Selective Inhibition by Adenosine of mGluR IPSPs in Dopamine Neurons After Cocaine Treatment

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Fiorillo, Christopher D. and John T. Williams. Selective inhibition by adenosine of mGluR IPSPs in dopamine neurons after cocaine treatment. J. Neurophysiol. 83: 1307–1314, 2000. With repeated exposure to psychostimulants such as cocaine and amphetamine, long-lasting changes occur in the mesolimbic dopamine system that are thought to underlie continued drug-seeking and relapse. One consequence of repeated cocaine treatment is an increase in extracellular adenosine in the ventral tegmental area (VTA), which results in tonic inhibition of synaptic input to dopamine neurons. The synapse specificity of this increased adenosine tone was examined on glutamate- and GABA-mediated responses using the selective A1 receptor antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX). The slow, metabotropic glutamate receptor (mGluR)-mediated inhibitory postsynaptic potential (IPSP) was enhanced by DPCPX only in slices from psychostimulant-treated animals. Under resting conditions, DPCPX was without effect on fast excitatory postsynaptic currents (EPSCs) in slices from saline- or cocaine-treated animals. However, in the presence of amphetamine, DPCPX did augment fast EPSCs in slices from cocaine-treated rats. Although DPCPX increased GABA_A IPSPs, the magnitude of the increase was not altered by cocaine pretreatment, even in the presence of amphetamine. This suggests that the elevated adenosine tone induced by cocaine treatment acts preferentially on glutamate terminals. Furthermore, the inhibition of the mGluR IPSP by endogenous adenosine may result in more effective burst firing mediated by glutamate afferents in cocaine-treated rats, a phenomenon known to enhance dopamine release.

INTRODUCTION

The mesolimbic dopamine system, originating in the ventral tegmental area (VTA), is thought to play a central role in reward, incentive learning, and motivational processes. Consistent with this, the mesolimbic dopamine system is the target of addictive drugs, many or all of which increase extracellular dopamine levels, particularly in the nucleus accumbens (Di Chiara and Imperato 1988). The addictive drugs best studied with respect to the dopamine system are the psychostimulants, especially cocaine and amphetamine (reviewed by Pierce and Kalivas 1997). Repeated use of psychostimulants by humans can result in the development of profound craving for the drug, as well as sensitization to the drug’s psychotomimetic effects. These effects of drug use are sustained even after long periods of abstinence. In rodents, repeated administration of cocaine or amphetamine results in a long-lasting sensitization to the locomotor stimulant effects of the drug. This sensitization is mediated at least in part by an enhanced ability of the stimulant to increase dopamine levels in the nucleus accumbens. The sensitization of the mesolimbic dopamine system may render it hypersensitive not only to psychostimulants, but also to stress and to environmental stimuli associated with drug use, all of which are known to promote craving and relapse to drug use (Robinson and Berridge 1993).

Although the behavioral consequences of repeated psychostimulant administration are reasonably well understood, far less is known about the underlying cellular and molecular changes. Perhaps the best established, persistent change at the cellular level is an alteration in signal transduction by dopamine D1 receptors, which are positively coupled to adenyl cyclase. This has been shown postsynaptically in the nucleus accumbens both in vitro (Higashi et al. 1989) and in vivo (Henry and White 1991, 1995) and presynaptically in the VTA in vitro (Bonci and Williams 1996).

D1 receptors in the VTA are found on afferent terminals from the nucleus accumbens and ventral pallidum containing GABA (Lu et al. 1997b; Mansour et al. 1991), and glutamatergic afferents from the prefrontal cortex (PFC) express D1 receptor mRNA (Lu et al. 1997a). Stimulation of D1 receptors in drug-naive animals enhances release of both GABA (Cameron and Williams 1993) and glutamate (Fiorillo and Williams 1998; Kalivas and Duffy 1995) onto dopamine neurons. In drug-naive guinea pigs, D1 receptor agonists enhance the slow inhibitory postsynaptic potential (IPSP). However, after 7–10 days withdrawal from repeated injections of cocaine or morphine, D1 agonists inhibited the IPSP (Bonci and Williams 1996). It was found that in treated animals, the cAMP produced by D1 activation was metabolized to extracellular adenosine, which then inhibited transmitter release through activation of adenosine A1 receptors. As a result, the adenosine tone was elevated in slices from drug-treated animals and was entirely dependent on tonic activation of D1 receptors. After blocking A1 receptors or the metabolism of cAMP, the modulation of transmitter release by D1 receptor agonists or antagonists was identical at all concentrations in slices from saline- and drug-treated animals (Bonci and Williams 1996). This suggests that the metabolism of cAMP to adenosine has been increased by repeated cocaine treatment, but that D1 receptors and their coupling to adenyl cyclase have not been changed.

