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Blockade of adenosine A_{2A} receptors prevents staurosporine-induced apoptosis of rat hippocampal neurons

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Since adenosine A_{2A} receptor (A_{2A}Rs) blockade protects against noxious brain insults involving apoptosis, we directly tested if A_{2A}R blockade prevents apoptosis induced by staurosporine (STS). Exposure of rat hippocampal neurons to STS (30 nM, 24 h) decreased neuronal viability while increasing the number apoptotic-like neurons and de-localizing mitochondria and cytochrome *c* immunoreactivities. This was prevented by the selective A_{2A}R antagonists, SCH58261 and ZM241385 (50 nM). Shorter incubation periods (6 h) with STS caused no neuronal loss but decreased synaptophysin and MAP-2 immunoreactivities, which was prevented by SCH58261. Furthermore, STS (100 nM) decreased MTT reduction and increased caspase-3 activity in rat hippocampal nerve terminals, which was prevented by SCH58261. These results show that A_{2A}R blockade inhibits STS-induced apoptotic-like neuronal cell death. This begins with an apoptotic-like synaptotoxicity, which later evolved into an overt neurotoxicity, and A_{2A}Rs effectively control this initial synaptotoxicity, in agreement with their predominant synaptic localization in the hippocampus.

Keywords: Adenosine; A_{2A} receptor; Staurosporine; Apoptosis; Neuroprotection; Mitochondria; Hippocampus; Cultured neurons; Synaptosomes; Caspase-3

Introduction

Adenosine is a ubiquitous neuromodulator in the brain, which mainly acts through two metabotropic adenosine receptors with opposite functions, namely the predominant inhibitory A_1 and facilitatory A_{2A} receptors (A_{2A}Rs) (Fredholm et al., 2005). Adenosine plays an important role in controlling neuronal survival in noxious brain conditions in accordance with its pivotal homeostatic role signaling metabolic imbalance (reviewed in Cunha, 2001). Thus, the levels of adenosine rapidly rise as a result of increased workload or decreased metabolic competence of brain tissue (Latini and Pedata, 2001) and the extracellular levels of adenosine produce biphasic effects in the control of neuronal viability in the adult brain: the initial activation of A_1Rs acts as a hurl increasing the threshold required for brain damage (de Mendonça et al., 2000); the over-activation of A_1Rs leads to their desensitization and adenosine then mainly acts through A_{2A}Rs which mainly seem to contribute for the aggravation of brain damage in chronic noxious brain conditions in adults (reviewed in Cunha, 2005). Accordingly, the blockade of A_{2A}Rs affords protection against different brain insults (Cunha, 2005; Xu et al., 2005), namely in chronic neurodegenerative conditions such as Parkinson's, Alzheimer's, or Huntington's disease, which involve apoptotic neuronal features (Rego and Oliveira, 2003; Vila and Przedborski, 2003). The mechanism underlying this neuroprotection remains unraveled, but the ability of A_{2A}R antagonists to directly protect cultured neurons against noxious insults that trigger neuronal damage featuring apoptotic-like characteristics (Dall'Igna et al., 2003; Mojsilovic-Petrovic et al., 2006) prompts the hypothesis that A_{2A}Rs might directly control apoptosis in neurons. We now directly tackled this question by testing if the blockade of A_{2A}Rs might prevent neuronal damage caused by the prototypic apoptotic inducer, staurosporine (e.g. Bertrand et al., 1994; Koh et al., 1995).

Materials and methods

Primary cultures of hippocampal neurons

Hippocampal neurons were cultured from 17- to 19-day-old Wistar rat embryos, handled according to European guidelines (86/609/EEC), as previously described (Rebola et al., 2005a,b), and plated on poly-D-lysine-coated 16-mm-diameter coverslips or 6-well dishes at densities of 5×10^4/coverslip (viability and immunocytochemistry assays) or 1×10^6/well (Western blot analysis). Neurons were grown at 37 °C in a 5% CO_2 humidified atmosphere in

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neurobasal medium with B-27 supplement, glutamate (25 μM), glutamine (0.5 mM) and gentamicin (0.12 mg/ml).

Staurosporine (STS)-induced neuronal damage was evaluated after culturing the neurons for 7 days by directly adding STS (30 nM) to the medium followed by incubation for periods between 6 and 24 h. To test the ability of SCH58261 or ZM241385 to modify the effects of STS, they were added 15 min before the addition of STS onwards.

