Epilepsy & Behavior 22 (2011) 207–213

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

# Differential neuroprotection by $A_1$ receptor activation and $A_{2A}$ receptor inhibition following pilocarpine-induced status epilepticus

Fernanda Elisa Rosim [a], Daniele Suzete Persike [a], Astrid Nehlig [b], Rebeca Padrão Amorim [a], Daniela Mara de Oliveira [c], Maria José da Silva Fernandes [a,*]

[a] Departamento de Neurologia e Neurocirurgia, Neurologia Experimental, Universidade Federal de São Paulo, São Paulo, Brazil
[b] INSERM U666; Faculty of Medicine, Strasbourg, France
[c] Departamento de Genética e Morfologia, Instituto de Ciências Biológicas, Universidade de Brasília, Brasilia, Brazil

ARTICLE INFO

*Article history:*
Received 2 May 2011
Revised 16 June 2011
Accepted 5 July 2011
Available online 17 August 2011

*Keywords:*
Adenosine
$A_{2A}$ receptor
SCH58261
$A_1$ receptor
Neuroprotection
Pilocarpine
Status epilepticus

ABSTRACT

Aiming at a better understanding of the role of $A_{2A}$ in temporal lobe epilepsy (TLE), we characterized the effects of the $A_{2A}$ antagonist SCH58261 (7-(2-phenylethyl)-5-amino-2(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*] pyrimidine) on seizures and neuroprotection in the pilocarpine model. The effects of SCH58261 were further analyzed in combination with the $A_1$ agonist R-Pia (*R*(−)-$N^6$-(2)-phenylisopropyl adenosine). Eight groups were studied: pilocarpine (Pilo), SCH + Pilo, R-Pia + Pilo, R-Pia + SCH + Pilo, Saline, SCH + Saline, R-Pia + Saline, and R-Pia + SCH + Saline. The administration of SCH58261, R-Pia, and R-Pia + SCH58261 prior to pilocarpine increased the latency to SE, and decreased either the incidence of or rate of mortality from SE compared with controls. Administration of R-Pia and R-Pia + SCH58261 prior to pilocarpine reduced the number of Fluoro-Jade B-stained cells in the hippocampus and piriform cortex when compared with control. This study showed that pretreatment with R-Pia and SCH58261 reduces seizure occurrence, although only R-Pia has neuroprotective properties. Further studies are needed to clarify the neuroprotective role of $A_{2A}$ in TLE.



## 1. Introduction

Temporal lobe epilepsy (TLE), the most prevalent type of epilepsy in humans, is characterized by the frequent occurrence of focal epileptiform discharges originating in the temporal region [1]. TLE is frequently associated with loss of pyramidal cells in the prosubiculum, CA1 and CA3 fields, and hilus of the dentate gyrus, as well as with mossy fiber reorganization in the hippocampal formation [2]. The TLE syndrome can be mimicked in rodents as the consequence of status epilepticus (SE) induced by such chemical agents as kainic acid and pilocarpine [3–5].

The pilocarpine model mimics several features of human TLE [4,5]. In this model, systemic administration of high doses of pilocarpine (300–380 mg/kg) generates limbic motor seizures and status epilepticus (SE), which can last up to 12 hours (acute phase). A latent phase, characterized by a seizure-free period lasting approximately 14 days after SE, precedes a chronic phase. The chronic phase is characterized by the occurrence of spontaneous seizures [6]. The pilocarpine model has been widely used in studies exploring antiepileptic agents that either reduce or block neuronal death caused by SE.

Adenosine is a neuromodulator that exerts neuroprotective and anticonvulsant effects in several experimental models of epilepsy, including the pilocarpine model [7,8]. Adenosine is considered an important target for the treatment of epilepsy, but has many adverse effects when administered systemically [9]. Adenosine can either inhibit or facilitate synaptic transmission through $A_1$ or $A_{2A}$ receptors, respectively. By activating $A_1$ receptors at presynaptic terminals, adenosine can inhibit excitatory amino acid release [10] and block seizures [8–13]. At the postsynaptic level, the inhibitory effect of the $A_1$ receptor is mediated by the activation of $K^+$ channels and increases in the efflux of $K^+$, which lead to hyperpolarization of postsynaptic neurons [14–16]. On the other hand, the activation of presynaptic $A_{2A}$ receptors by high concentrations of extracellular adenosine generated by ATP catabolism during high-frequency stimulation results in an increase in glutamate release [14,17]. The $A_{2A}$ pharmacological blockade has been used as a neuroprotective strategy in animal models of ischemia, Parkinson's disease, and Alzheimer's disease [18–22], but little is known about the role of $A_{2A}$ receptors in TLE. Aiming at a better understanding of the role of $A_{2A}$ in TLE, we characterized the effects of blocking $A_{2A}$ receptors with the antagonist SCH58261 (7-(2-phenylethyl)-5-amino-2(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine) on seizure modulation and neuroprotection of the brain areas vulnerable to the injury provoked by pilocarpine. The effects of SCH58261 were further analyzed in combination with the activation of the $A_1$ receptors by the agonist R-Pia

* Corresponding author at: Departamento de Neurologia e Neurocirurgia, Neurologia Experimental, Universidade Federal de São Paulo, Rua Pedro de Toledo, 781–6° andar, CEP 04039–032, São Paulo, Brazil. Fax: +55 11 5573 9304.
*E-mail address:* fernandes.nexp@epm.br (M.J.S. Fernandes).

1525-5050 
doi:10.1016/j.yebeh.2011.07.004

($R(-)N^6$-(2)-phenylisopropyl adenosine), an effective anticonvulsant and neuroprotective molecule in the pilocarpine model [8,12].

