

Chapter 14

Adenosine and Oxygen/Glucose Deprivation in the Brain



Felicita Pedata, Ilaria Dettori, Lisa Gaviano, Elisabetta Coppi,
and Anna Maria Pugliese

Abstract Extracellular adenosine concentrations in the brain increase dramatically during ischemia in concentrations that are able to stimulate all (A_1 , A_{2A} , A_{2B} , and A_3) receptors. Adenosine exerts a clear neuroprotective effect through A_1 receptors during ischemia mainly by reducing precocious excitotoxic phenomena. Unfortunately, the use of selective A_1 agonists is hampered by undesirable peripheral effects. Evidence indicates that A_{2A} receptor antagonists administered early after ischemia provide protection centrally by reducing excitotoxicity. After ischemia, the primary damage due to the early massive increase of extracellular glutamate is followed by activation of resident immune cells, i.e., microglia, and production or activation of inflammation mediators and blood cell infiltration. Evidences are that agonists at A_{2A} , A_{2B} , and A_3 receptors mainly acting on blood and vascular endothelial cells provide protection by controlling neuroinflammation, endothelial leaking, and massive blood cell infiltration in the hours and days after brain ischemia. Since ischemia is a multifactorial pathology characterized by different events evolving in the time and protracted neuroinflammation is recognized as the predominant mechanism of secondary brain injury progression, adenosinergic drugs aimed at dampening damage in the hours/days after ischemia appear promising.

Keywords Brain ischemia · Oxygen/glucose deprivation · Adenosine receptors · Glutamate · Neuroinflammation

F. Pedata (✉) · I. Dettori · A. M. Pugliese
Department of Neuroscience, Psychology, Drug Research and Child Health
(NEUROFARBA), University of Florence, Florence, Italy
e-mail: felicita.pedata@unifi.it

L. Gaviano · E. Coppi
Department of Health Sciences, University of Florence, Florence, Italy

14.1 Introduction

Stroke is today evaluated as the second most common cause of death and a major cause of long-term disability worldwide. Ischemic stroke commonly accounts for approximately 80% of all stroke cases, and is caused from occlusion of a major cerebral artery by a thrombus or an embolism, which leads to loss of cerebral blood flow, a condition of hypoxia and glucose deprivation (oxygen/glucose deprivation: OGD) and subsequently tissue damage in the affected region. The only successful pharmacological treatment approved to date is tissue plasminogen activator (tPA) that aims to decrease ischemia-associated thrombosis risk. Yet, because of the narrow therapeutic time window involved, thrombolytic application is very restricted in clinical settings (Chen et al. 2014). Aspirin, other antiplatelets, and anticoagulants are used as preventive therapy of stroke (Macrez et al. 2011).

After stroke, brain injury results from a complex sequence of pathophysiological events consequent to hypoxia/ischemia that evolve over time (Dirnagl 2012). A primary acute mechanism of excitotoxicity and periinfarct depolarizations is due to increased extracellular concentration of glutamate (see Fig. 14.1). Excitotoxicity brings to activation of resident immune cells, i.e., microglia, and production or activation of inflammation mediators. In the hours and along days after ischemia, protracted neuroinflammation is recognized as the predominant mechanism of secondary brain injury progression (Tuttolomondo et al. 2009). Activated microglial cells proliferate, migrate, and, by production of inflammatory substances and chemokines, trigger an inflammatory response (Dirnagl et al. 1999). Pro-inflammatory mediators and oxidative stress give rise to the endothelial expression of cellular adhesion molecules and to an altered permeability of the blood-brain barrier (BBB) that allows infiltration of leukocytes that on their turn exacerbate neuroinflammation and ischemic damage (Haskò et al. 2008; Iadecola and Anrather 2011). A huge increase of extracellular adenosine concentrations matches the increase of glutamate in the first hours after ischemia (see Fig. 14.1) as demonstrated under OGD conditions *in vitro* in the hippocampus (Dale et al. 2000; Frenguelli et al. 2007; Latini et al. 1998; Pedata et al. 1993) and in the *in vivo* models of brain ischemia (Dux et al. 1990; Hagberg et al. 1987; Matsumoto et al. 1992; Melani et al. 1999; Sciotti et al. 1992). In the first minutes after ischemia, the increase of extracellular adenosine concentration is due to the major part of extracellularly released ATP that is hydrolyzed by ectonucleotidases, and then, in the hours after ischemia, adenosine *per se* is mainly released from cells (Melani et al. 2012). After *in vivo* ischemia, the extracellular concentrations of adenosine are high enough to stimulate all adenosine receptor subtypes (A_1 , A_{2A} , A_{2B} , and A_3 receptors) (Melani et al. 2012). All receptor subtypes are expressed at significant levels in neurons and glial cells and in peripheral blood inflammatory cells (Burnstock and Boeynaems 2014) (see Fig. 14.2). The wide distribution is consistent with the multifaceted neurochemical and molecular effects of adenosine and suggests that adenosine role in ischemia is the consequence of an interplay among different receptor activations in neuronal, glial, and inflammatory cells, which varies depending on the time-related development of the

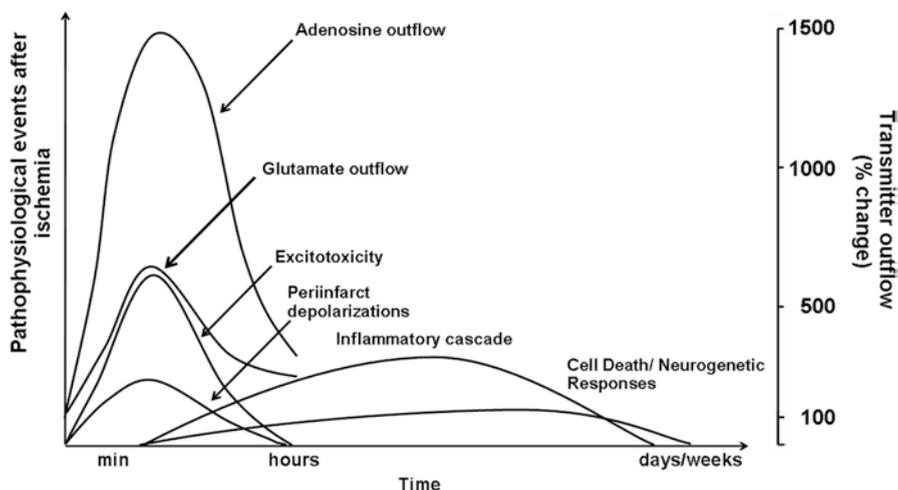


Fig. 14.1 Cascade of pathogenetic mechanisms after ischemia. Primary mechanisms of excitotoxicity lead to acute cell death in the ischemic core. Depolarization spreads in the periinfarct areas. Glutamate and extracellular adenosine concentrations increase in the first 4 h after ischemia (Melani et al. 1999, 2003, 2012). The curves of increases of glutamate and adenosine evoked by ischemia and induced by middle cerebral artery occlusion (MCAo) were drawn on the basis of values obtained by striatal microdialysis (Melani et al. 1999). In the following several hours, activation of resident immune cells, i.e., microglia and production of a cascade of inflammation mediators, occurs. Cell death/neurogenetic responses progress along days/weeks after ischemia (figure modified from Dirnagl et al. 1999). Putative therapeutic opportunities with purinergic drugs comprehend strategies aimed at reducing excitotoxicity in the first 4 h after ischemia with adenosine A_{2A} and A_{2B} receptor antagonists. In the hours and days after ischemia, agonists of adenosine A_{2A} , A_{2B} , and A_3 receptors peripherally located on vascular and blood cells may dampen vascular adhesion signals and neuroinflammation

pathological condition. Numerous authors have proposed adenosine and adenosine receptors as important targets for therapeutic implementation in the treatment of stroke.

14.2 Role of Adenosine Receptors in Ischemia

The increase in extracellular adenosine early after ischemia has long been known as an endogenous neuroprotective response (Pedata et al. 2007). In fact, adenosine infusion into the ischemic striatum has been shown to significantly ameliorate neurological outcome and reduce infarct volume after transient focal cerebral ischemia (Kitagawa et al. 2002). Adenosine protection has been attributed to stimulation of the A_1 receptor subtype; however important roles of the other three receptor subtypes have been outlined in the last 20 years.

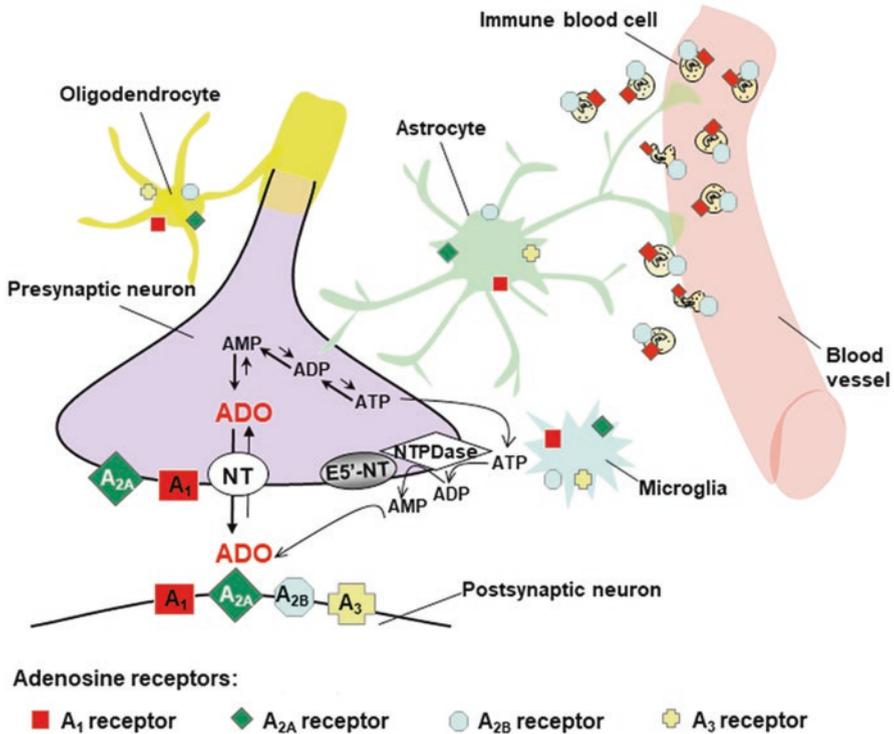


Fig. 14.2 Schematic drawing of adenosine receptors on different cell types. All adenosine receptor subtypes are expressed both at the central level on presynaptic and postsynaptic neurons, on astrocytes, on microglia, and on oligodendrocytes and at the peripheral level on leukocytes and vasculature. After cerebral ischemia, leukocytes infiltrate into ischemic tissue due to increased permeability of BBB. During ischemia, extracellular adenosine levels increase mainly due to (i) extracellular ATP degradation by NTPDase and ecto-5'-nucleotidase enzymes; (ii) release per se from cells likely by the equilibrative nucleoside transporter (ENT) (Melani et al. 2012); and (iii) inhibition of adenosine uptake processes due to downregulation of concentrative nucleoside transporters (CNT) 2 and 3 and of ENT. ADO, adenosine; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; E5'-NT, ecto-5'-nucleotidase; NT, nucleoside transporter; NTPDase, ectonucleoside triphosphate diphosphohydrolases. The proportions of the various components of the nervous tissue have not been kept

14.2.1 Adenosine A₁ Receptors Are Protective

One of the prime adaptive mechanisms in response to hypoxia-ischemia is the cellular activation of adenosine A₁ receptors that inhibit excitatory synaptic transmission as demonstrated *in vitro* and *in vivo* (Latini and Pedata 2001). Adenosine protective effects are greatly attributed to adenosine A₁ receptor activation that due to reduced Ca²⁺ influx, lower presynaptic release of excitatory neurotransmitters (Corradetti et al. 1984; Dunwiddie 1984) and in particular glutamate which exerts an excitotoxic effect during ischemia mainly by overstimulation of NMDA

(Choi 1990) and AMPA receptors (Stockwell et al. 2016). In addition, by directly increasing the K^+ and Cl^- ion conductances, adenosine stabilizes the neuronal membrane potentials, thus reducing neuronal excitability (Choi 1990). Consequent reductions in cellular metabolism and energy consumption (Greene and Haas 1991) and moderate lowering of the body/brain temperature (Tupone et al. 2013; Muzzi et al. 2013) protect against ischemia. A continuous infusion of the adenosine A_1 receptor agonist (6)*N*-cyclohexyladenosine (CHA) that maintains the body temperature between 29 and 31 °C for 24 h induces better survival and decreases the extent of brain damage in rats subjected to asphyxial cardiac arrest for 8 min (Jinka et al. 2015; Tupone et al. 2016).