Viability assays

Viability assays were performed by double labelling (3-min incubation) with the fluorescent probes Syto-13 (4 μM) and propidium iodide (PI, 4 μg/ml) (Molecular Probes, Leiden, The Netherlands) followed by fluorescence microscopy cell counting. As previously described (Rebola et al., 2005b), viable neurons present nuclei homogenously labelled with Syto-13 (green fluorescent nuclei), whereas apoptotic neurons show condensed and fragmented nuclei labelled with Syto-13 (primary apoptosis) or with Syto-13 plus PI (secondary apoptosis) and necrotic neurons present intact nuclei labelled with PI (red fluorescent nuclei). In parallel, the nuclear morphology of hippocampal neurons was analyzed by fluorescence microscopy using Hoechst 33342 (2 μg/ml for 10 min; from Molecular Probes), as previously described (Almeida et al., 2004). Each experiment was repeated using different cell cultures in duplicate, and cell counting was carried out in at least six fields per coverslip, with a total of approximately 300 cells. Results are expressed as mean ± SEM and statistical significance ($P < 0.05$) was evaluated by one-way ANOVA followed by Newman–Keuls multiple comparison test.

Immunocytochemical analysis

Immunocytochemistry assays were performed as previously described (Rebola et al., 2005a,b) after incubation with drugs, as described for the viability assays. For the double labelling with mitotracker-red and anti-cytochrome *c* antibody (Almeida et al., 2004), the neurons were incubated for 1 h in Krebs buffer with 500 nM mitotracker-red (Molecular Probes), a mitochondrial marker that is insensitive to the mitochondrial potential (Krohn et al., 1999). The following steps had to be performed protected from light. After fixation in 4% paraformaldehyde, the neurons were washed three times with PBS, incubated for 10 min with 20 mM glycine, permeabilized with 0.1% saponin, incubated for 30 min with a mouse anti-cytochrome *c* antibody (1:100; PharMingen, San Diego, USA) and, after washing, with an Alexa Fluor 488-labelled anti-mouse secondary antibody (1:200; Molecular Probes). For the immunocytochemical double labelling of MAP-2 and synaptophysin, the fixed permeabilized neurons were incubated for 1 h with rabbit anti-MAP-2 antibody (1:400; Santa Cruz Biotechnologies, Freelab, Lisbon, Portugal) and mouse anti-synaptophysin antibody (1:200; Sigma, Sintra, Portugal). The secondary antibodies used were Alexa Fluor 488-labelled anti-rabbit and Alexa Fluor 594-labelled anti-mouse antibodies (1:200; Molecular Probes).

The labelled neurons were visualized using either a fluorescence microscope (Zeiss Axiovert 2000, PG-HITEC, Portugal) or a confocal microscope (MRC 600, Bio-Rad, Hercules, USA). Immunoreactivities were evaluated using 3 representative fields per coverslip (magnification ×600). Quantitative assessment of synaptophysin immunoreactivity was calculated by comparison of the total number of labelled dots in control *versus* test conditions (approximately one thousand dots per field, with 3 fields counted per experimental condition). Results are expressed as mean ± SEM and statistical significance ($P < 0.05$) was evaluated by one-way ANOVA followed by Newman–Keuls multiple comparison test.

Western blot analysis

Hippocampal neurons, plated on 6 wells dishes, were gently scraped in lysis buffer (50 mM KCl, 50 mM PIPES, 10 mM EGTA, 2 mM $MgCl_2$, 0.5% Triton X-100, 1 mM PMSF, 1 mM dithiothreitol and 5 μg/ml of a mixture of protease inhibitors containing chymostatin, leupeptin, pepstatin A and antipain) and subject to 3 freezing cycles at −80 °C. Neuronal extracts were diluted at the final concentration of 1 μg protein/μl in SDS–PAGE buffer and 20 μg were separated by SDS–PAGE (7.5% with a 4% concentrating gel), as previously described (Rebola et al., 2005a). After electro-transfer, the membranes were incubated overnight at 4 °C with mouse anti-synaptophysin antibody (1:1000) or rabbit anti-MAP-2 antibody (1:400), washed and incubated with an alkaline phosphatase-conjugated anti-mouse secondary antibody (1:2000; Calbiochem, PG-HITEC). The membranes were then analysed with a VersaDoc 3000 (Biorad) after incubation with ECF (Amersham, Buckinghamshire, UK). The membranes were then re-probed and tested for tubulin immunoreactivity using a mouse anti-α-tubulin antibody (1:1000; Zymed, Lisbon, Portugal), as previously described (Rebola et al., 2005a).

