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Adenosine A_{2A} receptors inhibit the conductance of NMDA receptor channels in rat neostriatal neurons

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Summary. Whole-cell patch clamp experiments were carried out in rat striatal brain slices. In a subset of striatal neurons (70–80%), NMDA-induced inward currents were inhibited by the adenosine A_{2A} receptor selective agonist CGS 21680. The non-selective adenosine receptor antagonist 8-(p-sulphophenyl)-theophylline and the A_{2A} receptor selective antagonist 8-(3-chlorostyryl)caffeine abolished the inhibitory action of CGS 21680. Intracellular GDP-β-S, which is known to prevent G protein-mediated reactions, also eliminated the effect of CGS 21680. Extracellular dibutyryl cAMP, a membrane permeable analogue of cAMP, and intracellular Sp-cAMPS, an activator of cAMP-dependent protein kinases (PKA), both abolished the CGS 21680-induced inhibition. By contrast, Rp-cAMPS and PKI 14–24 amide, two inhibitors of PKA had no effect. Intracellular U-73122 (a phospholipase C inhibitor) and heparin (an inositoltriphosphate antagonist) prevented the effect of CGS 21680. Finally, a more efficient buffering of intracellular Ca^{2+} by a substitution of EGTA (11 mM) by BAPTA (5.5 mM) acted like U-73122 or heparin. Hence, A_{2A} receptors appear to negatively modulate NMDA receptor channel conductance via the phospholipase C/inositoltriphosphate/Ca^{2+} pathway rather than the adenylate cyclase/PKA pathway.

Keywords: Rat striatum – Medium spiny neuron – Adenosine A_{2A} receptor – NMDA receptor channel – Whole-cell patch clamp

Introduction

Four adenosine receptors have been cloned to date, and are referred to as the A_1, A_{2A}, A_{2B} and A_3 subtypes (Fredholm et al., 1994). In the brain, the occurrence of A_{2A} receptors is mostly restricted to the striatum (Jarvis and Williams, 1989), where they are expressed in the γ-aminobutyric acid (GABA)ergic medium-sized spiny projection neurons (Schiffmann et al., 1991). The striatum receives massive glutamatergic inputs from the cerebral cortex and related structures (Parent, 1990). It is well established that activity in glutamatergic

corticostriatal afferents induces excitation of the medium spiny neurons via postsynaptic N-methyl-D-aspartate (NMDA) and non-NMDA receptors (Calabresi et al., 1996). Furthermore, it has been shown by whole-cell patch clamp methods in a rat striatal brain slice preparation that adenosine and its A_{2A} receptor selective structural analogue CGS 21680 inhibit NMDA-evoked inward currents in a subset of medium spiny neurons (Nörenberg et al., 1997). High levels of A_{2A} binding were only detected in GABAergic striatal neurons containing also enkephalins (Schiffmann et al., 1991) and projecting to the external segment of the globus pallidus (Alexander and Crutcher, 1990). Another group of medium spiny neurons are positive for substance P (Penny et al., 1986) and project to the basal ganglia output nuclei such as the substantia nigra pars reticularis (Alexander and Crutcher, 1990). Hence, it was concluded that CGS 21680-sensitive neurons belong to the subpopulation which projects to the external segment of the globus pallidus. The aim of the present paper was to clarify the transduction mechanism by which A_{2A} receptors depress NMDA currents in these striatal neurons.

Methods

Membrane currents of medium spiny neurons in striatal brain slices were recorded in the whole-cell configuration by procedures similar to those described previously (Edwards et al., 1989; Nörenberg et al., 1997). Brain slices were superfused (3 ml/min) with artificial cerebrospinal solution 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, $NaHCO_3$ 26 and glucose 10; pH 7.4. The bath temperature was kept at room temperature (23–25°C). Patch pipettes were filled with intracellular solution of the following composition (mM): potassium gluconate 140, NaCl 10, $MgCl_2$ 1, HEPES 10, EGTA 11, MgATP 1.5 and GTP 0.3; pH 7.3 was adjusted with KOH. Data were recorded and filtered at 3–10 kHz with a patch clamp amplifier (EPC-7; List Electronic, Darmstadt, Germany). Then, data were digitized at 0.15–3 kHz (Model 1401, Cambridge Electronic Devices, Cambridge, U.K.) and stored on a laboratory computer. Further analyzis was performed by commercially available patch and voltage clamp software.

