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European Journal of Pharmacology 451 (2002) 161–164

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Short communication

Adenosine A_{2A} receptors inhibit the *N*-methyl-D-aspartate component of excitatory synaptic currents in rat striatal neurons

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Received 27 June 2002; received in revised form 9 August 2002; accepted 16 August 2002

Abstract

The effects of the adenosine A_{2A} receptor agonist 2-*p*-(2-carboxyethyl)phenethyl-amino-5′-*N*-ethylcarboxamidoadenosine (CGS 21680) on currents mediated by excitatory amino acid receptors were examined in rat striatal brain slices. In a Mg^{2+}-free superfusion medium, CGS 21680 decreased the amplitude of excitatory postsynaptic currents (EPSCs) in about 70% of striatal neurons. The inhibitory effect of CGS 21680 disappeared both in the presence of the adenosine A_{2A} receptor antagonist 8-(3-chlorostyryl) caffeine and the NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (AP-5). NMDA-induced currents were also depressed by CGS 21680 in a subset of striatal cells, whereas α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)-induced currents were not affected. The results suggest that adenosine A_{2A} receptor agonists inhibit the NMDA component of the EPSC.

Keywords: Patch-clamp, whole-cell; Adenosine A_{2A} receptor; NMDA receptor; Striatum

1. Introduction

The most notable cell type of the striatum is the γ-aminobutyric acid (GABA)ergic medium spiny neuron that constitutes 90–95% of the total cell population (Smith and Bolam, 1990) and plays a major role in the regulation of locomotor behaviour (Gerfen, 1992). These neurons receive massive glutamatergic inputs from the cerebral cortex (Alexander and Crutcher, 1990; Calabresi et al., 1996) and their axons constitute the two major striatal output pathways (Parent and Hazrati, 1995). The enkephalin positive striatopallidal neurons project to the globus pallidus and express adenosine A_{2A} receptors. The substance P positive striatonigral neurons project to the basal ganglia output nuclei and do not express adenosine A_{2A} receptors (Augood et al., 1999).

It has been shown that adenosine A_{2A} receptor agonists depress inward currents evoked by bath-applied *N*-methyl-D-aspartate (NMDA) in a subset of striatal neurons, which appear to belong to the striatopallidal subpopulation (Nörenberg et al., 1997; Wirkner et al., 2000). In the present study, whole-cell patch-clamp methods were applied to rat striatal neurons in brain slices in order to investigate whether adenosine A_{2A} receptor activation has a modulatory influence on excitatory postsynaptic currents (EPSCs) mediated by NMDA and non-NMDA receptors.

2. Materials and methods

2.1. Brain slice preparation

Young Wistar rats (8–13 days old) were anaesthetized with diethylether and decapitated, their brains were quickly removed and submerged in ice-cold artificial cerebrospinal fluid (ACSF) saturated with 95% O_2 and 5% CO_2 of the following composition (mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $CaCl_2$ 2.4, $MgCl_2$ 1.3, $NaHCO_3$ 26 and glucose 10; pH 7.4. Coronal slices (200 μM thick), containing parts of the neocortex–corpus callosum and the neostriatum, were prepared. Then, three to five slices were transferred to a holding chamber and stored in oxygenated ACSF at 36 °C for at least 1 h before use. Subsequently, single slices were continuously superfused (3 ml/min) with oxygenated, Mg^{2+}-free ACSF in a recording bath. Mg^{2+} was omitted

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PII: S0014-2999(02)02301-4

from the ACSF in order to augment NMDA-induced responses. Cells were visualized using an upright differential interference contrast microscope with a ×40 water immersion objective (Axioscope; Carl Zeiss, Oberkochen, Germany).

2.2. Electrophysiological techniques

Patch pipettes, produced from borosilicate glass capillaries, were filled with intracellular solution of the following composition (mM): potassium gluconate 140, NaCl 10, HEPES 10, EGTA 11, MgATP 1.5, LiGTP 0.3; pH 7.3 was adjusted with KOH. Pipette resistance was in the range of 3 to 6 MΩ. Stable series resistance values were consistently obtained within 20 min after seal formation and these values only slightly changed during the course of the experiments (<10%; see Wirkner et al., 2000). Currents were elicited while holding the membrane potential at −80 mV and filtering at 3 kHz with an EPC-7 amplifier (List Electronic, Darmstadt, Germany). Electrical stimulation (0.1 Hz; 0.1–0.5 ms; 1–10 V) was applied by means of bipolar tungsten electrodes to evoke EPSCs. NMDA (100 μM) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA; 30 μM) were administered for 1 s by a pressurized superfusion system (DAD-12, Adams and List, Westbury, NY, USA), in 2- and 3.3-min intervals, respectively. Data were digitized at 10 kHz and stored on a laboratory computer. Data analysis was performed by use of commercially available patch- and voltage-clamp software (Cambridge Electronic Devices, Cambridge, UK).

