Hypoxia in brain

In acute neurological conditions such as stroke severe injuries to the CNS occur (Honig and Rosenberg 2000) and stroke is the second most common cause of death and a major cause of long-term disability worldwide (Macrez et al. 2011). Hippocampus and cerebellar cortex are particularly sensitive to ischemia (Yue et al. 1997). Hypoxic–ischemic insult generally causes necrosis, although in most cases there exists also a process of delayed and apoptotic type injury in the region surrounding the area of most severe damage (Honig and Rosenberg 2000; Yuan and Yankner 2000; Schaller et al. 2003; Lo 2008). Lately, it was considered that this degeneration might be better regarded as an ‘apoptosis-necrosis cell death continuum’ (Northington et al. 2011). Neurons in the adult mammalian CNS, which are injured by stroke normally fail or have only limited ability to regenerate axons, which causes long-lasting disabilities in sensory, motor, or cognitive functions (Bennett and Carmichael 2010). In addition, cell death in the brain leads to the subsequent release of endogenous molecules termed ‘damage-associated molecular patterns’ from dying cells, triggering further cascades of inflammatory events that both have deleterious but also beneficial effects (Sitkovsky et al. 2004; Chen and Nunez 2010). As an immediate result, a disrupted microcirculation leads to local tissue hypoxia associated with an impaired adenosine 5’-triphosphate (ATP) production and energy status of neurons and glia. This is the basis of further insults including increased calcium, release of glutamate, synthesis of...
enzymes involved in free radical production and the accumulation of leukocytes (Barone and Feuerstein 1999; Lipton 1999; White et al. 2000; Hertz 2008) (Fig. 1). In the hope to improve clinical outcome after stroke, remarkable progress in understanding its pathophysiology has been made in the past 10 years and basic research yielded numerous pharmacologic agents leading to the identification of more than 1000 molecules with brain-protective effects from experimental models and to the implementation of more than 250 clinical trials. However, none has so far successfully completed phase III clinical development and the only acute pharmacological treatment approved to date is tissue plasminogen activator and aspirin, other antiplatelets, and anticoagulants are used as preventative therapy (Young et al. 2007; Ginsberg 2009; Moskowitz et al. 2010; Albers et al. 2011; Macrez et al. 2011).

Purine nucleosides in hypoxia

Following hypoxia–ischemia, dying, injured, and hypoxic cells release soluble purine- nucleotide and -nucleoside pools (Ciccarelli et al. 1999; Dale et al. 2000; Latini and Pedata 2001), normally regulated by ENTs, ectonucleotidases and ecto-adenosine deaminase (Delaney et al. 1998; Zimmermann et al. 1998; Dunwiddie and Masino 2001; Frenguelli et al. 2003; Fredholm et al. 2011; Ipata et al. 2011; Zhang et al. 2011). Purine nucleoside-mediated effects in hypoxia are therefore exceptionally interesting due to their endogenous regulatory mechanisms in stress situations. Growing evidence suggests that purine nucleosides, which may remain elevated for days after the insult (Uemura et al. 1991), might also act as trophic factors in both the CNS and PNS (Neary et al. 1996; Rathbone et al. 1999). In addition to ENTs regulating purine nucleoside concentrations, specific extracellular receptor subtypes for these compounds are expressed on neurons, glia, and endothelial cells, where they mediate strikingly different effects. Such effects range from induction of cell differentiation, apoptosis, mitogenesis, and morphogenetic changes, to stimulation of synthesis and/or release of cytokines and neurotrophic factors under both physiological and pathological conditions (Fields and Burnstock 2006; Burnstock 2008). Nucleosides, for example, adenosine, inosine and guanosine are therefore likely to be involved in the regulation of the nervous system’s development and plasticity (Neary et al. 1996).

Fig. 1 Biochemistry of ischemia–reperfusion injury. Hypoxic–ischemic brain injury starts with the insult but extends into a recovery-reperfusion period (Barone and Feuerstein 1999; Lipton 1999; White et al. 2000; Hertz 2008; Macrez et al. 2011). In case of prolonged ischemia, restricted blood flow leads to a reduction in ATP, causing severe impairment of cellular function by disruption of ATP-dependent processes. A key incidence is the increase in intracellular calcium, which is responsible for the release of neurotransmitters such as glutamate and the activation of many cytocidal enzymes. Activated endonucleases then lead to DNA damage and apoptosis. Though restoration of seized blood flow and oxygen delivery is essential for organ survival, damage is potentially amplified during this period by oxygen sensitive mechanisms, for example, by the activity of pro-inflammatory cytokines (Barone and Feuerstein 1999; Lipton 1999; White et al. 2000; Hertz 2008; Macrez et al. 2011). In parallel, hypoxia leads to the decreased production and enhanced breakdown of purine nucleotides to purine nucleosides (PN) (Jurkowski et al. 1998; Sitkovsky et al. 2004; Fredholm et al. 2007; Fredholm 2010), which may enter/leave cells via bidirectional nucleoside transporters (ENTs) or in the case of adenosine and inosine directly bind to adenosine receptors (Fredholm et al. 1994, 2001a; Schulte and Fredholm 2003b). To date it is not clear, whether the protective effect of guanosine is at least partly arbitrated by adenosine or adenosine receptors (Ciccarelli et al. 2000; D’Alimonte et al. 2007), or is mediated by its own specific G-coupled receptors (Traversa et al. 2003; Rathbone et al. 2008).
Adenosine is formed by stepwise dephosphorylation of ATP (Zimmermann 2000) and to a minor extent from hydrolysis of 5'-adenosyl homocysteine (Deussen et al. 1989). Normally, it is present in body fluids in concentrations 20–200 nM but in response to stress, for example, hypoxia, ischemia, inflammation and trauma, elevated levels of adenosine, up to 300 µM are produced and released (Fredholm et al. 2001a; Fredholm 2007, 2010; Burnstock 2008; Lopes et al. 2011). This observed increase in extracellular adenosine is due to a decreased production of intracellular ATP, accumulation of AMP, enhanced dephosphorylation of adenine nucleotides to adenosine by cytosolic-5'-nucleotidase and inhibition of adenosine kinase (Sitkovsky et al. 2004; Sitkovsky 2009) and liberation from cells via nucleoside transporters (Pastor-Anglada et al. 2001). However, extracellular adenosine accumulates through the activities of local tissue hypoxia-up-regulated ectonucleotidase activity (Braun et al. 1998) (Fig. 1). In conditions of profound hypoxia these could depict significant sources of extracellular adenosine, which was shown in other cell systems to generate immunsuppressive loops (Ohta and Sitkovsky 2001; Sitkovsky et al. 2004; Deaglio et al. 2007; Fredholm 2007; Sitkovsky 2009).

Adenosine then acts as a powerful endogenous neuroprotectant and intra- and intercellular messenger during ischemia-induced energy failure (Dunviddie and Masino 2001; Fredholm et al. 2005b) by decreasing neuronal metabolism and increasing cerebral blood flow and there is reduction in the release of excitotoxic neurotransmitters, attenuation of NMDA receptors, vasorelaxation, and anti-inflammatory effects (Sciotti et al. 1992; Yawo and Chuhma 1993; Soricelli et al. 1995; Johansson et al. 2001; Ohta and Sitkovsky 2001; Li et al. 2006; Kusano et al. 2010). But most importantly, there is, at least in animal models, an impressive reduction of neuronal damage and mortality (von Lubitz 1999). Adenosine also showed neuroprotective effects in vitro models of hypoxic neuronal cells (Bocklenger et al. 2004; Heftberger et al. 2005; Tomaselli et al. 2005a,b; Tomaselli et al. 2008; zur Nedden et al. 2008).

Adenosine effects are mediated by specific receptors (Rudolph et al. 1992; Sweeney 1997; Kobayashi et al. 1998; von Lubitz 1999; Fredholm et al. 2001a). In brain, high membrane adenosine receptor expression levels are found (Fredholm et al. 2005b; Hasko et al. 2005; Wei et al. 2010), and stimulation of adenosine receptors was hypothesized to result in an effective treatment of stroke (Dunwiddie and Masino 2001; Laubach et al. 2011). As discussed before, extracellular adenosine accumulates in inflamed areas with damaged microcirculation, diminished blood supply, and low oxygen tension. Under such conditions, adenosine serves as a marker of collateral immune damage and supports the prevention of additional injury through inhibition of activated immune cells (Sitkovsky et al. 2004; Sitkovsky 2009). Adenosine deaminase (converting adenosine to inosine), adenosine kinase (phosphorylates adenosine to 5'-AMP) and nucleoside transporters, are responsible for an extremely short half-life of adenosine in circulation (Fredholm et al. 2001a, 2011; Eltzschig 2009) and therefore some of its effects, are apparently due to its metabolites as was reported, for example, for inosine (Haun et al. 1996).

