

Pathophysiology of astroglial purinergic signalling

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Abstract Astrocytes are fundamental for central nervous system (CNS) physiology and are the fulcrum of neurological diseases. Astroglial cells control development of the nervous system, regulate synaptogenesis, maturation, maintenance and plasticity of synapses and are central for nervous system homeostasis. Astroglial reactions determine progression and outcome of many neuropathologies and are critical for regeneration and remodelling of neural circuits following trauma, stroke, ischaemia or neurodegenerative disorders. They secrete multiple neurotransmitters and neurohormones to communicate with neurones, microglia and the vascular walls of capillaries. Signalling through release of ATP is the most widespread mean of communication between astrocytes and other types of neural cells. ATP serves as a fast excitatory neurotransmitter and has pronounced long-term (trophic) roles in cell proliferation, growth, and development. During pathology, ATP is released from damaged cells and acts both as a cytotoxic factor and a proinflammatory mediator, being a universal “danger” signal. In this review, we summarise contemporary knowledge on the role of purinergic receptors (P2Rs) in a variety of diseases in relation to changes of

astrocytic functions and nucleotide signalling. We have focussed on the role of the ionotropic P2X and metabotropic P2YRs working alone or in concert to modify the release of neurotransmitters, to activate signalling cascades and to change the expression levels of ion channels and protein kinases. All these effects are of great importance for the initiation, progression and maintenance of astrogliosis—the conserved and ubiquitous glial defensive reaction to CNS pathologies. We highlighted specific aspects of reactive astrogliosis, especially with respect to the involvement of the P2X₇ and P2Y₁R subtypes. Reactive astrogliosis exerts both beneficial and detrimental effects in a context-specific manner determined by distinct molecular signalling cascades. Understanding the role of purinergic signalling in astrocytes is critical to identifying new therapeutic principles to treat acute and chronic neurological diseases.

Keywords Astroglia · Astrogliosis · ATP · P2X/Y receptors · Neurodegeneration · Pathophysiology · Purinergic signalling

Abbreviations

AA	Arachidonic acid
Aβ	Oligomeric β-amyloid peptide
AC	Adenylate cyclase
AD	Alzheimer’s disease
AKT	Serine-threonine kinase AKT
ALS	Amyotrophic lateral sclerosis
APP	Amyloid precursor protein
ATP	Adenosine 5′-triphosphate
BrdU	5-Bromo-2′-deoxyuridine
[Ca ²⁺] _i	Intracellular free calcium concentration
cAMP	Cyclic adenosine-3′,5′-monophosphate
cGMP	Cyclic guanosine-3′,5′-monophosphate
CNS	Central nervous system
COX	Cyclooxygenase

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DAG	Diacylglycerol
DRG	Dorsal root ganglion
EGF	Epidermal growth factor
EGFP	Enhanced green fluorescent protein
ERK	Extracellular signal regulated protein kinase
FGF	Fibroblast growth factor
GFAP	Glial fibrillary acidic protein
GSK3	Glycogen synthase kinase 3
IL	Interleukin
InsP ₃	Inositol (1,4,5)-trisphosphate
IR	Immunoreactivity
JNK	Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MCAO	Middle cerebral artery occlusion
MS	Multiple sclerosis
NAc	Nucleus accumbens
NGF	Nerve growth factor
NG2	Chondroitin sulphate proteoglycan
NF-κB	Nuclear factor-κB
NO	Nitric oxide
PD	Parkinson's disease
PDGF	Platelet-derived growth factor
PGE ₂	Prostaglandin E ₂
PKC	Protein kinase C
PI3K	Phosphatidylinositol 3-kinase
PL(A ₂)	Phospholipase (A ₂)
PPADS	Pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid
P2R	Purinergic receptor
SAPK	Stress-activated protein kinase
SE	Status epilepticus
STAT3	Signal transducer and activator of transcription 3
TBI	Traumatic brain injury
TNF	Tumor necrosis factor
UTP	Uridine 5'-triphosphate

Introduction

Neuroglia of the central nervous system (CNS) is represented by highly heterogeneous cellular populations generally classified as astrocytes, oligodendrocytes, microglia, and chondroitin sulphate proteoglycan NG2-positive glia (also known as synantocytes; [1]). Adult brain also contains radial Müller cells in the retina and pseudoradial Bergmann glial cells in the cerebellum [2, 3]. Astrocytes, oligodendrocytes and NG2-glial cells are of ectodermal/neural origin, whereas microglial cells of mesodermal origin invade the CNS during perinatal period. Neuroglial cells perform multiple functions being generally responsible for homeostasis of the nervous system at multiple levels including neurogenesis and development of the CNS, defining nervous

system cytoarchitecture, providing metabolic support, controlling molecular homeostasis of the extracellular space and mounting CNS defence. Extracellular adenosine 5'-triphosphate (ATP) plays a complex signalling role in the CNS, and its binding to P2 purinoceptors (P2Rs) can be neuroprotective or neurotoxic in various pathological conditions. In this review, we shall summarise the role of nucleotide signalling in astroglia under physiological and pathophysiological conditions.

The ubiquity of the extracellular signalling molecule ATP and diversity of purinoceptors allow multiple physiological roles (including development and growth) and pathological significance of the purinergic signalling system [4–12]. ATP, released from neurones and glial cells, contributes to synaptic transmission in many brain regions. Physiologically ATP is delivered by exocytosis of ATP-containing vesicles or vesicles co-storing ATP with other neurotransmitters such as glutamate, γ -aminobutyric acid (GABA), noradrenaline or acetylcholine (ACh) [9, 13–17]. Regulated release of ATP is relevant for neuronal–glial communications (for example regulating synaptic plasticity) as well as for glial–glial signalling (for example, by initiating propagating Ca²⁺ waves) [9, 18].

Pathological insults to the CNS, e.g., hypoxia, ischaemia, trauma or epilepsy-associated seizures are accompanied by the rapid increase in concentrations of extracellular purine nucleotides resulting from massive exit of ATP from damaged neural cells; in addition, purines can be released by exocytotic and transporter-mediated processes [19–23]. Increases in extracellular purines may assume cytotoxic proportions resulting in cell death.

The released ATP undergoes rapid and successive enzymatic degradation by ectonucleotidases, producing adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine. Adenosine also acts as a signalling molecule of its own right in the CNS and often has effects opposite to those of ATP [9, 24, 25].

Purine and pyrimidine nucleotides/nucleosides activate several families of purinoceptors, classified as metabotropic P1 adenosine receptors, ionotropic P2XRs and metabotropic P2YRs, which are broadly distributed in both neurones and glial cells; activation of these receptors mediate a remarkable variety of physiological and pathophysiological reactions [5, 11, 26–31]. Ionotropic (cationic) P2XRs are represented by seven subtypes (P2X₁ through P2X₇). Importantly, permeability of P2X₂, P2X₄, and P2X₇Rs to larger cations increases following repetitive or longer lasting exposure to ATP and (particularly in the case of P2X₇Rs) may open large pores in the cell membrane. There are eight metabotropic G-protein-coupled P2YRs, with different ligand preferences (P2Y₁: ADP; P2Y₂: ATP/UTP; P2Y₄: UTP; P2Y₆: UDP; P2Y₁₁: ATP; P2Y₁₂: ADP; P2Y₁₃: ADP and P2Y₁₄: UDP-glucose and other nucleotide sugars).

Astrocytes in the normal brain

Functions of astrocytes

Astrocytes are fundamental for normal brain physiology and, being homeostatic/defensive cellular elements of the brain, are deeply involved in neuropathology, to a very great extent determining the progress and outcome of various neurological diseases [9, 32–37].

Astrocytes have a highly heterogeneous morphological appearance. Main types of astrocytes are represented by (1) protoplasmic astrocytes of grey matter, which possess numerous highly branched fine processes, and (2) the fibrous astrocytes in the white matter, which have less branched and thicker processes. In the cortex, astrocytes occupy strictly delineated domains, within astrocytes form multiple contacts with neuronal structures, whereas astroglial perivascular end-feet envelop CNS blood vessels.

In the grey matter, astroglial perisynaptic processes ensheath synapses, whereas in the white matter astroglial processes terminate at nodes of Ranvier, the sites of action potential generation [37]. Astrocytic processes associated with synapses tightly regulate synapse formation, maintenance and plasticity [38, 39]. For example, in the hippocampus or cortex of rodents, finely branching processes emanating from a single astrocyte are estimated to contact several hundred dendrites from multiple neurones and to envelope 20,000–100,000 synapses [36]. Interest in astroglia function has increased steadily in recent years because of their newly discovered roles in synapse formation, maturation, efficacy and plasticity [40].

Astrocytes express intermediate filament proteins [e.g. glial fibrillary acidic protein (GFAP), vimentin, nestin] depending on their type and developmental stage. There are different isoforms and splice variants of GFAP, including GFAP α , β , γ , δ , and κ , which are expressed in a heterogeneous manner in healthy CNS and in pathological tissues including gliomas [36]. GFAP and vimentin, together with microtubules and actin filaments, constitute the cytoskeleton of astrocytes. Expression of GFAP is often up-regulated in pathophysiological stages; this up-regulation is generally considered as hallmark of reactive astrogliosis, the latter being evolutionary conserved and ubiquitous defensive reaction of astroglia [36, 41]. Some examples of astroglial activation after mechanical injury *in vitro* and *in vivo* are given in Fig. 1.

Astrocytes assume numerous and well-defined supportive functions, including structural support, providing for neurovascular coupling, regulation of extracellular K^+ , uptake of neurotransmitters, metabolic support of neurones, etc. Astroglia integrate glia, neurones and brain vasculature into functional networks. Pathological potential of astrocytes is similarly manifold, including the formation of neuroprotective scars following injury, homeostasis preservation, blood–brain

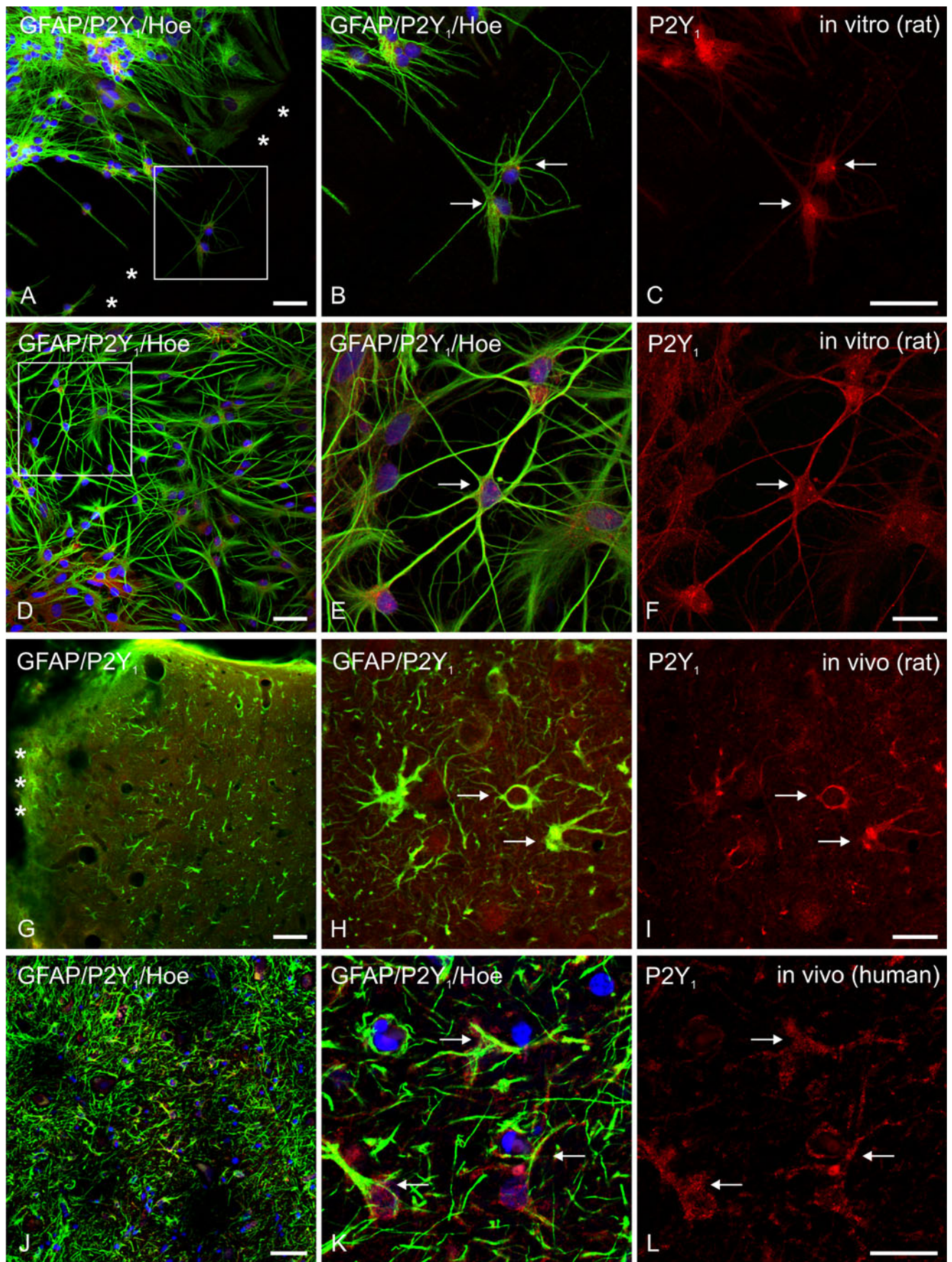
barrier maintenance and repair, axon guidance during regeneration as well as responses to neuronal activity and plasticity. Astrocytes are able to sense and modulate neuronal environment and to actively remodel themselves to meet constantly changing neuronal demands (e.g. [16, 42, 43]).

