A central role for ATP signalling in glial interactions in the CNS

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Abstract:

The purine ATP has a prominent regulatory role in CNS function and pathology due to its actions on glial cells - microglia, astrocytes and oligodendrocytes. ATP serves as an apparently ubiquitous 'gliotransmitter' that is released by astrocytes and other cells to activate purine receptors on neighbouring cells. In pathology, the release of ATP mediates both tissue damage and repair by its direct effects on glial cell integrity and survival. The actions of ATP on glia are mediated via a wide range of receptors, broadly divided into ionotropic P2X and metabotropic (G-protein coupled receptors (GPCR) P2Y receptors, of which there are multiple subtypes ($P2X_1$-$P2X_7$ and $P2Y_1$-$P2Y_{14}$). ATP-mediated interactions between astrocytes and microglia are at the centre of immune responses in the CNS, with prominent roles for the $P2X_4$, $P2X_7$, $P2Y_1$, $P2Y_6$ and $P2Y_{12}$ receptor subtypes. In oligodendrocytes, $P2X_7$ and $P2Y_1$ receptor subtypes have a bipartite function in respectively mediating oligodendrocyte destruction and protection. Purine receptors mediate glial pathology, with prominent roles in ischemia, neuroinflammation, Multiple Scelerosis, neuropathic pain and traumatic injury. Notably, glial ATP signalling may be altered with ageing and is implicated in impaired myelination and immunity in Alzheimer's disease. Hence, glial purine receptors provide potential therapeutic targets in multiple neuropathologies, but the 'Jeckyll and Hyde' nature of purine signalling underscores the importance of further research and a comprehensive understanding of the roles of the different purine receptors in mediating tissue damage and repair.

Keywords: glia, astrocyte, oligodendrocytes, ATP, purine receptor

BACKGROUND

The main types of glia in the central nervous system (CNS) are astrocytes, oligodendrocytes and microglia, each of which are specialised to perform specific functions to enable CNS function and are integral to all neuropathologies (1). Astrocytes are multifunctional cells with essential neuron supporting functions, including homeostasis, metabolic support, neurovascular coupling and forming the neuroprotective scar following injury. Furthermore, astrocytes encompass synapses and play a critical role in neurotransmitter uptake and are implicated in synaptic transmission as part of the tripartite synapse (2). Oligodendrocytes are responsible for myelination, which is essential for rapid axonal communication and higher cognitive function (3, 4). Microglia have a macrophage function and provide the innate immunity in the CNS (5). A universal property of glial cells is their responsiveness to ATP through their expression of multiple purine receptors (6). Purinoceptors mediate the characteristic responses of glia to CNS insults – astrocyte reactivity, microglial activation and oligodendrocyte/myelin loss. Hence, glial purinoceptors are potential therapeutic targets in multiple CNS disease states.

GLIAL PURINE RECEPTORS

Purine receptors are broadly divided into adenosine (P1) and ATP (P2) receptors, and there is evidence for expression of both groups in different glial cells (6). P2 receptors (P2R) are further divided into ionotropic P2XR and G-protein coupled metabotropic P2YR, which can be distinguished by a range of agonists and antagonists (7). There are seven P2XR subunits ($P2X_{1\text{-}7}$), which assemble as trimers to form homomeric and heteromeric receptors with a diverse range of properties (8). P2XR are cationic ligand-gated channels permeable to Na^+, K^+ and Ca^{2+}, and most are activated at low concentrations of ATP, with EC_{50} of 1–10 μM,

whereas $P2X_7R$ are activated at high concentrations of ATP, in the 0.1-1 mM range, and are capable of pore formation, resulting in sustained influx of Ca^{2+} (7). There are eight subtypes of P2YR, which exhibit different preferences for ATP/ADP ($P2Y_1$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$), ATP/UTP ($P2Y_2$), UTP ($P2Y_4$), UDP ($P2Y_6$) or UDP-glucose and other nucleotide sugars ($P2Y_{14}$) (7). P2YR may assemble as homodimers or heterodimers with other P2YRs or with other G-protein coupled receptors, which increases their diversity further (9).

