The Role of Adenosine A_{2a} Receptors in Regulating GABAergic Synaptic Transmission in Striatal Medium Spiny Neurons

Akihisa Mori, Tomomi Shindou, Michio Ichimura, Hiromi Nonaka, and Hiroshi Kase

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Nagaizumi-Cho, Sunto-Gun, Shizuoka 411, Japan

We demonstrated an adenosine A_{2a} receptor-mediated disinhibition of medium spiny projection neurons using intracellular recording and the whole-cell patch-clamp recording applied to these cells, visually identified in thin rat striatal slices. The A_{2a} receptor agonist 2-[*p*-(2-carboxyethyl) phenylethylamino]-5′-*N*-ethylcarboxamido adenosine (CGS-21680; 0.3–10 μM) suppressed GABAergic synaptic transmission onto these cells in a manner inhibited by the A_{2a} receptor-selective antagonist (*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine (0.1–1.0 μM). The A_1 receptor antagonists had no effect on the CGS-21680-induced suppression. Analysis of spontaneous miniature inhibitory synaptic currents indicated that suppression of intrastriatal GABAergic synaptic transmission was attributable to presynaptic, but not postsynaptic, A_{2a} receptors. Therefore, the A_{2a} receptor may regulate striatal output activity by relieving GABA-mediated inhibition of the medium spiny projection neurons, which explains the ability of purinergic agents to affect motor control.

Key words: adenosine; adenosine receptor; GABA; striatum; CGS-21680; KF17837; IPSP; IPSC; cAMP

There is a growing body of evidence that adenosine is a potent inhibitor of neuronal activity in the central and peripheral nervous systems (Nicoll et al., 1990). Four major subtypes of adenosine receptors, A_1, A_{2a}, A_{2b}, and A_3, have been characterized (for review, see Abbracchio et al., 1993). Among these subtypes, only A_1 receptors have been known to modulate synaptic transmission in the mammalian CNS (for review, see Fredholm and Dunwiddie, 1988).

In contrast to the widespread distribution of the A_1 and A_{2b} receptors in brain, A_{2a} receptors appear to be confined to the striatum, nucleus accumbens, and olfactory tubercle (Jarvis and Williams, 1989; Parkinson and Fredholm, 1990; Martinez-Mir et al., 1991). This discrete distribution of the A_{2a} receptor suggests a specific functional role of the A_{2a} receptor in neuronal communication in these areas. In the striatum, which is a major component of the basal ganglia, recent *in situ* hybridization studies have detected the mRNA encoding the A_{2a} receptor only in the medium spiny neurons (MSNs) (Schiffmann et al., 1990, 1991a,b).

The MSNs are the principal striatal output neurons, making up 90–95% of the neuronal population in this area, and they send GABAergic axons onto the globus pallidus and substantia nigra. They receive not only massive inputs from the cerebral cortex and other areas but also intrinsic inputs from cholinergic (for review, see Graybiel, 1990; Gerfen, 1992) and glutamatergic interneurons (Mori et al., 1994b,c). The GABAergic synapse onto MSNs is believed to be intrinsic and to originate from either extensive axon collaterals of MSNs or GABAergic interneurons, forming intrastriatal inhibitory circuits. In these circuits, the release of GABA appears to be regulated tightly by an unidentified neuroactive substance(s) (for review, see Kita, 1993). However, the precise roles of these collaterals and interneurons have not been determined; nor have the mechanisms regulating GABA release from their nerve terminals been described.

Because the A_{2a} receptor is restricted to the MSNs, we hypothesized that adenosine, via A_{2a} receptors, regulates GABA-containing synapse activity. To test this hypothesis, we examined GABAergic transmission onto the MSNs, using intracellular recording and whole-cell patch-clamp recording in striatal slices. To identify the A_{2a} receptor-mediated mechanism, we used the newly developed A_{2a} receptor-selective antagonist (*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine (KF17837) (Nonaka et al., 1994a,b) and the A_{2a} receptor-selective agonist 2-[*p*-(2-carboxyethyl) phenylethylamino]-5′-*N*-ethylcarboxamido adenosine (CGS-21680) (Jarvis et al., 1989).

In this study, we have found that the A_{2a} receptor serves to suppress GABAergic transmission in the striatal MSNs. The suppression was mimicked by membrane permeable cAMP analogs. Furthermore, we analyzed spontaneous miniature synaptic transmission to determine that this suppression is mediated via presynaptic A_{2a} receptors. The results provide evidence supporting the hypothesis that adenosine is a specific neuromodulator of the intrastriatal GABAergic circuits. The implications of these findings are discussed in terms of the physiopathological roles of adenosine in the striatum.

Preliminary results have been reported previously in abstract form (Kase et al., 1994; Mori et al., 1994a).

MATERIALS AND METHODS

Drugs. KF17837, 8-(dicyclopropylmethyl)-1,3-dipropylxanthine (KF15372), and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were synthesized at the Medicinal Chemistry Department of the Pharmaceutical Research Laboratories (Shizuoka, Japan) (Shimada et al., 1992). KF17837 was used as KF17837S (Nonaka et al., 1994a,b). Other reagents were from standard commercial sources.

