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Purinoceptors on Neuroglia

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Abstract Purinergic transmission is one of the most ancient and widespread extracellular signalling systems. In the brain, purinergic signalling plays a unique role in integrating neuronal and glial cellular circuits, as virtually every type of glial cell possesses receptors to purines and pyrimidines. These receptors, represented by metabotropic P1 adenosine receptors, metabotropic P2Y purinoceptors and ionotropic P2X purinoceptors, control numerous physiological functions of glial cells and are intimately involved in virtually every form of neuropathology. In this essay, we provide an in depth overview of purinoceptor distribution in two types of CNS glia—in astrocytes and oligodendrocytes—and discuss their physiological and pathophysiological roles.

Keywords ATP · Adenosine · P1 receptors · P2Y receptors · P2X receptors · Astrocytes · Oligodendrocytes

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Neuronal–Glial Circuitry

The mammalian brain, evolved through millions of years, is moulded from two functionally distinct cell populations, the electrically excitable neurones and electrically non-excitable glial cells. The neuroglia, the concept of which was introduced by Rudolf Virchow 150 years ago ([1] for historic overview, see [2, 3]), provides a three-dimensional canvas into which the synaptically connected neuronal networks are embedded [4, 5]. The main type of grey matter glia, the astroglia, divides, through the process of "tiling", the brain parenchyma into relatively independent structural units, determined by the territories occupied by individual astrocytes [6–8]. Within these territories, astrocytes provide structural and functional support to neurones [9, 10], create neuronal–glial–vascular units [11] and enwrap the synaptic contacts making the tripartite synapses [12, 13], characteristic for the central nervous system (CNS). Functional importance of these individual domains acquired experimental attention only very recently, and yet, these local neuronal–glial units may appear to be the basic structural elements of the grey matter, which shape integrative processes in the CNS. Furthermore, the astroglia, being the main cellular element of brain homeostasis, is intimately involved in neuropathology, determining to a very great extent the progress and outcome of various diseases of the CNS [14–17].

Purinergic Signalling System

The purinergic signalling system, which utilises purines and pyrimidines as extracellular messengers [18–20], appeared very early in evolution and became exceptionally widespread in both the animal and plant kingdoms [21, 22].

Indeed, purine- and pyrimidine-mediated information transfer can be found in virtually every type of cell and tissue [23] and throughout every developmental stage [24]. Adenosine 5'-triphosphate (ATP), which is the principal purinergic signaller, is released from cells by several mechanisms, which include exocytosis, diffusion through "maxi" plasmalemmal channels and probably transporters or even lysosomes [20, 25, 26]. In addition, ATP is released from damaged cells, being a universal "danger" signal. The released ATP is rapidly degraded by numerous endonucletidases [20, 27] that produces a trail of derivatives [adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine], which in turn also act as signalling molecules. At the receiving end, purines and pyrimidines activate several families of purinoceptors' broadly classified as metabotropic P1 adenosine receptors, metabotropic P2Y purinoceptors and ionotropic P2X purinoceptors [18, 28–33]. These receptors alone or in combination are expressed throughout living cells and tissues and mediate a remarkable variety of physiological and pathophysiological reactions.

Purinergic Signalling in Neuronal–Glial Networks

The purinergic signalling system plays a unique role in neuronal–glial interactions, as virtually every type of glial cell, be it the cells of ectodermal/neural origin (astrocytes and oligodendrocytes) or of mesodermal origin (microglia), displays sensitivity to ATP and its analogues (Fig. 1). In the nervous system, ATP is released from neurones, their axons and terminals as well as from neuroglia. Probably the predominant mechanism of neuronal ATP release in the CNS is vesicular [25, 34]. ATP is involved in synaptic transmission in many brain regions [20, 35], as it can be stored and released either on its own or together with other neurotransmitters such as glutamate, γ-aminobutyric acid (GABA), noradrenaline or acetylcholine (ACh). In addition, ATP is released from astrocytes and oligodendrocytes by not yet fully characterised pathways [36–38], which may include exocytosis, or diffusion through maxi-pore forming channels (such as hemichannels, pannexins, volume-sensitive anion channels or $P2X_7$ receptors). Regulated release of ATP from glial cells may play important roles in

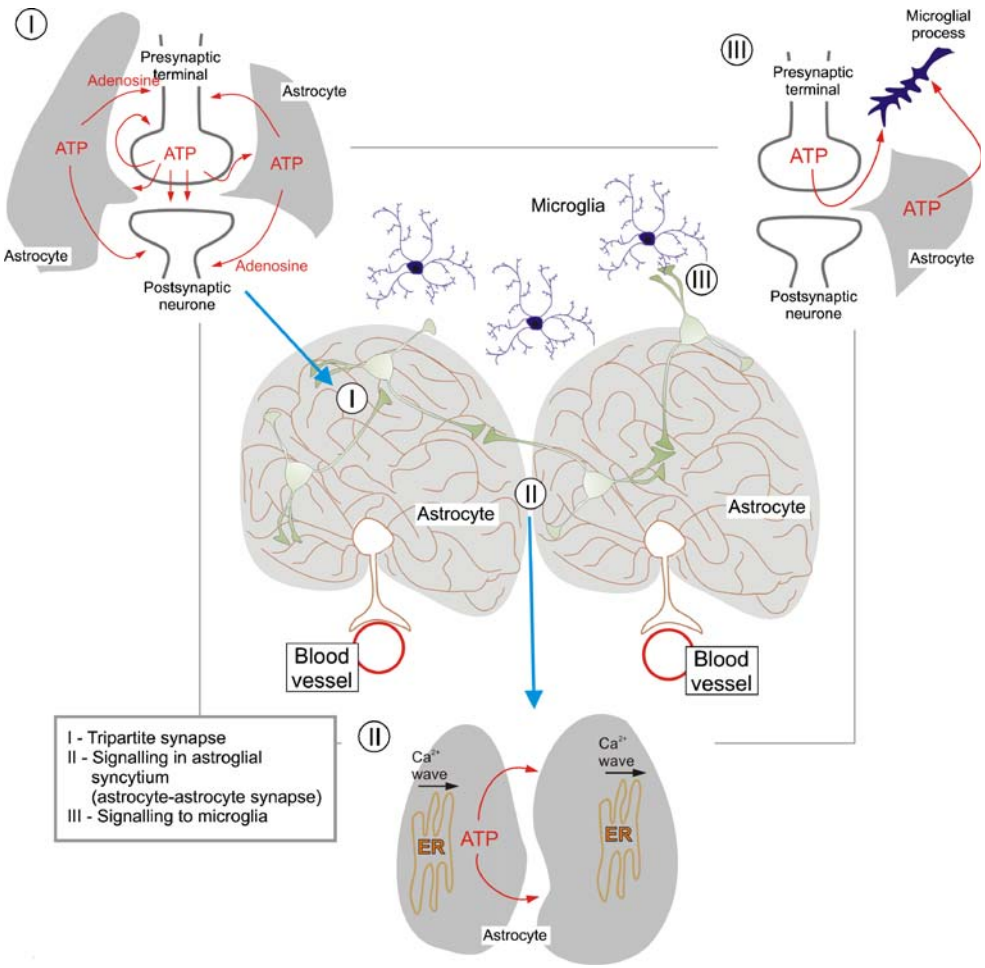

Fig. 1 Omnipresence of purinergic signalling pathways in neuronal–glial circuits in the grey matter. The microarchitecture of the grey matter (as shown in the centre) is defined by astroglial domains, composed of astrocyte, neighbouring blood vessel encompassed by astroglial endfeet and neurones residing within astroglial territory. The microglial cells (each also having its own territory) are constantly surveying these domains spying for damage. ATP and its derivatives act as an extracellular signalling molecule at all levels of communications within neuronal–glial networks. Within the tripartite synapse (*I*), ATP, released during synaptic transmission, activates astrocytes receptors, which in turn initiate Ca^{2+} signals and Ca^{2+} waves in astroglial syncytium. Astroglial Ca^{2+} signals induce release of ATP, which feeds back to neurones via activation of pre- and postsynaptic P1 and P2 receptors. ATP released from astrocytes (*II*) triggers and maintains astroglial Ca^{2+} waves. Finally, ATP released from all types of neural cells control activation (*III*) of microglia

both glial–glial signalling (for example by initiating propagating Ca^{2+} waves [37, 39]) and in glial–neuronal communications (for example regulating synaptic plasticity [40]). Finally, massive release of ATP inevitably accompanies neural tissue damage, being thus ultimately involved in many forms of neuropathology.

