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Extracellular ATP and P2X7 receptors in neurodegeneration

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Abstract

Neuronal injury and cell death in the central nervous system (CNS) are underlying features of neurodegenerative disorders. However, our understanding of the fundamental mechanisms involved is still limited. Inflammatory processes mediated by cytokines, and interleukin-1 (IL-1) in particular, play a significant role in neuronal death following pathological insults. Despite this growing area of research, very little is known about the factors regulating the expression, cleavage and release of interleukin-1 in the brain. Recent studies on immune cells demonstrate that extracellular ATP can act as a potent stimulus for the maturation and release of interleukin-1β, via activation of P2X7 receptors. Stimulation of P2X7 receptors with ATP has dramatic cytotoxic properties and a wider role in neurodegenerative processes is possible. This review discusses the potential involvement of extracellular ATP and P2X7 receptors as regulators of interleukin-1-mediated neuropathologies and thus as a mediator of cell death following pathological insults.

Keywords: ATP; P2X7; Neurodegeneration; Interleukin-1

1. Introduction—ATP and neurodegeneration

The role of extracellular ATP and purinoceptors in cytokine regulation and neurodegeneration is the focus of a rapidly expanding area of research. ATP can act as a neurotransmitter, whilst the presence of P2X7 receptors (purinergic receptor sub-class) on immune cells suggests that it also regulates immune function and inflammatory responses. In addition, activation of this receptor has dramatic cytotoxic properties, which together with its ability to regulate cytokine production and release, suggest that it can act as an important regulator of cell death in response to pathological insults.

Neurodegeneration is the underlying basis of many disorders including cerebral ischaemia, brain trauma, multiple sclerosis, Parkinson's, Alzheimer's and Huntington's diseases. Despite being the focus of considerable research over the past 20 years, our understanding of the fundamental mechanisms involved in neurodegeneration is still limited in terms of potential clinical therapies. This review will discuss some recent progress in the field of neurodegeneration, the involvement of inflammatory cytokines such as interleukin-1 (IL-1), and will elaborate new findings on their regulation through ATP stimulation of P2X7 receptors, which may offer novel therapeutic approaches.

2. Mechanisms of neurodegeneration

The mechanisms involved in neuronal death are complex and are likely dependent upon multiple signalling cascades. This review will focus on cerebral ischaemia, as a major clinical condition for which there is extensive experimental data. However, a diverse range of neurodegenerative diseases, trauma and excitotoxicity show common features. Cerebral ischaemia occurs when the blood supply to an area of the brain is reduced either permanently or transiently, resulting in oxidative and metabolic stress to the tissue. One consequence of such metabolic compromise is the depletion of cellular ATP reserves. With the failure of the Na^+/K^+ pump, neurones depolarise resulting in excess release and accumulation of the excitatory amino acid neurotransmitter, L-glutamate (Choi, 1994; Haddad and Jiang, 1992; Limbrick et al., 1995).Glutamate activates a number of post-synaptic ionotropic (e.g., NMDA, kainate and AMPA) and metabotropic receptors leading to disturbances in the intracellular ionic environment. High cytoplasmic Ca^{2+} concentrations, resulting from suppression of Ca^{2+} pumps and activation of

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agonist-dependent and voltage-gated Ca^{2+} channels, are central to the pathological process culminating in cell death (Siesjö, 1981).

Neurones, which survive the primary insult, may die hours or days later as increased intracellular Ca^{2+} or other second messenger systems initiate cascades with the activation of proteases, lipases, nitric oxide synthases and nucleases. Depending on the severity of the insult and the metabolic energy state of the cell, death may occur by either apoptotic or necrotic mechanisms (Lee et al., 1999; Nicotera, 2000). Increased apoptosis is reported in several neurodegenerative diseases (Forloni et al., 1996; Saudou et al., 1998; Kim et al., 1999). In experimentally induced cerebral ischaemia in rodents, apoptosis may be particularly important in the penumbra. This area, suffers only mild ischaemic insult, but may undergo apoptosis from actions of secondary mediators such as oxygen radicals, cytokines and lipid peroxidation products from the more severely stressed areas of the necrotic core (Mattson, 1998; Charriaut-Marlangue et al., 1996; Lipton and Rossenberg, 1994). During reperfusion of the tissue, excessive uptake of Ca^{2+} by mitochondria could play a key role in ischaemic cell death by activating the production of oxygen-free radicals and blocking the synthesis of high-energy phosphates (Kristian and Siesjö, 1998).