Two key features of glial P2R is the predominant role for $P2Y_1R$ in glial calcium signalling and for $P2X_7R$ in glial pathology (6, 10). In astrocytes, activation of $P2Y_1R$ results in an increase in cytosolic Ca^{2+}, often referred to as glial 'calcium signalling', which is propagated between cells by astroglial release of ATP to activate receptors on neighbouring cells in a 'spillover' or volume transmission manner (11, 12). Astroglial ATP release in physiological signalling may be primarily vesicular and through connexin-43 (Cx43) hemichannels, although they can also release ATP through pore-forming $P2X_7R$, pannexin-1 hemichannels, and volume-regulated anion channels, but the relative importance of the different mechanisms in physiological and pathological signalling is unclear (13-16). Microglia may also release ATP via Cx43 and vesicles (17, 18), and during ischemia oligodendrocytes can release ATP through pannexin hemichannels (19). Nonetheless, ATP release under physiological conditions may be principally from astrocytes and appears to act on astrocytes and oligodendrocytes predominantly via $P2Y_1R$, which cause a rise in $[Ca^{2+}]_i$ through IP_3-dependent release of Ca^{2+} from intracellular ER stores (11, 12). In microglia, UDP-preferring $P2Y_6R$ and ADP-preferring $P2Y_{12}R$ have prominent roles in calcium signalling, which mediates microglial activation and is heavily dependent on store-operated Ca^{2+} entry (SOCE) (20-22). $P2Y_{12}$ receptors are also expressed by oligodendrocytes/myelin and have

unresolved roles in demyelination (23-25). In the case of P2XR, heteromeric $P2X_1/P2X_5R$ mediate fast physiological signalling in astrocytes (26, 27), although this is not a universal feature of astrocytes (28), and it is not clear that P2XR mediate physiological signalling in oligodendrocytes or microglia under non-pathological resting conditions (10). Microglial $P2X_4R$ are activated by low micromolar ATP concentrations and are important in spinal microglia and their involvement in pain (29). The physiological role of P2XR in glia may be in doubt, but a universal feature is their expression of $P2X_7R$, which are activated by high levels of ATP and mediate pathological responses in all three glial cell types - astrogliosis, microglial reactivity, and oligodendrocyte/myelin damage (19, 30-32).

PURINE SIGNALLING IN GLIAL PATHOLOGY

Multiple P2R are involved in the characteristic responses of glial cells to neuropathology (1). Microglia are central to immune and injury responses in the CNS and their activation state is carefully controlled by multiple 'on' and 'off' signals (33). ATP is an 'on' signal and is released by astrocytes and by damaged cells following CNS injury to mediate microglial activation (34, 35). There is a prominent role for $P2X_7R$ in driving microglial activation and proliferation, and $P2X_7R$ play a major role in the microglial neuroinflammatory response (31, 36, 37). Reactive astrogliosis and glial scar formation is a more protracted process and involves prominent roles for both $P2X_7$ and $P2Y_1R$ subtypes (9). In addition, ATP stimulates the production of pathological levels of pro-inflammatory cytokines and prostaglandin E_2 (PGE_2) by astrocytes, resulting in a double hit and increased neuroinflammation and damage (38). The actions of ATP on astroglial and microglial purinoceptors results in a vicious cycle in which $P2Y_1R$ on reactive astrocytes stimulate production of several cytokines/chemokines that activate microglia (39, 40), and $P2X_7R$ on

activated microglia stimulate secretion of multiple cytokines, including IL-6, TNF-α, and IL-1β, which are key mediators of neurodegeneration, inflammation and pain (41, 42). The earliest response of microglia to injury is the rapid ATP-mediated extension of their processes to the area of damage and to seal it off from further damage (34, 43). ATP-induced microglial chemoattraction is principally mediated by $P2X_4R$ and $P2Y_{12}R$ (44). $P2Y_{12}R$ are expressed by resting microglia and mediate their immediate chemotactic response to injury (22), whereas $P2X_4R$ are expressed mainly in activated microglia (45) and control their fate and survival (46). $P2X_4R$ in activated microglia are also central to neuropathic pain (29, 47, 48). Nerve injury induces increased expression of $P2X_4R$ in spinal cord microglia, and their activation by ATP triggers calcium influx and the release of brain-derived neurotrophic factor (BDNF), which in turn induces neuronal hyperexcitability (49, 50). $P2Y_6R$ are also upregulated in activated microglia and specifically promote a phagocytic phenotype in response to UDP leaked from damaged cells (21, 51). Activation of $P2Y_6R$ can reduce the permeability of $P2X_4R$, suggesting the interplay between UDP- and ATP-activated receptors are important in regulating microglial responses to injury (52).