Inosine is formed by deamination of adenosine, mainly at high intracellular concentrations, which are associated with hypoxia, ischemia and other forms of cellular stress (Hasko et al. 2004). Inosine may be formed intra- and extracellularly and shunted across the cell membrane via ENTs (Pastor-Anglada et al. 2001) (Fig. 1). Inosine concentrations up to 6 µM have been detected in human myocardial ischemia, and many times higher concentrations may be observed in experimental models of ischemia–reperfusion injury (Hasko et al. 2004). Initially, inosine did not attract the same interest as adenosine. Yet, inosine was shown to effect neuronal (Benowitz et al. 1998; Litsky et al. 1999; Bocklenger et al. 2004; Heftberger et al. 2005; Tomaselli et al. 2005a,b, 2008; zur Nedden et al. 2008) and glial (Hu et al. 1996; Jurkowitz et al. 1998) cell viability and neurite outgrowth in cells subjected to glucose deprivation and/or mitochondrial respiratory chain inhibition or challenged with low oxygen. Moreover, inosine was shown to stimulate neurons to extend new projections to denervated areas in adult rats with unilateral cortical infarcts (Chen et al. 2002). Inosine was also shown to exert multiple anti-inflammatory effects such as reduction of the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha, macrophage inflammatory protein-2 and IL-6 (Hasko et al. 2004). These findings, coupled with the fact that inosine has very low toxicity, suggested that this agent may be useful in the treatment of inflammatory/ischemic diseases, and might help to restore essential circuitry after injury to the CNS (Jurkowitz et al. 1998; Benowitz et al. 1999, 2002; Chen et al. 2002; Hasko et al. 2004; Zai et al. 2009; Benowitz and Carmichael 2010).

Guanosine is metabolized from guanosine 5'-triphosphate (GTP) and guanosine 5'-monophosphate (GMP) (Schmidt et al. 2007) and is present in the brain under both physiological and pathological conditions (Uemura et al. 1991). In analogy to ATP, GTP concentrations decrease in ischemic tissue (Kinouchi et al. 1990) and guanosine concentrations showed significant increases at 2 h to 7 days (Uemura et al. 1991). Guanine derivatives may reach threefold higher levels than adenine-derivatives in cell injuries like hypoxia and hypoglycemia (Ciccarelli et al. 1999). Guanine-based purines are released from neurons and astrocytes (Rathbone et al. 2008). As discussed (Rathbone et al. 2008), extracellular guanosine stimulates mitosis, synthesis of trophic factors, and cell differentiation, including neurogenesis, is neuro- and glia-protective, and reduces apoptosis (Gysbers and Rathbone 1996a,b; Benowitz et al. 1998; Jurkowitz et al. 1998; Rathbone et al. 1998, 2008, 2011; Litsky et al. 2001).
Purine nucleoside receptors

Adenosine receptors (AR) belong to the superfamily of G-protein-coupled receptors characterized by seven transmembrane helices (Palmer and Stiles 1995). There are four G-protein-coupled ARs, namely A1R, A2AR, A2BR and A3R, all of them expressed in brain, with A1R and A2AR being the physiologically more important subtypes (Fredholm et al. 2001a, 2005a; Abbracchio et al. 2009; Wei et al. 2010). ARs, via its alpha subunit, either stimulate (Gα) or inhibit (Gβγ) adenylate cyclase, the enzyme that catalyzes the formation of cAMP, whereby A1R and A3R interact with Gαi/ Gβγ proteins, and A2A and A2B with G, (van Calker et al. 1979; Zhou et al. 1992; Palmer and Stiles 1997; Fredholm et al. 2001a). In addition to the classical adenylate cyclase–cAMP-protein kinase A signaling pathway, it is now apparent that other pathways, such as phospholipase C, Ca2+- and mitogen-activated protein kinases (MAPKs), are also relevant (Linden 1991; Abbracchio et al. 1995; Fredholm et al. 2001a; Schulte and Fredholm 2003b; Tomasselli et al. 2008).

The A1 high affinity receptor albeit expressed throughout the body reaches highest levels in brain especially in neurons of cortex, hippocampus, cerebellum and dorsal horn of spinal cord, eye, adrenal gland, and atria (Fredholm et al. 2005b; Wei et al. 2010) at pre-synaptic and post-synaptic sites (Rebola et al. 2003). A1R stimulation generally suppresses neuronal activity and efficiently controls the release of all the classical neurotransmitters (glutamate, acetylcholine and serotonin), leading to the idea that A1Rs mainly fulfill a synaptic neuromodulatory role, particularly in excitatory synaptic neurotransmission (Neary 2005). The increase in inosine during hypoxia was reported to be largely due to, either extracellular degradation of adenosine (Frenguelli et al. 2003), or else to the intracellular formation of inosine and subsequent release by ENTs (Parkinson and Xiong 2004). Likewise guanosine was shown to be transported into neurons and astrocytes by nucleoside transporters (Nagasawa et al. 2007).

Nucleoside transporters

During metabolic stress like hypoxia, intracellular adenosine is formed at the expense of ATP, leaves cells via nucleoside transporters and activate ARs (Fredholm et al. 2005a). Bidirectional transporters allow purine nucleosides to gain access to the intracellular space (Pastor-Anglada et al. 2001; Parkinson et al. 2005, 2011; King et al. 2006; Takahashi et al. 2010; Sebastiao 2011). Alternatively, ATP may be released from cells by cell lysis, exocytosis, transporters or channels, and dephosphorylated extracellularly to adenosine (Neary 2005). The increase in inosine during hypoxia was reported to be largely due to, either extracellular degradation of adenosine (Frenguelli et al. 2003), or else to the intracellular formation of inosine and subsequent release by ENTs (Parkinson and Xiong 2004). Likewise guanosine was shown to be transported into neurons and astrocytes by nucleoside transporters (Nagasawa et al. 2007).

Purine nucleoside-mediated neuroprotection and neuroregeneration

Evaluation of the effects of AR agonists and antagonists in stroke models indicates that adenosine acting through A1R has neuroprotective effects (Rudolph et al. 1992; Sweeney 1997; von Lubitz 1999), probably by control of glutamate release and inhibiting excitatory synaptic neurotransmission in the brain during hypoxia (Wei et al. 2010). In contrast, activation of A2AR may enhance neuronal damage, as mice...
lacking these receptors exhibited reduced damage following focal ischemia (Chen et al. 1999). Results by others (Kobayashi et al. 1998), however indicated that hypoxia-induced membrane responses of PC12 cells are likely to be mediated via activation of the A2AR.

Administration of adenosine to the brain at times of stroke was shown to ameliorate damage (Kitagawa et al. 2002), and transgenic over-expression of adenosine kinase, leads to increased vulnerability to ischemia-induced cell death (Pignataro et al. 2007). It is now at large believed and confirmed by genetic knockout models, that elevated extracellular adenosine levels exert an overall neuroprotective effect in injured brain; however, because of complex organ- and injury-type specific responses precise predictions are still difficult (Wei et al. 2010). Correspondingly, stroke animals receiving inosine pre-treatment demonstrated a higher level of locomotor activity and less cerebral infarction (Shen et al. 2005). Also guanosine was shown to prolong rat survival and decrease both neurological deficits and tissue damage resulting from middle cerebral artery occlusion (MCAo) (Chang et al. 2008). Other strategies to improve outcome after stroke-induced injuries are considering a potential-reinnervation of brain regions that are devoid of their normal inputs. Along this line, adenosine and guanosine were shown to inhibit injury-induced axonal degeneration in cultured dorsal root ganglion (DRG) neurons (Press and Milbrandt 2002). Along this line, adenosine and guanosine were shown to inhibit injury-induced axonal degeneration in cultured dorsal root ganglion (DRG) neurons (Press and Milbrandt 2002). Also inosine, proved to alter gene expression in dorsal root ganglion (DRG) neurons (Press and Milbrandt 2002; Tomaselli et al. 2005a; b) and physiological- (Kobayashi and Millhorn 1999; Frizzo et al. 2002; Chang et al. 2008; zur Nedden et al. 2008; Oleskovicz et al. 2008; Thomazi et al. 2008; Tomasselli et al. 2008; Thauerer et al. 2010; Dal-Cim et al. 2011) hypoxia (Table 1). Results in these models confirmed an important role for A1R as well as A2AR, because purine-mediated rescue was inhibited by A1R (in primary cerebellar granule neurons) or A2AR-antagonists (in neuronal PC12 cells) respectively (Heftberger et al. 2005; Tomasselli et al. 2005a).

