Review

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P2X7 receptor: an emerging target in central nervous system diseases

Beáta Sperlágh[1] and Peter Illes[2]

[1] Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, Hungary
[2] Rudolf Boehm Institute of Pharmacology and Toxicology, University of Leipzig, D-04107 Leipzig, Germany

The ATP-sensitive homomeric P2X7 receptor (P2X7R) has received particular attention as a potential drug target because of its widespread involvement in inflammatory diseases as a key regulatory element of the inflammasome complex. However, it has only recently become evident that P2X7Rs also play a pivotal role in central nervous system (CNS) pathology. There is an explosion of data indicating that genetic deletion and pharmacological blockade of P2X7Rs alter responsiveness in animal models of neurological disorders, such as stroke, neurotrauma, epilepsy, neuropathic pain, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease, and Huntington's disease. Moreover, recent studies suggest that P2X7Rs regulate the pathophysiology of psychiatric disorders, including mood disorders, implicating P2X7Rs as drug targets in a variety of CNS pathology.

P2X7Rs in the CNS

It has been known for almost a decade that P2X7Rs convey important pathophysiological functions in the CNS [1]. ATP is released in large quantities following any kind of cell injury, and the ensuing stimulation of the low affinity P2X7R results in necrosis/apoptosis or proliferation as the two opposing end-points of neuroinflammation. Therefore, P2X7R antagonists are potential therapeutics in disorders of neuroinflammation, such as traumatic brain injury, stroke, epilepsy, neuropathic pain, and neurodegenerative illnesses, because in these cases, secondary cell damage accompanies the primary pathological condition. In this review, we discuss the latest developments in the description of these functions, to redirect interest to those fields where there are still significant gaps in our present understanding, and to promote further development of those therapeutic areas in which P2X7R is the most promising as a potential drug target.

The structure and molecular physiology of P2X7Rs

P2X7Rs are ATP-gated, non-selective cation channels belonging to the family of ionotropic P2X receptors. P2X7Rs function in homo-trimeric form and most mammalian P2X7R subunits comprise 595 amino acids [2]. The common structural motifs of P2X7Rs are the two transmembrane domains (TM1, TM2), a large, glycosylated, cysteine-rich extracellular loop, a short intracellular N-terminal domain, and an intracellular C-terminal domain, which is longer than that of other P2X receptor subunits. Within the family of P2X receptors, so far only the crystal structure of zebrafish zfP2X4.1R has been solved in the closed [3] and ATP-binding, open state [4]; nevertheless, its considerable homology with mammalian P2X7Rs allowed for the structural modelling of the latter [2]. The molecular architecture of an individual P2X7R subunit is akin to a leaping dolphin, with the extracellular loop forming the body, and the TM domains forming the tail. When co-assembled as a trimeric unit, P2X7R has a chalice-like structure, overarching the channel pore (Figure 1A). There are three ATP binding sites localized at the interface of the three subunits; occupancy of at least two of the three sites is necessary for the activation of the receptors [5]. The adenine base and the β- and γ-phosphate groups of ATP form hydrogen bonds with the respective amino acid residues of the ATP binding pocket, as suggested for the zfP2X4.1R. However, because a residue corresponding to Leu217, which interacts with the ribose moiety, is missing in the mammalian P2X7R, the affinity of ATP to P2X7Rs is more than 100-fold lower than to other P2XR-subtypes [2]. By contrast, non-conserved residues surrounding the ATP binding site might confer differences in agonist sensitivity between mammalian P2XR species, (i.e., rat P2X7Rs display substantially higher sensitivity to ATP and 2′(3)-O-(4-Benzoylbenzoyl)ATP (BzATP) than their human and mouse counterparts [6]). A distinctive feature of the mouse P2X7R is that it can be activated by extracellular nicotinamide adenine dinucleotide (NAD^+) by ADP-ribosylation with the ADP-ribosyltransferase 2 ectoenzyme [7]. In contrast, less is known about the binding site of antagonists, although potent and selective antagonists of P2X7Rs are now widely available. Earlier data indicated that P2X7R subunits are able to form heterotrimers with P2X4Rs [8], but more recent studies did not confirm this (e.g., [9]).

There are several splice variants of mammalian P2X7Rs, all of which are widely expressed in the nervous system. Hence, a naturally occurring truncated isoform of the human P2X7R (P2X7B) has been found in the CNS [10]; a C-terminally truncated variant of mouse P2X7R has also been identified, which partly retains its functionality when expressed in tissues of the *P2rx7* gene deficient mice [11]. Another mouse isoform is the P2X7(k) variant, which, in contrast to the originally identified P2X7(a), is sensitive to ADP-ribosylation [12,13].

Corresponding author: Sperlágh, B. (sperlagh@koki.hu).
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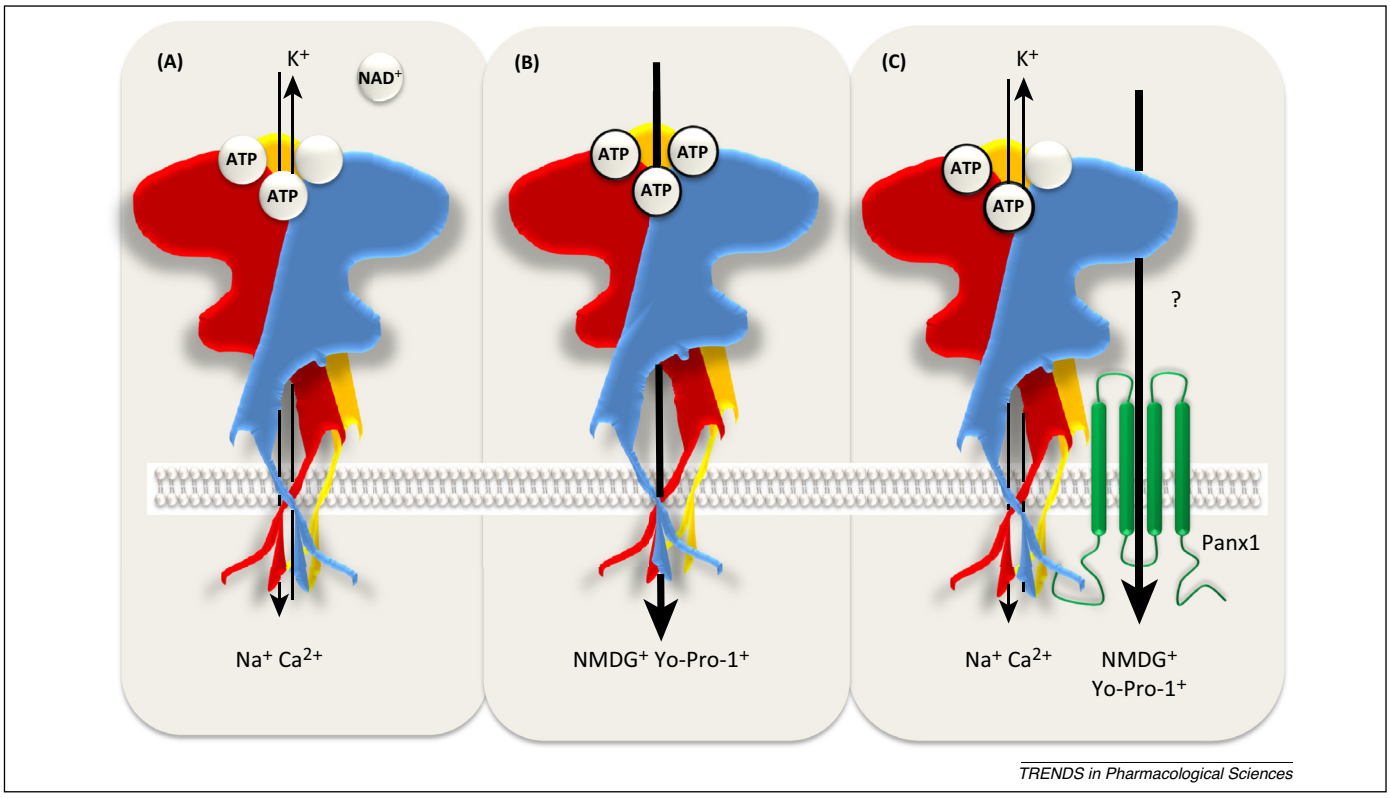

Figure 1. The simplified schematic structure of the P2X7 receptor (P2X7R) in open state **(A)** and during pore formation **(B and C)**. (A) The P2X7R functions as a homo-trimer, forming a chalice-like structure, while the individual P2X7R subunit is akin to a leaping dolphin. The agonist binding sites are located at the subunit interfaces and the occupation of two out of three binding sites is necessary for opening of the channel. In addition to ATP, which is the presumed endogenous agonist, the mouse P2X7R receptor could also be activated by NAD^+ through ADP-ribosylation. The activation of the receptor–ion channel leads to the inward flux of cationic current. Prolonged and/or repeated activation of P2X7R and occupation of the third agonist binding site renders the membrane permeable for high molecular weight organic cations and dyes, such as $NMDG^+$ and Yo-Pro-1^+ (B and C). (B) One potential mechanism of the pore formation is the dilation of the P2X7R-mediated channel pore itself. (C) Alternatively, but not exclusively, additional pore forming proteins, such as pannexin (Panx1) might be recruited, which seem to be indispensable for pore formation under certain circumstances.

The gene encoding the human P2X7R (*P2RX7*) is also well known to exhibit a number of non-synonymous single nucleotide polymorphisms (NS-SNPs), which results in a change in amino acid sequence and the expression of different human *P2RX7* variants, further increasing the structural diversity of P2X7Rs. The functional consequence of several individual NS-SNPs has been determined in native and recombinant systems and their association with various human CNS disease states has been extensively investigated in genetic linkage studies [14].

