7 receptor than expressing green fluorescent protein as control, which was attenuated by pretreatment of JNJ-54173717 via subcutaneous administration. PET imaging of the rhesus monkey brain demonstrated a relatively high initial binding and homogeneous binding of ^{11}C-JNJ-54173717 throughout the brain, which were reduced by pretreatment via intravenous injection of JNJ-54173717 or JNJ-42253432.

A distinctive series of 1,2,3-triazolopiperidines have been reported as P2X7 antagonists, including compound 12d with IC_{50} values of 4.2 nM, 6.8 nM and 4.0 nM at the human, rat and mouse P2X7 receptors, respectively, in a Ca^{2+} influx assay (Savall et al., 2015). Structurally related compound 12f was shown to be less potent with IC_{50} values of 24 nM, 3 μM and 600 nM at the human, rat and mouse P2X7 receptors, respectively, in a Ca^{2+} influx assay, but exhibit better target engagement and BBB penetration with a B/P of ~1 after oral dosing in rats (Savall et al., 2015). Another study has described methyl substituted 1-(5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl)methanones as CNS-penetrable P2X7 antagonists, exemplified by JNJ compound 7u with IC_{50} values of 7.7 nM and 10 nM at the human and rat P2X7 receptors, respectively, in a Ca^{2+} influx assay (Rudolph et al., 2015). The B/P ratio reached 0.35 for compound 7u in rats following oral administration. A recent study has characterized substituted 6,7-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-8(5H)-one P2X7 antagonists and identified several CNS-penetrable P2X7 antagonists to be extremely potent with IC_{50} values of < 1 nM at the human P2X7 receptor based on a Ca^{2+} influx assay, including JNJ compound 11d (Ameriks et al., 2016). This compound exhibits good BBB penetration with a B/P ratio of 0.71 in rats after oral administration. Another recent study has reported optimization of phenyl-substituted 5,6-dihydro-[1,2,4]triazolo[4,3-a]-pyrazine P2X7

antagonists, leading to identification of JNJ compound 25 and compound 26 (Chrovian et al., 2016). In a Ca^{2+} influx assay, compound 25 has IC_{50} values of 8.7 nM and 42 nM at the human and rat P2X7 receptors, respectively, and compound 26 has IC_{50} values of 8.8 nM and 82 nM at the human and rat P2X7 receptors, respectively. Both compounds exhibit good BBB permeability and high target engagement in the rat brain with a B/P ratio of 0.5 after oral dosing.

JNJ-54166060, identified recently from characterization of methyl substituted 4,5,6,7-tetrahydro-imidazo[4,5-c]pyridines, is a potent antagonist at the human, rat and mouse P2X7 receptors with IC_{50} values of 4 nM, 115 nM and 72 nM, respectively, in a Ca^{2+} influx assay, and exhibits strong target engagement and BBB penetration in rats with a B/P ratio of 0.84 following oral administration (Swanson et al., 2016). Another recent study has described substituted 5,6-(dihydropyrido[3,4-d]pyrimidin-7(8H)-yl)-methanones as CNS-penetrable P2X7 antagonists, such as JNJ compound 20 and compound 30. The human and rat P2X7 receptors were inhibited by compound 20 with IC_{50} values of 11 nM and 10 nM, and by compound 30 with IC_{50} values of 19 nM and 17 nM, respectively, in a Ca^{2+} influx assay (Ziff et al., 2016). Both compounds display good brain tissue binding and BBB permeability with B/P ratios of 0.3-0.4 and 0.3-0.36, respectively (Ziff et al., 2016).

