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Short communication

SCH 58261 differentially influences quinolinic acid-induced effects in striatal and in hippocampal slices

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Abstract

The influence of the adenosine A_{2A} receptor antagonist SCH 58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-trizolo[1,5-c] pyrimidine) (50, 200 nM, 1 μM) on quinolinic acid effects has been studied in rat striatal and hippocampal slices. Quinolinic acid induced disappearance of field potentials at concentrations of 500 μM and 2 mM in hippocampal and corticostriatal slices, respectively. We found that 1 μM SCH 58261 prevented quinolinic acid-induced field potential disappearance in corticostriatal but not in hippocampal slices. This finding demonstrates that the peculiar binding profile of SCH 58261 and the predominance in the hippocampus of ''atypical'' adenosine A_{2A} receptor population (not recognized by SCH 58261) could have a functional relevance in the occurrence of region-specific neuroprotective effects.

Keywords: Striatum; Hippocampus; Quinolinic acid; Adenosine A_{2A} receptor; Neuroprotection

1. Introduction

In the central nervous system adenosine interacts with at least four different G protein-coupled receptors, namely adenosine A_1, A_{2A}, A_{2B} and A_3 receptors (Fredholm et al., 1994).

Many evidence suggest that adenosine A_{2A} receptors are involved in the modulation of excitotoxic processes and that their blockade could be neuroprotective. In particular, adenosine A_{2A} knock-out mice have been found to be more resistant to ischaemia- and MPTP (1-methyl-4-phenyl-1,2,3,6, tetrahydropyridine)-induced neuronal damage (Chen et al., 1999, 2001). Moreover, selective adenosine A_{2A} receptor antagonists have shown neuroprotective effects in models of neurodegenerative diseases in which excitotoxic mechanisms are thought to play a pathogenetic role (Bona et al., 1997; Gao and Phillis, 1994; Impagniatiello et al., 2000; Monopoli et al., 1998; Popoli et al., 2002).

Adenosine A_{2A} receptors are mainly expressed in the striatum (Jarvis et al., 1989), although reduced levels of expression also exist at cortical and hippocampal level (Rosin et al., 1998). It has been reported that the striatal binding sites represent ''classical'' adenosine A_{2A} receptors, whereas the cortical and hippocampal sites may be considered as ''atypical'' adenosine A_{2A} receptors (Cunha et al., 1996).

SCH 58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-trizolo[1,5-c] pyrimidine) is a potent and selective non-xanthine-derivative adenosine A_{2A} receptor antagonist which is able to discriminate between the two different adenosine A_{2A} receptor binding sites. Specifically, unlike striatal adenosine A_{2A} receptors, ''atypical'' adenosine A_{2A} binding sites in the hippocampus are not recognised by SCH 58261 (Lindström et al., 1996).

Whether this different distribution of ''classical'' and ''atypical'' adenosine A_{2A} receptors could underlie a regional specificity in the neuroprotective effects of SCH 58261 has never been investigated.

The aim of the present work was to verify, on a functional ground, the relevance of the peculiar binding profile of SCH 58261 in the possible occurrence of region-specific effects. To this end, the ability of SCH 58261 to attenuate quinolinic acid-induced effects in striatal and hippocampal slices was compared.

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2. Materials and methods

2.1. Subjects

Adult male Wistar rats (250–280 g) were used. The animals were kept under standardised temperature, humidity and lighting condition, with free access to water and food. Animal care and use followed the directives of the Council of the European Communities (86/609/EEC). The experimental protocol was approved by our Institutional Ethics Committee.