The activation of P2X7Rs results in the opening of the channel pore, allowing the passage of small cations (Na^+, Ca^{2+}, and K^+). In addition, a hallmark feature of the P2X7R is the opening of a non-selective pore in response to repeated or prolonged activation, allowing the permeation of large molecular weight organic cations up to 600–800 Da. The pore forming property of P2X7Rs can be studied by the uptake of high molecular weight cations, such as $NMDG^+$, or dyes, such as Yo-Pro-1 or ethidium bromide; nevertheless, its molecular mechanism has remained a highly debated issue, with two alternative, but non excluding possibilities, both having substantial experimental support (Figure 1B,C). The first potential mechanism is the progressive dilation of the P2X7R-gated channel itself. A conformational change of the receptor protein could be the structural basis for channel dilation, as previously confirmed for other P2XRs (P2X2, P2X4) by electrophysiological methods [15]. In agreement with the pore dilation theory, the carboxyl terminal domain [16] and the TM2 region of the P2X7R protein are essential for pore formation [17]. Moreover, recent studies revealed that the open channel conformation of the P2X7R can allow the passage of negatively charged fluorescent dyes with molecular diameters of up to 1.4 nm [18], and occupation of one or two agonist binding sites favours transition to the desensitized state, whereas occupation of the third binding site favours the transition to the sensitized/dilated state [19].

The alternative mechanism involves the recruitment of an additional pore-forming protein, most likely the pannexin-1 hemichannel (Panx1). Evidence derived from studies using genetic knockdown of Panx1 indicate that this protein is indispensable for the pore formation (e.g., [20]) and can be selectively affected pharmacologically by colchicine [21]. However, other data conflict with the involvement of Panx1 in the formation of the membrane pore (e.g., [22]). Therefore, it appears that although recruitment of pannexin hemichannels is a downstream signalling event closely linked to P2X7R activation, it is not an absolute requirement [23]. A potential dissolution of conflicting results is that different P2X7R splice variants display distinct pore forming properties [12,23].

The opening of the large pore might eventually result in membrane blebbing and cell death; however, this is not an obligatory consequence of P2X7R activation. Pore

formation might gain significance in the pathological sensitization underlying chronic pain, as highlighted by a recent study [24]. This paper reported that mutations of the gene encoding the P2X7R, which result in hypofunctional pore formation, affect chronic pain sensitivity in both mice and humans. Moreover, treatment with a peptide corresponding to the P2X7R C-terminal domain, which blocks pore formation, but not cation channel activity, selectively reduced allodynia only in mice with the pore-forming *P2rx7* allele. These findings illustrate that the pore formation associated with P2X7R by itself could be a potential target of personalized therapy to combat chronic pain disorders.

Tissue and cell type specific distribution of P2X7Rs

P2X7Rs are expressed by many cell types, including cells of hematopoietic origin (lymphocytes, monocyte-macrophages, and microglia) and intrinsic cells of the nervous system (neurons, astrocytes, oligodendrocytes, and Schwann cells). P2X7R binding sites have been explored in autoradiographic studies using the radioligand [^{3}H]-A-804598, and a dense P2X7R binding was found throughout the brain and spinal cord [25], including hypothalamic nuclei, thalamic nuclei, hippocampus, spinal trigeminal nucleus and tract, cortical regions, cerebellum and caudate putamen [25]. Nevertheless, the cell-type specific localization of the P2X7Rs in the CNS has been the subject of a long-standing debate, which has not reached general consensus even after a decade: immunohistochemical findings are inhomogeneous and contradict findings obtained by physiological and neurochemical methods. Whereas early studies found a prominent expression of P2X7R immunoreactivity (IR) on excitatory nerve terminals [26], and later studies confirmed these findings throughout the CNS [27,28]; other groups questioned these findings, revealing P2X7R-IR in brain sections obtained from P2X7R deficient animals [29]. Subsequently, however, functional splice variants of rodent P2X7R [11,12] were identified which are likely to be responsible for P2X7-pseudo-IRs, found in the brain of P2X7R$^{-/-}$ mice. These variants represent either gain- or loss-of function P2X7Rs, and may explain the high variability of responses induced by P2X7R stimulation. Other studies reported an activity-dependent expression pattern of P2X7Rs, induced or upregulated following an insult such as a seizure [30], ischemia [31], sleep deprivation [32], undernourishment [33], or morphine tolerance [34]. A recent study utilizing single particle tracking photoactivated localization microscopy (sptPALM) revealed that Dendra2 tagged P2X7Rs transfected to hippocampal neurons formed two dynamic populations within the extrasynaptic membrane of proximal dendrites: one was composed of rapidly diffusing receptors and another stabilized within nanoclusters, both being rarely appositioned to synaptic sites [35].

In contrast to immunohistochemistry, the available evidence on functional P2X7Rs on different cell types of the CNS is convincing. Functional studies, verifying P2X7Rs on neurons, astrocytes, and microglia are presented in Table 1. The most parsimonious explanation for the contradictory findings is that the expression of P2X7Rs dynamically changes in response to experimental variables, such as age or different levels of stressful stimuli prior to sample collection (freshly prepared versus fixed sections). Moreover, under *in vivo* conditions even mild stimuli, such as saline injection, may cause a dramatic change in the expression level of P2X7Rs.

Table 1. Examples from recent studies verifying functional P2X7Rs on different cell types of the rodent central nervous system

Cell type/brain area, preparation	Technique	Refs
Neurons		
Cerebral cortex, purified synaptosomes	Neurochemistry, Ca^{2+} fluorimetry	[44]
Midbrain, synaptic terminals	Ca^{2+} microfluorimetry	[130]
Neurohypophysis, nerve terminals	Patch clamp electrophysiology	[131]
Caudal brainstem, nerve terminals	Neurochemistry	[132]
Hippocampus, isolated hilar neurons	Patch clamp electrophysiology	[51]
Retina, isolated ganglion cells	Patch clamp electrophysiology	[53]
Suprachiasmatic nucleus, isolated neurons	Ca^{2+} imaging	[133]
Embryonic spinal cord, cultured neurons	Neurochemistry	[134]
Cortex, cultured neurons	Neurochemistry	[135]
Astrocytes		
Cortex, *in situ*	Patch clamp electrophysiology	[136]
Cortex, cultured	Patch clamp electrophysiology	[137]
Cerebellum, cultured	Neurochemistry	[138]
Human, cultured	Ca^{2+} fluorimetry	[139]
Bergmann glia		
Cerebellum, *in situ*	Patch clamp electrophysiology, Ca^{2+} imaging	[140]
Satellite glia		
Immature dorsal root ganglion, isolated	Electrophysiology	[141]
Microglia		
Cortex, *in situ*	Patch clamp electrophysiology	[142]
N9 microglia, cultured	Neurochemistry	[143]

Physiopathology of P2X7 receptors

P2X7R function can be studied with a selection of pharmacological and genetic tools (Box 1). The activation of P2X7Rs is followed by Ca^{2+} influx and a variety of cellular responses depending on the cell type investigated (Figure 2). Outside the nervous system, the most prominent role of P2X7R is in the regulation of cytokine response to inflammatory challenge. In fact, P2X7R is a key regulatory element of the inflammasome molecular complex, providing the external stimulus necessary for the post-translational modification and subsequent release of the proinflammatory cytokine IL-1β. The role of P2X7Rs has been confirmed in the regulation of central cytokine response after lipopolysaccharide (LPS) priming [36]. This effect could be involved in physiological and pathological

Box 1. Tools to study P2X7 receptors

The continuously evolving interest in this receptor resulted in the generation of various tools to study its function. P2X7Rs could be identified based on the following distinctive pharmacological features:

- The affinity of the endogenous agonist ATP is low, in the high micromolar–millimolar range.
- BzATP is a more potent agonist than ATP itself. It has been frequently mistakenly used as a selective agonist of P2X7R. However, this is not valid, because BzATP also binds to other P2X receptors with high affinity.
- The effect of ATP and BzATP are potentiated by a low Ca^{2+}/no Mg^{2+}-containing external medium.
- There are several potent antagonists available, such as A-438079, A-740003, the negative allosteric modulator AZ-10606120 and Brilliant blue G (BBG); among these, BBG is selective in concentrations below 1 μM. This antagonist is also a useful tool in *in vivo* experiments. The penetration of BBG through the blood–brain barrier has already been determined, and using doses not higher than 50 mg/kg, the resultant brain concentration remains below 1 μM [105]. It should be noted, however, that many P2X7R antagonists, including BBG, also inhibit Panx1 channels. Therefore, BBG alone is inadequate to prove the involvement of P2X7Rs [144]. In this respect, a valuable compound could be Brilliant blue FCF, which inhibits Panx1, but not P2X7R [145].
- Novel radioligands, for example, [^{3}H]A-804598, are also available to characterize the affinity of newly developed compounds to rodent P2X7Rs [25].

In addition to pharmacological approaches:

- Genetic knockdown by siRNA has been increasingly used to silence P2X7Rs in the past years in both *in vitro* and *in vivo* studies (e.g., [34,39]).
- Mouse lines genetically deficient in P2X7Rs, initially generated by the companies Glaxo (*LacZ* gene and neomycin cassette insertion into exon 1; [146]) and Pfizer (Neo insertion in exon 13, close to the carboxyl terminal; [147]), have also been widely used. However, none of these mouse lines could be regarded as fully deficient in P2X7Rs, as individual splice variants evaded inactivation [11,12].
- For studies of P2X7R function in morphologically identified neurons, astrocytes, or microglia, the GFP-P2X7 reporter mouse seems to be a crucial tool [148].