Purinergic signalling in neuronal–glial communications has been discussed in many reviews [35, 41–50]; the pathophysiological importance of purinergic signalling for microglial activation was overviewed even more frequently [51–63]. In the present essay, we shall specifically focus on the purinergic receptors expressed in two major types of glial cells of neural origin, in astrocytes and oligodendrocytes, and consider their physiological and pathophysiological relevance.

P1 Receptors

P1 adenosine receptors are classical 7-transmembrane-spanning metabotropic receptors coupled to several families of G_i and G_o proteins. Four types of adenosine receptors (A_1, A_{2A}, A_{2B} and A_3) with distinct pharmacological and functional properties were cloned [33]. As a rule, the A_1 and A_3 receptors exert an inhibitory effect on adenylyl cyclase (mediated through $G_{i/o}$ proteins), whereas A_{2A} and A_{2B} receptors activate cyclic AMP (cAMP) production via G_s proteins. A_1 and A_3 receptors also regulate phospholipase C (PLC) and thus inositol 1,4,5-trisphosphate ($InsP_3$) synthesis; in some cells, A_1 receptors were reported to activate K^+ and/or Ca^{2+} channels [33, 64–66].

Astrocytes

All four P1 receptors have been identified in astrocytes (Table 1) using various functional assays applied to both in vitro and in situ preparations [66]. Initially, the functional role for adenosine receptors was demonstrated in electrophysiological experiments on cultured rat astrocytes [67]; this study showed that adenosine hyperpolarised a subpopulation of astroglial cells, and this hyperpolarisation was antagonised by a P1 receptor antagonist 8-phenyltheophylline. Subsequently, adenosine receptors with pharmacological profiles corresponding to A_1 and A_2 types were identified in human foetal astrocytes [68]. In these cells, the A_1 receptors inhibited, whereas A_2 receptors potentiated synthesis of cAMP. In primary cultures of rat astrocytes, A_{2B} receptors stimulated adenylyl cyclase and their activation caused dose-dependent accumulation of cAMP [69]. In the same cultures, stimulation of A_1 receptors in type 1 (but not in type 2) astrocytes lead to an inhibition of cAMP production [70].

Expression of A_1 receptor-specific messenger RNA (mRNA) was demonstrated in rat-cultured astroglia [71]. Activation of A_1 receptors in rat-cultured astrocytes activated PLC; incidentally, this activation was observed only in cultures with high levels of A_1 receptor expression [72]; up-regulation of A_1 receptors synthesis potentiated A_1-dependent PLC stimulation [71, 73].

In cortical astrocytes, acutely isolated from 4–12-day-old rats, adenosine triggered $[Ca^{2+}]_i$ responses, which were mediated through $InsP_3$-induced Ca^{2+} release and were blocked by the selective A_{2B} antagonist alloxazine [74]. The sensitivity of acutely isolated cells to adenosine was much higher as compared with the same cells maintained in culture, thus indicating modified adenosine receptors expression in the in vitro conditions [74]. In astroglial cultures obtained from neonatal rat forebrains, stimulation of A_1 receptors triggered both intracellular Ca^{2+} release and Ca^{2+} entry and potentiated histamine-induced Ca^{2+} mobilisation [75]. Similarly, adenosine, acting through P1 receptors, triggered $[Ca^{2+}]_i$ elevation in the majority of astrocytes in acute rat hippocampal slices [76]. In astrocytes from acutely isolated mouse olfactory bulb slices, adenosine, which occurred following enzymatic degradation of ATP released from olfactory nerve terminals, induced $[Ca^{2+}]_i$ elevation via activation of A_{2A} receptors [77].

In cultured mouse astrocytes adenosine triggered $[Ca^{2+}]_i$ transients in ~85% of cells via activation of A_3 receptors as judged by a specific sensitivity to A_3 receptor antagonists [78]; incidentally, treatment with guanosine induced $[Ca^{2+}]_i$ responses as well, and these responses are likely to be also mediated through A_3 receptors.

In primary cultured astroglia, adenosine was shown to modulate the amplitude and/or kinetics of $[Ca^{2+}]_i$ transients originated through $InsP_3$-induced Ca^{2+} release initiated by activation of metabotropic glutamate receptors, muscarinic ACh receptors or P2Y receptors [79–84]. The signalling systems involved, however, were different; in some cases, intracellular Ca^{2+} mobilisation was promoted through activation of A_1 receptors [79, 80, 82]; in others, Ca^{2+} release was up-regulated through A_{2B} receptors [81, 83], whereas stimulation of A_1 receptors suppressed the plateau phase of the Ca^{2+} signal, possibly through inhibition of store-operated Ca^{2+} entry [81]. Similarly, activation of A_1 receptors suppressed sustained Ca^{2+} influx following opening of $P2X_7$ receptors in cultured cortical astrocytes [85].

Astroglial adenosine receptors are also coupled with astrocyte-dependent regulation of extracellular glutamate. Activation of A_{2A} receptors in hippocampal astrocytes reduced glutamate uptake via inhibition of GLT-1 transporter and resulted in glutamate release from astrocytes through $[Ca^{2+}]_i$ and a protein kinase A-dependent pathway [86, 87]. This in turn potentiated neuronal activity in

Table 1 P1 adenosine receptors in neuroglia

Receptor type	Experimental preparation/species/technique	Properties/function	References
Astrocytes			
P1 receptors[a]	Cell culture/rat/electrophysiology	Cell hyperpolarization	[67]
	Hippocampal slices/Ca^{2+} imaging	Initiation of $[Ca^{2+}]_i$ transients due to Ca^{2+} release from the ER	[76]
A_1 receptor	Cell culture/rat	Inhibition of cAMP production	[69]
	Cell culture/rat	Activation of PLC	[71–73]
	Cell culture/rat forebrain/Ca^{2+} imaging	Initiation of $[Ca^{2+}]_i$ transients due to Ca^{2+} release from the ER, activation of Ca^{2+} entry and potentiation of histamine-induced Ca^{2+} release	[75]
	Cell culture/rat cortex/Ca^{2+} imaging	Potentiation of ACh-induced Ca^{2+} signalling	[82]
	Cell culture/rat hippocampus/Ca^{2+} imaging	Potentiation of glutamate (mGluRs)-induced Ca^{2+} signalling	[79]
	Cell culture/rat cortex/Ca^{2+} imaging	Inhibition of $P2X_7$-mediated Ca^{2+} influx	[85]
A_{2A} receptor	Acute slices/mouse olfactory bulb/Ca^{2+} imaging	Initiation of $[Ca^{2+}]_i$ transients due to Ca^{2+} release from the ER	[77]
	Cell culture/Slices/rat hippocampus/electrophysiology	Inhibition of astroglial glutamate transporter GLT-1 and activation of astroglial glutamate release	[86]
	Cell culture/rat striatum	Inhibition of astrogliosis	[90]
	Cell culture/rat	Inhibition of iNOS and NO production	[97]
A_{2B} receptor	Cell culture/rat	Stimulation of cAMP production	[69]
	Acutely isolated cells/rat cortex/Ca^{2+} imaging	Initiation of $[Ca^{2+}]_i$ transients due to Ca^{2+} release from the ER	[74]
	Cell culture/rat cerebellum/Ca^{2+} imaging	Potentiation of glutamate (P2Y)-induced Ca^{2+} signalling	[83]
A_3 receptor	Cell culture/mouse	Initiation of $[Ca^{2+}]_i$ transients due to Ca^{2+} release from the ER	[78]
	Cell culture/rat	Activation of apoptosis at intense stimulation; protective effects at low/moderate stimulation	[93–95]
	Cell culture/mouse	Up-regulation of CCL2 cytokine synthesis	[100]
Oligodendrocytes			
P1 receptors	Neuronal–glial co-culture/mouse/Ca^{2+} imaging	Initiation of $[Ca^{2+}]_i$ transients following axonal firing, inhibition of oligodendroglial precursors proliferation and promotion of differentiation and myelination	[101, 103]

