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Adenosine A_{2A} receptor blockade attenuates excitotoxicity in rat striatal medium spiny neurons during an ischemic-like insult

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From the Contents

Abstract

During brain ischemia, excitotoxicity and peri-infarct depolarization injuries occur and cause cerebral tissue damage. Indeed, anoxic depolarization, consisting of massive neuronal depolarization due to the loss of membrane ion gradients, occurs *in vivo* or *in vitro* during an energy failure. The neuromodulator adenosine is released in huge amounts during cerebral ischemia and exerts its effects by activating specific metabotropic receptors, namely: A_1, A_{2A}, A_{2B}, and A_3. The A_{2A} receptor subtype is highly expressed in striatal medium spiny neurons, which are particularly susceptible to ischemic damage. Evidence indicates that the A_{2A} receptors are upregulated in the rat striatum after stroke and the selective antagonist SCH58261 protects from exaggerated glutamate release within the first 4 hours from the insult and alleviates neurological impairment and histological injury in the following 24 hours. We recently added new knowledge to the mechanisms by which the adenosine A_{2A} receptor subtype participates in ischemia-induced neuronal death by performing patch-clamp recordings from medium spiny neurons in rat striatal brain slices exposed to oxygen and glucose deprivation. We demonstrated that the selective block of A_{2A} receptors by SCH58261 significantly reduced ionic imbalance and delayed the anoxic depolarization in medium spiny neurons during oxygen and glucose deprivation and that the mechanism involves voltage-gated K^+ channel modulation and a presynaptic inhibition of glutamate release by the A_{2A} receptor antagonist. The present review summarizes the latest findings in the literature about the possibility of developing selective ligands of A_{2A} receptors as advantageous therapeutic tools that may contribute to counteracting neurodegeneration after brain ischemia.

Key Words: adenosine A_{2A} receptors; anoxic depolarization; brain ischemia; glutamate excitotoxicity; medium spiny neurons; oxygen and glucose deprivation

Introduction

Stroke is the second highest cause of death and a leading cause of disability worldwide (Paul and Candelario-Jalil, 2021) but few therapeutic options are available (Campbell et al., 2019; Kuriakose and Xiao, 2020) and the only Food and Drug Administration-approved drug is the thrombolytic enzyme tissue plasminogen activator to be administered within the first 4 hours from the insult (Henderson et al., 2018). However, neurodegeneration occurs hours and days after the primary event and the lack of treatments able to counteract neuronal loss has prompted research towards those agents able to inhibit glutamate-induced excitotoxicity and consequent neurodegeneration. Loss of normal neuronal signaling capacity and substantial neuronal depolarization, named anoxic depolarization (AD), occurs during stroke in the ischemic core, due to energy failure and consequent loss of ion gradients (Kalia et al., 2021), which gives rise to overwhelming neurotransmitter release. This condition, together with the fact that the glutamate transporters reverse transport direction during hypoxia/ischemia (Rossi et al., 2000), leading to extracellular glutamate overload and excessive activation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl D-aspartate receptors, causes exaggerated neuronal depolarization and Ca^{2+} influx. On the whole, these phenomena bring about the initiation of repetitive waves of depolarization, the AD (see Obeidat and Andrew, 1998) propagating from the ischemic core to the neighboring "penumbral" areas, not only during acute infarction but even later, hours or days after the event (Binder et al., 2022). Whether ADs occur, and their timing after the insult, are crucial factors determining the extent of tissue damage and the severity of neurological impairment (Ayata, 2018). Hence, inhibiting or delaying the appearance of this hypoxia/ischemia-induced damage is considered an advantageous strategy to reduce neuronal loss after stroke.

Search Strategy

This narrative review was compiled by using "PubMed" with sources within the last 5 years, with an emphasis on the most recent, novel, and comprehensive papers. If the topic did not have relevant information within the last 5 years, we used the most recent paper. Due to the strict limit of 50–100 references, we could not cite all of the relevant publications.

