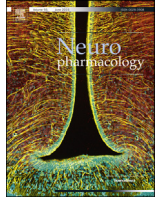




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Invited review

Purinergic mechanisms in neuroinflammation: An update from molecules to behavior

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ABSTRACT

The principle functions of neuroinflammation are to limit tissue damage and promote tissue repair in response to pathogens or injury. While neuroinflammation has utility, pathophysiological inflammatory responses, to some extent, underlie almost all neuropathology. Understanding the mechanisms that control the three stages of inflammation (initiation, propagation and resolution) is therefore of critical importance for developing treatments for diseases of the central nervous system. The purinergic signaling system, involving adenosine, ATP and other purines, plus a host of P1 and P2 receptor subtypes, controls inflammatory responses in complex ways. Activation of the inflammasome, leading to release of pro-inflammatory cytokines, activation and migration of microglia and altered astroglial function are key regulators of the neuroinflammatory response. Here, we review the role of P1 and P2 receptors in mediating these processes and examine their contribution to disorders of the nervous system. Firstly, we give an overview of the concept of neuroinflammation. We then discuss the contribution of P2X, P2Y and P1 receptors to the underlying processes, including a discussion of cross-talk between these different pathways. Finally, we give an overview of the current understanding of purinergic contributions to neuroinflammation in the context of specific disorders of the central nervous system, with special emphasis on neuropsychiatric disorders, characterized by chronic low grade inflammation or maternal inflammation. An understanding of the important purinergic contribution to neuroinflammation underlying neuropathology is likely to be a necessary step towards the development of effective interventions.

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Abbreviations: ASC, apoptosis-associated speck-like protein; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; BD, bipolar disorder; BBB, blood brain barrier; CNS, central nervous system; DAMPs, endogenous damage-associated molecular patterns; FST, forced swim test; LPS, bacterial lipopolysaccharide; MAPK, mitogen activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MDD, major depressive disorder; MPC, microglial process convergence; NLRP, nod-like receptor protein; OCD, obsessive compulsive disorder; PAMPs, pathogens-associated molecular patterns; Panx1, pannexin-1 hemichannels; PD, Parkinson's disease; PI3K kinase, phosphoinositide 3 kinase; PKA, protein kinase A; PKC, protein kinase C; sIL-2R, soluble interleukin (IL)-2 receptor; sIL-6R, soluble interleukin (IL)-6 receptor; SM, multiple sclerosis; sTNFR1, soluble tumor necrosis factor receptor type 1; TLRs, toll-like receptors; TNF- α , tumor necrosis factor- α ; TST, tail suspension test.

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1. Introduction – the evolution of the concept of neuroinflammation

The term 'inflammation' was coined by Celsus around the 1st century BC, defined by four cardinal signs; tumor, rubor, calor and dolor (Celsus, 1478) (i.e. oedema, redness, increased temperature and pain in the affected tissue) with a fifth sign, loss of function, attributed to Galen. By the identification of underlying molecular and cellular mechanisms, inflammation now assumes a substantially wider meaning, as a complex biological response to harmful stimuli, composed of three phases: initiation, propagation and resolution (Lister et al., 2007). While the principle functions of inflammation are to limit tissue damage and promote tissue repair (Nathan, 2002), inappropriate inflammatory responses, particularly when chronic, can lead to toxicity and cell loss (Hauss-Wegrzyniak et al., 2002).

The central nervous system (CNS), separated from the periphery by a specialized blood brain barrier (BBB) has long been considered an 'immuno-privileged' region (Carson et al., 2006), protected from systemic immune and inflammatory responses to pathology or injury. Behind the protection of the BBB, however, CNS-specific immune effector cells, particularly microglia (Streit et al., 2004), mediate neuroinflammatory responses to insult in response to a variety of triggers, including toxic metabolites, autoimmunity (Gendelman, 2002) or via the detection of pathogens or endogenous damage-associated molecular patterns (PAMPs and DAMPs) released in response to CNS damage, such as traumatic brain injury (Bernier, 2012). The outcome of a neuroinflammatory response depends, to a large extent, on its severity and duration (Vivekanantham et al., 2015), but pathological neuroinflammation, promoting apoptosis and necrosis and influencing the synaptic and intrinsic membrane properties of neurons (Yirmiya and Goshen, 2011), contributes to a host of CNS pathologies. A central role for neuroinflammation has been reported for primary or secondary neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (SM) amyotrophic lateral sclerosis (ALS), Huntington's disease, stroke and epilepsy (Frank-Cannon et al., 2009). Neuroinflammation has also been recognized as a pathological factor in psychiatric mood disorders, characterized by chronic mild neuroinflammation (Najjar et al., 2013), and developmental neuropsychiatric disorders, such as schizophrenia and autism (Meyer, 2013).

The main cellular effectors of neuroinflammation are astrocytes and microglia, as well as perivascular monocytes and macrophages invading to sites of insult from the circulation (Yamasaki et al., 2014). In addition, neurogenic neuroinflammation, the direct contribution of neuronal activity, is a unique feature of the nervous system (Xanthos and Sandkuhler, 2014). Chronic neuroinflammation involves the sustained activation of glial cells, chronic release of pro-inflammatory cytokines, increased permeability of the BBB and recruitment of systemic immune effector cells into the CNS (O'Callaghan et al., 2008).

Neuroinflammatory cascades rely on the activation of an inflammasome, a protein complex, consisting of caspase-1, apoptosis-associated speck-like protein (ASC) and nod-like receptor protein (NLRP1 or NLRP3) (Martinon et al., 2002). The inflammasome is the principle source of mature proinflammatory

cytokine IL-1 β , with caspase-1 activity necessary for the proteolytic cleaving of pro-IL-1 β (Gross et al., 2011). While NLRP inflammasomes are activated by the recognition of PAMPs or DAMPs (Bernier, 2012), the identification of host-derived DAMPs has become a major project, necessary for uncovering the pathway from insult to pathology.

