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Progress in Neurobiology 78 (2006) 327–346

progress in Neurobiology

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$P2X_7$ receptors in the nervous system

Beáta Sperlágh [a,*], E. Sylvester Vizi [a], Kerstin Wirkner [b], Peter Illes [b]

[a] *Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, Hungary*
[b] *Rudolf-Boehm-Institute of Pharmacology and Toxicology, University of Leipzig, D-04107 Leipzig, Germany*

Received 4 February 2006; accepted 27 March 2006

Abstract

$P2X_7$ receptors are ligand-gated ion channels, expressed as homo-oligomeric assemblies of individual subunits. They are widely distributed at immunocompetent cells of the central and peripheral nervous system and are believed to be primarily involved in host-defense reaction. However, a growing amount of evidence indicates that their signaling role in the brain is more widespread than previously anticipated. In this paper, we review the present knowledge on the structural and pharmacological features of the $P2X_7$ receptor, as well as its cell-type specific localization in the nervous system. Subsequently, the participation of $P2X_7$ receptors in distinct neuronal, astroglial and microglial functions are described. Finally, since they may play a prominent role in certain neurologic disorders, such as ischemia-reperfusion injury, Alzheimer's disease, spinal cord injury and sensory neuropathies, the pathological role and potential therapeutic exploitation of $P2X_7$ receptors are also discussed.

Keywords: $P2X_7$ receptor; ATP; Presynaptic; Neuron; Microglia; Astrocyte

Contents

Abbreviations: AP-1, activator protein-1; 2-AG, 2-arachidonoylglycerol; Aβ, amyloid beta peptide; APP, amyloid precursor protein; ABC, ATP binding cassette; LPS, bacterial lypopolisaccharide; BzATP, 2′; 3′-*O*-(4-benzoyl-benzoyl)adenosine 5′-triphosphate; CAMKII, Ca^{2+}/calmodulin dependent prtein kinase II; EPSC, excitatory postsynaptic currrent; ERK, extracellular signal regulated protein kinase; LTD, long-term depression; 2-MeSATP, 2-methylthioATP; α,β-meATP, α,β-methyleneATP; IC, immuncytochemistry; iNOS, inducible nitric oxide synthase; MRF-1, microglial response factor-1; IL-1β, interleukin-1β; ICE, interleukin-1 converting enzyme; MCAO, middle cerebral artery occlusion; mEPSC, miniature excitatory postsynaptic currrent; MAPK, mitogen activated protein kinase; MCP1, monocyte chemoattractant protein; NO, nitric oxide; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; OxiATP, periodate-oxidized ATP; IP3K, phosphatidylinositol 3-kinase; PLD, phospholipase D; PKA, protein kinase A; Akt, protein kinase B; PKC, protein kinase C; PPADS, pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid; RT-PCR, reverse-transcription-coupled-polymerase-chain-reaction; PVN, paraventricular nucleus; ROI, reactive oxygen intermediates; vAChT, vesicular acetylcholine transporter; SON, supraoptic nucleus; TGF-β1, transforming growth factor-beta 1; vGAT, vesicular GABA transporter; VGLUT1, vesicular glutamate transporter 1; VGLUT2, vesicular glutamate transporter 2; VMAT, vesicular monoamine transporter; WB, Western blotting

* Corresponding author at: Laboratory of Molecular Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1083 Budapest, Szigony u. 43., Hungary. Tel.: +36 1 210 9970; fax: +36 1 210 9423.
E-mail address: sperlagh@koki.hu (B. Sperlágh).

0301-0082/$ – see front matter
doi:10.1016/j.pneurobio.2006.03.007

1. Introduction: from the P2z purine receptor to the $P2X_7$ receptor

P2X receptors are ligand-gated ion channels conveying the ionotropic actions of extracellular ATP. Until now, seven different subunits of this receptor family have been identified which form functional receptor-ion channel-complexes in homo- and/or hetero-oligomeric assemblies. Within the P2X receptor family, the $P2X_7$ receptor has a distinguished role for several reasons. First, this receptor seems to function only in homo-oligomeric form, and is activated by relatively high concentrations of ATP. Second, this receptor was also known previously as P2z receptor, the ''cell death receptor''; its prolonged activation elicits the opening of a transmembrane pore, permeable to large molecular weight molecules up to 800 Da, which finally leads to cellular death. Last but not least, in spite of being cloned originally from the rat brain, it was suggested that $P2X_7$ receptors are expressed predominantly on antigen-presenting immune cells and epithelia, but not in neurons, and function as immunomodulatory receptors (Collo et al., 1997; Surprenant et al., 1996).

Supporting this concept, a wealth of data confirm that $P2X_7$ receptors regulate many aspects of immune function in immunocompetent cells (Di Virgilio et al., 2001a). Thus $P2X_7$ receptors participate in the regulation of the expression and secretion of cytokines and inflammatory mediators including IL-1β (Brough et al., 2003; Donnelly-Roberts et al., 2004; Ferrari et al., 1997b; Grahames et al., 1999; Gudipaty et al., 2003; Labasi et al., 2002; Mehta et al., 2001; Verhoef et al., 2003), IL-1ra (Wilson et al., 2004), IL-2 (Loomis et al., 2003), IL-4, IL-6, IL-13, IL-18 (Mehta et al., 2001; Sluyter et al., 2004), TNFα (Bulanova et al., 2005), NO (Hu et al., 1998; Sperlágh et al., 1998) and superoxide anions (Suh et al., 2001). In addition, $P2X_7$ receptors are also involved in the direct effector function of immune cells, such as multinuclear giant cell formation (Chiozzi et al., 1997; Falzoni et al., 1995), and mycobacterium killing (Fernando et al., 2005; Saunders et al., 2003; Sikora et al., 1999; Smith et al., 2001, but see Myers et al., 2005); moreover they transmit cell death signaling via membrane permeabilization, cytoskeletal reorganization (Pfeiffer et al., 2004; Wilson et al., 2002), and caspase 1 activation (Bulanova et al., 2005; Donnelly-Roberts et al., 2004; Kahlenberg and Dubyak, 2004; Kawamura et al., 2005; Tsukimoto et al., 2005) but also promote proliferation of lymphoid cells (Baricordi et al., 1996, 1999), depending on the intensity of receptor stimulation.

However, in the past few years the potential role of this receptor in neuronal functions has also received considerable attention. Although a major debate has emerged on the cell-type specific localization of $P2X_7$ receptors in the nervous system, most data indicate that they are involved in the regulation of diverse neural functions, such as modulation of neurotransmitter release, as well as microglial and astroglial activation. Moreover, $P2X_7$ receptors have been proposed to be potential therapeutic target sites in disorders of the nervous system, such as ischemia-reperfusion injury, Alzheimer's disease, spinal cord injury and neuropathic pain. Thus, in this review we will primarily focus on the localization and function of $P2X_7$ receptors in the nervous system, under normal and pathological conditions, whereas the survey of the $P2X_7$ receptor-related literature regarding other organs such as the haemopoietic system (Di Virgilio et al., 2001b) or the bones (Gartland et al., 2003; Gallagher, 2004) can be found in other recent reviews.

2. The structure of the $P2X_7$ receptor

The $P2X_7$ receptor has been isolated from rat superior cervical ganglia and medial habenula; its full length cDNAs were cloned for the first time from a rat brain cDNA library. The $P2X_7$ subunit is 595 amino acid long, having 35–40% homology with the other six members of the P2X receptor family (Surprenant et al., 1996). Although its structure is basically similar to the residual P2X receptor subunits, having two transmembrane domains (M1, M2) and a large extracellular loop, the $P2X_7$ receptor has a unique structural feature. Its intracellular carboxy terminal domain is much longer (239 amino acids) than those of other P2X receptor subunits (27–129 amino acids). Based on the characteristics of ATP evoked inward currents recorded from HEK293 cells, transfected with $P2X_7$ receptor-cDNA, the phenotype of this receptor was found to be identical with its native counterpart, the pore-forming P2X receptor responsible for the cytolytic action of ATP in immune cells. This receptor was first described by Buisman et al. (1988) and later named P2z receptor (Di Virgilio, 1995), until its molecular identification.

The activation of the $P2X_7$ receptor-gated ion channels leads to two distinct responses, depending on the exposure time of the agonist: following a single brief application, a non-selective inward cationic current can be recorded, which is similar to inward currents caused by the activation of homo- or hetero-oligomeric assemblies of other P2X receptor subunits, although with different deactivation kinetics. Upon repeated or prolonged application the opening of a membrane pore can be detected, which renders the membrane permeable to high molecular weight molecules and ions up to the size of 800 Da (Surprenant et al., 1996). The pore formation is characterized by the uptake of high molecular weight fluorescent dyes, such as YO-PRO, or Lucifer yellow, is followed by cytoskeletal rearrangement such as membrane blebbing (Virginio et al., 1997, 1999a), and eventually leads to cell death in immune cells. Although more recently it has been revealed that the permeability of other P2X subunits, such as $P2X_2$ and $P2X_4$ (Khakh et al., 1999; Virginio et al., 1999b), can also increase as a function of time, the property of pore dilation is generally associated with $P2X_7$ receptors. The long intracellular C-terminal domain has been shown to be instrumental for the pore-forming property of $P2X_7$ receptors (Adriouch et al., 2002; Surprenant et al., 1996). A lipopolysaccharide (LPS)-binding site has also been identified close to the carboxy terminus of the receptor (Denlinger et al., 2001, 2003), whereby the receptor could translate inflammatory signals to signal transduction events. Moreover, gene polymorphism studies indicated that a trafficking domain of the receptor lies within this region, between the residues 551 and 581 (Wiley et al.,

2003), and the same domain is essential for cell surface expression of the $P2X_7$ receptor (Smart et al., 2003). In contrast, the ATP binding site is suggested to be located within an antiparallel six-stranded beta-pleated sheet (Freist et al., 1998), nearby a cysteine-rich region of the extracellular domain of the receptor (Gu et al., 2004; Worthington et al., 2002), between the M1 and M2 transmembrane regions, similarly to other P2X receptor subunits.