More recently, it has been reported that the slow IPSP in dopamine neurons of rats consists of two components, an early component mediated by GABA_A receptors, and a late component mediated by metabotropic glutamate receptors (Fiorillo and Williams 1998). Furthermore, it is known that D1 receptors are present on glutamate as well as GABA terminals, so the observed changes could be present on either or both sets of terminals. It was therefore of interest to examine the synapse
specificity of the increased adenosine tone in drug-treated animals. The present study focused on adenosine tone in cocaine-treated rats. Certain experiments were also performed in rats treated with amphetamine or morphine.

**Methods**

**Treatment protocol**

Naive, male Wistar rats weighed 150–200 g. Treated rats received intraperitoneal injections of 1 ml/kg of 0.9% NaCl solution. Saline- and cocaine-treated (20 mg/kg cocaine HCl) animals were injected once daily for 14 days in their home cages. Other rats were given 2 mg/kg amphetamine sulfate every third day for 15 days (5 injections) or 10 mg/kg morphine sulfate every other day for 14 days (7 injections). They were then withdrawn for 10–20 days before killing, at which time they weighed 300–350 g. The care and killing of the rats complied with the guidelines of the National Institutes of Health.

**Slice preparation**

Intracellular or whole cell patch recordings were made in horizontal slices (250–300 μm for intracellular recordings, 200 μm for patch recordings) of ventral midbrain. Details of the method of slice preparation and recording have been published (Williams et al. 1984). Recordings were made from submerged slices in a chamber (0.5 ml) superfused with physiological saline at a rate of 1.5 ml/min and maintained at 35°C. The solution was equilibrated with 95% O2-5% CO2 and contained (in mM) 126 NaCl, 2.5 KCl, 1.2 MgCl2, 2.4 CaCl2, 1.4 NaH2PO4, 25 NaHCO3, and 11 D-glucose.

**Recordings**

Recordings were made with an Axoclamp 2A amplifier from dopamine neurons, identified by their electrical properties (Johnson and North 1992), in the VTA. The VTA was defined as the area medial and rostral, but not lateral or caudal, of the medial terminal nucleus of the accessory optic tract. Although some of these neurons are found close to the MT in the area of dense cell bodies termed the “compacta,” they are likely to be mesolimbic (Fallon and Loughlin 1995). Microelectrodes (50–80 MΩ) were filled with 2 M KCl. The membrane potential was adjusted to between −60 and −70 mV to prevent spontaneous action potentials. For whole cell patch recordings, cells were visualized using an upright microscope with infrared illumination and Nomarski optics. Patch electrodes (2–5 MΩ) contained (in mM) 125 KCl, 1 MgCl2, 1 EGTA, 5 HEPES, 1 ATP, and 0.3 GTP. For recordings of N-methyl-D-aspartate (NMDA) EPSCs, electrodes contained 125 Cs-glucionate, 10 NaCl, 1 MgCl2, 10 HEPES, 1 EGTA, 0.3 CaCl2, 1 ATP, and 0.3 GTP. A junction potential of approximately −10 mV with gluconate-containing electrodes was not corrected. The membrane potential was clamped between −70 and −80 mV for recording α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) excitatory postsynaptic currents (EPSCs), and at +30 mV for recording NMDA EPSCs. The access resistance was monitored with each stimulus by applying a 10-mV hyperpolarizing step. Membrane potential or holding current was recorded continuously at a slower sampling frequency in all experiments.

**Synaptic responses**

Synaptic potentials or currents were evoked with bipolar tungsten stimulating electrodes with a tip separation of 300–1,000 μm. For all synaptic responses, a train of 8–10 stimuli of 400 μs at 0.3–1.5 mA was delivered at 66 Hz (15-ms interval) every 60 s, except for NMDA EPSCs, which were evoked at 10 Hz. Stimulating electrodes were placed within 1 mm rostral of the recording site. By stimulating rostrally, descending afferents may be preferentially activated, many of which are presumed to originate in the prefrontal cortex (PFC) and nucleus accumbens.