Consistent data demonstrate that adenosine acting on adenosine A_1 receptor reduces the ischemia-evoked increase of excitatory transmission. In brain slices, the OGD-induced depression of synaptic transmission is reversed by administration of selective adenosine A_1 receptor antagonists (Pedata et al. 1993) that also increase OGD-evoked aspartate and glutamate efflux (Marcoli et al. 2003), impair the recovery of synaptic potentials (Sebastião et al. 2001), and shorten the onset of anoxic depolarization (AD) induced by hypoxia (Lee and Lowenkopf 1993). Depression of excitatory synaptic transmission brought about by adenosine A_1 receptors during hypoxia/ischemia involves AMPA receptor downregulation (Stockwell et al. 2016) in particular the internalization of GluA1 and GluA2 subunit-containing AMPA receptors (Stockwell et al. 2016). Depression of excitatory synaptic activity and a cross talk with A_{2A} receptor are crucial for the functional recovery of hippocampal circuits upon reoxygenation when adenosine A_{2A} receptors play a critical role by increasing excitatory amino acid efflux (Stockwell et al. 2017). The A_1 -mediated depression of excitatory synaptic transmission may also be due to the enhancement of inhibitory synaptic transmission in CA1 neurons (Liang et al. 2009).

In *in vitro* studies, both adenosine and selective A_1 receptor agonists reduce neuronal damage following hypoxia and/or OGD in primary cortical or hippocampal cell cultures (Daval and Nicolas 1994) and brain slices (Mori et al. 1992). A_1 receptor agonists increase survival in anoxia and anoxia/reoxygenation and decrease reactive oxygen species (ROS) production, while A_1 receptor blockade increases ROS release and cell death in primary neuronal cultures (Milton et al. 2007). Studies in support of the neuroprotective role of adenosine A_1 receptor stimulation demonstrate that hippocampal slices from A_1 receptor knockout (KO) mice showed a markedly reduced and delayed protective response to hypoxia compared to slices from wild-type (WT) mice (Johansson et al. 2001). In astrocytes prepared from A_1 receptor KO mice, more pronounced hypoxic cytotoxicity was observed (Bjorklund et al. 2008). In murine astrocytes exposed to hypoxic injury, adenosine, through activation of A_1 and A_3 receptors, inhibits accumulation of the lipopolysaccharide (LPS)-induced hypoxia-inducible factor-1 (HIF-1), a master regulator of oxygen homeostasis (Gessi et al. 2013).

In *in vivo* animal models of global cerebral ischemia, it has been demonstrated that local administration of an adenosine analogue, 2-chloroadenosine (CADO), and of a nonselective A_1 receptor agonist, N6-(*L*-2-phenylisopropyl) adenosine (L-PIA), attenuates neuronal loss in the CA1 region of the rat hippocampus

(Domenici et al. 1996; Evans et al. 1987). The acute systemic or intracerebroventricular (i.c.v.) injection of the A₁ agonists cyclohexyladenosine (CHA) and R-phenylisopropyl-adenosine (R-PIA) improves neurological deficits (Heron et al. 1994; Von Lubitz and Marangos 1990; Zhou et al. 1994), protects the CA1 region of the hippocampus (Von Lubitz et al. 1988), and prevents the reduction of adenosine A₁ receptors (Daval et al. 1989) in rats or gerbils. Similarly, acute administration of the A₁ agonists N⁶-cyclopentyladenosine (CPA) and 2-chloro-N(6)-cyclopentyladenosine (CCPA) reduces mortality and the loss of neurons after global forebrain ischemia in the gerbil (Von Lubitz et al. 1994a). Systemic administration of the A₁ receptor agonist adenosine amine congener (ADAC) after global ischemia in the gerbil increased survival, preserved neuronal morphology, and maintained spatial memory and learning ability (Phillis and Goshgarian 2001; von Lubitz et al. 1996).

Several intracellular mechanisms might account for adenosine A₁ receptor-mediated neuroprotection in hypoxia/ischemia. Postischemic intraperitoneal (i.p.) administration of adenosine amine congener (ADAC) resulted in preservation of microtubule-associated protein 2 (MAP-2) (von Lubitz et al. 1996). CCPA administered i.c.v. before focal ischemia reduces lipid peroxidation in the cerebral cortex (Sufianova et al. 2014). Chronic coadministration of CCPA and vitamin C i.p. after global ischemia, induced by common carotid arteries ligation, minimized ischemia-reperfusion damage by increasing the expression of antiapoptotic protein Bcl-2 and decreasing the expression of proapoptotic protein Bax in mice (Zamani et al. 2013).

In accordance with a protective role of adenosine A₁ receptors in ischemia, acute administration of adenosine A₁ antagonists exacerbates the damage (Phillis 1995). However, chronic administration of adenosine receptor antagonists administered before an ischemic insult reduced the neuronal injury (Rudolph et al. 1989), and chronic administration of A₁ agonists worsened survival and increased neuronal loss (Jacobson et al. 1996). It has been suggested these *phenomena* depend on A₁ receptor upregulation and desensitization, respectively.

Plastic changes in A₁ receptors are critical to understand the effects of adenosine A₁ agonists/antagonists but also whether adenosine maintains its neuroprotective efficiency after ischemia. Several studies have shown that short periods of focal or global ischemia produced a long-lasting decrease in the density of A₁ receptors (Lee et al. 1986). In rat hippocampal slices, hypoxia leads to a rapid (<90 min) desensitization of A₁ receptor that is likely due to an internalization of A₁ receptors in nerve terminals (Coelho et al. 2006), a process that may result in hyperexcitability and increased brain damage. In a chronic cerebral ischemic mouse model induced by common carotid artery occlusion, A₁ receptor downregulation, a decreased proteolipid protein (a marker of white matter myelination), inhibition of the anti-inflammatory interleukin-10 (IL-10) production, and cognitive impairment measured by the Morris water maze test have been reported (Cheng et al. 2015). However it has been reported that A₁ receptor KO mice, when exposed to global ischemia, do not show increased neuronal damage in the CA1 region of the hippocampus, in the cortex, or in the striatum (Olsson et al. 2004). These discrepancies may reflect development of compensatory mechanisms after genetic deletion.

In models of hypoxia-ischemia in neonatal rats, it was reported that A₁ receptors contribute to protection of hypoxic brain (Bona et al. 1997). In agreement, most

recently it has been reported that A₁ receptor KO neonatal mice, from 10 to 17 days after brain hypoxia/ischemia, displayed larger infarctions, cognitive impairment, and exaggerated activation of myeloid cells (Winerdal et al. 2016). Since inflammation greatly affects the outcome after neonatal brain injury, activation of myeloid cells is proposed as cause of the increased damage in A₁ receptor KO neonatal mice, (Winerdal et al. 2016). Thus, the decrease of adenosine A₁ receptors (Aden et al. 1994) and increase of adenosine deaminase (Pimentel et al. 2015) that has been described after rat neonatal hypoxia/ischemia would worsen hypoxic brain damage in neonatal period. On the other end, adenosine acting on A₁ receptors appears to mediate hypoxia-induced brain ventriculomegaly during early postnatal development (Turner et al. 2003). It should be remembered that in the formation of the central nervous system (CNS), A₁ receptor activation potently inhibits the development of axons and can lead to leukomalacia (Rivkees et al. 2001). Notably, caffeine, a competitive antagonist of adenosine A₁, A_{2A}, and A_{2B} receptors, that is commonly used in neonates against apnea of prematurity has become a candidate for neuroprotection (Schmidt et al. 2007).

Adenosine by stimulating A₁ receptors plays a crucial role in the “precondition phenomenon” consisting in protection by sublethal anoxic/ischemic insults from subsequent ischemic insults. The A₁ receptor agonist, CADO, markedly enhanced and A₁ receptor antagonists completely prevented the protective effect of ischemic preconditioning in rat hippocampal slices (Pugliese et al. 2003). In accordance with in vivo models of ischemia, the selective A₁ antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), attenuated the neuroprotective effect of ischemic preconditioning (Cui et al. 2013) and CCPA pretreatment-induced ischemic tolerance against cerebral ischemia/reperfusion injury induced by middle cerebral artery occlusion (MCAo) in the rat (Hu et al. 2012). Preconditioning induced also by limb remote ischemia contributes neuroprotective effects against rat focal cerebral ischemic injury induced by transient MCAo, and the selective A₁ antagonist DPCPX abolished the protective effects demonstrating the involvement of A₁ receptors (Hu et al. 2012). Interestingly ischemic preconditioning-induced neuroprotection appears transferable among cells through intervention of A₁ receptors as studied in human neuroblastoma SH-SY5Y cells (Yun et al. 2014). Peculiarly, adenosine A₁ receptors activation is involved in the ischemic tolerance in mice induced by a ketogenic diet (a high-fat, low-carbohydrate diet that increases acetyl-CoA that is involved in ketone body formation that represents an alternative energy source for brain cells under conditions of glucose deprivation) (Yang et al. 2017).

Although data, on the all, demonstrate a neuroprotective effect of adenosine through A₁ receptors during ischemia, the use of selective A₁ agonists is hampered by undesirable peripheral effects such as sedation, bradycardia, and hypotension. Interestingly, nowadays it is proposed that partial agonists at A₁ receptor may be devoid of hemodynamic effects being therefore valuable drugs in ischemia (Baltos et al. 2016). The possibility that new adenosine A₁ receptor partial agonists are protective in ex vivo and in vitro experimental models of ischemia was recently discussed by Martire and coworkers (personal communication 2016).

14.2.2 Adenosine A_{2A} Receptors in Brain Ischemia

14.2.2.1 Brain A_{2A} Receptors Increase Glutamatergic Excitatory Transmission

A_{2A} receptors play an important modulation of synaptic transmission counteracting depression brought about by A₁ receptor (Lopes et al. 2011). In the CA1 area of the rat hippocampus, the selective A_{2A} receptor agonist, 2-p-(2-carboxyethyl) phenethylamino-5'-Nethylcarboxamidoadenosine hydrochloride (CGS21680), clearly reduces the OGD-induced depression of synaptic activity (Latini et al. 1999). In agreement, the selective A_{2A} receptor antagonists, 4-(2-[7-amino-2-(2-furyl) [1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-amino]ethyl)phenol (ZM241385) and 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4, triazolo[1,5-c] pyrimidine (SCH58261), delay the appearance of AD, a phenomenon strictly related to cell damage and death (Somjen 2001), protect from the synaptic activity depression brought about by a severe (7 min) OGD period, and protect CA1 neuron and astrocyte from injury (Pugliese et al. 2009). The same effects of ZM241385 were observed after a severe 9 min OGD period in the *gyrus dentatus* of the hippocampus (Maraula et al. 2013).

Protective effects against OGD by A_{2A} receptor antagonists are greatly attributed to antagonism of excessive excitatory transmission. In fact adenosine A_{2A} receptor regulates glutamatergic excitatory transmission by several mechanisms. Adenosine by stimulating A_{2A} receptors located presynaptically on glutamatergic terminals can directly regulate glutamate outflow under normoxic (Lopes et al. 2002) and ischemic conditions (Marcoli et al. 2003). Moreover A_{2A} receptors modulate glutamate uptake transporter. In particular, A_{2A} receptors located on astrocytes mediate inhibition of glutamate uptake by glutamate transporter-1 (GLT-1) (Pinto-Duarte et al. 2005). An imbalance of A₁/A_{2A} receptor expression might also contribute to inhibition of excitatory synaptic transmission under ischemia. Short periods of global ischemia decrease A₁ adenosine receptor density in the brain likely due to an internalization of A₁ adenosine receptors in nerve terminals (Coelho et al. 2006), thus switching the balance toward A_{2A} receptor-mediated effects. Moreover, adenosine acting on A_{2A} receptor increases AMPA (Dias et al. 2012) and NMDA receptor function (Rebola et al. 2008).