Adenosine and Brain Ischemia: Role of A_{2A} Receptors

The neuromodulator adenosine is largely released during an ischemic episode and may activate four adenosine-sensitive metabotropic receptors: A_1, A_{2A}, A_{2B}, and A_3. The activation of A_1 receptors (A_1Rs) is known to be neuroprotective during brain ischemia (Muzzi et al., 2013; Liu et al., 2019; Chen et al., 2021) but, unfortunately, A_1R agonists are not devoid of important side effects, e.g., bradycardia and sedation (Deb et al., 2019). Hence, research has focused on the other three adenosine receptor subtypes, in particular A_{2A}Rs, which are Gs-coupled receptors located either at pre- or post-synaptic sites on neurons in many brain areas, included the hippocampus (Cunha et al., 1994) and the striatum (Ferrè et al., 2023), as well as on astrocytes (Matos et al., 2012). Striatal adenosine, released during brain ischemia, and consequent A_{2A}R activation are crucial players in mediating neuronal damage induced by energy failure (Ganesana and Venton, 2018). Indeed, evidence indicates that the selective A_{2A}R antagonist, SCH58261, acutely (5 minutes) or subchronically (5 minutes, 6, and 20 hours) administered in the *in vivo* rat model of permanent middle cerebral artery occlusion, is protective against excessive glutamate outflow, neurological deficit and brain damage evaluated 24 hours after the insult (for a review see: Pedata et al., 2014). However, the molecular mechanisms mediating this protective effect, as well as the possible involvement of other elements in A_{2A}R-mediated signaling such as voltage-dependent channels, are still unclear. Deeper knowledge is nevertheless available concerning a similar neuroprotective effect of A_{2A}R block in the rat hippocampus, where a model of brain ischemia, obtained *in vitro* by oxygen and glucose deprivation (OGD), demonstrated that A_{2A}R antagonism rescued hippocampal neurons from AD appearance and irreversible synaptic failure after an otherwise lethal OGD insult (Pugliese et al., 2009; Maraula et al.,

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2013). Importantly, it has been demonstrated, using *in vitro* rat primary cultured astrocytes or gliosomes (Matos et al., 2012) that prolonged A2AR activation by the selective agonist CGS21680 inhibits glutamate reuptake via a cyclic adenosine monophosphate and protein kinase A-dependent reduction of transcription of astrocytic glutamate transporters (excitatory amino acid transporters) which exert important neuroprotection in conditions of high energy demand (Todd and Hardi, 2020; Kovermann et al., 2022). Hence, based on these results, it appears that $A_{2A}R$ antagonism might prove neuroprotective in conditions of exaggerated glutamate release, such as brain hypoxia/ischemia, by promoting removal of this excitatory neurotransmitter from the extracellular space.

It is recognized that adenosine is released up to µM levels in the striatum during an ischemic insult (Coppi et al., 2021). In this brain region, $A_{2A}R$ activation enhances glutamate release, possibly by counteracting the inhibitory role of adenosine A_1Rs on neurotransmission (Ciruela et al., 2006). Similar results were found in the hippocampus, where the $A_{2A}R$ agonist CGS 21680 facilitated K^+-evoked glutamate release from synaptosomes only when applied in the presence of the A_1R agonist N^6-cyclopentyladenosine (Lopes et al., 2002). Accordingly, an in-depth investigation of $A_{2A}R$-mediated effects "per se" on neurotransmission confirmed the lack of efficacy of $A_{2A}R$ antagonists to modify basal synaptic transmission, whereas a clear inhibitory role is exerted by $A_{2A}R$ blockers on synaptic plasticity phenomena (Costenla et al., 2011). At variance with these observations, A_1Rs exert a "tonic" inhibition of hippocampal neurotransmission under basal (e.g., normoxic) conditions, as shown in the early 1980s by Dunwiddie and Hoffer, who demonstrated that adenosine deaminase, the enzyme which breaks down adenosine into inosine, significantly increased the amplitude of synaptic responses evoked in the CA1 area of rat hippocampal slices (Dunwiddie and Hoffer, 1980). In light of the above data, it is worth noting that $A_{2A}Rs$, which are sensitive to nM adenosine, are activated within the first minutes of an OGD event. Accordingly, the field excitatory post-synaptic potential decrease observed during an *in vitro* OGD, due to A1Rs activated by endogenous adenosine released during the insult, is significantly delayed in the presence of $A_{2A}R$ antagonists SCH58261 and SCH442416 (Pugliese et al., 2009). In addition, $A_{2A}R$ block also restored the number of newborn (5-bromo-20-deoxyuridine-positive: $BrdU^+$) neurons at 6 hours after OGD in the rat dentate gyrus (Maraula et al., 2013), attenuated CA1 neuronal and glial injury and inhibited astrocytosis (Pugliese et al., 2009).