Astrocytes are also central for energy consumption, neurotransmitter release and reactive oxygen species (ROS) production in the nervous tissue. Astroglia regulate (1) the uptake of glucose and the release of lactate (“astrocyte-neurone-lactate shuttle”), (2) the uptake of glutamate and the release of glutamine (“glutamate-glutamine cycle” maintaining both glutamatergic and GABAergic synaptic transmissions) and (3) the uptake of glutathione precursors and the release of glutathione [42–45]. Glutamate, released into the synaptic cleft during neuronal activity, is rapidly removed by surrounding astrocytes, which convert glutamate to glutamine (using the enzyme glutamine synthase) and release glutamine into the interstitial space for uptake by neurones [46]. The uptake of synaptic glutamate by astroglia is the major mechanism preventing accumulation of glutamate in the synaptic space and thus protecting neurones from excessive activation and excitotoxic cell death [42]. At the same time, glutamate–glutamine shuttle is the only pathway allowing neurones to replenish their stores of glutamate and GABA.

Nucleotide-induced modulation of astrocytic function

Astrocytes are arguably the main source for the physiologically released ATP in the CNS [47]. They release ATP to communicate with each other and with other types of glia by stimulating purinoceptors [9, 10, 17, 18, 48]. Astroglial ATP and its metabolite adenosine activate purinoceptors on neurones, microglia, oligodendrocytes and blood vessels [37]. Generally, ATP and other nucleotides are taken up by and stored in secretory and synaptic vesicles. Accumulation of ATP into neuronal synaptic vesicles is mediated by a Cl^- -dependent vesicular nucleotide transporter expressed in astroglia [49, 50]. Conceptually, ATP can be released from astrocytes in several ways by (1) Ca^{2+} -dependent exocytosis [14, 15, 51, 52], (2) from lysosomes [53], and (3) through hemichannels formed by connexins and pannexins (note activation by ATP of pannexin-1 channels and involvement of P2YRs [54]; P2X₇Rs [55], as well as connexin-43 channels and involvement of P2YRs [56, 57]). In addition, plasmalemmal voltage-dependent anion channels and P2X₇Rs can function as exit pathways for glutamate or ATP release [9, 14, 37, 58].

Astrocytes communicate with each other via intercellular “calcium waves” [9, 16, 18, 59–63]. These cells respond to a wide range of physiological and pathophysiological stimuli with increases in cytosolic Ca^{2+} ($[Ca^{2+}]_i$) mainly mediated through G_q protein-coupled receptors, including those of the P2Y type, with subsequent release of Ca^{2+} from intracellular



◀ **Fig. 1** From astroglial cell cultures to the human brain—up-regulation of P2Y₁R expression on activated GFAP-astrocytes. Confocal images of double immunofluorescence to characterise the expression of P2Y₁R subtypes on activated astrocytes after mechanical injury in **A–F** in vitro, **G–I** in vivo in rat brain and **J–L** in vivo in human brain. Under physiological conditions (data not shown) in rat and human tissue the expression of P2Y₁R on single astrocytes was observed, but with low intensity. In vitro (*primary cultures of rat cortical astrocytes*): **A, D** overview about GFAP-positive astrocytes after mechanical injury (marked by *asterisks* in **A**; **A** near the lesion, **D** far away from the lesion). **B, C** Single outgrowing astrocytes in the lesioned area with clear co-expression of the astrocytic marker GFAP with the P2Y₁R subtype (*arrows*); **D** a high number of GFAP/P2Y₁R-positive astrocytes at sites remote from the lesion; **E, F** one example of the colocalisation in higher magnification. In vivo (*rat brain, cortex, perilesion area*): **G** overview about the perilesion area after mechanical injury (stab wound injury; according [100]; marked by *asterisks*) in rat cortex. **H, I** Single activated GFAP-positive astrocytes in higher magnification with clear co-expression with the P2Y₁R subtype (*arrows*). In vivo (*human brain; removed at autopsy, pericontusion zone, cortex*): **J–L** After traumatic brain injury (**J**, overview, pericontusion zone), the P2Y₁R was found on activated GFAP-positive astrocytes (**K, L**; *arrows*) in the pericontusion zone around the injured area (K. Bremicker, M. Weber and H. Franke, unpublished data)

stores through inositol 1,4,5-trisphosphate (InsP₃) receptors. Propagation of Ca²⁺ waves results from the intercellular diffusion of InsP₃ through gap junctions or from the release of ATP in a single cell, this release then producing [Ca²⁺]_i transients in the neighbouring cell by activation of P2YR/InsP₃ signalling systems [47, 64, 65].

Astrocytes also release several non-nucleotide neurotransmitters, including adenosine, glutamate and D-serine, to stimulate their respective receptors at neighbouring neurones and glial cells [66–69]. Ca²⁺ waves underlie communication in astrocytic networks, being the substrate of glial excitability.

In the CNS, the synapses are generally organised according to “tripartite” architecture, according to which the synapse is composed of the presynaptic neuronal terminal, the postsynaptic membranes of dendrites or neuronal somata, and of the astrocytic perisynaptic process enwrapping neuronal structures. Within the “tripartite” synapse, ATP released during synaptic transmission activates astrocytic receptors, which initiate Ca²⁺ signals and Ca²⁺ waves in the astroglial networks. Astroglial Ca²⁺ signals in turn induce the release of ATP, which may feed back to neurones via activation of pre-, postsynaptic and extrasynaptic P1 and P2Rs [9, 70–74].

P2 receptors at astrocytes

Astrocytic P2X and P2Y receptors

Astrocytes express various types of functional P2X/Y purinoceptors (e.g. [4, 5, 37, 75–87]). Nucleotide signalling mediated by these receptors is of significance for both physiological regulation and pathophysiological processes

in the nervous system [6, 88, 89]. Ionotropic P2XRs are responsible for rapid astrocytic signalling, whereas G-protein-coupled P2YRs mediate long-term effects, including trophic responses through a variety of intracellular pathways including gene activation [7, 12, 90].

Glial P2X₇Rs are of special importance, since these receptors are involved in initiation of apoptosis [91–93], and act as “emergency” receptors aimed at restraining the excessive astrogliosis triggered by brain injuries [94, 95]. It is noteworthy that ionotropic P2X₇Rs, through their ability to open large pores in the cell membranes, are able to damage the cytoskeleton; they appear also to trigger necrotic/apoptotic death of neurones and glia by regulating the processing and release of interleukin-1β (IL-1β), a key mediator in neurodegeneration, chronic inflammation and chronic pain [96, 97].

Recently P2X₇R-mediated currents were identified in cultured rodent cortical astrocytes [58, 85] and in astrocytes patch-clamped in acute brain slices of rats and mice [86]. The discovery of functional P2X₇Rs in astrocytes of the prefrontal cortex and hippocampus is somewhat at odds to the previously reported failure of ATP and BzATP to activate ion currents in astrocytes in hippocampal slices of transgenic mice that express enhanced green fluorescent protein (EGFP) under the control of the human glial fibrillary acidic promoter [Tg(GFAP/EGFP)] [98]. There are at least two possible reasons for this discrepancy. First, in contrast to the prefrontal cortex, the BzATP-induced astrocytic currents in the hippocampus are minuscule, when measured in a normal Ca²⁺/Mg²⁺-containing external medium [86], although they can be greatly increased by exchanging the normal medium to one which contains a low Ca²⁺ concentration with no added Mg²⁺. Second, the BzATP responses are also surprisingly very small in cortical astrocytes of Tg(GFAP/EGFP) mice and might have therefore interfered with their identification in the hippocampus. These results are in agreement with the reported data that both BzATP and ATP activate mouse recombinant P2X₇R with much lower potencies than the corresponding rat receptor [99].

The presence of multiple P2X and P2Y receptors at the same astrocyte may lead to an abundance of effects, the common denominator arguably being an increase in [Ca²⁺]_i, which may trigger further second messenger pathways resulting in the neurodegenerative or proliferative reactions [80, 100, 101].

Interaction of astrocytic P2 receptors with other receptors

P2YRs may assemble as homodimers or heterodimers with other P2YRs or with other G protein-coupled transmitter receptors in glia and neurones [31, 102]. For example, adenosine A₁ and nucleotide P2Y₁Rs aggregate to form a multimeric complex in human astrocytes and the regulation

of A₁R function is induced by P2Y₁R activation [103]. A high level of co-localisation between A₁Rs and P2Y₁Rs at glutamatergic synapses and surrounding astrocytes and the functional interaction between these receptors in the hippocampus have been demonstrated [104]. P2Y₁R stimulation impaired the potency of A₁R coupling to G proteins, whereas the stimulation of A₁Rs increased the functional responsiveness of P2Y₁Rs. The interaction between A₁ and P2Y₁Rs is important for nucleotide signalling in astrocytes because it is involved in cell-to-cell communication and in the control of synaptic transmission, particularly during pathological conditions, when large amounts of purines are released [103]. In hippocampal astrocytes, P2Y₁ and P2Y₂R-mediated Ca²⁺ responses exhibit two forms of activity-dependent negative feedback mechanisms on synaptic transmission via the phospholipase (PL)Cβ-InsP₃ pathway [79].

P2YRs also interact with the ionotropic P2XRs either directly or indirectly. P2Y₂ and P2X₇R stimulation of protein kinase D (PKD) activation and localisation in primary rat cerebellar astrocytes was demonstrated [105], and the contribution of calcium-permeable heteromeric P2X_{1/5}R channels to the excitability of astrocytes appeared to be modulated by PI(4,5)P(2) through the P2X₁ lipid-binding domain [106].

Astrocytes in the injured CNS

Reactive gliosis and purinergic modulation

Astrogliosis is fundamental for regeneration following lesioning of the brain. In vitro and in vivo experiments corroborated the important role of nucleotides in the reactive responses of astrocytes to brain injury because purinoceptors regulate morphological remodelling, proliferation, up-regulation of GFAP synthesis as well as chemotaxis and chemokinesis in activated astrocytes.

Different kinds of astrogliosis have been described [44], which are classified as follows: (1) anisomorphic astrogliosis—often observed as an encapsulation of the damaged CNS region after neurotrauma; it is characterised by disappearance of astroglial domain organisation and the formation of a dense plexus or wall; (2) isomorphic astrogliosis—characterised by astroglial hypertrophy with preserved domain organisation and is often reversible and beneficial for post-traumatic regeneration.

Various glial cells and cellular signals are involved in the formation of the glial scar representing the end stage of anisomorphic gliosis. Within the first few hours after injury, neuronal and glial cells undergo cell death at the injury site, with a concurrent recruitment of CNS microglia and peripheral monocytes (including macrophages [107]). Astrocytes migrate from the adjacent undamaged parenchyma towards

the injured cells and start the repair, for example by protruding processes into the injury core [108–111].

Immediately after ischaemic or traumatic damage to the brain, extracellular ATP concentrations markedly increase not only because of lack of efficient metabolism by degrading enzymes but also due to active release from surviving cells [21, 23, 112]. One of the major hallmarks of nucleotide-induced reactive astrogliosis is that astrocytes react to ATP with hypertrophy, swelling of the cell body and main processes, and proliferation that ultimately result in full blown astroglial phenotype with subsequent formation of the glial scar [88, 113, 114]. Astrocytes react to nucleotides with up-regulation of the GFAP and vimentin. In cultured astrocytes, it was repeatedly shown that extracellular ATP and its structural analogues increase GFAP content and DNA synthesis [115, 116]. Morphological remodelling (e.g. “stellation” or an increase in the mean length of processes of GFAP positive cells; see also examples given in Fig. 1A, B) was also described in vitro [115, 117–119].

Within 3–5 days after injury, hypertrophic and elongated astrocytes at the injury border express high levels of vimentin and brain lipid-binding protein (reviewed in [35]). The mitogenic and morphogenic responses to extracellular ATP in cultured astrocytes have been well documented [88, 101, 113, 120–124]. Proliferation of reactive astrocytes, as indicated by bromodeoxyuridine (BrdU) incorporation or the expression of Ki67, is stimulated by ATP and P2R agonists in vivo and in vitro (e.g. [125–128]). The total number of astrocytes (identified e.g. by GFAP or S100β labelling) increases over time and in correlation with the expression of additional nucleotide receptors (e.g. [23, 129]).

Although it has been shown that nestin-positive progenitor cells differentiate into astrocytes at the site of injury [44], the extent to which these cells contribute to the formation of the astroglial scar remains open. Similarly, we do not know the intrinsic and extrinsic mechanisms by which mature astrocytes contribute to the formation of precursor cells in the process of reactive gliosis [35].

Mediators of reactive gliosis

Astrocytes exhibit both regional and local variability that is dynamically regulated by a large number of inter- and intracellular signalling molecules. Acute brain injury is a complex phenomenon and ATP is only one of many endogenous factors determining reactive astrogliosis (acting alone or in concert with other factors).

There are many potential intercellular signalling molecules that are able to trigger reactive astrogliosis or to regulate specific aspects of it; these molecules are large polypeptide growth factors and cytokines [such as interleukins (IL-1, IL-6, and IL-10), ciliary neurotrophic factor (CNTF), tumour necrosis factor-α (TNF-α), interferon-γ

(INF γ), transforming growth factor- β (TGF β), fibroblast growth factor-2 (FGF2), leukaemia inhibitory factor (LIF), etc.), mediators of innate immunity (e.g. lipopolysaccharide [LPS], other Toll-like receptor ligands), neurotransmitters (e.g. ATP, glutamate, noradrenaline), ROS, nitric oxide (NO), factors released in consequence of hypoxia and glucose deprivation [e.g. monocyte chemoattractant protein-1/chemokine ligand 2 (MCP-1/CCL2) and interferon-inducible protein-10/chemokine ligand 10 (IP-10/CXCL10)], products associated with neurodegeneration (e.g. β -amyloid) and eventually molecules associated with systemic metabolic toxicity or regulators of cell proliferation (e.g. endothelin-1) [36, 44, 101, 130–132]. Purines interact with these factors in complex manners.