All the above-described modulatory effects of the glutamatergic excitatory transmission by adenosine A_{2A} receptors might be relevant in in vivo ischemia. A definite overexpression of A_{2A} receptors was found in vivo in neurons of the striatum and cortex 24 h after focal ischemia (Trincavelli et al. 2008). The A_{2A} agonist CGS21680 increases excitatory amino acid outflow from the ischemic cortex during in vivo ischemia (O'Regan et al. 1992).

Several studies demonstrated that antagonists of adenosine A_{2A} receptors were protective in in vivo models of global ischemia. Gao and Phillis (1994) demonstrated for the first time that the nonselective A_{2A} receptor antagonist, 9-chloro-2-(2-furanyl)-[1,2,4] triazolo[1,5-c]quinazolin-5-amine (CGS15943),

reduced cerebral ischemic injury in the gerbil following global forebrain ischemia. Thereafter many reports have confirmed the neuroprotective role of A_{2A} receptor antagonists in different models of ischemia. The selective A_{2A} receptor antagonist, 8-(3-chlorostyryl)caffeine (CSC), and the less selective antagonists, CGS15943 and 4-amino [1,2,4] triazolo [4,3a] quinoxalines (CP66713), both administered preischemia and protected against hippocampal cell injury during global forebrain ischemia in gerbils (Phillis 1995; von Lubitz et al. 1995). The selective A_{2A} receptor antagonist, ZM241385, administered preischemia, reduced hippocampal injury, and improved performance in the Morris water maze in hyperglycemic four-vessel occluded rats (Higashi et al. 2002). In all these studies, adenosine A_{2A} receptor antagonists were administered preischemia. However, postischemic administration is more relevant to a possible clinical use of drugs in stroke. The selective A_{2A} receptor antagonist, SCH58261, acutely administered after hypoxia/ischemia in neonatal rats reduced brain damage (Bona et al. 1997) and acutely administered i.p. 5 min after focal ischemia in adult rats was protective from brain damage 24 h thereafter (Melani et al. 2003). The same antagonist, administered subchronically (i.p., 5 min, 6 and 15 h) after focal ischemia, was protective not only against brain damage but also from neurological deficit (Melani et al. 2006, 2009; Pedata et al. 2005) and disorganization of myelin (Melani et al. 2009) 24 h after focal cerebral ischemia in the adult rat. In the model of global ischemia (i.e., 7 min asphyxic cardiac arrest) in newborn piglets, posttreatment infusion with SCH58261 improved neurologic recovery and protected striatopallidal neurons 4 days after ischemia (Yang et al. 2013).

The ability of adenosine A_{2A} receptor antagonists in protecting against ischemic damage in vivo is largely attributed to the control of excessive glutamatergic transmission and of the ensuing acute excitotoxicity after ischemia. The low dose of SCH58261 that 24 h after ischemia has protected against tissue damage induced by MCAo (Melani et al. 2003) or quinolinic acid (QA) excitotoxicity (Popoli et al. 2002), has also reduced, in the first 4 h after ischemia, the increase of extracellular glutamate estimated by microdialysis in the striatum (Melani et al. 2003) and has reduced glutamate content in the hippocampus after occlusion of both carotids in the rat (Svenningsson et al. 1997). In agreement, adenosine A_{2A} receptor KO mice are protected from an excess of striatal glutamate outflow and damage induced by transient MCAo (Gui et al. 2009).

In addition, ZM241385, injected directly to intrahippocampus, is protective against excitotoxicity induced by kainate (Jones et al. 1998), and SCH58261 administered directly in the hippocampus (Mohamed et al. 2016) ameliorates infarct size, memory impairment, and motor incoordination 24 h after occlusion of both carotids in the rat. A further mechanism by which A_{2A} receptor antagonism is protective may be due to the capability of increasing brain GABA extracellular concentration during ischemia (Cristóvão-Ferreira et al. 2009).

SCH58261 behaves as a significant protective agent at a dose (0.01 mg/kg) that does not have cardiovascular effects. This low dose does not affect motor activity in naive animals but decreases contralateral turning behavior after MCAo induced by the monofilament technique (Melani et al. 2003, 2006). At a higher dose, in the

range that is effective in different models of Parkinson's disease (PD), the same drug significantly increases motility and rearing in the rat (Svenningsson et al. 1997).

Control of several intracellular pathways activated by ischemia might account for protection by A_{2A} receptor antagonism. Twenty-four hours after focal ischemia, the A_{2A} receptor antagonist SCH58261 has decreased the ischemia-induced activation of p38 mitogen-activated protein kinase (MAPK) in activated microglia (Melani et al. 2006) and of JNK MAPK that is mainly expressed in mature oligodendrocytes and in oligodendrocyte progenitors (OPCs) (Melani et al. 2009). p38 is considered a death factor in ischemia (Barone et al. 2001), and phospho-JNK is a factor involved in oligodendrocyte death (Jurewicz et al. 2006). JNK MAPK KO mice are in fact protected from damage following cerebral ischemia (Kuan et al. 2003). Reduced activation of JNK might be directly due to A_{2A} receptors located on OPCs (Coppi et al. 2015). In fact in primary OPC culture, selective stimulation of A_{2A} receptors by CGS21680 inhibits maturation of OPCs (Coppi et al. 2013) and inhibits "delayed rectifier" K^+ currents (K_{DR}) (Coppi et al. 2013) that are known to promote proliferation and differentiation of OPC to mature oligodendrocytes, thus preventing myelin deposition.

Direct intrahippocampus administration of SCH58261 after global ischemia, 24 h thereafter, has reduced also phospho-ERK 1/2 bringing to the reduction of different inflammation products and to the increase of the anti-inflammatory cytokine IL-10 (Mohamed et al. 2016).

The reduced MAPK activation by SCH58261 might be due to a direct effect of the A_{2A} receptor antagonists on A_{2A} receptors located on oligodendrocytes or microglia but also to the overall reduction of the excitotoxic cascade that in the initial hours after in vivo ischemia primes microglial activation and MAPK activation. In fact, oligodendroglial cells are extremely sensitive to glutamate receptor overactivation, and ensuing oxidative stress and p38 and ERK1/2 MAPK activation is definitely induced by glutamate receptor stimulation (Kurino et al. 1995).

The recent observation that the A_{2A} receptor antagonist SCH58261 chronically administered after ischemia has not maintained protection 7 days after transient focal ischemia (Melani et al. 2015) supports the idea that the early protection offered by A_{2A} antagonism is overwhelmed on time by the secondary damage due to blood cell infiltration and neuroinflammation.

14.2.2.2 Adenosine A_{2A} Receptor Agonists Are Protective against Ischemic Damage

Considering that A_{2A} receptor antagonists are protective after ischemia, in an apparent paradoxical manner, also adenosine A_{2A} agonists were found protective under hypoxia/ischemia. An early study demonstrated that the adenosine A_{2A} receptor agonist

2-[(2-aminoethylamino)-carbonylethylphenylethylamino]-5'-N-ethylcarboxoamidoadenosine (APEC), administered systemically and chronically for 13 days, before a global 10-min ischemia in the adult gerbil, ameliorated animal

and neuron survival (von Lubitz et al. 1995). Also the selective A_{2A} receptor agonist, CGS21680, administered immediately after 5 min of global ischemia in gerbil at the high dose of 10 mg/kg i.p., exhibited highly significant protection against neuronal loss (Sheardown and Knutsen 1996). In agreement, A_{2A} receptor KO mice subjected to chronic cerebral hypoperfusion by permanent stenosis of bilateral common carotid artery showed impairment in working memory, increased demyelination and proliferation of glia, and increased levels of pro-inflammatory cytokines (Duan et al. 2009). The same transgenic mice, at neonatal age, showed aggravated hypoxic/ischemic injury in comparison to WT littermates (Adén et al. 2003). Most recently, Melani et al. (2014) have demonstrated that the A_{2A} receptor agonist, CGS21680, administered at the low dose of 0.01 mg/kg, twice/day for 7 days i.p. (chronic protocol) starting from 4 h after transient (1 h) MCAo, induced protection from neurological deficit, weight loss, cortical infarct volume, myelin disorganization, and glial activation evaluated 7 days after ischemia.

In considering translation to clinic, a main problem of A_{2A} receptor agonists consists in their cardiovascular effect because adenosine A_{2A} receptors located on vascular smooth muscle and endothelial cells exert a vasodilatory effect. Relevantly, Melani et al. (2014) have demonstrated that the protective dose (0.01 mg/kg) of CGS21680 does not modify either mean blood pressure or heart frequency. Moreover, adenosine by stimulating $A_{2A}R$ G_s -coupled adenylate cyclase in platelets enhances the intracellular cAMP levels, a potent molecule that inhibits platelet activation (Cooper et al. 1995) having thus potential antithrombotic activity. Therapies under study in ischemia (i.e., neuroprotective drugs including hypothermia or antioxidant/anti-inflammatory strategies) need to be associated with thrombolytic drugs since restoration of oxygen and glucose, at the moment, is considered the best therapy to protect against cell death from stroke (Liu et al. 2017), although its efficacy may be limited by the potential hemorrhagic effects. Considering that tPA and/or antiplatelet drugs are also routinely used in prevention of the secondary stroke, administration of a further drug that has antiplatelet activity could potentiate a previous antiplatelet therapy increasing the hemorrhagic potential or on the contrary could be useful in maintaining an antiplatelet effect after the primary stroke. However we found (personal unpublished results) that a chronic treatment with CGS21680, twice/day for 7 days at the dose of 0.01 mg/kg administered i.p. in control rats, does not modify platelet aggregation induced by 10 μ M ADP (technique described by Ma et al. 2016; Yang et al. 2015) (50.9% \pm 11.5 of ADP induced aggregation in control $n = 3$ versus 49.4% \pm 1.3 in treated rats $n = 4$). In agreement, concentrations of CGS21680 that decrease production of free radicals of the oxygen from isolated human neutrophils were calculated three times lower (EC₅₀ 300 nM) than those that decrease human platelet aggregation (IC₅₀ 1090 nM) (Gessi et al. 2000). Data suggest that adenosine A_{2A} receptors exert antioxidant effects and inhibit granulocyte infiltration at doses /concentrations lower than those necessary to inhibit platelet aggregation.

Protection by CGS21680 after ischemia could be attributable to central effects because it easily crosses the BBB. As a vasodilator agent, adenosine acting on A_{2A} receptors is in fact implicated in cerebral blood flow regulation and might favor

brain reperfusion after ischemia. Recently, importance of adenosine receptors located on vasculature as therapeutic targets in cardiovascular pathologies including stroke was pointed out (Sousa and Diniz 2017). Moreover CGS21680 administered directly into the rat striatum immediately prior to the induction of intracerebral hemorrhage reduces parenchymal neutrophil infiltration and tissue damage: an effect that was related to the inhibition of tumor necrosis factor- α (TNF- α) expression (Mayne et al. 2001). Activation of central A_{2A} receptors is known to increase expression and release of neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) (Sebastião and Ribeiro 2009). The increase in neurotrophic factor expression by adenosine A_{2A} receptor stimulation may contribute to restore neurological functions and cerebral damage after brain ischemia.

A bulk of evidences however indicates that A_{2A} receptors located on blood cells greatly account for protective effects of adenosine A_{2A} agonists after ischemia. The adenosine A_{2A} receptors are expressed in fact both on cells of innate (microglia, macrophages, mast cells, monocytes, dendritic cells, neutrophils) and on adaptive (lymphocytes) immunity. After ischemia altered permeability of BBB allows infiltration of leukocytes (neutrophils, lymphocytes and monocytes) that on their turn exacerbate ischemic damage (Haskó et al. 2008). In the transient MCAo model in the rat, selective immunostaining for granulocytes, by anti-HIS-48 antibody, shows numerous infiltrated cells in ischemic striatal and cortical core, 2 days after transient MCAo (Melani et al. 2014). This is in agreement with observation that after transient MCAo, a peak of neutrophil infiltration occurs at 6 and 48 h thereafter (Zhang et al. 1994). Seven days thereafter, infiltrated blood cells were anymore observed (Melani et al. 2014). Chronic treatment with the A_{2A} adenosine receptor agonist, CGS21680, 2 days after transient MCAo, has definitely reduced the number of infiltrated blood cells in the ischemic areas (Melani et al. 2014). The importance of a protracted treatment with the A_{2A} agonist in order to achieve protection is proved by the observation that the A_{2A} agonist administered subchronically (4 and 20 h after induction of MCAo) did not prove protective 24 h after permanent MCAo nor 7 days after transient MCAo (Pedata et al. 2014).