We recently contributed to knowledge of $A_{2A}R$-mediated functions during energy failure in the brain by performing patch-clamp experiments from rat striatal slices in which an OGD was carried out, until the appearance of AD, in the absence or presence of different $A_{2A}R$ ligands or selective channel blockers (Coppi and Gibb, 2022). By this paradigm, we evaluated the effect of $A_{2A}R$ activation or blockade during energy failure and the respective contribution of different ion channels to AD timing. The results suggest that $A_{2A}Rs$ contribute to OGD-induced neuronal injury since the receptor antagonist SCH58261 significantly delayed AD appearance, measured as an increase in holding current in voltage-clamped medium spiny neurons (MSNs) at-60 mV, and mitigated ionic imbalance across the cell membrane, measured as a shift in the neuron "zero current potential" (Erev) during a depolarizing voltage ramp protocol in the same cell (Coppi and Gibb, 2022). Concomitant spontaneous excitatory post-synaptic current (sEPSC) measurements in the same slice revealed that, within the first 5 minutes from OGD start (before the appearance of the anoxic depolarisation), the frequency of sEPSCs was significantly reduced, demonstrating a decrease in neurotransmitter (i.e., glutamate) release in control OGD slices. This effect can likely be ascribed to the well-known presynaptic inhibition of synaptic transmission mediated by A_1Rs when activated by endogenous adenosine released during an hypoxia/ischemia event. The OGD-induced sEPSC decrease persisted in the presence of the $A_{2A}R$ antagonist SCH58261 but, notably, it was completely abolished by the $A_{2A}R$ agonist CGS 21680 as well as by the K^+ channel blocker Ba^{2+} (Coppi and Gibb, 2022). These results suggest that, in conditions of energy challenge, $A_{2A}R$ activation is deleterious since it counteracts those protective mechanisms engaged by neurons to mitigate the effects of energy failure and these effects match what was observed on application of Ba^{2+} to block K^+ channels. Hence, we propose that $A_{2A}Rs$ contribute to OGD-induced neuronal damage by inhibiting K^+ channel opening and thus, facilitating glutamate release.