The following antagonists were used to isolate the desired synaptic response. Picrotoxin (100 μM, GABA A), strychnine (1 μM, glycine), and eticlopride (100 nM, D2) were present in all experiments. 1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzof[1]quinoxaline (NBQX) (5 μM, AMPA) and MK-801 (50–100 μM, NMDA, pretreatment only) were used to isolate all slow IPSPs. The GABA A antagonist CGP 35348 (100–300 μM) or CGP 56999a (100–1,000 nM) was used to isolate mGluRI IPSPs. GABA A IPSPs were studied after treating the slice with apamin (100 nM, SK channels) or in cases in which a GABA A antagonist completely blocked the slow IPSP. Pretreatment with MK-801 was performed before studying AMPA EPSCs, and NBQX was present in experiments on the NMDA EPSC.

An mGluR-mediated slow excitatory postsynaptic potential (EPSP) can also be evoked in dopamine neurons and overlaps with the IPSP (Shen and Johnson 1997). The slow EPSP is infrequently observed with microelectrode recording and, when present, requires more stimuli than the IPSP (Fiorillo and Williams 1998). The depolarizing response to mGluR activation requires a more prolonged activation of the receptor than does the hyperpolarizing response, whereas the hyperpolarizing response desensitizes with prolonged receptor activation. It is therefore possible that a drug that increases glutamate release might enhance the EPSP and thereby mask or desensitize the IPSP. For this reason, after 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) was applied to mGluR IPSPs, apamin was superfused to reveal a slow EPSP if present. In the few cases in which an EPSP was present, the data were discarded.

**Drugs**

All drugs were applied to the slice by superfusion. The majority of drugs used, including DPCPX, had no effect on membrane potential or holding current. N6-cyclopentyladenosine (CPA) at high concentrations sometimes caused a hyperpolarization of up to 5 mV. Amphetamine (3 μM, in the presence of eticlopride) consistently produced a small depolarization (~3 mV), CGP 35348 (200 μM) and CGP 56999a (100–1,000 nM) consistently caused depolarizations of several millivolts, suggesting tonic activation of postsynaptic GABA A receptors.

Adenosine, adenosine triphosphate (ATP), S(+)–amphetamine sulfate, apamin, guanosine triphosphate (GTP), picrotoxin, and strychnine were from Sigma (St. Louis, MO). CPA, DPCPX, S(–)–eticlopride, and MK-801 were from Research Biochemicals International (Natick, MA). NBQX was from Toecis Cookson (St. Louis, MO). Cocaine HCl and morphine sulfate were from the National Institute on Drug Abuse (Rockville, MD). CGP 35348 and CGP 56999a were a gift from Novartis Pharmaceuticals (Basel, Switzerland).

**Data analysis**

Each specific experiment was performed only once per rat. Values are given as arithmetic means ± SE. The percent change produced by a drug was calculated as the mean amplitude of 5–10 synaptic responses after equilibrium had been reached (7–20 min) relative to the mean of five responses before drug superfusion. To construct concentration-response curves, two concentrations of CPA were superfused sequentially before reversal with DPCPX. To estimate the EC50 and maximal response, concentration-response curves were fit with a least-squares regression using the logistic equation. Statview software was used for performing statistical tests; *P* < 0.05 was considered as a significant difference. One- and two-way ANOVAs were performed with Fisher’s post hoc test. Unpaired comparisons between two groups were made with a Mann–Whitney *U* test, whereas paired comparisons were made using a Wilcoxon signed rank test.
RESULTS

Identification of dopamine neurons

Dopamine neurons in the VTA of horizontal slices were identified by their electrical properties (Johnson and North 1992). With intracellular, KCl-containing electrodes, these cells exhibited slow, spontaneous firing (~1–3 Hz), broad action potentials followed by large after hyperpolarizations, and a depolarizing “sag” in response to hyperpolarizing current injection. With patch electrodes, dopamine neurons were identified by their firing pattern in the cell-attached mode, and the presence of a large time- and voltage-dependent inward rectification in the current-voltage relation (Ih current) measured in the whole cell mode. As in previous studies, these neurons were found to respond to electrical field stimulation of the slice with both fast and slow synaptic responses mediated by both glutamate and GABA.