In hypoxic PC12 cells, viability is predominantly rescued by adenosine, whereas guanosine is more supportive of neurite outgrowth (Kobayashi et al. 1998; Tomasselli et al. 2005a). Hypoxia-induced membrane responses of PC12 cells are likely to be mediated via activation of the A2A adenosine receptors, and elevation of cAMP and inhibition of the A2A receptor itself induced death of PC12 cells (Kobayashi et al. 1998; Arslan et al. 1999; Tomasselli et al. 2005a). Therefore, it is not surprising that PKA is required for the A2 receptor modulation of both voltage-sensitive potassium $I_{K_{\text{voltage}}}$ and calcium $I_{Ca}$ currents in PC12 cells (Kobayashi et al. 1998) and that pharmacological inhibition of protein kinase A (PKA) with H89 superinduced chemical hypoxia-mediated cell death and inhibited the rescue of hypoxic PC12 cells by purine nucleosides (Tomasselli et al. 2005a). The role of nucleoside transport varies for different purine nucleosides and cell types. In PC12 cells, the inhibition of nucleoside transport with S-(4-nitrobenzyl)-6-thioinosine caused an increase in adenosine-mediated rescue of viability (Tomasselli et al. 2005a), presumably due to increased A2A receptor-mediated signaling (Parkinson et al. 2000). Our own results therefore confirm the hypothesis that adenosine mainly acts via adenosine receptor-mediated signaling mechanisms, whereas many aspects of the mechanisms involved in inosine- and guanosine-based protection still remain unclear. However, A1R-expressing primary cerebellar granule neurons were more effectively rescued by the adenosine metabolite inosine, than adenosine and guanosine (Bocklinger et al. 2004; Heftberger et al. 2005), whereby adenosine- and inosine-mediated rescue was sensitive to an A1R antagonist (8-cyclopentyl-1, 3-dipropylxanthine) whereas guanosine was largely unaffected (Heftberger et al. 2005). Nucleoside transport is apparently important for adenosine-
Table 1 Key molecules in Purine nucleoside-mediated signal transduction in hypoxic neuronal cells. This table summarizes *in vitro* data, collected from neuronal/hypoxia experiments. Data are separated in the effects of purine nucleosides (adenosine, inosine and guanosine) on (i) viability and (ii) neurite outgrowth.

| Purine nucleoside | Experimental model | Proposed key molecule | Reference |
|-------------------|--------------------|-----------------------|-----------|
| **Viability studies** |                    |                       |           |
| Adenosine–NECA    | PC12 cells, 1% O₂  | Ca²⁺ homeostasis       | Kobayashi and Millhorn (1999) |
| Adenosine         | Cerebellar granule neurons, rotenone | AR (DPCPX), ENT (NBTI) | Heftberger *et al.* (2005) |
| Adenosine         | PC12 cells, rotenone | AR (CSC)         | Tomaselli *et al.* (2005a) |
| Adenosine         | PC12 cells, rotenone | ENT (NBTI)        | Tomaselli *et al.* (2005a) |
| Adenosine         | PC12 cells, rotenone | PI3K (LY294002)    | Tomaselli *et al.* (2005a) |
| Adenosine         | PC12 cells, rotenone | MAPK (PD098059, U0126) | Tomaselli *et al.* (2008) |
| Adenosine         | PC12 cells, 1% O₂  | HIF-1α (siRNA)       | zur Nedden *et al.* (2008) |
| Adenosine         | Cerebellar granule neurons, 1% O₂ | MAPK (siRNA) | Tomaselli *et al.* (2008) |
| Adenosine         | Cerebellar granule neurons, 1% O₂ | HIF-1α (siRNA) | zur Nedden *et al.* (2008) |
| Inosine           | Murine spinal cord, rotenone | Purine nucleoside phosphorylase | Litsky *et al.* (1999) |
| Inosine           | Cerebellar granule neurons, rotenone | ENT (NBTI) | Heftberger *et al.* (2005) |
| Inosine           | PC12 cells, rotenone | AR (CSC)         | Tomaselli *et al.* (2005a) |
| Inosine           | Cerebellar granule neurons, 1% O₂ | MAPK (siRNA) | Tomaselli *et al.* (2008) |
| Inosine           | Cerebellar granule neurons, 1% O₂ | HIF-1α (siRNA) | zur Nedden *et al.* (2008) |
| Inosine           | PC12 cells, 1% O₂  | MAPK (PD098059)     | Tomaselli *et al.* (2008) |
| Inosine           | PC12 cells, 1% O₂  | HIF-1α (siRNA)       | zur Nedden *et al.* (2008) |
| Guanosine         | Murine spinal cord, rotenone | Purine nucleoside phosphorylase | Litsky *et al.* (1999) |
| Guanosine         | Cortical slices, OGD | PKA, PKC, MEK, PI3K | Oleskovicz *et al.* (2008) |
| Guanosine         | Cerebellar granule neurons, rotenone | ENT (NBTI) | Heftberger *et al.* (2005) |
| Guanosine         | Cerebellar granule neurons, rotenone | AR (CSC) | Tomaselli *et al.* (2005a) |
| Guanosine         | SH-SY5Y cells, OGD | PKR 1 (siRNA)       | Thauerer *et al.* (2010) |
| Guanosine         | Hippocampal slices, OGD-reox. | Ca²⁺-activated K⁺ channels, PI3K, AKT | Dal-Cim *et al.* (2011) |
| Guanosine         | Hippocampal slices, OGD-reox. | PKR 1 (siRNA) | Thauerer *et al.* (2010) |
| Guanosine         | PC12 cells, 1% O₂  | AR (SCH-58261)      | Thauerer *et al.* (2010) and unpublished data |
| Guanosine         | Cerebellar granule neurons, 1% O₂ | PRK 1 (siRNA) | Thauerer *et al.* (2010) and unpublished data |
| Guanosine         | PC12 cells, 1% O₂  | AR (SCH-58261)      | Thauerer *et al.* (2010) and unpublished data |
| **Neurite studies** |                    |                       |           |
| Adenosine         | Cerebellar granule neurons, rotenone | MAPK (PD098059, siRNA) | Thauerer *et al.* (2010) and unpublished data |
| Adenosine         | Cerebellar granule neurons, rotenone | HIF-1α (siRNA) | zur Nedden *et al.* (2008) |
| Inosine           | Cerebellar granule neurons, rotenone | MAPK (PD098059, siRNA) | Thauerer *et al.* (2010) and unpublished data |
| Inosine           | PC12 cells, 1% O₂  | HIF-1α (siRNA)       | zur Nedden *et al.* (2008) |
| Inosine           | Dorsal root ganglion neurons | Mtb3, MAPK | Lorber *et al.* (2009) |
| Inosine           | PC12 cells, 1% O₂  | AR (SCH-58261)      | Thauerer *et al.* (2010) and unpublished data |
| Guanosine         | Cerebellar granule neurons, rotenone | MAPK (PD098059, siRNA) | Thauerer *et al.* (2010) and unpublished data |
| Guanosine         | Cerebellar granule neurons, rotenone | PRK 1 (siRNA) | Thauerer *et al.* (2010) and unpublished data |
| Guanosine         | PC12 cells, 1% O₂  | PRK 1 (siRNA)       | Thauerer *et al.* (2010) and unpublished data |
| Guanosine         | PC12 cells, 1% O₂  | AR (SCH-58261)      | Thauerer *et al.* (2010) and unpublished data |
inosine- and guanosine-mediated rescue of hypoxic A1R positive cerebellar granule neurons (Heftberger et al. 2005).