2.3. Materials and statistics

N-methyl-D-aspartate sodium salt, 2-*p*-(2-carboxyethyl)-phenethyl-amino-5′-*N*-ethylcarboxamidoadenosine, 8-(3-chlorostyryl) caffeine, α-amino-3-hydroxy-5-methylisoxazole-4-propionate, 2-amino-5-phosphonopentanoic acid, and 6-cyano-7-nitroquinoxaline-2,3-dione were purchased from Sigma (Deisenhofen, Germany). Stock solutions (10–100 mM) of drugs were prepared with distilled water or dimethyl sulphoxide and aliquots were stored at −20 °C. Further dilutions were made daily with ACSF. Equivalent quantities of the solvent had no effect.

The percentage changes of EPSC amplitudes, measured in the presence of various drugs applied for 10 min, were compared with the time-matching, drug-free controls. Means ± S.E.M. are shown. Statistical comparison with controls was made by one-way analysis of variance (ANOVA) followed by the Bonferroni *t*-test.

3. Results

The EPSCs recorded in this study were mediated by the NMDA and AMPA/kainate receptor channels, since both the NMDA receptor antagonist D(−)-2-amino-5-phosphonopentanoic acid (AP-5; 50 μM) and the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM) inhibited the currents by 28.4 ± 10.6% ($n=7$; $P<0.05$) and 51.9 ± 8.3% ($n=7$; $P<0.05$) of their control amplitude, respectively; the co-application of the two antagonists completely abolished the EPSCs ($n=7$). In 8 out of 11 cells, the adenosine A_{2A} receptor agonist 2-*p*-(2-carboxyethyl)phenethyl-amino-5′-*N*-ethylcarboxamidoadenosine (CGS 21680; 0.1 μM) approximately halved the EPSC amplitude (Fig. 1A,B), whereas in the residual three cells, no change could be detected (Fig. 1B). This inhibitory effect of CGS 21680 was abolished in the presence of the adenosine A_{2A} receptor antagonist 8-(3-chlorostyryl) caffeine (CSC; 10 μM) (Fig. 1B). In addition, CGS 21680 did not alter the AP-5 (50 μM)-insensitive EPSC, which corresponds to the AMPA/kainate component (Fig. 1C). It is interesting to note that in contrast to AP-5 (50 μM), which markedly shortened the EPSC by facilitating its offset (not shown), CGS 21680 (0.1 μM) did not appreciably alter the time-course of the excitatory synaptic current (Fig. 1A). This may be due to the fact that whereas AP-5 (50 μM) abolished the amplitude of the current response to pressure-

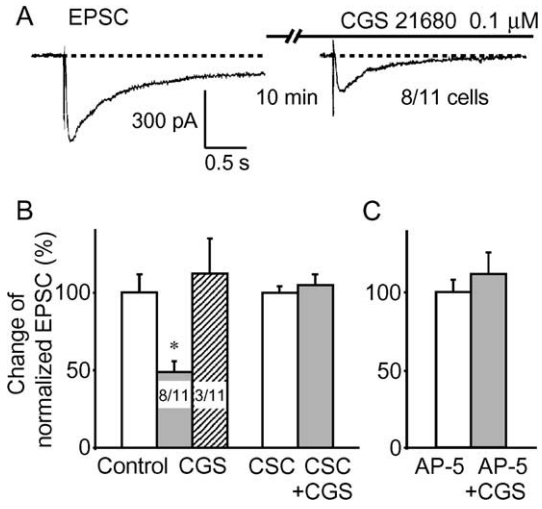

Fig. 1. Modulation by A_{2A} receptor activation of excitatory postsynaptic currents (EPSCs) evoked by electrical stimulation (0.1 Hz; 0.1–0.5 ms; 1–10 V) in striatal neurons. Series of six EPSCs were evoked during two stimulation periods separated by a 10-min interval. CGS 21680 (0.1 μM) was superfused immediately after the first stimulation period and was maintained until the end of the experiment. CSC (10 μM) and AP-5 (50 μM) were superfused either for 10 min immediately after the first stimulation period or were present 10 min before the beginning and also maintained until the end of the experiment. (A) Representative tracings of individual EPSCs are shown. Inhibitory effect of CGS 21680 on the amplitude of EPSCs in a subset (8 out of 11) of striatal neurons. (B) Percentage changes by CGS 21680 of normalized EPSC amplitudes both in the absence and presence of CSC. (C) Percentage changes by CGS 21680 of normalized EPSC amplitudes in the presence of AP-5. When given alone, CSC (10 μM) and AP-5 (50 μM) depressed the EPSC by 13.8 ± 3.7% ($n=7$; $P<0.05$) and 28.4 ± 10.6% ($n=6$; $P<0.05$), respectively (see the normalized empty columns in B and C). Each column represents the means ± S.E.M. of 6–11 experiments. *$P<0.05$; statistically significant difference from controls.