Adenosine tone on slow, mGluR IPSPs

We first measured the increase in slow IPSP amplitude in response to superfusion of the adenosine A1 receptor selective antagonist DPCPX (Fig. 1). In saline-treated animals, DPCPX (200 nM) did not cause a significant increase in IPSP amplitude (7.6 ± 6.6%, mean ± SE, n = 9). However, in cocaine- or amphetamine-treated rats, DPCPX caused a 52.1 ± 12.7% increase (n = 16) and 91.0 ± 24.0% (n = 6) increase (Fig. 1B), respectively (1-way ANOVA, main effect of treatment, F[2, 29] = 6.2, P = 0.006; effect of cocaine, P = 0.028, and amphetamine, P = 0.002). This confirms in rats previous results obtained in guinea pigs (Bonci and Williams 1996), showing that cocaine pretreatment potentiates the augmentation of slow IPSPs by A1 receptor antagonists.

In the above analysis of “slow IPSPs,” responses were selected in which the mGluR-mediated IPSP was present, as determined pharmacologically or by visual inspection. A GABA_A-mediated IPSP was present in most but not all cases. The increase produced by DPCPX was observed to be primarily due to an increase in the late (mGluR) component of the IPSP (Fig. 1A), although it was not possible under these conditions to accurately separate and measure the effect of DPCPX on the two synaptic components individually.

Adenosine tone on fast, glutamate-mediated EPSCs

Because it appeared that DPCPX had an effect on the mGluR IPSP, the adenosine tone was next examined on glutamate-mediated fast EPSCs. AMPA-mediated EPSCs were evoked using the same protocol as used to evoke slow IPSPs (10 stimuli at 66 Hz, as shown in Fig. 5A). The amplitudes of the first peak were 113 ± 19 pA (n = 22) and 120 ± 13 pA (n = 53) in slices from saline- and cocaine- or amphetamine-treated animals, respectively. Cocaine pretreatment did not alter the paired-pulse ratio of the third (saline, 0.72 ± 0.07; cocaine, 0.70 ± 0.05); or the 10th EPSC (saline, 0.44 ± 0.06; cocaine, 0.42 ± 0.04) relative to the first EPSC. The paired-pulse ratios measured here are very similar to those previously published for EPSCs in dopamine neurons from naive rats (Bonci and Malenka 1999). DPCPX was without effect on EPSCs in slices from either saline- or cocaine-treated rats (Fig. 2; 0.3 ± 4.5%, n = 10, and 0.9 ± 6.9%, n = 10, respectively, percent increase in amplitude of the 1st EPSC in the train). Another recent study also found a lack of adenosine tone on AMPA EPSCs in dopamine neurons of the VTA, in both naive and acutely morphine-withdrawn rats (Manzoni and Williams 1999).

It is generally thought that the same terminals release glutamate onto both AMPA and NMDA subtypes of glutamate receptor. However, because of the unique role that NMDA receptors are thought to play in the burst firing of dopamine neurons (Johnson et al. 1992b; reviewed by Overton and Clark 1997), as well as the possible presence of “NMDA-only” synapses, adenosine tone was also examined on NMDA-mediated EPSCs, studied at +30 mV to relieve Mg^2+ blockade. DPCPX had no effect or caused a small decrease in the amplitude of NMDA EPSCs in both saline- and cocaine-treated rats (−9.7 ± 2.4%, n = 6, and −9.7 ± 4.5%, n = 7, respectively). Therefore while adenosine tone is present on mGluR synaptic responses in cocaine-treated rats, it is absent on synaptic responses mediated by ionotropic glutamate receptors (iGluRs).

Having examined adenosine tone under resting conditions, we next investigated adenosine tone in the presence of d-amphetamine at a concentration known to be self-administered by rats (3 μM) (Clausing et al. 1995; Yokel and Pickens 1974). By releasing dopamine and activating D1 receptors, amphetamine may increase adenosine tone, as implied by Bonci and Williams (1996). Amphetamine (3 μM) itself did not have a
clear effect on AMPA EPSCs in slices from saline- or cocaine-treated rats after 10–15 min of superfusion (−0.6 ± 13%, n = 4 and −1.5 ± 5.0%, n = 12, respectively).

