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Cocaine and Amphetamine Depress Striatal GABAergic Synaptic Transmission through D2 Dopamine Receptors

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The striatum is a brain area implicated in the pharmacological action of drugs of abuse. To test the possible involvement of both cocaine and amphetamine in the modulation of synaptic transmission in this nucleus, we coupled whole-cell patch clamp recordings from striatal spiny neurons to the focal stimulation of glutamatergic or GABAergic nerve terminals. We found that neither cocaine (1–600 μM) nor amphetamine (0.3–300 μM) significantly affected the glutamate-mediated EPSCs recorded from these cells. Conversely, both pharmacological agents depressed GABA-mediated IPSCs in a dose-dependent manner. This effect was mediated by the stimulation of dopamine (DA) D2 receptors since it was prevented by 3 μM L-sulpiride (a DA D2-like receptor antagonist), mimicked by the DA D2-like receptor agonist quinpirole (0.3–30 μM), and absent in mice lacking DA D2 receptors. A presynaptic mechanism was likely involved in this action since both cocaine and amphetamine depress GABAergic transmission by increasing paired-pulse facilitation. Cocaine and amphetamine failed to affect GABAergic IPSCs after 6-OHDA-induced nigral lesion, indicating that both drugs cause their effects through the release of endogenous DA. The modulation of GABAergic synaptic transmission in the striatum might underlie some motor and cognitive effects of psychostimulants in mammalians.

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Following psychostimulant administration, markers of brain activity are altered in many areas (Stein and Fuller 1993; Lyons et al. 1996; Breiter et al. 1997), suggesting that the diverse cognitive, emotional, and motor effects of these drugs are caused by the interaction with multiple neuronal systems in the central nervous system. Increasing evidence indicates that not only cortical but also subcortical areas play a role in the cocaine- and amphetamine-mediated effects. In particular, the increased locomotor activity and stereotypy caused by psychostimulants seem to involve specifically the nucleus striatum (Kelly et al. 1975; Amalric and Koob 1993; Berke and Hyman 2000), a structure that receives virtually all the cortical information directed to the basal ganglia (Penney and Young 1983; Albin et al. 1989; McGeorge and Faull 1989; Calabresi et al. 1996;

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Kincaid and Wilson 1996; Parthasarathy and Graybiel 1997; Kaneko et al. 2000). The nucleus striatum also receives profuse dopaminergic innervation from the substantia nigra and has a very high density of D1 and D2 dopamine (DA) receptors but also of D3, D4 and D5 receptors (Mansour and Watson 1995; Surmeier et al. 1996; Bordet et al. 1997; LaHoste et al. 2000).

Amphetamine and cocaine cause in the striatum rapid induction of c-fos, a commonly used molecular marker for neuronal activity. Interestingly, this effect is sensitive to dopamine (DA) receptor blockade (Graybiel et al. 1990; Moratalla et al. 1993), suggesting that increased release of DA in the striatum is responsible, at least in part, for the action of these drugs. In this regard, while the full diversity of drug effects is mediated by multiple neurotransmitters acting in multiple brain regions, most drugs abused by humans share the common property of increasing DA release in this brain area (Di Chiara and Imperato 1988; Kuczenshi et al. 1991; Yamamoto and Spanos 1988; Koob et al. 1998; Ito et al. 2000). Cocaine increases DA availability in the striatum through the blockade of transporter-mediated reuptake of this transmitter, whereas amphetamine exerts its action by promoting DA efflux from DA-containing synaptic endings by reversing the DA uptake transporter (Seiden 1993). Other mechanisms, however, might be involved in the pharmacological actions of cocaine and amphetamine in the striatum. Both drugs, in fact, facilitate serotonin and norepinephrine release (Barker and Blakely 1995; Johanson and Schuster 1995) and cocaine has been found to modulate striatal neuron firing activity in vivo independently of transmitter release but through a direct interaction with sodium channels (Kiyatkin and Rebec 2000).