It has been suggested that under pathological conditions, such as chronic inflammation and neurodegeneration, astroglia switch their phenotypes from metabolically supportive cells to immunocompetent cells capable of inducing inflammation via the production of a variety of proinflammatory factors [44]. ATP release during neuronal excitation or injury can enhance the inflammatory effects of cytokines (IL-1 β , TNF- α , and IFN- γ) and prostaglandin E₂ (PGE₂) in cultures of astrocytes and may contribute to chronic inflammation [133, 134].

It has been demonstrated that the selective stimulation of astroglial P2Y₁Rs is involved in the secretion of glutamate (e.g. [68, 135]) and the regulation of several cytokines/chemokines (for example, IL-6 [136], glial cell line-derived neurotrophic factors (GDNF) [137], IL-6, TNF- α , monocyte chemoattractant protein-1/chemokine (MCP-1/CCL2) and IP-10/CXCL10 messenger RNA (mRNA) expression [132]).

ATP can act in combination with fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF) as well as nerve growth factor (NGF) to stimulate astrocytic proliferation, contributing to the process of reactive astrogliosis and to hypertrophic/hyperplastic responses [138]. ATP released from damaged cells and acting via P2YRs synergistically enhances the proliferative effects of FGF2, whereas P2XRs inhibit the ability of FGF2 to stimulate DNA synthesis in rat cortical astrocyte cultures [139]. Opposing effects of P2X and P2YRs on the ability of FGF2 to induce proliferation in primary cultures of rat cortical astrocytes were found. Low concentrations of ATP enhanced DNA synthesis induced by FGF2, whereas high concentrations inhibited FGF2-induced proliferation [140]. It was concluded that P2YRs enhance proliferation caused by FGF2 in astrocytes, whereas stimulation of P2X₇Rs inhibits proliferation by shifting cells to a state of reversible growth arrest that may be mediated by protein kinase signalling [140].

Trauma-induced activation of P2Y₄Rs in astrocytes stimulates synthesis and release of thrombospondin-1, an extracellular matrix molecule that induces synapse formation

during development and might have a role in CNS repair and remodelling after injury [141]. ATP stimulates N-cadherin expression, which is a Ca²⁺-dependent cell-adhesion molecule involved in glia–glia and axon–glia interactions [142]. Chondroitin sulphate proteoglycans [143], tenascin-R [144], keratin, vimentin [145] and Eph receptor tyrosine kinase [146] are among the repellent proteins expressed by reactive astrocytes.

Functional consequences of reactive astrogliosis

The glial scar demarcates the healthy brain from the wounded area. In fact, recent experimental evidence indicates that astroglial scars act as neuroprotective barriers to inflammatory cells and infectious agents and that they are formed in particular along borders to severe tissue damage, necrosis, and infection or autoimmune-triggered inflammatory infiltration [36]. However, such a demarcation has also the consequence that reparative processes such as axonal outgrowth are inhibited, and therefore, either beneficial or detrimental outcomes might be favoured [113, 114].

Conceptually the glial scar protects CNS tissue by (1) uptake of potentially excitotoxic glutamate, (2) by counteracting oxidative stress via glutathione production, (3) by the release of adenosine, (4) by the degradation of amyloid- β (A β) peptides, (5) by the facilitation of blood brain barrier repair, (6) by the reduction of vasogenic oedema after trauma, stroke or obstructive hydrocephalus, (7) by the stabilisation of extracellular fluid and ion balance and (7) by the limitation of the spread of inflammatory cells or infectious agents from the areas of damage or disease into healthy CNS parenchyma (e.g. [36, 43, 45, 130]). At the same time, in spite of these multiple protective functions, reactive astrogliosis and scar formation might delay or inhibit regenerative responses; therefore, this astrocytic reaction may play an important role in the pathogenesis and progression of diverse neuropathological conditions.

In chronic neuropathologies, such as for example Alzheimer's disease (AD), reactive astrocytes contribute and reinforce inflammatory cascades [45]. The inflammatory response occurs mainly around the amyloid plaques and is characterised by the release of proinflammatory substances from activated microglia and astroglia. The evolving general concept assumes that in the early stages of injury reactive astrocytes are needed to limit tissue damage including scar formation, which provides beneficial effects in shielding the brain, whereas the consequences of persistent reactive astrogliosis can be harmful.

ATP released by astrocytes mediates activation of microglia [147]. The functions of reactive astrocytes are partially overlapping with those of reactive microglia, the latter being specialised in phagocytosis and integration of inflammatory responses [35]. Microglial activation is essential for phagocytosis of cellular debris following injury and is important

for the immunoprotection of the CNS [148]. ATP may act as a chemoattractant for microglia, directing them to a site of injury [96]. ATP released from activated astrocytes stimulates microglia as well, and microglial cells encounter high levels of ATP near dying and disintegrating cells. Collectively, these observations indicate that ATP acts on microglia through P2X₇R, which serve as an important component of a neuro-inflammatory response. Additionally, astroglial death induced by ATP-mediated microglial activation may be an important pathophysiological pathway in epileptogenesis [149]. It has been speculated that antagonists of the P2X₇R could thus have therapeutic relevance in the treatment of AD, cerebral ischaemia and neuroinflammatory conditions by regulating pathologically activated glial cells [96].

Finally, microglia express multiple P2X/YRs, of which the P2X₄, P2Y₆ and P2Y₁₂R subtypes play important roles in the pathophysiology of microglial cells after acute injury or neurodegenerative disorders, contributing to the nucleotide responses [e.g. 31, 150, 151].

Astroglia and microglia may also exert opposite effects. Recent findings indicate that microglia control astroglial proliferation by preventing the proliferative response to ATP/ADP and favouring an inhibitory effect of UTP/UDP. Several microglial P2YRs may be involved by inducing the release of messengers that restrain astrogliosis [152].

P2 nucleotide receptors and signalling cascades

Intracellular signalling cascades: the P2X (P2X₇) receptor

Astroglial P2XRs can be activated by synaptic transmission and can mediate fast local signalling through the elevation of cytoplasmic Ca²⁺ and Na⁺ concentrations. A number of functional roles of astroglial P2X₇Rs might be related to Ca²⁺ signalling (e.g. [91–93, 153]) and secretory activities of astrocytes [46, 154], which are promoting the further release of purines [91] via the receptor channel itself or an accessory protein. ATP-stimulated glutamate [46, 58] and GABA release [155] may also occur [96]. These signals can be translated into various physiological messages by numerous pathways, including release of neurotransmitters, metabolic support of neurones and regulation of activity of postsynaptic glutamate and GABA_ARs [10, 17]. P2XRs can interact with cell-adhesion molecules, physically cross-talk with other receptor channels such as nicotinic acetylcholine, GABA_A or 5-HT₃ receptor-channels, or interact with G protein-coupled receptors. Activation of P2XRs can also trigger intracellular trophic signalling pathways [12].

The P2X₇R activates a particularly wide range of signal cues (Table 1 and Fig. 2). By virtue of its extended C terminus, it undergoes multiple protein interactions forming a signalling complex. P2X₇Rs activate several second messenger and enzyme cascades, including extracellular signal-regulated protein

kinases (ERKs), serine-threonine kinase Akt (Akt), c-Jun N terminal kinases (JNKs) and p38 kinase. The stimulation of this receptor also increases protein tyrosine phosphorylation, ultimately leading to mitogen-activated protein kinase (MAPK) pathway initiation. In addition, by dilation of the channel pore, P2X₇Rs can induce a collapse of ion gradients over the plasma membrane leading to cell death (see above [96, 156, 157]).

P2X₇Rs differentially modulate hippocampal and cortical astrocytes for example with respect to pore opening, p38 MAPK activation, caspase-1 activation and pro-IL-1 β release [158]. These results open the possibility that activation of P2X₇Rs influences the neuroinflammatory processes in distinct brain regions in different ways.

Recent studies identified several splice variants of the P2X₇R that may account for the differential outcome of P2X₇R activation in various experimental settings [159]. The splice variant P2X₇A relates to pore formation and cell death, whereas the P2X₇B variant does not induce plasma membrane permeabilisation and stimulates cell growth. All these data give evidence in favour of the versatility and plasticity of the P2X₇R, and its ability to adopt different dispositions and conformations to elaborate particular actions when expressed natively in different cell types and tissues [160].

Intercellular signalling cascades: the P2Y (P2Y₁) receptor

Four main heterotrimeric G protein subfamilies have been hitherto characterised (G_s, G_{i/o}, G_{q/11} and G_{12/13}) and individual P2YR subtypes may be linked to one or more of them [27, 30, 31, 102, 157]. The P2Y₁, P2Y₂, P2Y₄, P2Y₆ and P2Y₁₁Rs couple to G_q proteins stimulating membrane-bound phospholipase C (PLC), which then cleaves phosphatidylinositol-bisphosphate (PIP₂) in the membrane into two second messengers InsP₃ and diacylglycerol (DAG). InsP₃ mobilises [Ca²⁺]_i, while DAG activates protein kinase C (PKC), leading to various other intracellular events, [27, 78, 157, 161]. P2Y₁₂, P2Y₁₃ and P2Y₁₄Rs bind preferentially to the G_{i/o} subunit. G_i activation is classically associated with the inhibition of adenylylate cyclase (AC) and reduce intracellular levels of cyclic adenosine-3',5'-monophosphate (cAMP). The activation of the G_{i/o} subunit may also have other consequences [27, 31, 157].

P2YRs in astrocytes are linked to a variety of signal transduction mechanisms (Table 2 and Fig. 3). This receptor family can activate (1) the MAP kinase pathway, an obligatory step for the triggering and/or persistence of reactive astrogliosis, (2) the phosphoinositide 3-kinase (PI3K)/Akt-pathway, associated with cell growth, fibre regeneration, and inhibition of apoptosis and (3) the PKC pathway.

MAP kinases are a large protein family consisting of ERKs, the stress-activated protein kinases (SAPKs), the JNKs and p38/MAPKs, which are activated consecutively. The role of MAPK/ERKs is implicated in cell growth and

Table 1 Examples of P2X receptor mediated signalling cascades in astrocytes

P2XR subtype	Preparation/Model	Stimulation	Purinergic signalling/Transduction signals	References
P2X ₇ /Y	Primary cultures of astrocytes (rat, cortex)	ATP, bFGF	↑MAPK activity, cAMP, (Ras/Raf kinase)	[296]
P2X ₇ /Y	Primary cultures of astrocytes (rat, cortex)	ATP	ATP stimulated AP-1 DNA binding activity; PKC, c-Fos	[164]
P2X ₇ /Y	Optic nerves from juvenile rats	ATP, UTP, ADP, αβmeATP	reactive astrocytes, Ca ²⁺ entry	[297]
P2X ₇ /Y (P2X ₇)	Primary cultures of astrocytes (neonatal rat)	BzATP, UTP, FGF2	BzATP-induced increase in p38/MAPK and SAPK/JNK; FGF2-induced proliferation	[140]
P2X ₇	Primary cultures of astrocytes (rat, hippocampus; cortex)	BzATP	activation of p38 (MAPK)	[158]
P2X ₇	Primary cultures of astrocytes	BzATP	↑Ca ²⁺ levels, Lucifer Yellow permeation	[91]
P2X ₇	Rat brain-derived type 2 astrocyte cell line (RBA-2)	e.g. BzATP	PKC regulated PLD activation	[153]
P2X ₇	Primary cultures of astrocytes (Sprague–Dawley rat, hippocampus)	e.g. BzATP	ERK activation, ↑MCP-1 expression	[169]
P2X ₇	Rat brain-derived type 2 astrocyte cell line (RBA-2)	e.g. BzATP	PKC-γ, Ca ²⁺ signalling	[298]
P2X ₇	Primary cultures of astrocytes (Wistar rat, cortex)	ATP, BzATP, LPS	↑iNOS-NO expression	[299]
P2X ₇ (P2X ₇ /Y)	Primary cultures of astrocytes (rat, cortex)	ATP, BzATP, LPS	TNF-α increase	[300]
P2X ₇	Primary cultures of astrocytes (rat, cortex); 1321N1 astrocytoma cells (P2X ₇ R transfected)	UTP, BzATP	PI3K/Akt-pathway	[173]
P2X ₇	Human 1321N1 astrocytoma cells	BzATP	ERK1/2 phosphorylation	[301]
P2X ₇	Rat brain-derived type 2 astrocyte cell line (RBA-2)	ATP, BzATP	PLD activation (PKC-dependent/-independent)	[302]
P2X ₇ (P2X ₄)	Rat brain-derived type 2 astrocyte cell line (RBA-2)	ATP, BzATP	TGF-β1 mRNA expression; ERK1/2 activation; PKC/MAPK	[303]
P2X ₇	Primary cultures of astrocytes (mouse, hippocampus)	ATP, BzATP	ATP-mediated sAPPα release, ERK1/2- and JNK signalling,	[221]
P2X ₇	Human foetal astrocytes	BzATP	IL-1-induced NOS; ↑NO	[237]
P2X ₇	Primary cultures of astrocytes (rat, cortex)	BzATP, AA	phosphorylation of MAPK ERK1/2, JNK and p38	[304]
P2X ₇ /P2Y ₂	Primary cultures of astrocytes (rat, cerebellum)	BzATP, UTP	PKD(1/2) activation, PI-PLC, PLD	[105]
P2X ₇ /P2Y _{1,2,4}	Human foetal astrocyte cultures	ATP, UTP, BzATP	IL-1β-signalling, NF-κB- and AP-1 activation	[133]
Different kinds of injury				
P2X ₇ , P2Y _{1,2,4}	Primary cultures of astrocytes (rat, cortex)	UTP, BzATP; mechanical strain-induced trauma	astrogliosis, phosphorylation of serine 9 on GSK3β, ↓GSK3 activity	[183]
P2X ₇	Primary cultures of astrocytes (rat, spinal cord)	Amyotrophic lateral sclerosis (ALS)	SOD1G93A, increased ATP-dependent proliferation	[245]
P2X ₇	Primary cultures of astrocytes (rat, cortex)	Ischemia	opening Panx1 hemichannels is regulated by the activity of P2X ₇ R; negative-feedback regulation of ATP release	[55]
P2X ₇	Rat brain (Sprague–Dawley rat; dentate gyrus)	Status epilepticus (SE) (pilocarpine)	effects of PPADS, suramin on astroglial cell death	[149]
P2X ₇ /Y	Rat brain (cortex)	Ischemia (MCAO)	PPADS-reduced cell death (TUNEL, neurons and astrocytes) and infarct volume	[202]