## 2. Methods

### 2.1. Animals

Adult male Wistar rats weighing approximately 250 g were housed under standard controlled conditions (12-hour light/12-hour dark cycle, 20–22 °C, 45–55% humidity), with food and water ad libitum. All animal procedures were conducted in accordance with national and international guidelines (Guidelines of the Brazilian College of Animal Experimentation, COBEA; *NIH Guide for Care and Use of Laboratory Animals*) and approved by the ethics committee of the university (CEP: 0052/08).

### 2.2. Pilocarpine protocol

Pilocarpine hydrochloride (360 mg/kg, Sigma) was administered intraperitoneally 20 minutes after subcutaneous injection of methylscopolamine (ME) (1 mg/kg, Sigma). ME was used to minimize the peripheral consequences of pilocarpine such as diarrhea, piloerection, olfactory and gustatory automatisms associated with salivation, eye blinking, vibrissa twitching, and yawning, which usually start 5–10 minutes after pilocarpine injection [5]. To standardize the different experimental groups, in all animals receiving pilocarpine, pretreated or not with the adenosinergic agents, SE was stopped at 6 hours by the simultaneous injection of diazepam (10 mg/kg, ip) and pentobarbital (30 mg/kg, ip) [see 23]. Only animals that reached stage 5 of Racine's scale [24] and experienced 6 hours of SE were included in the epileptic group examined 24 hours and 7 days later. Control groups received the same doses of diazepam and pentobarbital, 6 hours after saline injection, and were studied at similar times.

### 2.3. Pharmacological treatments

The adenosinergic $A_1$ receptor agonist R-Pia (Sigma) was dissolved in dimethylsulfoxide (DMSO, Sigma) and saline, at a ratio of 1:5 (v/v), and a dose of 0.025 mg/kg was given intraperitoneally 15 minutes prior to pilocarpine or saline [8]. 8-*p*-Sulfophenyl theophylline (8pSPT), an adenosinergic receptor antagonist that does not cross the blood–brain barrier and is used to reduce peripheral effects of adenosine, was given intraperitoneally at the dose of 1.5 mg/kg, simultaneously with R-Pia, but using different syringes [13]. A pilot study performed to test the effect of 8pSPT in the pilocarpine model showed that at this dose, 8pSPT altered neither seizure pattern nor hippocampal neurodegeneration in comparison with rats that received only pilocarpine. The adenosinergic $A_{2A}$ receptor antagonist SCH58261 (Tocris Bioscience), dissolved in DMSO/saline (1:5, v/v), was administered intraperitoneally at the dose of 0.2 mg/kg 5 minutes prior to pilocarpine or saline [25]. The injection volume was 1 mL/kg body wt. Control animals were given an equivalent volume of the vehicle or saline at the respective time.

Eight groups of rats were studied: Saline ($N=5$) group rats were injected with saline following DMSO/saline injection; Pilo ($N=43$) group rats were treated with pilocarpine following DMSO/saline injection; R-Pia + Saline ($N=5$) animals were treated with saline following co-administration of R-Pia and 8pSPT; R-Pia + Pilo ($N=80$) animals were treated with pilocarpine following R-Pia + 8pSPT injection; SCH + Saline ($N=5$) animals were treated with saline following SCH58261 injection; SCH + Pilo ($N=95$) animals were treated with pilocarpine following SCH58261 injection; R-Pia + SCH + Saline ($N=5$) animals were treated with saline after co-administration of R-Pia, 8pSPT, and SCH58261 given simultaneously; R-Pia + SCH + Pilo ($N=94$) animals were treated with pilocarpine after co-administration of R-Pia, 8pSPT, and SCH58261.

### 2.4. Behavior

After pilocarpine treatment, latency to the onset of SE, number of animals displaying SE, and mortality rate were evaluated. SE was defined as continuous limbic motor seizures lasting more than 30 minutes [5].

### 2.5. Perfusion

Rats ($N=5$/group) from the eight groups (Pilo, R-Pia + Pilo, SCH + Pilo, R-Pia + SCH + Pilo, and the respective controls Saline, R-Pia + Saline, SCH + Saline; R-Pia + SCH + Saline) were perfused 24 hours and 7 days after SE onset or saline injection, for Fluoro-Jade B analysis. The animals were deeply anesthetized with a solution containing ketamine (80 mg/kg, ip) and xylazine (30 mg/kg, ip). They were fully heparinized before transcardiac perfusion–fixation with saline followed by 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4; 10 mL/min for 30 min) freshly depolymerized from paraformaldehyde salt. The brain tissue was allowed to fix in situ for 24 hours prior to removal from the skull to achieve full formaldehyde binding, then removed and kept in the same fixative solution (phosphate-buffered formaldehyde 1%) at 4 °C until being processed. The brains were sectioned at 40 μm in coronal slices with a vibratome.

### 2.6. Fluoro-Jade B staining

The Fluoro-Jade B (FJ-B) method was used to label dying and "suffering" neurons in brain slices. The brain sections were rinsed in distilled water, mounted on gelatin-coated slides, and immersed in a solution containing 1% sodium hydroxide in 80% ethanol for 5 minutes, then 70% ethanol for 2 minutes and distilled water for 2 minutes. The slides were then incubated in a solution of potassium permanganate 0.06% for 15 minutes and shaken gently on a rotating platform to ensure background suppression between sections. The slides were washed with distilled water for 2 minutes and then incubated with FJ-B staining solution (0.0004% FJ-B diluted in 0.1% acetic acid prepared 10 minutes before use) at room temperature for 30 minutes with gentle shaking. After staining, the slides were rinsed with distilled water, dried in a hot plate at 50 °C, dehydrated in ethanol, immersed in xylene, and mounted with Vecta Mount (Vector).

### 2.7. Cell counting

Cell counts were performed in two coronal slices spaced 400 μm apart and stained with FJ-B. FJ-B-positive cells were manually quantified in the dorsal hippocampus (pyramidal layer of CA3 and hilus), piriform cortex (3300–3800 μm posterior to bregma), and entorhinal cortex (4800–5300 μm posterior to bregma), defined according to the atlas of Paxinos and Watson [26].