[a] This refers to experiments were P1 receptors subtypes were not identified

hippocampus due to an increase in glutamate concentration in the synaptic zones [86].

P1 Receptors in Neuropathology Brain injury results in a massive release of ATP (a substantial part of which is rapidly converted into adenosine) and adenosine; the latter can significantly increase in the cytosol upon conditions of cell stress and hypoxia. As a consequence, adenosine concentrations can attain 10–50 μM [88]. Direct injections of adenosine or the adenosine analogue 5′-(*N*-cyclopropyl)-carboxamidoadenosine into the rat cortex triggered prominent reactive astrogliosis, which was antagonised by the A_2 receptor blocker 1,3-dipropyl-7-methylxanthine [89]. In primary rat striatal astroglial cultures, pharmacological blockade of A_{2A} receptors inhibited astrogliosis induced by basic fibroblast growth factor [90]. Short-term (3 h) treatment of cultured human astrocytes and astrocytoma cells with the astrogliosis-promoting factor tumour necrosis factor (TNF)-α caused phosphorylation of A_{2B} receptors, which reduced their positive coupling to adenylyl cyclase and in turn suppressed A_{2B} receptor-mediated cAMP production [91, 92].

Adenosine receptors are also involved in the regulation of astroglia survival and death. Selective activation of A_3 but not A_1/A_2 receptors triggered apoptosis in primary cultured rat astrocytes and in C6 glioma cell line through down-regulation of BCL2 expression and increase in caspase 3 activity [93]. In fact, activation of A_3 receptors modulated astroglial cell death in a concentration-dependent manner; low doses of A_3 agonists induced reorganisation of the cytoskeleton and increased cell survival, whereas over-stimulation of A_3 receptors induced astroglial death [94, 95].

Adenosine also exerted a general glio-protective action in conditions of glucose deprivation by maintaining mito-

chondrial potential and ATP synthesis [96]. Activation of A_{2A} receptors was reported to inhibit inducible nitric oxide (NO) synthase expression and NO production in cultured rat astrocytes [97], which also may contribute to glio-protection. Specific activation of A_1 receptors had an anti-apoptotic effect in primary astroglial cultures treated with staurosporine; this effect was mediated through the phosphatidylinositol 3-kinase (PI3K) pathway [98]. Both A_1 and A_3 receptors are involved in protection of astrocytes against hypoxic conditions. Hypoxia-induced cytotoxicity was much more pronounced in astroglial cultures obtained from A_1 or A_3 receptors knockout mice [99]. Stimulation of A_3 receptors also increased the synthesis of neuroprotective cytokine CCL2 in cultured mouse astroglia [100].

Oligodendrocytes

All four P_1 adenosine receptors were identified in cultured oligodendrocytes (Table 1) and their precursors at the mRNA level [101]. Adenosine receptors play an important role in differentiation of cells from the oligodendroglial lineage. In experiments in vitro, in co-cultures of sensory neurones and oligodendroglial precursor cells (OPCs), adenosine and ATP released during action potentials triggered Ca^{2+} signals in immature oligodendrocytes (represented by both NG2-positive cells and O4-positive OPCs). This action was mediated through both P2Y and P1 adenosine receptors [101] and allowed OPCs to detect electrical activity of non-myelinated nerve fibres. Activation of adenosine receptors, however, had another specific action; it inhibited OPCs proliferation, promoted their differentiation and initiated myelination, thus constituting a signalling loop between axon activity and oligodendrocyte function [101]. Incidentally, myelination was also promoted by the astroglia-derived cytokine, leukaemia inhibitory factor, which was released following stimulation of astroglial P2Y receptors [102]. All in all, ATP and adenosine released from axons represent a specific signalling system coordinating axonal/oligodendroglial development and interaction [103].

Adenosine receptors are also involved in various forms of oligodendrocyte pathology. Genetic deletion of A_1 adenosine receptors triggered severe demyelination and provoked the progressive-relapsing form of experimental allergic encephalomyelitis (EAE), which is generally considered to be a model for multiple sclerosis (MS) [104]. A substantial part of the pathology was associated with activation of microglia, although direct effects on oligodendrocytes can also play a relevant role. Similarly, sustained activation of A_1 receptors in early postnatal brain reduces expression of myelin basic protein and triggers white matter damage and ventriculomegaly [105].

P2X Receptors

Ionotropic ATP receptors, classified as P2X in 1985 [29, 32] are represented by archetypical ligand-gated cationic ($Na^+/K^+/Ca^{2+}$) channels, assembled in trimeric form by different subunits [106–108]. These subunits, encoded by distinct genes, are classified $P2X_1$ to $P2X_7$ according to historical order of cloning [31, 107]. Receptors, formed through homo- or heteromeric assembly of $P2X_1$ to $P2X_6$ subunits (so far, homomeric composition was shown for $P2X_{1\text{-}5}$ subunits, whereas $P2X_6$ subunits apparently cannot olygomerise; heteromeric compositions are represented by $P2X_{1/2}$, $P2X_{1/4}$, $P2X_{1/5}$, $P2X_{2/3}$, $P2X_{2/6}$ and $P2X_{4/6}$ channels), are activated by low micromolar ATP concentrations [20, 35, 109, 110]. In contrast $P2X_7$ receptors are much less sensitive to ATP (their full activation is reached at mM ATP concentrations) and demonstrate several properties, which readily distinguish them from other P2X receptors [110–112]. All P2X receptor subunits are expressed in the nervous system, although their expression varies in different regions of the peripheral nervous system and CNS.

Astrocytes

Surprisingly, our knowledge on the functional expression of P2X receptors in astrocytes is very limited (Table 2). Expression of various P2X subunits on the transcriptional level was found in several astroglial preparations. Studies on primary cultured rat cortical astrocytes identified the expression of mRNA for $P2X_{1–5}$ and $P2X_7$ receptors [113, 114]. In tissue extracts from rat nucleus accumbens, reverse transcriptase polymerase chain reaction (RT-PCR) revealed expression of all seven P2X mRNAs [115]. In freshly isolated retinal Müller cells, $P2X_3$, $P2X_4$, $P2X_5$ but not $P2X_7$ mRNAs were identified [116]. In contrast, $P2X_7$ receptor-specific mRNA was identified in Müller cells isolated from human retina [117]. In acutely isolated mouse cortical astrocytes, only $P2X_1$- and $P2X_5$-specific mRNAs were found [118]. Astroglial localisation of some P2X subunits was also corroborated by immunohistochemistry. In nucleus accumbens (where all seven receptors were present at transcriptional level), immunofluorescence revealed that only $P2X_{2–4}$ receptors were co-localised with glial fibrillary acidic protein (GFAP)-labelled astroglial profiles [115]. Mechanical lesion triggered up-regulation of $P2X_{1–4}$ and $P2X_7$ immunofluorescence in nucleus accumbens astrocytes [115]. Immunoreactivity for $P2X_1$ and $P2X_2$ receptors was detected in astroglial cells in cerebellum [119, 120]; in a similar way, $P2X_2$ receptors were found in spinal cord astrocytes [121], whereas $P2X_4$ receptors were identified in astrocytes from the brainstem [122]. In hippocampal astrocytes, immunostaining revealed expression of $P2X_{1–4}$, $P2X_6$ and $P2X_7$ subunits [123].