MCP-1 monocyte chemoattractant protein-1, SOD1G93A superoxide dismutase 1, TUNEL terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labelling

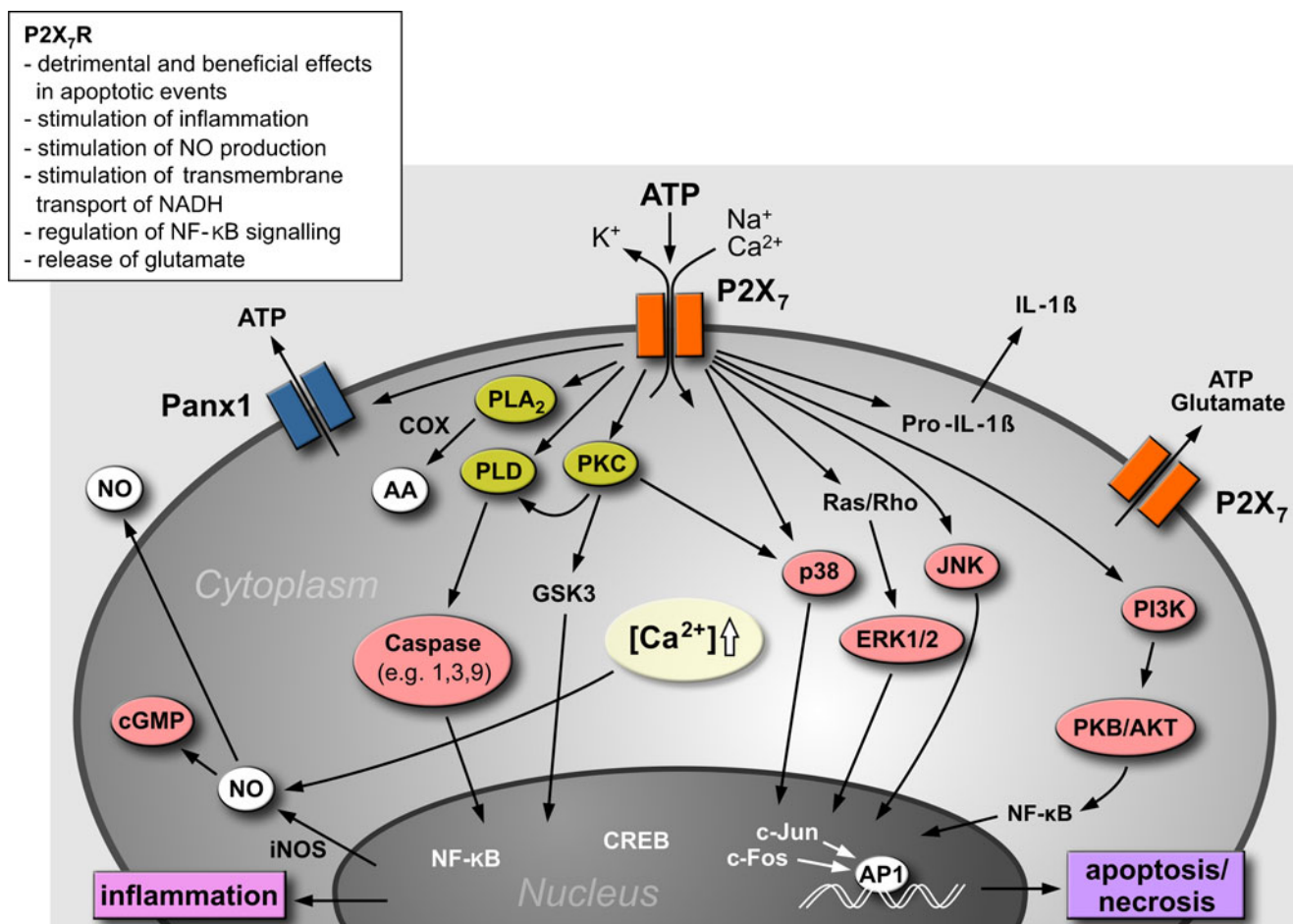


Fig. 2 Schematic illustration of examples of signal transduction pathways in astroglial cells following P2X₇R activation. After channel opening the P2X₇R is permeable for Na⁺, K⁺ and Ca²⁺. Activation of the P2X₇R triggers the efflux of K⁺ from cells and activates IL-1 converting enzyme, leading to cleavage of pro-IL-1β to mature IL-1β and release from the cell. Many events downstream of P2X₇R activation are dependent on extracellular calcium influx. Stimulation of ionotropic P2X₇Rs leads to activation of phospholipases A₂ and D (PLA₂, D) and protein kinase C (PKC), e.g. resulting in the activation of glycogen synthase kinase 3 (GSK3) or the activation of caspase cascades. Furthermore, the induction of second messenger and enzyme cascades promoted e.g. the activation of mitogen activated protein kinase (MAPK) pathway proteins (ERK1/2), p38 MAPK, and c-Jun

N-terminal kinase (JNK) as well as PI3K/Akt activation. The activity of transcription factors, such as nuclear factor κB (NF-κB), cyclic element-binding protein (CREB), and activator protein (AP-1) are also up-regulated, leading to the expression of proinflammatory genes, such as cyclooxygenase-2 (COX-2) or inducible nitric oxide synthase (iNOS); this in turn causes the production of arachidonic acid (AA) or nitric oxide (NO), respectively. Finally, the release of ATP via pannexin-1 (Panx1) hemichannels as well as of ATP and glutamate via P2X₇Rs was also found to take place. The present data suggest that astroglial P2X₇R stimulation is associated with neurological disorders leading to neuroinflammation, and apoptosis. The *inset* summarises examples of P2X₇R mediated effects in astrocytes (artwork by courtesy of Dr. Jens Grosche)

differentiation, whereas SAPKs and p38 appear to regulate the cell death machinery. The importance of the ERK-cascade in mitogenic signalling by P2YRs in cultured astrocytes has been demonstrated repeatedly [162]. MAPKs are activated by phosphorylation at threonine and tyrosine residues [163] and phosphorylate themselves transcription factors, which control early gene expression, such as that of c-Fos [139, 164].

The involvement of MAPK/ERK in P2YR-mediated astrogliosis [165, 166], as well as caspase-3 activation and subsequent apoptosis [22, 167], have been described *in vitro*. The proliferation of astrocytes induced by traumatic injury *in vivo* and their modulation by pharmacological ligands suggest the

involvement of the MAPK/ERK1/2- and PI3K/Akt-pathways in astroglial proliferation also *in vivo* [129].

ATP released by stretch-induced injury *in vitro* triggers ERK activation in astrocytic response to brain trauma [166]. Studies with P2R antagonists indicated a role for P2X₂ and P2Y₁Rs in injury-induced ERK-activation. P2Y₂ [168] and P2X₇Rs [169] are coupled to astroglial ERK. Sustained ERK1/2-activation is often associated with neuronal death, whereas acute ERK1/2-activation is typically a defence mechanism of cells in response to injury [170]. Stab wound and P2R stimulation by adenosine-5'-O-(2-thiodiphosphate) (ADPβS) caused ERK1/2-phosphorylation, which was inhibited by

Table 2 Examples of P2Y receptor mediated signalling cascades in astrocytes

P2YR subtype	Preparation/model	Stimulation	Purinergic signalling/Transduction signals	References
P2(X)/Y	Neuron-glia culture (rat, corpus striatum)	$\alpha\beta\text{meATP}$	astroglial proliferation, BrdU	[125]
P2U, P2Y	Primary astrocytes (rat cortex); <i>Xenopus</i> oocytes	ATP, UTP, $\alpha\beta\text{meATP}$, 2MeSATP	$[\text{Ca}^{2+}]_i$, MAPK	[305]
P2X/Y	Primary cultures of astrocytes (rat, cortex)	P2R agonists	Ca^{2+} release	[92]
P2Y	Primary cultures of astrocytes (rat, cortex)	ATP, FGF2	proliferation, ERK, MAPK, Raf	[124]
P2Y	Primary cultures of astrocytes (Wistar rat, dorsal horn)	ATP	Ca^{2+} wave propagation	[80]
P2Y	Primary cultures of astrocytes (rat, striatum)	ATP, UTP, $\alpha\beta\text{meATP}$, 2MeSATP	PLA ₂ activation, AA release, c-Fos, c-Jun	[178]
P2Y	Primary cultures of astrocytes (rat, striatum)	ATP, UTP, $\alpha\beta\text{meATP}$, 2MeSATP, ADP βS	PLC activation, Ca^{2+} release	[306]
P2Y	Primary cultures of astrocytes (Fischer rat, cortex)	ATP	PI-PLC/ Ca^{2+} and ERK pathways, Ca^{2+} -independent PKC isoform, PLD	[162]
P2Y	Primary cultures of astrocytes (rat, striatum)	$\alpha, \beta\text{meATP}$	astroglial proliferation, COX-2 and PGE ₂ activation	[188]
P2Y	Primary cultures of astrocytes (rat, striatum, cortex)	ATP, $\alpha\beta\text{meATP}$	COX-2 activation, astroglial proliferation	[119]
P2Y	Primary cultures of astrocytes (rat, cortex)	$\alpha\beta\text{meATP}$	astroglial proliferation, ERK1/2-mediated COX-2 induction	[165]
P2Y ₍₁₎	Astrocyte cultures (rat)	ADP βS	induction of c-Fos, c-Jun, junB, TIS11mRNA; cAMP accumulation, PKC	[307]
P2Y ₁	Dorsal spinal cord astrocytes; 1321N1 astrocytoma cells	P2R agonists	propagation of intercellular Ca^{2+} waves	[308]
P2Y _{1,2}	Primary cultures of astrocytes (rat, cerebellum)	2MeSATP, UTP, ATP	AA release, PKC, MAPK, cPLA ₂	[309]
P2Y ₂	321N1 astrocytoma cells	e.g. UTP	PKC-dependent mechanism, MAPK; metalloprotease activation	[216]
P2Y ₂	1321N1 astrocytoma cells	e.g. UTP	Src activation, EGFR	[310]
P2Y _{2,4} , P2Y ₍₁₎	Primary cultures of astrocytes (rat, cortex)	e.g. ATP, UTP, P2Y _{2,4,6R} agonists	proliferation, astroglial proliferation, STAT3 signalling	[186]
P2Y ₂	1321N1 astrocytoma cells (recombinant P2Y _{2R})	UTP	proliferation, bcl-2, bcl-xl, bax, CREB phosphorylation; PI3K/Akt	[311]
P2Y ₂	Primary cultures of astrocytes (rat, cortex)	ATP, UTP	astrocyte migration; expression of $\alpha_v\beta_{3/5}$ integrins; PI3K/Akt- and MEK/ERK cascades	[312]
P2Y ₂	Rat brain slices, hippocampus, cerebellum	ATP, UTP	proliferation, migration, (via RGD (Arg-Gly-Asp) motif), $\alpha_v\beta_{3/5}$ integrins	[172]
P2Y ₂ /P2Y ₄	Primary cultures of astrocytes (rat, cortex)	ATP, P2R agonists	Nucleotide-stimulated production and release of APP; ERK-, p38/MAPK-pathways	[217]
P2Y _{1,2,4}	Primary cultures of astrocytes (rat, cortex)	2MeSATP, ATP, ADP	ERK cascade, MEK activation	[313]
P2Y _{1,2} /P2X ₇	Human foetal astrocyte cultures	ATP, BzATP	IL-1 β -mediated signal transduction	[133]
P2Y ₁ , P2Y ₁₂	1321N1 astrocytoma cells (hP2Y _{1R} or hP2Y _{12R} transfected)	TNF- α , ADP, 2MeSATP	TNF- α -induced apoptosis, ERK1/2-, Akt-, JNK phosphorylation	[314]
P2Y	Human foetal astrocytes	IL- β treatment	\downarrow Cx43 mRNA expression after IL-1 β treatment	[315]
P2Y ₂	Primary cultures of astrocytes (mice, cortex)	IL- β treatment	inhibition of gap junction communication	[316]
P2Y ₂	Primary cultures of astrocytes (rat)	UTP, guanosine	ERK1/2 (MAPK) signalling cascade	[317]
P2Y ₆	1321N1 astrocytoma cells (rP2Y ₆ transfected)	UDP, TNF- α	P2Y _{6R} (by UDP) protected astrocytes from TNF- α -induced apoptosis; PKC, PLC, ERK	[291]
		Different kinds of injury		
P2X/Y	Primary cultures of astrocytes (rat, cortex)	mechanical strain	Akt-phosphorylation	[22]
P2Y ₁		H ₂ O ₂ -evoked oxidative injury	IL-6 release	[136]

Table 2 (continued)

P2YR subtype	Preparation/model	Stimulation	Purinergic signalling/Transduction signals	References
	Primary cultures of astrocytes (rat); mixed astrocyte-neuronal cocultures			
P2Y ₁	In vivo (rat, NAc)	stab wound injury	PI3K/Akt; MEK/ERK cascade	[129]
P2Y ₁	In vivo (rat)	transient MCAO	p-RelA-mediated NF-κB-pathway; chemokine/cytokine release; cerebral infarct volume	[132]
P2Y ₁	In vivo (rat brain), In vitro systems	MCAO	JAK2/STAT3 and PI3K/Akt/CREB	[137]
P2Y ₁	In vivo (male mice brain)	Photothrombosis	↑mitochondrial metabolism, ↑IP3-dependent Ca ²⁺ release	[204]
P2Y ₂	1321N1 astrocytoma cells (recombinant P2Y ₂ R)	Mechanical strain	p38 phosphorylation, ERK1/2 and Akt/PKB signalling	[174]
P2Y _{1,2,4,6}	In vivo (rat, NAc, cortex)	Stab wound injury	astrogliosis, proliferation	[100, 128]
P2Y _{1,2,4} ; P2X ₇	Primary cultures of astrocytes (rat, cortex)	UTP, BzATP; mechanical strain	astrogliosis, phosphorylation of serine 9 on GSK3β, ↓GSK3 activity	[183]
P2Y ₁ , P2X ₂	Primary cultures of astrocytes (rat, cortex)	Mechanical strain	ERK-activation	[166]
P2Y ₄	Primary cultures of astrocytes (rat, cortex)	Mechanical strain	↑Thrombospondin (TSP)-1 expression	[141]

CREB cyclic element-binding protein

pretreatment with the P2X/YR antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; [129]). After retinal detachment, the P2YR-mediated ERK1/2- and PI3 kinase-activation are necessary for mitogenic effects during proliferative vitreoretinopathy [171]. Weisman et al. [172] reported that proliferation of primary astrocytes by P2Y₂R-stimulation depends on PI3K and ERK1/2.