As electrophysiology is the favored method to study neuronal functionality, it is worth noting that there are aspects of neuronal activity during OGD, which may be useful to improve our understanding about cell excitability and, most importantly, over-excitability due to an OGD insult. As an example, neurons, either in slices or *in vivo*, might display hyperexcitability phenomena such as action potential (AP) bursts, and these episodes may signal a "border line" between reversible and irreversible functional changes (Karunasinghe and Lipski, 2013). It has long been known that hippocampal or cortical neurons experiencing energy deprivation might produce such a burst of APs which, if energy supply is not restored, is usually followed by irreversible depolarization and loss of function (Rossi et al., 2000). However, such phenomena, also reported during *in vivo* middle cerebral artery occlusion (Rasheed et al., 2022), do not always occur in *in vitro* brain tissue preparations even if experimental conditions are suitably reproduced, and the reason for that is still unknown. In our work, we made the same observation since most, but not all, MSNs investigated displayed spontaneous AP bursts just before AD appearance. An interesting aspect of this is that MSNs are GABAergic inhibitory neurons and so while MSNs have a powerful glutamatergic input from cortico-striatal afferents, MSN axon collaterals will tend to inhibit local neuronal activity. As stated above, the reason why some neurons show spontaneous hyperactivity before irreversible loss of ionic balance and some others do not is still under debate. However, it can be hypothesized that subtle changes in recording conditions, such as the deepness in the brain slice of the patched cell, which may influence the intensity of local changes in extracellular K^+ concentration, as well as differences in MSN sub-populations (e.g., we did not distinguish in our recording conditions, between D1-direct *vs*. D2-indirect pathway striatal MSNs) could generate the variation in susceptibility to AP burst firing during OGD. Whatever the reasons, we can adopt these events as indicators of overall cell responsiveness to excessive environmental stress. In our case, AP bursts (on average 7.2 Hz frequency) were observed around the 12^{th} minute of OGD in 67% (10 out of 15) of MSNs exposed to control OGD and lasted for approximately 15 seconds. In contrast, when OGD was carried out in the presence of the $A_{2A}R$ agonist CGS21680, almost all MSNs generated spontaneous AP bursts. This observation suggests that, in addition to enhancement of presynaptic glutamate release, the activation of $A_{2A}Rs$ may also promote neuronal excitability in other ways.

Finally, in order to describe the sequence of hypoxia/ischemia-induced events, we measured the timeline in the striatal brain slice of electrophysiological changes induced by the OGD in MSNs. This could be of help in delineating preferred molecular targets that might interrupt the sequence of cell failures during overwhelming energy loss. Among the parameters analyzed, we measured the increase in holding current at –60 mV as an index of AD appearance, the inflection point of Erev (the zero current potential) during a voltage ramp protocol, due to extracellular K^+ overload, and the decrease in Rm, due to overall K^+ conductance increase typical of hypoxic conditions (Obeidat and Andrew, 1998). Of note, the first of these events to be recorded, in our experimental conditions, was the decrease in R_m, appearing on average 7.6 minutes from OGD start. This means that the primary injury during an OGD insult is the opening of membrane ion channels and, possibly, not K^+ channels at the very beginning but more likely ligand-gated ion channels, e.g., GABA and glutamate-activated ionotropic receptors. Indeed, intense GABA release with associated fall in membrane resistance has been described during OGD in the hippocampus (Allen et al., 2004). The fact that K^+ channel opening may not be the primary event involved in R_m decrease during OGD is confirmed by: (i) the even larger and faster R_m decrease observed when OGD is performed in K^+ channel block by Ba^{2+} (2 mM) and (ii) the longer latency to achieve Erev inflection point (likely mostly due to extracellular K^+ increase) observed in control OGD slices (Coppi and Gibb, 2022).

Hence, on the basis of above data, the sequence of events taking place in striatal MSNs during an OGD insult (**Figure 1**) might be summarized as follows: (1) adenosine released at the beginning of the insult activates metabotropic A_1Rs which inhibit glutamate release reflected by a decrease in sEPSC frequency between 3–5 minutes OGD; (2) extracellular adenosine also activates presynaptic $A_{2A}Rs$ on neurons, thus inhibiting the A_1R-mediated reduction of glutamate release, as well as $A_{2A}Rs$ on astrocytes, thus inhibiting glutamate reuptake: these are key events that may concur in the participation of $A_{2A}Rs$ to brain hypoxic/ischemic damage; (3) as energy challenge persists, membrane ion gradients start to be compromised causing gradual neuronal depolarization which induces Ca^{2+} influx and overwhelming neurotransmitter release, included GABA causing activation of GABAA Cl^- channels that contribute to R_m decrease (after OGD for approximately 7.5 minutes); (4) at this point, a number of events take place in a short time: the decrease in sEPSC release is reversed (possibly by extracellular glutamate accumulation due to reversed transporter activity, which overrides adenosine A_1R-mediated inhibition of synaptic release) and E_{rev} shifts towards more positive values due to K^+ ion accumulation in the extracellular milieu (approximately 8 minutes from OGD start); (5) these events combine in eliciting AD appearance measured as a sudden increase in holding current at –60 mV (approximately 10 minutes from OPC start). This is the limiting point beyond which an OGD insult turns into irreversible neuronal damage.

