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P2Y and P2X purinoceptor mediated Ca^{2+} signalling in glial cell pathology in the central nervous system

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Abstract

Activation of purinoceptors by extracellular ATP is an important component of the glial response to injury in the central nervous system (CNS). ATP has been shown to evoke raised cytosolic $[Ca^{2+}]$ in astrocytes, oligodendrocytes, and microglia, the three major glial cell types in the CNS. Glial cells express a heterogenous collection of metabotropic P2Y and ionotropic P2X purinoceptors, which respectively mobilise Ca^{2+} from intracellular stores and trigger Ca^{2+} influx across the plasmalemma. It is likely that different receptors have distinct roles in glial cell physiology and pathology. Our studies on optic nerve glia in situ indicate that $P2Y_1$ and $P2Y_{2/4}$ receptors are activated at low ATP concentrations, suggesting they are the predominant purinoceptors mediating physiological Ca^{2+} signalling. Glia also express $P2X_1$ and $P2X_3$ purinoceptors, which mediate fast, rapidly desensitising current and may also be important in signalling. At high concentrations, such as occur in CNS injury, ATP induces large and prolonged increases in glial $[Ca^{2+}]_i$ with a primary role for P2Y purinoceptors and inositol trisphosphate (IP_3)-dependent release of Ca^{2+} from intracellular stores. In addition, we found that high concentrations of ATP activated a significant P2X component that did not desensitise or saturate and was dependent on extracellular Ca^{2+}. These are characteristic properties of the $P2X_7$ subtype, and we provide in situ evidence that application of the $P2X_7$ receptor agonist benzoyl-benzoyl ATP (BzATP) evokes raised $[Ca^{2+}]_i$ in optic nerve glia, and that the dye YO-PRO-1, which passes through pore-forming $P2X_7$ receptors, is taken up by astrocytes, oligodendrocytes and microglia. Glia also express $P2X_2$ and $P2X_4$ receptors that are also pore-forming in the presence of sustained high ATP concentrations and which may also be important in the glial injury response. There is evidence that activation of P2 purinoceptors is a key step in triggering reactive changes in glial cells, including expression of immediate early genes, induction of extracellular signal regulated kinase and cyclooxygenase-2, synthesis of phospholipase A_2, release of arachidonic acid, production of prostaglandins and release of interleukins. We show that the ATP-mediated increase in glial $[Ca^{2+}]_i$ is potentiated by arachidonic acid and reduced by the inhibition of phospholipase A_2 inhibition. Together, the results implicate ATP as a primary signalling molecule in glial cells and indicate specific roles for P2Y and P2X purinoceptors in glial cell pathology.

Keywords: Glia; Ca^{2+}; ATP; Purinoreceptor; Astrocyte; Oligodendrocyte; Microglia; Axon; Signalling

1. Glial cells and CNS pathology

The mammalian central nervous system (CNS) consists of two main cell types, neurons and glia. Glia are subdivided into macroglia, namely astrocytes and oligodendrocytes, and microglia, together with Bergmann glia and Müller cells, which are specialised radial astrocytes of the cerebellum and retina, respectively. Astrocytes form multiple contacts with neurons at synapses and nodes of Ranvier, as well as with blood vessels and other glia, and have numerous functions, including structural support, regulation of extracellular K^+ levels, uptake of neurotransmitters and metabolic support of neurons. Oligodendrocytes exclusively form myelin in the CNS, whereas microglia are phagocytic and have an immunoprotective function. In addition, the adult CNS contains a small but significant population of NG2 glia, which have the antigenic phenotype of oligodendrocyte progenitor cells, but have the morphological phenotype and some functional characteristics of astrocytes (Butt et al., 1999). All glia exhibit characteristic responses in CNS pathology: (1) astrocytes and NG2 glia undergo reactive gliosis, characterised by proliferation and cellular hypertrophy, and form the glial scar that inhibits axon regrowth in the CNS; (2) oligodendrocytes undergo degenerative changes, including the disintegration of myelin and cell

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death; (3) microglia become activated and change into motile, phagocytic and cytotoxic cells, which invade the site of injury (reviewed by Berry et al., 2002). Although the loss of oligodendrocytes and myelin is clearly destructive, and is the underlying basis of the demyelinating disease multiple sclerosis, the exact significance of reactive astrogliosis and microglial activation is less clear. The glial scar is a major impediment to axon regrowth after injury, but it is also an advantage, since it isolates the lesion site from the intact CNS tissue and protects it against secondary damage. Similarly, microglial activation is essential for phagocytosis of cellular debris following injury and is important in the immunoprotection of the CNS. Moreover, the inflammatory response of reactive astrocytes and activated microglia can be neuroprotective, but eventually is destructive for neurons and oligodendrocytes. A key issue, therefore, is to identify endogenous molecules that regulate glial cell pathology and to determine their modes and sites of action.

2. Ca^{2+} signalling in glial cells

Glia express plasmalemmal receptors that enable them to respond dynamically to extracellular factors (Verkhratsky and Kettenmann, 1996). The primary response of glial cells is an increase in intracellular calcium ($[Ca^{2+}]_i$) and glial Ca^{2+} signalling occurs in response to physiological and pathological stimuli. Astrocytes have been shown to signal to each other using Ca^{2+} waves, which propagate intercellularly through a network of gap junctions and also using ATP as a diffusible signal (Dani et al., 1992; Giaume and McCarthy, 1996; Venance et al., 1997; Guthrie et al., 1999; Cotrina et al., 2000; Fam et al., 2000; Newman, 2001). In addition, Ca^{2+} acts as an intracellular messenger regulating growth, proliferation, and the secretion of cytokines, growth factors, peptides and nitric oxide (Finkbeiner, 1993; Cornell-Bell and Finkbeiner, 1991; Verkhratsky and Kettenmann, 1996; Verkhratsky et al., 1998). Astrocytes and neurons form intimate associations at synapses and nodes of Ranvier (Butt et al., 1994, 1999; Grosche et al., 1999), and the evidence that glia respond to neuronal activity and that astrocytes can release neurotransmitters to act on neurons, has led to the proposal that glial Ca^{2+} signalling is an extraneuronal information processing system (Dani et al., 1992; Parpura et al., 1994; Clark and Barbour, 1997; Newman and Zahs, 1998; Grosche et al., 1999; Fields and Stevens, 2000; Araque et al., 2000, 2001; Parpura and Haydon, 2000). It is exciting to speculate on the possible physiological role for astrocytes in modulating synaptic activity, but glial Ca^{2+} signalling and the release of potentially cytotoxic factors may have greater significance in neuropathology. Moreover, the release of neurotransmitters by astrocytes and gap junctional communication between glial cells provides a potential communication pathway for long range glial Ca^{2+} signalling, which may be the basis of the extensive reactive astrogliosis, myelin disintegration and microglial cell activation that characterise the glial response to CNS injury.