2.2. Preparation and maintenance of the slices

The animals were decapitated under ether anesthesia and the brain was quickly removed from the skull. Transverse hippocampal slices (400–450 μm thick) and coronal corticostriatal slices (300 μm thick) were cut with a tissue chopper and a vibratome, respectively. Slices were maintained at room temperature (22–24 °C) in artificial cerebrospinal fluid (ACSF) containing (mM): 126 NaCl, 3.5 KCl, 1.2 NaH_2PO_4, 1.3 $MgCl_2$, 2 $CaCl_2$, 25 $NaHCO_3$, 11 glucose (pH7.3) saturated with 95% O_2 and 5% CO_2. After

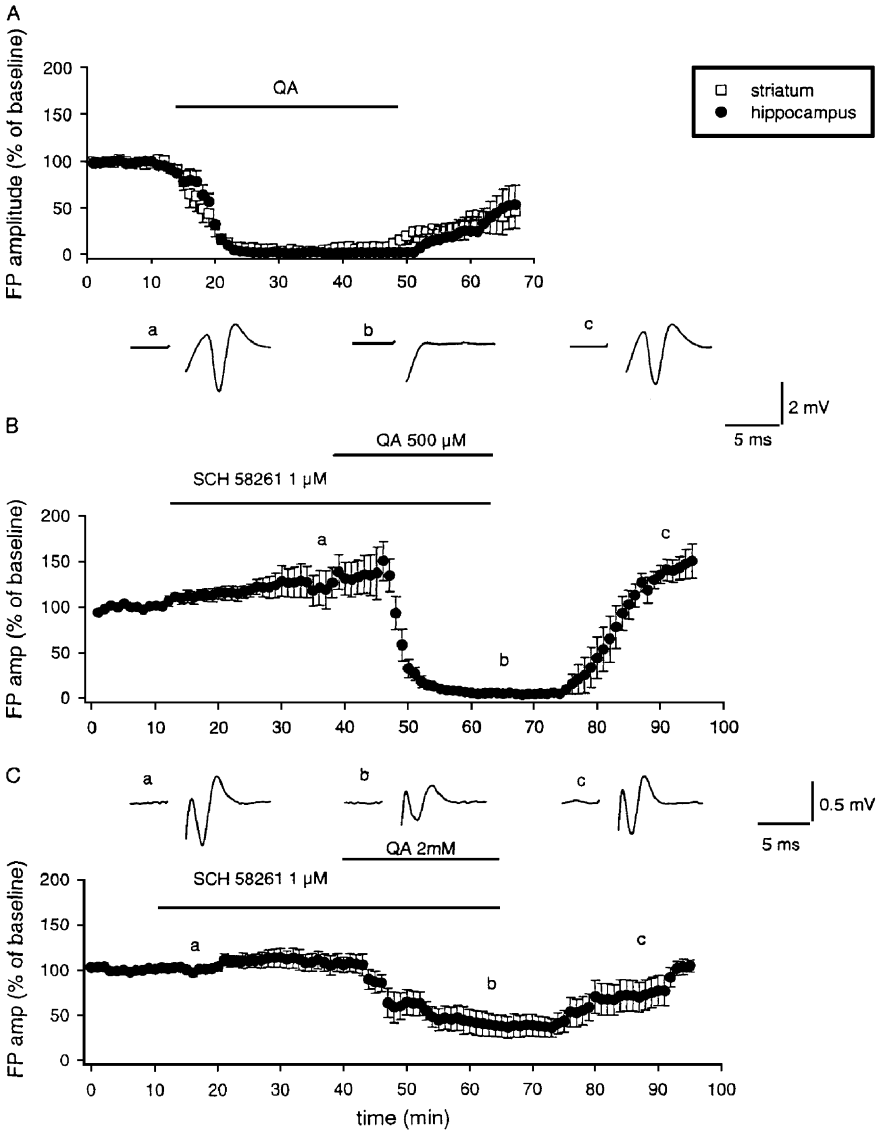

Fig. 1. Influence of SCH 58261 on quinolinic acid-induced effects in hippocampal and corticostriatal slices. In (A) is shown the time course of the effects of quinolinic acid in hippocampal (500 μM) and corticostriatal (2 mM) slices. Each point represents the mean of five (hippocampus) and six (striatum) slices. In (B) and (C) is shown the effect of 1 μM SCH 58261 on quinolinic acid-induced disappearance of field potential in hippocampal and corticostriatal slices. Note that SCH 58261 did not modify the quinolinic acid response in hippocampal slices ($N=4$) (B) but it prevented the disappearance of field potential induced by quinolinic acid in corticostriatal slices ($N=6$) (C). The period of drug application is indicated by the horizontal bars. All the values are expressed as percentage of baseline values (mean ± S.E.M.). Insets show field potentials recorded at the time indicated by the letters on the graphs. Each trace is the average of three successive field potentials (artefacts of stimulation have been truncated).

incubation in ACSF for at least 1 h, a single slice was transferred to an interfaced recording chamber and continuously superfused at 32–33 °C with ACSF at rate of 2 ml/min. Extracellular field potentials were recorded in the medio-dorsal striatum or in the stratum pyramidale of the CA1 area of the hippocampus with a glass microelectrode filled with 2 mM NaCl (pipette resistance 2–5 MΩ). A bipolar twisted NiCr-insulated electrode (50 μm o.d.) was used to stimulate the Schaffer collaterals (hippocampal slices) or the corticostriatal fibers (striatal slices). Stimulus was delivered every 20 s (frequency 0.05 Hz, duration 100 μs) and three responses were averaged. Signals were acquired with a DAM-80 AC differential amplifier (WPI) and analysed with the "LTP software" (courtesy of Dr W.W. Anderson, University of Bristol, UK).