Alterations in Ca^{2+} homeostasis result in changes in protein synthesis and secretion, which involve the activation of nuclear factor κB (NF-κB) and hence, NF-κB-dependent genes, such as interferons and cytokines, and activation of death related genes that increase caspase expression (Paschen and Doutheil, 1999; Nicholson and Thornberry, 1997). Multiple caspases are activated during ischaemia (Bhat et al., 1996; Chen et al., 1997; Harrison et al., 2001). Pan-caspase inhibitors are neuroprotective in a number of experimental neurodegenerative paradigms (Schneider et al., 2000), whilst ischaemic lesions are significantly reduced in mice lacking the caspase-1 gene (Li et al., 1995; Liu et al., 1999).

For more than 15 years, extracellular glutamate accumulation has been suspected as the initiator of immediate and delayed neuronal death following stroke (McCulloch et al., 1993). Despite this, inhibition of excess glutamate by antagonism of receptors, or the enhancement of glutamate re-uptake mechanisms have not provided effective stroke therapies (Doble, 1999). Since glutamate transmission, Ca^{2+} homeostasis and nitric oxide (NO) synthesis are all involved in normal brain function, therapeutic strategies aimed at these areas may be accompanied by unacceptable side effects (Iadecola, 1997; Doble, 1999).

3. Neurodegeneration and cytokine regulation

Evidence produced over the last decade has demonstrated an important role for inflammatory processes in the pathogenesis of cerebral ischaemia and many forms of neurodegeneration (Kogure et al., 1996; Allan and Rothwell, 2001). Cytokines are a group of polypeptides, which include interleukins, tumour necrosis factors, chemokines, interferons and growth factors/neurotrophins, and are key mediators of inflammation. Members of each of these polypeptide families have been implicated in stroke either as mediators of damage or potential neuroprotective/neurotrophic factors. Since cytokines appear to play little or no role in normal brain function therapeutic approaches to block their actions may prove successful. Indeed, the involvement of cytokines in central nervous system (CNS) disease, is now a rapidly growing area of biological and clinical research (Barone and Feuerstein, 1999; del Zoppo et al., 2000). An early ischaemic event is the infiltration of leukocytes and the activation of resident brain cells including astrocytes, microglia and endothelial cells (Stoll et al., 1998). This inflammatory reaction is triggered by expression of a complex array of genes regulated by NF-κB, including the inflammatory cytokines (interleukin-1β (IL-1β), tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS) (see Feuerstein et al., 1998 for review)), which modulate inflammation, apoptosis and neuronal plasticity.

Cytokine bioactivity can be regulated at the levels of transcription, translation, cleavage and cellular release as well as through receptor and post-receptor signalling mechanisms. Excitatory amino acids can regulate cytokine expression directly during excessive release following CNS injury, through phosphorylation of C-jun N-terminal kinase (JNK) (Sattler et al., 1999; Savinainen et al., 2001) and the activation of the transcriptional factor c-*jun* which promotes expression of cytokines (e.g., IL-1, IL-6 and TNF).

The cytokine interleukin-1 has received the most attention as a potential mediator of neurodegeneration. The interleukin-1 family comprises two agonists, interleukin-1α and -1β, and a naturally occurring interleukin-1 receptor antagonist (IL-1ra) (Dinarello, 1998; Fantuzzi and Dinarello, 1999; Hannum et al., 2002). Interleukin-1's actions have so far been ascribed to binding to the interleukin-1 receptor 1 (IL-1R1, 80 kDa) which then interacts with a specific accessory protein (AcP) to recruit intracellular adapter molecules and signal through mitogen-activated protein (MAP) kinases and NF-κB (Sims et al., 1993; O'Neill and Dinarello, 2000). However, the possibility of other ligands and receptors exists (Smith et al., 2000; Touzani et al., 2002).