3. Glial P2 purinoceptors

It may be no exaggeration to state that ATP is the primary extracellular signalling molecule for glial cells in the CNS, where it has both physiological and pathological functions. ATP mediates raised intracellular $[Ca^{2+}]_i$ via P2 purinoceptors in all glial cell types in vitro and in situ to activate a number of signal transduction pathways (Table 1). Sources of extracellular ATP in the CNS include (1) neuronal release at synapses and along axons (Edwards et al., 1992; Hamann and Attwell, 1996), (2) release from astrocytes by a poorly understood mechanism (Guthrie et al., 1999), but could possibly involve pore-forming P2 purinoceptors (Ballerini et al., 1996), and (3) the "flooding" of the extracellular space with cytosolic ATP following cellular damage (White and Hoehn, 1991). It is likely that the latter two processes are the most important in glial cell pathology. However, it is often difficult to distinguish the functions of different glial P2 purinoceptors, not least because glial cells express heterogenous purinoceptors, but also because of the varied techniques and preparations used to study them. In addition, the pharmacology of cloned purinoceptors may not always reflect the endogenous receptors expressed by glial cells. P2 receptors are divided into metabotropic P2Y and ionotropic P2X receptors (reviewed by Ralevic and Burnstock, 1998), and both mediate glial Ca^{2+} signalling and have identified roles in glial cell physiology and pathology. P2Y receptors are G-protein linked receptors that increase intracellular inositol trisphosphate (IP_3), triggering release of Ca^{2+} ions from thapsigargin-sensitive stores into the cytosolic compartment. P2X receptors are ligand-gated non-specific cation channels, which increase $[Ca^{2+}]_i$ both by allowing direct Ca^{2+} ion entry, and by an inward Na^+ current, which depolarises the cell and opens voltage-operated Ca^{2+} channels (Soto et al., 1997). Purinoceptors that have now been cloned include the ionotropic purinoceptors $P2X_{1-7}$ (North and Surprenant, 2000), and the metabotropic receptors $P2Y_{1,2,4,6,11,12}$ (Ralevic and Burnstock, 1998; Hollopeter et al., 2001). This terminology has replaced earlier nomenclature that was based on pharmacological characteristics (e.g. P_{2Y}, P_{2U}, P_{2Z}, etc.). Additionally, some P2X purinoceptors are capable of a conformational change that results in larger pore diameter following prolonged (seconds) exposure to ATP. This property was initially believed to be exclusive to the $P2X_7$ subtype (Surprenant et al., 1996), but has been described in $P2X_2$ and $P2X_4$ subtypes, and may be a universal characteristic of P2X receptors (Khakh et al., 1999). In general, P2 receptors can transmit very fast (<10 ms) to long-term (>60 s) signals, depending on the type of receptor, length of exposure to and concentration of ATP, which in turn affects the dynamics of the increase in $[Ca^{2+}]_i$ (Ralevic and Burn-

Table 1
Purinoceptor expression in CNS glia

Purinoceptor subtype	Preparation	Techniques	Transduction signal	Reference
Astrocytes				
P2	in situ	A	Ca^{2+} release	Kriegler and Chiu, 1993
P2	in situ	A, B, E	Ca^{2+} release, SOCC activation	Bernstein et al., 1996
P2	culture	A, F	propagation of intercellular Ca^{2+} waves	Guthrie et al., 1999
P2	culture	A, B, E, F	propagation of intercellular Ca^{2+} waves	Cotrina et al., 2000
P2Y	culture	A	Ca^{2+} release	Kastritsis et al., 1992
P2Y	culture	F, M	complex actions on proliferation	Ciccarelli et al., 1994
P2Y	culture	C, F, J	PLA_2 activation, AA release, Fos/Jun	Bolego et al., 1997
P2Y	culture	A	PLC activation, Ca^{2+} release	Centemeri et al., 1997
P2Y	culture	A	Ca^{2+} release	Bernstein et al., 1998
P2Y	culture	H, J	COX2 dependent astrogliosis	Brambilla et al., 2000
P2Y	culture	F	inhibition of cRaf-1 activation	Lenz et al., 2001
$P2Y_1$	culture	F, G	induction of c-fos, c-jun, junB, TIS11	Priller et al., 1998
$P2Y_1$	in situ	C, I		Morán-Jiménez and Matute, 2000
$P2Y_1$	culture	A, I	propagation of intercellular Ca^{2+} waves	Fam et al., 2000
P2U and P2Y	culture	A, F, H	IP_3, TXA production	Bruner and Murphy, 1993
P2U and P2Y	culture	A, F	MAPK activation	King et al., 1996
P2U > P2Y	culture	F	PKC regulated Ca^{2+} entry	Chen and Chen, 1996
$P2Y_1$, $P2Y_2$	culture	A, F, H	PLC-MAPK-PLA_2 cascade, AA release	Chen and Chen, 1998
$P2Y_1$, $P2Y_2$	isolated	A, I	Ca^{2+} release	Zhu and Kimelberg, 2001
$P2Y_1$, $P2Y_{2/4}$, $P2Y_6$	culture	A, G	Ca^{2+} release	Jiménez et al., 2000
$P2Y_1$, $P2Y_2$, $P2Y_4$	culture	F, I	Erk, Mek activation	Lenz et al., 2000
P2X	culture	F, I		Magowski and Walz, 1992
$P2X_1$	in situ	C		Loesch and Burnstock, 1998
$P2X_2$	in situ	C, D		Kanjhan et al., 1999
$P2X_{1,2,3,4,7}$	in situ	C, H		Franke et al., 2001a
$P2Y_{1,12}$>P2X	in situ	C, H	astrogliosis	Franke et al., 2001b
$P2X_{1,2,3,4,6,7}$	in situ	C		Kukley et al., 2001
$P2X_7$	in situ	A	Ca^{2+} entry, YO-PRO-1 entry	James and Butt, 2001b; this study
$P2X_7$	culture	A, E	plasmalemmal pore formation	Ballerini et al., 1996
$P2X_7$	culture	A, F	PKC regulated PLD activation	Sun et al., 1999
$P2X_7$	culture	C, F, H	activation of Erk, MCP-1 upregulation	Panenka et al., 2001
$P2X_7$	culture	F, I	PLD activation	Hung and Sun, 2002
$P2Y_1$, $P2Y_2$, $P2X_7$	culture	F, I	potentiation of IL-1β activation of NF-κB	John et al., 2001
$P2Y_1 = P2Y_2 = P2X$	in situ	A	Ca^{2+} release	James and Butt, 1999
$P2Y_1$>$P2Y_{2/4}$>P2X	in situ	A	Ca^{2+} release	James and Butt, 2001a
P2Y = P2X	in situ	A	Ca^{2+} release	James and Butt, 2001b
	culture	G, H	reduces iNOS expression, NF-κB binding	Lin and Murphy, 1997
Müller glia				
$P2Y_1$	in situ	A, C	evokes intercellular calcium waves	Li et al., 2001
P2X (poss. P2Y)	in situ	K	reduces GABA uptake	Neal et al., 1998
$P2X_{3,4,5}$	isolated	I		Jabs et al., 2000
$P2X_7$	isolated	A, B, C, I	Ca^{2+} release	Pannicke et al., 2000
Bergmann glia				
P2Y	in situ	A, B	Ca^{2+} release	Kirischuk et al., 1995a
Microglia				
Heterogenous P2	in situ	A		Möller et al., 2000
P2Y	culture	D, G	induction of c-fos, c-jun, junB, TIS11	Priller et al., 1995
P2Y and pyrimidinoceptor	culture	B	Ca^{2+} release, SOCC activation	Norenberg et al., 1997
P2Y (non-G protein linked)	culture	F, I	p38/PKC dependent IL-6 release	Shigemoto-Mogami et al., 2001
$P2Y_{12}$	culture	F, J	membrane ruffling, chemokinesis/taxis	Honda et al., 2001
P2X	culture	A, B	nonspecific cation current	McLarnon et al., 1999
P2X	culture	B	cation conductance	Walz et al., 1993
$P2X_7$	culture	F	IL-1β release	Ferrari et al., 1997a
$P2X_7$	culture	F, J, L	apoptosis, necrosis	Ferrari et al., 1999a
$P2X_7$	culture	H	NFAT, NF-κB upregulation	Ferrari et al., 1999b
$P2X_7$	culture	B	plasmalemmal pore formation	Chessell et al., 1997
$P2X_7$	in situ	C, D		Collo et al., 1997
$P2X_7$	culture	A, F, H	plasminogen release	Inoue et al., 1998

(continued on next page)

Table 1 (*continued*)