To our knowledge, the main contribution of $A_{2A}Rs$ to this sequence of events is relevant during the central phases of the insult, as the $A_{2A}R$ antagonists reduce the magnitude of E_{rev} shift (point 3) and delay the appearance of AD (point 4) thus allowing MSNs to bear a longer energy deprivation insult before potentially undergoing irreversible cell impairment.

In conclusion, after an hypoxic/ischemic insult in the brain, neuronal damage results from a series of pathophysiological events evolving over time: a primary inhibitory (neuroprotective) role exerted by adenosine through A_1R activation is followed by an hyperexcitability period facilitated by $A_{2A}R$ activation in the striatum (where this adenosine receptor subtype is highly expressed), contributing to mechanisms of excitotoxicity and likely peri-infarct depolarization followed by initiation of secondary brain injury activation triggered by protracted neuroinflammation. Knowledge acquired up to now indicates that adenosine $A_{2A}Rs$ located on MSNs represent an important pharmacological target having an interesting therapeutic time window after stroke.

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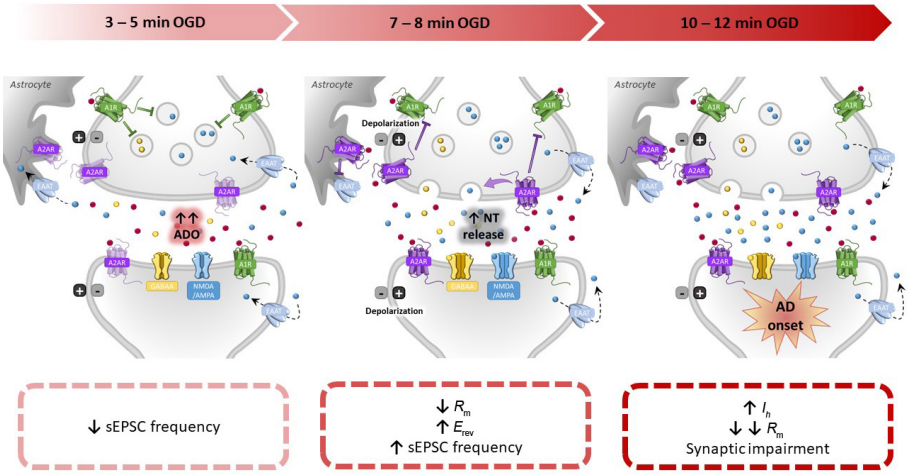

Figure 1 | Timeline of pathophysiological events estimated to occur during ischemia-induced neuronal damage in the striatum.
The cartoon is a schematic representation of neurochemical events occurring at striatal synapses at different times (expressed in minutes) after the onset of an OGD insult. Created with PowerPoint 97-2003. A1R: Adenosine A1 receptor; A2AR: adenosine A2A receptor; AD: anoxic depolarization; ADO: adenosine; AMPAR: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; EAAT: excitatory amino acid transporter; Erev: reversal potential of voltage ramp-evoked currents; GABAA: gamma-aminobutyric acid receptor A; I_h: holding current; NMDAR: N-methyl D-aspartate receptor; NT: neurotransmission; OGD: oxygen and glucose deprivation; R_m: membrane resistance; sEPSC: spontaneous excitatory post-synaptic currents.