$P2X_7R$ are central to white matter pathology and ATP-mediated loss of oligodendrocytes/myelin (53). Oligodendrocytes constitutively express $P2X_7R$ and their blockade protects against demyelination in experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS) (54), degeneration following neurotrauma (55), and oligodendrocyte loss in ischemia (19). Oligodendrocytes are highly susceptible to ischemia/hypoxia and enhanced ATP signaling during oxygen-glucose deprivation (OGD) activates oligodendroglial $P2X_7R$ and cytosolic Ca^{2+} overload, resulting in cell death (19). Similarly, the cytotoxic effects of $P2X_7R$ in OGD have been demonstrated in microglia (56).

In contrast, $P2Y_1R$ are protective against ischemic damage in astrocytes and treatment with 2-MeSADP reduces cytotoxic edema and brain infarction in mice (57-59). However, over-activation of $P2Y_1R$ induces astrocyte reactivity, indicating the 'Jeckyll and Hyde' nature of purine signalling (9). Oligodendrocytes and their progenitors (OPs) also express $P2Y_1R$ (6, 11, 12, 60-62), and both $P2Y_1R$ and $P2X_7R$ in OPs are likely to be involved in oligodendrocyte regeneration and remyelination, which are prominent features of neuropathology (63-65). ATP and its breakdown product adenosine regulate the migration, proliferation and differentiation of OPs, acting via $A_1\alpha R$, $P2Y_1R$ and $P2X_7R$ receptors (60, 66, 67). Moreover, ATP acting on astroglial P2R triggers their release of leukemia inhibitory factor (LIF) which promotes myelination (68). $P2Y_{12}R$ are also enriched in oligodendrocytes/myelin and are present in demyelinated lesions, but their precise functions in myelination/demyelination are unresolved (23-25).

PURINOCEPTOR SIGNALLING IN THE AGEING BRAIN

There is a loss of glial function in the ageing brain and glial cells are affected at early stages of neurodegenerative processes in Alzheimer's disease (AD) (69). $P2X_{1/5}R$-mediated calcium signalling in astrocytes was decreased in ageing (27, 70), and there is evidence in mouse models of AD of enhanced calcium signalling in astrocytes associated with $P2Y_1R$-mediated release of ATP from reactive astrocytes (71). Dysregulation of calcium homeostasis is a central thesis in AD (72), and increased astroglial calcium signalling is observed in animal models of AD and on exposure of astrocytes to β-amyloid (Aβ) (73). Aβ increases the amount of ATP released from the astrocytes (74), which would have diverse effects on glial cells in AD. Aged microglia express higher levels of $P2Y_{12}R$ and respond to extracellular ATP by becoming less dynamic and ramified, opposite to the response of young

microglia (75). These studies are consistent with evidence of microglial senescence and increased inflammation in the ageing brain (76, 77). In addition, there is up-regulation of $P2X_7R$ and increased levels of IL-6, TNF-α, and IL-1β in ageing microglia (78, 79). A vicious cycle of increased $P2Y_1R$-mediated release of ATP from astrocytes and increased expression of microglial $P2X_7R$ would enhance production of cytokines in microglia.

SUMMARY AND CONCLUSIONS

Purine receptors are central to glial signalling and mediate bipartite effects in glial physiology and pathology, and a common theme is that $P2X_7R$ and $P2Y_1R$ mediate damage and repair, respectively. Astrocytes release ATP to act on microglia and oligodendrocytes to integrate them within a glial network. In pathology, ATP released from astrocytes and damaged cells mediates astrocyte reactivity, microglial activation and oligodendrocyte/myelin loss. Moreover, purinergic signalling is likely to play an important role in glial dysfunction in the ageing brain and AD, with consequent effects on neurodegeneration. Hence glial purine receptors are emerging as drug targets and blockade of $P2X_7R$ reduces pathology and improves outcome in mouse models of stroke, MS, AD and hyperalgesia (54, 80-82). Furthermore, $P2X_7R$ drive microglial activation and increase production of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β, which are key mediators of neurodegeneration and oligodendrocyte/myelin damage. In addition, activated microglia express $P2Y_{13}R$ and $P2Y_{14}R$ (83-85) and $P2Y_6R$ specifically promote a phagocytic microglial phenotype, whereas $P2Y_{12}R$ are associated with demyelinating lesions. Overall, purinergic receptors are potentially key drug targets for modulating astrocytes and microglia and, either directly or indirectly, providing cytoprotection for oligodendrocytes. However, purinergic signalling has a 'Jekyll and Hyde' nature that is mimicked by glial pathology,

whereby they mediate both tissue destruction and repair. It may not be a simple matter of blocking glial P2R in human neuropathology, since this could result in increased neurodestruction.

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