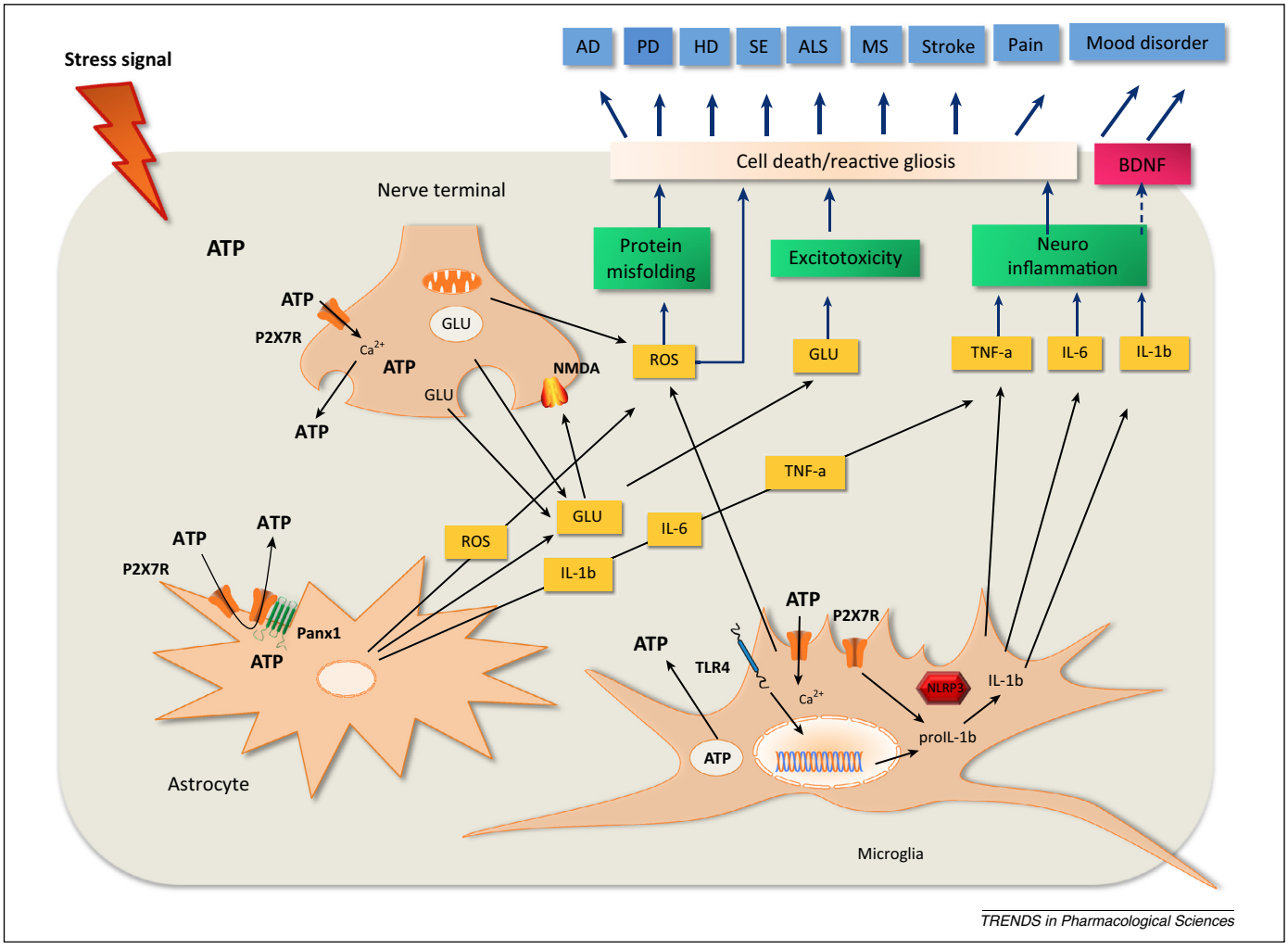

Figure 2. Common disease mechanism by P2X7 receptor (P2X7R)-mediated pathways in central nervous system (CNS) disorders of different etiology. P2X7Rs are expressed on nerve terminals, astrocytes, and microglia, and they are upregulated in various disease conditions. Stress signals, such as hypoxia/ischemia (metabolic limitations), mechanical injury, and bacterial or chemical toxins elicit the endogenous activation of P2X7R and leads to a self-amplifying ATP release and to further activation of P2X7 receptors on neighbouring cells. Following the influx of Ca^{2+} through the receptor ion channel complex, P2X7 receptor activation (i) releases glutamate from nerve terminals and astrocytes by both exocytotic and non-exocytotic mechanisms, which may give rise excitotoxicity; (ii) leads to the posttranslational processing of pro- IL-1β to the leaderless, mature IL-1 β and to its further release by the NLRP3 inflammasome and that of other cytokines, which contribute to neuroinflammation; (iii) enhance reactive oxygen species (ROS) production and thereby aggravate protein misfolding and neuronal damage; (iv) leads directly or indirectly to cell death and the following reactive astrogliosis; and (v) directly or indirectly downregulates the production of brain-derived neurotrophic factor (BDNF) and the subsequent neuroplasticity. These key mechanisms could be manifested and contribute to disease pathology in Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), status epilepticus (SE), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), stroke, pain, and mood disorders in different forms and proportion, depending on the etiology. Abbreviations: GLU, glutamate, ROS, reactive oxygen species.

actions controlled by P2X7Rs, such as memory formation [37], sleep [32], fever [38], hyperalgesia [39], and depression [40,41].

However, a major caveat in our understanding of the physiopathology of P2X7R function is how the endogenous activation of P2X7Rs is achieved, given the low affinity of the endogenous agonist ATP. ATP is present in the synaptic vesicles and is co-released as a co-transmitter with various other transmitters in the autonomic nervous system under physiological conditions [42]. This also holds true, to a certain extent, for central synapses, and the increase in extracellular ATP in response to normal neuronal activity might transiently reach the high micromolar concentration required for the activation of P2X7R, at least in the synaptic cleft. However, a more widespread activation of P2X7Rs is expected under pathological conditions, when tissue damage, trauma, or other pathological signals provide an ATP-rich extracellular milieu, which might lead to the activation of extrasynaptic and extraneuronal P2X7Rs. In addition, the possibility of constitutive activity without the presence of the endogenous agonist cannot be excluded either, and should be further investigated. In the CNS, the best characterized consequence of P2X7R activation is the release of neurotransmitters, in particular, of glutamate to the extracellular space [43]. This effect could be evoked both from synaptosomes [44] and from astrocytes [45]. In nerve terminals and cell lines expressing recombinant P2X7Rs, the P2X7R mediated glutamate release appears to be both exocytotic and non-exocytotic, [46,47]. P2X7R-mediated excitatory amino acid efflux can be detected in acutely prepared brain slices by neurochemical (e.g., [48,49]) and electrophysiological techniques [50]. In rat hippocampal (hilar neurons [51]; CA1 neurons [52]), and midbrain slices (locus coeruleus [50]), stimulation of P2X7Rs by BzATP elicited an increase of the frequency, but not amplitude, of spontaneous excitatory postsynaptic currents (sEPSCs) and miniature (m)EPSCs. Occasionally [49,50], the P2X7R-mediated glutamate release was sensitive to blockade by fluorocitric acid, a glia-selective metabolic poison, and to antagonists of glutamate receptors. These findings imply that glutamate release induced by P2X7R stimulation from neurons could also be indirect, mediated by glutamate release from astrocytes, acting subsequently on glutamatergic nerve terminals.

To add further complexity to neuron–glia and glia–neuron P2X7R signalling, P2X7R stimulation elicits or reinforces the release of ATP, thereby providing an autostimulatory loop. This effect was observed in retinal ganglion cells [53], hippocampal brain slices [49], and cultured spinal cord astrocytes [54]. The mechanism of P2X7R-driven ATP release could be exocytotic, as observed by total internal reflection microscopy in neuroblastoma cells [55], whereas in other studies it appears to involve connexin and/or pannexin hemichannels [49,54].

A further interesting function of P2X7Rs is to regulate differentiation and cell fate during development. P2X7Rs are expressed by both embryonic [56] and adult neural progenitor cells (NPCs) in the subventricular zone of the lateral ventricle [57]. Whereas stimulation of P2X7Rs induces neuronal differentiation in embryonic NPCs [56], other studies indicated that P2X7Rs stimulate gliogenesis [58]. By contrast, the activation of P2X7Rs on adult, cultured NPCs decrease cell proliferation and induce necrotic/apoptotic cell death [57].

Of note, a very recent study showed that P2X7Rs regulate ion channel density and protein composition/function of the axon initial segment, a key structural element of neuronal excitability and, in consequence, action potential initiation in cultured hippocampal neurons and brain slices [59].

It has been known for a long time that P2X7R activation might lead to cell death through pore formation, as has been described for peripheral immune cells. However, a more recently emerging view is that P2X7Rs also convey trophic function against cell death promoting physiological or pathological stimuli: for example the microglial 'suicide' P2X7R promotes cell cycle progression and proliferation [60,61], and this receptor might act as a scavenger for the removal of apoptotic cells in the absence of its ATP ligand [62,63].

P2X7R as a potential target in neurological diseases

Recent data indicate that genetic deletion and pharmacological blockade of P2X7Rs alter responsiveness in animal models of neurological disorders, such as stroke, neurotrauma, epilepsy, neuropathic pain, MS, ALS, Alzheimer's disease, Parkinson's disease, and Huntington's disease.