Photomicrographs were captured with a PixeLink digital camera connected to a Nikon E600 microscope under a 40× objective and displayed on the 21-in. CRT monitor. A system test (a transparency containing 37×37-μm counting frames spaced 47 μm apart) was superimposed on the images. Each counting frame has two lines of inclusion and two of exclusion. All FJ-B-positive cells inside the frame were counted within the delimited fields, except those touching the exclusion lines. At the end, the numbers of cells were summed to obtain the mean total number of FJ-B-positive cells sampled in each area of interest. All stained cells were counted for each set of brain sections by an observer who was experimentally blinded to the treatment groups.

### 2.8. Statistical analysis

Statistical comparisons of latencies and numbers of FJ-B-stained cells (means ± SD) were performed with one-way analysis of variance (ANOVA) followed by adjustment of $P$ values with Bonferroni's post

**Table 1**
Influence of adenosinergic agents given prior to pilocarpine on behavioral parameters.

| Group | Number of animals | Occurrence of SE | Latency to SE (min) | Mortality following SE |
|---|---|---|---|---|
| Pilo | 43 | 72% | 42 ± 12 | 65% |
| SCH + Pilo | 95 | 32%[a] | 58 ± 6[b] | 37%[c] |
| R-Pia + Pilo | 80 | 20%[a] | 74 ± 10[b] | 31%[c] |
| R-Pia + SCH + Pilo | 94 | 14%[a] | 80 ± 7[b] | 31%[c] |

*Note*. Behavioral parameters, occurrence of status epilepticus (SE), latency, and mortality were evaluated after pretreatment with SCH58261 (SCH) and R-Pia given prior to pilocarpine (360 mg/kg, ip). Pretreatment with the adenosinergic agents, injected alone or in combination, reduced the occurrence of SE and delayed the latency to seizure onset, when compared with pilocarpine or vehicle treatment of rats, and decreased mortality in rats experiencing SE in each treated group.

[a] $P<0.001$, statistically significantly different from the Pilo group ($\chi^2$ test).

[b] $P<0.0001$, statistically significantly different from the Pilo group (ANOVA, Bonferroni's test).

[c] $P<0.05$, statistically significantly different from the Pilo group ($\chi^2$ test).

hoc test. A $\chi^2$ test was used to assess treatment effects on incidence of SE, defined as the number of animals with or without identified seizures, and mortality ratio, defined as the number of animals that died following SE. $P<0.05$ was considered significant.

## 3. Results

### 3.1. Behavior

On administration of pilocarpine + vehicle (N = 43), the majority of the animals (72%) displayed SE within an average of 42 ± 12 minutes and with a mortality rate of 65% (Table 1). Pretreatment with R-Pia and SCH58261, given alone or simultaneously, prior to pilocarpine, diminished the convulsant effect of pilocarpine. Consequently, an increased number of rats were required in the studied group.

Of 95 rats treated with SCH58261 prior to pilocarpine, only 32% displayed SE with a latency of 58 ± 6 minutes and a mortality rate of 37%. Co-administration of R-Pia prior to pilocarpine ($N = 80$) resulted in 20% of rats displaying SE after a latency of 74 ± 10 minutes; the mortality rate in this group was 31%. Finally, in the R-Pia + SCH + Pilo group ($N = 94$), 14% of animals displayed SE after a latency of 80 ± 7 minutes and the mortality rate was 31%. These data are summarized in Table 1 and indicate that administration of the $A_1$ agonist R-Pia and the $A_{2A}$ antagonist SCH58261, prior to pilocarpine, increased the resistance of rats to the convulsant effect of pilocarpine.

### 3.2. Evaluation of neuronal degeneration

Positive staining of cells by FJ-B indicates neuronal degeneration. We did not observe any FJ-B-positive cells in the brain areas of the control groups studied 24 hours and 7 days after injection of R-Pia or SCH58261 given prior to saline. However, we observed FJ-B-positive cells in CA3, hilus of the dentate gyrus, amygdala, entorhinal cortex, and piriform cortex, 24 hours and 7 days after the onset of SE, in all experimental groups that received pilocarpine (Table 2).

Twenty-four hours after SE, there was no statistically significant difference in the number of FJ-B-positive cells in any region between the Pilo and SCH + Pilo groups. By 24 hours, pretreatment with R-Pia or R-Pia + SCH58261 led to a statistically significant reduction in the number of FJ-B-positive cells in CA3 ($P<0.001$), hilus of the dentate gyrus ($P<0.01$), and piriform cortex ($P<0.001$) (Table 2, Fig. 1). However, there was no significant difference in the number of FJ-B-positive cells between the R-Pia + Pilo and R-Pia + SCH + Pilo groups (Table 2, Fig. 1).

Seven days after the onset of SE, the difference in the number of positive cells between the Pilo and SCH + Pilo groups was not statistically significant. Both the R-Pia and R-Pia + SCH58261 treatments reduced the number of FJ-B-positive cells in CA3 ($P<0.001$) and hilus of the dentate gyrus ($P<0.01$), in comparison to the pilocarpine-treated group (Fig. 2). In addition, there was no statistically significant difference in the number of FJ-B positive cells between the R-Pia + Pilo and R-Pia + SCH + Pilo groups (Table 2, Fig. 2).