Many studies have reported that selective activation of A_{2A} receptors directly on blood cells, including platelets, monocytes, some mast cells, neutrophils, and T cells, inhibits pro-inflammatory responses, reduces production of adhesion cell factors, and reduces neutrophil activation, thereby exerting antioxidant and anti-inflammatory effects. A_{2A} receptor activation is known to reduce ischemia-induced rolling, adhesion, and transmigration of various peripheral inflammatory cells (such as lymphocytes, neutrophils) (Haskó et al. 2008). It has been reported that adenosine A_{2A} receptors are sensors of inflammatory disease and increase in number in blood cells in different human peripheral and central inflammation-based pathologies including rheumatoid arthritis multiple sclerosis and amyotrophic lateral sclerosis (Borea et al. 2016). Our (unpublished) results demonstrate that density of adenosine A_{2A} receptor assayed by RT-PCR in leukocytes isolated from sham-operated (mean \pm ES; sham-operated, 1.02 ± 0.02) is not modified 48 h after tMCAo (0.95 ± 0.01) but was significantly decreased after 7 days ($0.92 \pm 0.03^*$, unpaired

student's t-test, $*p < 0.03$ vs sham-operated rats; results are expressed as fold increase according to the $2^{-\Delta\Delta Ct}$ method, utilizing as target genes ADORA2A) when infiltrated blood cells were anymore observed (Adén et al. 2003).

In support that A_{2A} receptors on blood cells are greatly responsible of the protective effects of A_{2A} agonists, protection of motor deficits by A_{2A} receptor agonists systemically administered after spinal trauma is lost in mice lacking A_{2A} receptors on bone marrow-derived cells (BMDCs) but is restored in A_{2A} receptor KO mice reconstituted with A_{2A} receptors on BMDCs (Li et al. 2006). Moreover, in the spinal cord trauma model in the mouse, CGS21680 protected from damage when injected systemically but not when centrally injected into the injured spinal cord (Paterniti et al. 2011). Consistent with its anti-inflammatory and immunosuppressive role, the protective effect of adenosine A_{2A} receptor stimulation has been observed in different pathologies where inflammatory process has an important role in tissue damage such as ischemia/reperfusion liver injury (Day et al. 2004), spinal cord trauma (Day et al. 2004; Genovese et al. 2010; Paterniti et al. 2011), rheumatoid arthritis (Mazzon et al. 2011), acute lung inflammation (Impellizzeri et al. 2011), intestine ischemia/reperfusion injury (Di Paola et al. 2010; Odashima et al. 2005), and experimental autoimmune encephalomyelitis (Xu et al. 2013).

14.2.2.3 A_{2A} Receptor as Target of Protective Drugs after Ischemia

In conclusion information up to now indicates that stimulation or antagonism of A_{2A} receptors might be a protective strategy secondary to the time-related development of phenomena typical of trauma and ischemia. Protective effects of A_{2A} antagonists, at doses that do not modify hemodynamic parameters and inside a therapeutic window compatible with arrival in a stroke unit, would provide protection by dampening central excitotoxicity, while A_{2A} agonists, at doses that do not modify hemodynamic parameters or platelet activity, provide protection by controlling massive infiltration in the hours after ischemia. Since a major mechanism underlying reperfusion injury is that of poststroke inflammation, targeting anti-inflammatory targets as a combined therapy with pharmacological thrombolysis or mechanical thrombectomy after reperfusion is a potential useful strategy after stroke (Mizuma and Yenari 2017).

14.2.3 Adenosine A_{2B} Receptors in Brain Ischemia

Among adenosine receptors, the adenosine A_{2B} receptor subtype is the least studied and still remains the most enigmatic adenosine receptor subtype because of the relatively low potency of adenosine at this receptor (EC50 value of 24 μM) (Fredholm et al. 2011) and the very few specific agonists that have been described so far. Adenosine A_{2B} receptors, although scarcely, are uniformly expressed throughout the CNS (Dixon et al. 1996) including the hippocampus (Perez-Buirra et al. 2007). Their

expression in neurons, glial, and vascular endothelial cells increases after ischemia and mRNA protein expression of A_{2B} receptor increased to a greater extent after ischemia-reperfusion than did expression of the other three adenosine receptors (A_1 , A_{2A} , and A_3) 24 h after transient MCAo in the rat (Li et al. 2017). Thus, during conditions of hypoxia or ischemia when the extracellular adenosine levels rise, A_{2B} receptors might be well activated (Xu et al. 2013).

Due to the existence of selective antagonists of A_{2B} receptors, their role under OGD was most recently investigated. Our recent data demonstrate that, in the CA1 area of the rat hippocampus, the selective A_{2B} receptor antagonists, N-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl) phenoxy]-acetamide (MRS1754) and 8-[4-[4-(4-Chlorophenyl)piperazine-1-sulfonyl] phenyl] -1-propylxanthine (PSB603), prevent the appearance of AD, a phenomenon strictly related to cell damage and death (Pugliese et al. 2006), and protect from the synaptic activity depression, bringing to a significant recovery of an otherwise disrupted neurotransmission induced by 7-min OGD (see Fig. 14.3) (Fusco et al. 2017). The damage to CA1 pyramidal neurons, assessed by the decrease of immunofluorescence density of CA1 NeuN⁺ neurons, was completely antagonized by treatment with PSB603 (see Fig. 14.3) (Gaviano et al. 2017). A_{2B} receptors are present in mouse hippocampal glutamatergic terminals, where their selective stimulation counteracts the A_1 receptor-mediated inhibition of synaptic transmission (Goncalves et al. 2015). Moreover, in transfected cells, a synergy with A_{2A} receptors has been envisaged because adenosine A_{2A} receptor, when stimulated, facilitates A_{2B} receptor externalization from the endoplasmic reticulum to the plasma membrane, possibly increasing the formation of the A_{2A} - A_{2B} dimer which could regulate glutamate outflow (Moriyama and Sitkovsky 2010).

In primary murine astrocytes, the expression of A_{2B} receptor is strongly stimulated by LPS in concert with hypoxia (Gessi et al. 2013). In human astroglial cells, a selective A_{2B} antagonist, N-(4-acetylphenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl) phenoxy]acetamide (MRS1706), completely prevents elongation of astrocytic processes (a morphological hallmark of *in vivo* reactive astrogliosis) induced by selective stimulation of A_{2B} receptors (Trincavelli et al. 2004). The selective A_{2B} receptor antagonist, MRS1754, administered *i.c.v.*, reduced an early ceramide production from primary astrocytes isolated from the hippocampus of rats subjected to global cerebral ischemia (Gu et al. 2013). Specific secretion of ceramide from astrocytes has been associated with neuroinflammation and is considered a contributing factor to neuronal dysfunction and damage (Wang et al. 2012). Such effect of the A_{2B} antagonist might be due to an early reduction of p38 MAPK activation (Wei et al. 2013) or to reduced expression of the “regulators of G-protein signaling” (RGS) in particular RGS-3 as demonstrated in astrocytoma cells (Eusemann et al. 2015). A_{2B} receptor desensitization described on astroglia might represent a cell defense mechanism in ischemia (Trincavelli et al. 2008). Since A_{2B} receptors are activated only by high adenosine concentrations as can be reached under brain ischemia, they might represent a good selective therapeutic target for antagonists that, by reducing excitotoxicity and neuroinflammation, can subserve a protective mechanism early after ischemia (Popoli and Pepponi 2012).

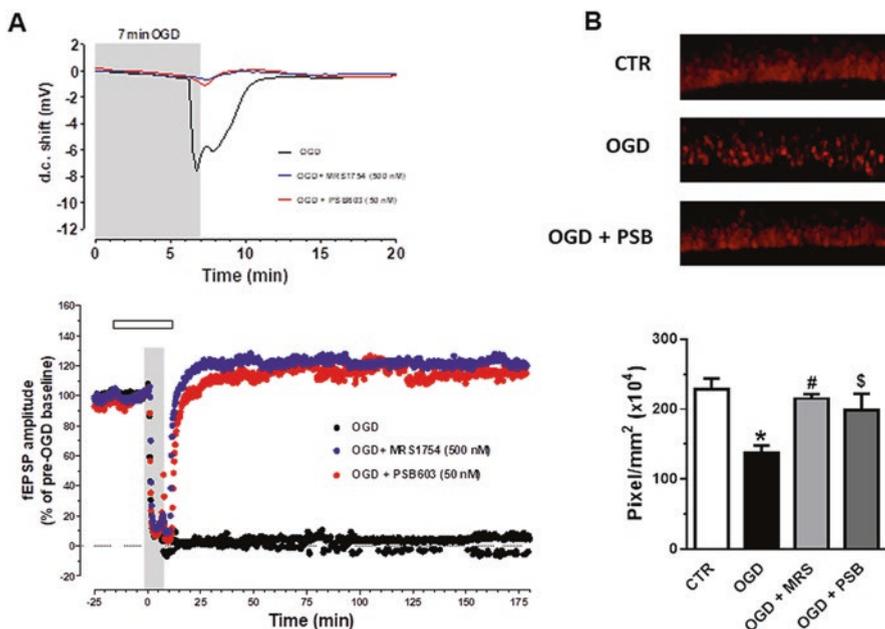


Fig. 14.3 The selective antagonism of adenosine A_{2B} receptors counteracts functional and histological damage induced by severe OGD. (A) *Upper panel*: AD was recorded as the negative d.c. shift in response to 7-min OGD in the absence (OGD) or in the presence of 500 nM MRS1754 or 50 nM PSB603. *Lower panel*: the graph shows the time course of 7-min OGD effects on fEPSP amplitude in OGD-untreated slice and in 500 nM MRS1754- or 50 nM PSB-603 treated slices. Amplitude of fEPSPs is expressed as percent of respective pre-OGD baseline. Note that, after reperfusion in oxygenated standard solution, a recovery of fEPSP was found in MRS1754 or PSB603 treated OGD slices. Gray bar, OGD time duration. Open bar, time of drug application. (B) Analysis of NeuN⁺ immunofluorescence in CA1 stratum pyramidale after the OGD insult. *Upper panels*: representative images of NeuN⁺ immunofluorescence in the region of interest of CA1 of a control slice (CTR), a slice where a 7-min OGD was performed (OGD), and a slice where a 7-min OGD was performed in the presence of 50 nM PSB-603 (OGD + PSB), all collected 3 h after the insult. Scale bar, 75 μ m. *Lower panel*: quantitative analyses of NeuN⁺ immunofluorescence in the four experimental groups. Each column represents the area, expressed in pixels ($\times 10^6$) above a threshold, maintained constant for all slices investigated. Statistical analysis: One-way ANOVA, Newman-Keuls multiple comparison test: * $P < 0.05$, OGD vs CTR; # $P < 0.05$, OGD + PSB vs OGD. CTR, $n = 6$; OGD, $n = 5$; OGD + PSB, $n = 3$. All data in the graphs are expressed as mean \pm S.E.M

Besides brain cells, A_{2B} receptors are present on blood immune cells, i.e., neutrophils and lymphocytes (Eckle et al. 2008; Gessi et al. 2005), where in most cases they are coexpressed with A_{2A} receptors. They are also expressed at low levels on platelets, where they are upregulated following injury and systemic inflammation *in vivo* and induce inhibition of platelet aggregation (Yang et al. 2010). Attenuation of hypoxia-associated increases in tissue neutrophil number in different tissues including brain largely depends on hematopoietic cell A_{2B} signaling (Eckle et al. 2008).