Hippocampal synaptosomes

Male Wistar rats (8 weeks old, 150–160 g, obtained from Charles River, Barcelona, Spain) were handled according to the EU guidelines for use of experimental animals (86/609/EEC), the rats being anesthetized under halothane atmosphere before being sacrificed by decapitation. Membranes from Percoll-purified hippocampal synaptosomes were prepared as previously described (Rebola et al., 2005a). Briefly, the two hippocampi from one rat were homogenized at 4 °C in sucrose solution (0.32 M) containing 10 mM HEPES, 1 mM EGTA and 1 mg/ml bovine serum albumin (fatty acid-free), pH 7.4, centrifuged at 3000×*g* for 10 min at 4 °C; the supernatants were collected, centrifuged at 14,000×*g* for 12 min at 4 °C and the pellet was resuspended in 1 ml of a 45% (v/v) Percoll solution made up in a Krebs solution (composition: 140 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM EDTA, 5 mM glucose, pH 7.4). After centrifugation at 14,000×*g* for 2 min at 4 °C, the top layer was removed (synaptosomal fraction), washed in 1 ml Krebs solution and resuspended in Locke's buffer (with 154 mM NaCl, 5.6 mM KCl, 5 mM HEPES, 3.6 mM $NaHCO_3$, 2.3 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, pH 7.2).

The mitochondrial reduction status of the synaptosomes (Mattson et al., 1998) was measured by a colorimetric assay for cell survival, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, from Sigma), as previously described (Almeida et al., 2004). The synaptosomes (1 mg/ml) were incubated for 2 h at 37 °C in Locke's buffer in the absence or presence of STS (100 nM) and/or SCH58261 (50 nM). We used a higher concentration of STS in the experiments using synaptosomes to compensate for the shorter exposure periods imposed by the limited period of viability of this preparation, which does not exceed 4 h. MTT (0.5 mg/ml) was then added and incubated for 1 h at 37 °C in the dark. As MTT is converted to a water-insoluble blue product (formazan) by viable terminals, the precipitated dye

can be spectrophometrically (570 nm) quantified after exposing the synaptosomes to isopropanol containing 0.04 M HCl. Values were expressed as the percentage of optical density of control synaptosomes, i.e. in the absence of drugs.

Caspase-3 activity was assessed in synaptosomes using a colorimetric pseudo-substrate of caspase-3, *N*-acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide (Ac-DEVD-pNA; Calbiochem), as previously described for cultured neurons (Almeida et al., 2004). After incubation for 2 h at 37 °C in Locke's buffer in the absence or presence of STS (100 nM) and/or SCH58261 (50 nM), the synaptosomes were pelleted and lysed by addition of lysis buffer before quantification of protein. The suspension (50 μg protein) was then incubated for 2 h at 37 °C in the dark with 100 μM Ac-DEVD-pNA in CHAPS buffer containing 25 mM HEPES–Na, 10 mM dithiothreitol, 10% (w/v) sucrose and 0.1% (w/v) CHAPS, pH 7.4 before reading the optical density at 405 nm.

Drugs and solutions

STS was purchased from Sigma and made up as a 5 mM stock in dimethylsulfoxide before diluting into working solutions at the desired concentration. The selective antagonists of adenosine A_{2A} receptors, SCH58261 (provided by S. Weiss, Vernalis, UK) or ZM241385 (Tocris, Northpoint, UK) were prepared as 5 mM stock solutions in dimethylsulfoxide and then dissolved (<0.001% dimethylsulfoxide) in the working solutions. All culture media were from GIBCO BRL (Life Technologies, Scotland, UK).

Results and discussion

In accordance with different studies confirming the ability of the non-selective protein kinase inhibitor staurosporine to trigger neuronal damage through an apoptotic-like process (e.g. Koh et al.,