Purinoceptor subtype	Preparation	Techniques	Transduction signal	Reference
Microglia				
$P2X_7$	culture	E, F, H	Erk/p38 activation, TNFα release	Hide et al., 2000
$P2X_7$	culture	A, C, F		Verderio and Matteoli, 2001
$P2X_7$	culture	B, E, F	IL-1β release, receptor priming	Chafke et al., 2002
$P2X_7$ and P2Y	culture	A, E	IL-1β release, morphology changes	Ferrari et al., 1996
$P2X_7$; other P2X; P2Y	culture	A, B		Visentin et al., 1999
$P2X_7$; other P2X; P2Y	culture	F, G	induction of iNOS, NO release	Ohtani et al., 2000
$P2X_7$; other P2X; P2Y	culture	F, H	caspase-1/ICE dependent IL-1β release	Sanz and Di Virgilio, 2000
$P2X_7$; other P2X; P2Y	culture	J	ramification	Wollmer et al., 2001
Oligodendrocytes				
P2	culture	A	Ca^{2+} release	Kastritsis and McCarthy, 1993
P2	in situ	A		Kriegler and Chiu, 1993
$P2Y_1$ and $P2Y_2$>P2X	in situ	A	Ca^{2+} release	Kirischuk et al., 1995b
P2	in situ	A, B, E	Ca^{2+} release, SOCC activation	Bernstein et al., 1996
$P2Y_1$	in situ	C, I		Móran-Jiménez and Matute, 2000
P2Y	in situ	A	Ca^{2+} release	James and Butt, 2001a
$P2Y_{12}$	in situ	N	$G_{i/o}$ activation	Laitinen et al., 2001
P2X	in situ	A	$[Ca^{2+}]_i$ increase	James and Butt, 2001a
$P2X_7$	in situ	A	Ca^{2+} entry, YO-PRO-1 entry	James and Butt, 2001b; this study

Techniques key: (A) $[Ca^{2+}]_i$ fluorimetry; (B) patch-clamp electrophysiology; (C) immunohistochemistry; (D) in situ hybridisation; (E) dye entry study; (F) appropriate biochemical assay; (G) Northern blotting; (H) Western blotting; (I) RT-PCR; (J) morphological analysis; (K) neurotransmitter assay; (L) cell death assay; (M) HPLC; (N) autoradiography.

Abbreviations: SOCC, store operated Ca^{2+} current; PLA_2, phospholipase A_2; AA, arachidonic acid; PLC, phospholipase C; COX, cyclooxygenase; IP_3, inositol trisphosphate; TXA, thromboxane; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; PLD, phospholipase D; MCP-1, monocyte chemoattractant protein-1; iNOS, inducible nitric oxide synthase; NFAT, nuclear factor of activated T cells; TNFα, tumour necrosis factor α; NO, nitric oxide; ICE, interleukin-converting enzyme.

stock, 1998; Khakh, 2001). This is important when considering the functions of different purinoceptors, since the large changes in glial $[Ca^{2+}]_i$ measured in most studies are characteristic of only a few purinoceptor subtypes.

3.1. Astrocytes

The focus on purinoceptor expression in astrocytes has been on the function of P2Y receptors in mediating Ca^{2+} signalling, mainly in culture. Early studies on cultured astrocytes showed activation of P2Y receptors mediated IP_3 production (Kastritsis et al., 1992) and UTP-preferring P2Y receptors ($P2Y_{2/4}$) activated several downstream events, including thromboxane production, IP_3 production and increased $[Ca^{2+}]_i$ (Bruner and Murphy, 1993). Further evidence for P2Y expression in cultured astrocytes came from the hippocampus (Bernstein et al., 1998), and a study showing that single cerebellar astrocytes express $P2Y_1$ receptors, plus either $P2Y_2$ or $P2Y_4$, with 30–40% also expressing the $P2Y_6$ receptor (Jiménez et al., 2000). However, a study comparing acutely isolated astrocytes with cultured astroglia found that glial fibrillary acidic protein (GFAP)-positive astrocytes lost their $[Ca^{2+}]_i$ response to ATP following acute isolation from hippocampus, while GFAP-negative cells retained their responses (Kimelberg et al., 1997). Zhu and Kimelberg (2001) recently provided evidence of a marked up-regulation of mRNA for $P2Y_2$ receptors during astrocyte development, whereas mRNA for $P2Y_1$ receptors was present at all ages. Conversely, a study in corpus callosum slices found ATP responses consistent with P2Y receptor expression in white matter glia, identified as a mixture of mature astrocytes and glial progenitors (Bernstein et al., 1996). Immunohistochemical confirmation of $P2Y_1$ expression by astrocytes has been demonstrated specifically in white matter areas such as corpus callosum, medullary tracts and optic nerve (Morán-Jiménez and Matute, 2000). Although earlier studies provided little evidence for P2X receptor expression by astrocytes, electrophysiological data did indicate astroglial membrane currents linked to extracellular ATP application (Magowski and Walz, 1992). P2X receptors have now been demonstrated in cultured astrocytes, notably the $P2X_7$ (P2Z) receptor, which increases $[Ca^{2+}]_i$ and causes purine release (Ballerini et al., 1996). There is also direct immunohistochemical evidence for glial expression of $P2X_1$ (Loesch and Burnstock, 1998) and $P2X_2$ (Kanjhan et al., 1999) subtypes, and hippocampal astrocytes have been shown to co-express $P2X_{1-4}$, $P2X_6$ and $P2X_7$ subunits (Kukley et al., 2001). We have shown that mature, immature and reactive astrocytes express both P2Y and P2X purinoceptors in situ (James and Butt, 1999, 2001a,b).

Functional P2 purinoceptors have also been demonstrated in Bergmann glia and Müller cells (Kirischuk et al., 1995a; Newman and Zahs, 1997). Bergmann glia and Müller cells, like astrocytes, express P2Y purinoceptors, which release Ca^{2+} from intracellular IP_3-regulated stores (Kirischuk et al., 1995a; Newman and Zahs, 1997). However, while no P2X-like responses could be elicited from

Bergmann glia, a great deal of interest has focussed on P2X ionotropic receptors in Müller cells and their possible role in modulation of retinal neuronal activity (Neal et al., 1998). The reports are conflicting. One group of investigators, using single-cell reverse transcription polymerase chain reaction (RT-PCR) in isolated rat Müller cells, found expression of $P2X_3$, $P2X_4$ and $P2X_5$ mRNA, but no evidence for $P2X_7$ (Jabs et al., 2000), whereas a study of isolated human Müller cells found pharmacological and molecular evidence for $P2X_7$ receptor expression (Pannicke et al., 2000). Interestingly, the authors found no evidence in Müller cells that $P2X_7$ forms a large-diameter membrane pore, which is usually associated with activation of this receptor (Virginio et al., 1999).

3.2. Microglia

Walz et al. (1993) first reported that cultured microglia responded to ATP with an inward cation current and outward K^+ current, with a concurrent increase in $[Ca^{2+}]_i$, consistent with P2X expression. Later investigations have determined that cultured microglia expressed P2Y receptors and the pore-forming receptor $P2X_7$, which was associated with Ca^{2+} influx and also interleukin-1β release (Ferrari et al., 1996; Chafke et al., 2002), as well as another as yet unidentified P2X component (Visentin et al., 1999). Microglia also express P2X-like and P2Y-like receptors that, among other effects, act together to regulate membrane potential (Illes et al., 1996; Norenberg et al., 1997). The different kinds of receptors may act synergistically to mediate the effects of ATP on microglia. For example, it has been shown that applying ATP to these cells causes an initial $[Ca^{2+}]_i$ peak via release from intracellular stores, followed by an elevated $[Ca^{2+}]_i$ plateau caused by Ca^{2+} entry from the extracellular milieu (McLarnon et al., 1999). The presence of $P2X_7$ receptors on microglia has generated the greatest interest because of its possible role in pathology. However, immunohistochemical and Ca^{2+} measurement studies are conflicting. The former indicated that $P2X_7$ receptors were only strongly expressed in situ following microglial activation by brain ischaemic injury (Collo et al., 1997), whereas the latter provided evidence that resting microglia expressed heterogenous P2 purinoceptors, but that activated microglia lost their sensitivity to ATP (Möller et al., 2000). Microglia also express an unknown P2Y receptor, possibly the $P2Y_{12}$ subtype (Honda et al., 2001), and regulate hydrolysis of ATP and its breakdown products via expression of the enzyme ectonucleoside triphosphate diphosphohydrolase (Braun et al., 2000).