Moreover, A_{2B} receptors are expressed on the surface of endothelial cells (Feoktistov et al. 2004) where they are upregulated by the hypoxia-inducible factor (HIF-1 α) (Eltzschig et al. 2004). Studies in mice deleted of A_{2B} receptors on bone marrow cells indicate an important contribution of vascular A_{2B} receptors in attenuating vascular leakage during hypoxia (Eckle et al. 2008). The A_{2B} receptor antagonist MRS1754 increases adhesion in human microvascular endothelial cells (HMEC-1 s) exposed to hypoxia (Eltzschig et al. 2004), and adenosine A_{2B} receptor KO mice show increased basal levels of TNF- α and expression of adhesion molecules in lymphoid cells, resulting in increased leukocyte rolling and adhesion (Yang et al. 2006). Evidences indicate that A_{2B} receptors are a valuable target to protect heart (Eltzschig et al. 2013) and kidney from ischemia (Grenz et al. 2008). Recent introduction of new pharmacological tools (Hinz et al. 2014) led to understand a role of A_{2B} receptors in ischemia. The selective A_{2B} receptor agonist BAY60–658380 systemically administered in mice before *in vivo* normobaric hypoxia exposure decreases vascular leak in the lung, liver, and colon (Eckle et al. 2008). It has also been demonstrated (Li et al. 2017) that treatment with BAY60–6583 (1 mg/kg intravenously), at the start of reperfusion after brain ischemia induced by 2-h transient MCAO, 24-h thereafter, reduced lesion volume and attenuated brain swelling and BBB disruption. In the presence of tPA (administered after ischemic stroke to dissolve intravascular clots), BAY60–6583 also mitigated sensorimotor deficits and reduced tPA-induced hemorrhages at 24 h (Li et al. 2017). The neurovascular protection afforded by BAY60–6583 appears to derive from stimulation of the tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) production, inhibition of tPA-induced matrix metalloprotease (MMP) activation, and prevention of tight junction protein degradation. In fact overactivation of MMP leads to increased cerebrovascular permeability after ischemia-reperfusion injury (Mishiro et al. 2012). It is proposed that A_{2B} receptor agonists might be adjuvant to tPA and could be a promising strategy for decreasing the risk of hemorrhages during treatment for ischemic stroke (Li et al. 2017).

All together these studies point toward a role of central A_{2B} receptors, in synergy with A_{2A} receptors in promoting brain excitotoxicity, while A_{2B} receptors located on vascular endothelial cells would play a pivotal role in attenuating hypoxia-induced increases in vascular leak. A_{2B} receptor has been described as implicated in dampening vascular adhesion signals and hypoxia-induced inflammation (Koeppen et al. 2011).

A further possible role of A_{2B} receptors in hypoxia/ischemia might be secondary to promotion of an angiogenic response because activation of A_{2B} receptors by adenosine increases endothelial cell proliferation, chemotaxis, capillary tube formation, and release of vascular endothelial growth factor (VEGF) (Feoktistov et al. 2004).

14.2.4 Adenosine A₃ Receptors in Brain Ischemia

Adenosine A₃ receptor has an affinity of 300 nM in his widespread in the rat and mouse brain but compared to A₁ and A_{2A} receptors has less affinity for adenosine (10–30 nM versus 1 μM) and is detected at relatively low levels (Gessi et al. 2008). However, since extracellular adenosine concentrations in the first hours after ischemia reach a μM range (Latini and Pedata 2001; Melani et al. 1999), also adenosine A₃ receptor is involved in the tonic adenosine effects in ischemia.

Studies currently in the literature concerning the role of adenosine A₃ receptor in the pathophysiology of cerebral ischemia are rather contradictory (Borea et al. 2009; Pedata et al. 2010). The use of mice with genetic deletion of the A₃ receptors has pointed out a neuroprotective function of adenosine A₃ receptors. Mice lacking A₃ receptors showed in fact increased neurodegeneration in response to repeated episodes of moderate hypoxia (Fedorova et al. 2003) and an increase in cerebral infarction after transient ligation of MCA (Chen et al. 2006). Accordingly, a chronic administration (10-day pre-ischemic) of the A₃ agonist N(6)-(3-iodobenzyl)-adenosine-5'-N-methylcarboxamide (IB-MECA) reduced ischemic damage after global forebrain ischemia in the gerbil (von Lubitz et al. 1994b), and pretreatment with a selective A₃ agonist, 1-[2-Chloro-6[[[3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-N-methyl-β-D-ribofuranuronamide] (Cl-IB-MECA), intracerebroventricularly or repeatedly intravenously administered before MCA ligation decreased the size of infarction-induced by transient MCAo (Chen et al. 2006).

Under in vitro OGD (5 min), selective activation of adenosine A₃ receptors by a brief (5 min) application of IB-MECA brings about an inhibition of excitatory neurotransmission on cortical neurons (Hentschel et al. 2003), and application of the selective A₃ receptor antagonist, 3-propyl-6-ethyl-5-[(ethylthio)carbonyl]-2-phenyl-4-propyl-3-pyridine carboxylate (MRS1523), before a brief (2 min) OGD reduces the OGD-induced depression of fEPSP in the CA1 hippocampal area (Pugliese et al. 2007). These findings indicated an inhibitory role of A₃ receptors on synaptic transmission during brief OGD periods and have suggested that A₃ receptors have a synergistic role with A₁ receptors in decreasing synaptic transmission, thus sustaining the neuroprotective effect of A₁ receptors.

On the other hand, when hippocampal slices are submitted to a severe (7-min) OGD, the selective antagonists of adenosine A₃ receptors abolish or delay the occurrence of AD and significantly protect from the irreversible disruption of neurotransmission caused by the severe ischemic episode in the CA1 region of rat hippocampal slices (Colotta et al. 2007, 2008, 2009; Poli et al. 2017; Pugliese et al. 2006, 2007). Depression of synaptic transmission following 15-min OGD was prevented by A₃ receptor antagonists also in the CA3 hippocampal area (Dennis et al. 2011).

To explain results above reported, we should consider that rat cortical neurons exposed to hypoxia in vitro show an increase in activation of protein kinase C (PKC) after selective adenosine A₃ receptor stimulation (Nieber and Hentschel 2006). If

OGD is applied long enough to be considered severe, PKC activation induced by adenosine A₃ receptor could account for an increase in intracellular calcium, which may participate in increasing tissue excitability and thus lead to irreversible synaptic failure. Thus while initially after OGD, massive excitotoxicity may be controlled by adenosine A₃ receptors, later the ensuing cascade of cytotoxic events could be potentiated by prolonged adenosine A₃ receptor stimulation. Moreover ischemia-induced plasticity of A₃ receptors might be relevant to explain the A₃ agonist effects in ischemia. A desensitization of A₃ receptors might account for the effect of a long application (before and during OGD) of CI-IB-MECA and of new selective A₃ agonists (Volpini et al. 2002, 2007) that like A₃ antagonists protect from the depression of synaptic activity brought about by prolonged OGD and delay the appearance of AD in the CA1 region of rat hippocampal slices (Pugliese et al. 2007).

A₃ receptor mRNA has been identified in mouse astrocytes, in microglia, and in oligodendrocytes. In human D384 astrocytoma cells, CI-IB-MECA at relatively low concentration (0.8 μM) reduced ATP depletion and apoptosis caused by hypoxic conditions (Bjorklund et al. 2008). Primary astrocytes prepared from adenosine A₃ receptor KO mice were more affected by hypoxia than those prepared from WT mice (Bjorklund et al. 2008). In cultured murine astrocytes, stimulation of A₃ receptors decreases HIF-1 expression induced by LPS under hypoxic conditions (Gessi et al. 2013), leading to inhibition of genes involved in inflammation injury (Gessi et al. 2013). In the *in vivo* model of transient MCAo, IB-MECA administered after ischemia proved to decrease the intensity of reactive gliosis involving microglia and astrocytes as evaluated 7 days after ischemia (von Lubitz et al. 1996).

Besides being localized on central cells, adenosine A₃ receptors are also localized on blood cells (Gessi et al. 2013). The state of the art about the role of adenosine A₃ receptors in inflammatory responses appears conflicting because exposure of blood peripheral cell lines to selective adenosine A₃ receptor agonists results in both anti- and pro-inflammatory effects (Borea et al. 2009). Choi et al. (2011) have demonstrated that treatment with 2-chloro-N(6)-(3-iodobenzyl)-5'-N-methylcarbamoyl-4'-thioadenosine (LJ529), a selective A₃ agonist administered by intraperitoneal injection 2 and 7 h after transient MCAo, markedly reduced cerebral ischemic injury 24 h thereafter. LJ529 also prevented the infiltration of monocytes and migration of microglia occurring after MCAo. A₃ receptor agonists can mediate their protective effects via anti-inflammatory signaling (inhibition of pro-inflammatory cytokines) and/or concomitant inhibition of innate immune cell trafficking because of A₃ receptor desensitization (Butler et al. 2012).

As adenosine A_{2A} receptor, also A₃ receptors are upregulated in lymphocytes obtained from patients affected by chronic autoimmune inflammatory rheumatic diseases, i.e., rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis (Ravani et al. 2017; Varani et al. 2011) raising the possibility to exploit adenosine A_{2A} and A₃ receptors as therapeutic targets to limit the inflammatory responses.

A₃ agonists, under clinical evaluation for the treatment of inflammatory diseases and cancer, demonstrated excellent safety and efficacy (Fishman et al. 2012).

Overall, results raise the question of the time-related utility of A₃ receptor antagonists/agonists for treatment of ischemia. It may be speculated, that after ischemia,

a prolonged treatment with adenosine A_3 receptor agonists protects first by reducing glutamate-mediated excitotoxicity and later on after ischemia, by desensitizing central A_3 receptors and via anti-inflammatory effects mediated by A_3 receptors on blood cells.

14.3 Conclusions

Information up to now acquired indicate that adenosine receptors located on any cell type of the brain and on vascular and blood cells partake in either salvage or demise of the tissue after a stroke. They thus represent important targets for drugs having different therapeutic time windows after stroke.

One of the prime adaptive mechanisms in response to hypoxia-ischemia is the cellular activation of adenosine A_1 receptors which inhibits excessive excitatory synaptic transmission. At the same time but, on the contrary, adenosine A_{2A} and A_{2B} receptors contribute to excessive excitotoxicity. Unfortunately the use of selective A_1 agonists is hampered by undesirable peripheral effects such as sedation, bradycardia, and hypotension. Early neuroprotective strategies with antagonists of adenosine A_2 receptors would be aimed at targeting the brain parenchyma to antagonize excitotoxicity and ensuing production of harmful molecular events responsible for acute brain damage.

In the hours and days after ischemia, adenosine A_{2A} , A_{2B} , and A_3 receptors peripherally located on vascular and blood cells may be the targets of drugs aimed at dampening vascular adhesion signals and neuroinflammation.

Overall, a therapeutic strategy with adenosine receptor antagonists/agonists should be carefully evaluated in terms of time after ischemia due to the balance of central versus peripheral adenosine receptor-mediated effects over time after ischemia. Besides early neuroprotective strategies with A_{2A} and A_{2B} receptor antagonists, strategies aimed at targeting events in a longer time window of days/weeks after ischemia appear promising in antagonizing inflammation and neurovascular protection and promoting neuroplasticity and neurogenesis. Considering that tPA is routinely used after ischemic stroke to dissolve intravascular clots, most recent data indicate that A_{2B} receptor agonists, by providing neurovascular protection, might be a promising strategy against BBB damage and permeability and for decreasing the risk of hemorrhages after stroke.

Compounds active at adenosine receptors are drugs under development and already exist in therapy or in clinical experimentation for other indications; some of them could enter in a reasonable time in clinical trials for stroke. Still there is urgent need of novel compounds to be developed with higher selectivity, oral bioavailability, stability in vivo, longer half-life, and better capability to cross the BBB.