The NLRP3 inflammasome and its downstream pathway has been recognized as a central mediator of systemic inflammation and a key mechanistic link between psychological stress and the emergence of depression and other psychiatric illnesses (Iwata et al., 2013). Much evidence has accumulated that ATP, found at higher extracellular concentrations following insult, potently induces NLRP-mediated IL-1 β processing (Burnstock, 2008) and that its metabolite, adenosine, is also heavily involved in the modulation of this signaling pathway. ATP and other nucleotides, such as UTP are released from induced cells and provide "find-me" and "eat-me" signals contributing to different phases of neuroinflammation by activating purinoceptors (Di Virgilio et al., 2009). Purinergic involvement in neuroinflammatory processes include the mediation of early events such as chemotaxis, microglia activation and the secretion of pro-inflammatory cytokines, phagocytosis, reactive astrogliosis and repair mechanisms such as neurogenesis.

Here we review the current understanding of the role of purinergic signaling in mediating the three phases of inflammation, the effect of the neuroinflammatory response on neuronal survival and functionality and the etiology and progression of CNS disease. Because the role of purinergic mechanisms in neurodegenerative diseases, such as AD, PD, SM, ALS and neuropathic pain is widely covered by other articles in this special issue; see (Burnstock, 2015), we will emphasize selected aspects which highlight the purinergic regulation of astrocytic and microglial cellular neuroinflammatory responses and their peculiar role in psychiatric disorders. Further elucidation of the participation of purinergic receptors in neuroinflammation will lead to a better of understanding of its role in neuropathology and may also pave the way towards more targeted and innovative therapies to combat CNS disorders.

1.1. The role of P2X receptors in neuroinflammation

P2X receptors are ligand-gated cation channels, rendered permeable to Na⁺, K⁺ and Ca²⁺ upon the binding of ATP (Abbracchio et al., 2009). Seven P2X subunits are expressed in the brain (P2X1-7), with P2X1-5 combining to form functional receptors with a heterotrimeric or homotrimeric quaternary structure, P2X6 forming only as a part of heterotrimeric receptors and P2X7 forming only homotrimers (North, 2002). Each combination of subunits forms a functional channel with different affinities for ATP and different desensitization dynamics conferring a variety in physiological responses to ATP; while P2X1 and P2X3 receptors desensitize within hundreds of milliseconds, P2X2 and P2X4 receptors desensitize an order of magnitude slower, and P2X7 receptors show very little desensitization, even over minutes (North and Jarvis, 2013). Both levels of subunit expression and receptor composition vary according to cell type and brain region. ATP-affinity and receptor function are also modulated by phosphorylation state and a host of allosteric and non-allosteric modulators, including heavy metals and reactive oxygen species (Coddou et al.,

2011).

Much evidence exists for changes in P2X subtype expression in neuroinflammatory conditions in various *in vitro* and *in vivo* models. For instance, early studies reported that P2X1 was strongly and transiently upregulated following ischemic insult in the hippocampus (Cavaliere et al., 2003), while P2X2 and P2X4 are upregulated in the dentate gyrus following oxygen and glucose deprivation in an organotypic hippocampal slice culture. Another study revealed an upregulation of P2X1 and P2X2 at the site of neurodegeneration following axotomy (Viscomi et al., 2004). P2X4 is expressed at increased concentrations in response to tissue injury following spinal cord injury (Schwab et al., 2005), traumatic brain injury (Zhang et al., 2006) and in spinal cord microglia following peripheral nerve injury (Tsuda et al., 2003; Ulmann et al., 2008). After stab wound injury, P2X1 and P2X7 receptor immunoreactivity was observed on astrocytes that were previously absent (Frankel et al., 2004). Cerebellar lesions produce up-regulation of P2X1 and P2X2 receptors in precerebellar nuclei (Florenzano et al., 2002). The majority of these studies, however, lack evidence for a causative relationship between P2X receptor activation and neuroinflammation.

Of the seven P2X subtypes, by far the strongest body of evidence for involvement in mediating neuroinflammation exists for P2X7 (Lister et al., 2007). Gene-linking and epidemiological studies have implicated P2X7 in a host of CNS diseases (Hansen et al., 2008; Ursu et al., 2014). *In vivo* studies, meanwhile, have been used to demonstrate the involvement of the P2X7 receptor in activating the inflammasome in a broad variety of rodent disease models, including cerebral ischemia (Kuan et al., 2015), epilepsy (Engel et al., 2012), Parkinson's disease (Marcellino et al., 2010), Alzheimer's disease (Diaz-Hernandez et al., 2012), depression and anxiety (Basso et al., 2009) and multiple sclerosis (Sharp et al., 2008). Systemic administration of bacterial lipopolysaccharide (LPS) markedly increases the expression of P2X7 receptors in the CNS (Choi et al., 2007), offering a mechanism for changes in CNS function in response to systemic infection.

A couple of features of the P2X7 receptor make it an optimal mediator of cellular responses to pathology. Firstly, the low-affinity of the receptor for ATP and its slow desensitization dynamics mean that it is unresponsive to micromolar fluctuations in extracellular ATP concentrations. This allows ATP signaling to function in different ways at different concentrations, with phasic micromolar ATP signaling operating via other P2X receptors to modulate a number of physiological pathways, e.g. functioning as a neuromodulator at glutamatergic synapses (Gu and MacDermott, 1997), while millimolar concentrations, released into the extracellular milieu in response to injury, can act, via P2X7, as a DAMP, initiating neuroinflammatory cascades (Fiebich et al., 2014). Secondly, the pore-forming functionality of the P2X7 receptor facilitates the release of larger hydrophilic molecules, up to 900 Da (Surprenant et al., 1996), a process which may be important for initiating neuroinflammation. Indeed, the formation of the P2X7 receptor pore seems to be necessary for the role of the molecule in activating the inflammasome (Monif et al., 2009).