Middle cerebral artery occlusion, the most widely used animal model of cerebral ischemia, results in cell death in the core of the affected neuronal tissue, while around it, in the so called penumbra, the cellular damage is reversible. Both infarct size and neurological deficits were reduced by P2X7R antagonists [64,65]. In combination with the sequential upregulation of P2X7R-IR in microglia and then in astrocytes and neurons, this receptor type was considered to be a primary target of the considerable amounts of ATP released. Similar results were reported for subarachnoid haemorrhage [66], traumatic brain [67,68] or spinal cord injury [69], and ischemic retina degeneration [70]. However, a later study failed to reconfirm the protective action of P2X7R in spinal cord injury [71]. Reperfusion after transient global cerebral ischemia exacerbates the consequences of oxygen/glucose deprivation (OGD) due to microglial and astroglial activation [72]. The ensuing neuroinflammatory reaction is also alleviated by P2X7R antagonists [73,74]. Brilliant blue G (BBG) partially reversed the OGD-induced anoxic depolarization and cell damage in cultured oligodendrocyte cells [75]. Accordingly, left common carotid artery occlusion decreased P2X7R-IR at oligodendrocyte precursor cells in the cerebral cortex, subcortical white matter, and hippocampus [76].

Status epilepticus (SE)-like seizures, modelled in rodents by pilocarpine or kainate, upregulate P2X7R-IR in microglial cells [77], astrocytes, and neurons [78]; quantification by Western blotting confirmed these results [79,80]. Utilizing the intra-amygdala application of kainate as an epileptic stimulus [79,80], it was shown that: (i) BzATP facilitated and prolonged the EEG activity caused by seizures; and (ii) P2X7R antagonists had a neuroprotective effect after epilepsy due to suppression of IL-β

production and microglial response. More recent findings suggest that the effect of P2X7Rs during SE depends on the nature of the chemical stimulus used. A-438079 increased pilocarpin-induced seizure susceptibility in mice by interrupting a direct inhibitory interaction between P2X7- and muscarinic receptors [81] or blockade of the release of the protective TNF-α [82]. P2X7R activation also influenced leukocyte infiltration [83] and reactive astrogliosis following SE [84].

The involvement of P2X7Rs in different models of inflammatory and neuropathic pain, and the potential therapeutic effect of P2X7R antagonists are well documented [85]. Downregulation of P2X7Rs with siRNA or BBG prevented the induction of spinal long-term potentiation *in vitro*, and at the same time, alleviated mechanical allodynia in naive rats *in vivo* [39]. Central sensitization of nociceptive neurons could be produced by intrathecal superfusion of BzATP and was depressed by P2X7R antagonists [86]. Additional studies extended these findings to mechanisms participating in the development of neuropathic or orofacial pain [87–89], bone cancer pain [90], and migraine [91]. Recent studies highlighted the association between human *P2RX7* variants with chronic pain sensitivity [24].

MS is a chronic degenerative disease of the CNS that is characterized by focal lesions with inflammation, infiltration of immune cells, demyelination, oligodendroglial death, and axonal damage [92]. A putative association of the *P2RX7* gene with this illness was indicated by the most frequent expression of the gain-of-function T allele of the *rs17525809* polymorphism of the receptor, which yields an Ala-76 to Val change in its extracellular domain [93]. The overexpression of P2X7Rs was detected in experimental autoimmune encephalomyelitis (EAE), an animal model of MS [94], whereas the amelioration of EAE was found in P2X7R deficient animals [95,96]; however, see also [97]. Furthermore, pannexin-1 knockout mice with restricted ability to mediate pore development/dye uptake after P2X7R stimulation, also displayed a delayed onset of clinical signs of EAE and decreased mortality when compared with wild type mice [98].

ALS is characterized by the progressive degeneration of motor neurons in the spinal cord, brainstem, and motor cortex, leading to respiratory failure and death of the affected patients within a few years of diagnosis [99]. Microglia and astrocytes are major contributors to motor neuron dysfunction in ALS through the maintenance of a chronic inflammatory response. Transgenic mice expressing a mutant protein Cu^{+}/Zn^{+} superoxide dismutase SOD1-G93A, which directly enhances the activity of the main reactive oxygen species producing enzyme in microglia (NADPH oxidase 2; NOX2), is used widely as a model of ALS [100]. P2X7R activation by BzATP induced the death of motor neurons in mixed astrocytic/neuronal cultures prepared from wild type mice [101]. Furthermore, apyrase, an enzyme degrading ATP or BzATP, decreased neuronal death observed in cultures prepared from SOD1-G93A transgenic mouse spinal cord. BzATP also increased the activity of NOX2, leading to motor neuron damage, an effect which did not occur in primary microglia cultures of SOD1-G93A mice lacking P2X7Rs [102].

A neuropathological hallmark of Alzheimer's disease (AD) is the appearance of plaques consisting of extracellular β-amyloid peptide (Aβ) surrounded by reactive microglial cells [103]. Aβ triggered increases in intracellular Ca^{2+}, ATP release, IL-1β secretion, and plasma membrane permeabilization in microglia from wild type, but not $P2X7R^{-/-}$ mice [104]. These findings, and the neuroprotective effects of BBG against intrahippocampally injected Aβ, suggest that Aβ activates a purinergic autocrine/paracrine stimulatory loop of which the P2X7R is an obligatory component. In fact, *in vivo* inhibition of the P2X7R in mice transgenic for mutant human APP indicated a significant decrease of the number of hippocampal amyloid plaques [105].

Parkinson's disease (PD) is a motor disease affecting the striatal dopaminergic system by damaging dopaminergic neurons in the substantia nigra. In the disease model induced by unilateral intrastriatal injection of 6-hydroxydopamine, BBG, and A-438079 prevented the ensuing synaptotoxicity, gliosis, and neurotoxicity [106]. In another study, A-438079 prevented the depletion of striatal dopamine stores by 6-hydroxydopamine treatment, but this was not associated with a reduction of dopaminergic cell loss [107]. Similarly, the effects of P2X7R antagonists appeared to depend on the neurotoxin used, because in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- or rotenone-induced Parkinson models, the genetic deletion of the P2X7R did not increase survival rates of mice compared to wild type counterparts [108].

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a triplet repeat expansion coding for a polyglutamine sequence in the N-terminal region of the huntingtin protein. A higher P2X7R level was documented by Western blot analysis in the striatum of transgenic mice models of this disease [109]. In addition, P2X7R antagonists prevented neuronal apoptosis and attenuated body weight loss and motor coordination deficits.

P2X7R as a potential target in psychiatric disorders

Mood disorders arise from complex interactions between genetic, developmental, and environmental factors [110,111]. Linkage studies suggested that variations of the chromosome *12q24.31* containing candidate genes for P2X7R, P2X4R, and calmodulin-dependent protein kinase b (CaMKKb) may be associated with major depressive, bipolar, and anxiety disorders. It has repeatedly been reported that the NS-SNP *rs2230912* coding for Glu460Arg–P2X7R is associated with major depressive disorder [112,113]. Furthermore, relevant SNP mutations identified by linkage studies were introduced into the human recombinant *P2X7R* and were expressed in human embryonic kidney cells [114]. The measurement of their functional properties by the patch-clamp technique indicated that some of them, exhibited a strong impairment of the current response to ATP, while other mutants demonstrated significant increases in sensitivity; however, Glu460Arg- and wild type P2X7Rs responded with comparable currents to ATP. By contrast, other studies failed to confirm the allelic or genotypic association of *rs2230912* or other SNPs of *P2X7R* with mood

disorders [115,116]. The reasons for this discrepancy are presently unknown. Eventually, variations in the *P2RX7* gene were described to be associated with cognitive manic symptoms in bipolar disorders [117], but not in schizophrenia [118].

Production of TNF-α and IL-6 is initiated by the activation of Toll-like receptors (TLRs) by, for example, bacterial lipopolysaccharide. The formation of IL-1β also requires TLR4 induction of gene transcription, but requires an additional step, the processing of pro-IL-1β to the mature form of IL-1β, which is then released via NLRP3 referred to as the 'inflammasome' [110,119]. P2X7Rs are indispensable activators of NLRP3. Inflammatory cytokines have been suggested to play key roles in the development of depressive behaviour. Their levels are elevated in depressed patients [110,120] and rodents exposed to stressful stimuli [111]. These cytokines are potent activators of the hypothalamic–pituitary–adrenal axis through which the secretion of hypothalamic corticotropin releasing hormone (CRH), pituitary adrenocorticotropic hormone (ACTH), and corticosterone are stimulated. In this respect, it is interesting to note that P2X7R stimulation also directly leads to increased ACTH secretion from the terminals of hypothalamic magnocellular neurons [121].

The interrelationship between inflammatory cytokines, P2X7Rs, and mood related behaviour has been intensively studied in animal models. The genetic deletion of P2X7Rs resulted in antidepressive-like behaviour in the forced swim and tail suspension tests and alleviated amphetamine induced hyperactivity [40,41]. Although P2X7Rs are present at peripheral/central immunocytes, glial cells, and neurons, it was shown that macrophages and microglia are not responsible for the detected changes in mood measured by tail suspension test and amphetamine-induced hyperlocomotion in P2X7R$^{-/-}$ mice [41]. On a larger scale, several potential mechanisms were identified for the antidepressant phenotype of P2X7R$^{-/-}$ mice, such as the absence of P2X7R-mediated glutamate release, elevated basal brain-derived neurotrophic factor (BDNF) production, enhanced neurogenesis, and increased serotonin bioavailability in the hippocampus [48]. It has also been observed that P2X7Rs are downregulated in the hippocampus in response to chronic stress [122] and P2X7R$^{-/-}$ mice exhibited impaired adaptive coping responses to repeated stress [123], which enlighten the potential role of P2X7Rs as a protective adaptive mechanism in the process leading to mood disorders.