Following the cloning and expression of the rat $P2X_7$ receptor, the genes encoding the human (Rassendren et al., 1997), mouse (Chessell et al., 1998) and *Xenopus laevis* (Paukert et al., 2002) $P2X_7$ receptors were also identified, having 80%, 85% and 50% amino acid sequence homology with the rat receptor, respectively. As for the human receptor, several splice variants have been discovered recently (Cheewatrakoolpong et al., 2005). Interestingly, the splice variant, in which the cytoplasmic tail is deleted, seems to be the dominant variant expressed in human tissues. It is now also clear, that unlike other P2X receptor subunits, the $P2X_7$ subtype does not hetero-oligomerize and functions only in homo-oligomeric form (Torres et al., 1999), most likely as a homo-trimer. Other observations indicate that whereas native $P2X_7$ receptors appear to exist in hexameric form in macrophages of the periphery, they are expressed as monomers in the resting microglia of the brain (Kim et al., 2001). Recently, $P2X_7$ receptor gene polymorphism has been suggested to contribute to individual cytokine responsiveness (Denlinger et al., 2004, 2005; Sluyter et al., 2004), and susceptibility against infection (Fernando et al., 2005; Saunders et al., 2003), autoimmune diseases (Elliott and Higgins, 2004; Elliott et al., 2005) and chronic lymphoid leukemia (Thunberg et al., 2002), although epidemiological studies have not confirmed a close relationship of $P2X_7$ receptor mutations with the higher risk of lymphoid leukemia (Sellick et al., 2004).

3. The pharmacological fingerprint of the $P2X_7$ receptors

Although homo- and hetero-oligomeric assemblies of different P2X receptor subunits have overlapping ligand binding profiles, and there are no ligands showing absolute selectivity towards the $P2X_7$ receptor, the pharmacological phenotype of the $P2X_7$ receptor is relatively well established and distinguishable from other members of the P2X receptor family.

The main distinguishing properties of the $P2X_7$ receptor are the following (North, 2002; North and Surprenant, 2000; Ralevic and Burnstock, 1998):

(1) A relatively low potency of endogenous ATP, which is above 100 μM even in recombinant systems and native tissues with good penetration properties, whereas the potency of ATP at other P2X receptor subunits is much higher, in the low micromolar range. These values might change in preparations, where the tissue penetration is slower and ATP is subject to fast catabolism by ectoNTPDases, but the potency of ATP is always at least one order of magnitude lower at the $P2X_7$ receptor, than at other members of the P2X family.

(2) Of various agonists, 2′, 3′-*O*-(4-benzoyl-benzoyl)adenosine 5′-triphosphate (BzATP) is at least 10–30-fold more potent than ATP at the $P2X_7$ receptor. Although BzATP is not a selective agonist for $P2X_7$, and can bind to other P2X receptors, in particular $P2X_1$, $P2X_2$ and $P2X_3$, with similar potency (Bianchi et al., 1999), it is only at the $P2X_1$ and the $P2X_7$ receptors where BzATP is substantially more potent than ATP (Bianchi et al., 1999). As for other commonly used agonists of P2X and P2Y receptors, ADP and AMP have lower affinities to this receptor than ATP itself, whereas 2-methylthioATP (2-MeSATP), ATP-γ-S, ADP-β-S, α,βmethyleneATP (α,β-meATP) and UTP are weak agonists or even inactive. Since α,βmeATP is a potent agonist at the $P2X_1$ receptor, the agonist binding profile of $P2X_7$ receptors is quite unique.

(3) Of divalent cations Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+} and low pH inhibit the current flow through the receptor ion channel complex. Again, although other P2X receptor subtypes are also modulated by divalent cations, it is only at the $P2X_7$ receptor where Zn^{2+} has an inhibitory action. The strong potentiation of the response to ATP in the absence of Mg^{2+} has been already observed in early studies (Cockcroft and Gomperts, 1980) and led to the suggestion that not ATP itself but ATP^{4-} is the active ligand at the receptor, which represents about 10% of the total ATP concentration under normal divalent cation concentration, but is increased under Mg^{2+} free conditions. However, this hypothesis, although it supplies a plausible explanation for the low affinity of ATP at the $P2X_7$ receptor, has not received further proof yet.

(4) Of various antagonists, Brilliant Blue G is a potent, non-competitive antagonist displaying remarkable selectivity towards $P2X_7$ receptors in the nanomolar range, having IC_{50} values of about 10 nM at the rat $P2X_7$ receptor (Jiang et al., 2000). Thus, Brilliant Blue G is about 100-fold less potent at $P2X_2$ than at $P2X_7$ receptors and is even less active at the residual P2X receptors. At 100 nM it completely blocks the ATP evoked currents at recombinant rat $P2X_7$ receptor, and even at 1 μM only slightly inhibits other P2X receptor subunits, although it is less potent at the mouse (IC_{50}: 100 nM) and at the human $P2X_7$ receptors (IC_{50}: 200 nM) than at their rat counterparts (Hibell et al., 2000). Besides Brilliant Blue G, other polysulfonated dyes such as Chicago sky blue or Cibacron brilliant red are also relatively potent antagonists at $P2X_7$ receptors (Hibell et al., 2001). Another compound, which has been regarded as a selective $P2X_7$ receptor antagonist for a considerable time, is periodate-oxidized ATP (oxiATP), which is a slowly equilibrating and irreversible antagonist at this receptor (Murgia et al., 1993). OxiATP has been widely used as a probe to identify $P2X_7$ receptor-mediated actions, especially in the immune system. Caution is required with its use, however, because it also interferes with inflammatory signaling processes, independently from the activation of $P2X_7$ receptors (Beigi et al., 2003; Di Virgilio, 2003). Furthermore, oxiATP by itself is severely cytotoxic when

used in high micromolar concentrations in cerebellar granule cells (Craighead et al., 2001) and macrophages (B. Sperlágh; unpublished observation). A third antagonist, which may be useful for the pharmacological identification of $P2X_7$ receptors is KN-62, also known as a CAM-kinase-II inhibitor, which is a selective antagonist at the human $P2X_7$, but not at the rat $P2X_7$ receptor (Gargett and Wiley, 1997). More recently, new analogues of KN-62 have also been synthesized including those devoid of CAM-kinase-II inhibitory activity (Baraldi et al., 2004). A novel series of cyclic imide analogues (Alcaraz et al., 2003) and adamantine amides (Baxter et al., 2003) may represent further new generations of potent $P2X_7$ receptor antagonists for therapeutic exploitation, although their selectivity is yet to be determined.

Of the other P2X receptor antagonists, pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) is a potent antagonist at the human $P2X_7$ receptor (pIC_{50}: 7.3); it is still fairly potent at the rat (pIC_{50}: 6.4) and mouse (pIC_{50}: 5.5) $P2X_7$ receptors (Hibell et al., 2001). Suramin is usually inactive or less potent at the $P2X_7$ receptor in comparison with other suramin sensitive P2X subunits (IC_{50}: >300 μM).

As compared to other subunit assemblies of P2X receptors, $P2X_7$ receptors belong to the slowly desensitizing type, showing no or very little desensitization during several seconds of application (North, 2002). Therefore, $P2X_7$ receptors can be also distinguished by this feature from rapidly desensitizing P2X subunit assemblies, i.e., from the homomeric $P2X_1$ and $P2X_3$ receptors in electrophysiological recordings.

Thus, taken into account the criteria outlined above, $P2X_7$ receptor-mediated functions were primarily identified based on their pharmacological profile and physiological characteristics (e.g., kinetics of the currents measured). However, only few studies used the full repertoire of pharmacological tools for the identification of $P2X_7$ receptors in the nervous system. Therefore, given the overlapping ligand binding profiles of various P2X receptor-subtypes, it is quite possible that responses assigned to $P2X_7$ receptors are due to the activation of other P2X subunits or their heteromeric combinations. Nevertheless, unless there were unequivocal reasons for critical discussion, we tentatively accepted the authors' claims for $P2X_7$ receptor-mediated effects. It is also important to note that the pharmacological properties of the recombinant and native $P2X_7$ receptors might differ. Furthermore, one should keep in mind that species differences exist in the agonist and antagonist sensitivity and kinetics of $P2X_7$ receptor orthologues (Bianchi et al., 1999; Hibell et al., 2001). In case of antagonists, the IC_{50} values may also vary depending on the type of agonist used (e.g., ATP versus BzATP) (Hibell et al., 2000). Finally, the possibility cannot be excluded that yet unidentified P2X receptor subunits or subunit combinations exist with unknown pharmacological profiles mimicking $P2X_7$ receptor-like pharmacological features.

In addition to these pharmacological approaches, the identity of the $P2X_7$ receptor can also be investigated by a genetical approach, i.e., by the use of $P2X_7$ receptor deficient mice (Solle et al., 2001). However, with a few exceptions (Brough et al., 2002; Chessell et al., 2005; Kukley et al., 2004; Le Feuvre et al., 2002a; Papp et al., 2004b) this technique was predominantly utilized to explore non-neuronal functions (Brough et al., 2003; Ke et al., 2003; Korcok et al., 2004; Labasi et al., 2002; Le Feuvre et al., 2002b; Sikora et al., 1999; Solle et al., 2001) and the majority of our knowledge on $P2X_7$ receptor function in the nervous system relies on the pharmacological identification.

4. Expression of $P2X_7$ receptors in different cell types of the nervous system

Initial Northern blotting studies detected a widespread tissue distribution of mRNA encoding the $P2X_7$ receptor, showing strong expression in the immune system such as the thymus or the spleen, but also in other organs, including the brain, spinal cord, skeletal muscle, lung and placenta (Rassendren et al., 1997; Surprenant et al., 1996). More recently, however, in situ hybridization studies suggested that the expression of mRNA encoding $P2X_7$ receptors is restricted to the ependymal layer of the third ventricle of the adult brain and to activated microglia following middle cerebral artery occlusion (MCAO) (Collo et al., 1997). Thus, despite being isolated from superior cervical ganglia and rat brain, it has been proposed that the $P2X_7$ receptor is absent in neurons; its limited distribution in the brain was suggested to derive from its expression in non-neuronal cells of the nervous system. The lack of identification of $P2X_7$ receptor mRNA in the early study of Collo et al. (Collo et al., 1997), however, can be explained by the relatively low sensitivity of in situ hybridization techniques. With the more sensitive RT-PCR analysis, we and others could observe strong mRNA expression for $P2X_7$ receptors as well as for other P2X receptor subunits in several areas of the CNS such as the cortex, hippocampus (Sperlágh et al., 2003), brainstem (Papp et al., 2004a), nucleus accumbens (Franke et al., 2001) and spinal cord of the rat (Deuchars et al., 2001) and human brain (Cheewatrakoolpong et al., 2005). Moreover, it is also evident that at least at the mRNA level, $P2X_7$ receptors may appear both in neurons (Table 1) and in astrocytes (Duan et al., 2003) or microglial cells (Collo et al., 1997). The list of neuronal cells expressing $P2X_7$ receptor mRNA includes peripheral neurons, such as cultured sympathetic (Allgaier et al., 2004), and dorsal root ganglion (Kobayashi et al., 2005) neurons, and central neurons, such as retinal ganglion cells (Brandle et al., 1998) and cerebellar granule cells (Hervas et al., 2003) (Table 1). It remains to be investigated, however, whether all these cell types shown to express $P2X_7$ receptor mRNA under in vitro conditions exhibit a similar receptor endowment under in situ conditions. Experimental manipulations such as cell culturing may change the expression pattern and cellular localization of receptors. Although a recent study failed to confirm the expression of mRNA encoding $P2X_7$ receptors in rat hippocampal pyramidal neurons (Rodrigues et al., 2005), further single cell PCR studies from intact brain slices or from in situ ganglia are needed to resolve this issue.