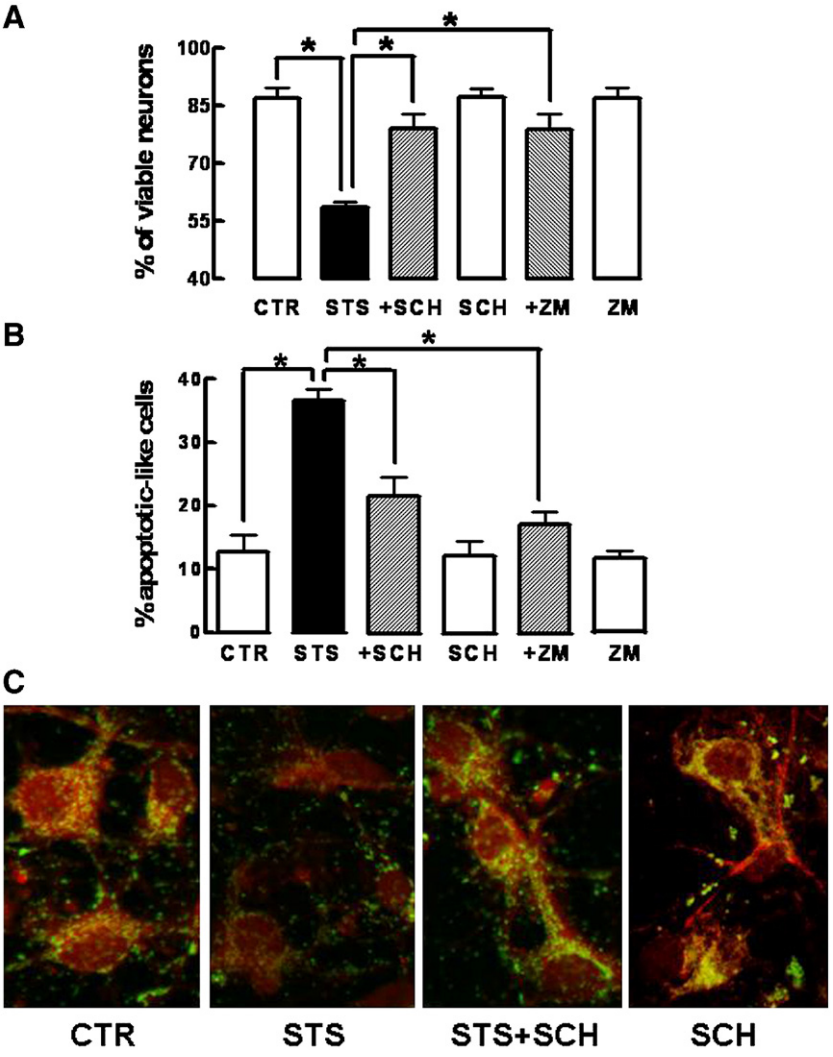

Fig. 1. Blockade of A_{2A} receptors prevents the apoptosis induced by staurosporine in cultured hippocampal neurons. Staurosporine (STS, 30 nM, 24 h) decreased neuronal viability (A) and increased the number of apoptotic-like neurons (*i.e.* displaying a condensed nucleus simultaneously labeled with Syto-13 and PI) (B). This was prevented by the A_{2A} receptor antagonists, SCH58261 (SCH, 50 nM) or ZM241385 (ZM, 50 nM), that were devoid of effects in the absence of STS. Results are mean ± SEM from 6 independent hippocampal cultures and a total of at least 300 neurons per coverslip were counted. *P<0.05 versus control (CTR, one-way ANOVA followed by Newman–Keuls test). Blockade of A_{2A} receptors also prevented STS (30 nM, 24 h)-induced release of cytochrome *c* from mitochondria in cultured hippocampal neurons (C) as shown by the loss of co-localization of mitotracker-red (red) and cytochrome *c* (green) immunoreactivities in the displayed merged confocal images (magnification ×600). These results are representative of 4 experiments.

1995; Prehn et al., 1997), rat cultured hippocampal neurons exposed to staurosporine (30 nM) displayed morphological characteristics of neuronal apoptosis such as neuronal shrinkage, chromatin condensation and nuclear pyknosis. At its reported EC_{50} concentration (30 nM, see Koh et al., 1995; Prehn et al., 1997), staurosporine decreased the number of viable neurons (from a control value of 84±3% to 57±2% of viable cells; Fig. 1A) and increased the number of primary (from 0% to 6±1%) and secondary apoptotic cells (from 15±3% to 36±2%) after 24 h of incubation ($n=6$; Fig. 1B). As illustrated in Fig. 1C, STS also triggered the loss of cytochrome *c* from mitochondria, a key feature of commitment to apoptotic death in neurons (Ahlemeyer et al., 2002; Krohn et al., 1999). In 4 experiments, we observed a 33±4% decrease in the number of cell displaying a co-localization of a mitochondrial marker (mitotracker-red) and cytochrome *c* immunoreactivity.