Electrophysiology. Intracellular recordings from striatal neurons were obtained following methods described previously (Kita et al., 1984; Shindou et al., 1994) using a Neurodata IR 183 amplifier (Cabin John, MD). Striatal

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Correspondence should be addressed to Hiroshi Kase, Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Company, 1188 Shimotogari, Nagaizumi-Cho, Sunto-Gun, Shizuoka 411, Japan.

slices (400 μm) were prepared from adult Wistar rats. Slices were preincubated in an extracellular solution containing (in mM): 120 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 23 $NaHCO_3$, 1.3 NaH_2PO_4, and 11 D-glucose with 95% O_2/5% CO_2 at 32°C for 1.5 hr. Slices then were perfused with this solution supplemented with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM), DL-2-amino-5-phosphonovalerate (APV; 100 μM), and atropine (10 μM). Drugs were dissolved in dimethylsulfoxide (DMSO) and bath-applied; final concentration of DMSO was adjusted to 0.11%. The extracellular solution containing the same concentration of DMSO was used as a vehicle. At this concentration, DMSO had little effect on IPSPs and IPSCs. The surface of the striatal slice was free of extracellular solution (flow rate 1.5 ml/min) and directly exposed to a humidified O_2/CO_2 gas mixture. The recording electrode contained 2 M KCl. Synaptic potentials were evoked by focal stimulation (0.1 Hz, 0.1 msec, 2–5 V) using a bipolar electrode placed within 1.5 mm of the recording site. All potentials were sampled at 5 Hz and were stored to a PC (PC9801DS, NEC).

The whole-cell patch-clamp recordings, as described in detail previously (Mori et al., 1994b), were performed in thin horizontal slices (180–200 μm) prepared from the striatum of 9- to 12-d-old Wistar rats, directly visualizing cells using an upright Nomarski microscope with a 40× water immersion objective (Optiphoto, Nikon, Tokyo, Japan). The conditions of slice preincubation, the composition of extracellular solution, and the procedure for drug application were similar to those of intracellular recordings described above. The speed of bath application was ~2 ml/min in a slice chamber volume of 0.7 ml. The pipette (6–12 MΩ) contained (in mM): 140 CsCl, 10 HEPES, 1 $MgCl_2$, 2 Mg-ATP, and 0.2 Cs-EGTA, pH-adjusted to 7.2 with CsOH. The series resistance during the whole-cell configuration was 15–40 MΩ. Membrane currents were recorded at −70 mV and were amplified and filtered at 2 kHz (Axopatch 1-D, Axon Instruments, Foster City, CA). Synaptic currents were evoked by focal stimulation (0.2 Hz, 0.2 msec, 20–100 V) delivered via a glass micropipette (tip diameter 5–10 μm) filled with the extracellular solution and positioned within 150 μm of the recorded cell. Under these conditions, the currents were inverted at 0 mV, close to the chloride equilibrium potential (E_{Cl}). All currents were sampled at 10 kHz and stored on a PC (PC9801DS, NEC). Drug effects on IPSCs were evaluated from the averaged IPSC amplitude. The experiments showing no recovery of IPSC amplitude after washout of the drugs were omitted.

Spontaneous miniature IPSCs (mIPSCs) in the MSNs were recorded with 1.0 μM tetrodotoxin (TTX) added to the extracellular solution. These currents almost completely disappeared after application of bicuculline (data not shown). Onset of IPSCs was determined by eye, and events that had amplitudes of <4.5 pA or that showed time-to-peak longer than decay were omitted from the analysis. Events were ranked by amplitude for preparation of cumulative probability distributions. Statistically significant difference was assessed by the Kolmogorov–Smirnov test (Van der Kloot, 1991), which considers both the maximum difference between the two distributions and the number of sampled events. The test calculates the likelihood that the two data sets are drawn from the same distribution, that is, the probability (p) that a difference larger than that observed would occur by chance. The higher the calculated p, the more likely that the observed curves are not different. We have chosen to define stringently two distributions to be not significantly different only at $p > 0.05$. All measurements were performed at room temperature (23–26°C). All values are expressed as mean ± SEM.

cAMP assay in striatal slices. cAMP level in rat brain slices was measured by a method described previously (Garthwaite and Garthwaite, 1987), with modifications. Perahorizontal slices (400 μm thick) of the striatum were prepared from male 35- to 40-d-old Wistar rats. The slices were incubated at 32°C in a Krebs'–Ringer's solution, gassed with 95% O_2/5% CO_2 at 32°C. The slices were preincubated for 2 hr. Drugs were dissolved in DMSO and then added at appropriate concentrations (final concentration of DMSO, 0.1%). After a 5 min exposure to drugs, treated slices were withdrawn and inactivated by boiling for 5 min in 50 mM Tris/4 mM EDTA buffer, pH 7.6. cAMP levels in the supernatants obtained after sonication and centrifugation (7000 × g, 10 min, 4°C) were measured with radioimmunoassay kits (Yamasa, Tokyo, Japan). Proteins were measured with a protein assay kit (Bio-Rad, Richmond, CA) with bovine serum albumin as the standard.