Table 2 P2X receptors in neuroglial cells

Receptor type	Experimental preparation/species/technique	Properties/function	References
Astrocytes			
$P2X_{1\text{-}5}$, $P2X_7$	Cell culture/rat cortex/RT-PCR	Specific mRNAs detected	[113, 114]
$P2X_{1\text{-}7}$	Tissue extracts/rat nucleus accumbens/ RT-PCR	Specific mRNAs detected	[115]
$P2X_{2\text{-}4}$	Rat nucleus accumbens/immunostaining	Immunoreactivity detected	[115]
$P2X_{3\text{-}5}$	Acutely isolated Müller cells/rat retina/ RT-PCR	Specific mRNAs detected	[116]
$P2X_1$, $P2X_5$	Acutely isolated cells/mouse cortex/RT-PCR	Specific mRNAs detected	[118]
$P2X_1$, $P2X_2$	Rat, guinea pig cerebellum/immunostaining	Immunoreactivity detected	[119, 120]
$P2X_4$	Rat brainstem/immunostaining	Immunoreactivity detected	[122]
$P2X_{1\text{-}4}$, $P2X_6$, $P2X_7$	Rat hippocampus/immunostaining	Immunoreactivity detected	[123]
$P2X_{1/5}$	Acute slices/mouse/electrophysiology	Specific currents through $P2X_{1/5}$ heteromeric receptors	[118]
P2X?, $P2X_7$?	Acutely isolated optic nerve/mouse/Ca^{2+} imaging	$[Ca^{2+}]_i$ transients associated with receptor mediated Ca^{2+} entry	[133, 145]
$P2X_7$	Acutely isolated Müller cells/human retina/ electrophysiology	Specific mRNAs and immunoreactivity as well as currents through $P2X_7$ receptors were detected	[144]
$P2X_7$	Cell culture/rat/Ca^{2+} imaging	$[Ca^{2+}]_i$ transients associated with receptor mediated Ca^{2+} entry	[85, 113, 143]
$P2X_7$	Cell culture/mouse cortex/electrophysiology	Specific currents through $P2X_7$ receptors; activation of $P2X_7$ receptors resulted in release of excitatory amino-acids	[138]
$P2X_7$	Cell culture/mouse	Activate synthesis of endocannabinoid 2-arachidonoylglycerol	[150]
$P2X_7$	Cell culture/human	Stimulation of NO production	[139]
$P2X_7$	Cell culture/human	Regulation of NF-κB signalling	[142]
$P2X_7$	Cell culture/rat	Up-regulation of P2Y receptors expression	[157]
$P2X_7$	Cell culture/rat	Down-regulation of aquaporin-4 expression	[158]
Oligodendrocytes			
$P2X_{1,2,3,4,7}$	Cell culture/rat/Western blot	Specific proteins detected	[161, 162]
$P2X_7$	Cell culture/rat/Ca^{2+} imaging	$[Ca^{2+}]_i$ transients associated with receptor mediated Ca^{2+} entry	[162]
$P2X_7$	Cell culture/rat optic nerve/electrophysiology	Specific currents through $P2X_7$ receptors	[165]

At a functional level, however, the activity and role of astroglial P2X receptors remains virtually uncharacterized. ATP-induced depolarisation and membrane currents with accompanying $[Ca^{2+}]_i$ rises were recorded from cultured astrocytes [124, 125], although the subunit composition of underlying receptors was not investigated. In hippocampal astrocytes, voltage- and concentration-clamped in slices or in isolation, an exhaustive series of experiments failed to identify P2X-mediated currents [126], despite immunohistochemical evidence suggestive of astroglial expression of P2X subunits [123]. Similarly, ATP-induced currents were not observed in Bergmann glial cells in acute cerebellar slices [127]. Nonetheless, absence of ATP-induced currents in the in situ experiments is not conclusive, as complex geometry of glial cells, diffusional barriers and rapid degradation of ATP in slice tissue may prevent detection of functional responses.

In acutely isolated cortical astrocytes, $P2X_{1/5}$ heteromeric receptor-mediated currents were discovered and characterised [118]. The $P2X_{1/5}$ heteromeric receptors were initially described in heterologous expression systems [128–131]; surprisingly, cortical astrocytes remain the only "real" cells where operational $P2X_{1/5}$ receptors were hitherto found. The $P2X_{1/5}$ combination exhibits several unique features, which include a very high sensitivity to ATP (currents are activated at nM ATP concentrations), biphasic kinetics with distinct peak and steady-state components and very little desensitisation in response to the repetitive agonist applications. Using these $P2X_{1/5}$ receptors, cortical astrocytes therefore are able to detect extremely low levels of extracellular ATP.

In acutely isolated optic nerves, ATP triggered large $[Ca^{2+}]_i$ transients in astrocytes, which, at least in part, were mediated through Ca^{2+} influx; these $[Ca^{2+}]_i$ responses demonstrated

sensitivity to the P2X receptor antagonist NF023 and could be mimicked by the P2X agonist α,β-methylene ATP (αβ-meATP); yet, the subunit composition of the underlying P2X receptors remains unknown [132, 133].

Functional roles of astroglial $P2X_7$ receptors deserve special attention. The $P2X_7$ receptors are unique in their low ATP sensitivity (in all probability, it is ATP^{4-} that acts as a true agonist), almost complete absence of desensitisation and ability to produce large transmembrane pores upon intense stimulation [31, 110, 112]. The mechanism of the pore formation remains obscure [111]; it may involve the dilatation of the channel or activation of other proteins (such as, for example, pannexins [134]) closely associated with $P2X_7$ receptors. Expression and distribution of $P2X_7$ receptors in healthy brain remains controversial; initial experiments using in situ hybridization found that specific mRNA was restricted to the ependymal layer of the third ventricle [135]. Subsequent RT-PCR studies, however, detected $P2X_7$ mRNA in many areas of the brain including hippocampus, cortex and brain stem (see [112] for detailed account). Similarly, immunoreactivity for $P2X_7$ receptors was demonstrated in hippocampus, medulla oblongata, cerebellum, thalamus and amygdala [112]. At the same time, the specificity of many antibodies used for $P2X_7$ receptors immunostaining remains far from ideal [136], thus making many previous observations questionable. A recent in-depth analysis of the cellular distribution of $P2X_7$ mRNA in the rat brain using isotopic in situ hybridization found its presence in microglia, oligodendrocytes and neurones in many brain areas; yet it failed to detect any presence of $P2X_7$ mRNA in astroglia [137].