Two more recent studies have described development of JNJ-54175446 (compound 14: (Letavic et al., 2017)) and JNJ-55308942 (compound 35: (Chrovian et al., 2018)) as P2X7 antagonists with a great capacity of penetrating CNS. JNJ-54175446 was developed from characterization of 4-methyl-6,7-dihydro-4H-triazolo[4,5-c]pyridines as P2X7 antagonists, with IC_{50} values of 1.5 nM, 7.9 nM, 3.5 nM, 16 nM and 13 nM at the human, monkey, rat, mouse and dog P2X7 receptors, respectively, in a Ca^{2+} influx assay (Letavic et al., 2017). JNJ-54175446 shows excellent partitioning into the CNS and target engagement after oral dosing, with a B/P ratio of ~1. JNJ-55308942 inhibits P2X7 receptor-mediated Ca^{2+} responses with IC_{50} values of approximately 10 nM and 15 nM at the human and rat receptors, respectively, and also shows robust partitioning into the CNS and target engagement after oral dosing with a B/P ratio of 0.82-0.95 (Chrovian et al., 2018). In addition, oral dosing with JNJ-55308942 significantly inhibited BzATP-induced release of IL-1β in hippocampus of freely moving rats (Chrovian et al., 2018).

Finally, a preliminary study has reported JNJ-64413739 as potent P2X7 antagonist, with IC_{50} values of 1.9 nM and 1.0 nM at the rat and human P2X7 receptor, respectively (Kolb et al., 2017). In addition, ^{18}F-JNJ-64413739 has been developed as a PET ligand for the P2X7 receptor. Furthermore, PET imaging on rhesus monkeys showed dose-dependent receptor

occupancy of ^{18}F-JNJ-54175446, indicating that it is a promising P2X7 receptor ligand for imaging.

4.4 Pfizer compounds

CNS-penetrable P2X7 antagonists were reported by Pfizer, for example, compound 4k and compound 7f (Chen et al., 2010) (Tab.1). These two compounds are potent in inhibiting human P2X7 receptor-mediated dye uptake with IC_{50} values of 16 nM and 27 nM, and has excellent BBB penetration with B/P ratios of 0.7 and1.3 in rats, respectively.

4.5 Other compounds

Researchers from academia in addition to pharmaceutical companies have been interested in developing CNS-penetrating P2X7 antagonists as a PET imaging tracer and as an anti-depressant. Wilkinson et al modified the AstraZeneca's adamantane benzamide P2X7 antagonist by replacing adamantanyl with polycyclic frameworks (cubanyl, trishomocubanyl, *closo*-1,2-carboranyl) or carborane cages (*nido*-1,2-carboranyl Cs) (Wilkinson et al., 2014). These derivatives exhibited various potency of inhibiting the human P2X7 receptor in a dye uptake assay, with IC_{50} values of 8 nM, 32 nM, 400 nM and 370 nM, respectively. In addition, treatment with trishomocubanyl and *nido*-1,2-carboranyl Cs significantly reduced depressive-like behaviours in mice in the FST, suggesting that these compounds are able to penetrate BBB and elicit antidepressant activity (Wilkinson et al., 2014). The same group has recently characterized a series of bioisosteres of adamantane benzamide and shown that several compounds with a BBB penetrability after administration via intravenous injection, including trifluorinated benzamide (compound 34) (Wilkinson et al., 2017). This compound blocks the human and mouse P2X7 receptors with IC_{50} values of 28 nM and 158 nM, respectively, in a Ca^{2+} influx assay, or the human P2X7 receptor with IC_{50} value of 34 nM in a dye uptake assay. In addition, this compound shows a similar potency (24-62 nM) of inhibiting the human P2X7 receptors carrying NS-SNP mutations (V76A, H155T, H270R, A348T, T357S and Q460R) in a dye uptake assay. They also have characterized in another recent study a series of adamantyl cyanoguanidine hybrid compounds by combining AstraZeneca's adamantyl amide scaffold and Abbott's cyanoguanidine scaffold, for example, compound 35 (Tab.1) (O'Brien-Brown et al., 2017). This compound inhibits the P2X7 receptor in a dye uptake assay with an IC_{50} value of 69 nM and via intraperitoneal administration depressive-like behaviours in mice examined in the FTS, demonstrating its BBB penetrability (O'Brien-Brown et al., 2017). Fantoni et al have developed a structural derivative of A-804598, i.e., ^{18}F-EFB as a PET tracer designed based on

molecular docking (Fantoni et al., 2017). EFB (Tab.1) has IC_{50} values of 1.8 μM, 7.2 μM and 6 μM at the human, rat and mouse P2X7 receptors, respectively, in a Ca^{2+} influx assay. ^{18}F-EFB exhibited limited, albeit quantifiably different, uptake in LPS-inflamed and control rat brains.