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References

- Adén U, Lindström K, Bona E et al (1994) Changes in adenosine receptors in the neonatal rat brain following hypoxic ischemia. *Brain Res Mol Brain Res* 23:354–358
- Adén U, Halldner L, Lagercrantz H et al (2003) Aggravated brain damage after hypoxic ischemia in immature adenosine A_{2A} knockout mice. *Stroke* 34:739–744
- Baltos JA, Gregory KJ, White PJ et al (2016) Quantification of adenosine A₁ receptor biased agonism: implications for drug discovery. *Biochem Pharmacol* 99:101–112
- Barone FC, Irving EA, Ray AM et al (2001) Inhibition of p38 mitogen-activated protein kinase provides neuroprotection in cerebral focal ischemia. *Med Res Rev* 21:129–145
- Bjorklund O, Shang M, Tonazzini I et al (2008) Adenosine A₁ and A₃ receptors protect astrocytes from hypoxic damage. *Eur J Pharmacol* 596:6–13
- Bona E, Adén U, Gilland E et al (1997) Neonatal cerebral hypoxia-ischemia: the effect of adenosine receptor antagonists. *Neuropharmacology* 36:1327–1338
- Borea PA, Gessi S, Bar-Yehuda S et al (2009) A₃ adenosine receptor: pharmacology and role in disease. *Handb Exp Pharmacol* 193:297–327
- Borea PA, Gessi S, Merighi S et al (2016) Adenosine as a multi-Signalling Guardian angel in human diseases: when, where and how does it exert its protective effects? *Trends Pharmacol Sci* 37:419–434
- Burnstock G, Boeynaems JM (2014) Purinergic signalling and immune cells. *Purinergic Signal* 10:529–564
- Butler M, Sanmugalingam D, Burton VJ et al (2012) Impairment of adenosine A₃ receptor activity disrupts neutrophil migratory capacity and impacts innate immune function in vivo. *Eur J Immunol* 42:3358–3368
- Chen GJ, Harvey BK, Shen H et al (2006) Activation of adenosine A₃ receptors reduces ischemic brain injury in rodents. *J Neurosci Res* 84:1848–1855
- Chen F, Qi Z, Luo Y et al (2014) Non-pharmaceutical therapies for stroke: mechanisms and clinical implications. *Prog Neurobiol* 115:246–269
- Cheng P, Ren Y, Bai S et al (2015) Chronic cerebral ischemia induces Downregulation of A₁ adenosine receptors during white matter damage in adult mice. *Cell Mol Neurobiol* 35:1149–1156
- Choi DW (1990) Possible mechanisms limiting N-methyl-D-aspartate receptor overactivation and the therapeutic efficacy of N-methyl-D-aspartate antagonists. *Stroke* 21:III20–III22
- Choi IY, Lee JC, Ju C et al (2011) A₃ adenosine receptor agonist reduces brain ischemic injury and inhibits inflammatory cell migration in rats. *Am J Pathol* 179:2042–2052
- Coelho JE, Rebola N, Fragata I et al (2006) Hypoxia-induced desensitization and internalization of adenosine A₁ receptors in the rat hippocampus. *Neuroscience* 138:1195–1203
- Colotta V, Catarzi D, Varano F et al (2007) New 2-arylpyrazolo[3,4-c]quinoline derivatives as potent and selective human A₃ adenosine receptor antagonists. Synthesis, pharmacological evaluation, and ligand-receptor modeling studies. *J Med Chem* 50:4061–4074
- Colotta V, Catarzi D, Varano F et al (2008) Synthesis, ligand-receptor modeling studies and pharmacological evaluation of novel 4-modified-2-aryl-1,2,4-triazolo[4,3-a]quinoxalin-1-one derivatives as potent and selective human A₃ adenosine receptor antagonists. *Bioorg Med Chem* 16:6086–6102
- Colotta V, Lenzi O, Catarzi D et al (2009) Pyrido[2,3-e]-1,2,4-triazolo[4,3-a]pyrazin-1-one as a new scaffold to develop potent and selective human A₃ adenosine receptor antagonists. Synthesis, pharmacological evaluation, and ligand-receptor modeling studies. *J Med Chem* 52:2407–2419
- Cooper DM, Mons N, Karpen JW (1995) Adenylyl cyclases and the interaction between calcium and cAMP signalling. *Nature* 374:421–424
- Coppi E, Cellai L, Maraula G et al (2013) Adenosine A_{2A} receptors inhibit delayed rectifier potassium currents and cell differentiation in primary purified oligodendrocyte cultures. *Neuropharmacology* 73:301–310

- Coppi E, Cellai L, Maraula G et al (2015) Role of adenosine in oligodendrocyte precursor maturation. *Front Cell Neurosci* 9:155
- Corradetti R, Lo Conte G, Moroni F et al (1984) Adenosine decreases aspartate and glutamate release from rat hippocampal slices. *Eur J Pharmacol* 104:19–26
- Cristóvão-Ferreira S, Vaz SH, Ribeiro JA et al (2009) Adenosine A_{2A} receptors enhance GABA transport into nerve terminals by restraining PKC inhibition of GAT-1. *J Neurochem* 109:336–347
- Cui M, Bai X, Li T et al (2013) Decreased extracellular adenosine levels lead to loss of hypoxia-induced neuroprotection after repeated episodes of exposure to hypoxia. *PLoS One* 8:e57065
- Dale N, Pearson T, Fringuelli BG (2000) Direct measurement of adenosine release during hypoxia in the CA1 region of the rat hippocampal slice. *J Physiol* 526:143–155
- Daval JL, von Lubitz DK, Deckert J et al (1989) Protective effect of cyclohexyladenosine on adenosine A_1 -receptors, guanine nucleotide and forskolin binding sites following transient brain ischemia: a quantitative autoradiographic study. *Brain Res* 491:212–226
- Daval JL, Nicolas F (1994) Opposite effects of cyclohexyladenosine and theophylline on hypoxic damage in cultured neurons. *Neurosci Lett* 175:114–116
- Day YJ, Marshall MA, Huang L et al (2004) Protection from ischemic liver injury by activation of A_{2A} adenosine receptors during reperfusion: inhibition of chemokine induction. *Am J Physiol Gastrointest Liver Physiol* 286:G285–G293
- Dennis SH, Jaafari N, Cimarosti H et al (2011) Oxygen/glucose deprivation induces a reduction in synaptic AMPA receptors on hippocampal CA3 neurons mediated by mGluR1 and adenosine A_3 receptors. *J Neurosci* 31:11941–11952
- Di Paola R, Melani A, Esposito E et al (2010) Adenosine A_{2A} receptor-selective stimulation reduces signaling pathways involved in the development of intestine ischemia and reperfusion injury. *Shock* 33:541–551
- Dias RB, Ribeiro JA, Sebastião AM (2012) Enhancement of AMPA currents and GluR1 membrane expression through PKA-coupled adenosine A_2A receptors. *Hippocampus* 22:276–291
- Dirnagl U, Iadecola C, Moskowitz MA (1999) Pathobiology of ischaemic stroke: an integrated view. *Trends in Neuroscience* 22:391–397
- Dirnagl U (2012) Pathobiology of injury after stroke: the neurovascular unit and beyond. *Ann N Y Acad Sci* 285:39–45
- Dixon AK, Gubitza AK, Sirinathsinghji DJ et al (1996) Tissue distribution of adenosine receptor mRNAs in the rat. *Br J Pharmacol* 118:1461–1468
- Domenici MR, de Carolis AS, Sagratella S (1996) Block by N6-L-phenylisopropyladenosine of the electrophysiological and morphological correlates of hippocampal ischaemic injury in the gerbil. *Br J Pharmacol* 118:1551–1557
- Duan W, Gui L, Zhou Z et al (2009) Adenosine A_{2A} receptor deficiency exacerbates white matter lesions and cognitive deficits induced by chronic cerebral hypoperfusion in mice. *J Neurol Sci* 285:39–45
- Dunwiddie TV (1984) Interactions between the effects of adenosine and calcium on synaptic responses in rat hippocampus *in vitro*. *J Physiol* 350:545–559
- Dux E, Fastbom J, Ungerstedt U et al (1990) Protective effect of adenosine and a novel xanthine derivative propentofylline on the cell damage after bilateral carotid occlusion in the gerbil hippocampus. *Brain Res* 516:248–256
- Eckle T, Faigle M, Grenz A et al (2008) A_{2B} adenosine receptor dampens hypoxia-induced vascular leak. *Blood* 111:2024–2035
- Eltzschig HK, Thompson LF, Karhausen J et al (2004) Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: coordination by extracellular nucleotide metabolism. *Blood* 104:3986–3992
- Eltzschig HK, Bonney SK, Eckle T (2013) Attenuating myocardial ischemia by targeting A_{2B} adenosine receptors. *Trends Mol Med* 19:345–354

- Eusemann TN, Willmroth F, Fiebich B et al (2015) Adenosine receptors differentially regulate the expression of regulators of G-protein Signalling (RGS) 2, 3 and 4 in astrocyte-like cells. *PLoS One* 11:e0134934
- Evans MC, Swan JH, Meldrum BS (1987) An adenosine analogue, 2-chloroadenosine, protects against long term development of ischaemic cell loss in the rat hippocampus. *Neurosci Lett* 83:287–292
- Fedorova IM, Jacobson MA, Basile A et al (2003) Behavioral characterization of mice lacking the A₃ adenosine receptor: sensitivity to hypoxic neurodegeneration. *Cell Mol Neurobiol* 23:431–447
- Feoktistov I, Ryzhov S, Zhong H et al (2004) Hypoxia modulates adenosine receptors in human endothelial and smooth muscle cells toward an A_{2B} angiogenic phenotype. *Hypertension* 44:649–654
- Fishman P, Bar-Yehuda S, Liang BT et al (2012) Pharmacological and therapeutic effects of A₃ adenosine receptor agonists. *Drug Discov Today* 17:359–366
- Fredholm BB, IJzerman AP, Jacobson KA et al (2011) International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptor—an update. *Pharmacol Rev* 63:1–34
- Frenguelli BG, Wigmore G, Llaudet E et al (2007) Temporal and mechanistic dissociation of ATP and adenosine release during ischaemia in the mammalian hippocampus. *J Neurochem* 101:1400–1413
- Fusco I, Coppi E, Dettori I et al (2017) The selective block of adenosine A_{2B} receptors protects synaptic transmission from damage induced by oxygen and glucose deprivation in the CA1 rat hippocampus. *Purinergic Signalling* 13:1–53
- Gao Y, Phillis JW (1994) CGS15943, an adenosine A₂ receptor antagonist, reduces cerebral ischemic injury in the Mongolian gerbil. *Life Sci* 55:PL61–PL65
- Gaviano L, Fusco I, Coppi E et al (2017) The selective block of adenosine A_{2B} receptors prevents neuronal death in CA1 hippocampus after oxygen glucose deprivation. *Purinergic Signalling* 13:1–53
- Genovese T, Melani A, Esposito E et al (2010) Selective adenosine a(2A) receptor agonists reduce the apoptosis in an experimental model of spinal cord trauma. *J Biol Regul Homeost Agents* 24:73–86
- Gessi S, Varani K, Merighi S et al (2000) A(2A) adenosine receptors in human peripheral blood cells. *Br J Pharmacol* 129:2–11
- Gessi S, Varani K, Merighi S et al (2005) Expression, pharmacological profile, and functional coupling of A_{2B} receptors in a recombinant system and in peripheral blood cells using a novel selective antagonist radioligand, [3H]MRE 2029-F20. *Mol Pharmacol* 67:2137–2147
- Gessi S, Merighi S, Varani K et al (2008) The A₃ adenosine receptor: an enigmatic player in cell biology. *Pharmacol Ther* 117:123–140
- Gessi S, Merighi S, Stefanelli A et al (2013) A₁ and A₃ adenosine receptors inhibit LPS-induced hypoxia-inducible factor-1 accumulation in murine astrocytes. *Pharmacol Res* 76:157–170
- Gonçalves FQ, Pires J, Pliassova A et al (2015) Adenosine A_{2B} receptors control A₁ receptor-mediated inhibition of synaptic transmission in the mouse hippocampus. *Eur J Neurosci* 41:878–888
- Greene RW, Haas HL (1991) The electrophysiology of adenosine in the mammalian central nervous system. *Prog Neurobiol* 36:329–341
- Grenz A, Osswald H, Eckle T et al (2008) The Reno-vascular A_{2B} adenosine receptor protects the kidney from ischemia. *PLoS Med* 5:e137
- Gu L, Huang B, Shen W et al (2013) Early activation of nSMase2/ceramide pathway in astrocytes is involved in ischemia-associated neuronal damage via inflammation in rat hippocampi. *J Neuroinflammation* 10:109
- Gui L, Duan W, Tian H et al (2009) Adenosine A_{2A} receptor deficiency reduces striatal glutamate outflow and attenuates brain injury induced by transient focal cerebral ischemia in mice. *Brain Res* 1297:185–193