**Table 2**
Number of Fluoro-Jade B-positive cells in rats studied 24 hours and 7 days following treatment with the $A_1$ receptor agonist or $A_{2A}$ receptor antagonist, given alone or at the same time, prior to pilocarpine.

| | CA3 | Hilus | Amygdala | Entorhinal cortex | Piriform cortex |
|---|---|---|---|---|---|
| *24 hours after SE* | | | | | |
| Pilo | 22 ± 2 | 31 ± 7 | 120 ± 8 | 47 ± 7 | 93 ± 15 |
| SCH + Pilo | 22 ± 3 | 29 ± 6 | 120 ± 9 | 44 ± 8 | 91 ± 16 |
| R-Pia + Pilo | 6 ± 1[a] | 11 ± 7[b] | 123 ± 4 | 45 ± 8 | 35 ± 2[a] |
| R-Pia + SCH + Pilo | 8 ± 1[a] | 12 ± 6[b] | 122 ± 11 | 43 ± 14 | 34 ± 4[a] |
| *7 days after SE* | | | | | |
| Pilo | 26 ± 5 | 23 ± 3 | 95 ± 12 | 36 ± 7 | 25 ± 10 |
| SCH + Pilo | 24 ± 5 | 26 ± 6 | 96 ± 12 | 33 ± 8 | 28 ± 11 |
| R-Pia + Pilo | 7 ± 4[a] | 11 ± 1[b] | 95 ± 12 | 34 ± 7 | 14 ± 2 |
| R-Pia + SCH + Pilo | 6 ± 6[a] | 11 ± 2[b] | 96 ± 13 | 33 ± 8 | 16 ± 8 |

*Note*. Values represent means ± SD. SCH, SCH58261; R-Pia, $R(-)$-$N^6$-(2)-phenylisopropyl adenosine; Pilo, pilocarpine.

[a] $P<0.001$, statistically significantly different from the Pilo group (ANOVA, Bonferroni's test).

[b] $P<0.01$, statistically significantly different from the Pilo group (ANOVA, Bonferroni's test).

## 4. Discussion

### 4.1. Seizure modulation

Adenosine, an endogenous regulator of hippocampal activity, can inhibit or stimulate synaptic transmission via $A_1$ or $A_{2A}$ receptors, respectively [10,14]. Although the antiepileptic properties of the $A_1$ receptor have been widely reported in scientific studies [9], relatively little is known concerning the role of $A_{2A}$ receptors in seizure modulation. The present investigation was undertaken to study this relationship further.

Both, $A_{2A}$ and $A_1$ receptors are present in the hippocampus on the glutamatergic terminals [14]. Stimulation of presynaptic $A_1$ receptors decreases glutamate release, whereas activation of presynaptic $A_{2A}$ receptors enhances neurotransmitter release [14]. In addition to their presynaptic location in hippocampal neurons, $A_{2A}$ receptors can be found postsynaptically, where they facilitate depolarization of the membrane [27]. Therefore, the anticonvulsive effect mediated by SCH58261 observed in this study could be due to a decrease in excitatory synaptic transmission in the hippocampus, a decrease in outflow of glutamate from the presynaptic terminal, or a decrease in postsynaptic membrane depolarization. Our findings are in agreement with other studies on the effect of $A_{2A}$ antagonists in experimental epilepsy models. Porciúncula et al. [28] found that SCH58261 is able to prevent seizures in the kainic acid model. Etherington and Frenguelli [29] showed that another antagonist ligand for the $A_{2A}$ receptor, ZM241385, reduces epileptiform activity in the hippocampus in vitro. Li and Henry [27] demonstrated that DMPX, another $A_{2A}$ antagonist, is able to block the depolarization effects provoked by CGS21680, an $A_{2A}$ agonist, in the hippocampus in in vitro assays. $A_{2A}$-deficient mice were not protected against seizures originating from brainstem structures, but displayed a significant reduction in the intensity of seizures as well as in the number of mice developing limbic seizures induced by pilocarpine [30]. On the other hand, previous studies by our group using the $A_{2A}$ antagonist DMPX given prior to pilocarpine showed that this $A_{2A}$ antagonist reduces the latency to the onset of seizures in the majority of animals and increases the number of apoptotic cells in the hippocampus of rats displaying SE [8]. These opposite effects of $A_{2A}$ antagonists, as