Data availability statement: *Not applicable.*

References

Allen NJ, Rossi DJ, Attwell D (2004) Sequential release of GABA by exocytosis and reversed uptake leads to neuronal swelling in simulated ischemia of hippocampal slices. J Neurosci 24:3837-3849.

Ayata C (2018) Monitoring anoxic depolarization at the bedside: a step closer to the 24th century. J Cereb Blood Flow Metab 38:1123-1124.

Binder NF, Glück C, Middleham W, Alasoadura M, Pranculeviciute N, Wyss MT, Chuquet J, Weber B, Wegener S, El Amki M (2022) Vascular response to spreading depolarization predicts stroke outcome. Stroke 53:1386-1395.

Campbell BCV, De Silva DA, Macleod MR, Coutts SB, Schwamm LH, Davis SM, Donnan GA (2019) Ischaemic stroke. Nat Rev Dis Primers 5:70.

Chen ZH, Han YY, Shang YJ, Zhuang SY, Huang JN, Wu BY, Li CH (2021) Cordycepin ameliorates synaptic dysfunction and dendrite morphology damage of hippocampal CA1 via A1R in cerebral ischemia. Front Cell Neurosci 15:783478.

Ciruela F, Casadó V, Rodrigues RJ, Luján R, Burgueño J, Canals M, Borycz J, Rebola N, Goldberg SR, Mallol J, Cortés A, Canela EI, López-Giménez JF, Milligan G, Lluis C, Cunha RA, Ferré S, Franco R (2006) Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J Neurosci 26:2080-2087.

Costenla AR, Diógenes MJ, Canas PM, Rodrigues RJ, Nogueira C, Maroco J, Agostinho PM, Ribeiro JA, Cunha RA, de Mendonça A (2011) Enhanced role of adenosine A(2A) receptors in the modulation of LTP in the rat hippocampus upon ageing. Eur J Neurosci 34:12-21.

Coppi E, Dettori I, Cherchi F, Bulli I, Venturini M, Lana D, Giovannini MG, Pedata F, Pugliese AM (2020) A2B adenosine receptors: when outsiders may become an attractive target to treat brain ischemia or demyelination. Int J Mol Sci 21:9697.

Coppi E, Dettori I, Cherchi F, Bulli I, Venturini M, Pedata F, Pugliese AM (2021) New insight into the role of adenosine in demyelination, stroke and neuropathic pain. Front Pharmacol 11:625662.

Coppi E, Gibb AJ (2022) Selective block of adenosine A2A receptors prevents ischemic-like effects induced by oxygen and glucose deprivation in rat medium spiny neurons. Br J Pharmacol 179:4844-4856.

Cunha RA, Johansson B, Van der Ploeg I, Sebastião AM, Ribeiro JA, Fredholm BB (1994) Evidence for functionally important adenosine A2A receptors in the rat hippocampus. Brain Res 649:208-216.

Deb PK, Deka S, Borah P, Abed SN, Klotz KN (2019) Medicinal chemistry and therapeutic potential of agonists, antagonists and allosteric modulators of A1 adenosine receptor: current status and perspectives. Curr Pharm Des 25:2697-2715.

Dunwiddie TV, Hoffer BJ (1980) Adenine nucleotides and synaptic transmission in the in vitro rat hippocampus. Br J Pharmacol 69:59-68.

Ferré S, Sarasola LI, Quiroz C, Ciruela F (2023) Presynaptic adenosine receptor heteromers as key modulators of glutamatergic and dopaminergic neurotransmission in the striatum. Neuropharmacol 223:109329.

Ganesana M, Venton BJ (2018) Early changes in transient adenosine during cerebral ischemia and reperfusion injury. PLoS One 13:e0196932.