- Hagberg H, Andersson P, Lacarewicz J et al (1987) Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia. *J Neurochem* 49:227–231
- Haskó G, Linden J, Cronstein B et al (2008) Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nat Rev Drug Discov* 7:759–770
- Hentschel S, Lewerenz A, Nieber K (2003) Activation of A_3 receptors by endogenous adenosine inhibits synaptic transmission during hypoxia in rat cortical neurons. *Restor Neurol Neurosci* 21:55–63
- Heron A, Lekieffre D, Le Peillet E (1994) Effects of an A_1 adenosine receptor agonist on the neurochemical, behavioral and histological consequences of ischemia. *Brain Res* 641:217–224
- Higashi H, Meno JR, Marwaha AS et al (2002) Hippocampal injury and neurobehavioral deficits following hyperglycemic cerebral ischemia: effect of theophylline and ZM241385. *J Neurosurg* 96:117–126
- Hinz S, Lacher SK, Seibt BF et al (2014) BAY60-6583 acts as a partial agonist at adenosine A_{2B} receptors. *J Pharmacol Exp Ther* 349:427–436
- Hu S, Dong H, Zhang H et al (2012) Noninvasive limb remote ischemic preconditioning contributes neuroprotective effects via activation of adenosine A_1 receptor and redox status after transient focal cerebral ischemia in rats. *Brain Res* 1459:81–90
- Iadecola C, Anrather J (2011) Stroke research at a crossroad: asking the brain for directions. *Nat Neurosci* 14:1363–1368
- Impellizzeri D, Di Paola R, Esposito E et al (2011) CGS 21680, an agonist of the adenosine (A_{2A}) receptor, decreases acute lung inflammation. *Eur J Pharmacol* 668:305–316
- Jacobson KA, Von Lubitz DK, Daly JW et al (1996) Adenosine receptor ligands: differences with acute versus chronic treatment. *Trends Pharmacol Sci* 17:108–113
- Jinka TR, Combs VM, Drew KL (2015) Translating drug-induced hibernation to therapeutic hypothermia. *ACS Chem Neurosci* 6:899–904
- Johansson B, Halldner L, Dunwiddie TV et al (2001) Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A_1 receptor. *Proc Natl Acad Sci U S A* 98:9407–9412
- Jones PA, Smith RA, Stone TW (1998) Protection against kainate-induced excitotoxicity by adenosine A_{2A} receptor agonists and antagonists. *Neuroscience* 85:229–237
- Jurewicz A, Matysiak M, Andrzejak S et al (2006) TRAIL-induced death of human adult oligodendrocytes is mediated by JNK pathway. *Glia* 53:158–166
- Kitagawa H, Mori A, Shimada J et al (2002) Intracerebral adenosine infusion improves neurological outcome after transient focal ischemia in rats. *Neurol Res* 24:317–323
- Koeppen M, Eckle T, Eltzschig HK (2011) Interplay of hypoxia and A_{2B} adenosine receptors in tissue protection. *Adv Pharmacol* 61:145–186
- Kuan CY, Whitmarsh AJ, Yang DD et al (2003) A critical role of neural-specific JNK3 for ischemic apoptosis. *Proc Natl Acad Sci U S A* 100:15184–15189
- Kurino M, Fukunaga K, Ushio Y et al (1995) Activation of mitogen-activated protein kinase in cultured rat hippocampal neurons by stimulation of glutamate receptors. *J Neurochem* 65:1282–1289
- Latini S, Bordoni F, Corradetti R et al (1998) Temporal correlation between adenosine outflow and synaptic potential inhibition in rat hippocampal slices during ischemia-like conditions. *Brain Res* 794:325–328
- Latini S, Bordoni F, Corradetti R et al (1999) Effect of A_{2A} adenosine receptor stimulation and antagonism on synaptic depression induced by in vitro ischaemia in rat hippocampal slices. *Br J Pharmacol* 128:1035–1044
- Latini S, Pedata F (2001) Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *J Neurochem* 79:463–484
- Lee KS, Tetzlaff W, Kreutzberg GW (1986) Rapid down regulation of hippocampal adenosine receptors following brief anoxia. *Brain Res* 380:155–158

- Lee KS, Lowenkopf T (1993) Endogenous adenosine delays the onset of hypoxic depolarization in the rat hippocampus *in vitro* via an action at A_1 receptors. *Brain Res* 609:313–315
- Li Y, Oskouian RJ, Day YJ et al (2006) Mouse spinal cord compression injury is reduced by either activation of the adenosine A_{2A} receptor on bone marrow-derived cells or deletion of the A_{2A} receptor on non-bone marrow-derived cells. *Neuroscience* 141:2029–2039
- Li Q, Han X, Lan X et al (2017) Inhibition of tPA-induced hemorrhagic transformation involves adenosine A_{2B} receptor activation after cerebral ischemia. *Neurobiol Dis* 108:173–182
- Liang R, Pang ZP, Deng P et al (2009) Transient enhancement of inhibitory synaptic transmission in hippocampal CA1 pyramidal neurons after cerebral ischemia. *Neuroscience* 160:412–418
- Liu S, Feng X, Jin R et al (2017) Tissue plasminogen activator-based nanothrombolysis for ischemic stroke. *Expert Opin Drug Deliv* 28:1–12
- Lopes LV, Cunha RA, Kull B et al (2002) Adenosine $a(2A)$ receptor facilitation of hippocampal synaptic transmission is dependent on tonic $a(1)$ receptor inhibition. *Neuroscience* 112:319–329
- Lopes LV, Sebastião AM, Ribeiro JA (2011) Adenosine and related drugs in brain diseases: present and future in clinical trials. *Curr Top Med Chem* 11:1087–1101
- Ma N, Liu XW, Yang YJ et al (2016) Evaluation on antithrombotic effect of aspirin eugenol ester from the view of platelet aggregation, hemorheology, TXB2/6-keto-PGF1 α and blood biochemistry in rat model. *BMC Vet Res* 12:108
- Macrez R, Ali C, Toutirais O et al (2011) Stroke and the immune system: from pathophysiology to new therapeutic strategies. *Lancet Neurol* 10:471–480
- Maraula G, Traini C, Mello T et al (2013) Effects of oxygen and glucose deprivation on synaptic transmission in rat dentate gyrus: role of A_{2A} adenosine receptors. *Neuropharmacology* 67:511–520
- Marcoli M, Raiteri L, Bonfanti A et al (2003) Sensitivity to selective adenosine A_1 and A_{2A} receptor antagonists of the release of glutamate induced by ischemia in rat cerebrocortical slices. *Neuropharmacology* 45:201–210
- Martire A, Pepponi R, Tebano MT et al (2016) Neuroprotective potential of adenosine A_1 receptor partial agonists in *ex vivo* and *in vitro* experimental models of ischemia. Communication to the annual meeting of the Italian Purine Club. Rome, January 15
- Matsumoto K, Graf R, Rosner G et al (1992) Flow thresholds for extracellular purine catabolite elevation in cat focal ischemia. *Brain Res* 579:309–314
- Mayne M, Fotheringham J, Yan HJ et al (2001) Adenosine A_{2A} receptor activation reduces proinflammatory events and decreases cell death following intracerebral hemorrhage. *Ann Neurol* 49:727–735
- Mazzon E, Esposito E, Impellizzeri D et al (2011) CGS 21680, an agonist of the adenosine (A_{2A}) receptor, reduces progression of murine type II collagen-induced arthritis. *J Rheumatol* 38:2119–2129
- Melani A, Pantoni L, Corsi C et al (1999) Striatal outflow of adenosine, excitatory amino acids, gamma-aminobutyric acid, and taurine in awake freely moving rats after middle cerebral artery occlusion: correlations with neurological deficit and histopathological damage. *Stroke* 30:2448–2455
- Melani A, Pantoni L, Bordoni F et al (2003) The selective A_{2A} receptor antagonist SCH58261 reduces striatal transmitter outflow, turning behavior and ischemic brain damage induced by permanent focal ischemia in the rat. *Brain Res* 959:243–250
- Melani A, Gianfriddo M, Vannucchi MG et al (2006) The selective A_{2A} receptor antagonist SCH58261 protects from neurological deficit, brain damage and activation of p38 MAPK in rat focal cerebral ischemia. *Brain Res* 1073-1074:470–480
- Melani A, Cipriani S, Vannucchi MG et al (2009) Selective adenosine A_{2A} receptor antagonism reduces JNK activation in oligodendrocytes after cerebral ischaemia. *Brain* 132:1480–1495
- Melani A, Corti F, Stephan H et al (2012) Ecto-ATPase inhibition: ATP and adenosine release under physiological and ischemic *in vivo* conditions in the rat striatum. *Exp Neurol* 233:193–204
- Melani A, Corti F, Cellai L et al (2014) Low doses of the selective adenosine A_{2A} receptor agonist CGS21680 are protective in a rat model of transient cerebral ischemia. *Brain Res* 1551:59–72

- Melani A, Dettori I, Corti F et al (2015) Time-course of protection by the selective A_{2A} receptor antagonist SCH58261 after transient focal cerebral ischemia. *Neurol Sci* 36:1441–1448
- Milton SL, Nayak G, Kesaraju S et al (2007) Suppression of reactive oxygen species production enhances neuronal survival *in vitro* and *in vivo* in the anoxia-tolerant turtle *Trachemys scripta*. *J Neurochem* 101:993–1001
- Mishiro K, Ishiguro M, Suzuki Y et al (2012) A broad-spectrum matrix metalloproteinase inhibitor prevents hemorrhagic complications induced by tissue plasminogen activator in mice. *Neuroscience* 205:39–48
- Mizuma A, Yenari MA (2017) Anti-inflammatory targets for the treatment of reperfusion injury in stroke. *Front Neurol* 8:467
- Mohamed RA, Agha AM, Abdel-Rahman AA et al (2016) Role of adenosine A_{2A} receptor in cerebral ischemia reperfusion injury: signaling to phosphorylated extracellular signal-regulated protein kinase (pERK1/2). *Neuroscience* 314:145–159
- Mori M, Nishizaki T, Okada Y (1992) Protective effect of adenosine on the anoxic damage of hippocampal slice. *Neuroscience* 46:301–307
- Moriyama K, Sitkovsky MV (2010) Adenosine A_{2A} receptor is involved in cell surface expression of A_{2B} receptor. *J Biol Chem* 285:39271–39288
- Muzzi M, Blasi F, al MA (2013) Neurological basis of AMP-dependent thermoregulation and its relevance to central and peripheral hyperthermia. *J Cereb Blood Flow Metab* 33:183–190
- Nieber K, Hentschel S (2006) Signalling pathways of adenosine A₃ receptors in rat cortical neurons. In: Proceedings of the 8th international symposium on adenosine and adenine nucleotides. Ferrara, May 24–28
- O'Regan MH, Simpson RE, Perkins LM et al (1992) The selective A₂ adenosine receptor agonist CGS 21680 enhances excitatory transmitter amino acid release from the ischemic rat cerebral cortex. *Neurosci Lett* 138:169–172
- Odashima M, Bamias G, Rivera-Nieves J et al (2005) Activation of A_{2A} adenosine receptor attenuates intestinal inflammation in animal models of inflammatory bowel disease. *Gastroenterology* 129:26–33
- Olsson T, Cronberg T, Rytter A et al (2004) Deletion of the adenosine A₁ receptor gene does not alter neuronal damage following ischaemia *in vivo* or *in vitro*. *Eur J Neurosci* 20:1197–1204
- Paterniti I, Melani A, Cipriani S et al (2011) Selective adenosine A_{2A} receptor agonists and antagonists protect against spinal cord injury through peripheral and central effects. *J Neuroinflammation* 8:31
- Pedata F, Latini S, Pugliese AM et al (1993) Investigations into the adenosine outflow from hippocampal slices evoked by ischemia-like conditions. *J Neurochem* 61:284–289
- Pedata F, Gianfriddo M, Turchi D et al (2005) The protective effect of adenosine A_{2A} receptor antagonism in cerebral ischemia. *Neurol Res* 27:169–174
- Pedata F, Pugliese AM, Coppi E et al (2007) Adenosine in the central nervous system: effects on neurotransmission and Neuroprotection. *Immunol Endocr Metab Agents Med Chem* 4:304–321
- Pedata F, Pugliese AM, Sebastião AM et al (2010) Adenosine A₃ receptor signaling in the central nervous system. In: Borea PA (ed) A₃ adenosine receptors from cell biology to pharmacology and therapeutics. Springer, Netherlands, pp 165–188
- Pedata F, Pugliese AM, Coppi E et al (2014) Adenosine A_{2A} receptors modulate acute injury and neuroinflammation in brain ischemia. *Mediat Inflamm* 2014:805198
- Perez-Buira S, Barrachina M, Rodriguez A et al (2007) Expression levels of adenosine receptors in hippocampus and frontal cortex in argyrophilic grain disease. *Neurosci Lett* 423:194–199
- Phillis JW (1995) The effect of selective A₁ and A_{2A} adenosine receptor antagonists on cerebral ischemic injury in the gerbil. *Brain Res* 705:79–84
- Phillis JW, Goshgarian HG (2001) Adenosine and neurotrauma: therapeutic perspectives. *Neurol Res* 23:183–189
- Pimentel VC, Moretto MB, Oliveira MC et al (2015) Neuroinflammation after neonatal hypoxia-ischemia is associated with alterations in the purinergic system: adenosine deaminase 1 isoenzyme is the most predominant after insult. *Mol Cell Biochem* 403:169–177