In each experiment, the mean basal field potential amplitude was expressed as mean from the values obtained over the 10-min period immediately preceding drug application. A reduction of at least 90% of basal field potential amplitude after quinolinic acid application was defined a field potential disappearance.

2.3. Drugs

SCH 58261 was a gift from Schering-Plough (Milan, Italy). Quinolinic acid was purchased from RBI/Sigma (Natick, MA). Drugs were applied in the perfusing solution after at least 10 min of baseline recording. Quinolinic acid was applied to the slices over 20–25 min. SCH 58261 was applied over 30 min before quinolinic acid and then both drugs were applied together. The washout period lasted 30 min.

3. Results

The stimulation of afferent fibers in hippocampal and striatal slices resulted in field potentials of mean basal amplitude of 2.26 ± 0.3 and 0.87 ± 0.13 mV, respectively. The perfusion of rat hippocampal slices with 500 μM quinolinic acid caused, within 11.2 ± 1.16 min, a complete depression (− 97% in mean) of the field potential amplitude which was preceded by the appearance of one to three secondary spikes. In order to achieve a comparable effect in corticostriatal slices (i.e. 94.3% depression of field potential within 13.5 ± 2.4 min), a concentration of 2 mM quinolinic acid was needed. No secondary spikes were evoked by quinolinic acid in striatal slices. The responses recovered, after 30 min of washout, to 66.83 ± 25.48% of the basal field potential amplitude in four of five hippocampal slices and to 56.47 ± 19.14% of the basal field potential amplitude in five of six corticostriatal slices. The recovery started in mean after 15 ± 4.56 and 11.8 ± 3.61 min in hippocampal and striatal slices, respectively (Fig. 1A).

SCH 58261 was tested at concentrations of 50 nM, 200 nM and 1 μM. No effects on basal field potential amplitude were observed after perfusion of SCH 58261 50 and 200 nM either in hippocampal and in striatal slices. When applied at 1 μM, SCH 58261 tended to increase the field potential basal amplitude in both areas (see Fig. 1B,C).

In hippocampal slices, SCH 58261 was unable to prevent quinolinic acid-induced secondary spikes or field potential, disappearance throughout the concentration range tested (50 nM to 1 μM) (Fig. 2). Specifically, in slices perfused with SCH 58261 1 μM plus quinolinic acid, field potential response disappeared in four of the four experiments with a mean latency of 14.25 ± 3.1 min (Fig. 1B). The responses recovered to 98.91 ± 34.3% (not significantly different versus quinolinic acid alone).