The p42/44 mitogen-activated protein kinase (MAPK) pathway, serine–threonine kinases constitute a convergent pathway for the regulation of multiple modalities involved in O₂ sensing (Seta et al. 2002). They are part of a signaling module that transduces signals from the cell membrane to the nucleus in response to a vast range of external stimuli (Irving and Bamford 2002; Colucci-D’Amato et al. 2003; Cheung and Slack 2004; Wada and Penninger 2004) and regulate proliferation, neuronal survival, differentiation, long-term memory and synaptic plasticity and apoptosis (Boulton et al. 1991; Segal and Greenberg 1996; Alessandrini et al. 1999; Johnson and Lapadat 2002; Sweatt 2004; Wada and Penninger 2004). Emerging evidence suggests that MAPKs are highly related to processes that promote neuron survival (e.g. by neuroprotective growth factors (Nicole et al. 2001), and plasticity (Impey et al. 1999). Stimulation of the adenosine receptors A1R, A2A- and A2BR was shown to activate MAPK (Sexl et al. 1997; Dickenson et al. 1998; Arslan and Fredholm 2000; Fredholm et al. 2001a; Charles et al. 2003; Schulte and Fredholm 2003a; b; Tomaselli et al. 2008). Authors reflect on different activation pathways of MAPK, as diverse as coupling of ARs to G₁₂/₁₃ proteins instead of Gₛ or Gₛ- and cAMP-independent affects on MAPK involving the Ras module (Sexl et al. 1997; Seidel et al. 1999; Schulte and Fredholm 2003b). Along this line another group (Faure et al. 1994) showed transiently expressed A1R in COS-7 cells mediated MAPK activation via release of βγ subunits. Although it appears from these and from many other cases (Hetman and Gozdz 2004), that MAPK activation is neuroprotective, mediating the effects of several extrinsic survival signals, MAPK activation in hypoxia/ischemia still remains a controversial issue. MAPKs are activated by small increases in calcium during survivable degrees of hypoxia (Mino et al. 2000; Bickler and Donohoe 2002) and studies in perinatal cerebral hypoxia–ischemia showed MAPK activation in neurons, mainly in cells displaying signs of damage (Wang et al. 2003). Authors therefore debate, whether MAPK is either trying, unsuccessfully, to rescue cells, or actually contributing to harmful cell signals (Wang et al. 2003). Recent results, however, suggested a vital role of the MAPK pathway in purine nucleoside-mediated protection of neuronal cells and primary neurons following hypoxic insult (Tomaselli et al. 2005b, 2008). In cells subjected to hypoxia, an increased phosphorylation of MAPK, was detected, that was further increased upon addition of purine nucleosides. Vice versa, upon blocking this pathway with a pharmacological inhibitor of MEK-1 (PD098059) viability and neurite outgrowth were decreased (Tomaselli et al. 2008). Further evidence came from experiments with small interference RNA constructs. Knockdown of MAPK severely affected purine nucleoside-mediated rescue of hypoxic PC12 cells and cerebellar granule neurons (Tomaselli et al. 2008; Fig. 2).

MAPK was shown to positively modulate the hypoxia-inducible factor-1 alpha (HIF-1α) by phosphorylation control (Minet et al. 2000). HIF-1α, a transcription factor that plays an essential role in cellular and systemic responses to hypoxia, appears especially interesting and relevant for hypoxia induced signaling. HIF-1α is a heterodimer composed of a 120-kDa HIF-1α subunit complexed to a 91- to 94-kDa HIF-1-β subunit. Under hypoxic conditions HIF-1α is stabilized and constitutes a key role in the cellular defense against hypoxic injury, including the regulation of genes involved in energy

© 2012 The Authors
Journal of Neurochemistry © 2012 International Society for Neurochemistry, J. Neurochem. (2012) 121, 329–342
metabolism, angiogenesis, and apoptosis (Sitkovsky and Lukashev 2005; Semenza 2011). Direct HIF-1 target genes are involved in energy metabolism and cell viability and thus HIF-1 is causally involved in human disease pathophysiology such as cerebral ischemia (Semenza 2000). Adenosine was hypothesized to have the ability to engage HIF-1 activation towards the cellular and systemic responses to hypoxia it mediates (Sitkovsky 2009). Fitting to these data, it was later reported that the nuclear HIF-1α signal in neuronal cells is increased by adenosine and that HIF-1α is apparently critical for purine-mediated neuroprotection (zur Nedden et al. 2008). Authors conclude from their results that the adenosine receptor/MAPK/HIF-1α pathway is tightly interwoven as proven by pharmacological inhibition or siRNA knockdown and plays a critical role for adenosine- and to a lesser degree also for inosine and guanosine-mediated neuro-protection (Fig. 2).

Next to the clear-cut effects of adenosine receptor-mediated activation of MAPK-HIF-1α, neuroprotection of hypoxic neuronal cells apparently does involve other pathways that deserve future attention. Amongst purine nucleosides guanosine attracted attention for its strong neurite-stimulating capacity (Bau et al. 2005; Jiang et al. 2007; Schmidt et al. 2007; Chang et al. 2008; Rathbone et al. 2008; Thauerer et al. 2010). Neuroprotective effects of guanosine were reported to involve an augmentation of glutamate uptake modulated by K+ channels and the activation of the phosphatidylinositol 3-kinase/Akt pathway (Dal-Cim et al. 2011). Recently, another protein kinase, namely protein kinase N alpha (PNKα)/protein kinase C-related kinase1 (PRK1) (Mellor and Parker 1998; Mukai 2003), made a name of itself in purine-mediated neuroprotection (Tomaselli et al. 2005a; Thauerer et al. 2010). PRK1 is a lipid-activated serine/threonine protein kinase and a member of the protein kinase C superfamily (Mellor and Parker 1998; Mukai 2003) of potential key regulators orchestrating physiological responses, and is involved in regulation of the actin cytoskeleton (Modha et al. 2008). PRK1 is activated by interacting with the Rho and Rac families of small G-proteins and arachidonic acid, or by caspase cleavage (Takahashi et al. 1998; Lu and Settleman 1999; Mukai 2003). Adenosine, inosine and guanosine up-regulated its activity in hypoxic neuronal cells (Tomaselli et al. 2005a; Thauerer et al. 2010). Vice versa, loss of functional PRK1 initiated a significant loss of viability and inhibition of neurite formation (Thauerer et al. 2010), which apparently involved a disturbance of the F-actin-associated cytoskeleton and the expression of the plasticity protein growth-associated protein-43 (Thauerer et al. 2010). Up-regulation of growth-associated protein-43 was also reported for inosine (Petrusch et al. 2000). To what extent inosine’s ability to induce neurite outgrowth (Zurn and Do 1988; Benowitz et al. 1998) is due to the activity of Mst3b, a Ste-20-like purine-sensitive protein kinase (Irwin et al. 2006; Lorber et al. 2009) or on PRK1 remains to be shown (Table 1).

**Conclusion**

Hypoxic–ischemic brain injury begins with the insult but extends into a recovery–reperfusion period (Barone and Feuerstein 1999; Lipton 1999; White et al. 2000; Hertz 2008; Macrez et al. 2011). In case of prolonged ischemia, restricted blood flow leads to a reduction in ATP, causing severe impairment of cellular function by disruption of ATP-dependent processes. Brain exposure to hypoxia in ischemia/reperfusion injuries often causes devastating and irreversible loss of function (Chen et al. 2002) and is linked to long term neurological shortages (Berger and Garnier 1999; El-Khodor and Boksa 2000). In parallel hypoxia leads to the decreased production and enhanced breakdown of purine nucleotides to purine nucleosides (Jurkowitz et al. 1998; Sitkovsky et al. 2004; Fredholm et al. 2007; Fredholm 2010). Earlier studies showed that in response to hypoxia, adenosine is produced intracellularly and released into the medium (Meighji et al. 1989; Lloyd et al. 1993; Parkinson and Xiong 2004; Takahashi et al. 2010), from where it triggers different actions through the activation of ARs (Fredholm et al. 1994, 2001a; Schulte and Fredholm 2003b). Growing evidence suggests that purine nucleotides and nucleosides might act as trophic factors in both the central and peripheral nervous systems and are involved in the regulation of the nervous system’s development and plasticity (Neary et al. 1996). Adenosine was reported to act as a powerful endogenous neuroprotectant during ischemia-induced energy failure by decreasing neuronal metabolism and increasing cerebral blood flow, and by playing a variety of different roles as an intra- and intercellular messenger (Dunwiddie and Masino 2001; Fredholm et al. 2005a; b). Guanosine and inosine resemble the like, were shown to induce neurite outgrowth (Benowitz et al. 1998; Rathbone et al. 2008) and in vivo studies demonstrated inosine’s ability to stimulate neurons to extend new projections to denervated areas in adult rats with unilateral cortical infaracts (Chen et al. 2002). In vitro studies confirmed the amazing neuroprotective capability of purine nucleosides in several neuronal hypoxia systems e.g. PC12 cells and cerebellar granule neurons (Bocklinger et al. 2004; Heftberger et al. 2005; Tomaselli et al. 2005a,b, 2008; zur Nedden et al. 2008; Thauerer et al. 2010) and prompted the investigation of purine-mediated hypoxia sensitive signaling. Amongst the multiple pathways and sophisticated mechanisms that have evolved and regulate gene expression during hypoxia, the MAPK module constitutes a convergent pathway for the regulation of multiple modalities involved in O2 sensing (Seta et al. 2002). Stimulation of the A1R, A2A- and A2BR was shown to activate MAPK (Sexl et al. 1997; Dickenson et al. 1998; Arslan and Fredholm 2000; Fredholm et al. 2001a; Charles et al. 2003; Schulte and Fredholm...
2003a; b; Tomaselli et al. 2008) and recent results suggested a vital role of the MAPK pathway plays in purine nucleoside-mediated protection of neuronal cells following hypoxic insult (Tomaselli et al. 2005b, 2008). These results are very relevant to understand the mechanisms by which purine nucleosides modulate neuronal signaling and should support the therapeutic approaches investigated by other groups, which claim that brief ischemia activates MAPK whereas its blockade inhibits ischemic tolerance (Meller et al. 2005). MAPK activation may thus act as a defensive mechanism that helps to compensate for deleterious effects of a damaging insult (Hetman and Gozdz 2004). Amongst MAPK-associated (Minet et al. 2000) downstream effector molecules the transcription factor HIF-1α appears to be most interesting. Adenosine was hypothesized to collaborate with HIF-1α in triggering the production of immunosuppressive molecules (Sitkovsky 2009). Likewise adenosine augmented hypoxia-mediated HIF-1α translocation to the nucleus and HIF-1α was shown to be critical for purine-mediated neuroprotection (zur Nedden et al. 2008).