Table 1
Expression and function of $P2X_7$ receptors in neurons

Tissue	mRNA	Protein	$P2X_7^{-/-}$	Function	$P2X_7^{-/-}$	Refs.
Sensory nerve terminals	ND	+/−IC	ND	Inflammatory pain	Positive	Chessell et al. (2005), Dell'Antonio et al. (2002a,b)
Sympathetic neurons	+	+IC	ND	$[Ca^{2+}]_i$[a],	ND	Allgaier et al. (2004)
Myenteric and submucosus neurons	+	+IC	ND	Inward current, depolarization response	ND	Hu et al. (2001)
Dorsal root ganglion	+	+IC	ND	ND	ND	Kobayashi et al. (2005), Ruan et al. (2005)
Retinal ganglion cells, photoreceptor terminals	+	+IC	Positive	$[Ca^{2+}]_i$, cell death	ND	Brandle et al. (1998), Wheeler-Schilling et al. (2000, 2001), Ishii et al. (2003), Kaneda et al. (2004), Puthussery and Fletcher (2004), Zhang et al. (2005), Franke et al. (2005)[b], Innocenti et al. (2004)
Neuromuscular junction	ND	+IC	ND	Vesicle release	ND	Deuchars et al. (2001), Deng and Fyffe (2004), Moores et al. (2005)
Spinal cord	ND	+IC	ND	$[Ca^{2+}]_i$, cell death, high frequency spiking	ND	Atkinson et al. (2004), Deng and Fyffe (2004), Wang et al. (2004)
Hippocampus	+	+IC	Negative	Facilitation of GABA and glutamate release	Positive	Atkinson et al. (2004), Papp et al. (2004b), Sperlágh et al. (2002), Kang et al. (2004), but see Rodrigues et al. (2005), Sim et al. (2004)
		+IC	Negative	Depression of mossy fiber EPSC, LTD	ND	Armstrong et al. (2002), but see Kukley et al. (2004)
		+IC	ND	Cell death	ND	Cavaliere et al. (2004)[c]
		+IC	ND	$[Ca^{2+}]_i$	ND	Vianna et al. (2002)[d]
			ND	In vivo brain injury	Negative	Le Feuvre et al. (2002a, 2003)
Brainstem		+IC	ND	Presynaptic increase in EPSCs	ND	Atkinson et al. (2004), Deuchars et al. (2001), Ireland et al. (2004)
Paraventricular nucleus			ND	Postsynaptic increase in mEPSC amplitude	ND	Gordon et al. (2005)
Cerebral cortex	+	+IC	ND	ND		Atkinson et al. (2004), Franke et al. (2004)[e]
Cerebellum, striatum, thalamus, amygdala	ND	+IC	ND	ND		Atkinson et al. (2004)
Supraoptic nucleus	+/−[f]	ND		ND		Shibuya et al. (1999)
Cerebellar granule neurons	+	+IC	ND	$[Ca^{2+}]_i$	ND	Cavaliere et al. (2002)[g], Hervas et al. (2003)
Midbrain synaptosomes	ND	+IC	ND	$[Ca^{2+}]_i$,	ND	Miras-Portugal et al. (2003)
Cortical synaptosomes	ND	+IC, WB	ND	$[Ca^{2+}]_i$,	ND	Lundy et al. (2002)
Cerebellar synaptosomes	ND	+IC	ND	$[Ca^{2+}]_i$,	ND	Hervas et al. (2005)
Neuronal progenitor cells	ND	ND	ND	$[Ca^{2+}]_i$,	ND	Hogg et al. (2004)
SH-SY5Y neuroblastoma cells	ND	+WB	ND	$[Ca^{2+}]_i$,	ND	Larsson et al. (2002)
NG108-15 cells	ND	ND		$[Ca^{2+}]_i$,	ND	Brater et al. (1999), Watano et al. (2002)
hippocampal neuroblastoma cells (HN2)	ND	ND		Non-selective cation current	ND	El-Sherif et al. (2001)
N1E-115 neuroblastoma cells	+	+IC	ND	Cell death	ND	Schrier et al. (2002)
Cochlea hair cells, ganglion cells	ND	+IC	ND	ND		Nikolic et al. (2003), Szucs et al. (2004)
Inner ear (organ of Corti, vestibular and spiral ganglion	+	ND	ND	ND		Brandle et al. (1999)

+, Indicates the presence of $P2X_7$ receptor mRNA/protein determined by IC (immuncytochemistry) or WB (Western blotting). ''Positive'', evidence in favor of the presence/involvement of $P2X_7$ receptors obtained in studies using $P2X_7^{-/-}$ mice; ''Negative'', evidence against the presence/involvement of $P2X_7$ receptors obtained in studies using $P2X_7^{-/-}$ mice; ND, no data; LTD, long-term depression; mEPSC, miniature excitatory postsynaptic currrent; EPSC, excitatory postsynaptic current.

[a] Not mediated by $P2X_7$ receptors.
[b] Upregulation in retinopathy.
[c] Upregulation after in vitro ischemia.
[d] Upregulation in chronic epileptic rats.
[e] Appeared only after in vivo ischemia.
[f] Stronger in non-neuronal cells.
[g] Upregulation after glucose deprivation.

Immunhistochemical studies showing the $P2X_7$ receptor specific immunoreactivity in different cells and its co-localization with cell-type specific markers at the light and electronmicroscopic level are also powerful tools to demonstrate the cell-type specific localization of this receptor protein. Indeed, using antibodies raised against the intra or extracellular epitopes of the $P2X_7$ receptor protein, a number of studies explored the distribution of $P2X_7$ receptors in the nervous system. $P2X_7$ receptor immunoreactivity has been reported to selectively target excitatory nerve terminals of the spinal cord (Atkinson et al., 2004; Deng and Fyffe, 2004; Deuchars et al., 2001; Wang et al., 2004), medulla oblongata (Atkinson et al., 2004; Deuchars et al., 2001), cerebellum, striatum, thalamus, amygdala (Atkinson et al., 2004; Hervas et al., 2005) and hippocampus of rats (Armstrong et al., 2002; Atkinson et al., 2004; Sperlágh et al., 2002) and gerbils (Kang et al., 2004). Moreover, co-localization studies revealed the co-expression of $P2X_7$ receptor immunoreactivity with vesicular glutamate transporter 1 (VGLUT1) and vesicular glutamate transporter 2 (VGLUT2) immunoreactivity as well as with vesicular GABA transporter (vGAT) and vesicular acetylcholine transporter (vAChT) in many areas of the brain and spinal cord, whereas no evidence was found for the co-localization of $P2X_7$ receptor immunoreactivity with the catecholaminergic marker vesicular monoamine transporter (VMAT) (Atkinson et al., 2004). Interestingly, $P2X_7$ receptor immunolabeling was also targeted to the nuclear envelope in hippocampal neurons, but appeared at the presynaptic nerve terminals only in excitatory neurons (Atkinson et al., 2002). $P2X_7$ receptor immunoreactivity was also found to be present on motor nerve terminals (Deng and Fyffe, 2004; Deuchars et al., 2001; Moores et al., 2005) sympathetic (Allgaier et al., 2004) and enteric neurons (Hu et al., 2001; Vanderwinden et al., 2003), dorsal root ganglion neurons (Ruan et al., 2005) and on sensory nerve fibers of the rat hindpaw (Dell'Antonio et al., 2002a). However, a recent study failed to confirm the immuncytochemical expression of $P2X_7$ receptors in human sensory nerves (Chessell et al., 2005). $P2X_7$ receptors appeared to be also strongly represented in the neural elements of sensory organs, since strong $P2X_7$ receptor immunostaining labeled retinal ganglion cells of the rat (Kaneda et al., 2004; Puthussery and Fletcher, 2004), mouse (Franke et al., 2005), and monkey (Ishii et al., 2003), and outer hair cells of the guinea-pig cochlea (Szucs et al., 2004).

Unfortunately, however, the specificity of the most commonly used antibodies has been questioned by two recent studies (Kukley et al., 2004; Sim et al., 2004), demonstrating a pseudo-immunoreactivity for $P2X_7$ receptors in hippocampal sections of $P2X_7^{-/-}$ mice, whereas the pseudo-immunoreactivity was absent in the peripheral organs, such as the submandibular gland. Moreover, these studies utilised a $P2X_7$ receptor deficient mouse line, in which a LacZ gene was inserted into the transgene construct, and the product of this gene was detected by x-gal staining only in ependymal cells around the third ventricle, but not in the hippocampus, indicating that the expression of $P2X_7$ receptor protein is either absent or below the detection limit in this brain area (Sim et al., 2004). In contrast, a more recent study with the same antibody demonstrated that the $P2X_7$ receptor immunoreactivity, at least in the retinal ganglion cells, disappeared in the $P2X_7^{-/-}$ mice (Franke et al., 2005). It is important to point out that the pseudo-staining found in $P2X_7$ receptor deficient animals in the above studies indicates only that the antibody recognizes a protein in the brain which is not identical with $P2X_7$ receptors; this finding by no way excludes the existence of neuronal $P2X_7$ receptors. Moreover, a very recent study revealed that the commercially available antibodies bind to a "$P2X_7$-like" protein in the brain, but not in the macrophages of $P2X_7^{-/-}$ mice; this protein is identical with the genuine $P2X_7$ receptor regarding the epitopes recognized by the antibodies, but differs in the sequence disrupted by the genetical manipulation (Sanchez-Nogueiro et al., 2005). The "$P2X_7$-like" protein is expressed in cerebellar granule neurons and partly retains its functionality responding to agonist application with Ca^{2+} transients, although with altered deactivation kinetics (Sanchez-Nogueiro et al., 2005). It remains to be investigated, whether this protein is an alternative splicing product of the $P2X_7$ receptor coding gene or a novel "$P2X_7$-like" protein, and whether it is also expressed in genetically intact animals. Other potential reasons leading to the detection of pseudo-immunoreactivity could be a different dilution of the antibody by different groups. Hence in the study of Sim et al. (2004) 1:1000–1:2000, whereas in the study of Kukley et al. (2004) 1:50–1:1000 dilutions were used, whilst in previous studies using the same antibody much higher dilutions (1:5000–1:18,000) were applied for the same brain area, i.e., the hippocampus (Armstrong et al., 2002; Sperlágh et al., 2002). Thus it is unknown whether the (pseudo)immunoreactivity also persists using more dilute solutions and it is also unclear whether it also appears in other areas and cell types of the brain, where previous studies indicated its expression. Although the majority of the previous studies used the commercially available rabbit antibody (Glaxo, Alomone, Chemicon, Biotrend, Sigma) raised against the intracellular carboxy terminus of the recombinant $P2X_7$ receptor (576–595), antibodies with non-commercial or unknown origin were also utilized by a few investigators (e.g., Collo et al., 1997; Wang et al., 2004).