In the present in vitro study, therefore, we investigated the cellular and synaptic mechanisms implicated in the pharmacological actions of both cocaine and amphetamine on striatal neurons, addressing in detail the dependency of their effects upon the integrity of the DAergic system in this brain area. To achieve these goals we coupled whole-cell patch clamp recordings from striatal projection neurons to the focal stimulation of either corticostriatal glutamatergic or intrinsic GABAergic nerve terminals. We found that both cocaine and amphetamine selectively modulate GABAergic inputs to striatal neurons. This effect was dependent on the release of DA from nigrostriatal nerve terminals since it was absent in corticostriatal slices prepared from rats given severe lesion of the substantia nigra. Finally, we unequivocally identified the DA receptor subtype implicated in the cocaine-, amphetamine-, and DA-mediated depression of striatal GABAergic synaptic transmission by comparing the effects of these pharmacological agents in normal mice with those obtained in transgenic mice selectively lacking D2 DA receptors.

METHODS

Corticostriatal slices 200–270 μm thick were prepared from adult Wistar rats and mice. The preparation and maintenance of coronal slices have been described previously (Calabresi et al. 1997; Centonze et al. 1999). Briefly, coronal slices were prepared from tissue blocks of the brain with the use of a vibratome. A single slice was placed onto the glass coverslip that formed the base of the recording chamber and continuously superfused with Krebs solution (30°C, 2–3 ml/min) gassed with 95% O_2 + 5% CO_2. The composition of the control solution was (in mM): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 11 glucose, 25 $NaHCO_3$.

Individual striatal neurons were visualized in situ using an Olympus BX50WI (Tokyo, Japan) non-inverted microscope with 40× water immersion objective combined with an infra-red filter, a monochrome CCD camera (Cohu 4912), and a PC compatible system for analysis of images and contrast enhancement (WinVision 2000, Delta Sistemi, Italy).

Recordings were made with borosilicate glass pipettes (1.8 mm O.D.; 3–5 Mμ) containing (mM) K^+-gluconate (125), NaCl (10), $CaCl_2$, (1.0), $MgCl_2$ (2.0), 1,2-bis (2-aminophenoxy) ethane-N,N,N,N-tetraacetic acid (BAPTA; 1), N-(2-hydroxyethyl)-piperazine-N-2-ethanesulfonic acid (HEPES; 19), guanosine triphosphate (0.3), Mg-adenosine triphosphate (2.0), adjusted to pH 7.3 with KOH. Recording pipettes were advanced towards individual cells in the slice under positive pressure and, on contact, tight G seals were made by applying negative pressure. The membrane patch was then ruptured by suction and membrane current and potential monitored using an Axopatch 1D patch clamp amplifier (Axon Instruments, Foster City, CA). Whole-cell access resistances measured in voltage clamp were in the range of 5–30 Mμ prior to electronic compensation (60–80% was routinely used).

Voltage steps were generated using pCLAMP software (version 8.0; Axon Instruments, Foster City, CA). For synaptic stimulation, bipolar electrodes were used.

Values given in the text and in the figures are mean ± SEM of changes in the respective cell populations. Wilcoxon's test or Student's *t*-test (for paired and unpaired observations) were used to compare the means and ANOVA was used when multiple comparisons were made against a single control group. For dose-response curves, the sigmoidal equation best fitting the experimental data was calculated by using the commercially available software Graph Pad Prism (version 2.01), which yielded values for IC_{50} ± SEM.

Drugs were applied by dissolving them to the desired final concentration in the saline and by switching the perfusion from control saline to drug-containing saline. Amphetamine and cocaine were generous gifts from Dr. N. B. Mercuri. 6-cyano-7-nitroquinoxaline-

2,3-dione (CNQX), D-2-amino-5-phosphonovalerate (APV), and (+)-MK 801 maleate (MK-801) were from Tocris (U.K.). Bicuculline (BMI), quinpirole, SCH 23390, and SKF38393 were from RBI (USA). L-sulpiride was from Sigma (Italy).

To obtain unilateral nigrostriatal lesions, rats (anaesthetized with 45 mg/kg body weight pentobarbitone i.p.) were injected with 6-OHDA (8 μg/4 μL of saline containing 0.1% ascorbic acid) via a Hamilton syringe through a cannula inserted just rostral to the substantia nigra using stereotaxic coordinates (Paxinos and Watson 1986). Twenty days later, the rats were tested with 0.5 mg/kg s.c. of apomorphine and contralateral turns were counted for 1 hr. Only those rats that consistently made at least 400 contralateral turns were used for the electrophysiological studies. In some cases, 6-OHDA-lesioned rats were anaesthetized with diethyl ether and brain dissection confirmed that the nigrostriatal pathway was lesioned. This was monitored by using a monoclonal antibody for tyrosine-hydroxylase.