The PI3K/Akt pathway is associated with controlling the balance between survival and cell death. Akt activation after trauma, axotomy and ischaemia is well established. The purinergic stimulation (e.g. P2X_{1,2,3}, P2Y₂Rs) of Akt phosphorylation in astrogliosis was studied in vitro (e.g. [22, 139, 173, 174]) and in vivo in the rat brain [129]. The involvement of P2Y₁Rs in Akt activation in vivo suggests that the PI3K/Akt cascade stimulates astroglial proliferation and prevents apoptosis, indicating contribution of specific P2R subtypes during neurodegenerative diseases associated with excessive gliosis [129].

P2YRs may be coupled to phosphatidylinositol-specific phospholipase C (PI-PLC) via G_q and to ERK-signalling via G_o or G_i. In cultured cortical astrocytes these receptors are coupled to the PI-PLC/calcium pathway [162]. Activation of the MAP kinase pathways can, in turn, activate transcription factors such as c-Fos, c-Jun, Elk1, ATF2 or the signal transducer and activator of transcription 3 (STAT3). Depending on the P2YR subtype, differences in the induction of signalling cascades were described. These signalling pathways may act synergistically for example by phosphorylating the immediate early gene products c-Fos and c-Jun, which both increase astrocyte proliferation and GFAP expression. c-Fos is an early gene product that contains in its promoter a binding site for the

activating protein 1 (AP-1) and heterodimerizes to form an AP-1 complex [175–177]. Triple-labelling studies demonstrated c-Fos expression in P2Y₁R-positive GFAP-labelled astrocytes (Fig. 4). Previous in vitro experiments reported an extracellular ATP-induced up-regulation of c-Fos protein and the formation of AP-1 transcriptional complexes [164, 178]. Additionally, PPADS reduced the incision-evoked c-Fos expression in a model of postoperative pain [179]. A role of the ERK-signalling cascade in gene regulation has intensively been studied [180, 181]. The activation kinetics of ERK signalling were associated with specific biological outcomes, whereas c-Fos was shown to function as a sensor for ERK1/2-signal duration; the use of the MAPK/ERK-inhibitor PD98059 suggest an ERK-mediated c-Fos expression [182].

P2Y_{1,2,4} and P2X₇Rs also couple to glycogen synthase kinase 3 (GSK3), a signalling molecule involved in cell survival, cell cycle regulation, proliferation and differentiation. The coupling of nucleotide receptors to GSK3 signalling was initially reported in cortical astrocytes [183]. P2Rs are coupled to GSK3β by a PKC-dependent pathway that is independent of Akt, p70 S6 kinase and the ERK pathways. These findings suggest that nucleotide signalling contributes to the regulation of GSK3 functions, one of which may be the response of astrocytes to CNS injury on the release of ATP [183].

P2X/YRs are also involved in the activation of NO signalling pathways and in the ATP/NO cross-talk. The presence of the Ca²⁺-dependent NO synthase (NOS) isoforms in astrocytes makes it conceivable that similarly to neurones, P2XR-mediated rises in Ca²⁺ may directly trigger NO production. Indeed, in purified cortical astrocytes in culture,

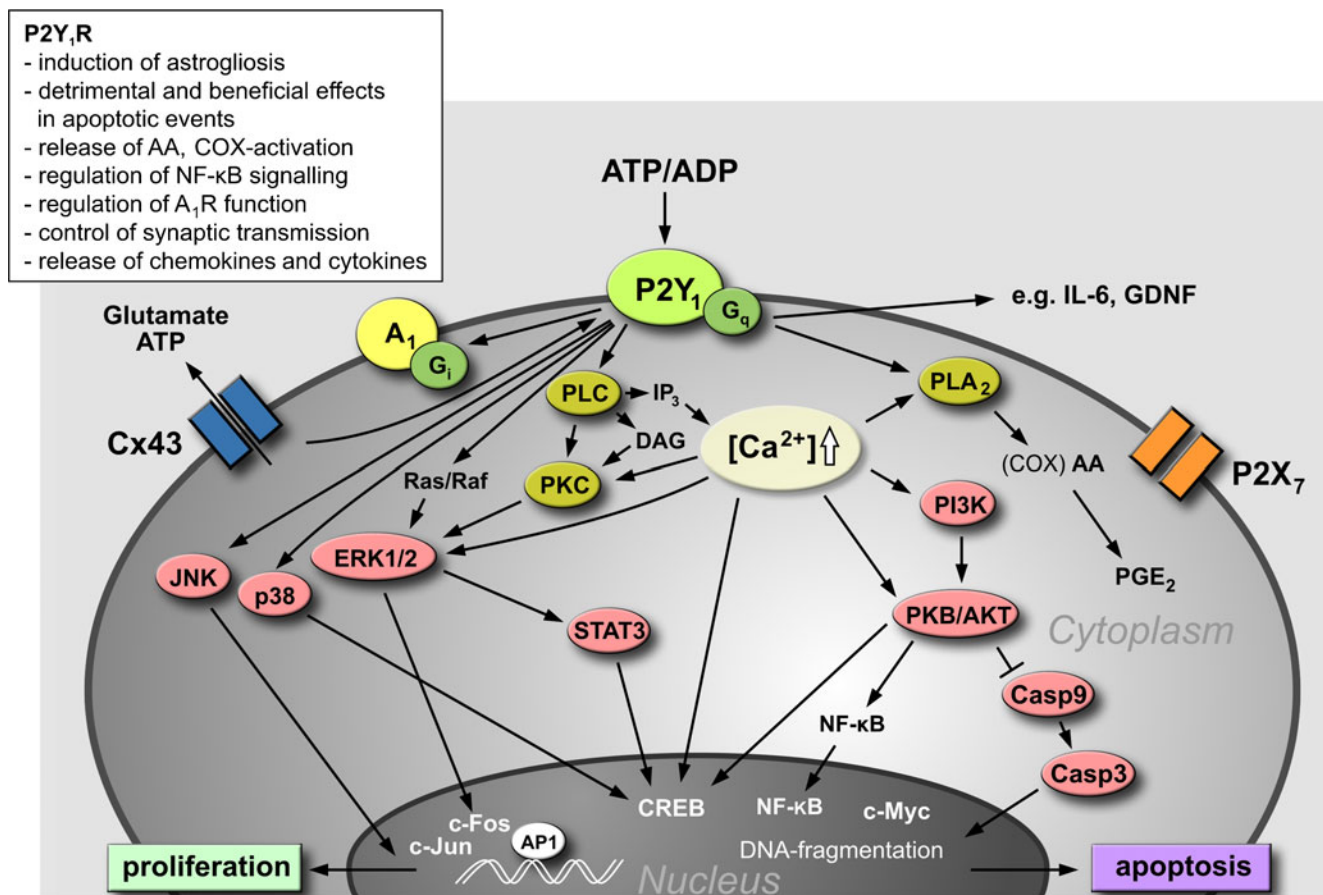


Fig. 3 Schematic illustration of examples of signal transduction pathways in astroglial cells following P2Y₁R activation. Stimulation of P2Y₁Rs leads to the activation of phospholipases A₂ and C (PLA₂, C) and protein kinase C (PKC), as well as an increase in intracellular calcium ([Ca²⁺]_i). The activation of P2Y₁Rs result in the induction of second messenger and enzyme cascades, e.g. activation of the mitogen activated protein kinase (MAPK) pathway proteins (ERK1/2), p38 MAPK, c-Jun N-terminal kinase (JNK), and PI3K/Akt activation. P2Y₁R-mediated signal transducer and activator of transcription 3 (STAT3) signalling may play a role in astrocyte proliferation and reactive astrogliosis. P2Y₁R activation appeared to be involved in the activation of caspase (Casp) cascades and the release of arachidonic acid and increase in

prostaglandin E₂ (PGE₂) levels. In addition, P2Y₁R activation induces the activity of transcription factors such as nuclear factor κB (NF-κB), cyclic element-binding protein (CREB) and activator protein (AP-1) (which up regulate the expression of proinflammatory genes, e.g. c-Fos, c-Jun, c-Myc). Interaction between adenosine A₁ and P2Y₁Rs may alter the nucleotide signalling cascades. Modulation of astrocytic P2Y₁Rs by the C-terminal domain of the gap junction protein connexin-43 (Cx43) appears to be involved in release of ATP and glutamate. The present data suggest that astroglial P2Y₁R stimulation is associated with neurological disorders leading to neuroinflammation, and apoptosis. The *inset* summarises examples of P2Y₁R-mediated effects in astrocytes (artwork by courtesy of Dr. Jens Grosche)

ATP triggered the formation of NO through P2X₂R-mediated [Ca²⁺]_i elevation [184, 185].

Astroglial P2Y₂Rs are coupled to α_vβ₃/β₅ integrin signalling pathways, which control cytoskeletal remodelling and motility [172]. The activation of P2Y₂ and P2Y₄Rs regulates astrogliosis through ERK1/2 and STAT3 signalling cascade, mostly active in reactive astrocytes [35, 186]. P2Y₂R activation induces the phosphorylation of the epidermal growth factor receptor (EGFR), response dependent upon the presence of a SH3 binding domain in the intracellular C terminus of the P2Y₂R that promotes Src binding and transactivation of EGFR, e.g. regulating the proliferation of cortical astrocytes [187].

ATP triggers reactive astrogliosis by activation of a P2YR linked to the induction of cyclooxygenase-2 (COX-2) [119,

188], and P2YR antagonists might counteract excessive COX-2 activation in both acute and chronic neurological diseases [188]. Transductional signalling to COX-2 induction involves an early activation of phospholipase A₂ (PLA₂) and arachidonic acid (AA) release; this may, hence, act as an inducer of the COX-2 gene via the PKC/MAPK pathway [113, 164, 189]. The different intracellular signalling pathways associated, e.g. with STAT3, nuclear factor κB (NF-κB), signalling molecules suppressor of cytokine signalling 3 (SOCS3), cAMP, JNK/c-Jun, FGF and MAPK are implicated in mediating different aspects and/or different degrees of reactive astrogliosis such as GFAP up-regulation, cell hypertrophy, proliferation and pro- or anti-inflammatory effects [for review see 130].

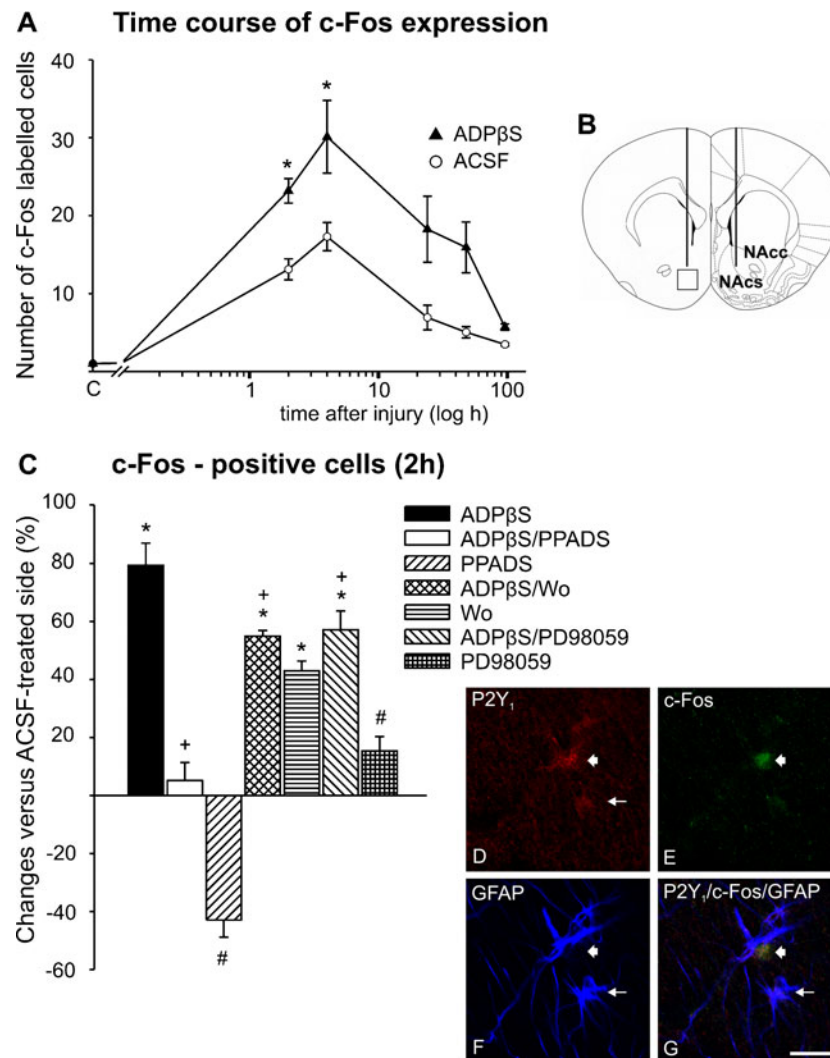


Fig. 4 Changes of c-Fos expression after stab wound injury in the NAc of rats. **A** Time course of c-Fos expression 2 h, 4 h, 24 h, 48 h and 4 days after ACSF- and ADPβS-microinjection in comparison to untreated controls (C) and plotted against log time (hours). **B** Schematic illustration of the localisation of the stab wound in the NAc of rats and the area (*square*) used for quantification of labelled cells (according to [127]; NAcc, core; NAcS, shell). **C** Quantification of the effects of ACSF, ADPβS (100 μM), PPADS (30 μM), PD98059 (50 μM; a MAPK inhibitor), wortmannin (Wo, 40 μM; a PI3K inhibitor), and the

combination of ADPβS with the respective inhibitors, on the number of c-Fos-positive cells in the NAc of rats after a post-injection time of 2 h. The values are expressed as a percentage of ACSF-treated controls and represent the mean±SEM of five animals per group (* $P<0.05$, vs. ACSF group; + $P<0.05$, agonist ADPβS vs. agonist ADPβS/inhibitor group; # $P<0.05$, inhibitor vs. agonist/inhibitor group). **D–G** Confocal images of triple-immunofluorescence of **D** the P2Y₁R subtype, **E** c-Fos-labelling and **F** GFAP-positivity (**G**, merge); *thick arrow* co-localisation with c-Fos; *thin arrow* no co-localisation with c-Fos. Scale bar: C–F=20 μm

Astroglial response to CNS injury

Reactive astrogliosis and glial scar formation are prominent features of brain pathophysiology. However, the involvement of P2X/YRs was not characterised for all diseases, where astrogliosis was found (for example in metabolic disorders, infection, hepatic encephalopathy or Huntington's disease). Here we present examples for several neurological diseases in relation to changes of astrocytes and nucleotide signalling. Some initial data on astrogliosis reaction in human tissue are also discussed (Table 3, Fig. 5).

