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European Journal of Pharmacology 421 (2001) 177–180

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Short communication

SCH 58261 (an adenosine A_{2A} receptor antagonist) reduces, only at low doses, K^+-evoked glutamate release in the striatum

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Received 4 April 2001; received in revised form 10 May 2001; accepted 15 May 2001

Abstract

The aim of the present work was to determine whether systemic administration of the adenosine A_{2A} receptor antagonist, SCH 58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4,triazolo[1,5-c]pyrimidine), could modulate striatal glutamate outflow in the rat. Microdialysis experiments were performed in male Wistar rats implanted with microdialysis probes in the striatum. Pretreatment (15 min before) with SCH 58261 (0.01 and 0.1, but not 1 mg/kg intraperitoneally) significantly prevented K^+-stimulated glutamate release. These results suggest that SCH 58261 could possess neuroprotective effects in the low dose range, while, at higher doses, the occurrence of additional mechanisms may limit the neuroprotective potential of this drug.

Keywords: SCH 58261; Glutamate; Striatum; Microdialysis

1. Introduction

Excitotoxic mechanisms are thought to be involved in the pathogenesis of several central nervous system (CNS) diseases, such as ischaemia, epilepsy and chronic neurodegenerative disorders. In particular, it has been postulated that an abnormal release of glutamate may play a crucial role in triggering the cellular events leading to neuronal death (Choi and Rothman, 1990; Rossi et al., 2000). In the past few years, the classical concept of high extracellular glutamate as a key and common pathogenetic mechanism in neurological disorders has been questioned (Obrenovitch and Urenjak, 1997). In particular, it has been observed that the levels of glutamate necessary to induce neuronal death are largely higher than those measured in models of neurodegenerative diseases, and that an increase in glutamate extracellular levels may not be a good index of excitotoxicity (Obrenovitch et al., 2000). Moreover, it has been shown that the efficacy of some neuroprotective drugs cannot be simply ascribed to their ability to inhibit glutamate release (Calabresi et al., 2000). Although the above observations suggest that a decrease in extracellular glutamate levels may not necessarily be neuroprotective, they do not rule out a contribution of increased glutamate release in inducing neuronal death. Inhibition of evoked glutamate release has been indeed reported to parallel the neuroprotective effects of some compounds (Mauler et al., 2001; O'Neill et al., 2000). Thus, the modulation of glutamate release can still be regarded as a potential neuroprotective mechanism.

In the past few years, increasing evidence has suggested that antagonists of adenosine A_{2A} receptors have neuroprotective properties in different models of neurodegenerative diseases (Abbracchio and Cattabeni, 1999; Impagniatiello et al., 2000; Ongini et al., 1997). The selective adenosine A_{2A} receptor antagonist, SCH 58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4,triazolo[1,5-c]-pyrimidine) has been reported to significantly reduce brain injury in a model of focal cerebral ischaemia in the rat (Monopoli et al., 1998b) and to prevent the striatal damage induced by intrastriatal injection of quinolinic acid in the rat, a rodent model of Huntington's disease (Popoli et al., 2000a). In both models, SCH 58261 was found to be effective after systemic administration of doses as low as 0.01 mg/kg. Although the mechanism underlying the neuroprotective effects of adenosine A_{2A} receptor antagonists has yet to be elucidated, a modulation of glutamate release may well be involved. An involvement of adenosine A_{2A} receptors in the regulation of glutamatergic transmission has indeed been suggested by the findings that, when perfused through microdialysis probes, selective adenosine A_{2A} receptor ligands significantly influence stri-

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01058-5

atal glutamate outflow (Corsi et al., 1999, 2000; Popoli et al., 1995). The possible effects of systemic SCH 58261 on striatal glutamate release have never been investigated.

The aim of the present work was to verify whether the above reported neuroprotective effects of SCH 58261 could be ascribed, at least, in part, to a reduction of extracellular glutamate levels. To this end, the influence of SCH 58261, administered by the same route and in the same range of doses reported to exert neuroprotective effects, on K^+-stimulated glutamate outflow was studied by striatal microdialysis.

2. Materials and methods

2.1. Animals

Young (3 months) male Wistar rats (Charles-River, Como, Italy) weighing 280–320 g were used. The animals were kept under standardized temperature, humidity and lighting conditions, and had free access to water and food. Animal care and use followed the directives of the Council of the European Communities (86/609/EEC).