The generation of mice lacking D2 receptors has been reported previously (Baik et al. 1995). The intrinsic and synaptic membrane properties of neurons recorded in vitro from D2 lacking striatal slices were similar to those recorded from wild-type mice and control rats, as previously reported (Calabresi et al. 1997).

RESULTS

Intrinsic and Synaptic Properties of Striatal Neurons

Rat striatal medium spiny neurons were identified in corticostriatal slices by morphological and electrophysiological criteria. Striatal spiny neurons had significantly smaller somata than interneurons (15–25 μm vs. 30–60 μm) and displayed high resting membrane potential (−82 ± 3 mV), action potential discharge with little adaptation during depolarizing current pulses and, when recorded in voltage-clamp mode, a typical current-voltage relationship (Figure 1A). These electrophysiological properties were similar to those reported previously by our group and by others for medium spiny neurons of the striatum (Kita et al. 1984; Jiang and North 1991; Wilson and Kawaguchi 1996; Calabresi et al. 1998).

Both glutamate-mediated post-synaptic inward currents (EPSCs) and GABA-mediated post-synaptic outward currents (IPSCs) were inducible in striatal spiny neurons. To evoke corticostriatal EPSCs, the stimulating electrode was placed close to the recording electrode in the white matter between the cortex and the striatum, whereas it was placed within the striatum to obtain IPSCs. In the presence of the GABAA receptor antagonist BMI (3 μM), a single activation of corticostriatal fibres produced EPSCs. At the holding potential (HP) of –80 mV, these synaptic currents (amplitude 330 ± 148 pA) were not affected by the NMDA glutamate receptor antagonists MK-801 (30 μM, n = 6) or APV (50 μM, n = 5), while they were fully abolished by 10 μM CNQX, an AMPA glutamate receptor antagonist (n = 8) (Figure 1B). GABA-mediated synaptic currents (amplitude 160 ± 50 pA) were conversely recorded following intrastriatal stimulation in the presence of MK-801 (30 μM) or APV (50 μM) and CNQX (10 μM). These currents were completely sensitive to BMI (3 μM; n = 25) and were detected as outward deflections when the membrane potential of the cells was depolarized to –40 mV (Figure 1C). At HP of –80 mV, conversely, the bicuculline-sensitive GABAA-mediated synaptic currents were usually detected as inward events of small amplitude (~20–50 pA). In this study, therefore, all glutamate-mediated EPSCs and GABAA-dependent IPSCs were recorded at HPs of −80 mV and −40 mV, respectively. In these experimental conditions, the amplitude of both inward and outward currents recorded from striatal spiny neurons depended on the intensity

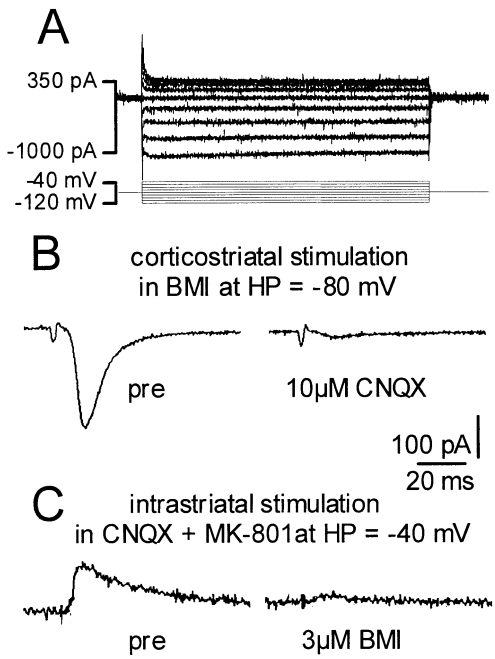

Figure 1. Intrinsic and synaptic properties of striatal neurons. **(A)** The electrophysiological traces show a typical current-voltage relationship recorded from a striatal spiny neuron in vitro. Plots were obtained from voltage-clamp experiments, holding the cell at −80 mV and applying positive and negative voltage steps. **(B)** The electrophysiological traces are corticostriatal EPSCs recorded in the presence of the GABAA receptor antagonist BMI at HP of −80 mV before (left) and 5 min after the application of the glutamate AMPA receptor antagonist CNQX (right). **(C)** The electrophysiological traces are striatal IPSCs recorded from another striatal neuron in the presence of the glutamate receptor antagonists MK-801 and CNQX at HP of −40 mV before (left) and 5 min after the application of BMI (right).