RESULTS

The adenosine A_{2a} receptor agonist CGS-21680 reduces evoked GABAergic IPSPs in striatal neurons

Evoked IPSPs were elicited by focal stimulation, and intracellular recordings were made from neurons in slices of adult rat striatum.

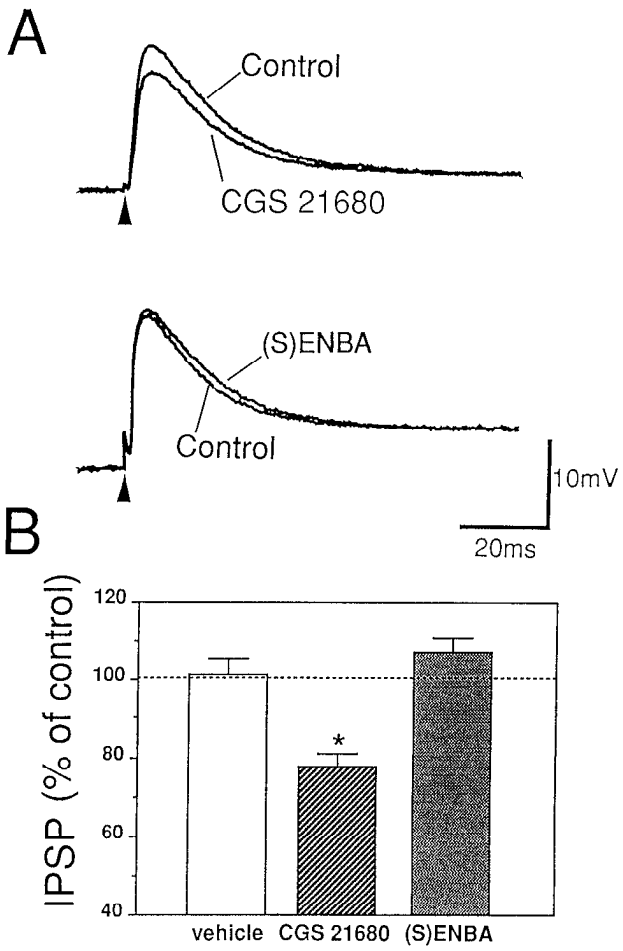

Figure 1. The adenosine A_{2a} receptor agonist suppresses GABAergic IPSPs in striatal neurons. Effects of the A_1 receptor agonist (*S*)ENBA and the A_{2a} receptor agonist CGS-21680 on GABAergic IPSPs recorded using intracellular recordings from striatal slices. The recordings were made in current clamp with the resting membrane potential maintained between −70 and −75 mV. *A*, Typical superimposed averaged traces of 10 consecutive IPSPs before (*Control*) and during the application of 1 μM CGS-21680 (*top*) or 0.3 μM (*S*)ENBA (*bottom*). *B*, CGS-21680 (1 μM) reduced peak amplitude of IPSPs, and (*S*)ENBA (0.3 μM) had no effect. Data represent percentage of control and mean ± SEM (*bars*) values. Vehicle application did not affect the amplitude of IPSPs (101 ± 4% of control; $n = 4$); *$p < 0.05$ versus vehicle by Scheffe test. All pooled data were obtained comparing the peak amplitude of averaged IPSPs (10 traces) in control and during drug application.

Excitatory glutamate and muscarinic acetylcholine receptors were blocked by the addition of CNQX (10 μM), APV (100 μM), and atropine (10 μM). Recorded IPSPs were shown to be mediated by $GABA_A$ receptors because they were blocked by bicuculline (30 μM; data not shown). These GABAergic IPSPs were reduced in amplitude by depolarization, increased by hyperpolarization, and reversed polarity between −50 and −60 mV. The average value of the peak IPSP amplitude in controls was 12.6 ± 0.7 mV ($n = 29$). The A_{2a} receptor-selective agonist CGS-21680 (1 μM) significantly reduced the peak IPSP amplitude to 78 ± 3% of control ($p < 0.05$; $n = 12$) without changing the resting membrane potential or the time course of the IPSP (Fig. 1*A*, *top*, *B*). The amplitude, however, was barely affected by the A_1 receptor-selective agonist N^6-(2*S*)-[2-*endo*-norbornyl]adenosine [(*S*)ENBA] (Trivedi et al., 1989) at