In corticostriatal slices, SCH 58261 did not influence the effects of quinolinic acid when administered at 50 or 200 nM (Fig. 2). In slices perfused with 1 μM SCH 58261 plus quinolinic acid, the field potential response disappeared in two ot the six experiments with a latency of 20 and 25 min, respectively. In the remaining four experiments, the response was reduced to 53.70 ± 10.57% of the basal field potential amplitude. When all the percentage reductions obtained in the six experiments were considered, the mean field potential amplitude after quinolinic acid was 38.16 ± 11.89% of the basal amplitude ($P < 0.05$ versus quinolinic acid alone according to Mann–Whitney U-test) (Figs. 1C and 2). The responses fully recovered (107 ± 11.4% of the basal field potential amplitude, $P < 0.05$ versus quinolinic acid alone according to Mann–Whitney U-test) after 30 min of washout.

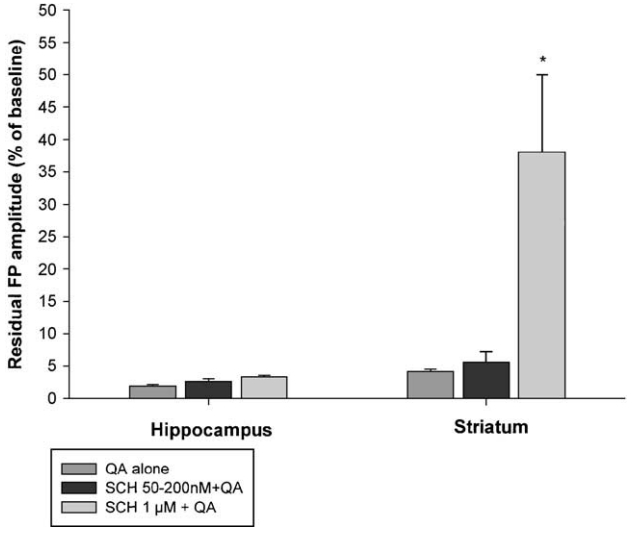

Fig. 2. Influence of SCH 58261 on quinolinic acid-induced field potential disappearance. In hippocampal slices, SCH 58261 was unable to influence the residual field potential amplitude, calculated at the end of quinolinic acid application. In corticostriatal slices, SCH 58261 1 μM significantly increased the residual field potential amplitude with respect to quinolinic acid alone. All values are expressed as percentage of baseline values (mean ± S.E.M.; $N = 5–6$ in each group). Data from SCH 58261, 50 and 200 nM in hippocampal and striatal slices were cumulated. * $P < 0.05$ versus quinolinic acid alone and SCH 58261 50–200 nM + quinolinic acid (Mann–Whitney U-test).

4. Discussion

The main finding of the present study is that SCH 58261, a selective adenosine A_{2A} receptor antagonist, significantly reduced the effects of quinolinic acid in corticostriatal but not in hippocampal slices, thus indicating that the effects of this compound are region-specific.

As previously demonstrated (Perkins and Stone, 1983), and in line with the well-known susceptibility of the hippocampus to excitotoxins, the hippocampal slices were much more sensitive than striatal slices to the actions of quinolinic acid. In spite of the different concentrations of quinolinic acid used in hippocampal and in striatal slices, however, the effects observed in the two areas were clearly superimposable, thus allowing a proper comparison between the effects of SCH 58261.