Interleukin-1α, interleukin-1β, interleukin-1 receptor antagonist and the enzyme responsible for interleukin-1β maturation, caspase-1 (originally termed interleukin-1 converting enzyme—ICE), are all up regulated rapidly in response to experimentally induced ischaemic brain damage in rodents (Loddick et al., 1997; Davies et al., 1999; Legos et al., 2000; Touzani et al., 2002) and raised interleukin-1 and interleukin-1 receptor antagonist levels have been reported in human stroke patients (see Touzani et al., 1999). Administration of interleukin-1 to the brain of normal rodents or to neurones in culture is not cytotoxic, but in vivo administration of low doses of interleukin-1 (i.c.v.) exacerbates ischaemic and excitotoxic damage in rat

and mouse models (Lawrence et al., 1998; Stroemer and Rothwell, 1998; Loddick and Rothwell, 1996; Yamasaki et al., 1995). A number of studies have now demonstrated that inhibiting the release or actions of interleukin-1 markedly reduces damage in permanent focal ischaemia in the rodent (Relton and Rothwell, 1992; Garcia et al., 1995; Touzani et al., 2002). Furthermore, ischaemic damage is reduced by over 70% in mice lacking genes for both interleukin-1α and -1β (Boutin et al., 2001). Targeting interleukin-1 could thus offer therapeutic strategies, and interleukin-1 receptor antagonist is now being tested in phase II clinical trials.

Interleukin-1 is a key mediator of host responses, inflammation and tissue injury, and is produced abundantly by macrophages and monocytes. In the brain, Interleukin-1β is expressed and released mainly by microglia, although astrocytes and invading macrophages may also contribute to interleukin-1β production some time after the insult (Pearson et al., 1999; Mabuchi et al., 2000). Microglia are the phagocytic inflammatory mediators within the CNS and they respond to diverse insults by adopting an ameboid morphology, proliferating and producing reactive oxygen species as well as a number of inflammatory mediators such as interleukin-1 and TNFα (Kreutzberg, 1996; Raivich et al., 1999).

Interleukin-1β is expressed as an inactive precursor (pro IL-1β) which is increased by pro-inflammatory stimuli such as bacterial endotoxin (lipopolysaccharide—LPS; (Dinarello, 1991)), other inflammatory cytokines (including interferon-γ (IFNγ) and TNFα (Dinarello and Krueger, 1986; Roux-Lombard, 1998), or activated T-lymphocytes (Vey et al., 1997). In vitro, the cleavage of pro-interleukin-1β and release of the mature active cytokine in lipopolysaccharide-stimulated macrophages, monocytes or microglia, requires a secondary stimulus (e.g., ATP) for the activation of caspase-1 (Hogquist et al., 1991; Thornberry et al., 1992; Cerretti et al., 1992; Sanz and Di Virgilio, 2000) and subsequent release of the mature cytokine.

4. ATP and the regulation of IL-1

In addition to its key roles as a ubiquitous enzyme cofactor and a source of cellular energy, the purine nucleotide ATP also functions as a potent extracellular messenger and neurotransmitter via activation of members of the P2 receptor family (Ralevic and Burnstock, 1998). P2 receptors comprise both metabotropic P2Y and ionotropic P2X receptor families. Seven members of the P2X receptor family have been described, although a P2X8 receptor has been suggested (Ralevic and Burnstock, 1998; Bo et al., 2000).