3.3. Oligodendrocytes

While astrocytes, especially cultured astrocytes, have been well studied, comparatively little work has been done on purinoceptor expression in oligodendroglia. ATP has been shown to increase $[Ca^{2+}]_i$ in cultured cortical oligodendrocytes (Kastritsis and McCarthy, 1993) and in oligodendroglia in situ in corpus callosum slices and the intact optic nerve (Kirischuk et al., 1995b; James and Butt, 2001a). The $[Ca^{2+}]_i$ response was mediated primarily via $P2Y_1$ purinoceptors triggering Ca^{2+} release from IP_3-sensitive stores, but there was evidence for heterogenous P2 receptors, and differences between oligodendrocytes from different brain areas, and between the soma and processes of individual cells. The demonstration of $P2Y_1$ immunoreactivity in oligodendroglia in vivo supports the possibility that this receptor subtype has similar roles in oligodendrocyte and astrocyte signalling (Morán-Jiménez and Matute, 2000). Interestingly, cultured oligodendrocyte progenitor cells were found not to respond to ATP (Kastritsis and McCarthy, 1993; Kirischuk et al., 1995b), whereas a study in the corpus callosum slice showed that ATP triggered Ca^{2+} release from IP_3-sensitive stores in a high proportion of precursor-like cells (Bernstein et al., 1996). The $P2Y_{ADP}$ receptor (also called the $P2T_{AC}$ receptor), previously thought to be expressed solely on platelets and a few other blood cell types, has also now been reported to be expressed on oligodendrocyte progenitor-like cells in the CNS (Laitinen et al., 2001). This receptor, recently identified as the cloned $P2Y_{12}$ receptor (Hollopeter et al., 2001), is linked to $G_{i/o}$ and has unresolved functions.

4. ATP-evoked glial Ca^{2+} signalling in situ is mediated via both P2Y and P2X purinoreceptors

In order to investigate P2 receptor signalling in glial cells in situ, we have used the optic nerve, because it is a simple white matter tract that contains axons and the glial cells that support them, but not neurons or synapses (James and Butt, 2001a). Accordingly, all cellular responses to agents superfused over the nerve surface are those of glial cells, which can be identified by their characteristic morphology and positions in the nerve (Butt and Ransom, 1993). We found that application of high concentrations of ATP (>100 μM) for 30–60 s causes a rapid and prolonged increase in cytosolic Ca^{2+} in astrocytes and oligodendrocytes, which peaks within 5 s and falls to a slowly decaying level in the continued presence of external ATP (Fig. 1A). Both the P2Y receptor agonist 2-methylthioATP (2MeSATP) and the P2X selective agonist α,β-methylene ATP (α,β-metATP) effected an increase in $[Ca^{2+}]_i$ in both glial cell types (Fig. 1B). In addition, the response was shown to be equivalent in immature and mature nerves and in enucleated optic nerves (Fig. 2A), indicating it is not dependent on age or neuronal contact (James and Butt, 1999, 2001a,b). A similar P2 receptor mediated increase in $[Ca^{2+}]_i$ has been demonstrated in situ for retinal astrocytes, Bergmann glia, Müller cells, oligodendrocytes and microglia (Kirischuk et al., 1995a,b; Möller et al., 2000; Newman, 2001).

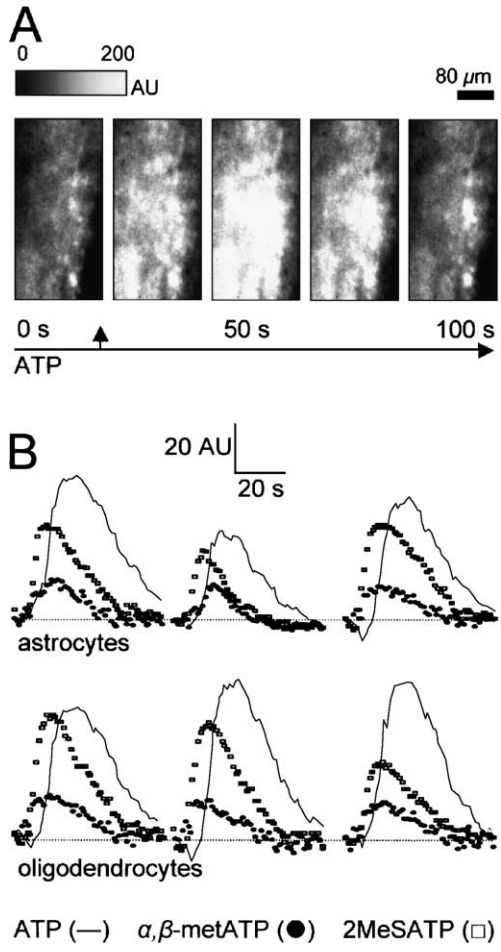

Fig. 1. P2Y and P2X purinoceptor-mediated Ca^{2+} signalling in optic nerve astrocytes and oligodendrocytes in situ. Optic nerves were loaded with fluo-3 and imaged during application of extracellular ATP, the P2Y purinoceptor agonist 2MeSATP and P2X purinoceptor agonist α,β-metATP and individual glial cells were analysed offline. (A) Sequence of images illustrating the rapid and transient rise in glial $[Ca^{2+}]_i$ following application of ATP (1 mM). The response of individual cells can be resolved and classified as either oligodendrocytes or astrocytes, on morphological criteria (James and Butt, 2001a). (B) Traces of fluo-3 intensity from astrocytes and oligodendrocytes, following application of ATP (continuous line), MeSATP (open squares) and α,β-metATP (closed circles; all 1 mM). AU = arbitrary unit.

The ATP-mediated increase in glial $[Ca^{2+}]_i$ in optic nerve glia is mediated predominantly by metabotropic P2Y purinoreceptors, with a small but significant ionotropic P2X component. This has been confirmed by the actions of thapsigargin and removal of extracellular Ca^{2+} (James and Butt, 2001a). Thapsigargin markedly reduced the ATP-mediated increase in glial $[Ca^{2+}]_i$, indicating that major source of Ca^{2+} was IP_3-dependent release from intracellular stores. Removal of extracellular Ca^{2+} also caused an initial small reduction in the ATP evoked increase in $[Ca^{2+}]_i$, consistent with its actions on P2X receptors. To investigate this further, we have used a number of agonists and antagonists to help distinguish between purinoceptor subtypes in immature, mature and axon-free nerves (Fig. 2B). Optic nerve glia are most responsive to ADP and 2MeSATP (James and Butt, 2001a), which are potent and selective agonists of $P2Y_1$ purinoreceptors (Leon et al., 1997). In addition, low (10 μM) concentrations of UTP evoke large increases in $[Ca^{2+}]_i$ in optic nerve glia, indicating they also express $P2Y_2$ and/or $P2Y_4$ receptors (King et al., 1996), which have been shown to activate an increase in $[Ca^{2+}]_i$ in cultured astrocytes (Bruner and Murphy, 1993; Ho et al., 1995; Zhu and Kimelberg, 2001), oligodendrocytes (Kirischuk et al., 1995b) and microglia (Möller et al., 2000). Application of α,β-metATP also evoked a large increase in glial $[Ca^{2+}]_i$ and, interestingly, this was increased following injury (Fig. 2B). α,β-MetATP is a potent agonist of both $P2X_1$ and $P2X_{2/3}$ receptors (North and Surprenant, 2000), and all three subtypes have been demonstrated immunohistochemically on glia in the brain (Loesch and Burnstock, 1998; Kanjhan et al., 1999; Kukley et al., 2001). However, the relatively low sensitivity of optic nerve glia to α,β-metATP, compared to ATP or 2MeSATP, and the fact that both $P2X_1$ and $P2X_3$ receptors desensitise rapidly, indicates that they are unlikely to be a major component of the ATP-mediated increases in $[Ca^{2+}]_i$ measured in optic nerve glia.