5. Concluding remarks

Mood disorders are projected to become the first leading cause of disease burdens worldwide in the coming decade that will impose immense challenges to healthcare provision. For such unmet clinical needs, basic research into the disease mechanisms, identification of novel drug targets and development of more effective therapeutics are required. As discussed in this review, both clinical and preclinical studies have accumulated a large body of evidence to support that the innate immune system dysfunction leading to brain inflammation is crucial in the pathogenesis and development of mood disorders. The P2X7 receptor has been identified as a key molecule in mediating stress-induced innate immune system dysfunction and alterations in synaptic and neuronal network functions, including synaptic transmission, neuron-glia communications, neurogenesis and neuroplasticity. It is of huge importance, both scientifically and therapeutically, to gain a clear understanding of the signalling mechanisms underlying such complicated deficiencies, due to P2X7 receptor-mediated generation of IL-1β, IL-6 and TNF-α, that translate into behavioural and cognitive impairments. With persistent interests in the development of CNS-penetrable P2X7 antagonists, one can hope in the foreseeable future that such compounds can be used as therapeutics mitigating the progression of mood disorders as well as clinically useful PET imaging tracers for monitoring the progression of diseases.

Conflict of interest statement

The author declares no conflict of interest.

Acknowledgments

The research works from the authors' laboratory were supported by the Disciplinary Group of Psychology and Neuroscience Xinxiang Medical University (2016PN-KFKT-06), Department of Education of Henan Province (16IRTSTHN020), Biological Science and Biotechnology Research Council (BB/C517317/1), Wellcome Trust (099758/Z/12/Z) and visiting professorship from University François-Rabelais of Tours (L-HJ), and the "Ministère de la

Recherche et des Technologies", the Inserm, the "Ligue Nationale Contre le Cancer-Interrégion Grand-Ouest", the Région Centre (grant "CancerInflamm", project "ARD2020 Biomédicaments") and the "Association CANCEN" (SR).

Figure Legend

Figure 1 Proposed P2X7 receptor mechanisms in the pathogenesis of mood disorders
Psychological stress induces presynaptic release of glutamate that in turn causes release of ATP from astrocytes and ATP induces release of glutamate via activation of the P2X7 receptor, leading to enhanced levels of ATP and glutamate at the synapse (not depicted). ATP acts on the P2X7 receptor on microglia, resulting in activation of the NLRP3 inflammasome and caspase-1. Capsase-1 generates IL-1β that subsequently induces generation of IL-6 and TNF-α. These proinflammatory cytokines further alter synaptic, neuron-glial and neuronal network functions, leading to mood disorders. The supporting evidence is discussed in the text.