There is ample evidence for $P2X_7$ receptor expression in cultured astrocytes at both the transcriptional and protein levels [113, 114, 138–143]. Immunoreactivity for $P2X_7$ receptors was also reported for freshly isolated astrocytes and astrocytes in brain slices [123, 143]. On a functional level, experiments on cultured astrocytes demonstrated both $P2X_7$-mediated Ca^{2+} signalling [85, 113, 144] and characteristic $P2X_7$ ion currents [138]. Similarly, $P2X_7$ currents were identified in Müller cells freshly isolated from human retina [145]. In isolated optic nerve, activation of $P2X_7$ receptors underlie Ca^{2+} influx and astroglial release of ATP, which subsequently acted upon neighbouring glial cells [133, 146]. This astroglia-originated signalling was absent in a $P2X_7$ knockout model [133].

Activation of $P2X_7$ receptors in cultured astrocytes has numerous functional consequences. First and foremost, opening of $P2X_7$ channels triggers release of gliotransmitters glutamate, GABA, ATP and associated purines through exocytosis, $P2X_7$ associated transmembrane pore or through Cl^-/HCO_3^--dependent mechanism of an as yet unidentified nature [111, 138, 144, 147, 148]. In hippocampal astrocytes, prolonged activation of $P2X_7$ receptors lead to a sustained glutamate release, which obviously may have pathological relevance [149]. High concentrations of ATP, acting most likely through $P2X_7$ receptors, were also shown to remarkably (~60 times) increase production of endocannabinoid 2-arachidonoylglycerol in cultured astroglia [150]. Furthermore, stimulation of $P2X_7$ receptors in cultured astrocytes or astroglial cell lines was shown to affect other signalling pathways, for example, modulate release of TNF-α [151], stimulate NO production [139, 152], induce phosphorylation of AKT [153] and p38MAPK/ERK1/ERK2 [154], stimulate transmembrane transport of NADH [155] and regulate NF-κB signalling [142]. Stimulation of $P2X_7$ receptors increased production of lipid mediators of inflammation cysteinyl leukotrienes, this action being mediated through $P2X_7$-mediated Ca^{2+} signalling [156]. Activation of $P2X_7$ receptors in the astroglial cell line RBA-2 rapidly decreased glutamate uptake via Na^+-dependent transporter and reduced expression and activity of glutamine synthetase [157]. Furthermore, $P2X_7$ receptors are involved in the control of expression of other purinoceptors and channels; in particular, $P2X_7$ stimulation up-regulates expression of $P2Y_2$ receptors [158] and down-regulates expression of aquaporin-4 [159] in cultured rat astrocytes.

Nonetheless, the crucial data on the functional expression of $P2X_7$ receptors in astroglial cells in undisturbed grey matter are yet to be obtained. Indeed, astrocytes in tissue culture are certainly different from the in vivo state, as the procedure for isolation triggers astrogliosis, which launches various programmes of functional remodelling, including, most likely, changes in $P2X_7$ receptors expression. There are certain hints that brain injury does induce expression of $P2X_7$ channels. For example, $P2X_7$ immunoreactivity in nucleus accumbens was observed only after mechanical damage [115], similarly focal cerebral ischemia resulted in an appearance of astroglial $P2X_7$ receptors in the rat cortex [160]. Vitreoretinopathy resulted in a significant increase in $P2X_7$ current density in freshly isolated human Müller cells [161]. The immunoreactivity for $P2X_7$ receptors was also found in reactive astrocytes from brain autopsies obtained from MS patients [139].

In conclusion, $P2X_7$ receptors are associated with astroglial responses to brain lesions and most likely constitute a part of global functional remodelling, which accompanies reactive astrogliosis. In this process, $P2X_7$ receptors play an important role regulating both pathologically relevant signalling events (for example, underlying massive Ca^{2+} influx or regulation of various kinases) and production and release of numerous inflammatory factors. The full pathological profile of astroglial $P2X_7$ receptors is still to be uncovered, although they might be considered as potentially important therapeutic targets.

Oligodendrocytes

Oligodendroglial precursor cells in purified postnatal cultures expressed $P2X_{1,2,3,4,7}$ proteins [162, 163]. There is little evidence, however, about functional expression of $P2X_{1–6}$ receptors in both OPCs and mature oligodendrocytes (Table 2). In the isolated optic nerve, the broad agonist of P2X receptors αβ-meATP triggered a small $[Ca^{2+}]_i$ elevation, thus suggesting possible involvement of $P2X_{1–6}$ receptors [164]. In contrast, in oligodendrocytes from corpus callosum slices, ATP failed to activate measurable currents [165].

At the same time, $P2X_7$ receptors may be operational in oligodendrocytes from other areas of the CNS and in white matter tracts. For example, functional $P2X_7$ receptors were found in the cells of oligodendroglial lineage in vitro. In cultured OPCs, the specific $P2X_7$ agonist 2′,3′-*O*-(benzoyl-4-benzoyl)-ATP (BzATP) triggered large Ca^{2+} transients, which were effectively inhibited by the $P2X_7$ receptor agonist oxidised ATP (oxATP) [163]. In cultured oligodendrocytes from optic nerve ATP in high concentrations (EC_{50}, ~8.8 mM) and BzATP (EC_{50}, ~0.5 mM) triggered sustained inward currents. These currents were potentiated in divalent cation-free extracellular solutions and were inhibited by oxATP. In addition high concentrations of ATP and BzATP induced a rapid increase in $[Ca^{2+}]_i$, which was almost exclusively dependent on transmembrane Ca^{2+} entry [166]. These data taken together are indicative of activation of $P2X_7$ receptors [166]. In addition $P2X_7$ receptor immunoreactivity was detected in oligodendrocytes from the optic nerve and the spinal cord [166, 167]. Stimulation of $P2X_7$ receptors for 15 min (with 1 mM ATP or BzATP) induced significant oligodendroglial death in culture and in situ in the optic nerve.

The $P2X_7$-dependent death of oligodendrocytes may have pathophysiological relevance for demyelinating diseases and for MS in particular. Indeed, in EAE, which is considered a model for MS, treatment with the $P2X_7$ antagonists oxATP or brilliant blue G inhibited demyelination and restored axon conduction velocity [166, 167]. Moreover, the levels of $P2X_7$ expression appeared to be increased in white matter of MS patients [166]. These data may indicate the relevance of $P2X_7$ receptors as a therapeutic target for treatment of demyelinating diseases.

P2Y Receptors

Metabotropic P2Y purinoceptors are (similar to P1 receptors) 7-transmembrane domain G protein-coupled receptors [20, 45]. They can be broadly divided into the $P2Y_{1,2,4,6,11}$ and $P2Y_{12,13,14}$ groups based on phylogenetic similarity and G protein preference [30]. The $P2Y_{1,2,4,6,11}$ are coupled to G_q/G_{11} proteins and regulate activity of PLC, thus controlling $InsP_3$-mediated Ca^{2+} release from the endoplasmic reticulum (ER) [30]. The $P2Y_{12,13,14}$ receptors modulate ion channels and inhibit adenylyl cyclase via $G_{i/o}$ proteins [30]. This general scheme has exceptions, and in some cases, the same receptor can couple to different G proteins [168] or exert effector action without any G proteins involvement [169].