5. P2Y receptors and the propagation of glial Ca^{2+} signalling

We have found that the minimal concentrations that evoked a measurable increase in glial $[Ca^{2+}]_i$ in the optic nerve were 100 nM for ATP and 2MeSATP, compared to 10 μM for α,β-metATP (Fig. 2C). The high sensitivity component of the response of optic nerve glia to ATP is mediated by both $P2Y_1$ and $P2Y_{2/4}$ receptors (James and Butt, 2001a), consistent with reports that glial cells in vitro and in slices express a heterogeneity of P2Y receptors (Kirischuk et al., 1995a,b; Jiménez et al., 2000). This suggests that P2Y receptors have a specific role in $[Ca^{2+}]_i$ signalling at physiological concentrations of ATP in astrocytes and oligodendrocytes in situ (Fig. 2C). The evidence that ATP release and purinoceptor activation form an extracellular pathway for intercellular $[Ca^{2+}]_i$ signalling came first from studies on astrocyte cultures (Guthrie et al., 1999), and a novel imaging method has shown waves of ATP release from stimulated cultured astrocytes (Wang et al., 2000). Interestingly, ATP was released by a Ca^{2+}-independent mechanism, a finding which is supported by the observation in single astrocytes that autocrine ATP release is responsible for the $[Ca^{2+}]_i$ increase in response to mechanical stimulation (Shiga et al., 2001). The evidence is that $P2Y_1$ receptors are specifically involved in propagation of $[Ca^{2+}]_i$ waves in cultured spinal cord astrocytes (Fam et al., 2000). In situ evidence for propagation of intercellular $[Ca^{2+}]_i$ waves via ATP release comes from studies on the retina. It has been demonstrated in retinal slices that ATP can cause intra- and intercellular $[Ca^{2+}]_i$ waves, probably via $P2Y_1$ receptors (Li et al., 2001). A study on the eyecup preparation revealed

that $[Ca^{2+}]_i$ waves are propagated both by intracellular diffusion through gap junctions between astrocytes, and by extracellular ATP between astrocytes and Müller (Newman, 2001).

Newman (2001) calculated that the level of extracellular ATP released by retinal astrocytes following mechanical stimulation as 78 μM at the site of stimulation and 6.8 μM at 100 μm away from this site. The dose–response curves in optic nerve glia (Fig. 2C) indicate that the latter concentration would primarily activate high-affinity P2Y receptors, which may be representative of localised, brief and intermittent increases in extracellular ATP during physiological signalling. However, 78 μM ATP would activate both P2Y and P2X purinoceptors and induce an increase in glial $[Ca^{2+}]_i$ of over 100 μM, which is near maximal (Fig. 2C). This may reflect a pathological situation, when there is a prolonged and high increase in extracellular ATP concentration. Although it is generally considered that this occurs due to cell lysis, the study of Newman (2001) raises the possibility the release of ATP by astrocytes would activate glial P2X receptors and initiate glial cell reactivity. Moreover, astrocytes would amplify the initial stimulus and spread it throughout the glial syncytium, thereby starting a 'vicious' cycle. This would help explain why astrocytes, NG2 glia and microglia are activated at long (mm) distances from the site of any CNS damage, far from any direct pathological challenge. Two recent studies have provided direct evidence of this phenomenon in vitro (Verderio and Matteoli, 2001) and in situ (Schipke et al., 2001). In the latter study, astrocytes in corpus callosum slice were loaded with Ca^{2+}-sensitive dye that did not enter microglial cells, which were recorded from electrophysiologically. Following stimulation of single astrocytes, either electrically or by highly localised ATP release, there was a purinoceptor-mediated propagation of an intercellular Ca^{2+} wave that activated P2X-mediated currents when it passed microglial cells. Similarly, Verderio and Matteoli (2001), showed that intracellular Ca^{2+} waves induced the release of ATP by astrocytes, which in turn triggered a Ca^{2+} response in microglia via $P2X_7$ receptors. These elegant studies demonstrate that astroglial Ca^{2+} waves not only signal between astrocytes, but also modulate the behaviour of other glial cell types and potentially induce reactive changes distant from the site of CNS damage.

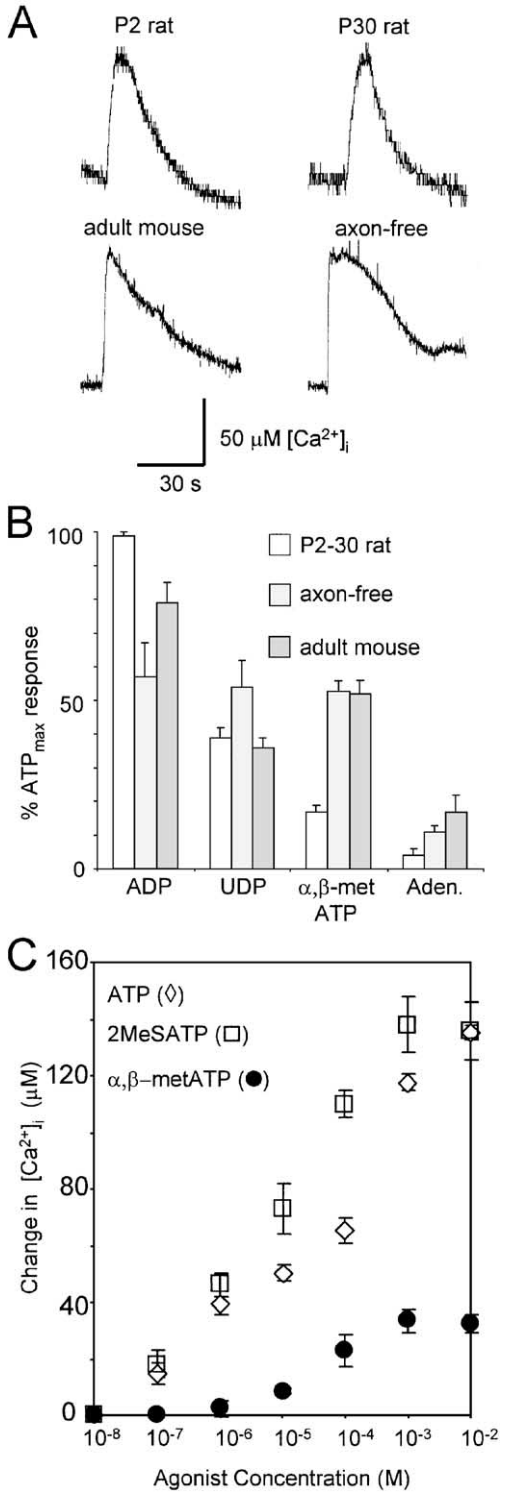

Fig. 2. Optic nerve glia express heterogenous P2Y and P2X purinoceptors in situ that are activated at different concentrations of ATP. Nerves were loaded with fura-2 and the change in the F_{340}/F_{380} ratio was measured during application of purinoceptor agonists, and the change in $[Ca^{2+}]_i$ was calibrated using commercial standards. (A) Application of ATP (1 mM) evoked a rapid increase in $[Ca^{2+}]_i$ by approximately 100 μM in neonatal rat (P2), juvenile rat (P30), adult mouse and enucleated rat (axon-free) optic nerves. Experiments in the enucleated nerve confirm that the fura-2 response is glial (James and Butt, 1999, 2001a,b). The ATP-mediated increase in $[Ca^{2+}]_i$ was not related to age, but was significantly longer in duration in reactive glia of the enucleated nerve, compared to normal nerves. (B) The rank order of potency for purinoceptor agonists in normal juvenile, axon-free and adult rat and mouse optic nerves, indicating that optic nerve glia express heterogenous purinoceptors, including $P2Y_1$, $P2Y_{2,4}$ and P2X, and A_1 receptors. Reactive glia exhibited a relative increase in $P2Y_{2,4}$, P2X and A_1 purinoceptors. Bars represent means ± S.E.M. ($n = 3$). (C) Dose–response curves for ATP, 2MeSATP and α,β-metATP in rat optic nerves, plotted as agonist concentration against evoked rise in $[Ca^{2+}]_i$. The results indicate that P2Y and P2X purinoceptors evoked a large increase in glial $[Ca^{2+}]_i$ at μM and mM concentrations, respectively. The response to ATP was not saturated even at 10 mM. Points represent means ± S.E.M. ($n = 3$).