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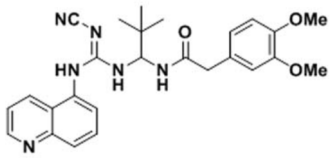
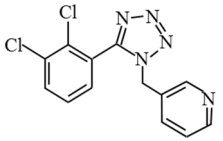
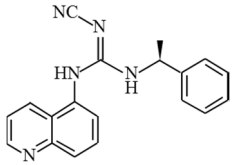
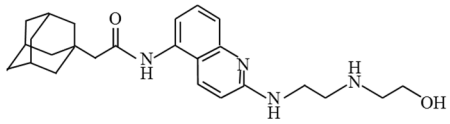
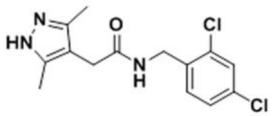
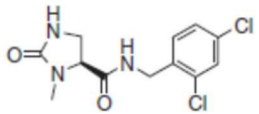
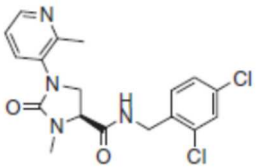
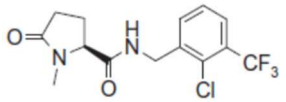
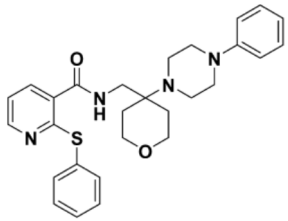
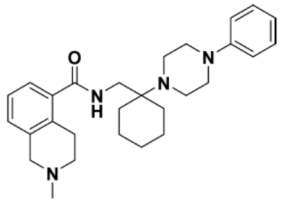
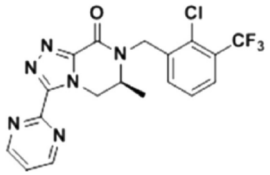
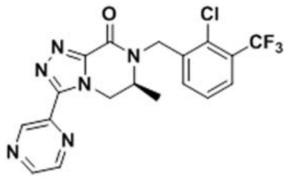
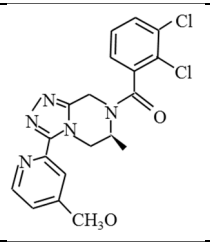
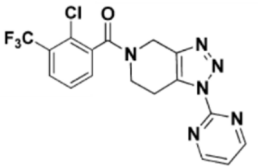
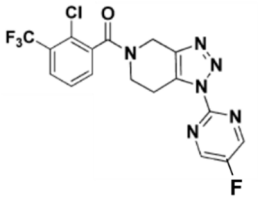
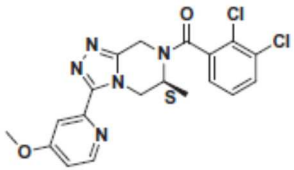
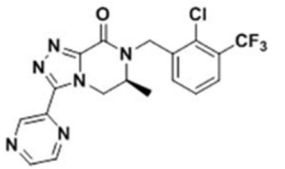
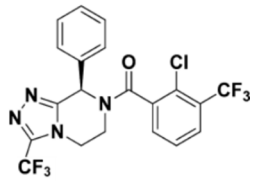
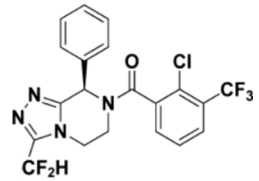
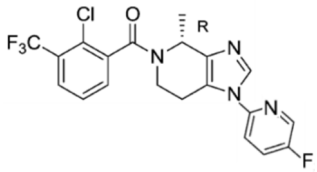
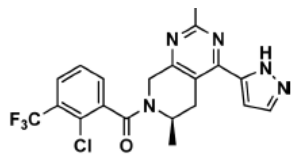
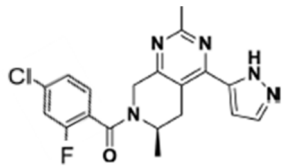
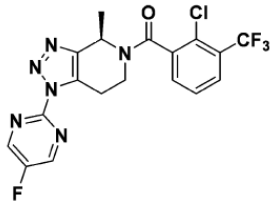
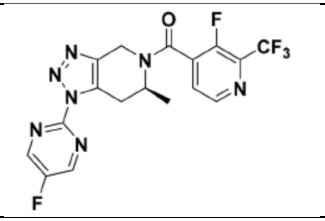
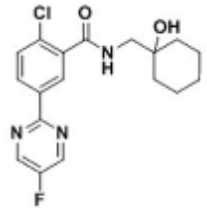
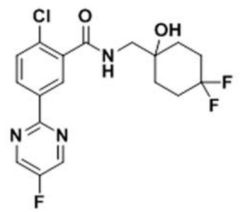
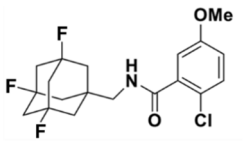
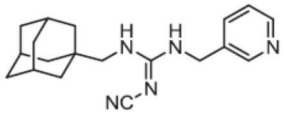
Table 1 Pharmacological properties of CNS-penetrable P2X7 antagonists