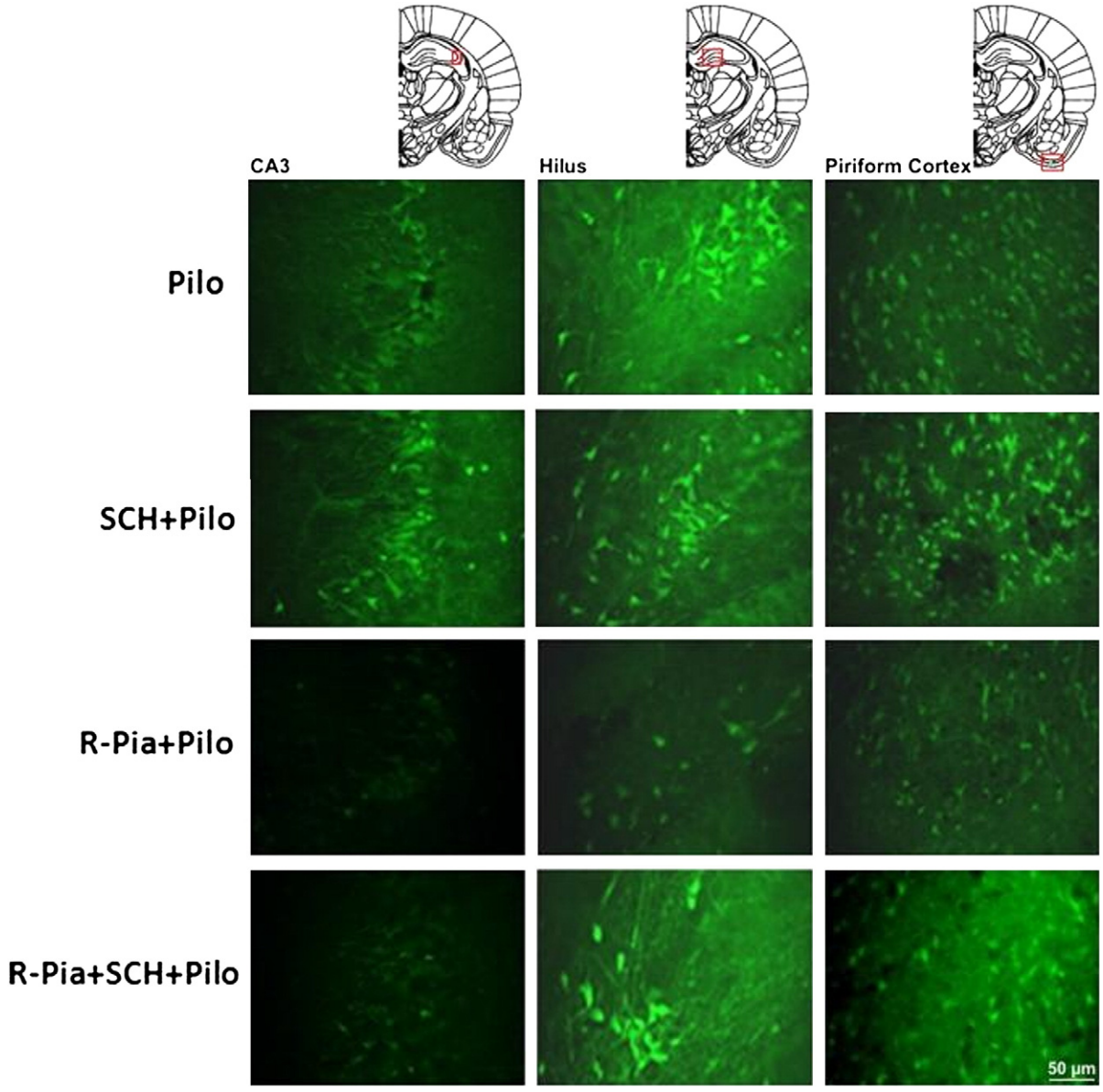


**Fig. 1.** Fluoro-Jade B histochemistry indicative of neuronal injury in rat brain areas (hippocampal formation and piriform cortex) of the pilocarpine (Pilo), SCH + Pilo, R-Pia + Pilo, and R-Pia + SCH + Pilo groups studied 24 hours after status epilepticus onset. Pretreatment with R-Pia and R-Pia + SCH58261 reduced the number of positive cells in CA3, hilus, and piriform cortex of rats. Similar staining was observed in the SCH + Pilo and Pilo groups. Scale bar: 50 µm.

compared with those described in the literature [21,25,31], could be related to the specificity, the dose used, and the different models.

In the present study, we also observed the anticonvulsive effect of R-Pia, an $A_1$ receptor agonist. This effect is in agreement with the current literature that describes the inhibitory effect of the $A_1$ receptor on ictal activity [8,9,11,13,32]. The hippocampus has high $A_1$ receptor density localized both pre- and postsynaptically [14]. Presynaptic activation of this receptor modulates excitatory synaptic transmission by decreasing glutamate release through the inhibition of voltage-dependent $Ca^{2+}$ channels. In parallel, postsynaptic activation reduces neuronal excitability by increasing membrane conductance to $K^+$ ions, thereby causing hyperpolarization of the cell [10,14,15]. Our results—significant reductions in the percentages of animals that exhibited SE after each pharmacological treatment and in mortality rates after the insult—are in agreement with the known effect of modulation of glutamatergic neurotransmission by $A_1$ and $A_{2A}$ adenosine receptors. The latter effect leads to reduced sensitivity to pilocarpine and less deleterious consequences of SE translating into lower mortality rates after pilocarpine-induced seizures.

### *4.2. Neuroprotection of brain areas that are vulnerable to injury*

Analysis of the pattern of FJ-B staining in animals treated with pilocarpine demonstrated substantial neurodegeneration in CA3, hilus of the dentate gyrus, basolateral amygdala, entorhinal cortex, and piriform cortex, 24 hours and 7 days after the insult. FJ-B has been used as a fluorescence marker for neurodegeneration caused by both pilocarpine and lithium–pilocarpine. Fabene et al. [33] found a large number of FJ-B-positive neurons in the hippocampus 8 and 24 hours after SE induced with pilocarpine [33]. Voutsinos-Porche et al. [34] reported the presence of FJ-B-positive cells in several brain regions analyzed in the acute (12 and 24 hours after the SE) and latent (3 and 6 days after SE) phases of the lithium–pilocarpine model. The neuronal death observed in the pilocarpine model can be triggered by various mechanisms, especially excitotoxicity. Prolonged SE causes the activation of excitotoxic cascades in which $Ca^{2+}$ ions are key mediators. Once activated, these cascades lead to an excessive release of glutamate that acts on kainate, AMPA, and NMDA receptors, promoting a massive $Ca^{2+}$ influx into the cell that triggers activation