Many stroke patients fail clinical time windows for acute effective treatment, hence making approaches that promote repair and recovery essential for integrated stroke therapy (Moskowitz et al. 2010). Growing evidence suggests that the biological processes underlying stroke are driven by the interaction of neurons, glia, vascular cells, and matrix components; all actively participating in tissue injury and repair and therefore trophic factor treatments that amplify and augment endogenous processes of neuroplasticity are pre-destined to support recovery (Moskowitz et al. 2010). Furthermore, the detection of continuous neurogenesis in the adult mammalian brain has encouraged a new perception of the plasticity of the mature nervous system (Ming and Song 2011). Thus, as data on the competence of purine nucleosides to support neuroprotection and regeneration accumulate, increasing levels of pro survival proteins may be a promising new strategy to reduce cell damage after ischemia (Cao et al. 2002). In light of recent developments for adenosine in epilepsy (Van Dycke et al. 2011), purine nucleoside augmentation techniques or localized delivery may facilitate possible approaches for neuroprotection and/or enhanced neuroregeneration in stroke.

Acknowledgements

This work was supported by a grant of the Austrian FWF grants P19578-B05 and T421-B18. We are grateful to Dr C. Bandtlow for helpful discussion and support.

References

Abbracchio M.P., Brambilla R., Ceruti S., Kim H.O., von Lubitz D.K., Jacobson K.A. and Cattabeni F. (1995) G protein-dependent activation of phospholipase C by adenosine A3 receptors in rat brain. Mol. Pharmacol. 48, 1038–45.

Abbracchio M.P., Burnstock G., Verkhraisky A. and Zimmermann H. (2009) Purinergic signalling in the nervous system: an overview. Trends Neurosci. 32, 19–29.

Albers G.W., Goldstein L.B., Hess D.C., Wechsler L.R., Furie K.L., Gorelick P.B., Hurn P., Liebeskind D.S., Nogueira R.G. and Saver J.L. (2011) Stroke Treatment Academic Industry Roundtable (STAIR) recommendations for maximizing the use of intravenous thrombolytics and expanding treatment options with intra-arterial and neuroprotective therapies. Stroke 42, 2645–50.

Alessandrini A., Namura S., Moskowitz M.A. and Bonventre J.V. (1999) MEK1 protein kinase inhibition protects against damage resulting from focal cerebral ischemia. Proc. Natl Acad. Sci. USA 96, 12666–9.

Arlsan G. and Fredholm B.B. (2000) Stimulatory and inhibitory effects of adenosine A2A receptors on nerve growth factor-induced phosphorylation of extracellular regulated kinases 1/2 in PC12 cells. Neurosci. Lett. 292, 183–6.

Arlsan G., Bull K. and Fredholm B.B. (1999) Signaling via A2A adenosine receptor in four PC12 cell clones. Naunyn. Schmiedebergs. Arch. Pharmacol. 359, 28–32.

Ballerni P., Ciccarelli R., Di Iorio P. et al. (2006) Guanosine effect on cholesterol efflux and apolipoprotein E expression in astrocytes. Purinergic Signal. 2, 637–49.

Barone F.C. and Feuerstein G.Z. (1999) Inflammatory mediators and stroke: new opportunities for novel therapeutics. J. Cereb. Blood Flow Metab. 19, 819–34.

Bau C., Middelmiss P.J., Hindley S., Jiang S., Ciccarelli R., Caciagli F., Diiorio P., Wierstuk E.S. and Rathbone M.P. (2005) Guanosine stimulates neurite outgrowth in PC12 cells via activation of heme oxygenase and cyclic GMP. Purinergic Signal. 1, 161–72.

Benowitz L.I. and Carmichael S.T. (2010) Promoting axonal rewiring to improve outcome after stroke. Neurobiol. Dis. 37, 259–66.

Benowitz L.I., Jing Y., Tabibiazar R., Jo S.A., Petrasch B., Stuermer A., Rosenberg P.A. and Irwin N. (1998) Axon outgrowth is regulated by an intracellular purine-sensitive mechanism in retinal ganglion cells. J. Biol. Chem. 273, 29626–34.

Benowitz L.I., Goldberg D.E., Madsen J.R., Soni D. and Irwin N. (1999) Inosine stimulates extensive axon collateral growth in the rat corticospinal tract after injury. Proc. Natl Acad. Sci. USA 96, 13486–90.

Benowitz L.I., Goldberg D.E. and Irwin N. (2002) Inosine stimulates axon growth in vitro and in the adult CNS. Prog. Brain Res. 137, 389–99.

Berger R. and Garnier Y. (1999) Pathophysiology of perinatal brain damage. Brain Res. Brain Res. Rev. 30, 107–34.

Bickler P.E. and Donohoe P.H. (2002) Adaptive responses of vertebrate neurons to hypoxia. J. Exp. Biol. 205, 3579–86.

Bocklinger K., Tomaselli B., Heftberger V., Podhraski V., Bandtlow C. and Baier-Bitterlich G. (2004) Purine nucleosides support the neurite outgrowth of primary rat cerebellar granule cells after hypoxia. Eur. J. Cell Biol. 83, 51–4.

Boulton T.G., Nye S.H., Robbins D.J., Ip N.Y., Radziejewska E., Morgenbesser S.D., DePinho R.A., Panayotatos N., Cobb M.H. and Yancopoulos G.D. (1991) ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell 65, 663–75.

Braun N., Zhu Y., Krieglstein J., Culmsee C. and Zimmermann H. (1998) Upregulation of the enzyme chain hydrolysing extracellular ATP after transient forebrain ischemia in the rat. J. Neurosci. 18, 4891–900.

Burnstock G. (2008) Purinergic signalling and disorders of the central nervous system. Nat. Rev. Drug Discov. 7, 575–90.

van Calker D., Muller M. and Hamprecht B. (1979) Adenosine regulates result from focal cerebral ischemia. Proc. Natl Acad. Sci. USA 96, 12666–9.

van der Velden N. and Bandtlow C. (1998) Adenosine regulates tension and/or enhanced neuroregeneration in stroke.
Cao Y.J., Shibata T. and Rainov N.G. (2002) Liposome-mediated transient focal cerebral ischemia in an animal model. *Gene Ther.* **9**, 415–9.

Chang R., Algird A., Bau C., Rathbone M.P. and Jiang S. (2008) Neuroprotective effects of guanosine on stroke models in vitro and in vivo. *Neurosci. Lett.* **431**, 101–5.

Charles M.P., Adamski D., Kholler B., Pelletier L., Berger F. and Wion D. (2003) Induction of neurite outgrowth in PC12 cells by the bacterial nucleoside N6-methyleneadenosine is mediated through adenosine A2a receptors and via CAMP and MAPK signaling pathways. *Biochem. Biophys. Res. Commun.* **304**, 795–800.

Chen G.Y. and Nunez G. (2010) Sterile inflammation: sensing and reacting to damage. *Nat. Rev. Immunol.* **10**, 826–37.

Chen J.F., Huang Z., Zhu J., Moratalla R., Standaert D., Mormino E.C. and Slack R.S. (2004) Emerging role for ERK as a key regulator of neuronal apoptosis. *Sci. STKE* **2004**, PE14.

Ciccarelli R., Di Iorio P., Giuliani P., D’Alimonte I., Ballerini P., Caciagli F. and Rathbone M.P. (1999) Rat cultured astrocytes release guanine-based purines in basal conditions and after hypoxia/hypoglycemia. *Glia* **25**, 93–8.