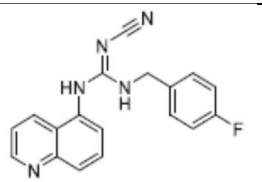
Compound	Chemical structure	IC_{50} in nM	PET tracer	B/P ratio	References
A-740003		44 (h); 93 (h)[a] 18 (r); 100 (r)[a]	^{11}C-A-740003	0.11	Honore et al., 2006; Janssen et al., 2014
A-438079		126-1000 (h) 1000 (mm) 316-1259 (r) 3162 (m) 3980 (d)		0.36	Nelson et al., 2006; McGaraughty et al., 2007; Bhattacharya et al., 2013
A-804598		11-20 (h) 40 (mm) 9.9-158 (r) 8.9-100 (m) 32 (d)		Yes	Donnelly-Roberts et al., 2009; Bhattacharya et al., 2013; Iwata et al., 2016
AZ10606120		1.3 (h); 2.6 (h)[a] 4 (mm) 1995 (r); 28 (r)[a] 630 (m) 50 (d); 58 (d)[a]		Yes	Roman et al., 2009; Bhattacharya et al., 2013; Csolle et al., 2013b
GSK cpd 16		8 (h)[a] 80 (r)		0.23	Beswick et al., 2010
GSK cpd 18		10 (h)[a] 398 (r)[a]		0.33	Abberley et al., 2010;

GSK cpd 21		10 (h)[a] 63 (r)[a]		< 0.1	Abberley et al., 2010;
GSK1482160		3.1 (h)[a] 316 (r)[a]	^{11}C-GSK1482160	2.1	Abdi et al., 2010; Gao et al., 2015; Territo et al., 2016
JNJ-47965567		5 (h) 2.5 (mm) 63 (r) 32 (m) 3.2 (d)		0.5-0.58	Letavic et al., 2013; Bhattacharya et al., 2013
JNJ-42253432		20 (h) 16 (r) 79 (m)		~1	Letavic et al., 2013
JNJ-54232334		0.3 (h) 32 (r)		unknown	Lord et al., 2015
JNJ-54140515		79 (r)		Yes	Lord et al., 2015

JNJ-54173717		4.2 (h) 7.6 (r)	^{11}C-JNJ-54173717	Yes	Ory et al., 2016
JNJ cpd 12d		4.2 (h) 6.8 (r) 4 (m)		unknown	Savall et al., 2015
JNJ cpd 12f		24 (h) 3000 (r) 588 (m)		~1	Savall et al., 2015
JNJ cpd 7u		7.7 (h) 10 (r)		0.35	Rudolph et al., 2015
JNJ cpd 11d		0.7 (h) 79 (r)		0.71	Ameriks et al., 2016