In the presence of amphetamine, DPCPX was without effect on AMPA-mediated EPSCs in slices from saline-treated animals (0.0 ± 3.2%, n = 10), but augmented EPSCs by 31 ± 9.7% (n = 10) in slices from cocaine-treated animals (Fig. 2; 2-way ANOVA, effect of pretreatment, F[1,36] = 5.6, P = 0.024; amphetamine, F[1,36] = 4.9, P = 0.033; and a pretreatment X amphetamine interaction, F[1,36] = 4.7, P = 0.037). Although the first EPSC in the train was significantly augmented, the third (Fig. 2, inset) and 10th EPSCs were not (14 ± 11%, n = 10, and 14 ± 14%, n = 8, respectively), consistent with a presynaptic effect on glutamate release. Amphetamine thus reveals an increased adenosine tone on glutamate terminals caused by cocaine pretreatment.

One concern was the lack of effect of amphetamine on EPSC amplitude. This observation may suggest that D1 receptors were not activated. Multiple effects of amphetamine in the VTA have recently been described. At slightly higher concentrations amphetamine decreases EPSC amplitude in the VTA through 5-HT–mediated presynaptic inhibition (Jones and Kauer 1999). In addition, amphetamine results in the activation of postsynaptic alpha-1-adrenoceptors on dopamine cells to cause a potent inhibition of the mGluR IPSP (C. D. Fiorillo, C. Paladini, and J. T. Williams, unpublished observations). Also note that amphetamine decreased the amplitude of the GABA B IPSP (Fig. 4). Thus the lack of effect of amphetamine on the EPSC amplitude may be complicated by multiple effects.

GABA B receptors may be necessary for adenosine tone

As illustrated by in Fig. 1, DPCPX significantly augmented mGluR IPSPs under conditions where both GABA B and mGluR IPSPs were present. The effect of DPCPX on pharmacologically isolated mGluR IPSPs were examined in the presence of a GABA B antagonist (CGP 35348, 100–300 μM or CGP 56999a, 0.1–1 μM). In these experiments the augmentation of the mGluR IPSP by DPCPX was quite variable, and a significant augmentation was not observed in either saline- or cocaine-treated rats (7.0 ± 14.0%, n = 5 and 20.6 ± 13.4%, n = 8, respectively, Fig. 3). It appears that GABA B antagonists prevent or reduce the augmentation of glutamate release by DPCPX.

We therefore examined the effect of a GABA B antagonist (CGP 56999a, 0.3–1.0 μM) on amphetamine-induced adenosine tone on AMPA EPSCs in slices from cocaine-treated rats. CGP 56999a was itself without significant effect on the amplitude of the AMPA EPSCs in naive or cocaine-treated rats, similar to previous results in naive and morphine-treated rats (Manzoni and Williams 1999). In the presence of CGP 56999a, DPCPX was without effect on the amplitude of EPSCs in cocaine-treated animals (0.4 ± 5.8%, n = 7), even in the presence of amphetamine (2.4 ± 4.0%, n = 10, not shown). This suggests that tonic activation of GABA B receptors is necessary for adenosine tone on glutamate terminals.

The mechanism by which CGP 56999a blocks adenosine tone was further investigated by measuring EPSC inhibition by exogenous adenosine in slices from cocaine-treated rats. Initially adenosine (50 μM) produced an inhibition of −20 ± 7.0% (n = 5). After superfusion of CGP 56999a (0.5–1.0 μM), adenosine caused a similar inhibition of −28 ± 4.5% in the same cells. This indicates that blockade of GABA B receptors does not decrease the sensitivity of presynaptic A1 receptors. It may therefore be the case that tonic activation of GABA B receptors is necessary for adenosine production, although it is not clear through what mechanism this could occur.

Adenosine tone on GABA B IPSPs

Isolated GABA B IPSPs were studied after exposure of the slice to apamin (100 nM), or in cases in which the entire IPSP could be blocked by CGP 35348 (100–300 μM). DPCPX augmented the isolated GABA B IPSP by 19.3 ± 3.8% (n = 8, Fig. 3).

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**FIG. 2.** Adenosine tone on fast excitatory postsynaptic currents (EPSCs) is present only in slices from cocaine-treated animals in the presence of amphetamine. Averaged data from the 1st EPSC in the train, showing that DPCPX produces an augmentation only in slices from cocaine-pretreated animals, and only in the presence of amphetamine (3 μM). Inset: superimposed traces from a single cell, illustrating the effect of DPCPX in the presence of amphetamine (3 μM) in a slice from a cocaine-pretreated rat. Only the 1st 3 of 10 EPSCs in the train are shown.