The principle function of the inflammasome is the cleavage, by the protease, caspase-1, of precursor interleukin molecules into the forms, IL-1 β and IL-18 and the subsequent release of these pro-inflammatory cytokines into the extracellular space (Watanabe et al., 2007). This process involves three steps: firstly, synthesis of precursor molecules, e.g. pro-IL-1 β , secondly, the cleavage of the precursors into the active form, and thirdly, the release of the active form into the extracellular milieu. Synthesis of precursors is upregulated via the activation of toll-like receptors (TLRs) (He et al., 2013). Subsequently, NLRP1 or NLRP3 inflammasomes activated by PAMPs or DAMPs, induce the protease, caspase-1, which then

cleaves pro-IL-1 β into the active form (alongside IL-18). Thirdly, release of the active pro-inflammatory cytokines requires loading of the inflammasome complex into the secretory lysosome, or the formation of membrane blebs (Di Virgilio et al., 2009; Ferrari et al., 2006). ATP functions as a DAMP, via P2X7, activating the inflammasome and caspase-1 (Deplano et al., 2013) and is also involved in cytokine release, with modulation of cell membrane K⁺ permeability being a key step in both processes. In this way, TLRs load the gun, while P2X7 receptors pull the trigger (Fig. 1).

P2X7 receptor activation of the inflammasome in response to high extracellular concentrations of ATP seems to rely primarily on K⁺ efflux through the cell membrane (Bernier, 2012; Lister et al., 2007). P2X7 receptor activation leads to an increase in permeability to K⁺, either directly through the P2X7 receptor pore, or through the opening of pannexin-1 hemichannels (Panx1). Reduced intracellular K⁺ concentration is the primary trigger of caspase-1 activation (Munoz-Planillo et al., 2013). While some reports indicate that Panx1 channel opening is an obligatory part of both NLRP1 and NLRP3 inflammasome activation (de Rivero Vaccari et al., 2008; Pelegrin and Surprenant, 2006; Silverman et al., 2009), other groups have reported P2X7 receptor-induced IL-1 β release, independent of Panx1 (Pelegrin et al., 2008; Qu et al., 2011). A further study postulated that Panx1 is responsible for the release of ATP from dying cells, upstream of P2X7 activation in the signaling cascade (Dahl and Keane, 2012). Alternatively, Panx1 hemichannels may open in response to an increase in extracellular K⁺ as a result of P2X7 pore opening (Bernier, 2012), acting to amplify K⁺ efflux.

P2X7 receptors are found in highest concentrations on microglia, but are also expressed on astrocytes, oligodendrocytes and neurons, particularly at presynaptic terminals (Weisman et al., 2012). P2X7 receptor expression on different cell types, upregulated in response to CNS insult, combines to mediate a neuro-inflammatory response. ATP-activated microglia are key regulators of the neuroinflammatory response, releasing IL-1 β in response to insult (Ferrari et al., 1997; Silverman et al., 2009), adopting an activated morphology, proliferating and migrating to the site of insult, to form an inflammatory focus. It has been demonstrated that P2X7 receptor overexpression is both necessary and sufficient to drive these processes (Monif et al., 2009). Other studies (de Rivero Vaccari et al., 2012), however, revealed the importance of IL-1 β release from neurons, while Silverman et al. (Silverman et al., 2009) report that in neurons, high extracellular K⁺ can activate the inflammasome via Panx1-dependent efflux. These latter data indicate that neurons may be the first site of IL-1 β release following an insult (Fig. 1).

Further, P2X7 receptor stimulation of enteric neurons elicits a direct release of ATP onto glia through Panx1 (Gulbransen et al., 2012). By way of this paracrine signaling, the P2X7 receptor can function as a gatekeeper between glia and neurons to regulate inflammatory cascades (Browne, 2013). Astrocytic P2X7 receptor expression has also been implicated in contributing to the inflammatory response, with the activation of astroglial P2X7 receptors leading to a neurotoxic phenotype in a model of ALS (Gandelman et al., 2010), while following trauma, astroglial P2X7 receptor activation leads to upregulation of the chemokine, monocyte chemoattractant protein-1 (MCP-1) and, subsequently an increased invasion of systemic immune cells into the site of insult (Panenka et al., 2001).

As previously described, there is plenty of evidence for changes in P2X receptor expression during neuroinflammation, but other than P2X7, there is little evidence that these receptors mediate the process. Evidence is accumulating, however, that P2X4 receptors may also play a role. P2X4 knockouts show less microglial activation and the loss of the prostaglandin E2-mediated inflammatory pathway (Ulmann et al., 2010). P2X4 receptors, similarly to P2X7,

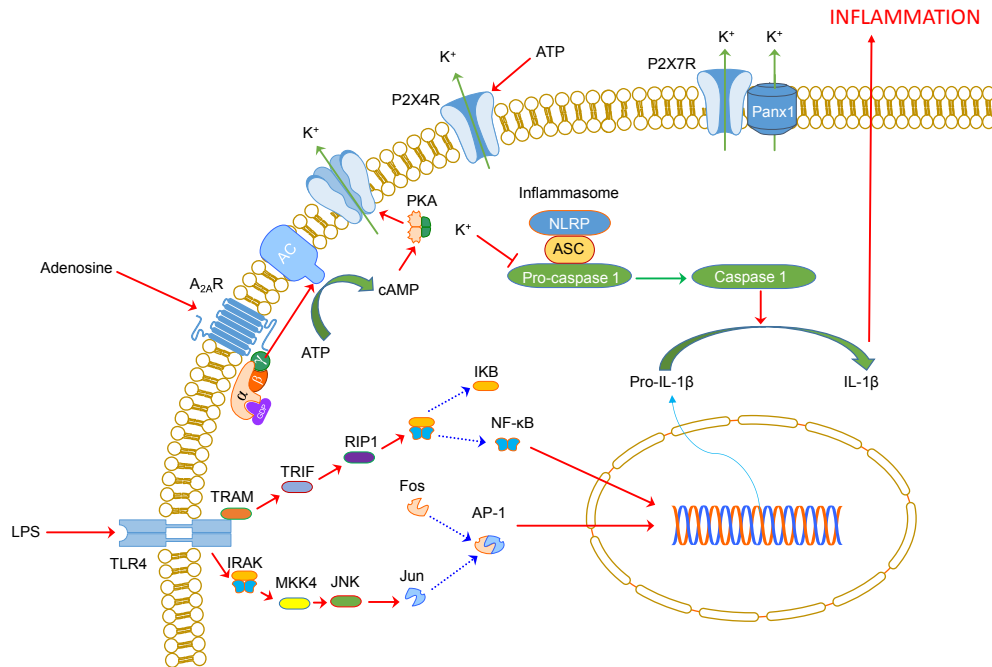


Fig. 1. Adenosine and ATP signalling both activate inflammasome via modulating cell membrane K^+ permeability. Toll-like receptors mediate pro-IL-1 β synthesis, via the activation of a host of transcription factors, including AP-1 and NF- κ B. $A_{2A}R$ activates adenylyl cyclase, increasing intracellular [cAMP], which can open K^+ channels via protein kinase A (PKA). P2X4 receptor opens to allow K^+ efflux directly. The P2X7 receptor, opens in response to higher extracellular [ATP] and facilitates K^+ efflux through a pore and through recruitment of Panx1.