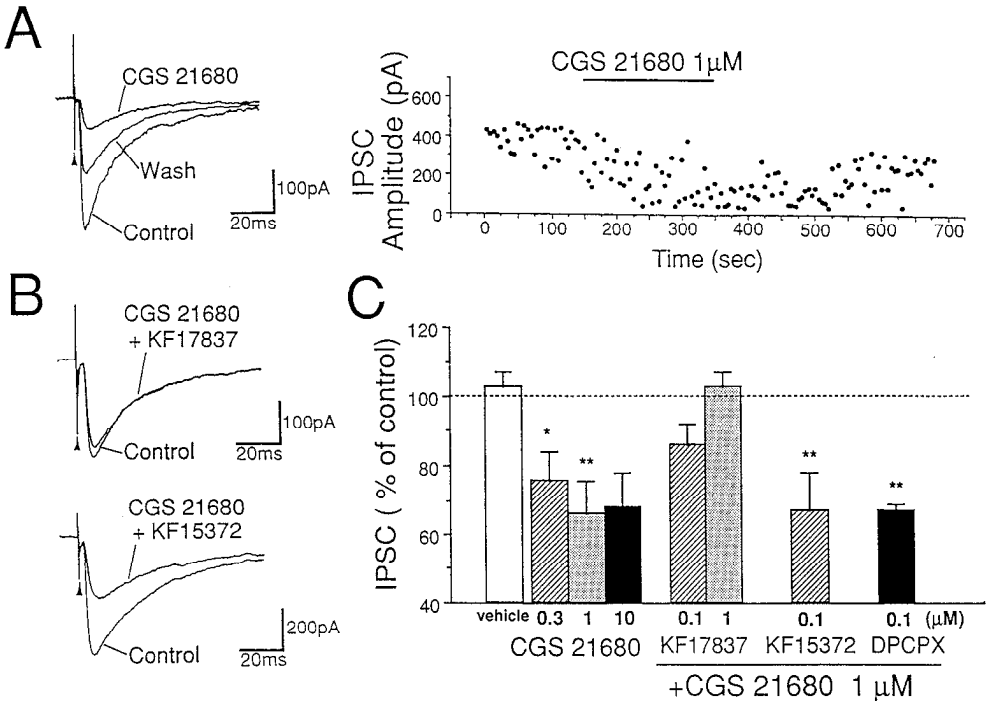

Figure 2. The adenosine A_{2a} receptor selectively suppresses GABAergic IPSCs in striatal MSNs. Effects of CGS-21680 alone and coapplication of CGS-21680 with the A_1 receptor antagonists KF15372 or DPCPX or with the A_{2a} receptor antagonist KF17837 on striatal GABAergic IPSCs recorded by using the whole-cell patch-clamp method from the MSNs in striatal slices. *A*, Superimposed traces of an average of consecutive IPSCs (15 traces) before (*Control*), during, and after (*Wash*) application of CGS-21680 (1 μM; *left*) and a representative time course (*right*). The slow onset and incomplete recovery may be attributable to solution exchange. *B*, Typical superimposed traces of average of consecutive IPSCs (12 traces) before (*Control*) and during coapplication of CGS-21680 (1 μM) with KF17837 (0.1 μM; *top*) or with KF15372 (0.1 μM; *bottom*). *C*, Pooled data showing that the suppression by CGS-21680 of IPSCs was dose-dependent (0.3–1 μM) and was blocked selectively by KF17837. Data represent percentage of control and mean ± SEM (*bars*) values. Vehicle application had no effect on amplitude of IPSCs (103 ± 4% of control; $n = 15$); $^*p < 0.05$; $^{**}p < 0.01$ versus vehicle by Scheffe test. All pooled data were obtained by comparing averaged IPSCs (12–15 traces) before and during drug application.

0.3 μM (107 ± 4% of control, $n = 4$; Fig. 1*A*, *bottom*, *B*), a concentration that is shown to be effective on striatal A_1 receptors via its action on glutamatergic synaptic transmission in the striatum (our unpublished data). These results suggest that adenosine A_{2a} receptors serve to suppress GABA receptor-mediated inhibitory inputs onto striatal neurons.

The adenosine A_{2a} receptor mediates inhibition of evoked GABAergic IPSCs in the MSNs