2.2. Microdialysis experiments

Under Equithesin anaesthesia, the animals were placed in a stereotaxic frame and implanted with a concentric dialysis probe (mod CMA/12, 4 mm length, Carnegie Medicine, Sweden) into the striatum. Stereotaxic coordinates in millimeters from the bregma, sagittal suture and dura, respectively, were as follows: A = +2.0, L = +2.5, V = −6.8. Twenty-four hours later, the probe was perfused at a rate of 2 μl/min with Ringer's solution (NaCl, 147; $CaCl_2$, 2.3 and KCl, 4.0 mM). After a washout period of at least 1 h, samples were collected every 5 min into a refrigerated fraction collector (mod CMA/170) and then frozen until assayed. SCH 58261 (0.01, 0.1 and 1 mg/kg) was administered intraperitoneally (i.p.) either without high-K^+ stimulation or 15 min before starting probe perfusion with Ringer's solution containing 100 mM K^+. Each experimental group was made up of four to five animals. Each rat was killed with an overdose of Equithesin. The brain was fixed with 4% paraformaldehyde, and coronal sections (20 μm thick) were cut to verify probe location. The glutamate content of all samples was measured by reverse-phase high-performance liquid chromatography coupled to a fluorometric detector (Perkin Elmer LC240 at wavelength of 335 nm and emission cut-off filter of 425 nm) using a 15-min gradient elution program (methanol from 20% to 80% with 50 mM NaH_2PO_4 and CH_3COONa) and automatic precolumn derivatization with ophthalaldehyde and β-mercaptoethanol. Cysteic acid was used as an internal standard. The concentration of the standard was linear ($r^2 = 0.99$) between 0.2 and 25 ng/10 μl. Basal glutamate levels were calculated by comparison of sample peak height with external standard peak height, both corrected for the internal standard peak height and expressed as ng/10 μl without probe recovery correction. Data were processed by two-way analysis of variance (ANOVA) followed by post hoc Student's *t*-test. The accepted level of statistical significance was $P < 0.05$.

3. Results

Basal extracellular levels of glutamate were 6.7 ± 0.35 μM. Probe perfusion with 100 mM K^+ over 20 min

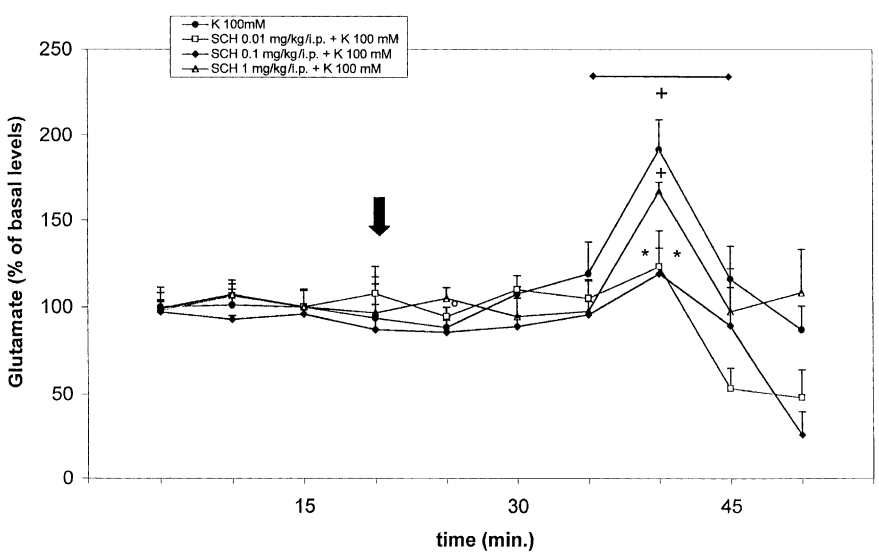

Fig. 1. Influence of SCH 58261 (0.01, 0.1 and 1 mg/kg i.p.) on K^+-stimulated glutamate outflow in the rat striatum. SCH 58261 was administered 15 min before the start of probe perfusion with 100 mM K^+. The arrow and the bar indicate the time of SCH 58261 injection and of high-K^+ perfusion, respectively. Each group was composed of four to five animals. * = $P < 0.05$ versus K^+ 100 mM alone.

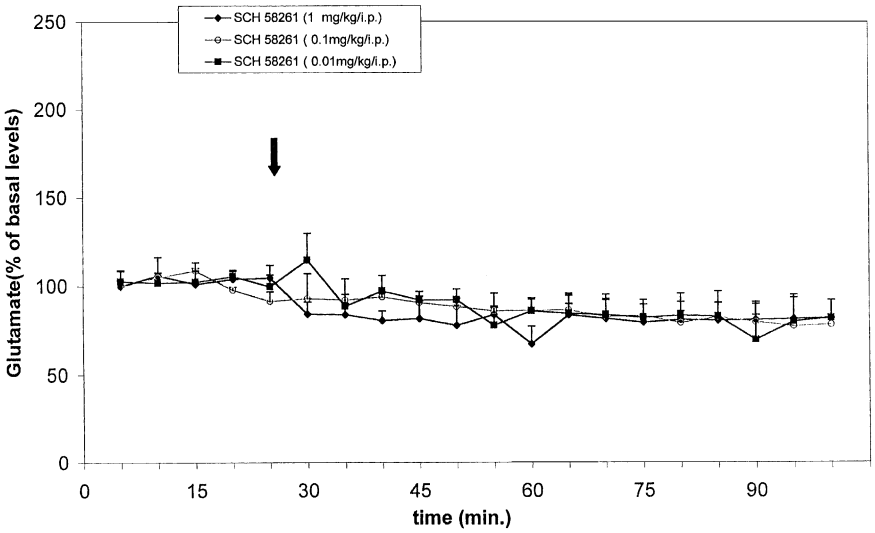

Fig. 2. SCH 58261 (0.01, 0.1 and 1 mg/kg i.p.) does not influence basal glutamate levels in the rat striatum. The arrow indicates the time of SCH 58261 injection. Each group was composed of four animals.

elicited a significant increase in glutamate extracellular levels with respect to basal values (mean effect: +190 ± 17%, $P = 0.01$ versus the mean of basal samples, Fig. 1).