Astrocytes

The majority of astrocytes studied in situ or in isolation express metabotropic P2Y purinoceptors (Table 3 and [41]). Primary cultured rat cortical astrocytes express mRNA for $P2Y_{1,2,4,6,12,\ 13}$ and UDP-glucose $P2Y_{14}$ receptors [41, 113, 114]. Similarly, in cerebrocortical glial cultures, RT-PCR found the predominant expression of $P2Y_{1,4,6}$ mRNAs [170]. Spinal cord astrocytes predominantly express $P2Y_{1,2}$-specific mRNA [171]. In astrocytes freshly isolated from the CA1 area of 8–12-day-old rat hippocampi, $P2Y_1$ receptors were identified in about 50% of cells at both transcriptional and protein levels [172], although some cells also expressed $P2Y_{2,4}$ receptors. The proportion of astrocytes expressing $P2Y_2$ receptors in CA1 area increased from ~5% at P8–P12 to ~38% at P25 [173]. Isolated rat Müller retinal glial cells expressed mRNA for $P2Y_{1,2,4,6}$ receptors [174], expression of these receptors was further corroborated by immunostaining as well as by functional and pharmacological analysis [175]. Interestingly, in tiger salamander Müller cells, the palette of P2Y receptors was somewhat richer: They express functional $P2Y_{1,2,6,11}$ and possibly $P2Y_4$ and $P2Y_{13}$ receptors [176]. In nucleus accumbens, the immunoreactivity for $P2Y_{1,4}$ receptors in astroglial cells was detected; whereas in cortex, astrocytic profiles were positively stained for $P2Y_{1,2,4,6}$ receptors [177].

Treatment of cultured astrocytes from various brain regions with ATP induced $[Ca^{2+}]_i$ transients, which were, as a rule, a consequence of P2Y receptor activation, production of $InsP_3$ and subsequent Ca^{2+} release from the ER [178–181]. Stimulation of rat brain astroglial cultures with ATP and UTP induced rapid and dose-dependent increase in PLC-dependent inositol phosphate production [182–184] accompanied with $[Ca^{2+}]_i$ increase due to intracellular Ca^{2+} release [185–188]. In rat striatal astrocytes in vitro ATP induced fast $[Ca^{2+}]_i$ transients, which were independent from extracellular Ca^{2+} and were inhibited by the SERCA blocker thapsigargin or by the PLC blocker U-73122 [189]. Likewise, ATP triggered $[Ca^{2+}]_i$ rise in pituitary folliculo-stellate cells (pituitary glia) and in rat neurohypophysial astrocytes through generation of $InsP_3$-induced Ca^{2+} release [190, 191]. The very same $InsP_3$-induced intracellular Ca^{2+} release occurred in cultured spinal

Table 3 P2Y receptors in neuroglial cells

Receptor type	Experimental preparation/species/technique	Properties/function	References
Astrocytes			
$P2Y_{1,2,4,6,12,\ 13}$ and UDP-glucose $P2Y_{14}$ receptor	Cell culture/rat/RT-PCR	Specific mRNAs detected	[41, 113, 114]
$P2Y_{1,4,6}$	Cell culture/rat cortex/RT-PCR	Specific mRNAs detected	[169]
$P2Y_{1,2}$	Cell culture/rat spinal cord/RT-PCR	Specific mRNAs detected	[170]
$P2Y_{1,2,4}$	Cell culture/rat hippocampus/RT-PCR, Western blot	Specific mRNAs and proteins detected; ~50% of cells express $P2Y_1$, some cells also express $P2Y_{2,4}$	[171]
$P2Y_{1,2,4,6}$	Acutely isolated Müller cells/rat retina/RT-PCR, immunostaining, electrophysiology	Specific mRNAs and immunoreactivity detected; stimulation of P2Y receptors triggered Ca^{2+}-dependent K^+ currents	[173, 174]
$P2Y_{1,2,4,6,11,13}$	Acutely isolated Müller cells/tiger salamander/Ca^{2+} imaging	$[Ca^{2+}]_i$ transients associated with Ca^{2+} release of the ER; receptors subtypes were identified using specific pharmacology	[175]
$P2Y^a$	Cell culture/rat/biochemical assays, Ca^{2+} imaging	Increase in $InsP_3$ production; $[Ca^{2+}]_i$ transients associated with Ca^{2+} release of the ER	[181–183, 185–187]
$P2Y^a$	Cell culture/rat, striatum/Ca^{2+} imaging	$[Ca^{2+}]_i$ transients associated with Ca^{2+} release of the ER	[188]
$P2Y^a$	Cell culture/rat, neurohypophysis/Ca^{2+} imaging	$[Ca^{2+}]_i$ transients associated with Ca^{2+} release of the ER	[189, 190]
$P2Y^a$	Cell culture/rat, spinal cord	$[Ca^{2+}]_i$ transients associated with Ca^{2+} release of the ER	[191, 192]
$P2Y^a$	Acute slices/mouse, cerebellum, Bergmann glial cells/Ca^{2+} imaging, electrophysiology	$[Ca^{2+}]_i$ transients associated with Ca^{2+} release of the ER	[126, 203]
P2Y	Cell culture/rat, hippocampus,/Ca^{2+} imaging	$[Ca^{2+}]_i$ transients associated with Ca^{2+} release of the ER	[204]
$P2Y_1$	Acute slices/mouse, olfactory bulb/Ca^{2+} imaging	$[Ca^{2+}]_i$ transients associated with Ca^{2+} release of the ER	[77]
$P2Y_1$, $P2Y_4$	Acutely isolated optic nerve/mouse/Ca^{2+} imaging	$[Ca^{2+}]_i$ transients associated with Ca^{2+} release of the ER	[163]
$P2Y_1$	Cell culture/mouse, cortex	Stimulation of ATP release through volume-sensitive anion channels	[36]
$P2Y^a$	Cell culture/rat	Stimulation of ATP release via exocytosis	[37, 208, 209, 211]
$P2Y^a$	Cell culture/cell lines	Stimulation of ATP release through hemichannels	[213, 214]
$P2Y^a$	Cell culture/rat	Stimulation of glutamate release via exocytosis	[218]
$P2Y_1$	Cell culture/rat hippocampus; Acute slices/mouse hippocampus	Stimulation of glutamate release via exocytosis	[219]
$P2Y^a$	Cell culture/rat	Promotion of astroglial differentiation	[230, 231]
$P2Y^a$	Cell culture/rat, human	Initiation of astrogliosis	[234–237]
$P2Y^a$	Retina preparations/rat, rabbit	Initiation of astrogliosis	[240–242]
$P2Y_1$, $P2Y_{12}$	Rat, Nucleus accumbens, cortex/in vivo	Initiation of astrogliosis	[176, 245]
Oligodendrocytes			
$P2Y_{1,2,4}$	Cell culture/rat/Western blot	Specific proteins detected	[161, 162]
$P2Y_1$	Rat, rabbit/brain/immunostaining	Immunoreactivity detected in oligodendrocytes throughout the CNS	[257]
$P2Y^a$	Cell culture/mouse, rabbit, retina/Acute slices/mouse, corpus callosum/Ca^{2+} imaging, electrophysiology	$[Ca^{2+}]_i$ transients associated with Ca^{2+} release of the ER	[164]

[a] This refers to experiments were P2Y receptors subtypes were not identified

cord astrocytes [192, 193]. Sometimes, P2Y-mediated Ca^{2+} release from the ER in cultured astrocytes also triggered secondary store-operated Ca^{2+} entry [194].

Functionally, $P2Y_1$ and/or $P2Y_2$ receptors assume the leading role in triggering ATP-induced Ca^{2+} signalling in astroglia. In embryonic glial cultures, the agonist profile for initiating $[Ca^{2+}]_i$ transients was 2-methylthioADP (2-MeSADP)>2-methylthioATP(2-MeSATP)>ADP>ATP> adenosine 5′-O-(3-thiotriphosphate), which is characteristic for $P2Y_1$ receptor [170]. Similarly, $P2Y_1$ receptors drive ATP-induced $[Ca^{2+}]_i$ responses in astrocytes from the supraoptic nucleus in acutely isolated slice preparations

[195]. The ER-release component of ATP-induced $[Ca^{2+}]_i$ elevation in rat cortical astrocytes in culture was significantly inhibited by the $P2Y_1$ antagonist MRS2179 [113], although in the same cells, sugar nucleotides triggered $[Ca^{2+}]_i$ rise by activation of UDP-glucose $P2Y_{14}$ receptor [113].