To verify the above finding, we measured evoked IPSCs with whole-cell patch-clamp recording from an MSN. We chose only small spiny neurons with a diameter of <13 μm. Injection of Lucifer yellow (Sigma, St. Louis, MO) into these neurons revealed the following characteristic features of the MSNs. The cells projected several fine, 100- to 250-μm-long dendrites in all directions. Fine, spiny structures were observed when cells were located close to the surface. In the presence of CNQX, APV, and atropine in the external solution and a high concentration of Cl^- in the pipette solution, the MSNs displayed inward currents evoked by the focal stimulation at a holding potential of −70 mV. Under these conditions, recorded intrastriatal IPSCs were confirmed to be mediated by $GABA_A$ receptors via antagonism with bicuculline (30 μM) or picrotoxin (100 μM; data not shown). The average values (mean ± SEM) of peak amplitude (in pA), latency (in msec), time-to-peak (in msec), and time constant of decay (in msec) at −70 mV in control were 406 ± 25 ($n = 67$), 2.9 + 0.1 ($n = 33$), 3.1 ± 0.1 ($n = 33$), and 30.6 ± 0.9 ($n = 33$), respectively. CGS-21680 significantly reduced the averaged amplitude of evoked IPSCs within 2 min of application of the drug (Fig. 2*A*, *right*) without affecting the time course of the IPSCs (Fig. 2*A*, *left*). After drug removal, the IPSC amplitude slowly recovered. The suppression of IPSCs by CGS-21680 was concentration-dependent and saturated at 1 μM: 76 ± 8% ($p < 0.05$; $n = 11$), 66 ± 10% ($p < 0.01$; $n = 7$), and 68 ± 10% ($n = 3$) of control at 0.3, 1.0, and 10 μM CGS-21680, respectively (Fig. 2*C*). The A_{2a} receptor-selective antagonist KF17837, when applied together with 1 μM CGS-21680, blocked the inhibitory effect of CGS-21680 in a concentration-dependent manner: the IPSC amplitudes were 86 ± 7% ($n = 6$) and 103 ± 4% ($n = 8$) of control at 0.1 and 1 μM KF17837, respectively. Two different A_1 receptor-selective antagonists, DPCPX and KF15372, had no effect on the CGS-21680 (1 μM)-induced suppression of the IPSCs at 0.1 μM concentration (Fig. 2*B*,*C*). For both antagonists, this concentration is sufficient to block A_1 receptors (Lohse et al., 1987; Suzuki et al., 1992). These results demonstrate the existence of A_{2a} receptor-mediated modulation of striatal GABA transmission in the MSNs. Furthermore, KF17837 alone dose-dependently enhanced the IPSC amplitude: 107 ± 16% ($n = 3$), 122 ± 14% ($n = 4$), and 140 ± 19% ($n = 4$) of control at 1, 3, and 10 μM of KF17837, respectively. This indicated that endogenous adenosine, present within the striatal slice, suppressed GABA receptor-mediated inhibitory input onto striatal neurons.

Modulation of GABAergic synaptic transmission in the MSNs is mediated by presynaptic adenosine A_{2a} receptors

To investigate whether the regulation of the striatal GABAergic synaptic transmission in the MSNs was mediated by post- and/or presynaptic A_{2a} receptors, we analyzed the spontaneous mIPSCs in the striatal slices. Spontaneous mIPSCs were recorded as inward currents from MSNs in the striatal slices (see Fig. 3*A*, *left*). These currents ranged from 5 to >75 pA in amplitude at a holding potential of −70 mV, and the basal mean frequencies were