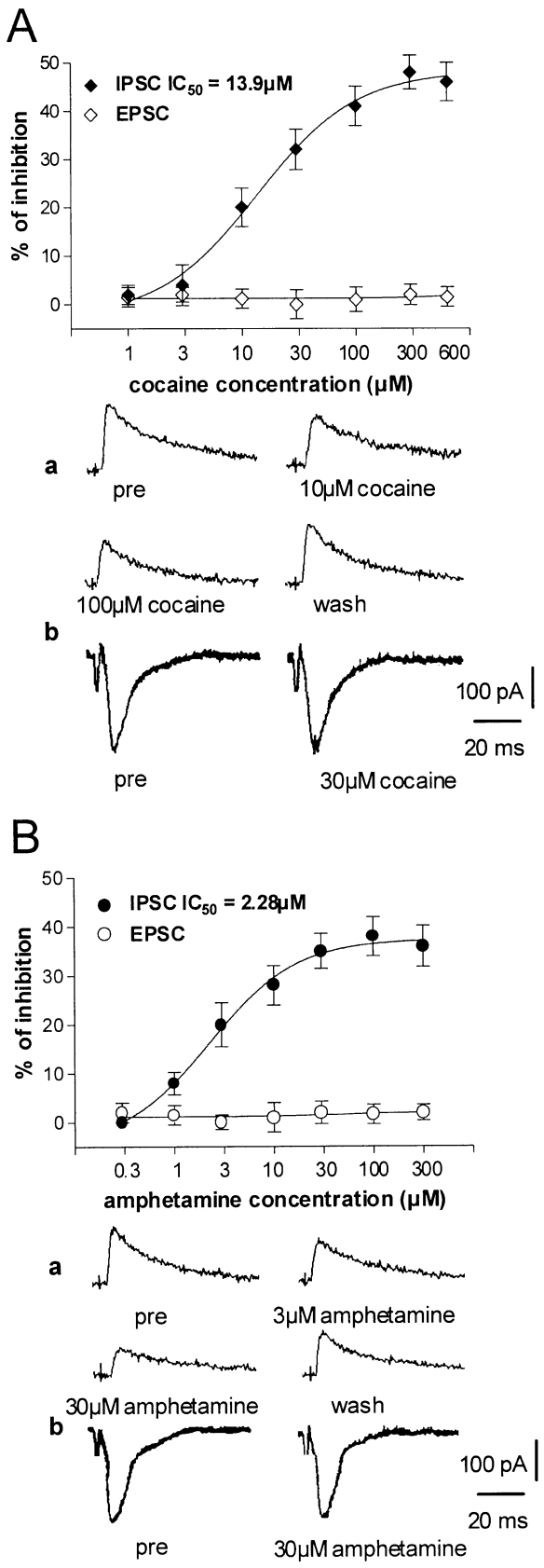

Figure 2. The inhibitory effects of both cocaine and amphetamine on striatal GABAergic IPSCs are dose-dependent. **(A)** Dose-response relationships of the effects of cocaine on striatal IPSCs and EPSCs. The electrophysiological traces represent IPSCs (a) and EPSCs (b) recorded from two different striatal neurons in control medium (pre), in the presence of 10 μM cocaine, in the presence of 100 μM cocaine, and 15 min after the wash of the drug. **(B)** Dose-response relationships of the effects of amphetamine on striatal IPSCs and EPSCs. The electrophysiological traces represent IPSCs and EPSCs recorded from two other striatal neurons in control medium, in the presence of 3 μM amphetamine, in the presence of 30 μM amphetamine, and 15 min after the wash out of the drug.

of stimulation and the distance between the stimulating and the recording sites.