**FIG. 3.** Cocaine pretreatment does not significantly alter adenosine tone on the mGluR IPSP when it is isolated by either of the GABA B antagonists CGP 35348 (100–300 μM) or CGP 56999 (100–1,000 nM). The bar graph on the left shows the average increases in amplitude of mGluR IPSPs in response to DPCPX (200 nM) in saline- and cocaine-pretreated rats. Control peak amplitudes were 9.8 ± 0.9 mV in saline-treated rats and 9.7 ± 0.9 mV in cocaine-pretreated rats. Numbers above the error bars indicate the number of rats in which each experiment was performed. At the right are mGluR IPSPs from a cocaine-pretreated animal in the absence and presence of DPCPX.
FIG. 4. Adenosine tone on GABA_B IPSPs is not altered by cocaine pretreatment. Left: bar graph shows the mean augmentation of GABA_B IPSP peak amplitude by DPCPX in slices from saline- or cocaine-treated animals, in the presence (right) or absence (left) of amphetamine (3 μM). Numbers above each error bar represent the number of animals in which each experiment was performed. Control peak amplitudes were 11.3 ± 1.3 mV in saline-treated rats, 8.6 ± 1.1 mV in cocaine-treated rats, 10.4 ± 0.6 mV in saline-treated rats in the presence of amphetamine, and 8.4 ± 0.9 mV in cocaine-treated rats in the presence of amphetamine. Right: GABA_B IPSPs from a cocaine-treated rat in the absence and presence of DPCPX.

Wilcoxon signed-rank test, P = 0.008, Fig. 4). However, the facilitation of GABA_B IPSPs by DPCPX was unchanged by cocaine pretreatment (20.4 ± 2.8%, n = 10, Fig. 4). This is in apparent contradiction to previously published results in cocaine-treated guinea pigs (Bonci and Williams 1996). However, in the present study it was found that in slices taken from rats 10 or more days withdrawn from repeated morphine injections, DPCPX produced a substantial increase in the amplitude of GABA_B IPSPs (47.9 ± 6.9%, n = 5). This is significantly greater than its effect in slices from saline-treated rats (P = 0.008, Mann-Whitney U test) and is similar to results from guinea pigs (Bonci and Williams 1996).

Although cocaine-pretreatment did not elevate adenosine tone on GABA terminals in slices under resting conditions, there may be elevated adenosine tone in the presence of amphetamine. Unexpectedly, amphetamine reduced the augmentation produced by DPCPX in both treatment groups (Fig. 4). This inhibition was not mediated by an increase in adenosine synthesis because DPCPX had no additional effect. In addition, in the presence of amphetamine the effect of DPCPX was not different between slices from saline- and cocaine-pretreated rats (6.1 ± 2.0%, n = 4 and 9.6 ± 3.7%, n = 10, respectively, 2-way ANOVA, effect of amphetamine only, F[1,28] = 10.2, P = 0.004). The inhibition by amphetamine may result from the release of 5-HT, which causes a potent presynaptic inhibition of the GABA_B IPSP (Cameron and Williams 1994; Johnson et al. 1992a). Although the interpretation of the present result is complicated by the inhibition by amphetamine, it suggests that the increased adenosine in the VTA caused by repeated cocaine treatment may be restricted to glutamate terminals.

Sensitivity of synaptic responses to A1 receptor inhibition

It has been reported that repeated cocaine treatment does not alter the inhibition by CPA (a metabolically stable, A1 receptor-selective analogue of adenosine) of either GABA_B IPSPs in the VTA (Bonci and Williams 1996) or glutamate EPSPs in the nucleus accumbens (Manzoni et al. 1998). However, the potency of adenosine in the nucleus accumbens (at least at room temperature) is decreased following cocaine withdrawal due to enhanced uptake (Manzoni et al. 1998). In the present study an approximately half-maximal concentration of adenosine (50 μM) caused an inhibition of the AMPA EPSC of −32 ± 9.8% (n = 6) in slices from saline-treated rats, −35 ± 2.4% (n = 5) in cocaine-treated rats, and −33 ± 4.9% (n = 7) in amphetamine-treated rats (Fig. 5C). This suggests that in the VTA, repeated psychostimu-
sufficiently high frequency (Brenowitz et al. 1998). The presence of amphetamine, at a concentration known to be effective in increasing adenosine tone on glutamate terminals, was increased by amphetamine, which enhances dopamine release. Furthermore, inhibition of EPSCs by exogenous adenosine was not altered by cocaine or amphetamine pretreatment, suggesting that increased adenosine tone must be dependent on enhanced adenosine production (Bonci and Williams 1996).