Acute CNS injury

Ischaemia/hypoxia

After hypoxia/ischaemia of the CNS, intracellular levels of ATP rapidly fall, whereas extracellular purine nucleotide concentrations are elevated due to cell damage [19–21, 190]. During in vitro ischaemia in hippocampal slices, ATP release occurred only after the anoxic depolarisation, whereas adenosine release was apparent almost immediately after the onset of ischaemia [191]. This suggests that ATP and adenosine release during ischaemia are independent

Table 3 Human brain—examples of the involvement of astrocytes in pathophysiological events

Disease	Receptor subtype	Characteristics	References
Trauma	P2Y ₁ ↑	Postmortem brain, IR, reactive astrocytes, astrogliosis	[214]
Multiple sclerosis (MS)	P2X ₇ ↑	IR, reactive astrocytes	[237]
	P2Y ₁₂ (↑)	Postmortem brain (MS); interlaminar astrocytes in cerebral cortex	[238]
Epilepsy	(P2X/Y)	Patients with epileptic episodes; ATP, ADP and AMP hydrolysis rates; soluble nucleotide phosphodiesterase (PDEase) activity	[254]
Alzheimer's disease (AD)	P2Y ₁ (↑)	Neurofibrillary tangles, neuritic plaques and neuropil threads	[226]
	P2Y ₂ ↓ (no change of P2Y _{4,6})	Reduced IR, parietal cortex	[225]
	P2X ₇ ↑	Microglia, β-amyloid plaques	[227]
	P2X ₇ ↑	Adult microglia obtained from AD	[224]
Parkinson's disease (PD)	A _{2A} ↑	Postmortem brain of patients with PD and dyskinesias, in situ hybridisation and receptor binding autoradiography	[318]
Glioma	P2X ₇ , P2Y ₁ , P2Y ₂ , P2Y ₁₄	Examples see in Tables 1 and 2	

processes with distinct underlying mechanisms, which consequently confer temporally distinct influences on neurones and glia in the ischaemic brain. Adenosine has an important protective role against ischaemic damage in the brain, possibly via A₁ and A₂R subtypes [89]. After all, these receptors are also able to influence P2YR activity, e.g. P2Y₁R, as described above.

Ischaemic events result in membrane depolarisation and the release of glutamate and ATP that induces cytosolic calcium overload, mitochondrial damage and the activation of intracellular enzymes with deleterious consequences for cell function and viability. Ultimately, cell death and membrane damage cause additional massive release of glutamate and ATP, aggravating the initial damage [4, 9]. Ischaemic insults can open pannexin hemichannels and contribute to ATP release and the post-anoxic depolarisation in cultured neurones, although these results have not been confirmed in brain slices or in the in vivo animal studies [192]. ATP is hydrolysed by ATP diphosphohydrolase and 5'-nucleotidase; up-regulation of these enzymes after ischaemia has been

reported and was considered as an indicator of an accelerated ATP release [19].

As frequently described, ischaemia may cause cellular degeneration and death, mediated through P2X and P2YRs [6, 88]. A post-ischaemic time- and region-dependent up-regulation of P2X (P2X_{2,4,7}) and P2Y (P2Y_{1,2}) receptors in neurones and glial cells was shown, suggesting the involvement of purinoceptors in the pathophysiology of cerebral ischaemia in vitro and in vivo.

In the cerebral cortex of spontaneously hypertensive rats, middle cerebral artery occlusion (MCAO) led to the up-regulation of P2X₇R-immunoreactivity (IR) in the penumbra surrounding the necrotic region [193]. This IR was co-localised with astroglial and neuronal markers; immunoelectron microscopy revealed the expression of P2X₇R on presynaptic elements of neurones and on glial cells. In order to clarify the mechanism of the ischaemic up-regulation, HEK293 cells were transfected with the recombinant human P2X₇R, the C-terminus of which was labelled with EGFP; the transfected cells were afterwards subjected to in vitro hypoxia/ischaemia [194]. The combined use of confocal laser scanning microscopy, flow cytometry and electrophysiology suggested that 12 h of in vitro ischaemia increased the integration of intracellularly localised P2X₇R into the plasma membrane of HEK293 cells. Neuronal changes observed under ischaemia [195, 196] may be due to an indirect effect via the P2X₇R-induced release of an astrocytic, neuroactive signalling molecule (e.g. [197]), although direct effects at neurones cannot be excluded either [198, 199].

P2Y₁Rs are strongly expressed in Purkinje cells, in the deep layers of the cerebral cortex and in ischaemia-sensitive areas of the hippocampus [200]. Astroglial P2Y₁R expression and activity under ischaemic/hypoxic conditions possess a dual effect on cell survival/death, which depends on the extent of damage, the time course and levels of ATP release and the modulator influences of other cells. Intensive accumulation of

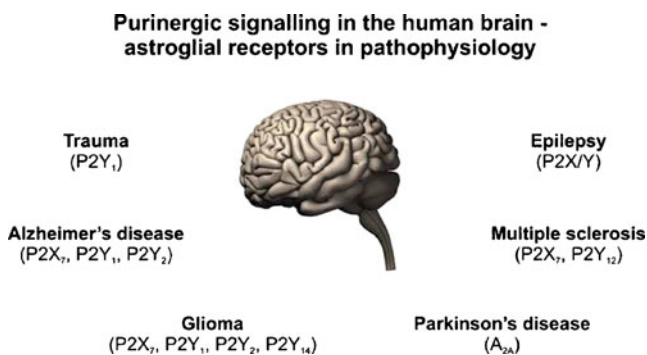


Fig. 5 Schematic diagram illustrating examples of P2X/YR expression in human brain on glial cells (especially astrocytes) in CNS neuropathological events (artwork by courtesy of Dr. Jens Grosche). For details, see also the Table 3

P2Y₁Rs was observed in the penumbra around the necrotic tissue [132]. In our own experiments, an increase in P2Y₁R-IR was also found after mechanical injury in the peritraumatic area of the rat cortex and nucleus accumbens (NAc [100]) and after MCAO in the periinfarct region (H. Franke, U. Krügel and A. Lämmer, unpublished data). Additionally, using the MCAO model, we have demonstrated that the inhibition of P2Rs by PPADS improved the morphological and functional alterations induced by hypoxia/ischaemia [201]. In particular, the recovery of the cortical electrophysiological and motor functions after permanent MCAO in rats was considerably accelerated by PPADS. PPADS pretreatment also led to a decrease in the infarct volume and reduced cell death (neurons and astrocytes) for up to 7 days after MCAO compared to ACSF-treated controls [202]. In the periinfarct area, we have found the expression of P2X₇Rs and observed a PPADS-sensitive up-regulation of P2Y₁Rs (H. Franke, U. Krügel and A. Lämmer, unpublished data). In a rat model of cerebral ischaemia, the intracerebroventricular administration of the P2Y₁R agonist MRS 2365 increased cerebral infarct volume 72 h after transient MCAO, whereas the P2Y₁ antagonist MRS 2179 significantly reduced infarct volume and led to recovered motor coordination [132]. Furthermore, the P2Y₁R agonist 2-methylthioladenosine 5'-diphosphate (2-MeSADP) has been reported to induce apoptosis by activating the human recombinant P2Y₁R heterologously expressed in the 1321N1 astrocytoma cell line [167]. Otherwise, a significant cerebroprotection was observed with the P2Y₁R selective antagonists MRS2279 and MRS2179, which allowed for the maintenance of motor coordination following transient MCAO [132].

P2Y₁Rs in cultured astrocytes are involved in the protection against hydrogen peroxide (reactive oxygen species, generated in ischaemic stroke)-induced damage [136]. Data of Sun and co-workers [137] suggested that P2Y₁Rs play a role in the production of GFAP and GDNF in astrocytes under transient MCAO and influence the related signalling pathways (Janus kinase 2/signal transducer [JAK2/STAT3], PI3K/Akt/CREB). Furthermore, it has been shown that after MCAO, inhibition of astrocytic P2Y₁Rs results in cytokine/chemokine transcriptional suppression and cerebroprotection via a p-RelA-mediated NF- κ B pathway [132].

Several studies have suggested roles for P2Y₁Rs in the regulation of astrocyte swelling in particular due to an autocrine release of ATP from cultured astrocytes, (e.g. [136, 203]). Glial mitochondrial metabolism appears to be a key determinant of cytotoxic oedema and necrosis during cerebral ischemic stroke. The inhibition of glial mitochondria by ischaemia increases astrocyte swelling that leads to oncotic cell death [204]. P2Y₁R stimulation reduces astrocyte cytotoxic oedema, necrosis and neuronal cell death in mice with Rose Bengal (RB)-induced brain lesions. In a RB-induced photothrombotic model of focal cerebral ischaemia, treatment with the P2Y₁R agonist 2-MeSADP showed that

the magnitude of ischaemic lesion was significantly reduced and the neurological outcome of oedema and cerebral ischaemic stroke was improved [204]. These data suggest that astrocyte mitochondria are a key energy source in the post-ischaemic penumbra. In fact, the mitochondrial metabolism can be stimulated by InsP₃-mediated intracellular Ca²⁺ release, which significantly improves the neurological outcome of brain injuries [204]. This is in a good agreement with the finding that P2R-stimulated InsP₃-mediated Ca²⁺ release enhances neuroprotection by an increase of the astrocytic mitochondrial metabolism during aging [205].

It should be noted that P2Y₁Rs, found on the glia limitans and on vascular endothelium, are also important for ischaemic regulatory processes; for example, endothelial P2Y₂R-mediated dilatations of rat middle cerebral artery in response to uridine-5'-triphosphate (UTP) were potentiated after ischaemia-reperfusion, while P2Y₁R-mediated dilatation was simultaneously attenuated [206, 207]. Not only purine but also pyrimidine nucleotides appear to be released during ischaemia; the UTP-induced activation of P2Y₄Rs causes cell death in human neuroblastoma cells [208].

An important question relates to the protective role of activated astrocytes and the P2Y₁R expression in the penumbra after brain ischaemia. Li and co workers [41] showed in a GFAP^{-/-}Vim^{-/-} mice model that reactive astrocytes protect the penumbra during brain ischaemia. Mice deficient in both intermediate filament proteins, exhibited attenuated posttraumatic reactive astrogliosis. Seven days after MCA transection, infarct volume was considerably higher in GFAP^{-/-}Vim^{-/-} than in wild-type (WT) mice [41]. The absence of immunofluorescence for astrocytic markers was linked to changes in glutamate transporter, endothelin B receptor (ET_BR)-mediated control of gap junctions, and plasminogen activator 1 (PAI-1) expression.

Mechanical trauma

Pathophysiological changes in traumatic brain injury (TBI) include cerebral ischaemia, oedema, release of excitatory neurotransmitters, oxidative stress, inflammation and apoptosis [36, 209]. Mechanical strain of rat cortical cultured astrocytes causes ATP release [22]; similarly, mechanical lesion of the brain increases extracellular ATP and glutamate [23].

A useful in vitro model for brain trauma was developed by Neary and co-workers (for review, see [139]). Astrocytes were cultured on deformable SILASTIC membranes and were subjected to rapid, reversible strain (stretch)-induced injury. These experiments demonstrated that ATP released by mechanical injury triggers ERK activation and suggested a role for extracellular ATP, P2Rs and calcium-dependent ERK signalling in astroglial responses to brain trauma [22, 166]. Studies with P2R antagonists indicated a role for P2X₂ and P2Y₁, but not P2X₁, P2X₃ or P2X₇Rs for injury-

induced astroglial ERK activation. The injury-induced activation of PKB/Akt in cultured astrocytes was also demonstrated [22]. Mechanical strain activates P2Y₄Rs, which in turn stimulate the synthesis and release of thrombospondin-1, an extracellular matrix molecule that induces synaptogenesis during development and repair [141]. In another study, it was shown that P2Y₂Rs promote the activation of ERK1/2 and PKB/Akt through PI3K dependent mechanisms after traumatic injury in astrocytic cells and enhance cell survival [174].