Effects of Cocaine and Amphetamine on Striatal Synaptic Transmission and Cell Membrane Properties

We investigated the possible modulatory action of psychostimulants on both excitatory and inhibitory synaptic transmission on the striatum. Cocaine (30–600 μM, 10 min bath application, $n = 8$) and amphetamine (30–300 μM, 10 min bath application, $n = 9$) produced no detectable effects on the amplitude of corticostriatal EPSCs ($p > .05$ for each compound at each concentration), while both drugs were found capable of causing a marked depression of IPSC amplitude (n=24 for cocaine and n = 26 for amphetamine). The cocaine and amphetamine inhibition of GABAergic IPSCs was concentration dependent with minimal effects produced by 10 μM cocaine ($p < .01$) or 1 μM amphetamine ($p < .05$) and maximal inhibition caused by 100–600 μM cocaine ($p < .001$ for each concentration) or 30–300 μM amphetamine ($p < .001$ for each concentration). The calculated IC_{50} was 13.9 ± 1.4 μM for cocaine and 2.28 ± 1.2 μM for amphetamine (Figure 2). This effect was fully reversible after 15–20 min wash and was not associated with changes either in the reversal potential of the GABA-mediated synaptic currents nor in the holding current and membrane conductance of the recorded striatal neurons ($p > .05$ for each electrophysiological parameter) (Figure 3). This latter electrophysiological parameter was calculated by measuring the current drops produced from the HP of –40 mV or –80 mV by 5 mV voltage steps in hyperpolarizing direction.

Effects of DA Denervation and of Pharmacological Blockade of DA D1- and D2-like Receptors on the Cocaine- and Amphetamine-induced Inhibition of Striatal GABAergic Transmission

Although it is generally believed that psychostimulants exert their physiological actions by increasing the levels of endogenous DA in critical brain areas, it has been re-

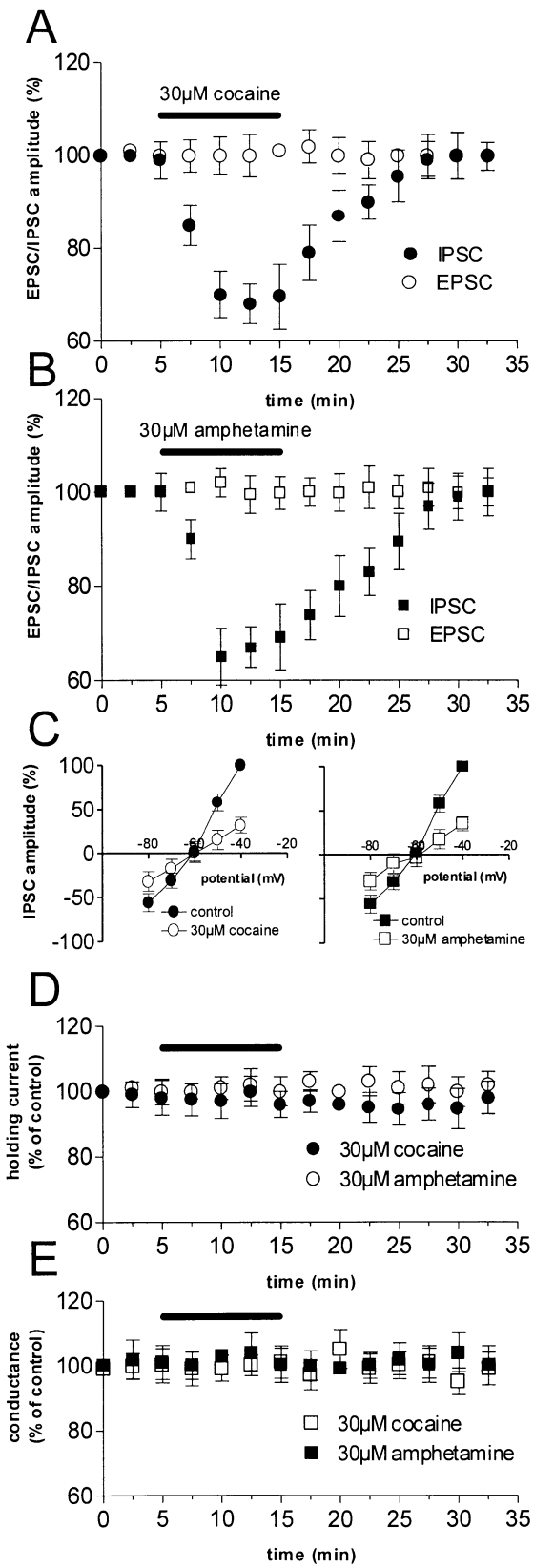

Figure 3. Psychostimulants depress striatal IPSCs. **(A)** The graph shows that bath application of cocaine causes a marked and reversible inhibition of striatal GABAergic transmission. Corticostriatal EPSCs are conversely unaffected by this pharmacological agent. **(B)** Similarly, the application of amphetamine depresses striatal GABAergic transmission without affecting corticostriatal glutamate-mediated synaptic currents. **(C)** Both cocaine (left) and amphetamine (right) depress GABA-mediated IPSCs without altering the reversal potential of these currents recorded in control medium. **(D, E)** The graphs show that both cocaine and amphetamine affected neither the holding current **(D)** nor the membrane conductance **(E)** of the recorded striatal neurons.