In summary, it appears to be premature to draw final conclusions on the neuron-specific localization of $P2X_7$ receptors from the above studies; nonetheless, they provoke the obligatory use of $P2X_7$ receptor-deficient mice in further immunocytochemical studies exploring the distribution of these receptors in the nervous system. Moreover, a systematic re-evaluation of previous studies with antibodies with proven specificity at the light and the electronmicroscopic level together with alternative approaches (e.g., in situ hybridization) could help to establish the precise cell-type specific localization of $P2X_7$ receptors in the brain and the periphery.

5. ATP availability for $P2X_7$ receptor activation under normal and pathological conditions

Given the low affinity of the endogenous agonist ATP, a crucial question in the evaluation of the physiological/

pathological role of $P2X_7$ receptors is to identify conditions, which result in accumulation of extracellular ATP in concentrations sufficiently high to activate them. Extracellular ATP availability in the nervous system is basically determined by the balance of release, and removal by enzymatic degradation. Since ATP is ubiquitous, all metabolically active cells of the nervous system are able to synthesize this nucleotide, which provides a large potential pool for release. Therefore, the cellular source of released ATP for $P2X_7$ receptor activation could be neurons, astrocytes, endothelial tissue and microglial cells. The majority of cellular ATP is formed in the mitochondria by oxidative phosphorylation, which results in approximately 1–10 mM ATP concentration in the cytoplasm under normal metabolic conditions (Gribble et al., 2000). In addition, ATP is taken up and stored in synaptic vesicles of nerve terminals and astrocytes.

Basically a number of different stimuli could elevate ATP concentrations in the vicinity of $P2X_7$ receptor-expressing neurons, as well as astroglial and microglial cells (for ref., see Sperlágh and Vizi, 1996). Although the stimulation-dependent release of ATP upon conventional (e.g., Cunha et al., 1996) and high frequency (e.g., Wieraszko et al., 1989) neuronal activity is well documented, these stimuli probably result in a spatially restricted, localized increase in extracellular purine levels, which serve the synaptic transmission and the modulation of pre- and postsynaptic functions within the synaptic cleft. ATP-metabolizing ectoenzymes, present at the nerve terminal membrane, such as ectoNTPDases (Zimmermann et al., 1998), may limit ATP availability under these conditions. Nevertheless, since the synaptic cleft is narrow, ATP might still reach millimolar concentration and could activate $P2X_7$ receptors upon neuronal activity, provided these receptors are located nearby the release sites. On the other hand, pathological events such as mechanical or metabolic stress, inflammation, cellular injury or changes in the ionic environment are also known to powerfully stimulate ATP release. This might result in an ATP-rich extracellular milieu leading to a more widespread activation of receptors reaching also the neighboring or distant cells such as astrocytes and microglia. These pathological signals include hypotonic (Wang et al., 1996) and mechanical stimuli (Coco et al., 2003; Verderio and Matteoli, 2001; Wang et al., 2004), energy deprivation (Juranyi et al., 1999), such as anoxia (Hisanaga et al., 1986; Lutz and Kabler, 1997), and inflammatory signals, such as bacterial LPS (Ferrari et al., 1997c; Sperlágh et al., 1998), concanavalin-A (Filippini et al., 1990), or interleukin-1β (IL-1β) (Sperlágh et al., 2004). Moreover, nucleotides and nucleosides by themselves (including ATP) may promote the further release of purines, by a homo- or heteroexchange mechanism, if they reach relatively high concentration in the extracellular space (Sperlágh et al., 2003). Finally, taken into account that the free ATP concentration within living cells under normal metabolic rate is in the millimolar range, cellular damage is usually hypothesized to result in very high, millimolar ATP concentrations in the extracellular space.

However, a paucity of information exists on the actual concentration of extracellular ATP at the vicinity of its receptors. When measured in superfusion systems, the amount of nerve stimulation-evoked ATP release varies between 1 and 1000 pmol/g tissue (see Sperlágh and Vizi, 1996). Since ecto-nucleotidases are present throughout the nervous system (Zimmermann et al., 1998), only a proportion of ATP, escaped from the extracellular degradation could be measured this way, which probably underestimates the biophase level of endogenous ATP. Moreover, any in vitro condition necessarily represents a metabolically impaired state and lower ATP levels in the cytoplasma, in comparison to in vivo conditions. This idea is supported by results obtained with $P2X_7$ receptor antagonists (e.g., Gordon et al., 2005; Sperlágh et al., 2002) and $P2X_7$ receptor deficient mice (Chessell et al., 2005; Papp et al., 2004b), which strongly indicate that $P2X_7$ receptors could be endogenously activated, presuming a localized high concentration of ATP under certain types of stimuli.

6. The role of $P2X_7$ receptors in the modulation of neurotransmitter release

Although the precise cell-type specific distribution of $P2X_7$ receptors in the brain is yet to be determined, a series of functional data strongly supports the role of this receptor in the information processing of the normal and pathological nervous system (Table 1). It has been known for a long time that P2 receptors are involved in the modulation of neurotransmitter release (Sperlágh and Vizi, 1991). The very first evidence suggesting the involvement of $P2X_7$ receptors in the presynaptic neuronal function came from the study of Deuchars et al. (2001) who observed that the $P2X_7$ receptor agonist BzATP depolarized and increased the firing rate of glutamatergic neurons of the spinal cord, and elicited the vesicular destaining of the nerve terminals of the mouse neuromuscular junction. These effects were sensitive to the $P2X_7$ receptor antagonists Brilliant Blue G and oxiATP. Therefore, it has been proposed that a predominant function of $P2X_7$ receptors in the nervous system is to promote transmitter release at the presynaptic site (Deuchars et al., 2001). Since P2X receptors have relatively high Ca^{2+} permeability, transmitter release could be directly initiated through the receptor ion channel complex, without preceding action potentials and subsequent activation of voltage-sensitive Ca^{2+} channels, provided these receptors are located nearby the nerve terminal release sites (Boehm, 1999; Sperlágh et al., 2000). The application of the above hypothesis to $P2X_7$ receptors gained further experimental proof by showing an increase in $[Ca^{2+}]_i$ by $P2X_7$ receptor activation in isolated cortical (Lundy et al., 2002) midbrain (Miras-Portugal et al., 2003) and cerebellar (Hervas et al., 2005) nerve terminals, cerebellar granule neurons (Miras-Portugal et al., 2003), and $P2X_7$ receptor mediated glutamate and subsequent GABA release from rat hippocampal slices (Sperlágh et al., 2002). In the latter study a detailed pharmacological analysis was performed, which indicated the involvement of $P2X_7$ receptors in GABA release based on both the rank order of agonist potency and sensitivity to the blockade by Brilliant Blue G (IC_{50}: 1.6 nM), PPADS (IC_{50}: 6.8 μM), oxidized ATP (100 μM) and Zn^{2+} (IC_{50}: 0.29 μM), and on its potentiation by the absence of Mg^{2+}. Furthermore, the role of $P2X_7$ receptors

in these actions has been confirmed by the use of $P2X_7^{-/-}$ mice, in which ATP evoked GABA and glutamate release is almost abolished (Papp et al., 2004b).

In contrast, Armstrong et al. (2002) reported that BzATP elicits long-lasting depression of mossy fiber EPSCs underlying the inhibition, but not the facilitation of glutamate release. The inhibitory effect of BzATP was also sensitive to oxiATP, but not to PPADS; Brilliant Blue G was not tested. Later on Kukley et al. (2004) showed that the BzATP induced depression of mossy fiber transmission persisted in $P2X_7^{-/-}$ mice and was prevented by A_1 adenosine receptor antagonists indicating that this particular effect of BzATP is due to its breakdown to Bz-adenosine and the subsequent activation of adenosine receptors (Kukley et al., 2004). Thus, it appears that the facilitation, rather than the inhibition of transmitter release is the genuine function of $P2X_7$ receptors in the hippocampus (Sperlágh et al., 2002).

However, in view of the non-specific binding of antibodies raised against $P2X_7$ receptors, the exact localization of such hippocampal receptors responsible for the facilitation of glutamate and GABA release, is still uncertain. Since $P2X_7$ receptor activation could also release glutamate (Duan et al., 2003) and GABA (Wang et al., 2002) from cultured astrocytes, one possibility is that glia also contributes to $P2X_7$ receptor-mediated transmitter release. This assumption is supported by a recent study, which failed to find evidence for the involvement of $P2X_7$ receptors in the BzATP-induced facilitation of glutamate release in isolated hippocampal nerve terminals detected in the lower concentration range (Rodrigues et al., 2005). However, the glial origin may hold true at best for glutamate release because ATP-evoked [^{3}H]GABA release from brain slices was Na^+-dependent, sensitive to tetrodotoxin and to the GABA uptake blocker nipecotic acid (Sperlágh et al., 2002), whereas ATP-evoked [^{3}H]GABA release from cultured astrocytes is Na^+-independent, insensitive to nipecotic acid, and probably mediated by a Cl^-/HCO_3^- exchanger (Wang et al., 2002). Therefore $P2X_7$ receptors might be involved in the cross-talk between glial and neuronal cells.