Ciccarelli R., Di Iorio P., D’Alimonte I., Giuliani P., Florio T., Caciagli F., Middlemiss P.J. and Rathbone M.P. (2000) Cultured astrocyte proliferation induced by extracellular guanosine involves endogenous adenosine and is raised by the co-expression of microglia. *Glia* **29**, 202–11.

Colucci-D’Amato L., Perrone-Capano C. and di Porzio U. (2003) Chronic activation of ERK and neurodegenerative diseases. *BioEssays* **25**, 1085–95.

Conrad P.W., Conforti L., Kobayashi S., Beitner-Johnson D., Rust R.T., Yuan Y., Kim H.W., Kim R.H., Seta K. and Millhorn D.E. (2001) Redox and hypoxia signaling in PC12 cells. *Int. Rev. Neurobiol.* **72**, 221–57.

Dickenson J.M., Blank J.L. and Hill S.J. (1998) Human adenosine A1 receptor and P2Y2-purinoreceptor-mediated activation of the mitogen-activated protein kinase cascade in transfected CHO cells. *Br. J. Pharmacol.* **124**, 1491–9.

Dunwiddie T.V. and Masino S.A. (2001) The role and regulation of adenosine in the central nervous system. *Annu. Rev. Neurosci.* **24**, 31–55.

El-Khodor B.F. and Bolsa P. (2000) Transient birth hypoxia increases behavioral responses to repeated stress in the adult rat. *Behav. Brain Res.* **107**, 171–5.

Eltzschig H.K. (2009) Adenosine: an old drug newly discovered. *Anesthesiology* **111**, 904–15.

Faure M., Voyer-Vasenetska T.A. and Bourne H.R. (1994) cAMP and beta gamma subunits of heterotrimeric G proteins stimulate the mitogen-activated protein kinase pathway in COS-7 cells. *J. Biol. Chem.* **269**, 7851–4.

Fields R.D. and Burnstock G. (2006) Purinergic signalling in neuro-glial interactions. *Nat. Rev. Neurosci.* **7**, 423–36.

Fredholm B.B. (2007) Adenosine, an endogenous distress signal, modulates tissue damage and repair. *Cell Death Differ.* **14**, 1315–23.

Fredholm B.B. (2010) Adenosine receptors as drug targets. *Exp. Cell Biol.* **316**, 1284–8.

Fredholm B.B., Abbracchio M.P., Burnstock G., Daly J.W., Harden T.K., Jacobson K.A., Left P. and Williams M. (1994) Nomenclature and classification of purinoreceptors. *Pharmacol. Rev.* **46**, 143–56.

Fredholm B.B., AP I.J., Jacobson K.A., Klotz K.N. and Linden J. (2001a) International Union of Pharmacology XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* **53**, 527–52.

Fredholm B.B., Irenius E., Kull B. and Schulte G. (2001b) Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells. *Biochem. Pharmacol.* **61**, 443–8.

Fredholm B.B., Chen J.F., Cunha R.A., Svenningson P. and Vaugeois J.M. (2005a) Adenosine and brain function. *Int. Rev. Neurobiol.* **63**, 191–270.

Fredholm B.B., Chen J.F., Masino S.A. and Vaugeois J.M. (2005b) Actions of adenosine at its receptors in the CNS: insights from knockouts and drugs. *Annu. Rev. Pharmacol. Toxicol.* **45**, 385–412.

Fredholm B.B., Chern Y., Franco R. and Sitkovsky M. (2007) Aspects of the general biology of adenosine A2A signaling. *Prog. Neurobiol.* **83**, 263–76.

Fredholm B.B., AP I.J., Jacobson K.A., Linden J. and Muller C.E. (2011) International Union of Basic and Clinical Pharmacology LXXI. Nomenclature and classification of adenosine receptors—an update. *Pharmacol. Rev.* **63**, 1–34.

Feng B.G., Llaudet E. and Dale N. (2003) High-resolution real-time recording with microelectrode biosensors reveals novel aspects of adenosine release during hypoxia in rat hippocampal slices. *J. Neurochem.* **86**, 1506–15.

Ginsberg M.D. (2009) Current status of neuroprotection for cerebral ischemia: synoptic overview. *Stroke* **40**, S111–4.
Role of purine nucleosides in brain hypoxia | 339

Greene L.A. and Tischer A.S. (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc. Natl Acad. Sci. USA 73, 2424–8.

Gysbers J.W. and Rathbone M.P. (1996a) Neurite outgrowth in PC12 cells is enhanced by guanosine through both cAMP-dependent and -independent mechanisms. Neurosci. Lett. 220, 175–8.

Gysbers J.W. and Rathbone M.P. (1996b) GTP and guanosine synergistically enhance NGF-induced neurite outgrowth from PC12 cells. Int. J. Dev. Neurosci. 14, 19–34.

Hasko G., Kuhel D.G., Nemeth Z.H., Mabley J.G., Stachlewitz R.F., Virag L., Lohinai Z., Southan G.J., Salzman A.L. and Szabo C. (2000) Inosine inhibits inflammatory cytokine production by a posttranscriptional mechanism and protects against endotoxin-induced shock. J. Immunol. 164, 1013–9.

Hasko G., Sittkovsky M.V. and Szabo C. (2004) Immunomodulatory and neuroprotective effects of inosine. Trends Pharmacol. Sci. 25, 152–7.

Hasko G., Pacher P., Vizi E.S. and Illés P. (2005) Adenosine receptor signaling in the brain immune system. Trends Pharmacol. Sci. 26, 511–6.

Haun S.E., Segeleon J.E., Trapp V.L., Clotz M.A. and Horrocks L.A. (1992) A2A adenosine receptors from rat striatum and rat pheochromocytoma PC12 cells: characterization with radioligand binding and by activation of adenylate cyclase. Mol. Pharmacol. 41, 352–9.

Hetman M. and Gozdz A. (2004) Role of extracellular signal regulated kinases in ischemia-reperfusion injury. Expert. Opin. Ther. Targets 8, 103–18.

Irwin N., Li Y.M., O'Toole J.E. and Benowitz L.I. (2006) Mst3b, a purine-sensitive Ste20-like protein kinase, regulates axon outgrowth. Proc. Natl Acad. Sci. USA 103, 18320–5.

Johnson G.L. and Lapadat R. (2002) Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 298, 1911–2.

King A.E., Ackley M.A., Cass C.E., Young J.D. and Baldwin S.A. (2006) Nucleoside transporters: from scavengers to novel therapeutic targets. Trends Pharmacol. Sci. 27, 416–25.

Kobayashi S., Conforti L., Pun R.Y. and Millhorn D.E. (1998) Adenosine modulates hypoxia-induced responses in rat PC12 cells via the A2A receptor. J. Physiol., 508 (Pt 1), 95–107.

Koeppe M., Eickle T. and Eltzschig H.K. (2011) The hypoxia-inflammation link and potential drug targets. Curr. Opin. Anaesthesiol. 24, 363–9.

Laubach V.E., French B.A. and Okusa M.D. (2011) Targeting of PNS pathways. Nat. Med. 17, 9407–12.

López L.V., Sebastiao A.M. and Ribeiro J.A. (2011) Adenosine and related drugs in brain diseases: present and future in clinical trials. Curr. Top. Med. Chem. 11, 1087–101.

Lorber B., Howe M.L., Benowitz L.I. and Irwin N. (2009) Mst3b, an Ste20-like kinase, regulates axon regeneration in mature CNS and PNS pathways. Nat. Neurosci. 12, 1407–14.
Lu Y. and Settlement J. (1999) The Drosophila Pkn protein kinase is a Rho/Rac effector target required for dorsal closure during embryogenesis. *Genes Dev.* **13**, 1168–80.

von Lubitz D.K. (1999) Adenosine and cerebral ischemia: therapeutic future or death of a grave concept? *Eur. J. Pharmacol.* **371**, 85–102.

Macrez R., Ali C., Toutirais O., Le Mauff B., Defer G., Dirmagl U. and Vivien D. (2011) Stroke and the immune system: from pathophysiology to new therapeutic strategies. *Lancet Neurol.* **10**, 471–80.

Majumdar A.J., Wong W.J. and Simon M.C. (2010) Hypoxia-inducible factors and the response to hypoxic stress. *Mol. Cell** **40**, 294–309.

Meghji P., Tuttle J.B. and Rubio R. (1989) Adenosine formation and release by embryonic chick neurons and glia in cell culture. *J. Neurochem.* **53**, 1852–60.