cently reported that cocaine modulates striatal neuron firing activity independently of a DA-mediated mechanism (Kiyatkin and Rebec 2000). To test whether the inhibitory effect of both cocaine and amphetamine on striatal GABAergic transmission was caused by the action of these drugs on DAergic nerve terminals of the striatum, we recorded striatal neurons from rats with 6-OHDA-induced lesion of the homolateral substantia nigra. This brain area represents the main source of DAergic terminals in the striatum and the nigral injection of 6-OHDA causes virtually complete disappearance of DA-containing striatal fibers (Calabresi et al. 1993).

As shown in Figure 4A, cocaine (30 μM) failed to produce a significant inhibition of IPSCs in DA-denervated striatal slices ($n = 7$; $p > .05$), indicating that the integrity of DA innervation is a crucial requirement for the modulatory action of cocaine on GABAergic transmission in the striatum. Moreover, the electrophysiological effects of cocaine on striatal IPSCs were also fully prevented by the DA D2-like receptor antagonist L-sulpiride (3 μM, 15 min bath application) ($n = 6$; $p > .05$) but not by 10 μM SCH 23390 (15 min; $n = 6$; $p < .01$), a DA D1-like receptor antagonist (Figure 4A). Essentially similar results were obtained with 30 μM amphetamine (6-OHDA lesion: $n = 6$, $p > .05$; 7–15 min L-sulpiride 3 μM: $n = 7$, $p > .05$; 15 min SCH 23390 10 μM: $n = 4$, $p < .01$) (Figure 4C).

Since either a decreased release of neurotransmitter or a decreased postsynaptic sensitivity to GABA can be responsible for the cocaine- and amphetamine-induced inhibition of striatal IPSCs, we tried to distinguish these possibilities through paired pulse experiments performed with interstimulus interval of 40–80 ms. No change in IPSC2/IPSC1 ratio (normally positive to 1) would accompany the depression of IPSCs if this synaptic inhibition is caused by a reduced postsynaptic sensitivity to GABA. As shown in Figures 4B and 4D, however, both cocaine and amphetamine depressed striatal GABAergic transmission by increasing IPSC2/IPSC1 ratio ($n = 8$ and $p < .01$ for each experimental condition), strongly implying in this pharmacological effect a presynaptic site of action. Together, these data demonstrate that both cocaine and amphetamine increase intrastriatal levels of endogenous DA, which, in