Here we have to mention that in addition to $P2X_7$ receptors other P2X subunits seem to be also involved in the facilitation of excitatory neurotransmission. Thus, in an elegant study using the $P2X_2^{-/-}$ mice line, Khakh et al. (2003) demonstrated that endogenously released ATP facilitates excitatory transmission onto interneurones by the activation of $P2X_2$ receptors in the stratum radiatum of the hippocampus. In contrast, in a more recent study $P2X_1$, $P2X_3$ and $P2X_{2/3}$ receptors were found to be the predominant receptor assemblies responsible for the facilitation of K^+ depolarization induced [^{3}H]glutamate efflux, measured from hippocampal synaptosomes (Rodrigues et al., 2005). Nevertheless, given the overlapping ligand binding profile of different subunit compositions of P2X receptors, all these data do not exclude the $P2X_7$ receptor-mediated modulation of glutamate release at the higher concentration range. Notably, the study of Khakh et al. (2003) also identified a residual component of facilitation, persisting in the $P2X_2$ null mutant mice, which might represent $P2X_7$ receptor mediated modulation.

Electrophysiological data from other parts of the brain and the periphery also support the assumption that $P2X_7$ receptor activation presynaptically facilitates neurotransmission. Hence, in the presence of adenosine A_1 receptor antagonists, BzATP increases EPSC amplitudes recorded from hypoglossal motoneurons in the brainstem by a presynaptic mode of action (Ireland et al., 2004). This effect was inhibited by nanomolar concentrations of Brilliant Blue G, indicating that $P2X_7$ receptors directly elicit transmitter release from excitatory nerve terminals in this brain area (Ireland et al., 2004). Interestingly, however, BzATP did not alter miniature EPSC frequency in the presence of action potential blockade by TTX, suggesting that the underlying mechanism of the facilitation of glutamate release might be a subthreshold depolarization of presynaptic terminals, increasing the excitability of the presynaptic membrane, rather than a direct Ca^{2+} influx through the receptor ion channel complex. In addition, BzATP and ATP also elicit high frequency firing of spinal cord neurons (Deuchars et al., 2001; Wang et al., 2004) and depolarize myenteric and submucous neurons (Hu et al., 2001) in an oxiATP and Brilliant Blue G sensitive manner, respectively. Although the involvement of $P2X_7$ receptors in these latter responses have not been verified by the use of $P2X_7^{-/-}$ mice, based on their pharmacological profile they were considered to be $P2X_7$ receptor mediated responses.

In addition to presynaptic facilitation, a very recent report indicates that $P2X_7$ receptors also facilitate glutamatergic neurotransmission postsynaptically in the rat paraventricular nucleus (PVN) (Gordon et al., 2005). In this study, it has been reported that norepinephrine, acting on α-adrenoceptors, releases ATP from glial cells, surrounding magnocellular neurosecretory cells, which then act on $P2X_7$ receptors causing an enduring increase in the amplitude of mEPSCs by the phosphatidylinositol 3-kinase (IP3K) dependent insertion of AMPA receptors.

In summary, the present knowledge indicates that regardless of their localization, the activation of $P2X_7$ receptors can alter the pre and/or postsynaptic function of nerve terminals. However, in view of the low affinity of $P2X_7$ receptors to ATP, the question arises whether these receptors are involved in the modulation of synaptic transmission by endogenous ATP. Since ATP release from brain slices is highly frequency-dependent (Cunha et al., 1996; Wieraszko et al., 1989), one possibility is that $P2X_7$ receptors serve as molecular sensors of increased neuronal activity and contribute to short- or long-term plasticity phenomena, underlying e.g., memory formation. Another intriguing possibility is that $P2X_7$ receptors are physiologically silent and they are expressed and/or activated only during pathological conditions, when metabolic distress or cellular damage provide an ATP rich extracellular milieu in the vicinity of $P2X_7$ receptors.

7. Functional role of $P2X_7$ receptors in glia

Apart from conventional neurotransmission, P2X receptors, including $P2X_7$ receptors, appear to mediate communication between glial cells and neurons, between different glial cell types, and within glial networks. According to current view, the function of glia is far more than a simple support for the neuronal network. Glia plays an active role in the information

Table 2
Expression and function of $P2X_7$ receptors in glia

Cell type	mRNA	Protein	Function	$P2X_7^{-/-}$	Refs.
Astrocytes	+	+IC		ND	Duan et al. (2003), Kukley et al. (2001), Narcisse et al. (2005)[a], Franke et al. (2001)[b], Hung and Sun (2002), Wang et al. (2003), Dixon et al. (2004), Fumagalli et al. (2003), John et al. (2001), Panenka et al. (2001)
			$[Ca^{2+}]_i$	ND	Ballerini et al. (1996), Fumagalli et al. (2003), Nobile et al. (2003)
			Inhibition of LPS induced TNF-α release	ND	Kucher and Neary (2005)
			Stimulation of IL-1β induced NO production	ND	Narcisse et al. (2005)
			Glutamate and aspartate release	ND	Duan et al. (2003)
			$[^3H]$GABA release	ND	Wang et al. (2002)
			Purine release	ND	Ballerini et al. (1996)
			TGF-β1 expression-PKC/MAPK	ND	Wang et al. (2003)
			PLD activation	ND	Hung and Sun (2002), Sun et al. (1999)
			AKT phosphorylation	ND	Jacques-Silva et al. (2004)
			MCP-1 expression/p38MAPK/ERK1, ERK2 phosphorylation	ND	Gendron et al. (2003b), Panenka et al. (2001)
			Potentiation of IL-1β induced NF-κB and IL-8 expression, induction of AP-1 expression; downregulation of IL-1β induced IL-10 expression	ND	John et al. (2001)
			2-AG production	ND	Walter et al. (2004)
			LPS induced NO production	ND	Murakami et al. (2003)
			Ca signaling between astrocytes and microglia	ND	Verderio and Matteoli (2001)
Schwann cells	ND	ND	LPS induced IL-1β maturation and release	ND	Colomar et al. (2003), Marty et al. (2005)
			$[Ca^{2+}]_i$, inward current, K^+, Cl^- conductances	ND	Colomar and Amedee (2001), Grafe et al. (1999)
Intestinal glial cells	ND	+	ND	ND	Vanderwinden et al. (2003)
Muller glial cells	+	+	$[Ca^{2+}]_i$, inward current, inhibition of glutamate uptake	ND	Bringmann et al. (2001)[c], Pannicke et al. (2000), but see Jabs et al. (2000)
Microglia	+	+IC		ND	Collo et al. (1997)[d], Ferrari et al. (1997a), Gendron et al. (2003a)
			ATP and LPS induced IL-1β, secretion $[Ca^{2+}]_i$, caspase 1 activation	Positive	Brough et al. (2002), Chakfe et al. (2002), Ferrari et al. (1996, 1997b, c), Sanz and Di Virgilio (2000)
			Pore formation, membrane blebbing, cytoskeletal reorganization, caspase 1 activation, cell death	Positive	Brough et al. (2002), Chessell et al. (1997), Ferrari et al. (1997a, 1999a)
			TNF-α secretion	ND	Hide et al. (2000), Suzuki et al. (2004)
			NOS activation	ND	Gendron et al. (2003a)
			Inward current, $[Ca^{2+}]i$	ND	Visentin et al. (1999)
			Plasminogen secretion	ND	Inoue et al. (1998)
			2-AG production	ND	Witting et al. (2004)
			NFAT, and NF-κB transcription	ND	Chiozzi et al. (1997), Ferrari et al. (1999b, 1997d)

+, Indicates the presence of $P2X_7$ receptor mRNA/protein determined by IC (immuncytochemistry) or WB (Western blotting). ''Positive'', evidence in favor of the involvement of $P2X_7$ receptors obtained in studies using $P2X_7^{-/-}$ mice; ND, no data.
[a] In SM patients.
[b] Expression only after in vivo mechanical damage.
[c] Upregulation in proliferative vitreoretinopathy.
[d] Upregulated after MCAO.

processing, controlling the homeostasis of the neuronal microenvironment, the development and differentiation of neural cells and the host-defense response. The main glial cell types of the nervous system are astrocytes, oligodendrocytes, and microglia. Whereas resident microglia are well known as the immune effector cells of the CNS, astrocytes and Schwann cells in the periphery are regarded also partially immunocompetent as they are also involved in the immune response. To fulfill their diverse roles, glial cells have to communicate with each other and with neurons. For this purpose they utilize their own signaling system; $P2X_7$ receptors, besides other ATP sensitive receptors, seem to have a pivotal role in these processes.

In addition to other subtypes of the P2X and P2Y receptor families, astrocytes express mRNA encoding $P2X_7$ receptors (see Table 2 e.g., Dixon et al., 2004; Fumagalli et al., 2003;

Hung and Sun, 2002; John et al., 2001; Wang et al., 2003). $P2X_7$ receptor immunoreactivity is present on the cell surface of human fetal astrocytes (Narcisse et al., 2005) and is co-localized with astrocytic markers in acutely isolated and cultured astrocytes (Panenka et al., 2001) as well as in the juvenile rat hippocampus (Kukley et al., 2001). In contrast, Franke et al. (2001) observed cell surface located $P2X_7$ receptor-immunoreactivity in GFAP labeled astrocytes in the in situ nucleus accumbens of adult rats only following mechanical damage (Franke et al., 2001). $P2X_7$ receptor immunreactivity is also present in enteric glial cells (Vanderwinden et al., 2003) and both $P2X_7$ receptor transcripts and immunreactivity can be found in Müller glial cells of the human (Pannicke et al., 2000), but not rat (Jabs et al., 2000) retina. It has to be emphasized, however, that the validation of immunocytochemical data by the use of $P2X_7^{-/-}$ mice is still scarce (Franke et al., 2005).

Astroglial $P2X_7$ receptors participate in a wide array of normal and pathological functions of the astrocyte network (Table 2). However, the majority of these actions has been described in cultured astrocytes and further experimental work is necessary to prove that $P2X_7$ receptors indeed play such roles both in situ and in vivo.