form a large conductance pore on the cell membrane, facilitating ion efflux and subsequently inflammasome activation (Fiebach et al., 2014). Interestingly, this process appears to be Panx1-independent (Bernier, 2012). A recent study showed that P2X4 knockout mice displayed impaired inflammasome activation resulting in a decrease in extracellular IL-1 β and reduced infiltration of neutrophils and monocyte-derived M1 macrophages following spinal cord injury (de Rivero Vaccari et al., 2012). Further, Compan et al. (2012) report that receptors made up of P2X2 and P2X5 subunits, which are expressed in specific neuronal populations, also show an ability to form a P2X7 receptor-like dilated pore, suggesting that these receptors may also function as

gatekeepers of the inflammasome. Because P2X receptors other than P2X7 have lower affinity to extracellular ATP, sequential inflammasome activation by distinct P2X receptors might represent responses to insults of different intensity. While the P2X4 receptor may act as an initial trigger, the P2X7 receptor, in concert with Panx1, may amplify the signal (Fig. 2).

1.2. The role of P2Y receptors in neuroinflammation

P2Y receptors are metabotropic receptors activated by adenine and uridine nucleotides and belong to the superfamily of G protein coupled receptors. So far eight subtypes of P2Y subfamily have been

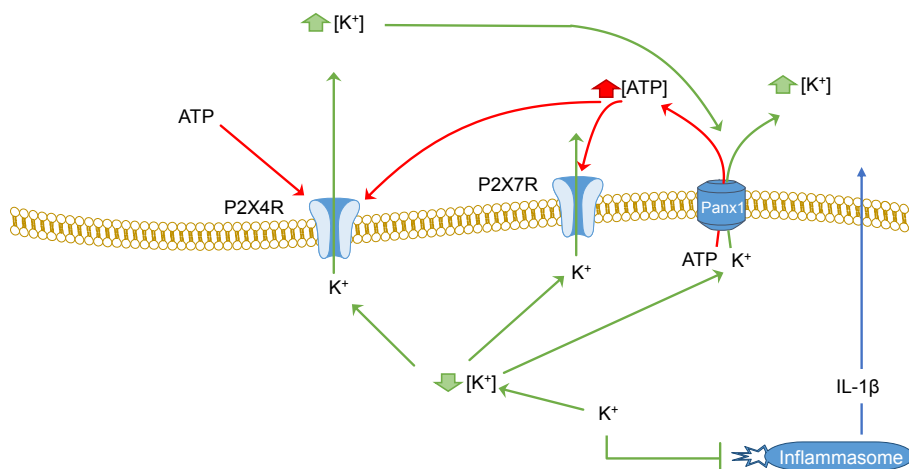


Fig. 2. P2X4, P2X7 and Panx1 act in unison to activate the inflammasome in response to high extracellular concentrations of ATP. With a lower threshold of activation, P2X4 receptors are activated by lower extracellular ATP concentrations than necessary for the direct activation of the P2X7 receptor. K^+ efflux through the P2X4 receptor channel can lead to increased extracellular concentrations of K^+ and activate the Panx1 channel, which amplifies the signal via release of both ATP and further K^+ . ATP released through Panx1 maintains the P2X receptors in their open state. K^+ efflux disinhibits the NLRP inflammasome, leading to caspase-1 activation, and the post-transcriptional modification and release of the pro-inflammatory cytokine IL-1 β .

identified (P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁, P2Y₁₂, P2Y₁₃ and P2Y₁₄), which are divided into two subgroups i.e. those coupled by Gq (P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁) and those coupled by Gi (P2Y₁₂, P2Y₁₃ and P2Y₁₄) (Pelegri et al., 2008; Qu et al., 2011). Like P2X, P2Y receptors also differ in their agonist binding profiles, with one group specific to adenine nucleotides (P2Y₁, P2Y₁₂, P2Y₁₃), another group (P2Y₂ and rodent P2Y₄) activated by both adenine and uridine nucleotides, a third group specific to uridine nucleotides (human P2Y₄ and P2Y₆) and P2Y₁₄ activated by UDP-glucose (Jokela et al., 2014). All known subtypes of P2Y receptors are expressed in the CNS with variable distribution among different cell types (i.e. neurons, astrocytes, microglia, oligodendrocytes) (Burnstock and Knight, 2004). These receptors are activated during pathological conditions and participate in neuroinflammation in many different ways. The majority of data are derived from cell culture models of neuroinflammation and are yet to be replicated in *in vivo* assays using specific probes to identify receptor subtypes (e.g. gene deficient mice, shRNA). Nevertheless, we highlight here the most important functions attributed to P2Y receptors in the regulation of neuroinflammation.

P2Y₁ receptors are expressed by neurons, astrocytes, oligodendrocytes and microglia and their primary postulated function is neuromodulation. In addition a recent study found that P2Y₁ receptors expressed on astrocytes can be activated under conditions of oxidative stress, stimulating the release of IL-6. In this way, P2Y₁ acts as a gatekeeper of astrocyte-mediated neuroprotection (Fujita et al., 2009).