The above data illustrate that P2X7R seems to be activated in a number of different pathological conditions, raising the possibility that the receptor is one common avenue of cellular stress signalling pathways (Figure 2). However, one should keep in mind that the pathophysiology of CNS diseases is very complex, involving a multiplicity of mediators and signalling pathways, and the P2X7R is only one among the multiple signalling pathways activated. Moreover, the significance of this avenue is probably not uniform in all CNS pathologies and could be more prominent in certain disease conditions (e.g., chronic pain, status epilepticus) than in other ones (e.g., Parkinson's disease), depending on the expression of P2X7Rs in the brain area afflicted. Finally, important physiological functions mediated by P2X7Rs should not be neglected. For instance, taking into account that the purportedly necrotic/apoptotic P2X7Rs also convey trophic and adaptive changes, their role might vary or even reverse during the course of the same disease, because neuroinflammation regulated by P2X7Rs has also a double-faced role. In fact, inflammation initially is a protective reaction and becomes detrimental only when it progresses to an excessive or chronic phase. These aspects serve as explanations to conflicting results with P2X7R inhibition on the disease outcome (e.g., [95–97]), and should also be addressed when P2X7R is considered as a potential human drug target.

Table 2. Non-comprehensive list of different classes of P2X7 receptor antagonists and allosteric modulators. For more information see [149]

Class/Compound	Function	Refs
Novel, small molecule		
(1H-pyrazol-4-yl) acetamides	Antagonist	[150,151]
Benzamides	Antagonist	[152,153]
Tetrasubstituted-imidazoles	Antagonist	[154]
2-oxo-N-(phenymethyl)-4-imidazolinecarboxamides	Antagonist	[155]
Novel, small molecule, CNS active		
JNJ-47965567	Antagonist	[128]
Polycyclic carboranes	Antagonist	[156]
Identified by screening compound libraries		
Clemastine	Positive allosteric modulator	[157]
Perazine-type antipsychotic drugs	Negative allosteric modulator	[129]
Ivermectin	Positive allosteric modulator	[158]
Natural compounds		
Teniposide	Antagonist	[159]

Current development of P2X7R ligands

Although end-products of the pioneering developments of P2X7R antagonists, such as CE-224,535 [124] and AZD 9056 [125] have not proved efficacious in Phase II trials in rheumatoid arthritis patients, clinical studies revealed an acceptable safety and tolerability profile of such antagonists as a whole [124–126], opening up the possibility of developing P2X7R-targeting compounds in new areas, such as CNS disorders.

In recent years, a number of different classes of small molecular weight, drug-like P2X7R ligands have been developed (Table 2), and P2X7Rs have been qualified as the most 'druggable' target within the P2X receptor family [85,127]. More recently, the development of centrally penetrating potent P2X7R antagonists has also been reported (Table 2). In addition, a systematic search through compound libraries resulted in the further discovery of novel P2X7R antagonists and allosteric modulators utilizable either for basic research or drug development. Analyses of natural compounds have also resulted in several valuable P2X7R ligands (Table 2).

Box 2. Outstanding questions

Despite the large interest in P2X7Rs and the correspondingly high number of publications dealing with this receptor, many questions still remain unresolved.

- The C terminus of the P2X7R has been implicated in regulating receptor function including signalling pathway activation, cellular localization, protein–protein interactions, and post-translational modification [160]. It would be important to learn the three-dimensional structure of the P2X7R C-terminal tail, which has yet to be determined [4].
- Although repetitive or long-lasting stimulation of P2X7Rs by ATP allows the passage of 600–800 Da organic molecules through the cell membrane, the mechanism of pore opening is still a matter of debate. There are good arguments favouring an accessory protein, with Panx1 hemichannels probably involved in this effect, but the cationic channel dilation theory is also an attractive alternative.
- Original work based on co-immunoprecipitation with epitope tagged subunits demonstrated that overexpressed recombinant P2X1-6 subunits could form hetero-oligomeric complexes, while P2X7 was able to form only homomeric receptor channels [161]. However, it remains to be established whether true functional P2X4/7 heteromers are formed in native systems, which might have great significance for CNS immune functions, for example, in microglia.
- A lot of controversy has arisen on the issue of whether P2X7Rs are located exclusively at microglia and astroglia in the CNS or also at neurons (see the discussion on 'tissue and cell type specific distribution of P2X7Rs'). The solution of this enigma might be that, under normal conditions, P2X7Rs are dormant, but after various types of damaging conditions (mechanical trauma, ischemia, inflammation, etc.) they become unmasked, mostly at central immunocytes, but probably also at neurons. Already the tissue damage afflicted to cells during the culturing procedure or the preparation of brain slices may be sufficient to induce the expression of previously absent P2X7Rs.
- Although endogenous activation of P2X7Rs under disease conditions has repeatedly been proven, its exact mechanism is not fully understood, given the low affinity of ATP. The possibility of constitutive activity of this receptor, as well as its potential endogenous ligands other than ATP, should be explored.
- Whereas available gene deficient mouse models are not fully deficient in P2X7Rs, more advanced mouse models, such as cell-type specific and/or inducible knockouts, optogenetic constructs, as well as humanized mouse models reproducing human gene polymorphisms in rodents are yet to be generated for probing P2X7R function.

Concluding remarks

In conclusion, P2X7R mediated pathways appear to be a common avenue of many CNS disorders of different aetiology, and P2X7R antagonists are potential drugs to treat them. Their immense advantage may lie in the absence or low density of P2X7Rs in healthy tissue and, therefore, in the limited systemic side effects of these compounds. However, major caveats in our understanding of the pathophysiological functions of central P2X7Rs should be further elucidated (Box 2). Although the majority of known antagonists fail to pass the blood–brain barrier, BBG and some new and high affinity P2X7R antagonists readily enter the CNS [128]. Furthermore, recently identified negative allosteric modulators of P2X7Rs (e.g., certain phenothiazine-type antipsychotic drugs), already registered for human use [129] may become important therapeutic tools.

The future development of new P2X7R antagonists has to take into consideration that P2X7R isoforms may exhibit large variability between different species in their agonist/antagonist sensitivities. Therefore, the classic search for new pharmacologically active compounds based on the use of laboratory animals, may lead to spurious negative or positive results. A further complicating factor is the presence of numerous splice variants and SNPs widely distributed in the animal and human organism; their sensitivities to pharmacological blockade is often different from that of the wild type receptor. Hence, the development of new and therapeutically valuable P2X7R antagonists is a tedious task, but the reward may be enormous.

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References

1 Sperlágh, B. *et al.* (2006) P2X7 receptors in the nervous system. *Prog. Neurobiol.* 78, 327–346
2 Jiang, L.H. *et al.* (2013) Insights into the molecular mechanisms underlying mammalian P2X7 receptor functions and contributions in diseases, revealed by structural modeling and single nucleotide polymorphisms. *Front. Pharmacol.* http://dx.doi.org/10.3389/fphar.2013.00055
3 Kawate, T. *et al.* (2009) Crystal structure of the ATP-gated P2X(4) ion channel in the closed state. *Nature* 460, 592–598
4 Hattori, M. and Gouaux, E. (2012) Molecular mechanism of ATP binding and ion channel activation in P2X receptors. *Nature* 485, 207–212
5 Stelmashenko, O. *et al.* (2012) Activation of trimeric P2X2 receptors by fewer than three ATP molecules. *Mol. Pharmacol.* 82, 760–766
6 Bradley, H.J. *et al.* (2011) Residues 155 and 348 contribute to the determination of P2X7 receptor function via distinct mechanisms revealed by single-nucleotide polymorphisms. *J. Biol. Chem.* 286, 8176–8187
7 Adriouch, S. *et al.* (2008) ADP-ribosylation at R125 gates the P2X7 ion channel by presenting a covalent ligand to its nucleotide binding site. *FASEB J.* 22, 861–869
8 Guo, C. *et al.* (2007) Evidence for functional P2X4/P2X7 heteromeric receptors. *Mol. Pharmacol.* 72, 1447–1456
9 Antonio, L.S. *et al.* (2011) P2X4 receptors interact with both P2X2 and P2X7 receptors in the form of homotrimers. *Br. J. Pharmacol.* 163, 1069–1077
10 Adinolfi, E. *et al.* (2010) Trophic activity of a naturally occurring truncated isoform of the P2X7 receptor. *FASEB J.* 24, 3393–3404
11 Masin, M. *et al.* (2012) Expression, assembly and function of novel C-terminal truncated variants of the mouse P2X7 receptor: re-evaluation of P2X7 knockouts. *Br. J. Pharmacol.* 165, 978–993
12 Nicke, A. *et al.* (2009) A functional P2X7 splice variant with an alternative transmembrane domain 1 escapes gene inactivation in P2X7 knock-out mice. *J. Biol. Chem.* 284, 25813–25822
13 Schwarz, N. *et al.* (2012) Alternative splicing of the N-terminal cytosolic and transmembrane domains of P2X7 controls gating of the ion channel by ADP-ribosylation. *PLoS ONE* http://dx.doi.org/10.1371/journal.pone.0041269
14 Fuller, S.J. *et al.* (2009) Genetics of the P2X7 receptor and human disease. *Purinergic Signal.* 5, 257–262
15 Chaumont, S. and Khakh, B.S. (2008) Patch-clamp coordinated spectroscopy shows P2X2 receptor permeability dynamics require cytosolic domain rearrangements but not Panx-1 channels. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12063–12068