Meller R., Minami M., Cameron J.A., Impey S., Chen D., Lan J.Q., Henshall D.C. and Simon R.P. (2005) CREB-mediated Bcl-2 protein expression after ischemic preconditioning. *J. Cereb. Blood Flow Metab.* **25**, 234–46.

Mellor H. and Parker P.J. (1998) The extended protein kinase C superfamily. *Biochem. J.* **332** (Pt 2), 281–92.

Nete E., Arnould T., Michel G., Roland I., Mottet D., Raes M., Remacle J. and Michels C. (2000) ERK activation upon hypoxia: involvement in HIF-1 activation. *FEBS Lett.* **468**, 53–8.

Ming G.L. and Song H. (2011) Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron* **70**, 687–702.

Modha R., Campbell L.J., Nietlispach D., Buhecha H.R., Owen D. and Mott H.R. (2008) The Rac1 polybasic region is required for interaction with its effector PRK1. *J. Biol. Chem.* **283**, 1492–500.

Moskowitz M.A., Lo E.H. and Iadecola C. (2011) Molecular biology of nucleoside transporters and their distributions and functions in the brain. *Curr. Top. Med. Chem.* **11**, 948–72.

Northington F.J., Chavez-Valdez R. and Martin L.J. (2011) Neuronal injury, adenosine levels, and adenosine receptor activity in rat forebrain ischemia. *J. Neurochem.* **75**, 795–802.

Palmer T.M. and Stiles G.L. (1995) Adenosine receptors. *Neuropharmacology* **34**, 683–94.

Palmer T.M. and Stiles G.L. (1997) Structure-function analysis of inhibitory adenosine receptor regulation. *Neuropharmacology* **36**, 1141–7.

Parkinson F.E. and Xiong W. (2004) Stimulus- and cell-type-specific release of purines in cultured rat forebrain astrocytes and neurons. *J. Neurochem.* **88**, 1305–12.

Parkinson F.E., Zhang Y.W., Shepel P.N., Greenway S.C., Peeling J. and Geiger J.D. (2000) Effects of nitrobenzylthioinosine on neuronal injury, adenosine levels, and adenosine receptor activity in rat forebrain ischemia. *J. Neurochem.* **75**, 1852–60.

Parkinson F.E., Xiong W. and Zamzow C.R. (2005) Astrocytes and neurons: different roles in regulating adenosine levels. *Neurot. Res.* **27**, 153–60.

Palmer T.M., Damaraju V.L., Graham K., Yao S.Y., Baldwin S.A., Cass C.E. and Young J.D. (2011) Molecular biology of nucleoside transporters and their distributions and functions in the brain. *Curr. Top. Med. Chem.* **11**, 948–72.

Pastor-Anglada M., Casado F.J., Valdes R., Mata J., Garcia-Manteiga J. and Molina M. (2001) Complex regulation of nucleoside transporter expression in epithelial and immune system cells. *Mol. Membr. Biol.* **18**, 81–5.

Peterfreund R.A., MacCollin M., Gasella J. and Fink J.S. (1996) Characterization and expression of the human A2a adenosine receptor gene. *J. Neurochem.* **66**, 362–8.

Petrausch B., Tabibiazar R., Roser T., Jing Y., Goldberg D., Steurer C.A., Irwin N. and Benowitz L.I. (2000) A purine-sensitive pathway regulates multiple genes involved in axon regeneration in goldfish retinal ganglion cells. *J. Neurosci.* **20**, 8031–41.

Pignataro G., Simon R.P. and Boison D. (2007) Transgenic overexpression of adenosine kinase aggravates cell death in ischemia. *J. Cereb. Blood Flow Metab.* **27**, 1–5.

Pastor-Anglada M., Casado F.J., Valdes R., Mata J., Garcia-Manteiga J. and Molina M. (2001) Complex regulation of nucleoside transporter expression in epithelial and immune system cells. *Mol. Membr. Biol.* **18**, 81–5.

Peterfreund R.A., MacCollin M., Gasella J. and Fink J.S. (1996) Characterization and expression of the human A2a adenosine receptor gene. *J. Neurochem.* **66**, 362–8.

Press C. and Milbrandt J. (2009) The purine nucleosides adenosine and guanosine delay axonal degeneration in vitro. *J. Neurochem.* **109**, 595–602.

Pastor-Anglada M., Casado F.J., Valdes R., Mata J., Garcia-Manteiga J. and Molina M. (2001) Complex regulation of nucleoside transporter expression in epithelial and immune system cells. *Mol. Membr. Biol.* **18**, 81–5.

Peterfreund R.A., MacCollin M., Gasella J. and Fink J.S. (1996) Characterization and expression of the human A2a adenosine receptor gene. *J. Neurochem.* **66**, 362–8.

Rathbone M.P., Middlemiss P., Andrew C., Herman C., Tomlinson A., McCormack D., Turnbull A., Goldstein J., Chung C., Chan P., Cao J., Murray C. and Neary J.T. (2005) Protein kinase signaling cascades in CNS trauma. *IUBMB Life* **57**, 711–8.

Rathbone M.P., Middlemiss P., Martin L.J., Saleh T.M., Connell B.J., Chang R., Su C., Worley B., Heslop P., Gormley P., Stuermer H., Negishi M., Pastia T., Stiles G.L. and Milbrandt J. (2011) The science of stroke: significant answers and significant questions. *J. Neurochem.* **66**, 362–8.

Rathbone M.P., Middlemiss P., Andrew C., Ciccarelli R., Di Iorio P. and Caciagli F. (2008) Transgenic overexpression of adenosine kinase aggravates cell death in ischemia. *J. Cereb. Blood Flow Metab.* **27**, 1–5.

Rathbone M.P., Middlemiss P., Di Iorio P. and Caciagli F. (2008) Transgenic overexpression of adenosine kinase aggravates cell death in ischemia. *J. Cereb. Blood Flow Metab.* **27**, 1–5.

Rathbone M.P., Middlemiss P., Di Iorio P. and Caciagli F. (2008) Transgenic overexpression of adenosine kinase aggravates cell death in ischemia. *J. Cereb. Blood Flow Metab.* **27**, 1–5.

Rathbone M.P., Middlemiss P., Di Iorio P. and Caciagli F. (2008) Transgenic overexpression of adenosine kinase aggravates cell death in ischemia. *J. Cereb. Blood Flow Metab.* **27**, 1–5.

Rathbone M.P., Middlemiss P., Di Iorio P. and Caciagli F. (2008) Transgenic overexpression of adenosine kinase aggravates cell death in ischemia. *J. Cereb. Blood Flow Metab.* **27**, 1–5.
nerve terminals and synapses of the rat hippocampus. *Brain Res.* **987**, 49–58.

Rudolphi K.A., Schubert P., Parkison F.E. and Fredholm B.B. (1992) Neurprotective role of adenosine in cerebral ischaemia. *Trends Pharmacol. Sci.* **13**, 439–45.

Schaller B., Graf R. and Jacobs A.H. (2003) Ischaemic tolerance: a window to endogenous neur protection? *Lancet* **362**, 1007–8.

Schmidt A.P., Lara D.R. and Souza D.O. (2007) Proposal of a guanine-based purinergic system in the mammalian central nervous system. *Pharmacol. Ther.* **116**, 401–16.

Schulte G. and Fredholm B.B. (2003a) The G(s)-coupled adenosine A(2B) receptor recruits divergent pathways to regulate ERK1/2 and p38. *Exp. Cell Res.* **290**, 168–76.

Schulte G. and Fredholm B.B. (2003b) Signalling from adenosine receptors to mitogen-activated protein kinases. *Cell. Signal.* **15**, 813–27.

Sciotti V.M., Roche F.M., Grabb M.C. and Van Wylen D.G. (1992) Adenosine receptor blockade augments interstitial fluid levels of excitatory amino acids during cerebral ischaemia. *J. Cereb. Blood Flow Metab.* **12**, 646–55.

Seidel M.G., Klinger M., Freissmuth M. and Holler C. (1999) Activation of mitogen-activated protein kinase by the A(2A)-adenosine receptor via a rap1-dependent and via a p21(ras)-dependent pathway. *J. Biol. Chem.* **274**, 25833–41.

Semenza G.L. (2000) HIF-1 and human disease: one highly involved factor. *Genes Dev.* **14**, 1983–91.

Semenza G.L. (2007) Life with oxygen. *Science* **318**, 62–4.

Semenza G.L. (2010) Hypoxia-inducible factor 1: regulator of mito chondrial metabolism and mediator of ischemic preconditioning. *Biochim. Biophys. Acta* **1813**, 1263–8.