We then tested if adenosine A_{2A}Rs directly controlled neuronal apoptosis. For this purpose, we tested the ability of two chemically distinct and selective antagonists of A_{2A}Rs, SCH58261 and ZM241385, used in supra-maximal concentrations of 50 nM, to modify STS-induced neuronal damage. As illustrated in Figs. 1A and B, the presence of either SCH58261 or ZM241385 significantly ($P<0.05$) attenuated STS-induced neurotoxicity. Furthermore, the presence of SCH58261 (50 nM) abrogated the STS-induced de-

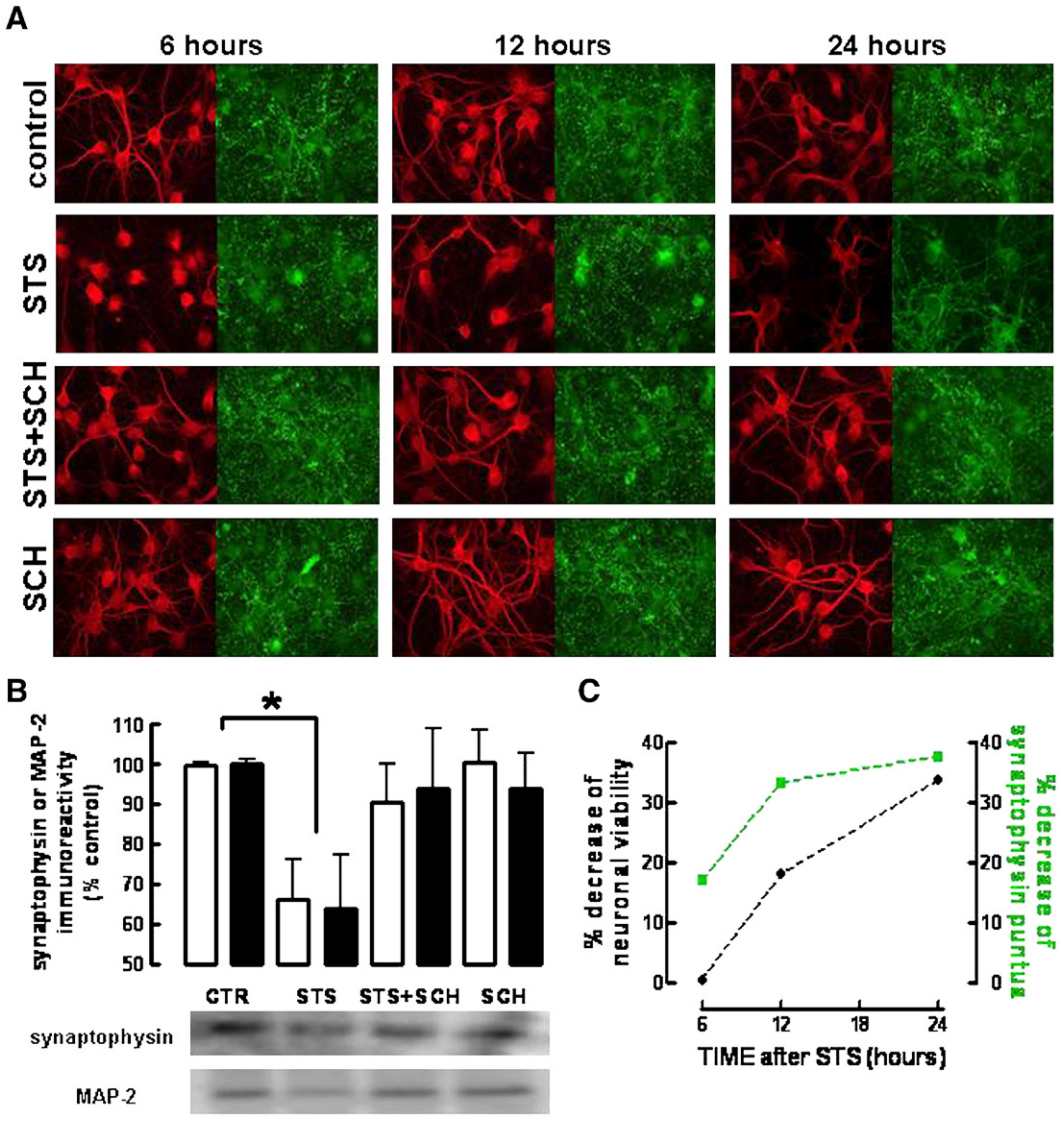

Fig. 2. Staurosporine (STS, 30 nM) causes a precocious synaptic degeneration, before overt neuronal loss occurred, which is prevented by A_{2A} receptor blockade. Panel A shows that STS decreased MAP-2 (red) and synaptophysin (green) immunoreactivity in cultured hippocampal neurons after 6 h (first column of paired photographs from the left), 12 h (second column) or 24 h (third column) of incubation with STS. This was abrogated at all time points upon blockade of A_{2A} receptor with SCH58261 (SCH, 50 nM). Similar qualitative results were obtained in 4 experiments. This STS-induced synaptic degeneration was confirmed by Western blot quantification, showing that the density of synaptic proteins (synaptophysin and MAP-2) in cultured hippocampal neurons is decreased by STS (30 nM, 24 h) and this decrease is prevented by SCH58261 (50 nM) (B). The results, presented as percentage of the densitometric values of control conditions (CTR, lack of added drugs), are mean±SEM of 3 experiments. *$P<0.05$ compared to control (one-way ANOVA followed by Newman–Keuls test). Panel C shows that at the early time point evaluated (6 h), STS (30 nM) caused a decrease of synaptic markers without evident modification of neuronal viability ($n=5$–6).

localization of mitotracker-red and cytochrome *c* immunoreactivity (Fig. 1C). Overall, these results indicate that the activation of adenosine A_{2A}Rs is an important event for the full expression of features of apoptotic damage induced by STS in cultured hippocampal neurons, since the blockade of A_{2A}Rs considerably reduced STS-induced membrane leakage and prevented the STS-induced release of cytochrome *c* from mitochondria.

We have previously found that A_{2A}Rs are mostly located in synapses in forebrain neurons (Lopes et al., 2004; Rebola et al., 2005a). This is in agreement with the neuroprotection afforded by A_{2A}Rs since it has been suggested that the damage of neurons in the course of neurodegenerative diseases might start with a synaptic dysfunction that gradually evolves into an overt pattern of neuronal degeneration (Herkenham et al., 1991; Li et al., 2001; Selkoe, 2002). Interestingly, it has