Only cells having a diameter smaller than 14 μm and a membrane potential more negative than –60 mV were included in this study. All drugs were applied by changing the superfusion medium by means of three-way taps.

Results

Possible actions of A_{2A} receptor agonists on NMDA-induced inward currents were evaluated by the following protocol: NMDA (10 μM) was superfused twice or three times for 1.5 min (T_1, T_2, T_3) to the same cell with a drug-free interval of 10 min. NMDA (10 μM) – evoked currents were stable under these conditions. However, when the A_{2A} receptor selective agonist CGS 21680 (0.1 μM) was present in the bath 5 min before and during the second application of NMDA (10 μM; T_2) an inhibition was observed which did not reverse after washout for 10 min at T_3 (Fig. 1A). As reported previously, CGS 21680 was active only in a subset of neurons (70–80%) (Nörenberg et al., 1997); non-responsive cells were not included in the calculation of the means. The CGS

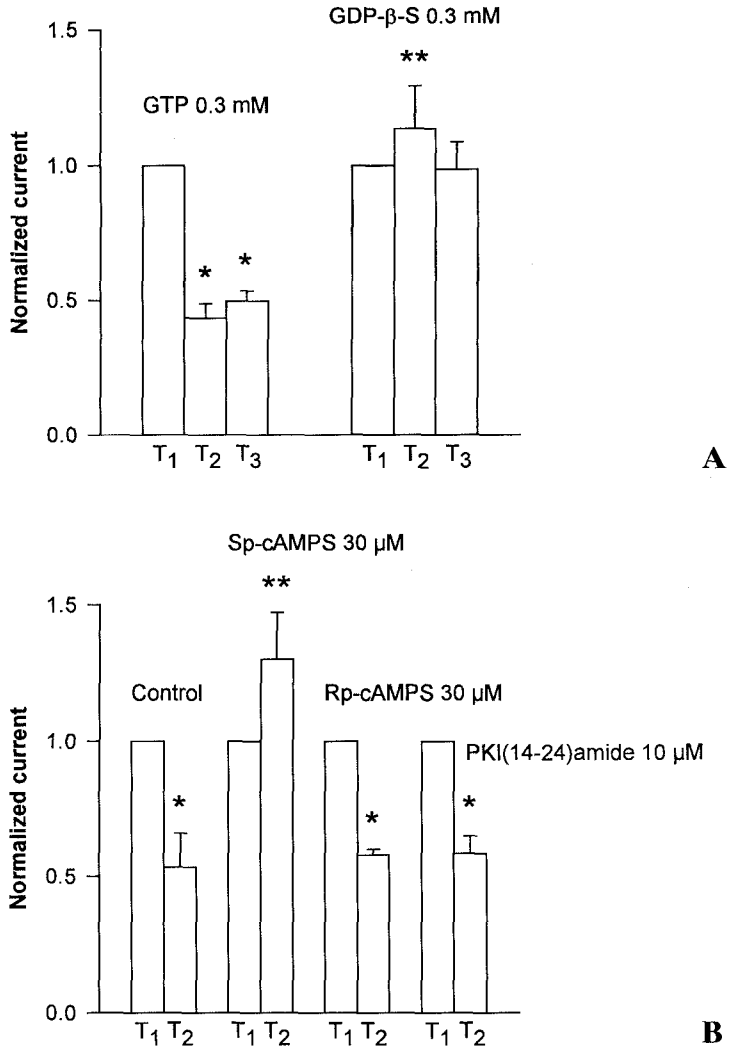

Fig. 1. Effect of CGS 21680 on NMDA currents in rat striatal neurons. NMDA (10 μM) was applied three times (**A** T_1, T_2, T_3) or twice (**B** T_1, T_2) for 1.5 min each and with a 10 min interval between applications. CGS 21680 (0.1 μM) was superfused for 5 min before and during T_2. **A** CGS 21680 (0.1 μM) inhibited the NMDA (10 μM) -evoked current when a standard pipette solution containing also GTP (0.3 mM) was used (n = 6). The substitution of GTP (0.3 mM) by GDP-β-S (0.3 mM) abolished the effect of CGS 21680 (n = 6). **B** Internal microperfusion with Sp-cAMPS (30 μM; n = 6) abolished the effect of CGS 21680 (0.1 μM), while microperfusion with Rp-cAMPS (30 μM, n = 6) or PKI (14–24)amide (10 μM; n = 6) did not alter it. CGS 21680 (0.1 μM) clearly depressed the current induced by NMDA (10 μM) when a standard pipette solution was used (Control; n = 5). The effects of T_2 were normalized with respect to the effect of T_1. The current induced by NMDA (10 μM) was -193.8 ± 27.5 pA (n = 35) at T_1. *$p < 0.05$; significant difference from zero. **$p < 0.05$; significant difference from the effect of CGS 21680 with a standard pipette solution

21680 (0.1 μM) -induced depression of the second (T_2) and third (T_3) NMDA (10 μM) currents was no longer detectable in cells internally microperfused with GDP-β-S (0.3 mM; Fig. 1A). Otherwise, the substitution of GTP (0.3 mM) in the standard pipette solution by GDP-β-S (0.3 mM) did not change the amplitude of the NMDA (10 μM) -induced currents at T_1.

