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Short communication

Adenosine A_{2A} receptor stimulation enhances striatal extracellular glutamate levels in rats

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Abstract

The influence of CGS 21680, an adenosine A_{2A} receptor agonist, on striatal glutamate extracellular levels was tested in a microdialysis study in rats. CGS 21680 (10 μM), infused intrastriatally through the microdialysis probe, greatly enhanced glutamate extracellular levels. These results show that striatal adenosine A_{2A} receptors are involved in the regulation of striatal glutamate extracellular levels. They also suggest that adenosine A_{2A} receptor antagonists may possess neuroprotective effects in models of striatal excitotoxicity.

Keywords: Adenosine A_{2A} receptor; Glutamate release; Striatum; (Rat)

1. Introduction

Adenosine has been reported to act as a potent neuromodulator (Williams, 1989). Several types of adenosine receptors (A_1, A_{2A}, A_{2B}, A_3, A_4) have been identified (Dalziel and Westfall, 1994). Among these, adenosine A_1 and A_{2A} receptors seem to be particularly involved in the regulation of neurotransmitter release.

As has been reported for acetylcholine (Brown et al., 1990), some evidence suggests that adenosine A_1 and A_{2A} receptors exert opposing influences on excitatory amino acid release (Simpson et al., 1992).

In particular, adenosine A_1 receptor agonists have been reported to decrease excitatory amino acid release from the ischemic rat cerebral cortex (Simpson et al., 1992), and kainate-induced glutamate release in the rat striatum (Arvin et al., 1989).

As for adenosine A_{2A} receptors, previous studies in rats showed that CGS 21680, an adenosine A_{2A} receptor agonist, enhanced excitatory amino acid release from the ischemic cerebral cortex (O'Reagan et al., 1992), as well as evoked glutamate release from the nucleus tractus solitarius (Castillo-Meléndez et al., 1994).

The aim of the present paper was to investigate the possible involvement of adenosine A_{2A} receptors in the regulation of striatal glutamate extracellular levels.

2. Materials and methods

2.1. Microdialysis procedure

Male Wistar rats (250–280 g) were anesthetized with Equithesin (3 ml/kg) and placed in a stereotaxic frame. Microdialysis probes (mod CMA/12, length 4 mm, Carnegie Medicine, Sweden) were inserted vertically in the striatum (stereotaxic coordinates: A = +1.7; L = +2.5; V = −6 mm from bregma, sagittal suture and dura, respectively). Perfusion was started 24 h after probe implantation at a flow rate of 2 μl/min with a Ringer solution composed as follows (mM): NaCl 147, $CaCl_2$ 2.3 and KCl 4.0 (Pazzagli et al., 1994). Sample collection (collection time = 10 min) started after 1 h of perfusion. CGS 21680 was dissolved in the Ringer solution and injected through the probe. The samples were collected in a refrigerated fraction collector (mod CMA/170, Carnegie Medicine), and then frozen until

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assay. Correct probe location was ascertained by post-mortem histological investigation.

2.2. Glutamate analysis

A high-performance liquid chromatographic method with coulometric electrochemical detection was used for the determination of glutamate. The dialysate samples were derivatized through a pre-column with phenylisothiocyanate (Sherwood et al., 1990) and analyzed on an isocratic system using a reversed-phase column (3.9 × 75 mm). The working parameters for the electrochemical detector were +400 mV for the first electrode and +600 mV for the second electrode. The signal generated by the second electrode was used for the quantitative determination. Detection limit was 10 pg/μl.

3. Results

Basal glutamate levels (mean ± S.E.M.) were 0.79 ± 0.09 ng/μl. In each experiment, the glutamate release observed after the administration of CGS 21680 was expressed as percentage of the pre-drug level (mean of 5 samples).

As shown in Fig. 1, the administration of CGS 21680 10 μM dramatically increased the striatal glutamate extracellular levels with respect to the pre-drug levels. Administered at a lower concentration (100 nM), CGS 21680 did not significantly affect the extracellular levels of glutamate ($n = 4$, data not shown).

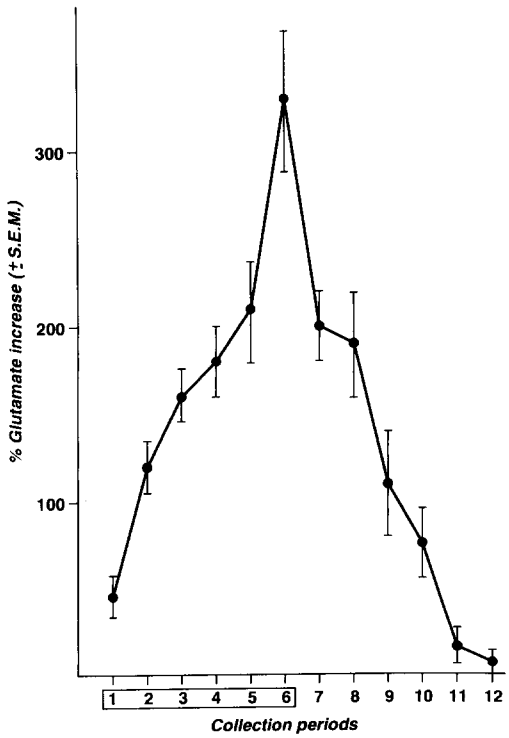

Fig. 1. Influence of intrastriatal perfusion with CGS 21680 (10 μM) on non-stimulated extracellular glutamate levels. Collection periods (10 min each) 1–6: administration of CGS 21680 through the dialysis probe. Collection periods 7–12: perfusion with Ringer. The flow rate was always 2 μl/min. Each point represents the mean value ± S.E.M. from 4 samples.

4. Discussion

The present results clearly show that stimulation of adenosine A_{2A} receptors increases the striatal extracellular glutamate levels in non-stimulated conditions. This finding is in line with the results of previous studies showing that CGS 21680 enhanced excitatory amino acid release from brain areas other than the striatum (see Introduction), and that CGS 15943, an adenosine A_2 receptor antagonist, reduced ischemic injury in the gerbil hippocampus (Gao and Phillis, 1994).

Even though CGS 21680 is effective at nM concentrations in in vitro studies (Ferré et al., 1991), the fact that remarkably higher doses are required under the present experimental conditions – in which poor diffusion through the dialysis probe is likely to occur – is not surprising. In fact, the same concentrations of CGS 21680 we used were needed in previous dialysis studies to stimulate adenosine A_{2A} receptors (Ferré et al., 1993; Castillo-Meléndez et al., 1994). A reduced selectivity of CGS 21680 at this dose would imply a concomitant stimulation of adenosine A_1 receptors and, as a consequence, an inhibitory effect on glutamate release. On the basis of these considerations, the potentiating effect of CGS 21680 10 μM on striatal glutamate release can be ascribed to a stimulation of adenosine A_{2A} receptors.

Even though conflicting data have been provided concerning their presence on corticostriatal terminals, dopamine D_2 receptors regulate striatal glutamate release (Yamamoto and Davy, 1992).

Since adenosine A_{2A} receptors have been reported to inhibit the activity of dopamine D_2 receptors (Ferré et al., 1991; Popoli et al., 1994), one possible explanation for the present results is that an inhibition of dopamine D_2 receptors may underlie the effects of CGS 21680 on extracellular glutamate levels. Alternatively, a presynaptic influence of adenosine A_{2A} receptors may be invoked.

Even though further studies are needed to elucidate the mechanisms responsible for the effects of CGS 21680, one possible implication of the present results is that adenosine A_{2A} receptor antagonists may possess neuroprotective effects in models of striatal excitotoxicity.

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