Astroglialosis in vivo was observed after direct infusion of ATP or ADP into one of the cerebral hemispheres, when compared with the contralateral hemisphere [210]. Intra-striatal microinjection of ATP causes P2XR-mediated cell death within 24 h in the striatum of rats, which supports the hypothesis that extracellular ATP mediates neuropathological events caused by brain injuries [211]. Finally, a single dose of the P2X/YR antagonist suramin injected intracerebrally through the needle used for producing a stab wound, reduced CNS inflammation, as judged by decreased cellular proliferation, GFAP levels and tenascin C-IR [212]. In addition, examination of the corpus callosum of saline-treated animals 30 days post-trauma revealed a disruption of the fibre tract within the lesion site, while suramin-treated animals displayed numerous fibres spanning the lesion. These results demonstrate that a single injection of suramin transiently inhibits the astroglial response, which may be sufficient to ameliorate subsequent tissue damage [212].

We have demonstrated that endogenous ATP participates in the in vivo astroglialosis caused by stab wound injury in the NAc and cortex of rats [127]; and we have found an increase in GFAP and astroglial proliferation following microinjection of ATP analogues into the NAc [127, 128]. P2X and P2Y receptors were expressed/co-expressed in astrocytes of untreated rats; the P2R expression (P2X_{1,7} and P2Y_{2,6}) was up-regulated by mechanical injury [76, 100, 213]. Pharmacological characterisation showed that astroglial activation was mediated specifically via P2Y₁Rs (Fig. 1G–I) [100, 128, 129]. Additionally, in this stab wound, paradigm P2Y₁R activation enhanced the early apoptosis marker caspase-3 in a PPADS reversible manner. Pretreatment with PPADS prevented the P2Y₁R mediated rise of the apoptotic pERK1/2 and the anti-apoptotic pAkt [129]. Our data indicated that traumatic injury in vivo is accompanied by a P2Y₁R-mediated modulation of PI3K/Akt- and MAPK/ERK-signalling pathways in astrocytes and neurones, resulting in astroglial proliferation and anti-apoptotic processes [129].

Finally, we also demonstrated the expression of the P2Y₁Rs in activated astrocytes in the pericontusion area after TBI in the prefrontal cortex of the human brain (post-mortem autopsy material, Fig. 1J–L [214]).

Neurodegenerative diseases

Alzheimer's disease

Astrocytes become activated in Alzheimer's disease (AD) and their accumulation has been observed around amyloid deposits in the brain, suggesting a role of these cells in the homeostasis of amyloid plaques and in modulating the neuronal environment in AD [34, 45].

The elevated levels of pro-inflammatory cytokines (e.g. IL-1 β) can enhance the non-amyloidogenic amyloid precursor protein (APP) processing through up-regulation of the P2Y₂Rs in neurones, as shown in primary cortical neuronal cell cultures [215]. The activation of P2Y₂Rs by UTP appears to be neuroprotective, since this nucleotide enhanced the non-amyloidogenic cleavage of APP [216]. Recent data obtained in primary cultures of rat cortical astrocytes indicate that APP production and secretion can be regulated by activation of heteromeric P2Y_{2/4}Rs coupled to protein kinase signalling pathways (MAPKs, but not Akt) and suggest that astrocytes can be a potential source of APP [217]. In conclusion, astrocytic P2Y₂R activation regulates a variety of signal transduction pathways, such as (1) PLC, activating different second messenger cascades (e.g. PKC, PLA₂, ERK1/2); (2) phosphorylation of the EGFR, depending on the direct interaction of a Src homology binding sequence, promoting the transactivation of EGFR and the regulation of some key functions (e.g. the proliferation of cortical astrocytes); and (3) activation of $\alpha_v\beta_{3/5}$ integrins that bind directly to an Arg-Gly-Asp (RGD) motif in the first extracellular loop of the P2Y₂R subtype, mediating cytoskeletal rearrangements and increasing astrocyte chemotactic activity, finally associated with reactive astroglialosis in AD brains [187, 218]. It should be noted that ATP and UTP in their interaction with the “sensor” P2Y₂R have been identified as critical “find-me” signals important for phagocyte chemoattraction by apoptotic cells [219]. Peterson and co workers [187] summarise the mechanisms underlying the pro-inflammatory and neuroprotective effects mediated by P2Y₂Rs in glial cells and neurones and their potential relationship to the pathophysiology of AD. The main hypothesis is that chronic stress or CNS damage causes an increased nucleotide release from astrocytes and the induction of pro-inflammatory responses in glial cells with subsequent up-regulation of neuronal P2Y₂Rs, which in turn mediate neuroprotective effects. The involvement of P2Y₂Rs in microglia during inflammatory processes was also emphasised. Recent data using brain slices indicated that activation of P2Y₂Rs with UTP results in an inflammatory response, measured as a rapid rise in intracellular Ca²⁺ and release of cytokines [220].

In mouse primary astrocytes, the specific activation of P2X₇Rs induced the release of the fragment soluble (s)APP α [221]. The capacity of P2X₇Rs to trigger non-amyloidogenic APP processing may tentatively suggest that astrocytes have a

neuroprotective effect in AD. It was demonstrated that the MAP-kinase modules ERK1/2 and JNK are involved in P2X₇R-dependent α -secretase activity. Extracellular ATP, acting through P2X₇Rs, can alter β -amyloid peptide (A β)-induced cytokine release from macrophages and microglia and makes this receptor-subtype to an important modulator of neuroinflammation in AD [222, 223]. Semiquantitative RT-PCR showed enhanced expression of P2X₇R mRNA in AD microglia in comparison with microglia from non-demented brains [224]. Immunohistochemical analysis showed prominent P2X₇R expression in association with A β plaques and a localisation to HLA-DR (class II major histocompatibility antigen)-immunoreactive microglia.

A possible role of P2Y_R alteration in regulating molecular processes, which underlie plaque formation was investigated using post-mortem materials from a cohort of longitudinally assessed AD patients and aged controls [225]. Interestingly, the receptor-deficit seems to be subtype-specific and was manifested by reduction in P2Y₂Rs, while P2Y₄ and P2Y₆Rs remained unchanged. P2Y₂R-IR was observed to be selectively reduced in the AD parietal cortex correlating with neuritic plaque and neurofibrillary tangle scores as well as synapse loss. Moreover, decreases in parietal P2Y₂R densities also correlated with lower synaptophysin-IR, a marker of synapse loss [225]. In addition to changes of the P2Y₂R expression in AD, alterations in the density of the P2Y₁R subtype [226] as well as prominent changes in the number of the P2X₇Rs (e.g. [227] were also observed. P2X₇Rs are up-regulated around senile plaques in animal models of AD and in patients with AD [224, 227]. P2Y₁Rs have been immunohistochemically localised at neuritic plaques and neurofibrillary tangles [226].

Parkinson's disease

In autopsies of the substantia nigra and putamen from Parkinson's disease (PD) patients, reactive astrogliosis is generally mild or moderate [228]. Astrocytes have been implicated as potentially exerting both neurotoxic and neuroprotective activities in PD [36].

Endogenous adenosine and ATP appear to modulate dopaminergic neurotransmission in the CNS. Exogenously applied ATP enhances the release of dopamine from the terminals of neurones projecting from the substantia nigra pars compacta to the striatum via P2Y_Rs [229] and modulate the release of dopamine from the respective axon terminals in the NAc [230–232]. The tonic dopamine release may be physiologically regulated by P2Y_Rs [231, 232]. The P2R-IR in the nigro-striatal and mesocortico-limbic dopaminergic systems *ex vivo* and *in vivo* has been documented [231, 233], as well as a trophic growth promoting effect of P2R agonists on fibre outgrowth in the dopaminergic projection system [233].

In an animal model of PD using 6-hydroxydopamine-lesioned substantia nigra, the expression of the P2X₇R in nigral microglial and astroglial cells was observed, but was absent in dopaminergic neurones. The enhanced neuroinflammatory process through the activation of microglia and the release of inflammatory agents was discussed in this context [234].

In the PD, the main focus of research is concentrating on striatal A_{2A}Rs being a target of adenosine generated by the enzymatic degradation of synaptically released ATP [235, 236]. Specific A_{2A}R antagonists are currently being investigated for the treatment of the PD. However, neuronal rather than astrocytic A_{2A}Rs appear to be drug targets for this purpose and are therefore not discussed here in detail.

Multiple sclerosis and amyotrophic lateral sclerosis

In human multiple sclerosis (MS) lesions of autopsy brain tissues, immunoreactivity for the P2X₇R was demonstrated on reactive astrocytes [237]. In cultures of primary human foetal astrocytes, P2X₇R stimulation increased the production of IL-1-induced NOS. These studies suggest that signalling *via* the P2X₇R may modulate the astrocytic response to inflammation [237]. An immunofluorescence and confocal analysis in sections of cerebral cortex from postmortem MS brain indicated that P2Y₁₂ protein is present in myelin and in interlaminar astrocytes; P2Y₁₂Rs were also highly enriched in oligodendrocytes [238].

Furthermore, astrocytes (via P2X₇Rs), infiltrating cells of the monocyte/macrophage lineage and activated microglia can release ATP (possible through P2X₇R pores; [239]) and thus contribute to further P2X₇Rs activation and cell death in oligodendrocytes [192, 240]. An increased expression of P2X₇Rs in axon tracts before lesions are formed indicating that this feature may constitute a risk factor associated with newly forming lesions in MS; the P2X₇R may thus prove to be a diagnostic and/or prognostic clinical biomarker for MS [192].

Finally, the P2Y-like receptor GPR17 has been identified as a modulator of oligodendrocyte differentiation during maturation and in myelinating disorders. In primary cultures of astrocytes and oligodendrocyte precursor cells (OPCs), the GPR17 expression was interpreted as a “danger signal” in the presence of high extracellular ATP concentrations, suggesting an important role of the receptor in brain disorders [241, 242].

Amyotrophic lateral sclerosis (ALS) is a neuromuscular disease causing progressive paralysis of all voluntary muscles. A pathological hallmark of ALS is neuroinflammation mediated by non-neuronal cells in the nervous system, such as microglia and astrocytes that accelerate the disease progression [243]. Recent data suggest that during the progression of ALS, microglia, astrocytes and motor neurones might cross-

talk via ATP release/degradation and P2X₇R activation, generating a feedback loop that drives a sustained pro-inflammatory and detrimental response [243, 244]. In ALS, extracellular ATP and P2X₇R are involved in astrocyte-mediated motor neurone death [245]. Mutant superoxide dismutase 1-positive astrocytes displayed increased ATP-dependent proliferation and basal increase in extracellular ATP degradation. The pharmacological inhibition of P2X₇R (e.g. by Brilliant Blue G) might reduce neuroinflammation in ALS through astrocytes [245].

Epilepsy

Epilepsy, characterised by recurrent unprovoked abnormal excessive or synchronous neuronal activity, is often accompanied by reactive astrogliosis [246, 247]. Glial alterations may contribute to the generation and spread of seizure activity. Recently astroglial Ca²⁺ waves have been shown to provoke seizures, and conventional antiepileptic drugs were able to attenuate these Ca²⁺ waves [65, 89, 192, 236, 248, 249].

Microinjection of ATP analogues into the prepiriform cortex induces generalised motor seizures [236]. Blocking ATP-mediated gliotransmission, therefore, represents a potential target for antiepileptic drugs. Adenosine is an endogenous inhibitor of excitatory synaptic transmission with potent anticonvulsive properties. Astrocytes are considered as the main regulators of adenosine release, which acts as the brain's natural anticonvulsant, and the basal synaptic levels are controlled by an astrocyte-based adenosine cycle in which a key element is the activity of adenosine kinase (ADK; "ADK hypothesis of epileptogenesis") [192, 250]. Adenosine accumulates from the hydrolysis of ATP (by ectonucleotidases) released by astrocytes and is believed to inhibit distant synapses by acting on adenosine P1 receptors [89, 192, 248].

Other potential roles for ATP signalling in the initiation and spread of epileptiform discharges may involve synaptic plasticity and coordination of synaptic networks [248]. Adenosine functions are consistent with a surround-inhibitory mechanism whose failure would predispose to seizures. Glutamate and extracellular ATP can activate astrocytes and trigger calcium signals modulating local excitability [68]. Glutamate exocytosis from astrocytes of the rat hippocampal dentate molecular layer enhances synaptic strength at excitatory synapses between perforant path afferents and granule cells. The NMDA receptor 2B subunits on the extrasynaptic portion of excitatory nerve terminals are spatially related to glutamate-containing synaptic-like microvesicles in the apposed astrocytic processes. This glial regulatory pathway is endogenously activated by neuronal activity-dependent stimulation of astrocytic P2Y₁Rs as an evidence for a physiological control of synaptic activity via exocytosis of glutamate from

astrocytes [68]. UTP, which is released during epileptic activity in the 4-aminopyridine-induced epilepsy model acts as an inhibitory neuromodulator by stimulating P2YRs [251].

Enhanced P2X₇-IR accompanied with mossy fiber sprouting at the dentate gyrus of epileptic animals, suggesting a role of nucleotide receptors in the pathophysiology of temporal lobe epilepsy. Otherwise, a decrease in presynaptic P2XRs in the hippocampus of rats after kainate-induced convulsions might be associated with the development of seizures and/or neurodegeneration during epilepsy (reviewed in [89]).

Increased expression of P2X₇Rs was found in the hippocampus of chronic epileptic rats which show enhanced responses to ATP [252]. After a status epilepticus (SE), an increased expression of P2X₇, P2Y₆, P2Y₁₂Rs mRNAs in the hippocampus has been observed [253]. Significantly elevated P2X₇R-IR in amoeboid or phagocytic microglia appeared in the dentate gyrus 7 days after SE and at this time point the loss of GFAP-IR was very pronounced, indicating that the network of astrocytes was disrupted [149]. The treatment with PPADS and suramin markedly, but not completely, inhibited microglial activation following SE and suggest that astroglial death induced by activated microglia may be pathophysiologically relevant for epileptogenesis [149].