16 Alloisio, S. *et al.* (2010) Evidence for two conductive pathways in P2X receptor: differences in modulation and selectivity. *J. Neurochem.* 113, 796–806
17 Sun, C. *et al.* (2013) The second transmembrane domain of P2X7 contributes to dilated pore formation. *PLoS ONE* http://dx.doi.org/10.1371/journal.pone.0061886
18 Browne, L.E. *et al.* (2013) P2X7 receptor channels allow direct permeation of nanometer-sized dyes. *J. Neurosci.* 33, 3557–3566
19 Khadra, A. *et al.* (2013) Dual gating mechanism and function of P2X7 receptor channels. *Biophys. J.* 104, 2612–2621
20 Suadicani, S.O. *et al.* (2012) ATP signaling is deficient in cultured Pannexin1-null mouse astrocytes. *Glia* 60, 1106–1116
21 Marques-da-Silva, C. *et al.* (2011) Colchicine inhibits cationic dye uptake induced by ATP in P2X2 and P2X7 receptor-expressing cells: implications for its therapeutic action. *Br. J. Pharmacol.* 163, 912–926
22 Alberto, A.V. *et al.* (2013) Is pannexin the pore associated with the P2X7 receptor? *Naunyn Schmiedebergs Arch. Pharmacol.* 386, 775–787
23 Xu, X.J. *et al.* (2012) Splice variants of the P2X7 receptor reveal differential agonist dependence and functional coupling with pannexin-1. *J. Cell Sci.* 125, 3776–3789
24 Sorge, R.E. *et al.* (2012) Genetically determined P2X7 receptor pore formation regulates variability in chronic pain sensitivity. *Nat. Med.* 18, 595–599
25 Able, S.L. *et al.* (2011) Receptor localization, native tissue binding and *ex vivo* occupancy for centrally penetrant P2X7 antagonists in the rat. *Br. J. Pharmacol.* 162, 405–414
26 Deuchars, S.A. *et al.* (2001) Neuronal P2X7 receptors are targeted to presynaptic terminals in the central and peripheral nervous systems. *J. Neurosci.* 21, 7143–7152
27 Atkinson, L. *et al.* (2004) Differential co-localisation of the P2X7 receptor subunit with vesicular glutamate transporters VGLUT1 and VGLUT2 in rat CNS. *Neuroscience* 123, 761–768
28 Puthussery, T. and Fletcher, E.L. (2004) Synaptic localization of P2X7 receptors in the rat retina. *J. Comp. Neurol.* 472, 13–23
29 Sim, J.A. *et al.* (2004) Reanalysis of P2X7 receptor expression in rodent brain. *J. Neurosci.* 24, 6307–6314
30 Henshall, D.C. *et al.* (2013) P2X receptors as targets for the treatment of status epilepticus. *Front. Cell. Neurosci.* http://dx.doi.org/10.3389/fncel.2013.00237
31 Milius, D. *et al.* (2008) Up-regulation of P2X7 receptor-immunoreactivity by *in vitro* ischemia on the plasma membrane of cultured rat cortical neurons. *Neurosci. Lett.* 446, 45–50
32 Krueger, J.M. *et al.* (2010) ATP and the purine type 2 X7 receptor affect sleep. *J. Appl. Physiol. (1985)* 109, 1318–1327
33 Girotti, P.A. *et al.* (2013) Differential effects of undernourishment on the differentiation and maturation of rat enteric neurons. *Cell Tissue Res.* 353, 367–380
34 Zhou, D. *et al.* (2010) Involvement of spinal microglial P2X7 receptor in generation of tolerance to morphine analgesia in rats. *J. Neurosci.* 30, 8042–8047
35 Shrivastava, A.N. *et al.* (2013) Dynamic micro-organization of P2X7 receptors revealed by PALM based single particle tracking. *Front. Cell. Neurosci.* http://dx.doi.org/10.3389/fncel.2013.00232
36 Csölle, C. and Sperlagh, B. (2010) Peripheral origin of IL-1beta production in the rodent hippocampus under *in vivo* systemic bacterial lipopolysaccharide (LPS) challenge and its regulation by P2X(7) receptors. *J. Neuroimmunol.* 219, 38–46
37 Labrousse, V.F. *et al.* (2009) Impaired interleukin-1beta and c-Fos expression in the hippocampus is associated with a spatial memory deficit in P2X(7) receptor-deficient mice. *PLoS ONE* http://dx.doi.org/10.1371/journal.pone.0006006
38 Barbera-Cremades, M. *et al.* (2012) P2X7 receptor-stimulation causes fever via PGE2 and IL-1beta release. *FASEB J.* 26, 2951–2962
39 Chu, Y.X. *et al.* (2010) Involvement of microglial P2X7 receptors and downstream signaling pathways in long-term potentiation of spinal nociceptive responses. *Brain Behav. Immun.* 24, 1176–1189
40 Basso, A.M. *et al.* (2009) Behavioral profile of P2X7 receptor knockout mice in animal models of depression and anxiety: relevance for neuropsychiatric disorders. *Behav. Brain Res.* 198, 83–90
41 Csölle, C. *et al.* (2013) The absence of P2X7 receptors (P2rx7) on non-haematopoietic cells leads to selective alteration in mood-related behaviour with dysregulated gene expression and stress reactivity in mice. *Int. J. Neuropsychopharmacol.* 16, 213–233
42 Burnstock, G. (2004) Cotransmission. *Curr. Opin. Pharmacol.* 4, 47–52
43 Sperlágh, B. *et al.* (2002) Involvement of P2X7 receptors in the regulation of neurotransmitter release in the rat hippocampus. *J. Neurochem.* 81, 1196–1211
44 Marcoli, M. *et al.* (2008) P2X7 pre-synaptic receptors in adult rat cerebrocortical nerve terminals: a role in ATP-induced glutamate release. *J. Neurochem.* 105, 2330–2342
45 Fu, W. *et al.* (2013) Activity and metabolism-related Ca2+ and mitochondrial dynamics in co-cultured human fetal cortical neurons and astrocytes. *Neuroscience* 250, 520–535
46 Cervetto, C. *et al.* (2013) The P2X7 receptor as a route for non-exocytotic glutamate release: dependence on the carboxyl tail. *J. Neurochem.* 124, 821–831
47 Cervetto, C. *et al.* (2012) Calmidazolium selectively inhibits exocytotic glutamate release evoked by P2X7 receptor activation. *Neurochem. Int.* 60, 768–772
48 Csölle, C. *et al.* (2013) Neurochemical changes in the mouse hippocampus underlying the antidepressant effect of genetic deletion of P2X7 receptors. *PLoS ONE* http://dx.doi.org/10.1371/journal.pone.0066547
49 Heinrich, A. *et al.* (2012) K^+ depolarization evokes ATP, adenosine and glutamate release from glia in rat hippocampus: a microelectrode biosensor study. *Br. J. Pharmacol.* 167, 1003–1020
50 Khakpay, R. *et al.* (2010) Potentiation of the glutamatergic synaptic input to rat locus coeruleus neurons by P2X7 receptors. *Purinergic Signal.* 6, 349–359
51 Cho, J.H. *et al.* (2010) P2X7 receptors enhance glutamate release in hippocampal hilar neurons. *Neuroreport* 21, 865–870
52 Ficker, C. *et al.* (2014) Astrocyte-neuron interaction in the substantia gelatinosa of the spinal cord dorsal horn via P2X7 receptor-mediated release of glutamate and reactive oxygen species. *Glia* http://dx.doi.org/10.1002/glia.22707
53 Xia, J. *et al.* (2012) Neurons respond directly to mechanical deformation with pannexin-mediated ATP release and autostimulation of P2X7 receptors. *J. Physiol.* 590, 2285–2304
54 Bennett, M.V. *et al.* (2012) Connexin and pannexin hemichannels in inflammatory responses of glia and neurons. *Brain Res.* 1487, 3–15
55 Gutierrez-Martin, Y. *et al.* (2011) P2X7 receptors trigger ATP exocytosis and modify secretory vesicle dynamics in neuroblastoma cells. *J. Biol. Chem.* 286, 11370–11381
56 Tsao, H.K. *et al.* (2013) PKC-dependent ERK phosphorylation is essential for P2X7 receptor-mediated neuronal differentiation of neural progenitor cells. *Cell Death Dis.* http://dx.doi.org/10.1038/cddis.2013.274
57 Messemer, N. *et al.* (2013) P2X7 receptors at adult neural progenitor cells of the mouse subventricular zone. *Neuropharmacology* 73, 122–137
58 Zou, J. *et al.* (2012) ATP-P2X7 receptor signaling controls basal and TNFalpha-stimulated glial cell proliferation. *Glia* 60, 661–673
59 del Puerto, A. *et al.* (2014) ATP-P2X7 receptor modulates axon initial segment composition and function in physiological conditions and brain injury. *Cereb Cortex* http://dx.doi.org/10.1093/cercor/bhu035
60 Bianco, F. *et al.* (2006) A role for P2X7 in microglial proliferation. *J. Neurochem.* 99, 745–758
61 Monif, M. *et al.* (2009) The P2X7 receptor drives microglial activation and proliferation: a trophic role for P2X7R pore. *J. Neurosci.* 29, 3781–3791
62 Gu, B.J. *et al.* (2011) P2X(7) is a scavenger receptor for apoptotic cells in the absence of its ligand, extracellular ATP. *J. Immunol.* 187, 2365–2375
63 Yamamoto, M. *et al.* (2013) P2X7 receptors regulate engulfing activity of non-stimulated resting astrocytes. *Biochem. Biophys. Res. Commun.* 439, 90–95
64 Arbeloa, J. *et al.* (2012) P2X7 receptor blockade prevents ATP excitotoxicity in neurons and reduces brain damage after ischemia. *Neurobiol. Dis.* 45, 954–961
65 Lämmer, A.B. *et al.* (2011) The P2 receptor antagonist PPADS supports recovery from experimental stroke *in vivo*. *PLoS ONE* http://dx.doi.org/10.1371/journal.pone.0019983
66 Chen, S. *et al.* (2013) P2X7 receptor antagonism inhibits p38 mitogen-activated protein kinase activation and ameliorates neuronal apoptosis after subarachnoid hemorrhage in rats. *Crit. Care Med.* 41, e466–e474