4.1. P2X7 receptors

Since their discovery in immune cells (Hogquist et al., 1991), P2X7 receptors have been proposed as mediators of inflammation, and a potential role in neurodegeneration has been suggested (Di Virgilio, 1995). The P2X7 receptor (previously referred to as the cytolytic P2Z receptor) has been implicated in signalling between macrophages and other immune target cells (Surprenant et al., 1996). The P2X7 receptor shares 35–40% homology with other P2X receptors. It has two hydrophobic membrane-spanning domains and an extracellular loop, and forms transmembrane ion channels. Stimulation of the P2X7 receptor with high concentrations of ATP (its major endogenous ligand) triggers massive transmembrane ion fluxes (particularly influx of Ca^{2+} and Na^{+}, and efflux of K^{+}) and the formation of non-selective plasma membrane pores (Rassendren et al., 1997) that result in cell death (Di Virgilio et al., 1998a). In addition, P2X7 receptor activation stimulates the induction of multiple cytokine pathways that may co-ordinate inflammatory responses.

Under normal conditions, extracellular nucleotides are present in only low concentrations. However, activated immune cells (lymphocytes (Filippini et al., 1990), macrophages (Sikora et al., 1999), microglia (Ferrari et al., 1997a), and platelets (Beigi et al., 1999)), and dying cells may release high concentrations of different nucleotide di- and tri-phosphates into the extracellular space (Dubyak and el-Moatassim, 1993). Extracellular ATP concentrations increase significantly under inflammatory conditions in vivo (Lazarowski et al., 2000; Di Virgilio et al., 1998b), and in response to tissue trauma (e.g., ischaemia/hypoxia; Nieber et al., 1999). ATP can be co-released with glutamate from pre-synaptic nerve terminals (Sperlágh and Vizi, 1996).

4.2. Interleukin-1 production

ATP was first shown to stimulate the release and processing of interleukin-1β and induce cell death in murine macrophages by Hogquist et al. (1991). ATP induces the processing and release of interleukin-1β by activation of P2X7 receptors (Surprenant et al., 1996; Ferrari et al., 1997b; Perregaux and Gabel, 1998; Solle et al., 2001) and cytokine release can be prevented by inhibition of caspase-1 (Watanabe et al., 1998). Caspase-1 is constitutively expressed in most cells as a 45-kDa inactive precursor, which requires two cleavages to form an active heterodimer (Wilson et al., 1994; Gu et al., 1995). The mechanism of caspase-1 activation was first suggested to be autoproteolysis (Thornberry et al., 1992). More recent evidence suggests that P2X7 receptor stimulation activates caspase-1 through the depletion of intracellular K^{+} (Perregaux and Gabel, 1994; Schulze-Lohoff et al., 1998; Ferrari et al., 1999a; Laliberte et al., 1999; Humphreys et al., 2000). Priming of cells with lipopolysaccharide renders cells particularly sensitive to the cytotoxic and cytokine releasing actions of ATP, possibly through the increased expression of pro-caspase-1 (Le Feuvre et al., 2002). ATP may also affect interleukin-1β production at transcriptional and translational levels, since P2X7 depend-

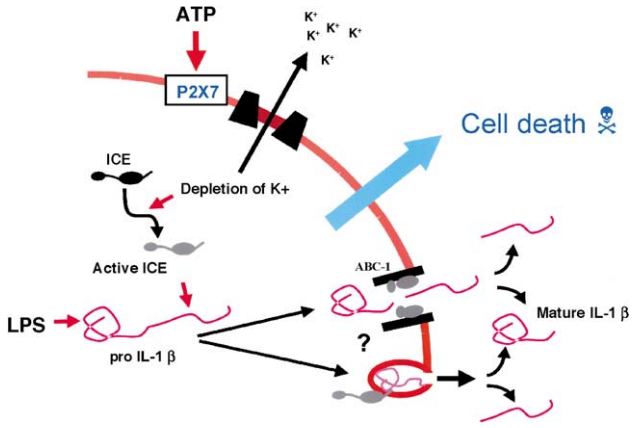

Fig. 1. Scheme depicting proposed mechanisms of ATP stimulated IL-1β processing and release.

ent induction of the transcriptional activator, NF-κB, has been observed in microglia (Ferrari et al., 1997c; Humphreys et al., 2000) (Fig. 1).