**DISCUSSION**

**Increased adenosine tone on glutamate terminals**

This study confirms previous work showing that repeated cocaine treatment increases adenosine tone in the VTA (Bonci and Williams 1996). The present results indicate that the increase in adenosine tone selectively inhibits glutamate release, whereas cocaine pretreatment does not alter adenosine tone on GABA<sub>B</sub> IPSPs. Although there was no adenosine tone on fast or slow glutamate-mediated synaptic responses in control animals, cocaine or amphetamine treatment resulted in substantial adenosine tone on slow, mGluR-mediated IPSPs. In the presence of amphetamine, at a concentration known to be self-administered (3 μM) (Clausing et al. 1995; Yokel and Pickens 1974), adenosine tone was also present on fast EPSCs in cocaine- but not saline-treated animals.

The lack of increase in adenosine tone on GABA<sub>B</sub> IPSPs resulting from cocaine treatment in the present study is in apparent contradiction with previously published results (Bonci and Williams 1996). Because the presence of an mGluR IPSP was not yet known at the time of the earlier study, it is possible that the effects observed on the slow IPSP were due to changes in release of glutamate rather than GABA. However, under the conditions of the previous study, the mGluR IPSP would have been less prominent than in the present study (unpublished observations). A species difference in adenosine tone could therefore account for the disparate results. For instance, if adenosine is produced in glutamate terminals, it could diffuse far enough to reach GABA terminals in slices from guinea pigs but not rats.

The present study did not address the mechanism by which adenosine tone is increased. Presumably, it is the result of dopamine activation of D1 receptors, production of cAMP, and subsequent metabolism of cAMP to adenosine (Bonci and Williams 1996; Shoji et al. 1999). In support of such a mechanism, adenosine tone on fast EPSCs in cocaine-pretreated rats was increased by amphetamine, which enhances dopamine release. Furthermore, inhibition of EPSCs by exogenous adenosine was not altered by cocaine or amphetamine pretreatment, suggesting that increased adenosine tone must be dependent on enhanced adenosine production (Bonci and Williams 1996).

D1 receptors are thought to be present on glutamate terminals of afferents from the prefrontal cortex (Lu et al. 1997a), as well as GABA terminals of afferents from the nucleus accumbens and ventral pallidum (Lu et al. 1997b; Mansour et al. 1991). In light of the present results, it is likely that the increased adenosine produced after cocaine pretreatment derives from D1 receptor activation on glutamate-containing terminals from the prefrontal cortex. Although D1 receptors on GABA terminals produced more adenosine than those on glutamate terminals in slices from control rats, this transduction mechanism appeared unaltered by repeated cocaine treatment. The present results also indicate that extracellular adenosine can be localized to specific synapses, because adenosine tone inhibited only GABA release in slices from control animals and was increased only on glutamate terminals by cocaine pretreatment.

**Selective inhibition of mGluR IPSPs**

Repeated cocaine treatment resulted in adenosine tone on the mGluR IPSP, but not on the AMPA or NMDA receptor-mediated EPSCs. Similarly, adenosine tone is enhanced on mGluR IPSPs (Williams, unpublished observations) but not EPSCs (Manzoni and Williams 1999) during acute morphine withdrawal. The reason for this selectivity of adenosine action is not known. Concentration-response curves to the metabolically stable A1 receptor agonist CPA suggest that the mGluR IPSP may be slightly more sensitive than the AMPA EPSP to A1 inhibition. It is possible that the difference in glutamate concentration and kinetics necessary for receptor activation, or the very different postsynaptic transduction mechanisms, could account for the slightly different sensitivities to A1 inhibition. However, the relatively small difference in sensitivity to A1 receptor occupation may not be sufficient to account for the disparity between the A1 inhibition of AMPA and mGluR IPSP (Williams, unpublished observations).
inhibition is unable to account for the substantial difference in adenosine tone on iGluR and mGluR synaptic responses.

There are a number of mechanisms through which the mGluR IPSP could be modulated postsynaptically (Fiorillo and Williams 1998). A postsynaptic effect of A1 receptors on the mGluR IPSP is possible but unlikely, because the maximal inhibition by CPA was the same for AMPA and mGluR synaptic responses. Furthermore, in guinea pigs acutely withdrawn from morphine, DPCPX enhanced mGluR IPSPs, but not mGluR-mediated hyperpolarizations by aspartate, in dopamine neurons of the VTA (Williams, unpublished observations). It is therefore concluded that the inhibition by A1 receptors of the mGluR IPSP occurs presynaptically, with little effect postsynaptically.