67 Kimbler, D.E. *et al.* (2012) Activation of P2X7 promotes cerebral edema and neurological injury after traumatic brain injury in mice. *PLoS ONE* http://dx.doi.org/10.1371/journal.pone.0041229
68 Roth, T.L. *et al.* (2014) Transcranial amelioration of inflammation and cell death after brain injury. *Nature* 505, 223–228
69 Peng, W. *et al.* (2009) Systemic administration of an antagonist of the ATP-sensitive receptor P2X7 improves recovery after spinal cord injury. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12489–12493
70 Niyadurupola, N. *et al.* (2013) P2X7 receptor activation mediates retinal ganglion cell death in a human retina model of ischemic neurodegeneration. *Invest. Ophthalmol. Vis. Sci.* 54, 2163–2170
71 Marcillo, A. *et al.* (2012) A reassessment of P2X7 receptor inhibition as a neuroprotective strategy in rat models of contusion injury. *Exp. Neurol.* 233, 687–692
72 Stoll, G. *et al.* (2010) Combating innate inflammation: a new paradigm for acute treatment of stroke? *Ann. N. Y. Acad. Sci.* 1207, 149–154
73 Chu, K. *et al.* (2012) Inhibition of P2X7 receptor ameliorates transient global cerebral ischemia/reperfusion injury via modulating inflammatory responses in the rat hippocampus. *J. Neuroinflammation* http://dx.doi.org/10.1186/1742-2094-9-69
74 Yu, Q. *et al.* (2013) Block of P2X7 receptors could partly reverse the delayed neuronal death in area CA1 of the hippocampus after transient global cerebral ischemia. *Purinergic Signal.* 9, 663–675
75 Domercq, M. *et al.* (2010) P2X7 receptors mediate ischemic damage to oligodendrocytes. *Glia* 58, 730–740
76 Wang, L.Y. *et al.* (2009) Downregulation of P2X7 receptor expression in rat oligodendrocyte precursor cells after hypoxia ischemia. *Glia* 57, 307–319
77 Kim, J.E. *et al.* (2009) Blockade of P2X receptor prevents astroglial death in the dentate gyrus following pilocarpine-induced status epilepticus. *Neurol. Res.* 31, 982–988
78 Dona, F. *et al.* (2009) Alteration of purinergic P2X4 and P2X7 receptor expression in rats with temporal-lobe epilepsy induced by pilocarpine. *Epilepsy Res.* 83, 157–167
79 Engel, T. *et al.* (2012) Seizure suppression and neuroprotection by targeting the purinergic P2X7 receptor during status epilepticus in mice. *FASEB J.* 26, 1616–1628
80 Jimenez-Pacheco, A. *et al.* (2013) Increased neocortical expression of the P2X7 receptor after status epilepticus and anticonvulsant effect of P2X7 receptor antagonist A-438079. *Epilepsia* 54, 1551–1561
81 Kim, J.E. and Kang, T.C. (2011) The P2X7 receptor-pannexin-1 complex decreases muscarinic acetylcholine receptor-mediated seizure susceptibility in mice. *J. Clin. Invest.* 121, 2037–2047
82 Kim, J.E. *et al.* (2011) P2X7 receptor activation ameliorates CA3 neuronal damage via a tumor necrosis factor-alpha-mediated pathway in the rat hippocampus following status epilepticus. *J. Neuroinflammation* http://dx.doi.org/10.1186/1742-2094-8-62
83 Kim, J.E. *et al.* (2010) P2X7 receptor regulates leukocyte infiltrations in rat frontoparietal cortex following status epilepticus. *J. Neuroinflammation* http://dx.doi.org/10.1186/1742-2094-7-65
84 Kim, J.E. *et al.* (2013) The effect of P2X7 receptor activation on nuclear factor-kappaB phosphorylation induced by status epilepticus in the rat hippocampus. *Hippocampus* 23, 500–514
85 North, R.A. and Jarvis, M.F. (2013) P2X receptors as drug targets. *Mol. Pharmacol.* 83, 759–769
86 Itoh, K. *et al.* (2011) Central sensitization of nociceptive neurons in rat medullary dorsal horn involves purinergic P2X7 receptors. *Neuroscience* 192, 721–731
87 Andó, R.D. *et al.* (2010) A comparative analysis of the activity of ligands acting at P2X and P2Y receptor subtypes in models of neuropathic, acute and inflammatory pain. *Br. J. Pharmacol.* 159, 1106–1117
88 He, W.J. *et al.* (2012) Spinal P2X(7) receptor mediates microglia activation-induced neuropathic pain in the sciatic nerve injury rat model. *Behav. Brain Res.* 226, 163–170
89 Ito, G. *et al.* (2013) P2X7 receptor in the trigeminal sensory nuclear complex contributes to tactile allodynia/hyperalgesia following trigeminal nerve injury. *Eur. J. Pain* 17, 185–199
90 Huang, Z.X. *et al.* (2014) Involvement of RVM-expressed P2X7 receptor in bone cancer pain: mechanism of descending facilitation. *Pain* 155, 783–791
91 Gölöncsér, F. and Sperlágh, B. (2014) Effect of genetic deletion and pharmacological antagonism of P2X7 receptors in a mouse animal model of migraine. *J. Headache Pain* http://dx.doi.org/10.1186/1129-2377-15-24
92 Amadio, S. *et al.* (2011) Purinergic signalling at the plasma membrane: a multipurpose and multidirectional mode to deal with amyotrophic lateral sclerosis and multiple sclerosis. *J. Neurochem.* 116, 796–805
93 Oyanguren-Desez, O. *et al.* (2011) Gain-of-function of P2X7 receptor gene variants in multiple sclerosis. *Cell Calcium* 50, 468–472
94 Grygorowicz, T. *et al.* (2010) Temporal expression of P2X7 purinergic receptor during the course of experimental autoimmune encephalomyelitis. *Neurochem. Int.* 57, 823–829
95 Matute, C. *et al.* (2007) P2X(7) receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. *J. Neurosci.* 27, 9525–9533
96 Sharp, A.J. *et al.* (2008) P2x7 deficiency suppresses development of experimental autoimmune encephalomyelitis. *J. Neuroinflammation* http://dx.doi.org/10.1186/1742-2094-5-33
97 Chen, L. and Brosnan, C.F. (2006) Exacerbation of experimental autoimmune encephalomyelitis in P2X7R$^{-/-}$ mice: evidence for loss of apoptotic activity in lymphocytes. *J. Immunol.* 176, 3115–3126
98 Lutz, S.E. *et al.* (2013) Contribution of pannexin1 to experimental autoimmune encephalomyelitis. *PLoS ONE* http://dx.doi.org/10.1371/journal.pone.0066657
99 Volonte, C. *et al.* (2011) ALS: focus on purinergic signalling. *Pharmacol. Ther.* 132, 111–122
100 Apolloni, S. *et al.* (2013) Ablation of P2X7 receptor exacerbates gliosis and motoneuron death in the SOD1-G93A mouse model of amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 22, 4102–4116
101 Gandelman, M. *et al.* (2010) Extracellular ATP and the P2X7 receptor in astrocyte-mediated motor neuron death: implications for amyotrophic lateral sclerosis. *J. Neuroinflammation* http://dx.doi.org/10.1186/1742-2094-7-33
102 Apolloni, S. *et al.* (2013) The NADPH oxidase pathway is dysregulated by the P2X7 receptor in the SOD1-G93A microglia model of amyotrophic lateral sclerosis. *J. Immunol.* 190, 5187–5195
103 Delarasse, C. *et al.* (2011) The purinergic receptor P2X7 triggers alpha-secretase-dependent processing of the amyloid precursor protein. *J. Biol. Chem.* 286, 2596–2606
104 Sanz, J.M. *et al.* (2009) Activation of microglia by amyloid {beta} requires P2X7 receptor expression. *J. Immunol.* 182, 4378–4385
105 Diaz-Hernandez, J.I. *et al.* (2012) *In vivo* P2X7 inhibition reduces amyloid plaques in Alzheimer's disease through GSK3beta and secretases. *Neurobiol. Aging* 33, 1816–1828
106 Carmo, M.R. *et al.* (2014) The P2X7 receptor antagonist Brilliant Blue G attenuates contralateral rotations in a rat model of Parkinsonism through a combined control of synaptotoxicity, neurotoxicity and gliosis. *Neuropharmacology* 81, 142–152
107 Marcellino, D. *et al.* (2010) On the role of P2X(7) receptors in dopamine nerve cell degeneration in a rat model of Parkinson's disease: studies with the P2X(7) receptor antagonist A-438079. *J. Neural Transm.* 117, 681–687
108 Hracskó, Z. *et al.* (2011) Lack of neuroprotection in the absence of P2X7 receptors in toxin-induced animal models of Parkinson's disease. *Mol. Neurodegener.* http://dx.doi.org/10.1186/1750-1326-6-28
109 Diaz-Hernandez, M. *et al.* (2009) Altered P2X7-receptor level and function in mouse models of Huntington's disease and therapeutic efficacy of antagonist administration. *FASEB J.* 23, 1893–1906
110 Iwata, M. *et al.* (2013) The inflammasome: pathways linking psychological stress, depression, and systemic illnesses. *Brain Behav. Immun.* 31, 105–114
111 Sperlagh, B. *et al.* (2012) The role of purinergic signaling in depressive disorders. *Neuropsychopharmacol. Hung* 14, 231–238
112 McQuillin, A. *et al.* (2009) Case–control studies show that a non-conservative amino-acid change from a glutamine to arginine in the P2RX7 purinergic receptor protein is associated with both bipolar- and unipolar-affective disorders. *Mol. Psychiatry* 14, 614–620
113 Soronen, P. *et al.* (2011) *P2RX7* gene is associated consistently with mood disorders and predicts clinical outcome in three clinical cohorts. *Am. J. Med. Genet. B: Neuropsychiatr. Genet.* 156B, 435–447
114 Roger, S. *et al.* (2010) Single nucleotide polymorphisms that were identified in affective mood disorders affect ATP-activated P2X7 receptor functions. *J. Psychiatr. Res.* 44, 347–355