6. A role for $P2X_7$ receptors in mediating the glial injury response

Our results indicated that P2X receptors only evoke large influxes of Ca^{2+} at high concentrations of ATP (Fig. 2C), suggesting they have a specific, but not exclusive, role in glial cell pathology. This possibility is supported by our observation that in reactive astrocytes, there is a relative down-regulation of P2Y receptors and up-regulation of P2X receptors (Fig. 2B). In addition, there are distinct differences in the response of glial cells depending on the concentration and duration of exposure to ATP (Gallagher and Salter, 1999). Low and high concentrations of ATP evoke two distinct Ca^{2+} responses in optic nerve glia (Fig. 3), and there is evidence that this is related to whether P2Y or P2X receptors are activated. The increase in glial $[Ca^{2+}]_i$ mediated by P2Y receptors is dependent on release from intracellular stores, and so it declines during exposure to ATP and is slow to recover when ATP is removed; as a result, the P2Y-mediated response to ATP is self-limiting (Fig. 3A). Moreover, compared to lower concentrations of ATP (<100 μM) (Fig. 3B), application of 10 mM ATP, which is the concentration that could be released following cell lysis, evokes an increase in glial $[Ca^{2+}]_i$ that is not only twice that induced by 100 μM ATP, but $[Ca^{2+}]_i$ remains elevated for minutes after 30 s exposure to ATP (Fig. 3C). Thus, a major component of the P2X-mediated response to high concentrations of ATP exhibits little saturation (Figs. 2C and 3C). The amplitude and temporal dynamics of the glial Ca^{2+} signal therefore depends on the duration of exposure to ATP and the purinoceptors activated. Different purinoceptors have different EC_{50} values for ATP (for review, see Ralevic and Burnstock, 1998), and the reason glial cells express such a diverse collection of purinoceptors may be so that physiological and pathological Ca^{2+} signalling have separate pathways. Such a "Jekyll and Hyde" action of ATP has been suggested in peripheral immune cells (Trams et al., 1989), with high ATP concentrations causing cell lysis via the pore-forming and relatively ATP-insensitive $P2X_7$ subtype (Di Virgilio, 2000).

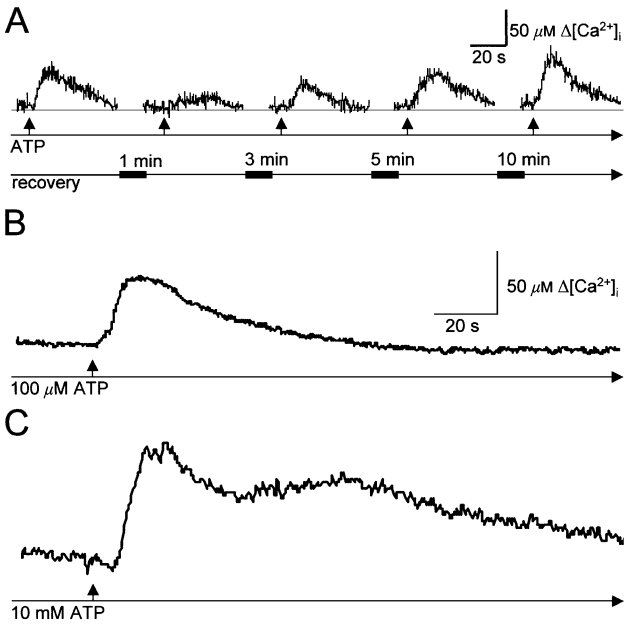

Fig. 3. The amplitude and temporal dynamics of the glial Ca^{2+} signal depends on the duration of exposure to ATP and the purinoceptors activated. (A) Recordings of changes in $[Ca^{2+}]_i$ following sequential pulses of 30 s administration of ATP (1 mM) given at increasing time intervals show that the response to ATP is self-limiting and takes 10 min to fully recover. There is a small but sustained component that does not desensitise and is activated even when two pulses of ATP are administered immediately one after the other. (B) Recording of changes in glial $[Ca^{2+}]_i$ following 30 s application of 100 μM ATP, equivalent to concentrations released during astroglial Ca^{2+} signalling, illustrating that the increase in $[Ca^{2+}]_i$ is transient and decays towards basal levels during agonist application. (C) Application of 10 mM ATP for 30 s, which could occur during CNS pathology and cell lysis, induced a much larger increase in glial $[Ca^{2+}]_i$, and which remained high and above baseline levels for 3 min.

The low sensitivity of the P2X component in optic nerve glia and the fact that the response to ATP did not saturate or desensitise at very high concentrations led us to suggest it was mediated by the pore-forming $P2X_7$ receptors (James and Butt, 2001a), which has been shown in astrocytes and microglia (Table 1). To investigate this further, we have begun to use the $P2X_7$ specific agonist benzoyl-benzoyl ATP (BzATP; Fig. 4A) and the dye YO-PRO-1 (Fig. 4B), which passes into cells through pore-forming $P2X_7$ receptors. Our results indicate that astrocytes, oligodendrocytes and microglia express $P2X_7$ in situ, and that they are specifically activated at very high extracellular concentrations of ATP to mediate a large and sustained increase in glial $[Ca^{2+}]_i$. Very recent data indicates that $P2X_7$ receptors in CNS cells may not be pore-forming, possibly due to their different configuration from those of the periphery (Kim et al., 2001). Future studies will need to determine whether this is the case in glial cells and to test the possibility that $P2X_7$ receptors mediate their distinctive injury responses. For example, pore-forming $P2X_7$ receptors in oligodendrocytes may trigger the myelin damage and cell death that is characteristic of CNS injury. Conversely, $P2X_7$ receptors may not be pore forming in astrocytes, in which cell death is not a major factor, and they may mediate the dramatic proliferation and cell growth that results in the formation of the glial scar. In microglia, $P2X_7$ receptors may initiate microglial activation, but it is unclear whether the receptors are subsequently downregulated (Möller et al., 2000) or upregulated (Collo et al., 1997). There is evidence that $P2X_7$ receptors are upregulated in activated microglia following brain ischaemic injury (Collo et al., 1997), and in Müller glial cells during proliferative vitreoretinopathy (Bringmann et al., 2001). A similar upregulation in reactive astrocytes has been indicated in the enucleated optic nerve (James and Butt, 2001b). These results are consistent with

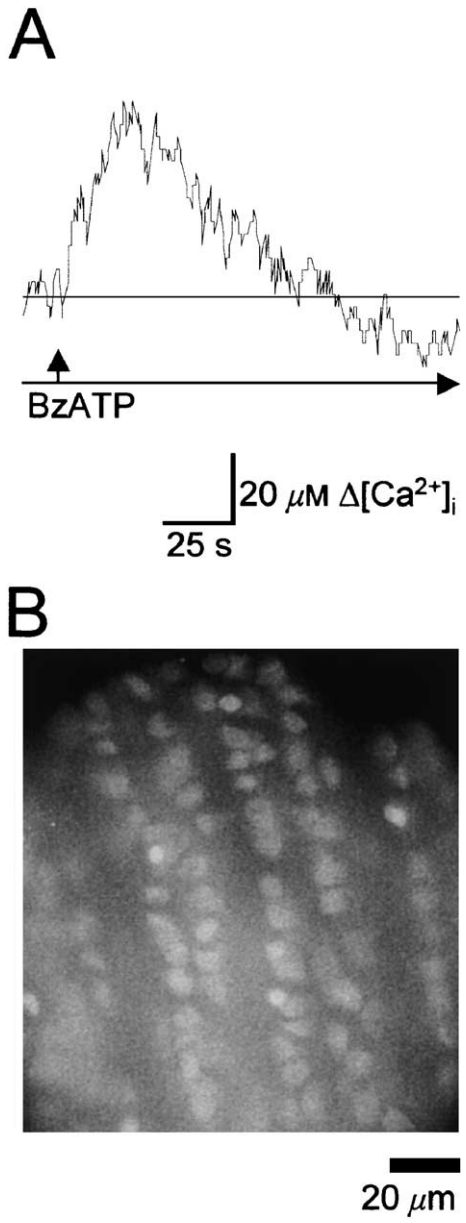

Fig. 4. Evidence for $P2X_7$ receptor expression in optic nerve glia in situ. (A) Brief application (<30 s) of the $P2X_7$ specific agonist BzATP (100 μM) evoked an increase in $[Ca^{2+}]_i$ that was reversible. (B) Photomicrograph of an isolated intact optic nerve following incubation in the fluorescent dye YO-PRO-1 iodide (1 μM) for 20 min in the presence of ATP (1 mM). Interfascicular rows of oligodendrocytes and astrocytes are clearly visible, together with smaller, more intensely labelled cells that are most likely microglia and/or NG2 glia. The dark areas between rows of glia are the unlabelled fascicles of axons.