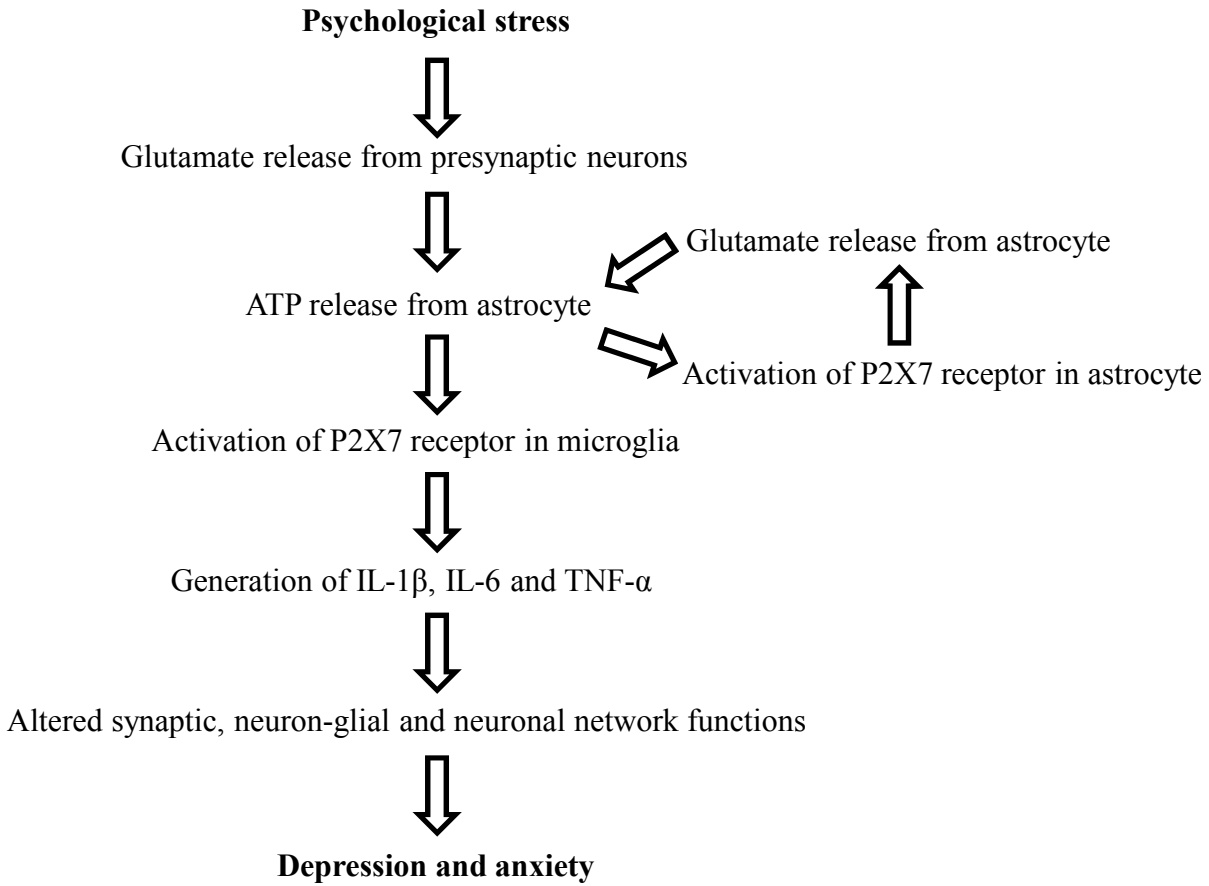
JNJ cpd 25		8.7 (h) 42 (r)		0.5	Chrovian et al., 2016
JNJ cpd 26		8.8 (h) 82 (r)		0.5	Chrovian et al., 2016
JNJ 54166060		4 (h) 115 (r) 72 (m)		0.84	Swanson et al., 2016
JNJ cpd 20		11 (h) 10 (r)		0.3-0.4	Ziff et al., 2016
JNJ cpd 30		19 (h) 17 (r)		0.3-0.36	Ziff et al., 2016
JNJ-54175446		1.5 (h) 7.9 (mm) 3.5 (r) 16 (m) 13 (d)		~1	Letavic et al., 2017

JNJ-55308942		10 (h) 15 (r)		0.82~0.95	Chrovian et al., 2018
Pfizer cpd 4k		16 (h)[a]		0.7	Chen et al., 2010
Pfizer cpd 7f		27 (h)[a]		1.3	Chen et al., 2010
Trifluorinated benzamide (cpd 34)		24 (h) 158 (m) 34 (h)[a]		Yes	Wilkinson et al., 2017
		69 (h)[a]		Yes	O'Brien-Brown et al., 2017
EFB		1820 (h) 7244 (r) 6026 (m)	^{18}F-EFB	Yes	Fantoni et al., 2017

Note: [a]IC_{50} determined in a dye uptake assay. h, human; mm; macaque monkey; r, rat; m, mouse; d, dog. B/P, the brain to plasma ratio.

Fig. 1


Psychological stress
Glutamate release from presynaptic neurons
ATP release from astrocyte
Glutamate release from astrocyte
Activation of P2X7 receptor in astrocyte
Activation of P2X7 receptor in microglia
Generation of IL-1β, IL-6 and TNF-α
Altered synaptic, neuron-glial and neuronal network functions
Depression and anxiety