$P2Y_1$ receptors played the major role in generating and maintaining propagating Ca^{2+} waves in hippocampal cultured astrocytes [37]. In cultured spinal cord astrocytes, however, ATP-mediated propagating Ca^{2+} waves required both $P2Y_1$ and $P2Y_2$ receptors, as pharmacological inhibition of either of them eliminated the wave propagation [196, 197]. Further investigations demonstrated that $P2Y_1$ and $P2Y_2$ receptors expressed in cultured dorsal spinal cord astrocytes have different functional kinetics. Activation of $P2Y_1$ receptors specifically resulted in $[Ca^{2+}]_i$ oscillations, which were the consequence of cyclic protein kinase C (PKC)-mediated depression of the said receptors [171, 198]. Incidentally, Ca^{2+} waves in astroglial syncytium can be generated by both $InsP_3$ diffusion through gap junctions and by a regenerative wave of ATP release. The contribution of these mechanisms differs between various brain regions and can be readjusted under various physiological conditions. In spinal cord astroglia, expression levels of connexion 43 regulate expression of P2Y receptors, which, in turn, maintain ATP-dependent Ca^{2+} wave propagation. Acute inhibition of Cx43 synthesis results in down-regulation of $P2Y_1$ receptor production and in an increase of $P2Y_4$ receptor expression, which in turn modified the mode of Ca^{2+} wave propagation [199]. Similar remodelling of P2Y receptor profile and Ca^{2+} wave propagation was also observed after treatment of astroglial cultures with the cytokine IL-1β [200]. Interestingly, in the inflammatory conditions (for example, in astroglial cultures chronically treated with IL-1β), activation of $P2Y_2$ receptors decreases gap junction communications [201].

Expression of $P2Y_2$ receptors in cultured rat astrocytes was up-regulated by guanosine and UTP; they increased both the levels of $P2Y_2$-specific mRNA and augmented $P2Y_2$-mediated $[Ca^{2+}]_i$ transients [202]. This regulatory action of guanosine and UTP involved ERK1-2/MAPK signalling cascade [202].

Functional P2Y receptors linked to $InsP_3$-induced Ca^{2+} release were also found in freshly isolated human Müller cells; Ca^{2+} released from the ER store activated Ca^{2+}-dependent (BK) K^+ channels and Ca^{2+}-gated cationic channels [203].

Activation of metabotropic purinoceptors was also instrumental in inducing Ca^{2+} signalling in astroglial cells in situ. In Bergmann glial cells in cerebellar slices, ATP triggered $[Ca^{2+}]_i$ transients, which did not require extracellular Ca^{2+} and were inhibited by incubation with thapsigargin or by intracellular perfusion with the $InsP_3$ receptor antagonist heparin, thus indicating involvement of a P2Y/PLC/$InsP_3$-dependent signalling cascade [127, 204]. Similarly, astroglial $[Ca^{2+}]_i$ transients mediated by P2Y receptors were observed in astrocytes from stratum radiatum region of mouse hippocampus [205]. In astrocytes imaged in acute mouse olfactory bulb slices, a significant part of ATP-induced $[Ca^{2+}]_i$ transients were mediated through MRS2179-sensitive $P2Y_1$ receptors [77]. In isolated optic nerve preparations, $P2Y_1$ and/or $P2Y_4$ receptors were responsible for the major part of $[Ca^{2+}]_i$ elevation following exposure to ATP [164].

P2Y Receptors and Regulation of Gliotransmitter Release

Stimulation of metabotropic ATP receptors triggers release of gliotransmitters from astroglia. In particular ATP stimulation of cultured astrocytes triggers release of ATP itself; this release occurs through several pathways. In astrocytes cultured from 1-day-old mouse cortex, for example, ATP-triggered ATP release was not affected by chelating $[Ca^{2+}]_i$ with BAPTA/AM but was inhibited by non-selective anion channel blockers [36]. Interestingly, stimulation of $P2Y_1$ receptors was found to activate volume-sensitive Cl^- channels in cultured astrocytes, thus indicating a direct link between activation of purinoceptors and ATP release via anion channel [206]. Similar Ca^{2+} independency of ATP release was found in rat cultured astrocytes; in this study, inhibition of the ER by thapsigargin also did not affect ATP secretion [207]. ATP can be also released from astrocytes through hemichannels [208].

At the same time, there is ample evidence in favour of exocytotic ATP release from astroglia. Exposure of rat cultured cortical astrocytes to 10 μM UTP triggered ATP release [209]. This release was inhibited by the P2 receptor antagonist suramin, by the inhibitor of ER Ca^{2+} accumulation thapsigargin, by the complex Golgi fragmenter brefeldin A, by cytoskeleton disruption with cytochalasin D and by the exocytosis inhibitor botulinum toxin A [209]. These data taken together suggested a role for $InsP_3$-mediated ER Ca^{2+} release triggering Ca^{2+}-regulated exocytosis [209]. Similarly, exocytotic ATP release was found to be the primary cause for generation of propagating Ca^{2+} signals in cultured rat hippocampal astrocytes [37]. The ATP-rich vesicles, which can be released following $[Ca^{2+}]_i$ elevation, were identified in rat cultured astrocytes; in fact, these astrocytes contained two pools of vesicles, containing either glutamate or ATP, with distinct properties [210]. The ATP release with subsequent activation of P2Y receptors was also the primary mechanism for Ca^{2+} wave propagation in corpus callosum slices [211]. Exocytotic $[Ca^{2+}]_i$- and vSNARE-dependent ATP release was also detected in astroglial progenitors [212]. In addition, release of ATP from astrocytes constitutes a powerful mechanism for astroglia–microglia signalling [213].

P2Y-induced $[Ca^{2+}]_i$ rise may trigger ATP release through connexins/hemichannels; in particular, overexpression of connexins in glial cell lines (normally devoid of gap junctions) significantly increased $[Ca^{2+}]_i$-regulated ATP release and produced propagating Ca^{2+} waves [214, 215].

ATP released from astroglia also acts as a source of adenosine, which rapidly builds up due to ATP degradation. This adenosine acts as an important gliotransmitter because it affects synaptic transmission through numerous synaptic and extrasynaptic P1 receptors expressed in neurones [216, 217]. In certain conditions, for example upon hypoxic stress, astrocytes are able to release adenosine from separate unidentified pools, which, in turn, suppresses overall synaptic activity and exerts a general cytoprotective action [218].

ATP, acting through metabotropic receptors, triggered release of glutamate and aspartate from cultured cortical astrocytes [219]. Secretion of excitatory amino acids in this preparation was inhibited by intracellular Ca^{2+} chelation and by thapsigargin, indicating a role for Ca^{2+} release from the ER [219]. The $P2Y_1$-mediated intracellular Ca^{2+} release also controls secretion of glutamate from astrocytes in culture and in situ in acute hippocampal slices [220]. This glutamate release occurs through exocytosis, which was directly shown by visualising fluorescence-labelled glutamatergic vesicles using total internal fluorescence reflection imaging. The $P2Y_1$ receptor activation also triggered release of TNFα and prostaglandins, which regulated glutamate release synergistically with intracellular Ca^{2+} signals [220]. In mouse prefrontal cortex, stimulation of astroglial $P2Y_4$ receptors triggered vesicular release of glutamate, which positively modulated NMDA receptors in layer V pyramidal neurones through activation of neuronal metabotropic glutamate receptors [221]. Furthermore, evidence exists suggesting that ATP may trigger glutamate release through activation or positive modulation of volume-regulated anion channels [222, 223].