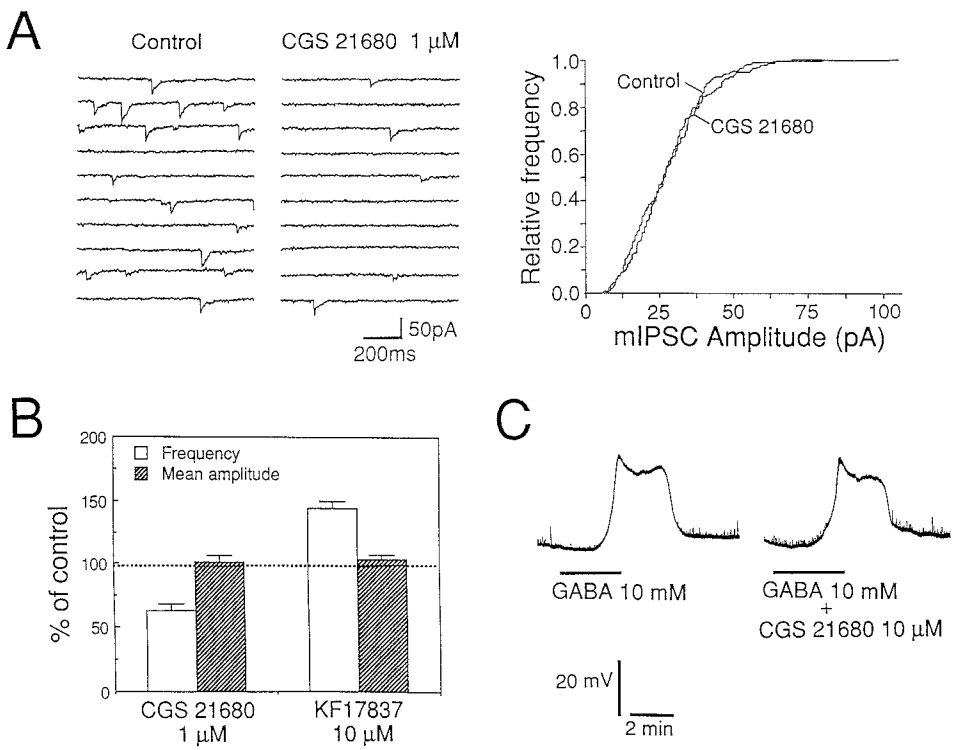

Figure 3. The adenosine A_{2a} receptor agonist and antagonist affect GABA synapses onto MSNs at presynaptic, but not postsynaptic, sites. *A: Left,* Typical traces of spontaneous mIPSCs recorded from an MSN are shown with each 10 sweeps at the *left* (*Control*) and 10 sweeps at the *right* (during 1 μM CGS-21680 application). Cumulative probability distributions of mIPSC amplitude in control (175 events) and in 1 μM CGS-21680 (142 events), constructed from the same data, are shown on the *right*. There was no statistically significant difference between these distributions, as assessed by the Kolmogorov–Smirnov test ($p > 0.4$). The frequencies (in Hz) and amplitudes (mean ± SD, in pA) of mIPSCs were 1.05 and 27.5 ± 11.8 in control and 0.66 and 27.7 ± 13.2 in CGS-21680 application, respectively. *B,* Pooled data showing that CGS-21680 (1 μM) decreased and KF17837 (10 μM) increased the mean frequency, without affecting the mean amplitude of mIPSCs. Data represent percentage of control and mean ± SEM (*bars*) values. Control values of the frequency (in Hz) and mean amplitude (in pA) of mIPSCs were 0.79 ± 0.13 and 23.4 ± 2.5 in the CGS21680 experiment (6 cells) and 0.71 ± 0.13 and 19.7 ± 1.00 in the KF17837 experiment (4 cells), respectively. *C,* Typical traces of $GABA_A$ receptor-mediated depolarizing action of striatal neurons recorded intracellularly showing that CGS-21680 (10 μM) had no effect on the actions. GABA (10 mM) was bath-applied.

between 0.4 and 1.4 Hz, depending on the slice preparations. These currents completely disappeared after application of bicuculline (data not shown), indicating that the mIPSCs were mediated entirely by $GABA_A$ receptors. CGS-21680 (1 μM) decreased the mean frequency of mIPSCs from 0.79 ± 0.13 to 0.50 ± 0.09 Hz (63 ± 5% of control, $p < 0.01$ by paired t test; $n = 6$), whereas the mean amplitude was not changed (mean amplitude in the presence of CGS-21680: 23.2 ± 2.0 pA, 101 ± 6% of control, $n = 6$; Fig. 3*B*). The decrease in the mean frequency slowly recovered after drug removal. In four cells, we were able to collect sufficient numbers of currents for the distribution analysis. There was no significant change in these mIPSC-amplitude distributions as determined by the Kolmogorov–Smirnov test. The typical data are shown in Figure 3*A*. In striking contrast to the effects of CGS-21680 on mIPSCs, 10 μM KF17837 increased the mean frequency of mIPSCs from 0.71 ± 0.13 to 1.00 ± 0.33 Hz (144 ± 6% of control, $p < 0.01$ by paired t test; $n = 4$). In these cells, KF17837 caused no significant change in either the mean amplitude (20.4 ± 1.24 pA, 104 ± 4% of control; Fig. 3*B*) or the amplitude distribution. These results indicated that CGS-21680 reduced and KF17837 increased the quantal release of transmitter from the presynaptic terminals. To support this conclusion, we examined the effect of CGS-21680 on GABA-induced depolarizing action using intracellular recordings from striatal slices. GABA was bath-applied and, thus, was significantly diluted before reaching the cell membrane. Therefore, 10 mM GABA was used so that a similar amplitude of evoked IPSPs and reproducible depolarizations could be obtained with repeated application. The depolarizing action by bath-applied GABA (10 mM) was confirmed to be mediated by $GABA_A$ receptors via antagonism with bicuculline (50 μM; data not shown). CGS-21680 had no effect on the peak amplitude of GABA-induced depolarizing action: 100 ± 5% ($n = 4$) and 98 ± 6% ($n = 3$) of control at 1 and 10 μM, respectively (Fig. 3*C*). These results demonstrate that the suppression of GABAergic synaptic transmission in the MSNs was made by presynaptic, but not postsynaptic, A_{2a} receptors.

Effect of cAMP analogs on IPSCs

The A_2 receptor is known to be coupled positively to adenylate cyclase (Van Calker et al., 1979). Corresponding with the results reported previously (Lupica et al., 1990; Hide et al., 1992), CGS-21680 (1 μM) actually increased cAMP accumulation in rat striatal slices from the control level of 15.1 ± 1.1 pmol/mg protein ($n = 12$) to 32.2 ± 4.8 pmol/mg protein ($n = 3$). KF17837 (0.1–0.3 μM) suppressed the increase of cAMP level to the control level dose-dependently (data not shown), indicating that CGS-21680-induced cAMP increase is mediated by the A_{2a} receptor. These results suggested that cAMP mediated the synaptic regulation of GABAergic transmission by the A_{2a} receptor. Therefore, we studied the effect of cAMP on GABAergic synaptic transmission using membrane-permeable cAMP analogs. 8-Bromo-cAMP and dibutyryl cAMP (0.5 mM) reversibly suppressed the IPSC amplitude to 69 ± 5% ($n = 8$) and 52 ± 7% ($n = 3$) of control, respectively (Fig. 4*A,B*).