Uridine nucleotide-sensitive P2Y₂ receptors are expressed by neurons, astrocytes and microglia and a growing number of studies implicate a role in neuroinflammation. Uniquely amongst the P2Y family, these receptors possess a consensus integrin-binding motif which interacts with avB3/5 integrin, regulating actin polymerization and cytoskeletal rearrangements through the Rac/Rho pathways (Weisman et al., 2012). P2Y₂ receptors are upregulated in animal models of inflammation and, interestingly, P2X₇ receptor activation on astrocytes and microglia act as a signal to upregulate P2Y₂ receptors through the release of the proinflammatory cytokine IL-1 β . The activation of P2Y₂ confers neuroprotection, particularly in AD, through several pathways: the promotion of neurite outgrowth, increases in cell motility, the processing of non-amyloidogenic APP and enhancement of phagocytosis and degradation of the A β peptide (Erb et al., 2015; Kim et al., 2012; Weisman et al., 2012). Using a TgCRND8 mouse model of AD, Ajit, et al. (2014) demonstrated both that P2Y₂ expression is enhanced in concert with the AD phenotype, and that genetic deletion of P2Y₂ enhances early AD pathology. In addition P2Y₂ receptors expressed on satellite glia participate in the sensitization leading to trigeminal pain (Magni et al., 2015).

In comparison with P2Y₂ receptors, the data on the involvement of P2Y₄ receptors in neuroinflammation is relatively sparse. Although the expression of P2Y₄ receptors in microglia is well documented, e.g. (Bianco et al., 2005; Fumagalli et al., 2003), the lack of specific tools to probe the receptor mean that a full understanding of its role remains elusive. In addition to P2Y₂ and P2Y₄, P2Y₆ receptors are also activated by uridine nucleotides, particularly UDP. These receptors regulate the phagocytic activity of microglia *in vivo* (Inoue, 2007). This process may be important under conditions of neuronal damage. UDP, leaking out from damaged hippocampal cells facilitates the uptake of cellular debris by microglia in a P2Y₆-dependent manner (Koizumi et al., 2007).

Because there is no rodent orthologue of the P2Y₁₁ receptor, our knowledge regarding its role in neuroinflammation is limited. While P2Y₁₁ mRNA can be found in the nervous system (Burnstock and Knight, 2004), and these receptors are expressed on human immunocytes and regulate neurotrophil apoptosis (Communi et al.,

1999; Vaughan et al., 2007), a potential immunomodulatory role in the CNS may also be hypothesized.

P2Y₁₂ receptors are G_i-coupled receptors and well-known as the target site of widely used antithrombotic drugs such as clopidogrel or ticagrelor, and are therefore of considerable interest to cardiovascular medicine. Activation of P2Y₁₂ receptors by ADP leads to rapid platelet aggregation. The inhibition of this effect is utilized in the clinic to prevent myocardial infarction and stroke. P2Y₁₂ receptors, expressed on platelets, regulate various forms of inflammation and could either improve or worsen the scenario (Liverani et al., 2014a). In the CNS, P2Y₁₂ receptors are expressed on astrocytes, oligodendrocytes and the resting, ramified microglia executing surveillant functions (Amadio et al., 2014; Franke et al., 2004).

The very first report indicating that P2Y₁₂ receptors regulate the reactivity of microglia showed that P2Y₁₂ receptors are instrumental for microglial chemotaxis in response to local brain injury. The expression of P2Y₁₂ decreases during the morphological change from the resting ramified to activated amoeboid state associated with microglial activation (Haynes et al., 2006). Recent investigation using *in vivo* two photon microscopy technologies confirmed a key role for P2Y₁₂ receptors in the regulation of microglial dynamics in response to other alarm signals. For example, during epileptic seizures, excess glutamate is released, which promotes microglial process extension towards the site of damage. This process involves the activation of neuronal NMDA receptors, calcium influx, subsequent ATP release, and the activation of P2Y₁₂ receptors (Eyo et al., 2014).

A similar phenomenon, described recently and termed microglial process convergence (MPC) involves the convergence of microglial processes on neuronal dendrites in response to a reduction of extracellular Ca²⁺ (Eyo et al., 2015). The induction of microglial migration, morphological transformation and process extension towards various pathological signals by P2Y₁₂ receptors probably represents a protective mechanism which could be beneficial during early stages of injury. Accordingly, the protective role of microglial P2Y₁₂ receptors against brain ischemia (Webster et al., 2013) or LPS induced sepsis (Liverani et al., 2014b) has been demonstrated. In the case of a more pronounced stroke model, however, the pro-inflammatory action of P2Y₁₂ receptor activation prevails and pharmacological blockade of the receptor in this case is protective (Gelosa et al., 2014). In addition to P2Y₁₂ receptor expression on microglia, oligodendrocytic expression may be an important marker of demyelinating lesions in the neuro-inflammatory disease, ALS (Amadio et al., 2014).

P2Y₁₃ receptors expressed by cerebellar astrocytes and neurons confer neuroprotection through the activation of the GSK3 ERK1/2 pathway (Perez-Sen et al., 2015). Finally P2Y₁₄ receptors are also expressed by microglia, however these receptors are exclusively sensitive to UDP-glucose and other conjugates of UDP but not adenine or uracil nucleotides (Bianco et al., 2005). Again, the participation of P2Y₁₄ receptors in neuroinflammation may be identified by future studies, as their role in the regulation of cell stress and repair in the periphery implicate a similar role in the nervous system (Jacobson et al., 2015).

1.3. The role of adenosine in neuroinflammation

Extracellular concentrations of adenosine are regulated by direct release from cells (Melani et al., 2012), extracellular metabolism of released ATP (Zimmermann, 2000) and reuptake into cells followed by intracellular metabolism (Bender and Hertz, 1986). Adenosine is principally metabolized in the CNS by adenosine kinase, an enzyme expressed in highest concentrations in astrocytes (Studer et al., 2006). Extracellular concentrations of

adenosine in the CNS are regulated by three nucleoside transporters expressed on astrocytes (Peng et al., 2005), with adenosine release, uptake and metabolism forming an 'adenosine-cycle', described in detail by Boison (2008). Following metabolic stress and cell damage, for example, under conditions of high neuronal activity or brain injury, the extracellular concentration of adenosine is dramatically elevated where it acts, in concert with ATP, as an alarm molecule, promoting or inhibiting neuroinflammation, depending on a host of complex factors, which are only beginning to be understood.