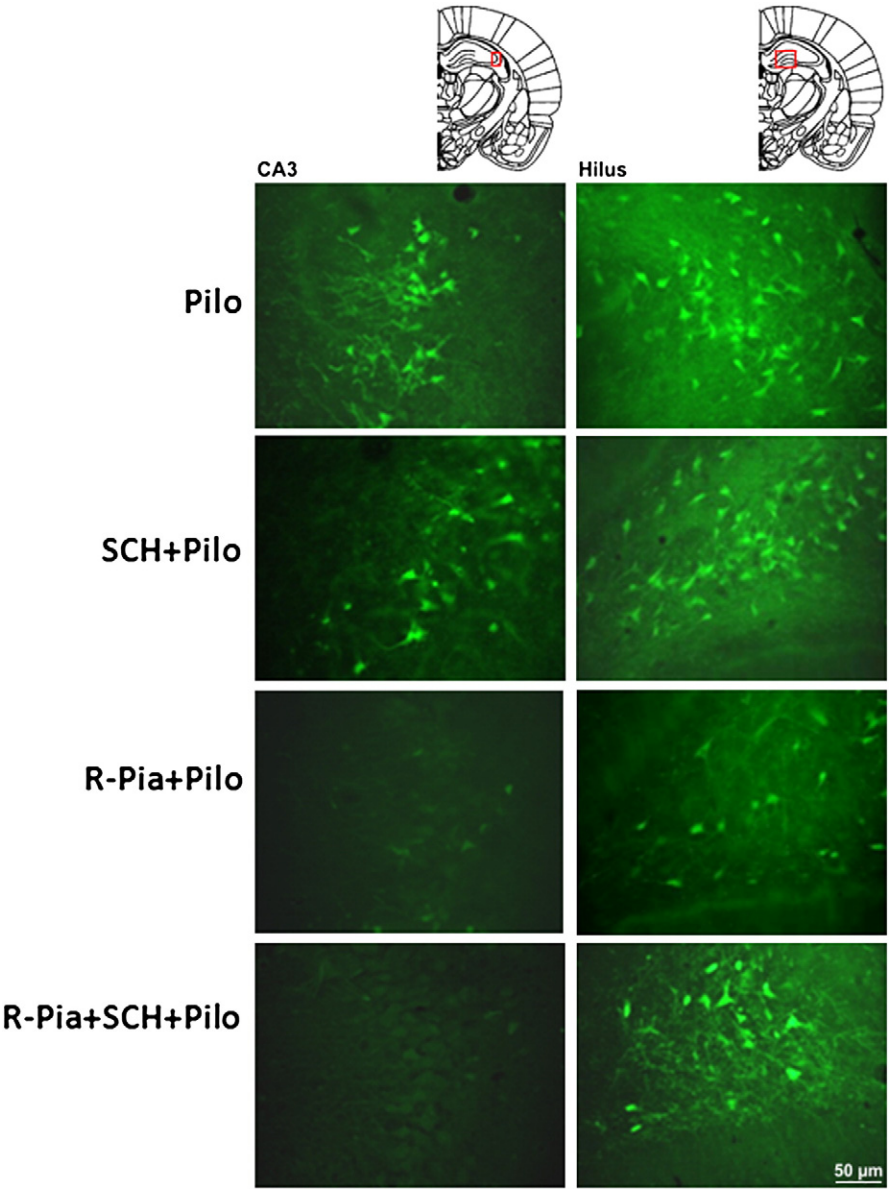


**Fig. 2.** Fluoro-Jade B histochemistry indicative of neuronal injury in hippocampal formation of the pilocarpine (Pilo), SCH + Pilo, R-Pia + Pilo, and R-Pia + SCH + Pilo groups analyzed 7 days after status epilepticus. Pretreatment with R-Pia and R-Pia + SCH58261 reduced the number of stained cells in CA3 and hilus. Similar staining was observed in the SCH + Pilo and Pilo groups. Scale bar: 50 µm.

of proteases, lipases, and endonucleotidases culminating in cell death [35].

The present work also showed that SCH58261 pretreatment did not alter the pattern of FJ-B labeling of cells in the brain regions studied when compared with pilocarpine alone. Thus, the $A_{2A}$ antagonist failed to confer neuroprotection in the pilocarpine model of TLE. These results are not in line with several reports employing different noxious brain stimuli that demonstrated the ability of $A_{2A}$ receptor blockade to confer neuroprotection [14,25,36–38]. Some authors used $A_{2A}$ receptor antagonists in the kainic acid model of neurotoxicity [39,40] or in a model of cerebral ischemia [31] and reported robust neuroprotection, particularly in the areas (i.e., cortex) where the density of these receptors is much lower than in basal ganglia [41–43]. In fact, $A_{2A}$ receptors can be upregulated during prolonged seizures and this response is most evident in extrastriatal regions (i.e., cortical area) [24].

On the other hand, SCH58261 combined with R-Pia led to neuroprotection in the hilus, CA3, and piriform cortex, suggesting that this effect must have occurred as a result of $A_1$ receptor inhibition. In fact, it appears that the neuroprotective effect of $A_1$ receptors is relevant at the onset of brain damage [14], and our data confirm this statement. Our data do not support an upregulation of $A_{2A}$ receptors at the time when the rats were studied. However, we suggest that our data could be related to the dose of SCH58261 employed in this study. Some authors have reported that the dose of this $A_{2A}$ receptor antagonist necessary to be effective in conferring neuroprotection is very low (nanomolar) and the neuroprotective effect could be lost with increasing dosages, that is, 1 mg/kg [18,21,25]. At high doses (i.e., millimolar), SCH58261 can block adenosine $A_{2A}$ receptor-mediated effects on blood pressure, reducing blood and nutrient supply to the compromised brain areas and further stimulating glutamate release [39,44]. The dose of SCH58261 used in the present study was an intermediate dose reported by Popoli and co-workers [25] as effective against the excitotoxic effects of quinolinic acid, that is, 0.2 mg/kg.

It must also be remembered that in the hippocampus, $A_1$ receptors are present on the presynaptic terminals and on the soma of postsynaptic neurons, whereas $A_{2A}$ receptors are present mainly on presynaptic terminals [14]. The striatum contains $A_{2A}$ receptors

postsynaptically, but in the hippocampus, their postsynaptic localization is minimal. Therefore, use of an $A_{2A}$ antagonist such as SCH58261 can be expected to block glutamate release in the hippocampus, but will not prevent activation of postsynaptic glutamate receptors. This dual effect might explain why pyramidal neurons were not protected by the $A_{2A}$ antagonist in the present study. In contrast, activating $A_1$ receptors will block glutamate release and activation of postsynaptic glutamate receptors involved in excitotoxicity. Furthermore, activation of $A_1$ receptors blocks the release of glutamate, but not of GABA, whereas $A_{2A}$ activation increases release of glutamate and GABA. Therefore, blocking $A_{2A}$ receptors will block the release of glutamate and GABA, which might precipitate SE as observed in the present study and by other groups [14].