4.3. IL-1 release

The mechanism underlying cellular release of mature interleukin-1β in response to ATP and indeed other stimuli is unknown. Pro-interleukin-1β is synthesized on free ribosomes, lacks a signal sequence and therefore accumulates in the cytosol of activated cells. A small fraction of pro-interleukin-1β is myristylated (Stevenson et al., 1993) and monocytes secrete small amounts of pro-interleukin-1β by a mechanism which appears to be distinct from the secretion of the mature form (Beuscher et al., 1990). Interleukin-1β release induced by ATP in lipopolysaccharide primed cells in vitro has proved a useful tool for studying these processes. ATP-induced release and maturation of interleukin-1β from monocytes and macrophages is suppressed by inhibition of the anion exchanger function of the ATP-binding cassette-1 (ABC-1) (e.g., by sulfonylurea glyburide or ethacrynic acid). This suggests that anion conductance is a necessary component of ATP promoted externalisation of interleukin-1 (Laliberte et al., 1994; Perregaux et al., 1996; Hamon et al., 1997; Sanz et al., 1998). These inhibitors do not affect the K^+ efflux, and thus caspase-1 activation would still occur (Perregaux and Gabel, 1998).

Bi-phasic changes in Ca^{2+} levels are observed in response to stimulation of P2X7 receptor expressing dendritic cells, with a rapid release of Ca^{2+} from intracellular stores and a delayed influx across the cell membrane (Ferrari et al., 2000). Current literature suggests that interleukin-1β release and processing is Ca^{2+}-independent but depends upon efflux of K^+ (Perregaux et al., 1992; Perregaux and Gabel, 1994; Walev et al., 1995). Studies with Ca^{2+} ionophores revealed that Ca^{2+} influx has no effect on the release of mature interleukin-1β, whilst agents which induce K^+ efflux, such as nigericin, stimulate interleukin-1 maturation and release. More recently K^+ efflux was shown to regulate interleukin-1β processing via the Ca^{2+}-independent phospholipase A_2 (Walev et al., 2000). Other studies have reported conflicting results. Ciesynski (1999) suggested that a Ca^{2+} ionophore augments interleukin-1 secretion by lipopolysaccharide in chick macrophages, and West et al. (1996) found that lipopolysaccharide-stimulated interleukin-1 release does not require Ca^{2+}-dependent signalling pathways.

Our recent research has demonstrated that ATP-induced IL-1β processing and release requires not only K^+ efflux, but also release of Ca^{2+} from intracellular stores (Brough et al., unpublished observations). The processing and release of IL-1β in macrophages and microglia is dramatically inhibited by addition of the intracellular Ca^{2+} chelator [1,2-bis(*o*-Aminophenoxyethan-*N*,*N*,*N*′,*N*′-tetraacetic acid tetra (acetoxymethyl) ester] (BAPTA-AM) (Fig. 2). Further