In line with the observation that the predominant adenosine A_{2A} receptor population in the hippocampus belongs to "atypical" receptors (Cunha et al., 1996), which are not recognised by SCH 58261 (Lindström et al., 1996), this compound (administered at a concentration that was effective in striatal slices) was totally ineffective in preventing quinolinic acid-induced field potential reduction in hippocampal slices. This finding suggests, for the first time, that the peculiar binding profile of SCH 58261 has a functional relevance in the occurrence of region-specific effects. In agreement with our findings, it was reported that SCH 58261 was unable to antagonise the effects of an adenosine A_{2A} receptor agonist in rat hippocampal slices (Latini et al., 1999). It should be noted, however, that in hippocampal slices treated with SCH 58261 plus quinolinic acid, after 30 min of washout the electrical response regained a mean amplitude very close to the basal values. Although such an effect does not reach the statistically significance with respect to quinolinic acid alone, this finding might indicate a sort of "partial" effect of SCH 58261 in hippocampal slices. Whether this might imply that the modest amount of typical adenosine A_{2A} receptors in the hippocampus could play a role in the modulation of quinolinic acid effects should be further investigated.

The finding that SCH 58261 significantly reduced quinolinic acid-induced effects in striatal slices is in line with a recent report from our group showing the ability of SCH 58261 to prevent quinolinic acid-induced striatal damage "in vivo" (Popoli et al., 2002). In the present report SCH 58261 was effective at a concentration of 1 μM, which is in line with a previous work showing protective effects of 1 μM SCH 58261 towards kainate toxicity in striatal slices (Chergui et al., 2000). Even though we cannot definitely exclude the possibility that adenosine receptors other than A_{2A} may be blocked by 1 μM SCH 58261, it should be noted that SCH 58261 has little or no affinity for adenosine A_{2B} and A_3 receptors, with interactions occurring at concentrations >1 μM (Varani et al., 1998; Ongini et al., 1999). As for the possibility that, besides adenosine A_{2A}, adenosine A_1 receptors may be contemporary blocked by SCH 58261 1 μM, this point deserves some consideration. In fact, adenosine A_1 receptor blockade has been reported to inhibit the depression of the electrical response induced by hypoxia and aglycemia in hippocampal and striatal slices, respectively (Calabresi et al., 1997; Latini et al., 1999; Sebastião et al., 2001). Thus, one could argue that the effects exerted by SCH 58261 in striatal slices (i.e. inhibition of quinolinic acid-induced field potential depression) are mediated, at least in part, by adenosine A_1 receptor blockade. This view, however, is at odds with the full recovery of the field potential amplitude occurring in slices treated with SCH 58261 plus quinolinic acid, since A_1 receptor antagonists have been reported to impair the recovery of the electrical response in hypoxic slices (Sebastião et al., 2001). Moreover, given the high level of expression of adenosine A_1 receptors in the hippocampus, if adenosine A_1 receptor blockade had been actually involved in the inhibition of quinolinic acid-induced field potential reduction by SCH 58261, such an effect should have been observed also in hippocampal slices. Thus, on the basis of the above considerations, it is likely that the present effects of SCH 58261 are actually mediated by a blockade of adenosine A_{2A} receptors.

As for the possible mechanism of the protective effects of SCH 58261 in the striatum, the experimental preparation used here does not allow to investigate it in depth. Quinolinic acid acts indeed both at pre-synaptic (by increasing the release of glutamate) and at post-synaptic (by stimulating NMDA receptors) levels (Stone, 1993), and SCH 58261 was found to exert opposite effects at these two sites (namely it reduced quinolinic acid-evoked glutamate release in the rat striatum, but it tended to amplify the increase in intracellular Ca^{2+} levels induced by quinolinic acid on striatal neurones, see Popoli et al., 2002). Although in the slice preparation, the post-synaptic component of the quinolinic acid effect is likely to play a major role, it was shown that the stimulation of glutamate release from corticostriatal terminal is indispensable for quinolinic acid toxicity to occur (Orlando et al., 2001). Moreover, the inhibition of quinolinic acid-induced glutamate release was enough to achieve neuroprotection (Popoli et al., 2002).

Further studies are needed in order to evaluate the real neuroprotective potential of SCH 58261 and other adenosine A_{2A} antagonists in models of excitotoxicity in which pre- and post-synaptic mechanisms play different relative roles.

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