To clarify the contradictory findings regarding the potential neuroprotective effect of the $A_{2A}$ receptor in the pilocarpine model, a new study using lower doses (nanomolar) of SCH58261 will be performed. One of the possible mechanisms involved in the genesis of the injury process triggered by SE is the imbalance between cerebral glucose consumption and blood flow that occurs during severe epileptic seizures or SE [45], where $A_{2A}$ receptors could play a role. These receptors are present in cerebral blood vessels and can promote vasodilation and self-regulation of cerebral blood flow [46], suggesting that $A_{2A}$ receptors might be directly contributing to the neurodegenerative process. By inhibiting $A_{2A}$ receptors, SCH58261 might alter blood flow homeostasis in the brain, worsening the imbalance between the metabolic demand and the energy supply during SE.

The administration of R-Pia prior to pilocarpine reduced FJ-B labeling in CA3, hilus of the dentate gyrus, and piriform cortex. This finding is in agreement with the literature concerning the neuroprotective properties triggered by the utilization of $A_1$ agonists in epilepsy models [8,12,22]. Activation of these receptors reduces the release of glutamate, inhibits NMDA receptors, and causes hyperpolarization on the neuronal membrane [14]. These effects are crucial to the intracellular $Ca^{2+}$ homeostasis maintenance mediated by $A_1$ receptors. In addition, injection of this $A_1$ receptor agonist prior to pilocarpine reduces the uncoupling between local cerebral blood flow and glucose metabolism caused by SE, resulting in neuroprotection [47].

## 5. Conclusion

This study showed that the $A_{2A}$ antagonist SCH58261 is not neuroprotective when administered prior to pilocarpine. Further studies aimed at clarifying the apparent discrepancy between the neuroprotective effects of $A_{2A}$ antagonists in different models of neurotoxicity and lack thereof in the pilocarpine model of temporal lobe epilepsy are warranted.

## Conflict of interest statement

The authors declare that they have no conflicts of interest.

## Acknowledgments

The authors acknowledge the financial support of CNPq, CAPES, CINAPCE/FAPESP, and INNT/CNPq/MCT. Special thanks to Iara R.Silva for his contribution to the experiments with R-Pia.

## References

[1] Engel Jr J. for the International League Against Epilepsy (ILAE). A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. Epilepsia 2001;42:796–803.
[2] Sloviter S. The neurobiology of temporal lobe epilepsy: too much information, not enough knowledge. Compt Rend Biol 2005;328:143–53.
[3] Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. Neuroscience 1985;14:375–403.
[4] Leite JP, Bortolotto ZA, Cavalheiro EA. Spontaneous recurrent seizures in rats: an experimental model of partial epilepsy. Neurosci Bioehav Rev 1990;14:511–7.
[5] Cavalheiro EA. The pilocarpine model of epilepsy. It J Neurol Sci 1995;16:33–7.
[6] Turski WA, Cavalheiro EA, Schwarz M, Czuczwar SJ, Kleinrok Z, Turski L. Limbic seizures produced by pilocarpine in rats: behavioral, electroencephalographic and neuropathological study. Behav Brain Res 1983;9:315–35.
[7] Bortolotto ZA, Mello LE, Turski L, Cavalheiro EA. Effects of 2-chloroadenosine on amygdaloid and hippocampal kindled seizures. Arch Int Pharmacodyn Ther 1985;277:313–20.
[8] Vianna EPM, Ferreira AT, Dona F, Cavalheiro EA, Fernandes MJS. Modulation of seizures and synaptic plasticity by adenosinergic receptors in an experimental model of temporal lobe epilepsy induced by pilocarpine in rats. Epilepsia 2005;5:166–73.
[9] Dunwiddie TV. Adenosine and suppression of seizures. Adv Neurol 1999;79:1001–10.
[10] Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 2001;24:31–55.
[11] Alasvand-Zarasvand M, Mirnajafi-Zadeh J, Fathollahi Y, Palizvan MR. Anticonvulsant effect of bilateral injection of $N^6$-cyclohexyladenosine into the CA1 region of the hippocampus in amygdala-kindled rats. Epilepsy Res 2001;47:141–9.
[12] MacGregor DG, Stones TW. Inhibition by adenosine analogue (*R*)-$N^6$-phenylisopropyladenosine of kainic acid neurotoxicity in rat hippocampus after systemic administration. Br J Pharmacol 1993;109:316–21.
[13] Zuchora B, Wielosz M, Urbanska EM. Adenosine $A_1$ receptors and the anticonvulsant potential of drugs effective in the model of 3-nitropropionic acid-induced seizures in mice. Eur Neuropsychopharmacol 2005;15:85–93.
[14] Cunha RA. Neuroprotection by adenosine in the brain: from $A_1$ receptor activation to $A_{2A}$ receptor blockade. Purinergic Signal 2005;1:111–34.
[15] De Mendonça A, Sebastião AM, Ribeiro JA. Adenosine: does it have a neuroprotective role after all? Brain Res Brain Res Rev 2000;33:258–74.
[16] Ongini E, Schubert P. Neuroprotection induced by stimulating $A_1$ or $A_{2A}$ adenosine receptors: an apparent paradox. Drug Dev Res 1998;45:387–93.
[17] Cunha RA. Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. Neurochem Int 2001;38:107–25.
[18] Blum D, Galas MC, Pintor A. A dual role of adenosine $A_{2A}$ receptors in 3-nitropropionic acid-induced striatal lesions: implications for the neuroprotective potential of $A_{2A}$ antagonists. J Neurosci 2003;23:5361–9.
[19] Chen JF, Huang Z, Ma J. $A_{2A}$ adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. J Neurosci 1999;19:9192–200.
[20] Chen JF, Xu K, Petzer JP. Neuroprotection by caffeine and $A_{2A}$ adenosine receptor inactivation in a model of Parkinson's disease. J Neurosci 2001;21:1–6.
[21] Fink JS, Kalda A, Ryu H. Genetic and pharmacological inactivation of the adenosine $A_{2A}$ receptor attenuates 3-nitropropionic acid-induced striatal damage. J Neurochem 2004;88:538–44.
[22] Tomé AR, Silva H, Cunha R. Role of the purinergic neuromodulation system in epilepsy. Open Neursoci J 2010;4:64–83.
[23] Lemos T, Cavalheiro EA. Suppression of pilocarpine-induced status epilepticus and the late development of epilepsy in rats. Exp Brain Res 1995;102:423–8.
[24] Racine RJ. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972;32:281–94.
[25] Popoli P, Pintor A, Domenici MR, et al. 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 2002;22:1967–75.
[26] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.
[27] Li H, Henry JL. Adenosine $A_{2A}$ receptor mediation of pre- and postsynaptic excitatory effects of adenosine in rat hippocampus in vitro. Eur J Pharmacol 1998;347:173–82.
[28] Porciúncula O, Canas P, Oliveira CR, Cunha RA. Blockade of adenosine $A_{2A}$ receptors prevents kainate-induced convulsions and neuronal cell death. Presented at the 4th International Symposium of Nucleosides and Nucleotides; 2004. p. 69T.
[29] Etherington LA, Frenguelli BG. Endogenous adenosine modulates epileptiform activity in rat hippocampus in a receptor subtype-dependent manner. Eur J Neurosci 2004;19:2539–50.
[30] El Yacoubi M, Ledent C, Parmentier M, Costentan J, Vaugeois JM. Adenosine $A_{2A}$ receptor deficient mice are partially resistant to limbic seizures. Arch Pharmacol 2009;380:223–32.
[31] Monopoli A, Lozza G, Forlani A. Blockade of adenosine $A_{2A}$ receptors by SCH 58261 results in neuroprotective effects in cerebral ischaemia in rats. Neuroreport 1998;9:3955–9.
[32] Mirnajafi-Zadeh J, Fathollahi Y, Pourgholami MH. Intraperitoneal and intraamygdala $N^6$-cyclohexyladenosine suppress hippocampal kindled seizures in rats. Brain Res 2000;858:48–54.
[33] Fabene PF, Andrioli A, Priel MR, Cavalheiro EA, Bentivoglio M. Fos induction and persistence, neurodegeneration, and interneuron activation in the hippocampus of epilepsy-resistant versus epilepsy-prone rats after pilocarpine-induced seizures. Hippocampus 2003;14:895–907.
[34] Voutsinos-Porche B, Koning E, Kaplan H, et al. Temporal patterns of the cerebral inflammatory response in the rat lithium–pilocarpine model of temporal lobe epilepsy. Neurobiol Dis 2004;17:385–402.
[35] DeLorenzo RJ, Sun DA. Basic mechanisms in status epilepticus: role of calcium in neuronal injury and the induction of epileptogenesis. Adv Neurol 2006;97:187–97.