the proposed role of $P2X_7$ receptors in maintaining proliferation in gliotic cells (Bringmann et al., 2001).

7. Transduction mechanisms activated by P2 purinoceptors

As well as mobilisation of $[Ca^{2+}]_i$, activation of astroglial purinoceptors in vitro induces activation of protein kinase C, extracellular signal regulated kinase (ERK), cyclooxygenase-2, phospholipase A_2 synthesis, arachidonic acid synthesis and release, eicosanoid release, stimulation of mitogen-activated protein (MAP) kinases, and induction of immediate early genes (Bruner and Murphy, 1993; Salter and Hicks, 1995; King et al., 1996; Bolego et al., 1997; Chen and Chen, 1998; Priller et al., 1998; Brambilla et al., 2000). Activation of microglial P2 receptors has also been shown to induce several downstream events and these may predominantly involve $P2X_7$ receptors (Ferrari et al., 1996; Chafke et al., 2002). Conversely, nothing is known about purinoceptor-mediated transduction signals in oligodendrocytes.

7.1. Astrocytes

ATP stimulates proliferation and changes in astrocyte morphology in culture, including increased GFAP expression and process extension and thickening (Ciccarelli et al., 1994). An atypical G-protein coupled P2Y-like receptor activated by α,β-metATP has been shown to induce similar changes in cultured astrocytes, and these are associated with induction of immediate early genes Fos and Jun (Bolego et al., 1997). Purinoceptor activation also increases cyclooxygenase-2 expression, and both the phenotypic change and cyclooxygenase-2 increase could be prevented by pre-treatment with a cyclooxygenase-2 inhibitor, but were unaffected by the $P2X_7$ receptor-specific antagonist periodate oxidised ATP (oATP; Brambilla et al., 2000). It was suggested that the effects are $[Ca^{2+}]_i$ independent and involved phospholipase A_2 and arachidonic acid synthesis acting via autocrine release of prostaglandin E_2 (reviewed by Abbracchio et al., 1999). Our experiments show that α,β-metATP evokes an increase in glial $[Ca^{2+}]_i$ in situ (Fig. 1), and we now provide evidence that the ATP-mediated increase in glial $[Ca^{2+}]_i$ is potentiated by arachidonic acid (Fig. 5A) and is reduced by the phospholipase A_2 inhibitor mepacrine (Fig. 5B). Moreover, arachidonic acid increased both the peak amplitude and the duration of the raised $[Ca^{2+}]_i$ (Fig. 5A), similar to the response of optic nerve glia to ultra high concentrations of ATP (Fig. 3C), and to that observed in reactive astrocytes (Fig. 2A). The results support a role for an arachidonic acid-mediated response to ATP in the glial injury response. The few studies that have been performed in vivo support a role for P2Y and P2X purinoceptors in reactive gliosis. Microinfusion of the purinoceptor agonist 2MeSATP and antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) respectively increased and decreased GFAP expression and bromodeoxyuridine (BrdU) uptake in astrocytes following injury in the rat nucleus accumbens (Franke et al., 1999). PPADS also blocked the gliotic effect of 2MeSATP when the two were given together. Similar effects were seen with α,β-metATP and ADP-β-S, while UTP-γ-S was ineffective (Franke et al., 2001b). Purinoceptor activation, as well as its possible direct role in stimulating astrogliosis, can also interact with other pathophysiological

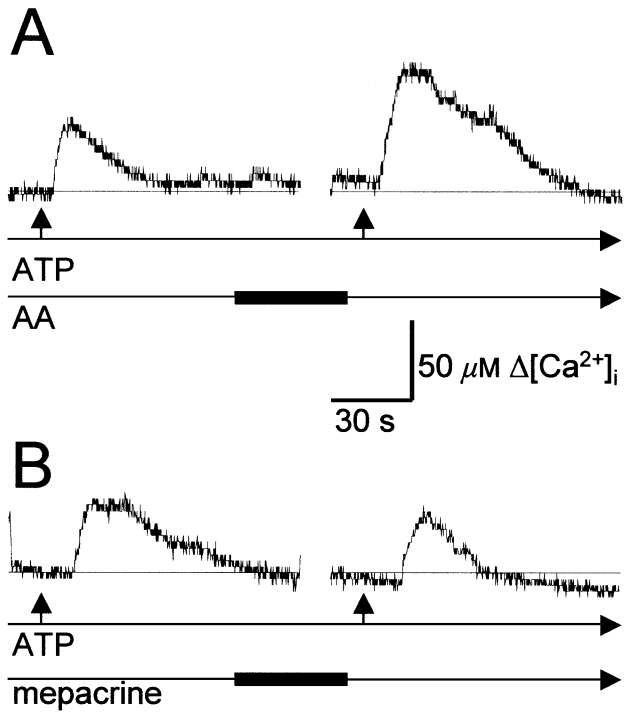

Fig. 5. The ATP-mediated increase in glial $[Ca^{2+}]_i$ is potentiated by arachidonic acid. (A) Incubation of nerves in arachidonic acid (100 μM) for 10 min increased both the amplitude and duration of the ATP mediated increase in $[Ca^{2+}]_i$ (right), compared to the same nerve pre-incubation (left). (B) Incubation of nerves in the phospholipase A_2 inhibitor, mepacrine (100 μM) for 10 min reduced both the amplitude and the duration of the ATP-mediated increase in $[Ca^{2+}]_i$ (right), compared to the same nerve prior to incubation (left).

pathways. Interleukin-1β, a cytokine known to induce activation of astrocytes (Lee et al., 1993), facilitates propagation of intercellular $[Ca^{2+}]_i$ waves in cultured astrocytes, via a down-regulation of the gap junctional pathway with a concurrent large increase in the extracellular purinergic pathway (John et al., 1999). Furthermore, ATP activates activator protein-1 (AP-1) and potentiates the interleukin-1β-mediated activation of the inflammatory transcription factors nuclear factor κB (NF-κB) and AP-1 (John et al., 2001). These effects were mediated by both P2Y and $P2X_7$ receptors.

7.2. Microglia

Microglia release a variety of pathological mediators in response to purinergic stimulation, including interleukin-1β via an unresolved $P2X_7$-mediated mechanism (Ferrari et al., 1996, 1997a,b; Sanz and Di Virgilio, 2000; Beigi and Dubyak, 2000; Chafke et al., 2002), interleukin-6 via a P2Y/protein kinase C pathway (Shigemoto-Mogami et al., 2001), tumour necrosis factor-α via $P2X_7$/ERK and p38 pathways (Hide et al., 2000), and plasminogen via $P2X_7$ activation (Inoue et al., 1998). ATP has also been shown to induce expression of nitric oxide (NO) synthase and cause NO release in microglia (Ohtani et al., 2000), as well as induction of immediate early genes (Priller et al., 1995). In addition, ATP stimulation of cultured microglia activates Caspase-1,3 and 8, which in turn mediate apoptotic cellular events, including chromatin condensation and DNA fragmentation (Ferrari et al. 1999a). ATP can also have direct effects on the phenotype and behaviour of microglial cells, causing membrane ruffling and chemotaxis, which are two classical microglial responses to CNS damage, acting via ADP-preferring, pertussis toxin-sensitive $P2T_{AC}$ ($P2Y_{12}$) receptors (Honda et al., 2001).