The primary intracellular signal following the activation of $P2X_7$ receptors is a sustained elevation of $[Ca^{2+}]_i$, caused by Ca^{2+} influx from the extracellular space through the receptor-ion channel complex (Ballerini et al., 1996; Fumagalli et al., 2003; Nobile et al., 2003) of astrocytes, which is then translated to various signal transduction pathways conveying diverse functions. Importantly, activation of $P2X_7$ receptors elicits glutamate (Duan et al., 2003), GABA (Wang et al., 2002), 2-arachidonoylglycerol (2-AG), (Walter et al., 2004) and purine (Ballerini et al., 1996) release from cultured astrocytes. The first data indicating $P2X_7$ receptor mediated transmitter release was obtained in primary cortical astrocytes, where BzATP increases the release of [^{3}H]purines, by an oxiATP sensitive mechanism (Ballerini et al., 1996). It is noteworthy, however, that despite the well documented action of $P2X_7$ receptor activation to elicit Ca^{2+} influx in cultured astrocytes (Ballerini et al., 1996; Fumagalli et al., 2003), $P2X_7$ receptor induced glutamate release is $[Ca^{2+}]_o$-independent, and appears to occur through the receptor ion channel complex itself (Duan et al., 2003). Moreover, $P2X_7$ receptor mediated [^{3}H]GABA release from type-2 astrocytes is also independent of $[Ca^{2+}]_o$ and is mediated by a Cl^-/HCO_3^--dependent mechanism that is regulated by protein kinase C (PKC), protein kinase A (PKA), extracellular signal regulated protein kinase (ERK) and phospholipase D (PLD) (Wang et al., 2002). On the other hand, $P2X_7$ receptor activation leads to a remarkable, approximately 25-fold increase in the production and release of the endocannabinoid 2-AG in astrocytes, which is the highest elevation of extracellular endocannabinoid level measured so far in the nervous system and is probably related to the sustained elevation of $[Ca^{2+}]_i$ caused by high concentrations of ATP (Walter et al., 2004). Finally, ATP and BzATP potentiate LPS-evoked inducible nitric oxide synthase (iNOS) expression and subsequent NO production by astrocytes with a pharmacological profile resembling that of the $P2X_7$ receptor (Murakami et al., 2003); these effects are similar to those detected in macrophages (Sperlágh et al., 1998) and microglia (Gendron et al., 2003a; Ohtani et al., 2000). Thus, $P2X_7$ receptor activation seems to be a common trigger for astrocytic transmitters and modulators to enter the extracellular space either $[Ca^{2+}]_o$-dependently or by other signaling mechanisms and, thereby, modulate synaptic activity.

Astrocytes also participate in neuroinflammation underlying a variety of CNS diseases including trauma, ischemia and neurodegeneration. Inflammatory stimuli rapidly induce the proliferation and hypertrophy of glial cells, a process called reactive gliosis, and respond with the expression and production of inflammatory cytokines, chemokines and other mediators. $P2X_7$ receptors are involved in these regulatory pathways; their activation promotes the expression of MCP1 protein, which is a critical factor in the early monocyte infiltration during the neuroinflammatory process (Panenka et al., 2001). Furthermore, it increases the phosphoryation of ERK1, ERK2 and p38 MAP kinase, proteins which have determinant role in the commitment of the cells to apoptosis (Panenka et al., 2001; Wang et al., 2003). The phosphorylation of ERK1/2 is mediated through a cellular pathway that is dependent on both $[Ca^{2+}]_o/[Ca^{2+}]_i$ and the expression of other intracellular signaling proteins, i.e., Pyk2, c-Src, IP3K and MEK1/2, respectively (Gendron et al., 2003b). Other astrocyte signaling proteins, regulated by $P2X_7$ receptors include PLD (Sun et al., 1999) transforming growth factor-beta 1 (TGF-β1) (Wang et al., 2003) and protein kinase B (Akt) (Jacques-Silva et al., 2004). The activation of PLD is dependent on extracellular Ca^{2+} (Sun et al., 1999), and is mediated by both PKC-dependent and PKC-independent intracellular pathways, as well as Ca^{2+}/calmodulin dependent protein kinase II (CAMKII) and tyrosine kinases (Hung and Sun, 2002), whereas the signaling pathway leading to TGF-β1 expression includes PKC and the subsequent activation of mitogen activated protein kinase (MAPK; ERK1/2) (Wang et al., 2003). The $P2X_7$ receptor-dependent phosphorylation of Akt is also $[Ca^{2+}]_o$- and $[Ca^{2+}]_i$-dependent, and potentially mediated by IP3K and a Src family kinase (Jacques-Silva et al., 2004). $P2X_7$ receptors have been suggested to mediate the effect of ATP to potentiate IL-1β-induced expression of nuclear factor-κB (NF-κB) and to promote activator protein-1 (AP-1) protein expression (John et al., 2001). A recent study revealed that the activation of $P2X_7$ receptors attenuate LPS induced TNF-α release from primary cortical astrocytes (Kucher and Neary, 2005); this contrasts with corresponding data in microglia, where $P2X_7$ receptors stimulate the production of the proinflammatory cytokine TNF-α (Suzuki et al., 2004).

Finally, $P2X_7$ receptor activation plays an important role in the Ca^{2+} signaling between astrocytes and microglial cells (Verderio and Matteoli, 2001). Astrocyte populations coordinate their functions via Ca^{2+} waves, and the spread of the Ca^{2+} signal is implemented by two ways: an intercellular pathway mediated by gap junctions, and an extracellular pathway mediated by ATP and P2 receptors (Guthrie et al., 1999). Astrocytes communicate by calcium-mediated signaling not only with each other but also with neighboring cells including neurons and microglia. Thus

astrocyte-derived ATP activates $P2X_7$ receptors on microglial cells and elicits Ca^{2+} signals in the microglia, which eventually leads to cytolysis of this cell type (Verderio and Matteoli, 2001). It is interesting to note that $P2X_7$ receptor-activation in astrocytes usually does not lead to cytolysis, whilst the same effect might cause cellular death in microglia. The reason for the different resistance of astrocytes and microglia against the $P2X_7$ receptor mediated cytolysis is unknown.

In the peripheral nervous system, Schwann cells express $P2X_7$ receptors both at the transcriptional (Colomar et al., 2003) and at the protein level (Colomar and Amedee, 2001). The activation of $P2X_7$ receptors results in an inward current, which comprises three ionic conductances: a Ca^{2+} activated K^+ conductance, a Cl^- conductance linked to K^+ ions, and an ion flux (including that of Ca^{2+}) through the $P2X_7$ receptor itself (Colomar and Amedee, 2001; Grafe et al., 1999). $P2X_7$ receptors and the K^+ conductance associated with their activation is responsible for the maturation and release of IL-1β through the interleukin-1 converting enzyme (ICE, also known as caspase 1) in this cell type, which is synthesized upon the priming of the primary inflammatory stimulus (e.g., LPS) (Colomar et al., 2003). This mechanism is analogous to the route whereby IL-1β is posttranslationally processed in microglia and other immune cells (e.g., Ferrari et al., 1997c). Moreover, recently it has been reported that the secretion of IL-1β by this pathway is carried out by an ATP binding cassette (ABC1) protein (Marty et al., 2005). Given the governing role of IL-1β in the propagation of the inflammatory signal along the cytokine network, this function sets the $P2X_7$ receptor to a strategically important central regulatory site of the immune response.

Microglial cells originate from monocyte/macrophage precursors and are regarded as the major immunocompetent cell type of the nervous system. Therefore, it is not surprising that they express $P2X_7$ receptors, which convey functions analogous to those of other cells of the monocyte/macrophage lineage upon inflammatory stimuli. Thus, they are rapidly activated in response to pathological signals such as ischemia and inflammation and respond with morphological changes transforming the resting ramified microglia to an amoeboid form with phagocytic activity, proliferation and the production of a wide array of inflammatory mediators. It has been known for a considerable time that microglial cells express both ionotropic and metabotropic receptors for ATP (Norenberg et al., 1994), and the presence of the ''pore-forming'' ATP receptor was also described before the molecular identification of $P2X_7$ receptors (Ferrari et al., 1996; Haas et al., 1996). The expression of $P2X_7$ receptor transcripts and of the corresponding protein has been confirmed later both in cultured microglial cells (Collo et al., 1997; Ferrari et al., 1997a; Gendron et al., 2003a) and in microglial cells activated following middle cerebral artery occlusion (Collo et al., 1997) (Table 2).

Primary microglial cultures and immortalized microglial cell lines respond to ATP and BzATP application with an inward current (Chessell et al., 1997; Haas et al., 1996; Visentin et al., 1999), membrane depolarization, a sustained increase in intracellular free Ca^{2+} (Ferrari et al., 1996), the uptake of Lucifer yellow (Ferrari et al., 1996) and ethidium bromide (Chessell et al., 1997), and the secretion of IL-1β upon an LPS stimulus (Ferrari et al., 1997c, 1996; Sanz and Di Virgilio, 2000), with a pharmacological profile resembling that of $P2X_7$ receptors. The central role of $P2X_7$ receptors, as co-stimulators of the posttranslational processing of IL-1β in microglial cells upon LPS challenge has been repeatedly proven (Brough et al., 2002; Ferrari et al., 1997c; Sanz and Di Virgilio, 2000). The mechanism underlying ATP dependent IL-1β maturation and release in this cell type involves an outwardly directed K^+ conductance and the activation of ICE (caspase 1) responsible for the cleavage of pro-IL1β to the mature, 17 kDA form (Sanz and Di Virgilio, 2000). This mechanism appears to participate not only in the exogenous but also in the endogenous activation of $P2X_7$ receptors, since LPS releases ATP from microglial cells, and the $P2X_7$ receptor selective antagonist oxiATP prevents the LPS-induced IL-1β release (Ferrari et al., 1997c).

Interestingly, ADP and AMP also act as agonists of $P2X_7$ receptors in microglial cells in terms of membrane currents and LPS induced IL-1β secretion, but only after ''priming'' of the cells by ATP challenge (Chakfe et al., 2002). The molecular mechanism underlying the priming effect of ATP could be a reversible conformational change leading to the modification of the agonist binding motif, or the gating properties of the $P2X_7$ receptor, or its activity-dependent phosphorylation. This priming effect, on the other hand, does not extend to the pore forming property of recombinant and microglial $P2X_7$ receptors (Chakfe et al., 2002).

In addition to IL-1β, the synthesis and release of other cytokines, are also stimulated by $P2X_7$ receptor activation in the microglia. Hence, ATP is a full stimulus (i.e., without the requirement of priming by LPS) to induce TNFα production via a Ca^{2+} dependent, ERK/JNK/p38 signalling pathway (Hide et al., 2000; Suzuki et al., 2004), although P2 receptors other than $P2X_7$ may also participate in ATP induced ERK activation (Suzuki et al., 2004). On the other hand, involvement of $P2X_7$ receptors in the regulation of the production of the antiinflammatory cytokine IL-6 is more controversial. Whereas Inoue et al. (Shigemoto-Mogami et al., 2001; Inoue, 2002) reported that ATP-induced IL-6 production is not mediated by $P2X_7$ receptors, a recent study found markedly elevated levels of IL-6 in inflamed hindpaw of $P2X_7^{-/-}$ mice (Chessell et al., 2005), implicating the participation of this receptor in shaping IL-6 levels. Moreover, Rampe et al. (Rampe et al., 2004) revealed that $P2X_7$ receptors play a role in the distinct modulation of cytokine secretory pathways not only after LPS but also upon amyloid beta peptide (Aβ) pre-activation. Whereas the production of IL-1β, IL-1α, TNFα and IL-18 was increased, that of IL-6, the anti-inflammatory cytokine was attenuated under these conditions, implicating the involvement of $P2X_7$ receptors in the pathogenesis of Alzheimer's disease (Rampe et al., 2004).