The non-selective adenosine receptor antagonist 8-(p-sulphophenyl)-theophylline (100 μM) and the A_{2A} receptor selective antagonist 8-(3-chlorostyryl)caffeine (1 μM) abolished the inhibitory action of CGS 21680 (0.1 μM) at T_2. Both antagonists were present 15 min before the start of and throughout the experiments.

In order to investigate a possible role of the adenylate cyclase/proteinkinase A pathway, the membrane permeable analogue dibutyryl cAMP (100 μM) was superfused together with CGS 21680 (0.1 μM) 5 min before and during the second application of NMDA (10 μM). The coapplication of dibutyryl cAMP suppressed the effect of CGS 21680 (0.1 μM). Interestingly, dibutyryl cAMP (100 μM) alone potentiated NMDA (10 μM) responses. When Sp-cAMPS (30 μM) an activator of cAMP-dependent protein kinases (PKA) was included in the pipette solution, CGS 21680 (0.1 μM) once more lost its inhibitory action on the NMDA (10 μM) current (Fig. 1B). On the other hand, the diastereomer Rp-cAMPS (30 μM) which inhibits the activation by cAMP of PKA did not alter the effect of CGS 21680 (0.1 μM) (Fig. 1B). Another PKA inhibitor (PKI 14–24 amide; 10 μM) had also no effect (Fig. 1B).

In a last series of experiments, the role of the phospholipase C/inositoltriphosphate ($InsP_3$)/Ca^{2+} pathway was investigated. For this purpose a phospholipase C inhibitor (U-73122) and an antagonist of $InsP_3$ (heparin) were included in the patch solution. Both U-73122 (1 μM) and heparin (10 mg/ml) abolished the effect of CGS 21680 (0.1 μM) (Fig. 2B). A more efficient buffering of intracellular Ca^{2+} by a substitution of EGTA (11 mM) by BAPTA (5.5 mM) also interfered with the CGS 21680 (0.1 μM) -induced inhibition of the NMDA (10 μM) current (Figs. 2A and B).

Discussion

As reported previously, the activation of A_{2A} receptors by CGS 21680 inhibited the conductance of NMDA receptor channels on striatal medium spiny neurons (Nörenberg et al., 1997). Inactivation of G proteins with GDP-β-S (Sternweis and Pang, 1990) abolished the inhibitory effect of CGS 21680, indicating the coupling of A_{2A} receptors to a G protein. A_2 receptors are defined by their ability to stimulate adenylate cyclase (Van Calker et al., 1979). Since, almost all neurotransmitter receptors examined to date have been shown to be regulated by protein phosphorylation (Huganir and Greengard, 1987; Swope et al., 1992), the most likely transduction mechanism of A_{2A} receptors is the activation of the adenylate cyclase/PKA system. One would expect that an increase in intracellular cAMP, as well as the activation of PKA should mimick the effects of A_{2A} receptor stimulation, whereas inhibitors of this enzyme should have the opposite effect. However, the