Cellular responses to extracellular adenosine are co-ordinated by four different metabotropic P1 receptor subtypes: A₁, A_{2A}, A_{2B} and A₃. Of these, the A₁ receptor and A₃ receptor inhibit the production of cAMP, via G_i protein signaling, while the A_{2A} receptor and A_{2B} receptor increase intracellular concentrations of cAMP via G_s (Fredholm et al., 2007). As such, these receptors in general act in opposition to each other and the cellular response depends on the concentration of receptors expressed, their affinity for adenosine, activation dynamics, extracellular adenosine concentrations and other complex factors (Chen et al., 2014). In addition to classical cAMP-mediated pathways, evidence is accumulating for P1 receptors operating through alternative signaling systems. Of particular note, evidence for protein kinase C (PKC), phosphoinositide 3 kinase (PI3K) kinase and mitogen activated protein kinase (MAPK) involvement in P1-mediated signaling cascades have been reported (Schulte and Fredholm, 2003). Further, the direct activation of K⁺ channels and inhibition of Ca²⁺ channels has been reported following A₁ receptor activation (Hasko and Pacher, 2008).

While P1 receptor expression in the CNS is dominated by A₁ and A_{2A}, the extent of A_{2B} and A₃ receptor expression is contentious, but as these receptors mediate inflammatory responses on immune effector cells in the periphery (Latini and Pedata, 2001), they may also play an important role in mediating neuroinflammatory processes, even at low concentrations. On microglia, van der Putten et al. reported A₁, A_{2A} and A₃ receptor expression, but not A_{2B} (van der Putten et al., 2009), whereas a more recent report revealed the presence of the A_{2B} receptor on microglia, and an important role for this receptor in augmenting the production of the cytokine, IL-10 (Kocsco et al., 2012). The difficulty in ascertaining P1 subtype expression on specific cell types lies in the possible introduction of experimental artifacts from cell culture methods, detection limits associated with anatomical methods and specificity issues relating to physiological approaches using pharmacological tools. Nevertheless, the available data collectively suggest that all four receptor subtypes are expressed on astrocytes, oligodendrocytes and microglia (Boison, 2012). Evidence also indicates that P1 receptor expression can be modulated by neuroinflammatory conditions themselves (Orr et al., 2009).

P1 receptors can play a role in inflammasome activation through the classical cAMP-pathway. Chiu et al. reported that, following A_{2A} receptor activation, K⁺ efflux is increased via an intracellular elevation of cAMP and, subsequent protein kinase A (PKA) activation (Chiu et al., 2014). As described previously, K⁺ efflux stimulates the activation of caspase-1 via increases in extracellular K⁺, decreased intracellular K⁺ concentrations, or both (Bernier, 2012). The relative concentration of K⁺ in the intra- and extracellular compartments appears to be a key point where P2X and P1 signaling converges to modulate inflammasome activation (Fig. 1). Moreover, an increase in extracellular adenosine concentration stimulates both caspase-1 activity and IL-1 β production (Chiu et al., 2014). As A₁ and A₃ receptors act in opposition to A_{2A} receptors, it is likely that the cellular response to high levels of extracellular adenosine is, to some extent dependent on the ratio of receptors expressed, however, alternative, non-cAMP-dependent pathways are also likely to be of importance.

Rebola et al. reported that the A_{2A} receptor is the primary receptor involved in mediating neuroinflammation in response to increases in extracellular concentrations of adenosine, not only via activation of the inflammasome and subsequent increases in IL-1 β production, but also by the recruitment and activation of microglia and alterations in astrocyte function (Rebola et al., 2011). Microglial process retraction and activation is of critical importance to the neuroinflammatory response, and P1 receptors are key modulators of this process. During chronic neuroinflammation, process retraction is mediated by the upregulation of the A_{2A} receptor (Orr et al., 2009). As an example, a recent study showed that microglial process retraction in a mouse model of Parkinson's disease is A_{2A} receptor-dependent (Gyoneva et al., 2014), while Ohsawa et al. postulate an important role for the A₃ receptor in mediating this process (Ohsawa et al., 2012). Further, A_{2A}R activation on microglia induces COX-2 expression (Fiebich et al., 1996), an important marker and mediator of inflammation. The effect of microglial A₁ receptor activation can be either pro- or anti-inflammatory, depending on other factors in the environment (Hasko et al., 2005); however, both neuroinflammation and microglial activity is enhanced in an A_{1A} knockout mouse strain under pathological conditions, suggesting that the primary role of A_{1A} receptor signaling is anti-inflammatory (Luongo et al., 2014).

Astrocytes also play an important role in mediating neuroinflammation, not least, by modulating extracellular concentrations of ATP and adenosine, as previously discussed. P1 receptors expressed on astrocytes are vital components in mediating this process. In addition, pharmacological A_{2A} receptor blockade inhibited astrogliosis in primary astroglial cultures (Brambilla et al., 2003). Because astrocytes are responsible for the maintenance of adenosine concentrations in the extracellular environment, through adenosine kinase, astrogliosis can lead to a decrease in extracellular adenosine. Fedele et al. (2005) demonstrated that astrogliosis contributes to ictogenesis via an ADK-dependent increased removal of adenosine from the extracellular space.

Whether increased extracellular adenosine concentrations have a pro- or anti-inflammatory effect is unclear, and appears to be dependent on other variables, such as relative expression of different P1 receptors. Hindley et al. report that direct cortical injections of an adenosine analog 5'-(N-cyclopropyl)-carbox-amidoadenosine, triggered reactive gliosis, which could be reversed with A₂ receptor blockade, suggesting the primary effect of high extracellular adenosine concentrations in the CNS is proinflammatory and mediated by A_{2A} receptor activation (Hindley et al., 1994). Another study, however, report that specific A₃ agonists provide neuroprotection against ischemia (Choi et al., 2011). The effect of glial activation on neuroinflammation and neuronal cell fate is likely to depend on specific conditions. A_{2A} receptor agonists reduce long-term neurologic injury after blunt spinal trauma (Reece et al., 2004), while a similar treatment improves recovery following experimental stroke (Pedata et al., 2014), in contrast to reports of the proinflammatory action of this receptor.