One explanation for the difference in adenosine tone is that different populations of terminals mediate the iGluR and mGluR synaptic responses. Although there is no evidence for “mGluR only” synapses, it is possible that a large proportion of terminals releasing glutamate onto AMPA and NMDA receptors do not release glutamate onto mGluRs. It appears that an analogous situation exists with respect to GABA terminals on dopamine neurons of the VTA. The vast majority of terminals releasing GABA onto GABA_A receptors in response to local stimulation of the VTA do not activate postsynaptic GABA_B receptors, because the GABA_B IPSP, but not the GABA_A IPSP, is sensitive to presynaptic regulation by D1 (Cameron and Williams 1993) and 5-HT1 agonists (Johnson et al. 1992a; Sugita et al. 1992). For glutamate terminals, however, the degree of modulation by maximally effective concentrations of A1, µ-opioid, 5-HT1, and D1 receptor agonists is similar for both AMPA and mGluR synaptic responses (present results; Fiorillo and Williams 1998; Manzoni and Williams 1999; unpublished observations); so at present there is little evidence for a difference in the pool of glutamate terminals responsible for the iGluR and mGluR synaptic responses.

Regardless of whether the iGluR and mGluR synaptic responses are mediated by identical or distinct glutamate terminals, it is anticipated that adenosine would cause greater inhibition of the mGluR response than of the iGluR response. Although A1 receptors caused substantial inhibition of the first EPSP in a train, subsequent EPSPs were inhibited little or not at all (Figs. 2A and 5A). Thus with a short train of presynaptic action potentials, adenosine would cause a greater inhibition of the mGluR IPSP than of the sum of the EPSPs. The net result would be a greater excitation of dopamine neurons for a given presynaptic fiber volley in cocaine-treated rats.

Implications for the mesolimbic dopamine system

Because of the difference in kinetics of the iGluR and mGluR synaptic potentials, it is predicted that the mGluR IPSP acts to limit the duration of the burst of action potentials driven by the iGluR EPSP (particularly the NMDA component of the EPSP). A selective inhibition of the mGluR IPSP would therefore be expected to prolong the duration of burst events. It has been reported that at 10 days withdrawal from repeated amphetamine treatment of rats, stimulation of prefrontal cortex more reliably elicits bursts in dopamine neurons of the VTA (Tong et al. 1995). The present results, with both cocaine and amphetamine treatment, suggest a mechanism that may account, at least in part, for the enhanced excitation of dopamine neurons after psychostimulant treatment.

A recent study has shown that systemic cocaine evokes greater glutamate release in the VTA of rats 21 days withdrawn from daily cocaine treatment (Kalivas and Duffy 1998). The glutamate release was blocked by prior infusion of a D1 antagonist into the VTA. However, the enhanced increase in glutamate release in response to cocaine was very transient and may have been due to a conditioned response of the behaving animal rather than a pharmacological action of cocaine.

It is well established that conditioned cues are a primary trigger of relapse to drug use in both human addicts and other species. It has been hypothesized that sensitization of the mesolimbic dopamine system may underlie the powerful craving elicited by external stimuli associated with drug effects (Robinson and Berridge 1993). The potential role of dopamine neurons in such a process has been advanced by studies of dopamine cell activity during the learning of cues predicting reward in monkeys (Ljungberg et al. 1992; Schultz et al. 1993; reviewed by Schultz 1998). Initially, dopamine cells are unaffected by a neutral stimulus, but respond to an unpredicted natural reward, such as juice, with a burst of action potentials.

With repeated pairings of the neutral stimulus and reward, the response of dopamine neurons is conditioned such that the formerly neutral stimulus now elicits a response, but the primary reward is no longer effective. Dopamine neurons therefore respond to errors in the prediction of reward. This finding promotes the theory that an increased activity of dopamine neurons underlies the craving and anticipation of rewards and not necessarily the response to rewards themselves (Robinson and Berridge 1993). It is not known which input(s) to dopamine cells mediates this excitation, but the glutamatergic inputs from the PFC are a likely candidate. If this is the case, then selective inhibition of the mGluR IPSP by adenosine might be one mechanism by which a conditioned cue could elicit a greater dopamine response, and presumably greater craving for drug, in a cocaine-experienced animal.

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REFERENCES


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