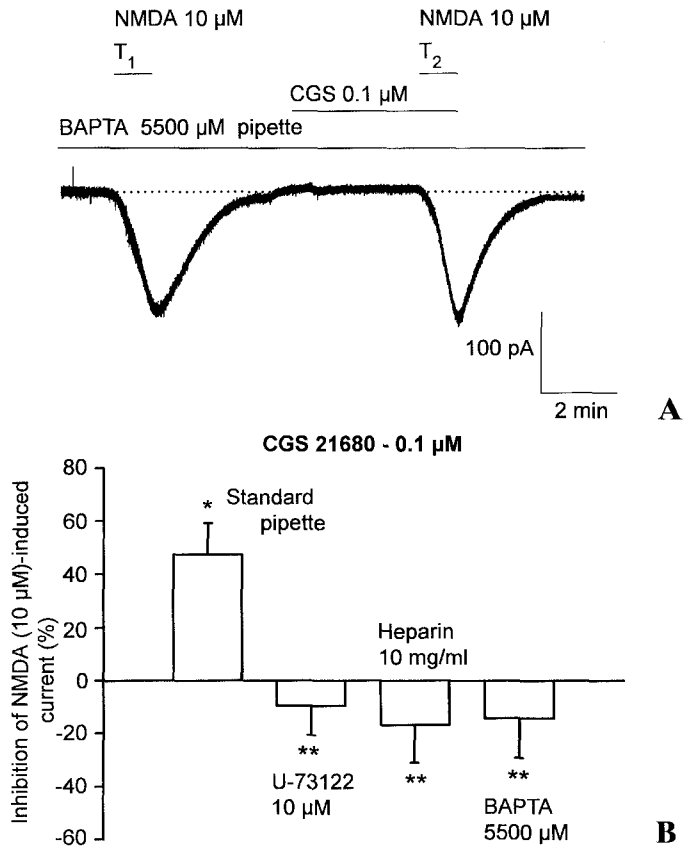

Fig. 2. Effect of CGS 21680 on NMDA currents in rat striatal neurons. NMDA (10 μM) was applied twice (T_1, T_2) for 1.5 min each and with a 10 min interval between applications. CGS 21680 (0.1 μM) was superfused for 5 min before and during T_2. **A** Representative recording with a pipette solution in which EGTA (11 mM) was substituted with BAPTA (5.5 mM). The dotted line indicates the zero current potential. All compounds were present in the superfusion medium (NMDA, CGS 21680) or the pipette solution (BAPTA) over the periods marked by the horizontal bars. **B** Internal microperfusion with U-73122 (10 μM; n = 8), heparin (10 mg/ml; n = 6) or BAPTA (5.5 mM; n = 7; see also **A**) abolished the effect of CGS 21680 (0.1 μM). CGS 21680 (0.1 μM) clearly depressed the current induced by NMDA (10 μM) when a standard pipette solution was used (n = 13). The current induced by NMDA (10 μM) was –159.6 ± 19.5 pA (n = 34) at T_1. *P < 0.05; significant difference from zero. **P < 0.05; significant difference from the effect of CGS 21680 with a standard pipette solution

membrane permeable structural analogue of cAMP, dibutyryl cAMP increased NMDA currents. In accordance with this finding, the activation of PKA was reported to augment rather than to inhibit NMDA receptor channel conductance (Raman et al., 1996). Moreover, dibutyryl cAMP and the PKA activator Sp-cAMPS (Van Haastert et al., 1984) abolished the effect of CGS 2168 in the present experiments, while PKA inhibitors (Rp-cAMPS, PKI 14–24 amide; Van Haastert et al., 1984; Cheng et al., 1986) did not interfere with CGS 21680.