115 Grigoroiu-Serbanescu, M. *et al.* (2009) Variation in *P2RX7* candidate gene (*rs2230912*) is not associated with bipolar I disorder and unipolar major depression in four European samples. *Am. J. Med. Genet. B: Neuropsychiatr. Genet.* 150B, 1017–1021
116 Viikki, M. *et al.* (2011) P2RX7 polymorphisms *Gln460Arg* and *His155Tyr* are not associated with major depressive disorder or remission after SSRI or ECT. *Neurosci. Lett.* 493, 127–130
117 Backlund, L. *et al.* (2011) Cognitive manic symptoms associated with the *P2RX7* gene in bipolar disorder. *Bipolar Disord.* 13, 500–508
118 Hansen, T. *et al.* (2008) Variation in the purinergic *P2RX(7)* receptor gene and schizophrenia. *Schizophr. Res.* 104, 146–152
119 Di Virgilio, F. (2013) The therapeutic potential of modifying inflammasomes and NOD-like receptors. *Pharmacol. Rev.* 65, 872–905
120 Dowlati, Y. *et al.* (2010) A meta-analysis of cytokines in major depression. *Biol. Psychiatry* 67, 446–457
121 Lemos, J.R. *et al.* (2012) Modulation/physiology of calcium channel sub-types in neurosecretory terminals. *Cell Calcium* 51, 284–292
122 Kongsui, R. *et al.* (2014) Chronic stress induces prolonged suppression of the P2X7 receptor within multiple regions of the hippocampus: a cumulative threshold spectra analysis. *Brain Behav. Immun.* http://dx.doi.org/10.1016/j.bbi.2014.05.017
123 Boucher, A.A. *et al.* (2011) Resilience and reduced c-Fos expression in P2X7 receptor knockout mice exposed to repeated forced swim test. *Neuroscience* 189, 170–177
124 Stock, T.C. *et al.* (2012) Efficacy and safety of CE-224,535, an antagonist of P2X7 receptor, in treatment of patients with rheumatoid arthritis inadequately controlled by methotrexate. *J. Rheumatol.* 39, 720–727
125 Keystone, E.C. *et al.* (2012) Clinical evaluation of the efficacy of the P2X7 purinergic receptor antagonist AZD9056 on the signs and symptoms of rheumatoid arthritis in patients with active disease despite treatment with methotrexate or sulphasalazine. *Ann. Rheum. Dis.* 71, 1630–1635
126 Ali, Z. *et al.* (2013) Pharmacokinetic and pharmacodynamic profiling of a P2X7 receptor allosteric modulator GSK1482160 in healthy human subjects. *Br. J. Clin. Pharmacol.* 75, 197–207
127 Gum, R.J. *et al.* (2012) P2X receptor antagonists for pain management: examination of binding and physicochemical properties. *Purinergic Signal.* 8, 41–56
128 Bhattacharya, A. *et al.* (2013) Pharmacological characterization of a novel centrally permeable P2X7 receptor antagonist: JNJ-47965567. *Br. J. Pharmacol.* 170, 624–640
129 Hempel, C. *et al.* (2013) The phenothiazine-class antipsychotic drugs prochlorperazine and trifluoperazine are potent allosteric modulators of the human P2X7 receptor. *Neuropharmacology* 75, 365–379
130 Marin-Garcia, P. *et al.* (2008) Synaptic terminals from mice midbrain exhibit functional P2X7 receptor. *Neuroscience* 151, 361–373
131 Cuadra, A.E. *et al.* (2014) P2X7 receptors in neurohypophysial terminals: evidence for their role in arginine-vasopressin secretion. *J. Cell. Physiol.* 229, 333–342
132 D'Amico, M. *et al.* (2010) AMPA- and P2X7-receptor-mediated facilitation of [^{3}H]D-aspartate release from nerve terminals isolated from the rat caudal brainstem. *Neurochem. Int.* 57, 623–628
133 Bhattacharya, A. *et al.* (2013) Potentiation of inhibitory synaptic transmission by extracellular ATP in rat suprachiasmatic nuclei. *J. Neurosci.* 33, 8035–8044
134 Gandelman, M. *et al.* (2013) P2X7 receptor-induced death of motor neurons by a peroxynitrite/FAS-dependent pathway. *J. Neurochem.* 126, 382–388
135 Nishida, K. *et al.* (2012) Mitochondrial dysfunction is involved in P2X7 receptor-mediated neuronal cell death. *J. Neurochem.* 122, 1118–1128
136 Oliveira, J.F. *et al.* (2011) Rodent cortical astroglia express *in situ* functional P2X7 receptors sensing pathologically high ATP concentrations. *Cereb. Cortex* 21, 806–820
137 Nörenberg, W. *et al.* (2010) Electrophysiological classification of P2X7 receptors in rat cultured neocortical astroglia. *Br. J. Pharmacol.* 160, 1941–1952
138 Carrasquero, L.M. *et al.* (2010) Mechanisms of protein kinase D activation in response to P2Y(2) and P2X7 receptors in primary astrocytes. *Glia* 58, 984–995
139 Hashioka, S. *et al.* (2014) Purinergic responses of calcium-dependent signaling pathways in cultured adult human astrocytes. *BMC Neurosci.* http://dx.doi.org/10.1186/1471-2202-15-18
140 Habbas, S. *et al.* (2011) Purinergic signaling in the cerebellum: Bergmann glial cells express functional ionotropic P2X7 receptors. *Glia* 59, 1800–1812
141 Chen, Y. *et al.* (2012) P2X7 receptors in satellite glial cells mediate high functional expression of P2X3 receptors in immature dorsal root ganglion neurons. *Mol. Pain* http://dx.doi.org/10.1186/1744-8069-8-9
142 Arnoux, I. *et al.* (2013) Adaptive phenotype of microglial cells during the normal postnatal development of the somatosensory 'Barrel' cortex. *Glia* 61, 1582–1594
143 Friedle, S.A. *et al.* (2011) The P2X7–Egr pathway regulates nucleotide-dependent inflammatory gene expression in microglia. *Glia* 59, 1–13
144 Patel, D. *et al.* (2014) Connexin hemichannel and pannexin channel electrophysiology: how do they differ? *FEBS Lett.* 588, 1372–1378
145 Wang, J. *et al.* (2013) The food dye FD&C Blue No. 1 is a selective inhibitor of the ATP release channel Panx1. *J. Gen. Physiol.* 141, 649–656
146 Sikora, A. *et al.* (1999) Cutting edge: purinergic signaling regulates radical-mediated bacterial killing mechanisms in macrophages through a P2X7-independent mechanism. *J. Immunol.* 163, 558–561
147 Solle, M. *et al.* (2001) Altered cytokine production in mice lacking P2X(7) receptors. *J. Biol. Chem.* 276, 125–132
148 Garcia-Huerta, P. *et al.* (2012) The specificity protein factor Sp1 mediates transcriptional regulation of P2X7 receptors in the nervous system. *J. Biol. Chem.* 287, 44628–44644
149 Chrovian, C.C. *et al.* (2014) P2X7 Antagonists as potential therapeutic agents for the treatment of CNS disorders. *Prog. Med. Chemistry* 53, 65–100
150 Beswick, P.J. *et al.* (2010) Structure–activity relationships and *in vivo* activity of (1H-pyrazol-4-yl)acetamide antagonists of the P2X(7) receptor. *Bioorg. Med. Chem. Lett.* 20, 4653–4656
151 Chambers, L.J. *et al.* (2010) Synthesis and structure–activity relationships of a series of (1H-pyrazol-4-yl)acetamide antagonists of the P2X7 receptor. *Bioorg. Med. Chem. Lett.* 20, 3161–3164
152 Chen, X. *et al.* (2010) Discovery of 2-chloro-N-((4,4-difluoro-1-hydroxycyclohexyl)methyl)-5-(5-fluoropyrimidin-2-yl)benzamide as a potent and CNS penetrable P2X7 receptor antagonist. *Bioorg. Med. Chem. Lett.* 20, 3107–3111
153 Subramanyam, C. *et al.* (2011) Discovery, synthesis and SAR of azinyl- and azolylbenzamides antagonists of the P2X(7) receptor. *Bioorg. Med. Chem. Lett.* 21, 5475–5479
154 Gleave, R.J. *et al.* (2010) Synthesis and biological activity of a series of tetrasubstituted-imidazoles as P2X(7) antagonists. *Bioorg. Med. Chem. Lett.* 20, 4951–4954
155 Abberley, L. *et al.* (2010) Identification of 2-oxo-N-(phenylmethyl)-4-imidazolidinecarboxamide antagonists of the P2X(7) receptor. *Bioorg. Med. Chem. Lett.* 20, 6370–6374
156 Wilkinson, S.M. *et al.* (2014) The first CNS-active carborane: a novel P2X receptor antagonist with antidepressant activity. *ACS Chem. Neurosci.* 55, 335–339
157 Nörenberg, W. *et al.* (2011) Clemastine potentiates the human P2X7 receptor by sensitizing it to lower ATP concentrations. *J. Biol. Chem.* 286, 11067–11081
158 Nörenberg, W. *et al.* (2012) Positive allosteric modulation by ivermectin of human but not murine P2X7 receptors. *Br. J. Pharmacol.* 167, 48–66
159 Fischer, W. *et al.* (2013) Natural compounds with P2X7 receptor-modulating properties. *Purinergic Signal.* http://dx.doi.org/10.1007/s11302-013-9392-1
160 Costa-Junior, H.M. *et al.* (2011) C terminus of the P2X7 receptor: treasure hunting. *Purinergic Signal.* 7, 7–19
161 Torres, G.E. *et al.* (1999) Hetero-oligomeric assembly of P2X receptor subunits. Specificities exist with regard to possible partners. *J. Biol. Chem.* 274, 6653–6659