In fact, co-release of glutamate and ATP/adenosine from astrocytes may provide for a coordinated regulation of synaptic transmission, where glutamate exerts general excitatory and adenosine general inhibitory action on synaptic transmission [48, 224, 225].

ATP also stimulates mobilisation of arachidonic acid and eicosanoid production in cultured astrocytes, an effect that depends on synergism between P2Y-mediated $[Ca^{2+}]_i$ elevation and direct coupling of subset of P2Y receptors with phospholipase A2 [226, 227]. Similarly stimulation of metabotropic P2Y receptors increased synthesis of prostaglandins [228]. Activation of $P2Y_1$ receptors induced expression of brain-derived neurotrophic factor in astroglial cell line [229]. Stimulation of $P2Y_4$ receptors in cultured cortical astrocytes induced a significant increase in expression and release of glycoprotein thrombospondin (TSP)-1, which is a potent stimulator of synaptogenesis [230].

Regulation of Growth and Differentiation In postnatal rat-cultured astrocytes, ATP and its analogues (α, β-meATP, β, γ-meATP, ADPβS, 2-MeSATP and UTP) promote astroglial differentiation and process growth, these being accompanied with *C-fos* and *C-jun* mobilisation and involving activation of phospholipase A2 [231, 232]. Interestingly, ATP effects on morphological differentiation were developmentally regulated [233]: In cultures from embryonic (E18) animals, ATP suppressed cAMP-dependent stellation via P2Y receptors, whereas in postnatal cells, it promoted differentiation (although most likely through P1 receptors).

P2Y Receptors in Neuropathology ATP triggered morphological gliosis in cultured rat and human astrocytes [234], which involved extracellular signal regulated protein kinases (ERK)1/2 mediated induction of cyclo-oxygenase-2 (COX-2), but was independent of $[Ca^{2+}]_i$ changes, thus suggesting a subset of P2Y receptors linked to ERK/COX-2 pathway [235–237]. In rat primary astroglial cultures, ATP, acting through P2Y receptors, stimulated astrocyte proliferative activity [238]. Activation of P2Y receptors also potentiated proliferation of astroglia induced by broad astrogliotic agent fibroblast growth factor 2 [239]. Mitogenic effects of ATP are mediated through the P2Y–PKC–ERK signalling pathway in a $[Ca^{2+}]_i$-independent fashion [240]. Reactive gliosis in retina, induced by intravitreal injection of the proteolytic enzyme dispase, triggered an up-regulation of P2Y receptors and increased Ca^{2+} signalling [241, 242]. Similar up-regulation of P2Y receptors and proportion of cells demonstrating P2Y-induced Ca^{2+} signals was observed in rabbit Müller cells following mechanical detachment of retina from pigmented epithelium [243]. Inhibition of P2 receptors with suramin attenuated reactive gliosis of Müller cells in the same model [244].

Likewise, injection of the P2Y agonist 2-MeSATP into the rat nucleus accumbens triggered astrogliosis manifested by increase in GFAP immunoreactivity and astroglial hypertrophy [245]; these effects were alleviated by the non-selective P2 receptor antagonists PPADS and reactive blue 2. Detailed pharmacological analysis led to a suggestion that purines induced astrogliosis in the nucleus accumbens mostly through activation of $P2Y_1$ and $P2Y_{12}$ receptors [246]; whereas in cortex, $P2Y_1$ receptors take the leading role [177]. The $P2Y_1$ receptors may also be involved in sensitization to D-amphetamine; the latter triggered up-regulation of $P2Y_1$ receptors in vivo, which enhanced ATP-induced astrogliosis [247]. In primary mouse astroglial cultures, stimulation of $P2Y_1$ and $P2Y_6$ receptors led to a $[Ca^{2+}]_i$-dependent activation of nuclear factor of activated T cells, a transcription factor believed to activate astrogliosis [248]. Astroglial $P2Y_2$ receptors are also coupled with αβ3/β5 integrin signalling pathways,

which control cytoskeletal remodelling and motility and are involved in regulation of various stages of astrogliosis [249, 250]. Activation of $P2Y_2$ and $P2Y_4$ receptors can also regulate astrogliosis via extracellular signal-regulated protein kinases (ERK) and signal transducer and activator of transcription 3 (STAT3) signalling cascade [251].

Brain lesions up-regulated P2Y expression in several regions of the brain, including cortex (where stab wound increased the immunoreactivity for $P2Y_{1,2,4,6}$ receptors) and in nucleus accumbens, where lesion increased expression of $P2Y_{1,4}$ proteins and triggered additional expression of $P2Y_{2,6}$ receptors [177].

Metabotropic P2Y receptors are linked to several glioprotective pathways. Activation of $P2Y_2$ receptors was reported to inhibit cell death in astroglial cell line [252]. In hippocampal astroglial cultures, stimulation of $P2Y_1$ receptors protected the cells against hydrogen peroxide-induced oxidative damage [253]. Brief stimulation of P2Y receptors significantly increased resistance of astrocytes isolated from old mice to oxidative stress, this effect being connected with Ca^{2+} release from the ER store [254]. In human glial cell lines, the glioprotective action of ATP was mediated through $P2Y_6$ receptors [255].

Stimulation of recombinant $P2Y_2$ receptors expressed in an astrocytoma cell line activated several cell survival mechanisms, including up-regulation of anti-apoptotic proteins BCL-2 and BCL-x and down-regulation of pro-apoptotic factor BAX; in addition, $P2Y_2$ receptors stimulated expression of various neurotrophins, neuropeptides and growth factors known for their neuroprotective abilities [256].

Oligodendrocytes

Cultured rat OPCs express $P2Y_{1,2,4}$ proteins (Table 3); the ATP-induced $[Ca^{2+}]_i$ elevation, however, is mediated mainly by $P2Y_1$ receptors (as suggested based on sensitivity to the $P2Y_1$ antagonist MRS2179 [257]). Using immunohistochemistry, $P2Y_1$ receptors were also localised in NG-positive glial cells in rat cortical sections [257]. Similarly, $P2Y_1$ immunoreactivity was detected in oligodendrocytes throughout the CNS [258]. Activation of P2Y receptors triggers intracellular Ca^{2+} release in cultured mature (O10 positive) and immature (O4 positive) oligodendrocytes but not in O4-negative precursor cells [165]. In oligodendrocytes in corpus callosum slices, ATP triggered robust $[Ca^{2+}]_i$ transients [165, 259], which originated exclusively through P2Y-activated/$InsP_3$-induced Ca^{2+} release from the ER stores [165]. Similarly, P2Y receptor-mediated ER Ca^{2+} release significantly contributes to Ca^{2+} signalling in oligodendrocytes from the optic nerve [260]. Stimulation of P2Y receptors control OPC migration and maturation in vitro [161].

Conclusions

The purinergic signalling system is, arguably, the main extracellular signalling system that integrates neuronal–glial and glial–glial circuits in the nervous system. Indeed, in the CNS, purines and pyrimidines mediate reciprocative signalling between neurones and astrocytes in the grey matter and between axons and oligodendrocytes in the white matter. In addition, purines and pyrimidines provide for multiple signalling pathways within glial syncytium, being responsible for propagating Ca^{2+} waves and for astroglial–oligodendroglial communications. Furthermore, the purinergic signalling system is intimately involved in neuropathology by mediating reactive astrogliosis, providing for glioprotection in stress conditions and assuming the main responsibility for microglial activation. The extended family of purinoceptors, universally expressed in glial cells, is coupled to numerous signalling cascades governing glial physiological and pathological responses. In-depth understanding of the molecular physiology and pathophysiology of these receptors may further our understanding of the integrative mechanisms within neural circuits and provide new strategies for curing neurological diseases.

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