High concentrations of ATP induce iNOS mRNA expression and increase NO production from rat microglia (Ohtani et al., 2000), an effect potentially mediated by $P2X_7$ receptors. In contrast, ATP by itself does not induce iNOS expression, but enhances IFNγ-induced iNOS expression and subsequent NO

production in the murine BV-2 microglial cell line through an ERK1/2 and tyrosine kinase mediated pathway (Gendron et al., 2003a). ATP and BzATP also promote the generation of reactive oxygen intermediates (ROI), in particular superoxide radicals, in a way depending on extracellular Ca^{2+}; PPADS, oxiATP and Brilliant Blue G all prevent this effect. The actions of ATP are mediated by the p38MAPK pathway (Parvathenani et al., 2003). Just as in astroglial cells, the activation of $P2X_7$ receptors elicits a pronounced increase in 2-AG secretion in primary microglial cell cultures by the activation of diacylglycerol lipase and the simultaneous inhibition of monoacylycerol lipase, the enzyme responsible for endocannabinoid degradation (Witting et al., 2004). This mechanism is unexpected, since lipases were thought to be primarily regulated by metabotropic, but not by ionotropic receptors. Finally, activation of $P2X_7$ receptors by low concentration of agonists stimulates the release of the neuroprotective mediator plasminogen from cultured microglia (Inoue et al., 1998). Therefore, regulation of the production of putatively protective (plasminogen, TNFα, 2-AG) and harmful (IL-1β, NO) mediators by $P2X_7$ receptors appears to follow a highly time- and concentration-dependent pattern (Inoue, 2002).

In addition to its role to regulate the production of inflammatory mediators, the activation of $P2X_7$ receptors elicits changes in microglia at the transcriptional level: it rapidly activates the transcription factor nuclear factor of activated T cells (NFAT) in a $[Ca^{2+}]_o$ dependent manner (Ferrari et al., 1999b) as well as causes the nuclear translocation of NF-κB via ROIs and caspase activation leading to the transcription of a subset of NF-κB target genes (Ferrari et al., 1997d). The expression of other transcription factors are regulated time-dependently upon $P2X_7$ receptor activation. While an acute exposition (10 min to 1 h) of microglial cells to ATP and BzATP upregulates the production of the inflammation related protein microglial response factor (MRF)-1 (Kaya et al., 2002), long-term (6 h) exposure suppresses its transcription and synthesis (Tanaka and Koike, 2002). Moreover, a conditioned medium from cerebellar granule cells undergoing apoptosis also upregulated MRF-1 release in an oxiATP sensitive manner, indicating that this mechanism plays a role in the neuron-microglia cross-talk during microglial activation in response to apoptotic signals (Tanaka and Koike, 2002). According to its pore-forming property, the activation of $P2X_7$ receptors leads to cytolysis in an apoptotic fashion in the microglia (Ferrari et al., 1997a). The $P2X_7$ receptor mediated apoptosis involves the activation of the proteolytic pathway of the caspase activation, which leads to nuclear DNA damage, but is not an absolute requirement for the membrane damage and cytolysis, i.e., if caspases are inhibited, cell death proceeds through the necrotic pathway (Ferrari et al., 1999a). Nevertheless, the $P2X_7$ receptor activated IL-1β secretion and cell death, although both processes involve caspases and both are eliminated in $P2X_7^{-/-}$ mice, appear to be independent from each other (Brough et al., 2002).

In contrast to the plethora of experimental data obtained in glial cells kept in culture, much less data has been accumulated on the role of glial $P2X_7$ receptors in more integrated systems, where the cell architecture and extracellular environment are retained. Non-selective cationic membrane currents could be recorded from identified resting microglial cells in acute brain slices in response to ATP (1 mM) and BzATP (0.2 mM) application, which presumably reflects the activation of $P2X_7$ receptors, although the responses have not been analyzed pharmacologically in detail (Boucsein et al., 2003). In addition, the presence of putative, functional $P2X_7$ receptors have also been demonstrated by the in situ optic nerve glia, where $P2X_7$ receptor activating concentrations of ATP and BzATP evoke a sustained increase in $[Ca^{2+}]_i$ and the uptake of YO-PRO into microglial cells (James and Butt, 2002). Finally, BzATP appears to permeabilize microglial cells though the activation of $P2X_7$ receptors in the isolated rat inner retina (Innocenti et al., 2004).

8. The pathological role of $P2X_7$ receptors in the nervous system: potential therapeutic implications

The widespread and profound effects of $P2X_7$ receptor activation on different aspects of neuronal excitability/survival and microglial/astroglial activation implicates their role in the pathology of CNS and PNS diseases and provokes their application as a therapeutic target. Indeed, rapidly emerging knowledge supports such a role, in particular the demonstration of (1) the activity-dependent expression of $P2X_7$ receptors under pathological conditions, and (2) the protective role of $P2X_7$ receptor ligands in animal disease models. The diseases, in which $P2X_7$ receptors may play either a harmful or protective role include neurodegenerative and neuroinflammatory diseases, such as ischemia-reperfusion and traumatic injury, Alzheimer's disease, sclerosis multiplex, rheumatoid arthritis and retinopathies. Besides its cytolytic property, the importance of $P2X_7$ receptors in these pathological situations is underscored by their determinant role in shaping the level of many other key regulators and signaling pathways of neurodegeneration and the subsequent repair process (e.g., glutamate, IL-1β, TNF-α, NO, ROI and endocannabinoids).

The upregulation of $P2X_7$ receptors has been observed in a number of pathological models, including energy deprivation (Cavaliere et al., 2004; Cavaliere et al., 2002), in vivo ischemia (Franke et al., 2004), epilepsy (Vianna et al., 2002), mechanical injury (Franke et al., 2001), transgenic models of Alzheimer's disease (Parvathenani et al., 2003) and retinitis pigmentosa (Franke et al., 2005), as well as in human tissue samples obtained from patients suffering from proliferative vitreoretinopathy (Bringmann et al., 2001), sclerosis multiplex (Narcisse et al., 2005), and sensory nerve injury (Chessell et al., 2005).

However, different stimuli increase the expression of $P2X_7$ receptors in these pathological models in different cell types and in a temporally distinct manner. The first study showing an upregulation of $P2X_7$ receptors in the brain was that of Collo et al. (1997) who found an increased immmunostaining for $P2X_7$ receptors in activated microglial cells of the zona penumbra in the MCAO model. The microglial up-regulation of $P2X_7$ receptors in response to oxygen deprivation has also been

documented in the retinal microglia (Morigiwa et al., 2000). On the other hand, in a more recent study, an early (1 day after MCAO occlusion) upregulation of the $P2X_7$ receptor protein was observed in microglial cells, and later (4–7 days after MCAO occlusion), the receptor also overexpressed in neurons and astrocytes of the perinfarct area in spontaneously hypertensive rats, as revealed by co-localization studies with neuronal, astroglial and microglial markers (Franke et al., 2004). Combined oxygen-glucose deprivation, which can be regarded as the in vitro model of ischemia, also strongly up-regulates $P2X_7$ receptor immunoreactivity in nerve terminals of cerebellar granule cells kept in culture (Cavaliere et al., 2002) and in organotypic hippocampal cultures (Cavaliere et al., 2004); the $P2X_7$ receptor antagonist oxiATP prevented both the upregulation and the neuronal death evoked by the metabolic impairment. This implies that ATP itself participates in the upregulation and has a self-perpetuary role in neurodegenerative processes. Indeed, ATP in high micromolar concentration increased the expression of $P2X_7$ receptors in cerebellar granular neurons and by itself initiated neuronal death, although this latter effect was not prevented by oxiATP (Amadio et al., 2002). Up-regulation of the $P2X_7$ receptor protein can also be observed in cultured cortical cells exposed to energy deprivation, and this upregulation is also manifested in functional terms, as increased efflux of [^{3}H]GABA and an increased facilitation of mIPSC frequency could be detected in response to BzATP after the treatment (Wirkner et al., 2005). $P2X_7$ receptors appear to be endogenously activated upon ischemia-like conditions to contribute to increased excitatory transmitter release and glutamatergic excitotoxicity, as both PPADS and the selective $P2X_7$ receptor antagonist Brilliant Blue G decreased glutamate release upon combined oxygen-glucose deprivation in the slice model of rat hippocampus (B. Sperlágh unpublished observation).

The altered responsiveness of neuronal $P2X_7$ receptors might also play a role in the pathogenesis of epilepsy, since Western blotting studies indicate a massive upregulation of $P2X_7$ receptor immunoreactivity in chronic pilocarpine-induced epilepsia of rats (Vianna et al., 2002). However, a decrease in $P2X_7$ receptor immunoreactivity was found in hippocampal CA1 neurons of seizure sensitive gerbils in comparison to their seizure resistant counterparts (Kang et al., 2004). $P2X_7$ receptors appear to be up-regulated in retinal ganglion cells, but not in microglial or Müller glial cells in a transgenic mouse model displaying retinal degeneration (Franke et al., 2005). Other pathological models implicate $P2X_7$ receptor overexpression in non-neuronal cells; mechanical injury leads to the expression of previously absent $P2X_7$ receptors in astrocytes, and $P2X_7$ receptor immunoreactivity appears to be up-regulated around amyloid plaques in activated microglia and astrocytes in the Tg2576 transgenic mice having mutant amyloid precursor protein (APP) (Parvathenani et al., 2003). Recently, $P2X_7$ receptor immunostaining was also identified in reactive astrocytes around the lesions in autopsy brain sections of sclerosis multiplex patients (Narcisse et al., 2005). Moreover, an increased $P2X_7$ receptor immunorecactivity was found in tissue sections of painful injured nerves correlating with the glial marker GFAP (Chessell et al., 2005).

Altogether these data indicate that the expression of the $P2X_7$ receptor or a $P2X_7$ receptor-like protein is strongly activity-dependent during pathological situations. Nonetheless, whether the change in their expression pattern and functional responsiveness is a simple adaptive change or plays a more active pathogenetic role warrants further investigation. It is also a largely open question whether the final outcome of versatile actions mediated by neuronal, microglial and/or astroglial $P2X_7$ receptors are protective or harmful. Since $P2X_7$ receptors seem to be situated at a rather upstream regulatory site of either neuronal or glial information processing, they may influence a number of events, which by themselves could be beneficial or toxic, and therefore could counterbalance each other's action. Thus, glutamate excitotoxicity, IL-1β, and ROIs are regarded to be clearly pathological factors deteriorating the disease states, GABA, endocannabinoids, anti-inflammatory cytokines, and plasminogen are protective, and, finally, TNFα and NO have a double-face role.