Finally, the variation in ATP, ADP and AMP hydrolysis rates and of the soluble nucleotide phosphodiesterase (PDEase) activity in the serum of patients suffering from epilepsy was investigated, and an increase in nucleotide hydrolysis was reported [254]. It was hypothesised that the higher nucleotidase activity might play a role in the modulation of epileptic events.

Chronic exposure to drugs

Ethanol

Chronic treatment with ethanol induces astrogliosis in rats (e.g. [255, 256]). The influence of ethanol on P2R-mediated PLD activity in primary astroglial cultures was postulated, since ADP was almost as efficient as ATP in inducing phosphatidylethanol formation, whereas AMP was significantly less potent [257]. Using cultured astrocytes, it was found that treatment with ethanol affects glutamate uptake but not adenosine uptake by affecting PKC modulation of transporter activity [258]. Moreover, the apoptotic effect of ethanol was potentiated by caffeine-induced calcium release [259], suggesting a correlation between cytosolic Ca²⁺ increase and the rate of apoptosis.

Acute exposure to ethanol elevates extracellular adenosine levels by selectively inhibiting the type 1 equilibrative nucleoside transporter (ENT1; [260]). Pharmacological inhibition or genetic deletion of ENT1 reduces the expression

of excitatory amino acid transporter 2 (EAAT2), the primary regulator of extracellular glutamate concentrations in astrocytes. These data support a central role for adenosine-mediated glutamate signalling and the involvement of astrocytes in regulating ethanol intoxication and preference.

Amphetamine

Dysregulation in the purinergic, dopaminergic and glutamatergic systems are possible causes for schizophrenia, PD or drug addiction. As described in “Parkinson’s disease”, extracellular ATP acting via P2YRs enhances dopamine release in the striatum and NAc. We have found that P2Y₁Rs are up-regulated in astrocytes of the striatum and NAc of rats treated with amphetamine (AMPH). Re-application of AMPH resulted in behavioural sensitisation and astrogliosis [261]. PPADS partly prevented the AMPH-induced astrogliosis as well as the up-regulation of P2Y₁ receptors and inhibited the development of sensitisation.

The expression of inducible NOS (iNOS) after AMPH-treatment was also observed and its inhibition after PPADS pretreatment suggests a link between P2Y₁R-induced astrogliosis, neuronal reactions and behavioural changes. The NO formation could be prevented by suramin and PPADS. The hypothesised interaction between NO and P2Y₁Rs was supported by the fact that the blockade of P2Rs by suramin or oxidised ATP (oxATP) in astrocytes resulted in inhibition of the NOS accumulation and the down-regulation of NO production [262]. In our own studies, we found a cellular co-localisation of P2Y₁Rs and iNOS, suggesting that astrocytes, expressing nucleotide receptors, may act as a link between the ATP-mediated P2Y receptor activation, NOS stimulation and NO induced up-regulation of cGMP [261, 263].

Neuropathic pain

The plasticity of spinal cord neurones and of the primary afferent nerves relaying peripheral information to the spinal cord was suggested to be the cause of chronic pain [89]. The occurrence of spinal cord astrogliosis in most pathological pain models is well known. Garrison et al. [264] used the sciatic constrictive injury (CCI) neuropathic pain model and found a strong correlation between elevated GFAP levels in the spinal cord dorsal horn and neuropathic pain measured in the same animals. In various types of neuropathic and inflammatory pain models, spinal astrogliosis usually spreading to all spinal cord lamina is described [265]. Furthermore, the degree of astrogliosis appeared to follow the increase in microglia numbers, although astrogliosis as well as hyperalgesia outlast the up-regulation of microglia [266]. These findings suggest that astrocytes are involved in pain modulation and pain processing.

ATP is known to be of major importance for the initiation and maintenance of pain symptoms [267]. It appears that astrocytes play critical roles in pathological pain (1) through the secretion of diffusible transmitters (e.g. interleukins, ATP, TNF, PGE and NO); (2) by augmenting primary afferent neuronal signalling or sensitising second order neurones in the spinal cord; (3) by directly modulating synaptic transmission between neurones in the nociceptive pathway; and (4) by creating astrocyte networks that convey signals across and along the spinal cord [36, 265, 268].

ATP release from Ca²⁺ wave propagating spinal astrocytes could play an important role in the conduction of nociceptive information, since the central terminals of dorsal root ganglion (DRG) neurones have been found to respond to ATP by depolarisation and glutamate release [269]. The amount of ATP secreted by astrocytes in response to glutamate stimulation *in vitro* was increased by substance P in a concentration-dependent manner [270], thus indicating a role for astrocytes in facilitating nociceptive transmission from peptidergic nociceptors.

The peripheral equivalents of astrocytes, the so-called satellite glial cells located in sensory ganglia, bear functional P2X₇Rs [271]. Thus, it was suggested that ATP released from DRG neurones may activate P2X₇Rs at satellite cells, which in consequence release TNF- α that potentiates P2X₃R-mediated responses at nearby neurones thereby regulating pain sensation [272]. Thus, DRG neurones and surrounding glial cells communicate with each other *via* signalling molecules; this communication has implications for the manifestation of chronic pain [273].

Furthermore, a role for astrocytes in pain modulation is supported by the therapeutic effects of fluorocitrate treatment, which specifically blocks astrocyte metabolism, thus revealing astrocytes as potential drug targets in clinical pain management [265]. Future research in astrocyte activation and signalling may therefore reveal novel drug targets for managing pathological pain.

Toxicology

Lead (Pb)

Exposure of the immature rat brain to lead (Pb), a potent neurotoxin, resulted in regional changes in the concentration of purines and selected nucleotide receptors [274]. In all examined brain structures (cortex, cerebellum, hippocampus), a decrease in ATP and ADP levels was found. Furthermore, an increase in A₁R and adenosine concentrations suggests protective mechanisms mediated by adenosine. In the hippocampus, most vulnerable to Pb, an enhanced expression of P2X₇R and connexin-43 in the astroglia was found, suggesting the involvement of astrocytes in pathophysiological changes observed after Pb-exposure in immature rat brain.

Trimethyltin

Trimethyltin (TMT) is a compound that generates neurotoxic effects and is used to model AD lesions in the brain. TMT causes astrogliosis and neuronal death in the hippocampus and other limbic regions [185]. These authors examined the expression of nitric oxide synthase (NOS) and P2XRs (P2X_{1,2,4,7}) that were induced by TMT administration at different time points. Massive glial activation and neuronal death in the CA1 and CA3 regions were observed after TMT treatment. Furthermore, expression of astrocytic P2X₂R and neuronal NOS were temporarily enhanced in association with the progression of neuronal death. TMT-induced hippocampal degeneration is associated with nitrergic and nucleotide activations. In this model, the two systems were active in segregated cell populations, namely, P2XRs in astrocytes and NOS in neurones. Finally, the temporal relations between P2XR and NOS expression and neuronal degeneration suggest interactions between P2XR/NO signalling and cell survival.

Glioma

Alterations in nucleotide signalling are involved in tumour generation [275, 276]. Glioblastomas are the most common form of primary tumours of the CNS and despite treatment, patients with these tumours have a very poor prognosis. Glioma C6 are chemically transformed astrocytes, often used as an experimental model system for studying glioblastoma growth and invasion [277–279]. However, glioma cell lines may not represent all the phenotypic and genetic characteristics of the primary tumours [280].

Recent data suggest that nucleotide signalling is involved in the growth and progression of gliomas [281]. ATP plays an important role in glioma proliferation *in vivo* and glioblastoma growth is reduced by the ectonucleotidase apyrase, indicating the role of nucleotide mechanisms in tumour development [282]. Glioma cell lines have altered extracellular ATP, ADP, and AMP catabolism, showing low rates of extracellular ATP hydrolysis and high rates of extracellular AMP hydrolysis when compared to primary astrocytes in culture [283]. ATP accumulation in the tumour periphery results in glioma cell proliferation and neuronal toxicity. Cell lysis caused by excitotoxic death or by tumour resection may liberate intracellular ATP, a known mitotic factor for glioma cells [277]. Adenine nucleotides induce cell proliferation in diverse human glioma cell lines and the majority of glioma cell lines are resistant to cell death induced by cytotoxic ATP concentrations [277, 284]. Morrone and co-worker [277] showed that the glioma cells exhibit resistance to death induced by ATP, when compared with a normal tissue. Indeed, high ATP concentrations induced astrocytic death after 24 h in organotypic cell cultures of the brain but not in glioma cell lines. In conclusion, ATP released in these situations can induce cell death of the normal tissue

surrounding the tumour, potentially opening space for the fast growth and invasion of the tumour tissue [277].

The effects of nucleotides on the growth of C6 glioma cells are regulated by ectonucleotidases, e.g. ecto-nucleotide pyrophosphatase (ecto-NPP) and by the activation of nucleotide receptors [285, 286]. Gliomas have low expression of all ecto-nucleotide diphosphohydrolases (E-NTPDases), particularly NTPDase2, when compared to astrocytes in culture. It has been shown that selective NTPDase2 expression modulates *in vivo* rat glioma growth, specifically, ADP derived from NTPDase2 activity stimulates platelet migration to the tumour area and NTPDase2, by regulating angiogenesis and inflammation, seems to play an important role in tumour progression [281].

A number of studies were performed in human astrocytoma cell lines. Analysis of the C6 glioma cell line and C6 *ex vivo* glioma cultures revealed distinct cell morphologies, although cell differentiation and angiogenic marker expressions were similar [280]. With regard to nucleotide receptor expression, it has been found that both the C6 cells and the C6 *ex vivo* glioma cultures express purinoceptors including P2X₄, P2Y₁, P2Y₂, P2Y₄, P2Y₁₂, P2Y₁₃ and P2Y₁₄ [280]. In addition, rat C6 glioma cell line and C6 *ex vivo* glioma cultures exhibited similar extracellular ATP metabolism and cell proliferation behaviour when exposed to cytotoxic ATP concentrations.

The P2Y₁R has a dual role; it has been implicated in decreased cell proliferation in different forms of cancer (e.g. melanomas) but it is also a cell proliferation stimulus in glioma cells [287, 288]. P2Y₁Rs coupled to PLC are responsible for the release of [Ca²⁺]_i. P2Y₂Rs are related to increased cell proliferation by activation of the PLC pathway, and P2Y₁₂Rs are negatively coupled to AC [276, 278, 287, 289]. P2Y₂R-mediated proliferation through the Ras/ERK-pathway as well as differential effects of the P2Y₁ and P2Y₁₂ receptor subtypes on ERK1/2, MAPK and PI3K signalling and cell proliferation in serum-deprived and non-starved glioma C6 cells were repeatedly reported [278, 287, 290]. In addition, the P2Y₆R activates PKC to protect 1321N1 astrocytoma cells against TNF-induced apoptosis [291].

Of interest are data indicating that P2Y₁, P2Y₂ and P2Y₁₂Rs can exist on intracellular membranes of mitochondria in rat astrocytes and glioma C6 cells [279]. Their role in these sub-cellular structures remains unclear. Expression and functional characterisation of nucleotide receptors in long-term serum-deprived glioma C6 cells showed an increased expression of P2Y₁₂Rs relative to P2Y₁Rs in relation to a reduced ERK1/2 phosphorylation and an enhanced Akt-phosphorylation [292]. Akt phosphorylation increased in parallel with the low expression of the P2Y₁R, indicating an inhibitory role of P2Y₁ in Akt pathway signalling. The reported shift in nucleotide receptor expression from P2Y₁ to

P2Y₁₂ is supposed to be a new and important self-regulating mechanism that promotes cell growth rather than differentiation and is a defence mechanism against effects of serum deprivation [292]. Finally, thrombin-promoted release of UDP-glucose from human 1321N1 astrocytoma cells, and the resulting activation of P2Y₁₄Rs are interesting observations [293]. Given the presence of P2Y₁₄ receptors in astrocytes, UDP-glucose may have important autocrine/paracrine functions in the brain at least under pathological conditions.

Immunohistochemical analysis of P2X₄R expression in the rat C6 glioma indicated specific expression in compact and infiltrative tumour growth areas, observed in a distinct subset of tumour-associated macrophages and activated microglia in glioma, whereas at GFAP- and nestin-immunopositive astrocytes P2X₄Rs were hardly found [294]. Interestingly, P2X₇Rs, which mediate cell apoptosis, are absent in both the C6 cell line and the C6 ex vivo glioma cultures [280]. Finally, cytotoxic ATP concentrations do not alter C6 ex vivo glioma cell proliferation [277], in agreement with the previously described absence of the P2X₇Rs [280, 295].

Conclusions

Astrocytes have important roles in the CNS supporting neurones during normal and pathological conditions. In neural injury caused by all types of traumatic, metabolic, hereditary and infectious diseases, astrocytic reactions are survivalistic and beneficial, but in case of excessive pathological stress, they turn into deleterious, aggravating the outcome of the insult.

Reactive astrogliosis and glial scar formation are activated by brain lesions both acute and chronic and are prominent factors in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis, and epilepsy-induced secondary neurodegeneration. Astrogliosis can be also induced by consumption of addictive drugs or certain neurotoxins such as lead or trimethyltin. The involvement of astrocytes in neuropathic pain is in the meantime also firmly established.

It is quite natural that in view of the plethora of neurological diseases in which extracellular nucleotides may be a pathological factor, P2X and P2Y receptor antagonists are considered to be possible therapeutic drugs. The finding of P2X₇Rs as critical mediators of neuronal and glial death in neurodegeneration of grey and white matter points to these receptors as key targets for the development of neuroprotective drugs. Hence, a lot of energy was brought up to synthesise new P2X₇R antagonists with high selectivity and improved bioavailability. These compounds belong to a series of chemical classes (e.g. benzamides, bicycloheteroaryls, acylhydrazines, biaromatics, and protoberberines).

The considerable activities in academia and pharmaceutical companies with the aim of introducing new therapeutically applicable P2X₇R antagonists underline the great need to use these compounds as an adjunct, non-causal therapy for the CNS illnesses discussed above. Hopefully, these efforts will lead to a major breakthrough in the near future.

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