It is not known hitherto whether there are G proteins other than G_S that interact with A_{2A} receptors, or whether the G protein coupled to A_{2A} receptors may influence effectors other than adenylate cyclase (Fredholm et al., 1994). The present results suggest that an increase in intracellular Ca^{2+} which is known to negatively modulate the conductance of NMDA receptors (Legendre et al., 1993; Jones and Westbrook, 1996) is the final step in the sequence of events initiated by A_{2A} receptor stimulation. In fact, a more efficient buffering of intracellular Ca^{2+} by BAPTA instead of EGTA abolished the CGS 21680 effect. The release of intracellular Ca^{2+} may be due to the activation of the phospholipase C/$InsP_3$ system by A_{2A} receptors. Indeed, both the phospholipase C inhibitor U-73122 (Smith et al., 1990) and the $InsP_3$ antagonist heparin (Salter and Hicks, 1995) prevented the effect of CGS 21680.

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References

Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13: 266–271

Calabresi P, Pisani A, Mercuri NB, Bernardi G (1996) The corticostriatal projection: from synaptic plasticity to the dysfunction of the basal ganglia. Trends Neurosci 19: 19–24

Cheng H-C, Kemp, BE, Pearson RB, Smith AJ, Misconi L, Van Patten SM, Walsh DA (1986) A potent synthetic peptide inhibitor of the cAMP-dependent protein kinase. J Biol Chem 261: 989–992

Fredholm BB, Abbracchio MP, Burnstock G, Daly JW, Harden TK, Jacobson KA, Leff P, Williams M (1994) Nomenclature and classification of purinoceptors. Pharmacol Rev 46: 143–156

Huganir RL, Greengard P (1987) Regulation of receptor function by protein phosphorylation. Trends Pharmacol Sci 8: 472–477

Jarvis MF, Williams M (1989) Direct autoradiographic localization of adenosine A_2 receptors in the rat brain using the A_2-selective agonist, [^{3}H]CGS 21680. Eur J Pharmacol 168: 243–246

Jones MV, Westbrook GL (1996) The impact of receptor desensitization on fast synaptic transmission. Trends Neurosci 19: 96–101

Legendre P, Rosenmund C, Westbrook GL (1993) Inactivation of NMDA channels in cultured hippocampal neurons by intracellular calcium. J Neurosci 13: 674–684

Nörenberg W, Wirkner K, Illes P (1997) Effect of adenosine and some of its structural analogues on the conductance of NMDA receptor channels in a subset of rat neostriatal neurones. Br J Pharmacol 122: 71–80

Parent A (1990) Extrinsic connenctions of the basal ganglia. Trends Neurosci 13: 254–258

Penny GR, Afsharpour S, Kitai ST (1986) The glutamate decarboxylase-, leucine enkephalin-, methionine enkephalin- and substance P-immunoreactive neurons in the neostriatum of the rat and cat: evidence for partial population overlap. Neuroscience 17: 1011–1045

Raman IM, Tong G, Jahr CE (1996) β-Adrenergic regulation of synaptic NMDA receptors by cAMP-dependent protein kinase. Neuron 16: 415–421

Salter MW, Hicks JL (1995) ATP causes release of intracellular Ca^{2+} via the phospholipase Cβ/IP_3 pathway in astrocytes from the dorsal spinal cord. J Neurosci 15: 2961–2971

Schiffmann SN, Jacobs O, Vanderhaeghen J-J (1991) Striatal restricted adenosine A_2 receptor (RCD8) is expressed by enkephalins but not by substance P neurons: an in situ hybridization histochemistry study. J Neurochem 57: 1062–1067

Smith RJ, Sam LM, Justen JM, Bundy GL, Bala GA, Bleasdale JE (1990) Receptor-coupled signal transduction in human polymorphonuclear neutrophils: effects of a novel inhibitor of phospholipase C-dependent processes on cell responsiveness. J Pharmacol Exp Ther 253: 688–697

Sternweis PC, Pang I-H (1990) The G protein-channel connection. Trends Neurosci 13: 122–126

Swope SL, Moss SJ, Blackstone CD, Huganir RL (1992) Phosphorylation of ligand-gated ion channels: a possible mode of synaptic plasticity. FASEB J 6: 2514–2523

Van Calker D, Müller M, Hamprecht B (1979) Adenosine regulates, via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. J Neurochem 33: 999–1005

Van Haastert PJM, Van Driel R, Jastorff B, Baraniak J, Stec WJ, De Witt RJW (1984) Competitive cAMP antagonists for cAMP-receptor proteins. J Biol Chem 259: 10020–10024

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