also been shown that STS is directly toxic to nerve terminals and STS-treated nerve terminals from the adult brain display apoptotic-like features (Gylys et al., 2002; Mattson et al., 1998; Xie et al., 2005). Thus, we decided to investigate if it was possible to temporally dissociate STS-induced synaptic and whole neuronal damage, as previously done by others using other noxious stimuli (e.g. Berliocchi et al., 2005; Cowan et al., 2001; Ikegami and Koike, 2003; Ivins et al., 1998), and to test if the synaptically located A_{2A}Rs might play a prominent role in the control of the putative synaptotoxicity caused by STS.

For that purpose, we began to test if STS caused initial synaptic alterations and a subsequent modification of neuronal viability. As illustrated in Fig. 2A, after 6 h of exposure to 30 nM STS, there was a reduction of synaptophysin (a presynaptic marker) and MAP-2 (a dendritic marker) immunoreactivities ($n=4$). Interestingly, at this time point (6 h), we found no statistical difference ($P>0.05$, $n=5$) between the number of viable and apoptotic-like neurons between control and STS-treated neurons whereas a 17.1±3.1% reduction ($n=4$) of the number of dots labelled with synaptophysin was observed (Fig. 2C). At a later time point (12 h), the STS-induced decrease of synaptophysin and MAP-2 immunoreactivities became more evident (Fig. 2A) and neuronal damage could now be measured (18.0±2.6% decrease of viable neurons, $n=5$). Finally, at the latest tested time point (after 24 h of exposure to STS), synaptotoxicity was also evident (37.7±1.4% decrease of the number of synaptophysin-labelled dots, $n=4$ and 33.8±9.9% and 36.3±13.8% decrease of synaptophysin and MAP-2 immunoreactivity evaluated by Western blot, $n=3$) and there was a more evident neuronal damage (33.7±2.0% decrease of viable neurons, $n=5$).

As displayed in Fig. 2, this early and sustained decrease of labelling of synaptic markers caused by STS was abrogated when A_{2A}Rs were blocked with SCH58261 (50 nM), which was devoid of effects in the absence of STS ($n=4$–5). This indicates that synaptic A_{2A}Rs play a prominent role in the control of early STS-induced synaptotoxicity, in accordance with their synaptic localization in cultured hippocampal neurons (Rebola et al., 2005a). Since A_{2A}Rs are also enriched in nerve terminals in the adult rat brain (Rebola et al., 2005a), we next tested if the blockade of A_{2A}Rs might also prevent the STS-induced features of synaptic apoptosis (see Gylys et al., 2002, Mattson et al., 1998; Xie et al., 2005), using the only preparation that allows studying nerve terminals independently of other brain elements, the synaptosomal preparation (Nicholls, 2003).