DISCUSSION

Presynaptic A_{2a} receptor-mediated suppression of GABAergic synaptic transmission in the MSNs

A fragile balance between excitation and inhibition maintains the neuronal functioning of the CNS. Modulation of excitation and/or inhibition has substantial implications for both normal physiolog-

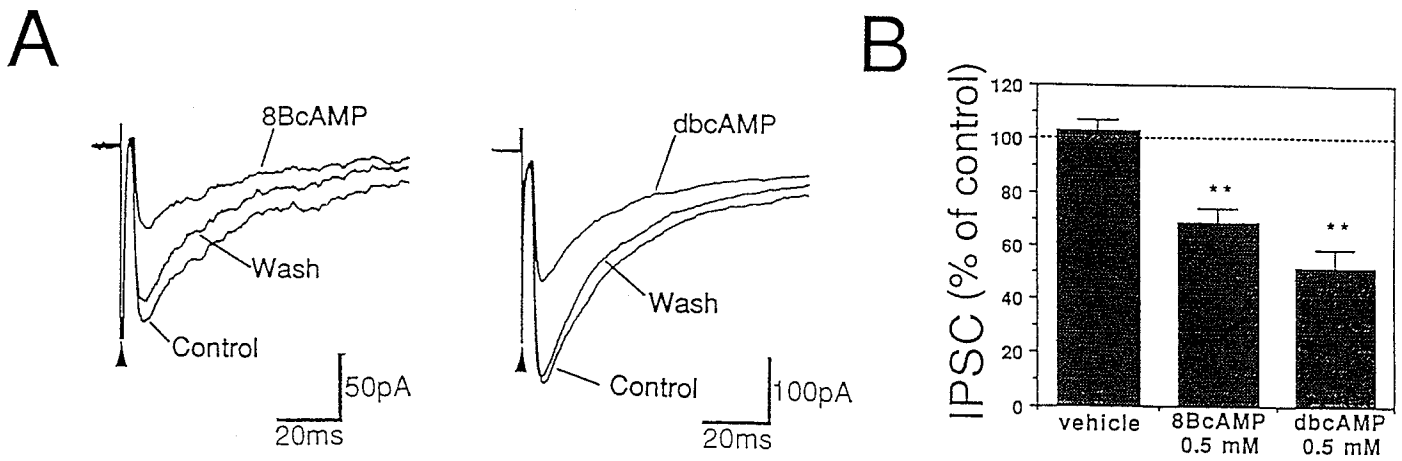

Figure 4. Involvement of cAMP-signaling pathway with the regulation of GABAergic synaptic transmission in the striatum. *A*, Typical superimposed traces of an average of consecutive IPSCs (12 traces) before (*Control*), during, and after (*Wash*) applications of 8-bromo-cAMP (*8BcAMP, left*) and dibutyryl cAMP (*dbcAMP, right*), each at 0.5 mM, showing that both analogs reversibly suppressed IPSCs. *B*, Pooled data for suppression of 8-bromo-cAMP ($n = 8$) and dibutyryl cAMP ($n = 3$) on IPSC amplitude; $^{**}p < 0.01$ versus vehicle by Scheffe test. Vehicle group is similar to that shown in Figure 2.

ical processes and pathological states of brain function. Many reports have shown that adenosine suppresses excitatory synaptic transmission through the A_1 receptors. In the present report, we demonstrate for the first time that the adenosine A_{2a} receptor suppresses inhibitory synaptic transmission in the mammalian CNS. One particularly important finding is that the inhibitory action of adenosine was shown in the major output neurons of the striatum.

Suppression of GABAergic synaptic transmission by an A_{2a} receptor-mediated mechanism in the MSNs was demonstrated in this study by adapting the intracellular recording and the high-resolution patch-clamp recording technique to striatal slices, directly visualizing these cells. In addition, we used CGS-21680 and KF17837, selective agents for A_{2a} receptors. Both of these agents have been shown to be selective for A_{2a} receptors in membrane-binding and autoradiographic assays (Jarvis and Williams, 1989; Parkinson and Fredholm, 1990; Martinez-Mir et al., 1991; Nonaka et al., 1994a,b). The $GABA_A$-mediated IPSPs or IPSCs were elicited by a stimulating electrode placed in the vicinity of the recording site, under conditions in which excitatory inputs were blocked, so that the effects of selective adenosine agonists and/or antagonists on GABA-mediated inhibition could be observed without complications caused by excitatory transmission. The GABAergic neurotransmission was examined further by analysis of mIPSCs caused by spontaneous release of GABA from presynaptic terminals in the presence of TTX to block the propagation of action potentials to the terminals. Both IPSPs and IPSCs were suppressed significantly by CGS-21680 (Figs. 1, 2, 3*A,B*). KF17837 blocked the effect of CGS-21680 (Fig. 2*B,C*) and alone enhanced the evoked IPSCs. The selective A_{2a} agonist and antagonist changed the frequency of mIPSCs without affecting either the mean amplitude or the amplitude distributions. This showed a presynaptic action of the A_{2a} receptor, regulating the quantal release of GABA (Fig. 3*A,B*). The absence of postsynaptic A_{2a} receptor-mediated control was supported by the finding that CGS-21680 had no effect on the peak amplitude of GABA-induced depolarizing action in striatal neurons (Fig. 3*C*). Recent neurochemical studies have shown that the A_{2a} receptor modulates [^{3}H]GABA release from globus pallidus slices (Mayfield et al., 1993) and from striatal synaptosomes (Kirk and Richardson, 1994), an effect that was blocked by KF17837 (Kurokawa et al., 1994). These results are consistent with the existence of presynaptic modulation by A_{2a} receptors of striatal GABA synapses onto MSNs.