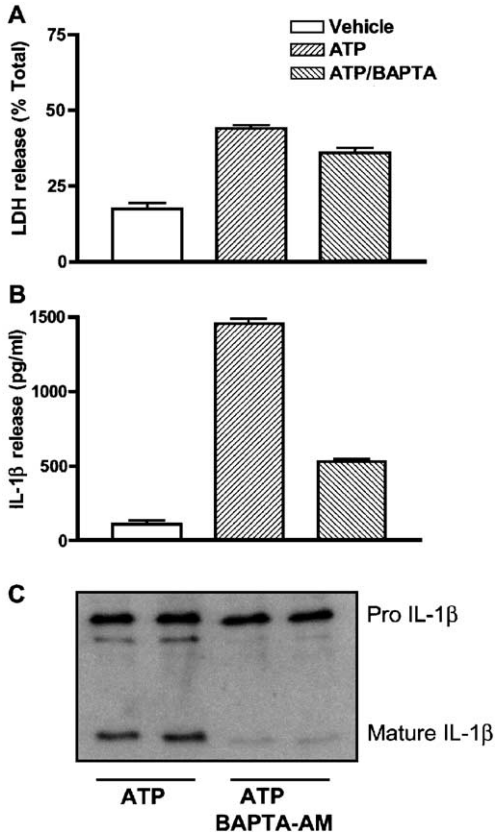

Fig. 2. The calcium chelator, BAPTA-AM, inhibits ATP induced interleukin-1 release in pure microglial cultures without affecting cell death. Primary microglia were primed with lipopolysaccharide (LPS, 0.1 μg) overnight then treated with 5 mM ATP in the presence of vehicle or BAPTA-AM (30 μM). (A) Cell death was assessed as lactate dehydrogenase (LDH) release and expressed as a % of total LDH (measured by complete cell lysis with Triton-X 100). (B) The concentration of IL-1β released into the medium was measured by a specific murine IL-1β ELISA (kindly supplied by Dr. S. Poole). (C) The presence of pro- and mature forms of IL-1β was tested using Western blot analysis.

experiments have revealed that it is the release of Ca^{2+} from intracellular stores rather than an influx of extracellular Ca^{2+} that is involved. BAPTA-AM did not affect caspase-1 activity since caspase-1-induced cell death was still apparent (Le Feuvre et al., 2002).

Although the mechanism of mature interleukin-1β release from the cell is not yet understood, there are a number of proposals resulting from work in monocytes. These currently suggest interleukin-1 release to depend upon a transmembrane pore gated by caspase-1 (Singer et al., 1995), secretion via late endosomes (Andrei et al., 1999) or secretion via microvesicles shed from the plasma membrane (MacKenzie et al., 2001). In contrast with our latest results, showing the importance of Ca^{2+} release from intracellular stores, the shedding of microvesicles in a human monocytic cell line (THP-1, MacKenzie et al., 2001) was dependent upon extracellular Ca^{2+}.

5. Cytotoxic effects of P2X7-receptor stimulation

Activation of P2X7 receptors is associated with cytotoxicity, and this receptor has been described as a death receptor (Di Virgilio et al., 1998a). It was initially suggested that the high concentrations of extracellular ATP required to stimulate cytotoxic actions of this receptor might not be reached in vivo. However, there is evidence that even short periods of P2X7 receptor activation are cytotoxic and that once activated, the P2X7 receptor sets in motion an irreversible death process (Hogquist et al., 1991; Laliberte et al., 1994). Cells primed with inflammatory mediators (e.g., lipopolysaccharide) are particularly susceptible to the toxic actions of ATP (Mehta et al., 2001; Le Feuvre et al., 2002) and this priming effect may alter the distribution or activation of P2X7 receptors in cell membranes (Denlinger et al., 2001).

We have demonstrated that the pan-caspase inhibitor *z*-Val-Ala-DL-Asp (OMe)-fluoromethylketone (zVAD-fmk) markedly inhibits cell death of macrophages exposed to ATP and LPS, and ATP fails to induce cell death of LPS primed macrophages from caspase-1 knock-out (KO) mice (Le Feuvre et al., 2002). These cytotoxic actions are independent of cytokine release (interleukin-1α, -β or interleukin-18), suggesting that caspase-1 has additional actions possibly through induction of other caspases involved in cell death. Indeed, stimulation of P2X7 receptors leads to activation of multiple caspases (e.g., caspases 3 and 9 (Ferrari et al., 1999a)) Prolonged exposure to ATP may reflect induction of other death pathways, such as the activation of stress activated protein kinases (Humphreys et al., 2000). Most importantly, P2X7 regulation of multiple caspases may act as a switch between death by necrosis or apoptosis (Ferrari et al., 1999a), which could affect pathological outcome.