As shown in Fig. 3, exposure of hippocampal synaptosomes obtained from adult rats to STS (100 nM) for 2 h decreased MTT reduction to 95.9±0.8% of control ($n=15$, $P<0.05$) and increased

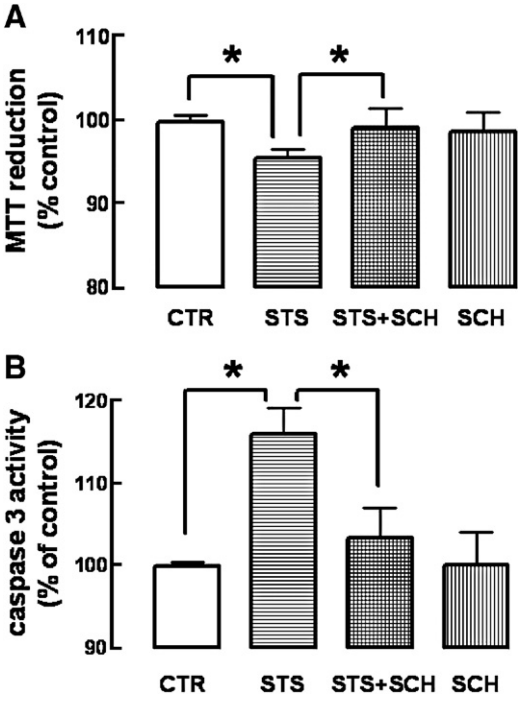

Fig. 3. Staurosporine (STS, 100 nM) decreased mitochondria function (evaluated by the reduction of MTT in panel A) and induced caspase-3 activation (evaluated by the increased fluorescence of its pseudo-substrate Ac-DEVD-pNA, panel B) in hippocampal nerve terminals (2 h of incubation). Both effects were prevented upon blockade of A_{2A} receptor with SCH58261 (SCH, 50 nM). Results are mean±SEM of 8–15 experiments. *$P<0.05$ using the paired Student's *t* test.

caspase-3 activity by 11.5±2.3% ($n=16$, $P<0.05$). According to our expectation based on the results obtained with cultured neurons, the STS-induced modifications observed in hippocampal synaptosomes were no longer observed when they were treated with 50 nM SCH58261 ($n=12$–16), which was devoid of effects by itself (Fig. 3).

This observed precocious susceptibility of nerve terminals to STS re-enforces the concept that synaptic toxicity might be an initial target of insults triggering a slowly developing apoptotic neuronal damage in the brain, such as these occurring in different neurodegenerative diseases. In fact, synaptic dysfunction and damage has been recognised as an early event in the course of different neurodegenerative diseases such as Alzheimer's (Selkoe, 2002), Parkinson's (Herkenham et al., 1991), Huntington's (Li et al., 2001) or prion's diseases (Ferrer, 2002) as well as in HIV infection (Garden et al., 2002), schizophrenia (Glantz et al., 2006) or motor neuropathies (Raff et al., 2002), which can be observed before overt neuronal loss occurs. However, it still remains debatable if this precocious synaptic degeneration can be considered a localised synaptic apoptosis (see Mattson et al., 1998). One consensual mechanism to explain the greater susceptibility of nerve terminal to insidious brain insults is the particular susceptibility to dysfunction of synaptic mitochondria. In fact, mitochondria located in nerve terminals have a different morphology and functional properties when compared to mitochondria located in other neuronal compartments or brain cells (e.g. Battino et al., 2000; Collins et al., 2002; Davey et al., 1998; Muller et al., 2005). Thus, nerve terminals are at particular catastrophic risk