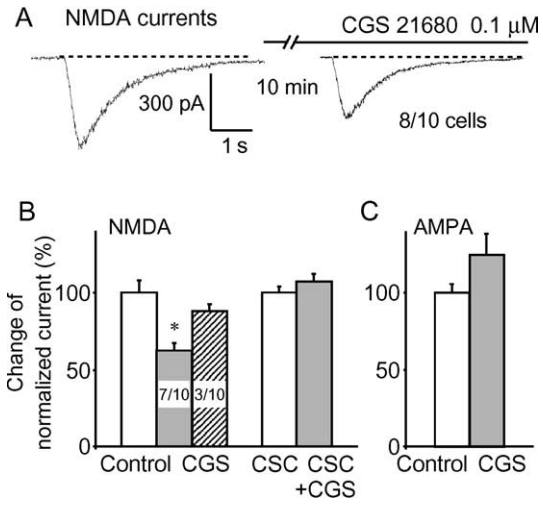

Fig. 2. Modulation by A_{2A} receptor activation of NMDA- but not AMPA-induced currents in striatal neurons. NMDA and AMPA currents were evoked by 1-s drug administration in 2- and 3.3-min intervals, respectively. At first, two responses of approximately similar size were produced by these excitatory amino acid agonists (NMDA, 100 μM; AMPA, 30 μM). CGS 21680 (0.1 μM) was superfused immediately after the second application period of NMDA or AMPA and was maintained until the end of the experiment. CSC (1 μM) was superfused either for 10 min immediately after the second application period of NMDA or was present 10 min before the beginning and also maintained until the end of the experiment. (A) Representative tracings of individual NMDA-induced currents are shown. Inward currents evoked by pressure-applied NMDA were inhibited in 7 out of 10 cells by CGS 21680. (B) Percentage changes by CGS 21680 of normalized NMDA currents both in the absence and presence of CSC. (C) Percentage changes by CGS 21680 of normalized AMPA currents. When given alone, CSC (1 μM) depressed the amplitude of the NMDA current by $14.2 \pm 3.4\%$ ($n = 8$; $P < 0.05$) (see the normalized empty column in B). Each column represents the means ± S.E.M. of 7–10 experiments. * $P < 0.05$; statistically significant difference from controls.

applied NMDA, CGS 21680 (0.1 μM) caused of about 35% inhibition only (Fig. 2A,B).

Pressure application of NMDA (100 μM) induced reproducible inward currents, which were decreased by CGS 21680 (0.1 μM) in 7 out of 10 cells (Fig. 2A). In the residual cells, CGS 21680 failed to affect the current amplitudes (Fig. 2B). The inhibitory effect of CGS 21680 (0.1 μM) was antagonized by CSC (1 μM; Fig. 2B). The AMPA (30 μM)-induced currents were not significantly changed by CGS 21680 (Fig. 2C).

4. Discussion

The main finding of the present study is that the adenosine A_{2A} receptor agonist CGS 21680 depressed the electrically evoked EPSCs mediated by NMDA but not by AMPA receptors in a subgroup of striatal neurons only. This subgroup might consist of the enkephalin-containing striatopallidal medium spiny neurons, since the other subgroup containing substance P fails to express adenosine A_{2A} receptors (Augood and Emson, 1994; Penny et al., 1986; Schiffmann et al., 1991). CGS 21680 was active irrespective of whether NMDA receptors were stimulated by exogenously applied NMDA or endogenous glutamate released on intrastriatal electrical stimulation.

In a first series of experiments, we confirmed that the electrically evoked EPSCs are due to the release of glutamate possibly from corticostriatal afferent fibers. A smaller fraction of the EPSC was inhibited by the NMDA receptor antagonist AP-5, whereas its major fraction was excluded by the AMPA/kainate receptor antagonist CNQX. The combination of AP-5 and CNQX abolished the EPSC, indicating that glutamate is the sole transmitter participating in the synaptic response (Jiang and North, 1991). Moreover, CGS 21680 has lost its inhibitory activity, when the NMDA receptor-mediated fraction of the EPSC was excluded by AP-5. Finally, CGS 21680 inhibited the current responses caused by locally applied NMDA but not AMPA. Hence, all these results indicate, as a whole, that modulatory adenosine A_{2A} receptors are situated postsynaptically at the medium spiny striatal neurons rather than presynaptically at the corticostriatal nerve terminals innervating them.

These results may be of functional significance, since the two groups of neurons project to divergent efferent targets. The enkephalin-containing striatopallidal cells express adenosine A_{2A} receptors, project to the external segment of the globus pallidus and are part of the indirect pathway, whereas the direct pathway is formed by the substance P positive neurons which connect directly the striatum with the basal ganglia output nuclei (the internal segment of the globus pallidus and the substantia nigra pars reticulata; Alexander and Crutcher, 1990; Penny et al., 1986). Activation of the indirect pathway reduces locomotor behaviour, while activity in the direct pathway increases it (Gerfen, 1992; Graybiel, 1990). In conclusion, agonists at striatal adenosine A_{2A} receptors may selectively interfere with excitatory amino acid neurotransmission onto the striatopallidal subgroup of medium spiny neurons and may, under certain conditions, alleviate locomotor depression, e.g. in Parkinson's disease (Köles et al., 2001).

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (IL 20/9-2).

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