Seta K.A. and Millhorn D.E. (2004) Functional genomics approach to hypoxia signaling. *J. Appl. Physiol.* **96**, 765–73.

Seta K.A., Spicer Z., Yuan Y., Lu G. and Millhorn D.E. (2002) Responding to hypoxia: lessons from a model cell line. *Sci. STKE* **2002**, RE11.

Sexti V., Mancusi G., Holzer C., Gloria-Maercker E., Schutz W. and Freissmuth M. (1997) Stimulation of the mitogen-activated protein kinase via the A2A-adenosine receptor in primary human endothelial cells. *J. Biol. Chem.* **272**, 5792–9.

Shen H., Chen G.J., Harvey B.K., Bickford P.C. and Wang Y. (2005) Inosine reduces ischemic brain injury in rats. *Stroke* **36**, 654–9.

Sitkovsky M.V. (2009) T regulatory cells: hypoxia-adenosinergic suppression and re-direction of the immune response. *Trends Immunol.* **30**, 102–8.

Sitkovsky M. and Lukashev D. (2005) Regulation of immune cells by local-tissue oxygen tension: HIF1 alpha and adenosine receptors. *Nat. Rev. Immunol.* **5**, 712–19.

Sitkovsky M.V., Lukashev D., Apasov S., Kojima H., Koshiba M., Caldwell C., Ohta A. and Thiel M. (2004) Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. *Ann. Rev. Immunol.* **22**, 657–82.

Soricelli A., Postiglione A., Cuocolo A., De Chiara S., Ruocco A., Brunetti A., Salvatore M. and Ell P.J. (1995) Effect of adenosine on cerebral blood flow as evaluated by single-photon emission computed tomography in normal subjects and in patients with occlusive carotid disease. A comparison with acetazolamide. *Stroke* **26**, 1572–6.

Su C., Picard P., Rathbone M.P. and Jiang S. (2010) Guanosine-induced decrease in side population of lung cancer cells: lack of correlation with ABCG2 expression. *J. Biol. Regul. Homeost. Agents* **24**, 19–25.

Sweatt J.D. (2004) Mitogen-activated protein kinases in synaptic plasticity and memory. *Curr. Opin. Neurobiol.* **14**, 311–7.

Sweeney M.I. (1997) Neurprotective effects of adenosine in cerebral ischemia: window of opportunity. *Neurosci. Biobehav. Rev.* **21**, 207–17.

Takahashi M., Mukai H., Toshimori M., Miyamoto M. and Ono Y. (1998) Proteolytic activation of PKN by caspase-3 or related protease during apoptosis. *Proc. Natl Acad. Sci. U S A* **95**, 11566–71.

Takahashi T., Otsguro K., Ohta T. and Itto S. (2010) Adenosine and inosine release during hypoxia in the isolated spinal cord of neonatal rats. *Br. J. Pharmacol.* **161**, 1806–16.

Thauerer B., zur Nedden S. and Baier-Bitterlich G. (2010) Vital role of protein kinase C-related kinase in the formation and stability of neurites during hypoxia. *J. Neurochem.* **113**, 432–46.

Thomazi A.P., Boff B., Pires T.D., Godinho G., Battu C.E., Gottfried C., Souza D.O., Salbego C. and Wofchuk S.T. (2008) Profile of glutamate uptake and cellular viability in hippocampal slices exposed to oxygen and glucose deprivation: developmental aspects and protection by guanosine. *Brain Res.* **1188**, 233–40.

Tomasselli B., Podhraski V., Bock G. and Baier-Bitterlich G. (2005a) Early cellular responses of purine nucleoside-mediated protection of hypoxia-induced injuries of neuronal PC12 cells. *Am. J. Biochem. Biotechnol.* **2**, 161–167.

Tomasselli B., Podhraski V., Heftberger V., Bock G. and Baier-Bitterlich G. (2005b) Purine nucleoside-mediated protection of chemical hypoxia-induced neuronal injuries involves p42/44 MAPK activation. *Neurochem. Int.* **46**, 513–21.

Tomasselli B., Nedden S.Z., Podhraski V. and Baier-Bitterlich G. (2008) p42/44 MAPK is an essential effector for purine nucleoside-mediated neuroprotection of hypoxic PC12 cells and primary cerebellar granule neurons. *Mol. Cell. Neurosci.* **38**, 559–68.

Traversa U., Bombi G., Camaioni E., Macchiarulo A., Costantino G., Palmieri C., Caciagli F. and Pelliccieri R. (2003) Rat brain guanosine binding site Biological studies and pseudo-receptor construction. *Biosw. Med. Chem.* **11**, 5417–25.

Uemura Y., Miller J.M., Matson W.R. and Beal M.F. (1991) Neurochemical analysis of focal ischemia in rats. *Stroke* **22**, 1548–53.

Van Dycke A., Raedt R., Vonck K. and Boon P. (2011) Local delivery strategies in epilepsy: a focus on adenosine. *Seizure* **20**, 376–82.

Volpini R., Marucci G., Buccioni M., Dal Ben D., Lamberti C., Lammi C., Mishra R.C., Thomas A. and Cristalli G. (2011) Evidence for the existence of a specific γ protein-coupled receptor activated by guanosine. *ChemMedChem* **6**, 1074–80.

Wada T. and Penninger J.M. (2004) Mitogen-activated protein kinases in apoptosis regulation. *Oncogene* **23**, 2838–49.

Wang X., Zhu C., Qiu L., Hagberg H., Sandberg M. and Blomgren K. (2003) Activation of ERK1/2 after neonatal rat cerebral hypoxia-ischaemia. *J. Neurochem.* **86**, 351–62.

Wei C.J., Li W. and Chen J.F. (2010) Normal and abnormal functions of adenosine receptors in the central nervous system revealed by genetic knockout studies. *Biochim. Biophys. Acta* **1808**, 1358–79.

White B.C., Sullivan J.M., DeGracia D.J., O’Neil B.J., Neumar R.W., Grossman L.I., Rafols J.A. and Krause G.S. (2000) Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. *J. Neurovirol. Sci.* **179**, 1–33.

Yawo H. and Chuhma N. (1993) Preferential inhibition of omega-conotoxin-sensitive presynaptic Ca2+ channels by adenosine autoreceptors. *Nature* **365**, 256–8.
Young A.R., Ali C., Duretete A. and Vivien D. (2007) Neuroprotection and stroke: time for a compromise. J. Neurochem. 103, 1302–9.

Yuan J. and Yankner B.A. (2000) Apoptosis in the nervous system. Nature 407, 802–9.

Yue X., Mehnert H., Penrice J., Cooper C., Cady E., Wyatt J.S., Reynolds E.O., Edwards A.D. and Squier M.V. (1997) Apoptosis and necrosis in the newborn piglet brain following transient cerebral hypoxia-ischaemia. Neuropathol. Appl. Neurobiol. 23, 16–25.

Zai L., Ferrari C., Subbaiah S., Havton L.A., Coppola G., Strittmatter S., Irwin N., Geschwind D. and Benowitz L.I. (2009) Inosine alters gene expression and axonal projections in neurons contralateral to a cortical infarct and improves skilled use of the impaired limb. J. Neurosci. 29, 8187–97.

Zhang D., Xiong W., Albensi B.C. and Parkinson F.E. (2011) Expression of human equilibrative nucleoside transporter 1 in mouse neurons regulates adenosine levels in physiological and hypoxic-ischemic conditions. J. Neurochem. 118, 4–11.

Zhou Q.Y., Li C., Olah M.E., Johnson R.A., Siles G.L. and Civelli O. (1992) Molecular cloning and characterization of an adenosine receptor: the A3 adenosine receptor. Proc. Natl Acad. Sci. USA 89, 7432–6.

Zhu W.H., Conforti L., Czyzyk-Krzeska M.F. and Millhorn D.E. (1996) Membrane depolarization in PC-12 cells during hypoxia is regulated by an O2-sensitive K+ current. Am. J. Physiol. 271, C658–65.

Zimmermann H. (2000) Extracellular metabolism of ATP and other nucleotides. Naunyn. Schmiedebergs. Arch. Pharmacol. 362, 299–309.

Zimmermann H., Braun N., Kegel B. and Heine P. (1998) New insights into molecular structure and function of ectonucleotidases in the nervous system. Neurochem. Int. 32, 421–5.

Zurn A.D. and Do K.Q. (1988) Purine metabolite inosine is an adrenergic neurotrophic substance for cultured chicken sympathetic neurons. Proc. Natl Acad. Sci. USA 85, 8301–5.

© 2012 The Authors
Journal of Neurochemistry © 2012 International Society for Neurochemistry, J. Neurochem. (2012) 121, 329–342