upon slight dysfunction of synaptic mitochondria due to the high metabolic demand imposed by the release of neurotransmitters (Davey et al., 1998; Nicholls, 2003) and to the higher calcium transients that are more poorly managed by synaptic mitochondria (Brown et al., 2006). This mitochondria dysfunction in synapses has the potential to trigger an apoptotic-like response, namely a restricted activation of caspase-3 in nerve terminals (Cowan et al., 2001; Mattson et al., 1998; Xie et al., 2005), as we also observed. However, it is still unclear if synaptic degeneration is indeed caused by caspase activation and it is not known what are the mechanisms involved in this dying-back process beginning as a synaptic degeneration and later evolving into overt neuronal loss (reviewed in Luo and O'Leary, 2005; Raff et al., 2002). In fact, some studies reported that inhibition of caspase-3 prevents the early synaptic damage (Cowan et al., 2001; Ivins et al., 1998; Mattson et al., 1998; Xie et al., 2005) and the antidromic propagation of activated caspases is associated with neuronal apoptosis (Cowan et al., 2001), whereas other elegant studies found that the inhibition of caspase-3 fails to affect the early synaptic damage, albeit it prevents the subsequent neuronal apoptotic death (Berliocchi et al., 2005; Finn et al., 2000; Ikegami and Koike, 2003). The elucidation of these mechanisms of precocious synaptic degeneration and dying-back process in neurons would certainly bolster the possibility of controlling this apparently reversible synaptic dysfunction which is now recognised as an effective strategy to arrest neurodegenerative diseases at their early stages before they evolve into overt irreversible neuronal loss (Coleman and Perry, 2002).

We now found that the blockade of the synaptically located adenosine A_{2A}Rs might achieve this goal. In fact, blockade of A_{2A}Rs was effective to directly prevent the STS-induced mitochondria dysfunction in purified nerve terminals and also abrogated the initial STS-induced synapto-toxicity and later apoptotic-like neuronal damage in cultured hippocampal neurons. This is in agreement with in vivo studies showing that the blockade of these synaptically located A_{2A}Rs (Lopes et al., 2004; Rebola et al., 2005a) prevented the initial synapto-toxicity caused by chronic noxious brain insults such as in animal models of Parkinson's disease (Chen et al., 2001; Ikeda et al., 2002) or restraint stress (Cunha et al., 2006). Furthermore, the current observation that A_{2A}R blockade directly prevents neuronal apoptosis caused by STS provides a mechanistic basis for the ability of A_{2A}R antagonists to prevent neurotoxicity and/or phenotypic burden in animal models of different neurodegenerative diseases known to involve apoptotic features such as Parkinson's (Chen et al., 2001; Ikeda et al., 2002), Alzheimer's (Dall'Igna et al., 2007) or Huntington's disease (Blum et al., 2003).

However, it still remains to be defined what are the mechanisms associated with this ability of A_{2A}Rs to control neuronal apoptosis. A_{2A}Rs are classically defined as G protein-coupled receptors selectively activating $G\alpha_S$- (or $G\alpha_{olf}$-) containing proteins and their main transducing system is assumed to be the increase of cAMP levels (Fredholm et al., 2005). Increased cAMP levels effectively counteract STS-induced neuronal apoptosis (Goswami et al., 1998; Park and Cho, 2006). However, we now report that it is the blockade of A_{2A}Rs (expected to limit cAMP accumulation) which affords protection against STS-induced damage. This makes it unlikely that the A_{2A}R neuroprotection might involve the cAMP transducing system. Accordingly, several reports have provided evidence supporting the possible coupling of A_{2A}Rs with other transducing systems (reviewed in Fredholm et al., 2005). Thus, A_{2A}Rs might control protein phosphatases (Murphy et al., 2003; Revan et al., 1996), which is of particular relevance since phosphatase activities play a prominent role in the control of mitochondria function and neuronal viability after noxious stimuli (Agostinho and Oliveira, 2003; Almeida et al., 2004). Another pathway potentially relevant to understand the neuroprotection afforded by A_{2A}R blockade is the control by A_{2A}Rs of MAPKs (Schulte and Fredholm, 2003; Seidel et al., 1999), which is independent of the cAMP pathway (Gsandtner et al., 2005; Klinger et al., 2002; Sexl et al., 1997), and is known to control the susceptibility of brain tissue to damage (Wang et al., 2004). Accordingly, it was recently proposed that the control by A_{2A}Rs of the ischemia-induced brain damage was related the ability of A_{2A}R antagonists to blunt the ischemia-induced accumulation of phosphorylated forms of p38 (Melani et al., 2006). However, the elucidation of the mechanism by which A_{2A}Rs control neuronal apoptosis might only be achieved after establishing the transducing systems operated by A_{2A}Rs in neurons, an issue that largely remains undefined.

In conclusion, we now report that A_{2A}R antagonists prevent STS-induced apoptosis, which provide a rationale to understand the ability of A_{2A}R antagonists to prevent the extent of neuronal damage caused by noxious brain stimuli (reviewed in Cunha, 2005).

Acknowledgments

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