The presynaptic A_{2a} receptor appears to regulate GABAergic inputs to the MSNs via cAMP signaling, because CGS-21680 at 1 μM induced both IPSC suppression (Fig. 2) and cAMP accumulation in the striatal slices, and the amount of IPSC suppression by cAMP analogs was similar to that by CGS-21680 (Figs. 2, 4). In addition, we had preliminary data indicating that dibutyryl cAMP reduces the frequency of mIPSCs, which suggests that the cAMP action is presynaptic. These data suggest that the presynaptic A_{2a} receptor stimulation is mediated by cAMP accumulation, although further, precise investigation is necessary to strengthen this point.

Physiological significance of the adenosine A_{2a} receptor-mediated modulation of GABAergic synaptic transmission in the MSNs

The GABA component of synaptic potentials in the MSNs is attributable either to intrinsic synaptic contact from other MSNs via axon collaterals (Jiang and North, 1991) or to interneurons. Consequently, the recorded IPSPs and IPSCs are attributable either to the GABA synapses caused by other MSNs or to another GABAergic input from the interneurons. GABA released from the axon collaterals would produce strong local mutual inhibition, comprising an intrastriatal inhibitory feedback circuit and thereby providing a mechanism for concentrating the diffused pattern of the various inputs onto the relevant striatum target system. Alternatively, GABA released from the interneurons would serve as part of a striatal feedforward circuit, providing another intrastriatal inhibition mechanism (Kita, 1993).

The modulation of GABAergic synaptic transmission onto the MSNs by adenosine in the striatum suggests, therefore, the following two physiological models. (1) Adenosine is a regulator of GABA release from the collateral axons, and the presynaptic A_{2a}

receptor activation causes a reduction in GABA-mediated synaptic inhibition within an intrastriatal feedback circuit. (2) Adenosine regulates GABA release from the interneurons and attenuates the feedforward regulation mechanism. These adenosine receptor-mediated disinhibition mechanisms may influence striatal processing of the information from cortex, from other regions of the brain, and from the striatal interneurons to the striatal output pathways on the basal ganglia–thalamocortical circuitry.

Although mRNA encoding the A_{2a} receptor has been detected in a number of MSNs, there is no evidence that GABAergic interneurons express the receptor (Schiffmann et al., 1991a,b). The MSN receives recurrent GABAergic inputs from axon collaterals of neighboring MSNs. Therefore, the presynaptic A_{2a} receptor-mediated suppression of GABAergic neurotransmission occurred in the recurrent inputs by the receptor-expressing axon collateral terminals of neighboring MSNs. However, Jaeger et al. (1994) have reported recently that the mutual inhibition among MSNs is weak or nonexistent in the rat striatum and have argued against the common view that the intrastriatal inhibitory feedback circuit is a central organizing principle of striatal function. If A_{2a} receptors were expressed in the axon terminal of GABAergic interneuron despite their absence in the cell body, the A_{2a} receptor-mediated suppression would have occurred in nerve terminals of GABAergic interneurons.

Both the intrastriatal GABAergic feedback circuit led by the MSNs and the feedforward circuit formed between MSNs and interneurons play an important role in temporal and spatial filtering of various input influences from cortex, from other regions of the brain, and from other striatal interneurons, and they regulate the striatal output activity via inhibition of the projection neurons (Groves, 1983; Kita, 1993). The A_{2a} receptor-mediated regulation mechanisms, therefore, may cause overactivity of the striatal output pathways. Corresponding with this hypothesis, we have demonstrated recently that KF17837 ameliorates motor dysfunction in 6-hydroxydopamine-lesioned rat (K. Koga, M. Kurokawa, S. Shiozaki, M. Ochi, J. Nakamura, and Y. Kuwana, unpublished data), although it remains to be determined whether KF17837 affects the activity of the globus pallidus and/or the substantia nigra.

In conclusion, these data show that presynaptic A_{2a} receptors modulate GABAergic synaptic transmission onto the MSNs and, therefore, regulate a major feedback circuit and/or feedforward circuit within the striatum. It is likely that this will provide new insights into the physiopathology of adenosine in this area of the brain. We also suggest that this modulation plays an important role in the control of voluntary movement and will provide a new approach to the therapy of Parkinson's disease.

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