[36] Pagonopoulou O, Efthimiadou A, Asimakopoulos B, Nikolettos NK. Modulatory role of adenosine and its receptors in epilepsy: possible therapeutic approaches. Neurosci Res 2006;56:14–20.
[37] Ribeiro JA. What can adenosine neuromodulation do for neuroprotection? Curr Drug Targets CNS Neurol Disord 2005;4:325–9.
[38] Wardas J. Neuroprotective role of adenosine in the CNS. Pol J Pharmacol 2002;54: 313–26.
[39] Jones PA, Smith RA, Stone TW. Protection against hippocampal kainate excitotoxicity by intracerebral administration of an adenosine $A_{2A}$ receptor antagonist. Brain Res 1998;800:328–35.
[40] Lee HK, Choi SS, Han KJ. Roles of adenosine receptors in the regulation of kainic acid-induced neurotoxic responses in mice. Mol Brain Res 2004;125:76–85.
[41] Cunha A, Johansson B, van der Ploeg I. Evidence for functionally important adenosine $A_{2A}$ receptors in the rat hippocampus. Brain Res 1994;649:208–16.
[42] Rebola N, Oliveira CR, Cunha RA. Transducing system operated by adenosine $A_{2A}$ receptors to facilitate acetylcholine release in the rat hippocampus. Eur J Pharmacol 2002;454:31–8.
[43] Rebola N, Sebastião AM, de Mendonça A. Enhanced adenosine $A_{2A}$ receptor facilitation of synaptic transmission in the hippocampus of aged rats. J Neurophysiol 2003;90:1295–303.
[44] Stella L, De Novellis V, Berrino L, d´Amico M, Rossi F. Evidence that $A_{2A}$ and not $A_{2B}$ purinoceptors are coupled to production of nitric oxide in the regulation of blood pressure. Environ Toxicol Pharmacol 1996;2:327–9.
[45] Fernandes MJS, Dubé C, Boyet S, Marescaux C, Nehlig A. Correlation between hypermetabolism and neuronal damage during status epilepticus induced by lithium and pilocarpine in immature and adult rats. J Cereb Blood Flow Metab 1999;19:195–209.
[46] Shi Y, Gebremedhin D, Falck JR, Harder DR, Koehler RC. Interaction of mechanisms involving epoxyeicosatrienoic acids, adenosine receptors, and metabotropic glutamate receptors in neurovascular coupling in rat whisker barrel cortex. J Cereb Blood Flow Metab 2008;28:111–25.
[47] Silva RI, Nehlig A, Rosim FE, et al. The $A_1$ receptor agonist R-Pia reduces the imbalance between cerebral glucose metabolism and blood flow during status epilepticus: could this mechanism be involved with neuroprotection? Neurobiol Dis 2011;41:169–76.