Pretreatment (15 min before the start of perfusion with 100 mM K^+) with SCH 58261 (0.01 and 0.1 mg/kg i.p.) significantly reduced the increase in glutamate levels elicited by high K^+ (Fig. 1). Conversely, the highest dose of SCH 58261 (1 mg/kg i.p.) was ineffective in preventing K^+-stimulated glutamate release (Fig. 1). None of the tested doses of SCH 58261 (0.01, 0.1 and 1 mg/kg i.p.) significantly influenced basal glutamate extracellular levels (Fig. 2).

4. Discussion

The new finding of this study is that SCH 58261, when given systemically in low doses (0.01 and 0.1 mg/kg i.p.), significantly prevented high K^+-stimulated glutamate release in the rat striatum. Since SCH 58261 was reported to exert neuroprotective effects when administered at the same doses and by the same route of administration in a model of striatal excitotoxicity (Popoli et al., 2000a), it is conceivable that an inhibition of glutamate outflow contributes to the effects of the drug. The same mechanism could also be invoked to explain the beneficial effects of SCH 58261 (0.01 mg/kg i.p. or i.v.) in a rat model of cerebral ischaemia (Monopoli et al., 1998b). The ability of low doses of SCH 58261 to prevent K^+-stimulated glutamate outflow is in line with a recent report by Corsi et al. (2000), showing that a very low concentration of this drug significantly inhibits the effects of 100 mM K^+ on glutamate outflow in the rat striatum.

The finding that the highest tested dose of SCH 58261 (1 mg/kg i.p.) was no longer able to prevent K^+-stimulated glutamate release deserves some consideration. Although the most obvious explanation is that adenosine receptors other than A_{2A} (i.e., adenosine A_1 receptors, which are known to be inhibitory for glutamate release) may also be blocked by higher doses of SCH 58261, this is most probably not the case. In fact, SCH 58261 has a great A_{2A}/A_1 selectivity (Zocchi et al., 1996), and it has been reported to behave as a selective adenosine A_{2A} receptor antagonist at doses up to 2 mg/kg i.p. in in vivo studies (Popoli et al., 2000b). Interestingly, after i.p. administration in rats, SCH 58261 did not induce haemodynamic changes up to the dose of 0.1 mg/kg, while it increased blood pressure and heart rate starting from a dose of 1 mg/kg (Monopoli et al., 1998a). Thus, the occurrence of peripheral effects after the administration of the higher dose of SCH 58261 might play a role in the inversely dose-related effects of the drug. This suggests that low doses of SCH 58261 might have protective effects against excitotoxic processes by inhibiting adenosine A_{2A}-stimulated glutamate release, while higher doses could also block adenosine A_{2A} receptor-mediated effects on blood pressure (Stella et al., 1996) and on platelet aggregation (Dionisotti et al., 1992), thus eventually reducing blood and nutrient supply to the compromised brain area (Jones et al., 1998) and further precipitating the excitotoxic cascade. The fact that in animal models of excitotoxicity adenosine A_{2A} receptor activation was found to be protective, most probably by means of peripheral mechanisms (Jones et al., 1998), supports the hypothesis that blocking adenosine A_{2A} receptors in the periphery may be detrimental. Moreover, the possibility that different doses of SCH 58261 differently affect other A_{2A} receptor-mediated effects, such as inflammatory mechanisms (Sullivan et al., 1999), energy metabolism (Nehlig et al., 1994) and modulation of glial cell activity (Brodie et al., 1998), should also

be considered. Finally, it should also be pointed out that the experimental evidence provided in the present study (in which no agonists were used) does not allow us to conclude with certainty that the effects of SCH 58261 are merely mediated by adenosine A_{2A} receptor blockade. Although the present results are largely in line with previous studies showing that adenosine A_{2A} receptor agonists stimulate striatal glutamate release (Corsi et al., 1999; Popoli et al., 1995), the possible involvement of additional mechanisms, besides adenosine A_{2A} receptor blockade, in the effects of SCH 58261 cannot be ruled out.

In conclusion, the present results suggest that the inhibition of glutamate release may be one of the mechanisms underlying the neuroprotective effects of SCH 58261, at least, in the low dose range. They also suggest that, at higher doses, the occurrence of additional mechanisms, which have yet to be elucidated, may limit or even abolish the neuroprotective potential of this drug.

Acknowledgements

The authors thank Dr. A. Monopoli (Schering-Plough, Milano, Italy) for generously providing SCH 58261.

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