Henderson SJ, Weitz JI, Kim PY (2018) Fibrinolysis: strategies to enhance the treatment of acute ischemic stroke. J Thromb Haemost 16:1932-1940.

Hernández-González O, Hernández-Flores T, Prieto GA, Pérez-Burgos A, Arias-García MA, Galarraga E, Bargas J (2014) Modulation of Ca2+-currents by sequential and simultaneous activation of adenosine A1 and A 2A receptors in striatal projection neurons. Purinergic Signal 10:269-281.

Kalia M, Meijer HGE, van Gils SA, van Putten MJAM, Rose CR (2021) Ion dynamics at the energy-deprived tripartite synapse. PLoS Comput Biol 17:e1009019.

Karunasinghe RN, Lipski J (2013) Oxygen and glucose deprivation (OGD)-induced spreading depression in the Substantia Nigra. Brain Res 1527:209-221.

Kovermann P, Engels M, Müller F, Fahlke C (2022) Cellular physiology and pathophysiology of EAAT anion channels. Front Cell Neurosci 15:815279.

Kuriakose D, Xiao Z (2020) Pathophysiology and treatment of stroke: present status and future perspectives. Int J Mol Sci 21:7609.

Liu YJ, Chen J, Li X, Zhou X, Hu YM, Chu SF, Peng Y, Chen NH (2019) Research progress on adenosine in central nervous system diseases. CNS Neurosci Ther 25:899-910.

Lopes LV, Cunha RA, Kull B, Fredholm BB, Ribeiro JA (2002) Adenosine A(2A) receptor facilitation of hippocampal synaptic transmission is dependent on tonic A(1) receptor inhibition. Neurosci 112:319-329.

Maraula G, Traini C, Mello T, Coppi E, Galli A, Pedata F, Pugliese AM (2013) Effects of oxygen and glucose deprivation on synaptic transmission in rat dentate gyrus: role of A(2A) adenosine receptors. Neuropharmacol 67:511-520.

Matos M, Augusto E, Santos-Rodrigues AD, Schwarzschild MA, Chen JF, Cunha RA, Agostinho P (2012) Adenosine A2A receptors modulate glutamate uptake in cultured astrocytes and gliosomes. Glia 60:702-716.

Muzzi M, Coppi E, Pugliese AM, Chiarugi A (2013) Anticonvulsant effect of AMP by direct activation of adenosine A1 receptor. Exp Neurol 250:189-193.

Obeidat AS, Andrew RD (1998) Spreading depression determines acute cellular damage in the hippocampal slice during oxygen/glucose deprivation. Eur J Neurosci 10:3451-3461.

Paul S, Candelario-Jalil E (2021) Emerging neuroprotective strategies for the treatment of ischemic stroke: an overview of clinical and preclinical studies. Exp Neurol 335:113518.

Pedata F, Pugliese AM, Coppi E, Dettori I, Maraula G, Cellai L, Melani A (2014) Adenosine A2A receptors modulate acute injury and neuroinflammation in brain ischemia. Mediat Inflam doi: 10.1155/2014/805198.

Pugliese AM, Traini C, Cipriani S, Gianfriddo M, Mello T, Giovannini MG, Galli A, Pedata F (2009) The adenosine A2A receptor antagonist ZM241385 enhances neuronal survival after oxygen-glucose deprivation in rat CA1 hippocampal slices. Br J Pharmacol 157:818-830.

Rasheed W, Wodeyar A, Srinivasan R, Frostig RD (2022) Sensory stimulation-based protection from impending stroke following MCA occlusion is correlated with desynchronization of widespread spontaneous local field potentials. Sci Rep 12:1744.

Rossi DJ, Oshima T, Attwell D (2000) Glutamate release in severe brain ischaemia is mainly by reversed uptake. Nature 403:316-321.

Todd AC, Hardingham, GE (2020) The regulation of astrocytic glutamate transporters in health and neurodegenerative diseases. Int J Mol Sci 21:9607.

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