In summary, the role of adenosine in mediating neuroinflammation is multi-factorial, involving activation of the inflammasome and the recruitment and activation of astrocytes and microglia. The A₁ receptor and A₃ receptor act in opposition to the A_{2A} and A_{2B} receptor in cAMP-mediated pathways, which are sufficient to explain much of the cellular response. The net effect of increased extracellular adenosine however is dependent on pathology, adenosine concentrations, receptor expression and cross-talk with pathways mediated by ATP and other signaling molecules. Conflicting reports of whether adenosine is pro- or anti-inflammatory underscore the complexity of this molecule's role in mediating neuroinflammatory cascades (Liang et al., 2014).

1.4. Purinergic regulation of neuroinflammation in CNS disorders

The role of purinergic receptors has been implicated in the majority of CNS diseases, characterized or accompanied by neuroinflammation. Because purinergic signaling in various neurodegenerative diseases are discussed in detail elsewhere in this issue (Burnstock, 2015), we focus here on non-organic psychiatric disorders, which are also coupled to inflammation and the repair process. Neuroimmunological alterations appear in classical psychiatric disorders, such as major depressive disorder (MDD), bipolar disorder (BD), obsessive compulsive disorder (OCD) and schizophrenia. Medical conditions associated with chronic inflammation, such as diabetes, obesity, or autoimmune diseases share comorbidity or increase the risk of psychiatric disorders, such as MDD. In contrast to neurodegenerative diseases, however, these disorders are characterized by chronic, low grade or intermittent inflammation, rather than a robust acute inflammation in the brain parenchyma, which is present e.g. after stroke or seizures.

As a reflection of inflammation in the peripheral blood, many human studies have revealed an altered innate immune status and cytokine profile in patients suffering from major depression and schizophrenia. For instance, a recent meta-analysis collecting data from 29 individual studies reported that serum IL-2 receptor, IL-6 and tumor necrosis factor- α (TNF- α) levels are elevated in MDD patients (Liu et al., 2012; Najjar et al., 2013). Among these, IL-6 and TNF- α , correlate with exacerbation and normalization of depressive episodes and the risk of suicide attempts, and are therefore proposed to not only be trait but also state markers of the disorders (Janelidze et al., 2011). This molecular profile is consistent with a Th1-proinflammatory immunophenotype. In contrast to acute neuroinflammatory conditions in MDD, however, astroglia and microglia do not proliferate (Najjar et al., 2013), consistent with a continuous, low grade inflammation. As for BPD, peripheral IL-2, IL-4 and IL-6 are found to be increased in manic episodes (Brietzke et al., 2011) whereas another meta-analysis revealed significant elevation of soluble interleukin (IL)-2 receptor (sIL-2R), TNF- α , soluble tumor necrosis factor receptor type 1 (sTNFR1) and soluble interleukin (IL)-6 receptor (sIL-6R) (Munkholm et al., 2013). In the case of schizophrenia, TNF- α , IFN- γ , IL-12 and IL-2 are consistently elevated in chronic schizophrenic patients, while IL-1 β , IL-6 and TGF- β correlate positively with disease activity (Miller et al., 2011).

Schizophrenia is also regarded as a neurodevelopmental disorder, and fetal neuroinflammation, which could be the consequence of maternal infection (Meyer, 2013), is implicated in its etiology. Fetal neuroinflammation is characterized by enhanced levels of proinflammatory cytokines in the brain and enhanced microglial activation, which changes neurodevelopmental trajectories and thereby predisposes the developing brain to long-term pathology, which is later manifested, during adolescence. During the postnatal period, further postnatal stressors can induce or maintain neuroinflammation during development leading to abnormal brain maturation and behavioral changes in the prodromal period, which eventually develop into an overt psychosis. Because purinergic mechanisms, in particular P2X7 receptors, regulate many aspects of neuroinflammation, it is unsurprising that emerging evidence indicates their activation and participation in animal models of psychiatric disorders.

In rodent studies mimicking depressive-like behaviors, both genetic deletion and pharmacological inhibition of P2X7 receptors lead to an antidepressant phenotype, which is manifested in the widely used forced swim (FST) and tail suspension tests (TST) (Basso et al., 2009; Boucher et al., 2011; Csolle et al., 2013a,b; Pereira et al., 2013; Wilkinson et al., 2014), but can also be measured using more complex paradigms such as learned helplessness (Iwata et al., 2013). Acute stress induced depressive-like

behaviors, following LPS challenge are also alleviated by P2X7 receptor antagonism, (Csolle et al., 2013b; Ma et al., 2014). When footshocks were used as a stressor, however, no change in behavior was found in the presence of a P2X7 antagonist (Catanzaro et al., 2014). Conflicting results were obtained using chronic stress models: chronic restraint leads to a downregulation of P2X7 mRNA throughout the hippocampus (Kongsui et al., 2014), whereas another study found a markedly upregulated Cx43 and Panx1 opening in the hippocampus under similar conditions, mediated by NMDA/P2X7 receptor signaling (Orellana et al., 2015). Interestingly, a very recent study of the same group revealed that a similar upregulation of the Cx43 and Panx1 pathway can be detected in offspring in response to maternal LPS treatment (Avendano et al., 2015).

Interestingly, ATP alone also elicits a rapid antidepressant-like effect if added directly to the CNS in the chronic social defeat model of depression, an effect probably mediated by P2X2 receptors in the medial prefrontal cortex (Cao et al., 2013). To study the manic pole of bipolar disorder, genetic deficiency and pharmacological blockade of P2X7 receptors attenuated hyperactivity induced by amphetamine in all studies examined (Bhattacharya et al., 2013; Csolle et al., 2013a; Gubert et al., 2014; Lord et al., 2014). These findings indicate that the presence and endogenous activation of P2X7 receptors contributes to behavioral changes induced by either negative or positive challenges, and the blockade of the receptor alleviates these fluctuations.