7.3. Oligodendrocytes

We are unaware of any studies on the downstream events initiated by purinoceptor activation in oligodendrocytes, but it is likely that many of those described in astrocytes and microglia are also applicable to oligodendrocytes. It is known that transient changes in cytosolic Ca^{2+} and IP_3 function as second messenger signalling mechanisms in oligodendrocytes and their progenitors (e.g. Haak et al., 2002), and our results indicate this would occur during activation of P2Y receptors at low ATP concentrations. Several growth factors induce Ca^{2+} influx in oligodendrocytes, including fibroblast growth factor-2 (FGF-2), which in turn activates the MAP kinase pathway and cyclic AMP response element binding protein (CREB) phosphorylation (Pende et al., 1997). In addition, the migration of oligodendrocyte progenitors in response to FGF-2 is dependent on Ca^{2+} influx (Simpson and Armstrong, 1999). Ca^{2+} influx also induces ERK activation in oligodendrocytes (Liu et al., 1999), which is required for oligodendrocyte process extension (Stariha et al., 1997). Oligodendrocyte proliferation is also functionally linked to growth factors and extracellular Ca^{2+} (Chattopadhyay et al., 1998). In astrocytes, activation of P2Y receptors synergises with the second messenger systems activated by FGF-2 to promote gliosis (Abbracchio et al., 1999), and a similar mechanism in oligodendrocytes would modulate the FGF-2 driven migration, proliferation and differentiation of oligodendrocytes. Furthermore, there are several studies showing that marked prolonged elevations in $[Ca^{2+}]_i$ can activate downstream events leading to cell death in oligodendrocytes (e.g. Knapp et al., 1999). In ischaemia, for example, glutamate-mediated Ca^{2+} influx mediates rapid cell death in immature oligodendrocytes in vitro (Fern and Möller, 2000) and mature oligodendrocytes in situ (Tekkök and Goldberg, 2001). In cultured oligodendrocytes, excessive increases in $[Ca^{2+}]_i$ can cause myelin retraction and cell death (Benjamins and Nedelkoska, 1996), and arachidonic acid has been shown to inhibit myelin production (Takeda and Soliven, 1997). We have provided evidence that oligodendrocytes express $P2X_7$ receptors and that pathological levels of ATP evoke a large and sustained influx of Ca^{2+} influx that is potentiated by arachidonic acid. However, it remains to be seen whether P2 purinoceptors mediate the myelin

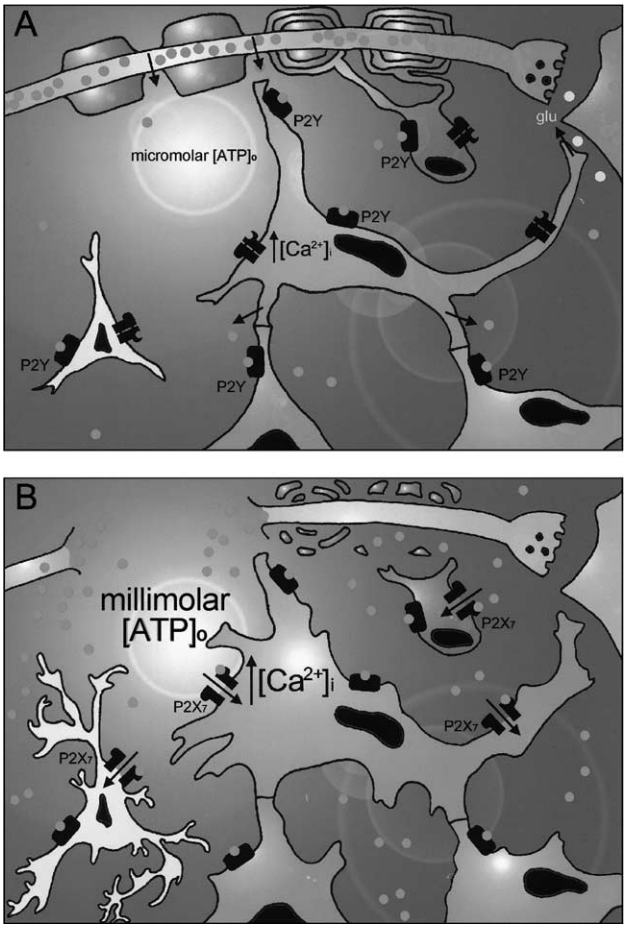

Fig. 6. Model of the dual roles for P2Y and P2X purinoceptors in CNS glial cell physiology and pathology. (A) During physiological signalling, axons and astrocytes release micromolar concentrations of ATP into the extracellular space. This will activate high-affinity purinoceptors (e.g. $P2Y_1$, $P2X_1$) and cause small and transient increases in glial $[Ca^{2+}]_i$, modulating physiological processes that may include glutamate and ATP signalling to neurons and other glia, via astroglial Ca^{2+} waves. (B) Following cellular damage in the CNS, millimolar ATP floods into the extracellular space. Such high ATP concentrations are sufficient to activate not only P2Y receptors but low-affinity $P2X_7$ receptors, which form pores allowing large and sustained $[Ca^{2+}]_i$ influx, causing manifold pathophysiological actions, such as activation of cyclooxygenase and NOS, upregulation of immediate early genes, and morphological and proliferative changes. Astrocytes propagate and amplifying the signal through the glial syncytium, which can initiate the injury response in astrocytes, microglia and oligodendrocytes at a distance from the initial insult. This vicious cycle can result in myelin degeneration, cell death, excessive inflammatory events and the production of an extensive nonpermissive glial scar.

destruction, oligodendrocyte cell death, and oligodendrocyte progenitor cell migration and proliferation that are seen in CNS injury.

8. Conclusions

One of the few undoubted functions of glial cells is their response to CNS damage, whereby astrocytes undergo reactive gliosis, oligodendrocytes and myelin degenerate, and microglia are activated. Activation of glial P2Y and P2X purinoceptors following CNS injury can initiate these events, and there may be at least three stages to the pathological process, depending on the level and duration of exposure to ATP and the purinoceptors activated, as summarised in Fig. 6. *Stage 1*: During physiological signalling, there are rapid, small and transient increases in extracellular levels of ATP that principally activate glial P2Y purinoceptors and rapidly desensitising P2X receptors. Activation of $P2Y_1$ purinoceptors propagates astroglial Ca^{2+} waves, which serves as a parallel processing pathway and couples glial and neuronal functions. *Stage 2*: Glial cells are sent to a second stage of activation following larger and more prolonged increases in extracellular ATP, which is reflected in a similarly large and prolonged increase in glial $[Ca^{2+}]_i$. This is sufficient to trigger astrocytes to release ATP and arachidonic acid, thereby spreading and amplifying the signal through the glial syncytium. In turn, this can initiate an injury response in astrocytes, microglia and oligodendrocytes, including upregulation of $P2X_7$ receptors. However, the Ca^{2+} signal is again mediated primarily via P2Y receptors and so it is not sustained and is self-limiting, since it depends on intracellular stores that are rapidly depleted and recover only slowly. *Stage 3*: Only in response to ultra high extracellular concentrations of ATP is there an activation of P2X receptors that do not desensitise or saturate, and glial cells may then reach a third stage of the injury response, involving cell death, excessive inflammatory events and the production of an extensive nonpermissive glial scar. There is evidence that $P2X_7$ receptors have specific functions in these events. In conclusion, ATP release by astrocytes and ATP-mediated glial Ca^{2+} signalling may be a critical step in initiating and propagating the injury response in glial cells. The small amount of work that has been done in situ shows that both P2Y and P2X purinoceptors are involved in these processes.

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