The transcriptional activator NF-κB has been implicated in the control of apoptosis and pro-inflammatory gene expression. A broad range of stimuli can activate NF-κB (e.g., lipopolysaccharide and inflammatory cytokines). In particular, ATP can lead to selective activation of NF-κB in microglial cells, through P2X7 stimulation and induction of caspase-1-like proteolytic activity (Ferrari et al., 1997c, 1999b). P2X7 receptors may also be involved in endotoxin signal transduction in macrophages (Beigi and Dubyak, 2000), since P2X7 receptor antagonists such as oxidised-ATP, attenuate a subset of endotoxin-induced effects including NF-κB activation and up-regulation of inducible NO synthase. Thus, an association between endotoxic events and P2X7 receptors appears to exist.

Finally, P2X7 receptors may affect immune function through antigen presentation (Mutini et al., 1999), nitric oxide production (Molloy et al., 1994), cell–cell communication (Inoue, 1998), and the formation of gap junctions and multinucleated giant cells (Chiozzi et al., 1996). P2X7 receptors have been identified at pre-synaptic nerve terminals, suggesting a role in synaptic transmission (Deuchars et al., 2001) where ATP may affect glutamate release (Nakatsuka and Gu, 2001) and vice versa. Our observations that ATP induces alterations in Ca^{2+} homeostasis which affect interleukin-1β processing and release, suggest that it could have a wider role in neurodegenerative processes.

6. Role of P2X7 in vivo

Involvement of P2X7 receptors in neurodegenerative conditions remains speculative since the role of the P2X7 receptor in vivo remains to be studied in detail. The only published reports so far have demonstrated that ATP increases release and processing of IL-1β in the peritoneum following LPS injections through a P2X7-receptor dependent mechanism (Griffiths et al., 1995; Solle et al., 2001). High concentrations of extracellular ATP have been observed at sites of tissue trauma (Nieber et al., 1999), and increased microglial P2X7 receptor immunoreactivity has been described in the penumbra following cerebral ischaemia in rodents (Collo et al., 1997). Whether this reflects an increase in P2X7 receptor density per se, or is due to infiltration of microglia to the site of inflammation has not been clarified. We are currently investigating a potential role for the P2X7 receptor in neurodegeneration in vivo, looking specifically at its role in mediating cell death following cerebral ischaemia and excitotoxic lesions induced by striatal infusion of the NMDA receptor agonist methano-glutamate (M-Glu). Owing to the lack of selective P2X7 antagonists and agonists, initial studies have been conducted comparing normal mice and those in which the gene for the P2X7 receptor has been deleted (P2X7 knockout mice). The P2X7 knockout mice exhibited "normal" responses to reperfusion and permanent ischaemia and to excitotoxic damage compared to their wild type counterparts (Fig. 3).