- Pinto-Duarte A, Coelho JE, Cunha RA et al (2005) Adenosine A_{2A} receptors control the extracellular levels of adenosine through modulation of nucleoside transporters activity in the rat hippocampus. *J Neurochem* 93:595–604
- Poli D, Falsini M, Varano F et al (2017) Imidazo[1,2-a]pyrazin-8-amine core for the design of new adenosine receptor antagonists: structural exploration to target the A_3 and A_{2A} subtypes. *Eur J Med Chem* 125:611–628
- Popoli P, Pintor A, Domenici MR et al (2002) Blockade of striatal adenosine A_{2A} receptor reduces, through a presynaptic mechanism, quinolinic acid-induced excitotoxicity: possible relevance to neuroprotective interventions in neurodegenerative diseases of the striatum. *J Neurosci* 22:1967–1975
- Popoli P, Pepponi R (2012) Potential therapeutic relevance of adenosine A_{2B} and A_{2A} receptors in the central nervous system. *CNS Neurol Disord Drug Targets* 11:664–674
- Pugliese AM, Latini S, Corradetti R et al (2003) Brief, repeated, oxygen-glucose deprivation episodes protect neurotransmission from a longer ischemic episode in the in vitro hippocampus. Role of adenosine receptors. *Br J Pharmacol* 140:305–314
- Pugliese AM, Coppi E, Spalluto G et al (2006) A_3 adenosine receptor antagonists delay irreversible synaptic failure caused by oxygen and glucose deprivation in the rat CA1 hippocampus in vitro. *Br J Pharmacol* 147:524–532
- Pugliese AM, Coppi E, Volpini R et al (2007) Role of adenosine A_3 receptors on CA1 hippocampal neurotransmission during oxygen-glucose deprivation episodes of different duration. *Biochem Pharmacol* 74:768–779
- Pugliese AM, Traini C, Cipriani S et al (2009) The adenosine A_{2A} receptor antagonist ZM241385 enhances neuronal survival after oxygen-glucose deprivation in rat CA1 hippocampal slices. *Br J Pharmacol* 157:818–830
- Ravani A, Vincenzi F, Bortoluzzi A et al (2017) Role and function of A_{2A} and A_3 adenosine receptors in patients with ankylosing spondylitis, psoriatic arthritis and rheumatoid arthritis. *Int J Mol Sci* 18(4):pii:E697
- Rebola N, Lujan R, Cunha RA et al (2008) Adenosine A_{2A} receptors are essential for long-term potentiation of NMDA-EPSCs at hippocampal mossy fiber synapses. *Neuron* 57:121–134
- Rivkees SA, Zhao Z, Porter G et al (2001) Influences of adenosine on the fetus and newborn. *Mol Genet Metab* 74:160–171
- Rudolph KA, Keil M, Fastbom J et al (1989) Ischaemic damage in gerbil hippocampus is reduced following upregulation of adenosine A_1 receptors by caffeine treatment. *Neurosci Lett* 103:275–280
- Schmidt B, Roberts RS, Davis P et al (2007) Long-term effects of caffeine therapy for apnea of prematurity. *N Engl J Med* 357:1893–1902
- Sciotti VM, Roche FM, Grabb MC et al (1992) Adenosine receptor blockade augments interstitial fluid levels of excitatory amino acids during cerebral ischemia. *J Cereb Blood Flow Metab* 12:646–655
- Sebastião AM, de Mendonca A, Moreira T et al (2001) Activation of synaptic NMDA receptors by action potential-dependent release of transmitter during hypoxia impairs recovery of synaptic transmission on reoxygenation. *J Neurosci* 21:8564–8571
- Sebastião AM, Ribeiro JA (2009) Triggering neurotrophic factor actions through adenosine A_{2A} receptor activation: implications for neuroprotection. *Br J Pharmacol* 158:15–22
- Sheardown MJ, Knutsen LJS (1996) Unexpected neuroprotection observed with the adenosine A_{2A} receptor agonist CGS21680. *Drug Dev Res* 39:108–114
- Somjen GG (2001) Mechanisms of spreading depression and hypoxic spreading depression-like depolarization. *Physiol Rev* 81:1065–1096
- Sousa JB, Diniz C (2017) The adenosinergic system as a therapeutic target in the vasculature: new ligands and challenges. *Molecules* 22:pii: E752
- Stockwell J, Chen Z, Niazi M et al (2016) Protein phosphatase role in adenosine A_1 receptor-induced AMPA receptor trafficking and rat hippocampal neuronal damage in hypoxia/reperfusion injury. *Neuropharmacology* 102:254–265

- Stockwell J, Jakova E, Cayabyab FS (2017) Adenosine A₁ and A_{2A} receptors in the brain: current research and their role in neurodegeneration. *Molecules* 22:676
- Sufianova GZ, Sufianov AA, Shapkin AG (2014) Effect of cyclopentyladenosine on lipid peroxidation during focal cerebral ischemia. *Bull Exp Biol Med* 157:228–230
- Svenningsson P, Nomikos GG, Ongini E et al (1997) Antagonism of adenosine A_{2A} receptors underlies the behavioural activating effect of caffeine and is associated with reduced expression of messenger RNA for NGFI-a and NGFI-B in caudate-putamen and nucleus accumbens. *Neuroscience* 79:753–764
- Trincavelli ML, Marroni M, Tuscano D et al (2004) Regulation of A_{2B} adenosine receptor functioning by tumour necrosis factor α in human astroglial cells. *J Neurochem* 91:1180–1190
- Trincavelli ML, Melani A, Guidi S et al (2008) Regulation of A_{2A} adenosine receptor expression and functioning following permanent focal ischemia in rat brain. *J Neurochem* 104:479–490
- Tupone D, Madden CJ, Morrison SF (2013) Highlights in basic autonomic neurosciences: central adenosine A₁ receptor - the key to a hypometabolic state and therapeutic hypothermia? *Auton Neurosci* 176:1–2
- Tupone D, Cetas JS, Morrison SF (2016) Hibernation, hypothermia and a possible therapeutic "shifted homeostasis" induced by central activation of A₁ adenosine receptor (A₁AR). *Nihon Shinkei Seishin Yakurigaku Zasshi* 36:51–54
- Turner CP, Seli M, Ment L et al (2003) A₁ adenosine receptors mediate hypoxia-induced ventriculomegaly. *Proc Natl Acad Sci U S A* 100:11718–11722
- Tuttolomondo A, Di Sciacca R, Di Raimondo D et al (2009) Inflammation as a therapeutic target in acute ischemic stroke treatment. *Curr Top Med Chem* 9:1240–1260
- Varani K, Padovan M, Vincenzi F et al (2011) A_{2A} and A₃ adenosine receptor expression in rheumatoid arthritis: upregulation, inverse correlation with disease activity score and suppression of inflammatory cytokine and metalloproteinase release. *Arthritis Res Ther* 13:R197
- Volpini R, Costanzi S, Lambertucci C et al (2002) N-(6-alkyl-2-alkynyl derivatives of adenosine as potent and selective agonists at the human adenosine A₃ receptor and a starting point for searching A_{2B} ligands. *J Med Chem* 45:3271–3279
- Volpini R, Dal Ben D, Lambertucci C et al (2007) N⁶-methoxy-2-alkynyladenosine derivatives as highly potent and selective ligands at the human A₃ adenosine receptor. *J Med Chem* 50:1222–1230
- von Lubitz DK, Dambrosia JM, Kemposki O et al (1988) Cyclohexyl adenosine protects against neuronal death following ischemia in the CA1 region of gerbil hippocampus. *Stroke* 19:1133–1139
- von Lubitz DK, Marangos PJ (1990) Cerebral ischemia in gerbils: Postischemic administration of cyclohexyl adenosine and 8-sulfophenyl-theophylline. *J Mol Neurosci* 2:53–59
- von Lubitz DK, Lin RC, Melman N et al (1994a) Chronic administration of selective adenosine A₁ receptor agonist or antagonist in cerebral ischemia. *Eur J Pharmacol* 256:161–167
- von Lubitz DK, Lin RC, Popik P et al (1994b) Adenosine A₃ receptor stimulation and cerebral ischemia. *Eur J Pharmacol* 263:59–67
- von Lubitz DK, Lin RC, Jacobson KA (1995) Cerebral ischemia in gerbils: effects of acute and chronic treatment with adenosine A_{2A} receptor agonist and antagonist. *Eur J Pharmacol* 287:295–302
- von Lubitz DK, Lin RC, Paul JA et al (1996) Postischemic administration of adenosine amine congener (ADAC): analysis of recovery in gerbils. *Eur J Pharmacol* 316:171–179
- Wang G, Dinkins M, He Q et al (2012) Astrocytes secrete exosomes enriched with proapoptotic ceramide and prostate apoptosis response 4 (PAR-4): potential mechanism of apoptosis induction in Alzheimer disease (AD). *J Biol Chem* 287:21384–21395
- Wei W, Du C, Lv J et al (2013) Blocking A_{2B} adenosine receptor alleviates pathogenesis of experimental autoimmune encephalomyelitis via inhibition of IL-6 production and Th17 differentiation. *J Immunol* 190:138–146
- Winerdal M, Winerdal ME, Wang YQ et al (2016) Adenosine A₁ receptors contribute to immune regulation after neonatal hypoxic ischemic brain injury. *Purinergic Signal* 12:89–101

- Xu J, Guo S, Jia Z et al (2013) Additive effect of prostaglandin E2 and adenosine in mouse experimental autoimmune encephalomyelitis. *Prostaglandins Other Lipid Mediat* 100-101:30–35
- Yang D, Zhang Y, Nguyen HG et al (2006) The A_{2B} adenosine receptor protects against inflammation and excessive vascular adhesion. *J Clin Invest* 116:1913–1923
- Yang D, Chen H, Koupenova M et al (2010) A new role for the A_{2b} adenosine receptor in regulating platelet function. *J Thromb Haemost* 8:817–827
- Yang ZJ, Wang B, Kwansa H et al (2013) Adenosine A_{2A} receptor contributes to ischemic brain damage in newborn piglet. *J Cereb Blood Flow Metab* 33:1612–1620
- Yang L, Chen X, Wang S et al (2015) N2 extenuates experimental ischemic stroke through platelet aggregation inhibition. *Thromb Res* 136:1310–1317
- Yang Q, Guo M, Wang X et al (2017) Ischemic preconditioning with a ketogenic diet improves brain ischemic tolerance through increased extracellular adenosine levels and hypoxia-inducible factors. *Brain Res* 1667:11–18
- Yun J, Li J, Zuo Z (2014) Transferred inter-cell ischemic preconditioning-induced neuroprotection may be mediated by adenosine A₁ receptors. *Brain Res Bull* 103:66–71
- Zamani M, Soleimani M, Golab F et al (2013) NeuroProtective effects of adenosine receptor agonist coadministration with ascorbic acid on CA1 hippocampus in a mouse model of ischemia reperfusion injury. *Metab Brain Dis* 28:367–374
- Zhang RL, Chopp M, Chen H et al (1994) Temporal profile of ischemic tissue damage, neutrophil response, and vascular plugging following permanent and transient (2H) middle cerebral artery occlusion in the rat. *J Neurol Sci* 125:3–10
- Zhou JG, Meno JR, Hsu SS et al (1994) Effects of theophylline and cyclohexyladenosine on brain injury following normo- and hyperglycemic ischemia: a histopathologic study in the rat. *J Cereb Blood Flow Metab* 14:166–173