Therefore, it is not surprising that when the protective role of $P2X_7$ receptor antagonism was tested in functional disease models the generated data were often ambiguous. The neuroprotective action of non-selective P2 receptor antagonists is well documented in in vitro models of glutamate- (Volonte et al., 1999; Volonte and Merlo, 1996) and kainate- (Zona et al., 2000) mediated excitotoxicity, glucose deprivation (Cavaliere et al., 2001b; Geng et al., 1997) and chemical hypoxia (Cavaliere et al., 2001a). By contrast, initial in vivo studies yielded discouraging results, since neither genetic deletion of $P2X_7$ receptors nor $P2X_7$ receptor antagonists affected the infarct volume in MCAO and in glutamate toxicity models of mice (Le Feuvre et al., 2002a, 2003). It has to be noted, however, that because alternative compensatory pathways could be activated, a simple genetic deletion of $P2X_7$ receptor might not be the right model to investigate the role of $P2X_7$ receptors in vivo in neurodegenerative diseases. Unfortunately, the utility of the pharmacological approach, on the other hand, is hampered by the limited selectivity of the available ligands.

In spite of these confounding factors, a series of recent studies supplied convincing proof on the involvement of $P2X_7$ receptors in several animal disease models. In an elegant study, Wang et al. (2004) reported that immediately following acute spinal cord injury a sustained, high amount of ATP release could be detected in the peritraumatic zone, which was strongly correlated with the degree of degeneration of nerve cells in the same area (Wang et al., 2004); the $P2X_7$ receptor antagonist oxiATP not only protected against neuronal death but also improved functional recovery after the traumatic insult.

Eventually, oxiATP has a well documented antinociceptive effect in the (FCA) induced neuropathy model (Dell'Antonio et al., 2002a,b), and another recent study found that the inflammatory and neuropathic hypersensitivity is completely absent in $P2X_7^{-/-}$ mice, whereas conventional nociception is preserved (Chessell et al., 2005). These intriguing findings set

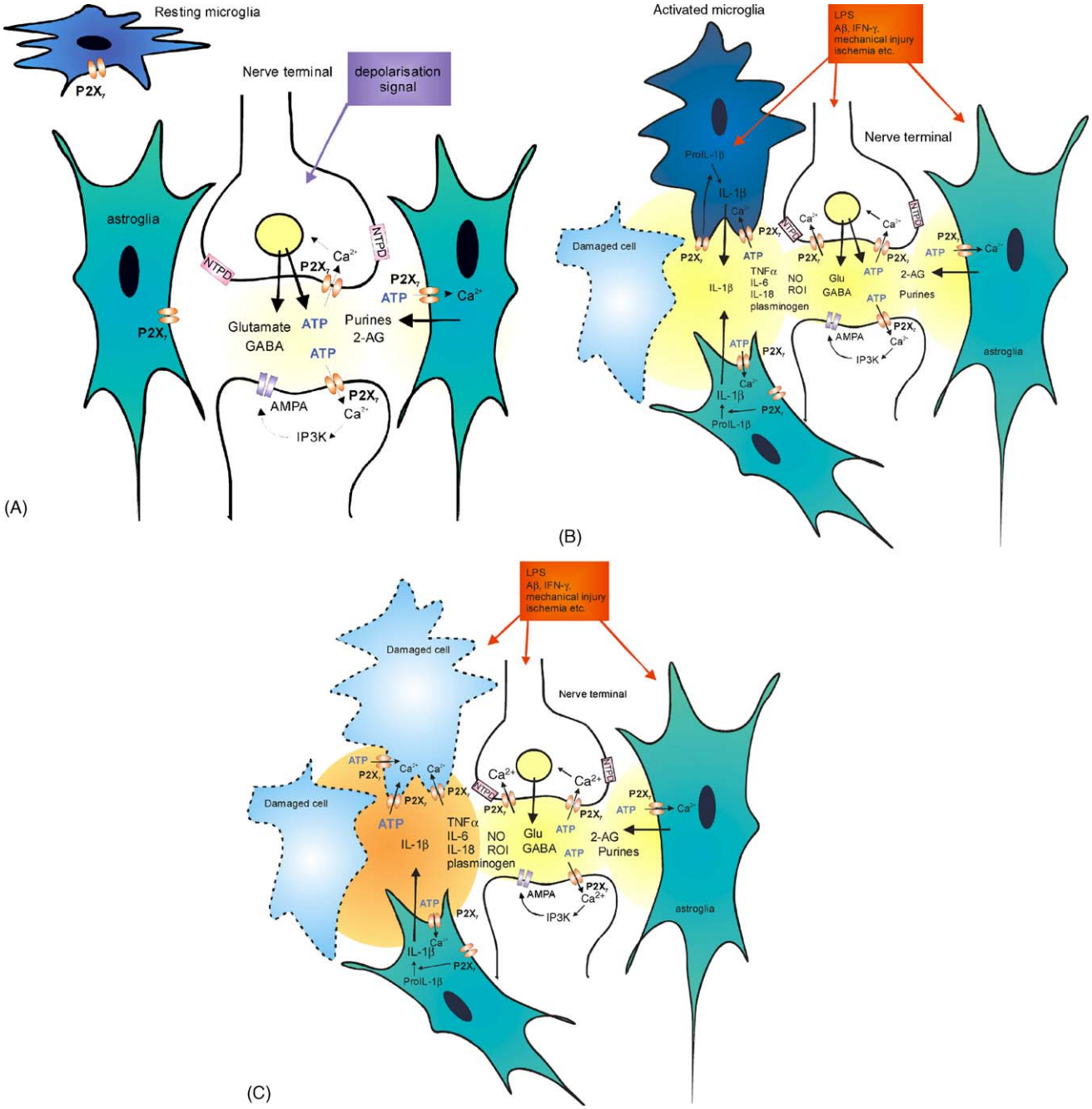

Fig. 1. Hypothetical pathways leading to endogenous activation of $P2X_7$ receptors. (A) Depolarisation signals invading the nerve terminals during physiological neuronal activity, result in a spatially restricted, localized increase of extracellular ATP concentration in the vicinity of $P2X_7$ receptors, which could directly affect neurotransmission by altering pre- and postsynaptic synaptic efficacy and thereby participate in plasticity events underlying diverse neuronal functions. The exact localization of $P2X_7$ receptors responsible for these actions are still uncertain: they could be located on neurons and/or astrocytes/microglia. However, ATP-metabolizing ectoenzymes, such as ectoNTPDases (NTPD), present on the nerve terminal surface, may limit ATP availability under these conditions. (B) Stressful situations, such as mechanical, metabolic or inflammatory stress, cellular injury and changes in the ionic environment might result in an ATP-rich extracellular milieu leading to the upregulation and widespread activation of $P2X_7$ receptors on astroglia and microglia and boosting diverse pathological cascades, such as glutamatergic excitotoxicity, oxidative damage, NO-, endocannabinoid- and IL-1β, TNF-α and other cytokine-mediated signaling and protective reactions such as sensory signaling and pain sensation. (C) The persistent activation of $P2X_7$ receptors could act as a suicide signal and might trigger apoptosis or necrosis of the cell-type expressing these receptors. Astroglia derived ATP might also participate in these processes and could elicit microglial cell death. The intensity of the shading of the extracellular space indicates different levels of extracellular ATP. Dashed borders represent the membrane of damaged cells subject to apoptosis/necrosis. 2-AG, 2-arachidonoyl-glycerol; Glu, glutamate; ROI, reactive oxygen intermediates; NO, nitric oxide; IP3K, phosphatidylinositol 3-kinase.

the $P2X_7$ receptor an attractive target in a previously unrecognized area, in the unresolved therapy of neuropathic and inflammatory pain. Interestingly, $P2X_7$ receptor deficient mice also show increased susceptibility to experimental autoimmune encephalomyelitis (EAE), an animal model of sclerosis multiplex (Chen et al., 2005), which is potentially due to the decreased microglial secretion of the protective endocannabinoid 2-AG (Witting et al., 2004).

9. Conclusions

Despite the continuous ambiguity on its exact localization, the overwhelming majority of data indicates that $P2X_7$ receptor or its brain analogue is an important signaling protein participating in the information processing of the normal and pathological nervous system. Nevertheless, the conditions, which lead to endogenous activation of $P2X_7$ receptors in the brain, need to be better explored. Collectively, the data suggest that at least three levels of the activation of $P2X_7$ receptors might exist (Fig. 1).

Firstly, under relatively ATP-poor conditions of physiological neuronal activity, which results in a spatially restricted, localized increase of ATP concentration in their vicinity, $P2X_7$ receptors could directly affect neurotransmission by altering pre- and postsynaptic synaptic efficacy and thereby participate in plasticity events underlying diverse neuronal functions (Fig. 1A). ATP-metabolizing ectoenzymes, present on the nerve terminal surface, may rapidly terminate the action of ATP under these conditions. Secondly, pathological events such as mechanical or metabolic stress, inflammation, cellular injury or changes in the ionic environment might result in a prolonged accumulation of ATP in the extracellular space leading to the upregulation and widespread activation of $P2X_7$ receptors on astroglia and microglia and boosting diverse pathological cascades, such as glutamatergic excitotoxicity, oxidative damage, NO-, endocannabinoid-, IL-1β and other cytokine-mediated signaling and protective reactions such as sensory signaling and pain sensation (Fig. 1B). Thirdly, under conditions of persistent activation, $P2X_7$ receptors might convey a cell death signal triggering the apoptosis or necrosis of the cell expressing it (Fig. 1C). To identify the relative importance of these events will pave the pathway to the much expected therapeutic utilization of $P2X_7$ receptors in nervous system diseases.

Acknowledgements

This study was supported by grants of the Hungarian Research Foundation (OTKA T037457: BS; TS049868: ESV), Hungarian Medical Research Council (472/2003: BS 123/2003: ESV), Hungarian Research and Development Fund (NKFP 1A/002/2004: BS), the Volkswagen Foundation (I777 854: PI and BS), the Bundesministerium für Forschung und Technologie (Deutsch-Polnische Zusammenarbeit in den Neurowissenschaften: PI), the Interdisciplinary Center of the Medical Faculty, University of Leipzig (Project C4: PI), and the European Community (Marie-Curie-Host-Fellowship: PI).

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