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

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https://doi.org/10.4103/1673-5374.375309

Date of submission: January 30, 2023
Date of decision: March 14, 2023
Date of acceptance: May 13, 2023
Date of web publication: May 31, 2023

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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-Jallo, 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 Obedat 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 ($A_1$Rs) is known to be neuroprotective during brain ischemia (Muzzi et al., 2013; Liu et al., 2019; Chen et al., 2021) but, unfortunately, $A_2A$Rs 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, including 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 Ventura, 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.,...
It is recognized that adenosine is released up to µM levels in the striatum during an ischemic insult (Coppi and Gibb, 2022). As electrophysiology is the preferred method to study neuronal functionality, it is a shift in the neuron “zero current potential” (Erev) during a depolarizing current step that provides a proxy of the field excitatory postsynaptic current (fEPSC) that can be measured as a sudden increase in holding current at –60 mV (approximately 8 mV in our recordings). This increase in holding current at –60 mV is caused by a decrease in neurotransmitter (i.e., glutamate) release from the extracellular space.

As adenosine receptor (A.R) activation is followed by an hyperexcitability period facilitated by A.R-mediated K+ channel opening and thus, facilitating glutamate release, it is worth noting that there are aspects of neuronal functionality that may be useful to improve our understanding about cell excitability and, most importantly, cell death. Apoptosis, as an example, is caused either in slices or in vivo, might display hypereexcitability phenomena such as action potential (AP) bursts, and these episodes may signal a “border line” between reversible and irreversible functional changes (Karunasinghe and Lipton, 2011). It is clear now known that hippocampal of the CA1 neurons will suffer cell death, 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 pathways in terms of evoked sEPSC). We measured a significant increase in holding current in voltage-clamped medium spiny neurons (MSNs) during the 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 12th minute of OGD in 28% (10 out of 35) of the neurons in the whole brain-OGD and lasted for approximately 15 seconds. In contrast, when OGD was carried out in the presence of the A.R agonist CGS21680, all almost MSNs generated spontaneous AP bursts. This observation suggests that, in addition to our previous study of presynaptic A.R-mediated release, the activation of A.Rs may also promote neuronal excitability in other ways.

In conclusion, after an ischemic/schismic insult in the brain, neuronal damage results from a series of pathophysiological events. Among these, the initial phase of the delayed primary inhibitory (neuroprotective) role exerted by adenosine through A.R activation is followed by a hypereexcitability period facilitated by A.R activation in the stratum (where this adenosine receptor subtype is highly expressed and contributes to excitatory neurotransmitter depletion followed by initiation of secondary brain injury activation triggered by protracted neuroinflammation. Knowledge acquired up to now indicates that adenosine A.Rs located on MSNs represent an important pharmacological target having an interesting therapeutic time window after stroke.

**Author contributions:** EC and A.JG conceived and wrote the manuscript and supported the research. FC revisited the manuscript and produced the Figure. All authors approved the final version of the manuscript.

**Conflicts of interest:** The authors declare no conflicts of interest.
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.

Data availability statement: Not applicable.

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