Several attempts have been made to correlate behavioral changes with neuroinflammatory parameters, in particular with IL-1 β levels in the CNS and periphery, given the well-established role of P2X7s in the regulation of posttranslational processing of this cytokine. However, it appears that the scenario is more complex as only LPS-induced, but not the basal IL-1 β levels are subject to regulation by P2X7 receptors in the rodent brain (Csolle and Sperlagh, 2010; Mingam et al., 2008) and the mood stabilizing phenotype detected in the absence of P2X7 receptors was not transferred with bone marrow transplantation (Csolle et al., 2013b). The contribution of P2X7 receptors present on cells of hematopoietic origin such as peripheral immune cells to behavioral changes detected in naïve animals, therefore, seems minimal.

Rather, P2X7-regulated intrinsic neuronal mechanisms such as the modulation of glutamatergic neurotransmission (Csolle et al., 2013b) or nitric oxide signaling (Pereira et al., 2013) may represent the underlying mechanisms of the antidepressant phenotype. On the other hand, amphetamine induced induction of IL-1 β and oxidative stress (Gubert et al., 2014) in the striatum were reversed by a P2X7 receptor antagonist coincidentally with the alleviation of amphetamine induced behavior and amphetamine induced striatal dopamine release is attenuated by the P2X7 receptor deficient genotype (Csolle et al., 2013a). The lack of local IL-1 β induction and subsequent modulation of dopamine release in the absence of P2X7 receptor could explain the alleviation of amphetamine induced hyperlocomotion. Interestingly, ATP-induced cell death in the hippocampus – a process probably mediated by P2X7 receptors – was reversed by the mood stabilizer drugs lithium and valproate (Wilot et al., 2007), therefore the action of currently used drugs against bipolar disorder might also involve P2X7 receptors.

Although less data have been accumulated, a recent study suggests that P2X7 receptors also regulate social behavior, an important aspect of psychiatric disorders. While JNJ-42253432, a novel brain-penetrant P2X7 receptor antagonist, did not affect footshock induced decreases in social interactions, it elicited a robust increase in overall social activity (Lord et al., 2014). Therefore, P2X7 receptor modulation may represent an intriguing possibility for interfering with neuropsychiatric conditions characterized by social deficit (e.g. schizophrenia, autism). In

addition, although the underlying receptor subtype has not been identified yet, a line of recent studies found that suramin, a broad spectrum purinergic antagonist, attenuates the whole variety of neurobiological and phenotypic alterations in different animal models of autism spectrum disorder, including the poly(I:C) model representing maternal immune activation (Naviaux et al., 2014, 2015, 2013).

Finally the neuroinflammatory response evoked by the recreational drug 3,4 -methylenedioxymethamphetamine (MDMA, ecstasy) also appear to involve the activation of P2X7 receptor: MDMA-induced BBB leakage, subsequent microglial activation and metalloproteinase induction were suppressed by *in vivo* treatment with specific P2X7 receptor antagonists (Rubio-Araiz et al., 2014).

Apart from the nucleotide-sensitive receptors detailed above, the role of adenosine receptors have also been examined in animal models of psychiatric disorders, although adenosine mediated control of neuroinflammatory mechanisms has not been envisaged in this respect. Adenosine has been reported to have an antidepressant-like effect in the forced swim paradigm, and this effect has been attributed to the activation of both A₁ and A_{2A} receptors (Kaster et al., 2004) and the subsequent involvement of K⁺ conductances (Kaster et al., 2007a) and the endogenous opioidergic system (Kaster et al., 2007b). Orally administered inosine also reproduces this effect (Muto et al., 2014). Interestingly A_{2A} receptor gene deficiency and A_{2A} receptor antagonists, including caffeine, also have antidepressant-like effect in the FST and TST (El Yacoubi et al., 2003; Hodgson et al., 2009) as well as in the chronic unpredictable stress paradigm of depression (Pechlivanova et al., 2012).

The antidepressant effect of A_{2A} antagonists are most likely mediated by D2 receptors in the ventral striatum (El Yacoubi et al., 2003). More recently, it was found that the rapid antidepressant-like effect of sleep deprivation is mediated by astrocyte-derived adenosine as it is absent in A₁ receptor knockout and astrocyte-deficient dnSNARE mice and can be mimicked by CCPA, an A₁-selective agonist (Hines et al., 2013). Further, astrocyte-specific deletion of A_{2A} receptors mimics certain features of the schizophrenia endophenotype, such as decreased working memory and enhanced psychomotor response to the NMDA antagonist MK-801 (Matos et al., 2015). These data point to the role glia-neuron cross talk in the action of endogenous adenosine on behavior. A potentially fruitful area of further investigation could be the identification of a link between the impact of adenosine receptors on neuroinflammatory mechanisms and its role in neuropsychiatric disorders.

The assumption underlying most research involving the role of purinergic signaling in neuroinflammation is that inflammatory responses exacerbate injury. However, the complex pathways leading to inflammasome activation are highly conserved, suggesting a strong adaptive value. It is likely, therefore, that reducing neuroinflammation associated with pathology is not necessarily always of value. Roth et al. (2014) investigated the role of purinergic signaling in neuroinflammatory responses following TBI and found that the P2X7-mediated inflammatory response contributes to better outcomes, with activated microglia protecting the parenchyma and myelomonocytic cells invading the damaged meninges. Both purinergic mechanisms and reactive oxygen species are key players in the response to injury. In this study, these pathways could be manipulated by delivering pharmacological agents transcranially.

2. Conclusion

Purines are ubiquitous extracellular mediators, which are released to the extracellular space under conditions of inflammation. Among various subtypes of purinergic receptors, both P1 and

P2 receptors participate in the regulation of the inflammatory response of the CNS in a complex way, i.e. in the activation of the inflammasome, the synthesis and posttranslational processing of IL-1 β and other pro- and anti-inflammatory cytokines, the recruitment and activation of microglia and in reactive astrogliosis. Under pathological conditions purinergic receptors are up-/or downregulated, and therefore the contribution of purinergic regulation dynamically changes during acute and chronic phases of neuroinflammation.

Conflicts of interest

None.

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