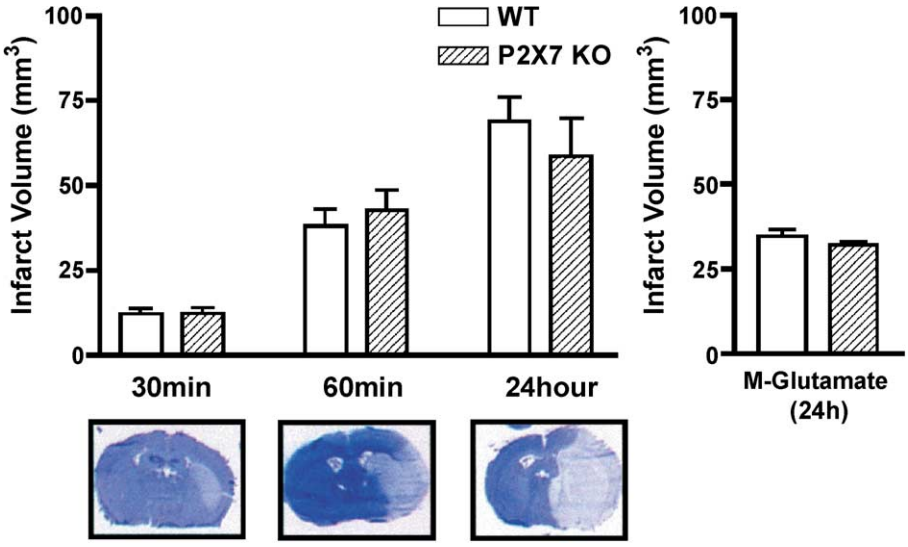

Fig. 3. Absence of the P2X7 receptor does not affect infarct volume following cerebral ischaemia or excitotoxic (methanoglutamate) infusions. Reperfusion (30–60 min) or permanent (24 h) cerebral ischaemia was induced by the intraluminal thread model and excitotoxic lesions by striatal infusion of M-glutamate, in normal mice and those lacking functional P2X7 receptors (P2X7 knockout). Infarct volumes were measured 24 h after surgery on brain sections.

Results generated by the use of knock-out mice must however be treated with caution since compensatory mechanisms may occur. Indeed other P2X receptors may be capable of pore formation by forming heterodimers (e.g., P2X2 and P2X3; Virginio et al., 1999). However, in vitro studies on primary cultures of macrophages and microglia demonstrated that the effects of ATP on cell death and interleukin-1β processing and release were completely absent in cells from these P2X7 knockout mice (Brough et al., 2002; Le Feuvre et al., 2002).

In contrast to the primarily pro-inflammatory activities of nucleotides on immune function, breakdown products of extracellular nucleotides, particularly adenosine, have immunomodulatory properties and neuroprotective roles (Chen et al., 1999; Dunwiddie and Masino, 2001). A balance between ATP/ADP and adenosine may thus regulate cell death in areas of immune attack by sparing adjacent tissues and possibly by switching the mode of cell death between necrosis and apoptosis. Alterations in P2X7 activation, the presentation/regulation of P2X7 receptors in the

7. Conclusions

It is now generally accepted that high levels of extracellular nucleotides such as ATP may be released under pathological conditions such as inflammation, trauma and stress. Signalling via P2X7 receptors may thus allow cells to sense and respond to events occurring in the extracellular environment, modulate the transcription of genes involved in cellular inflammatory responses and thus to regulate cytokine responses in the CNS (Liu et al., 2000). In addition to its role in cytokine regulation, it appears that extracellular ATP may act with glutamate and modify intracellular Ca^{2+} homeostasis, and thus have a wider role in neurodegenerative processes (Fig. 4).

Further research on P2X7 receptors and their actions may unveil novel therapeutic targets to combat neurodegenerative conditions. Future in vivo work is particularly important, as there is often a discrepancy between effects observed in vitro and in vivo. Indeed while interleukin-1 has a profound effect on cell death in vivo, neuroprotective actions of interleukin-1 have been described in vitro (Strijbos and Rothwell, 1995).

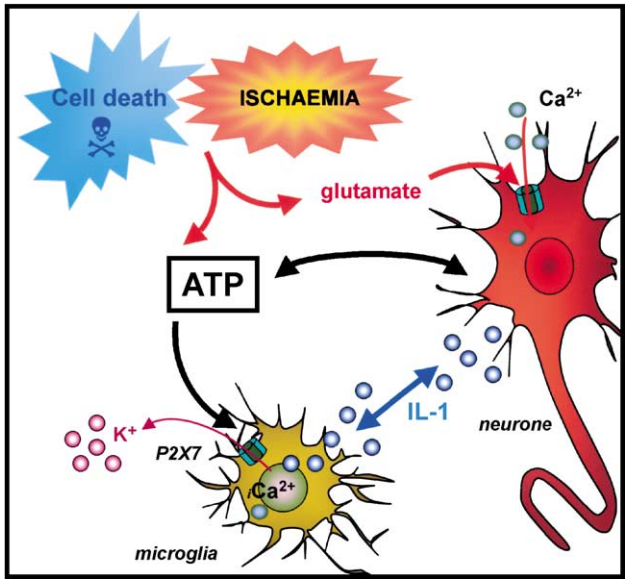

Fig. 4. A general scheme for a role of ATP and P2X7 receptors in neurodegeneration.

plasma membrane (Gu et al., 2000), and its resultant actions could provide interesting therapeutic opportunities.

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