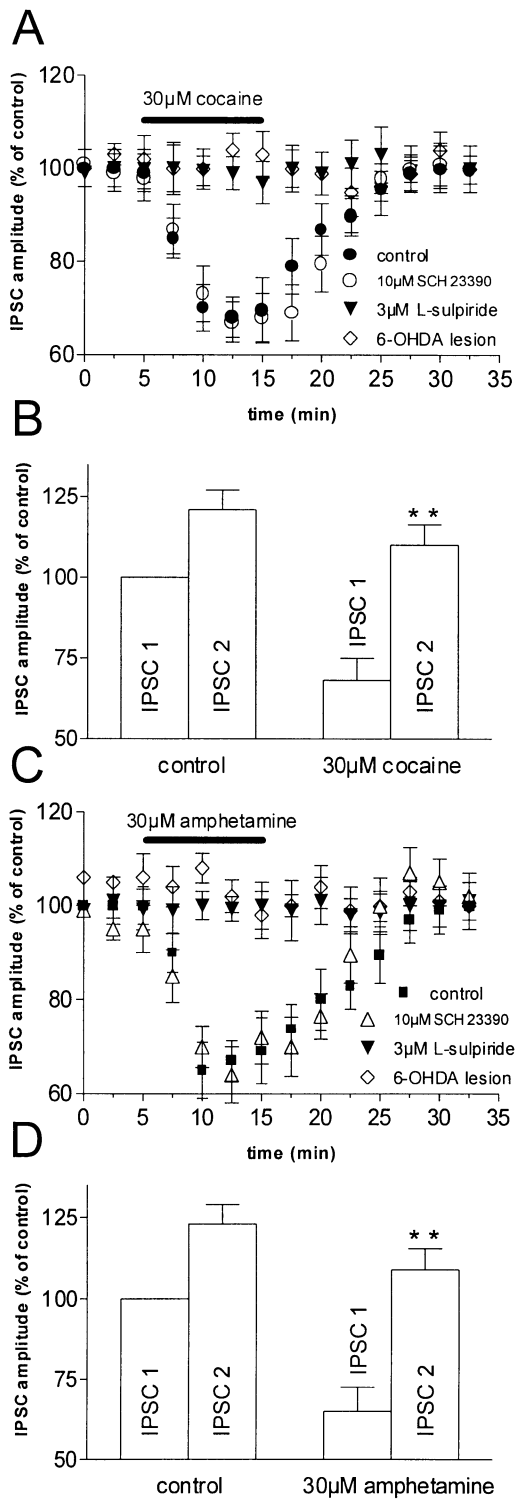

Figure 4. Pharmacological antagonists of D2-like DA receptors and chronic DA denervation of the striatum prevent cocaine- and amphetamine-induced presynaptic inhibition of IPSCs. **(A)** The graph shows the effects of cocaine on striatal IPSCs recorded in control condition (filled circles), in the presence of the D1-like DA receptor antagonist SCH23390 (10 μM, open circles), in the presence of the D2-like DA receptor antagonist L-sulpiride (3 μM, filled triangles), and in 6-OHDA-treated rats (open diamonds). **(B)** The histogram shows that cocaine depresses striatal IPSCs by increasing paired pulse facilitation evoked by a pair of synaptic stimuli delivered 40-80 ms apart (**$p < .01$). **(C)** The graph shows the effects of amphetamine on striatal IPSCs recorded in control condition (filled squares), in the presence of the D1-like DA receptor antagonist SCH23390 (10 μM, open triangles), in the presence of the D2-like DA receptor antagonist L-sulpiride (3 μM, filled triangles), and in 6-OHDA-treated rats (open diamonds). **(D)** The histogram shows that amphetamine depresses striatal IPSCs by increasing paired pulse facilitation (**$p < .01$).

turn, depresses GABAergic synaptic transmission by activating at least one subtype of presynaptic DA receptors composing the D2-like family (D2, D3, D4, and their variants).

Effects of DA Agonists on Striatal GABAergic Transmission and on Cocaine- and Amphetamine-induced Depression of IPSCs

To further strengthen the idea that psychostimulants exert their effect on striatal GABAergic transmission through a DA-dependent mechanism, we tried to mimic the electrophysiological actions of cocaine and amphetamine by using selective agonists of DA D1- and D2-like receptors. Bath application (7–10 min) of the DA D2-like receptor agonist quinpirole ($n = 12$; $p < .01$ at 3, 10 and 30 μM) but not of the DA D1-like receptor agonist SKF 38393 ($n = 5$; $p > .05$ at each concentration) produced a dose-dependent inhibition of striatal IPSCs ($IC_{50} = 3.38 \pm 0.8$ μM) (Figure 5A). This effect was not associated with changes in the intrinsic membrane properties of the recorded cells and was only partially reversible at the wash-out of the drug. In most cases, in fact, the administration of L-sulpiride (3 μM) was required to fully restore the IPSC amplitude obtained in control condition (not shown). As with cocaine and amphetamine and according to a previous report (Delgado et al. 2000), quinpirole-induced reduction of striatal GABAergic IPSCs was coupled to a significant increase in paired pulse facilitation ($n = 8$; $p < .01$) (Figure 5B), indicating that the inhibitory action of this DAergic agonist on GABAergic synaptic currents was mediated by the activation of DA D2-like receptors located on presynaptic nerve terminals. Moreover, quinpirole occluded the cocaine- or amphetamine-induced depression of striatal GABAergic transmission, as demonstrated by the finding that in the presence of 10 μM quinpirole no further depression of striatal IPSCs was inducible by cocaine (30 μM) ($n = 7$; $p > .05$) or by amphetamine (30 μM) ($n = 4$; $p > .05$) (Figure 5C). Similarly, when the application of cocaine (30 μM; $n = 4$) (Figure 5D) or amphetamine (30 μM; ($n = 5$) (not shown) preceded the application of quinpirole, this DA

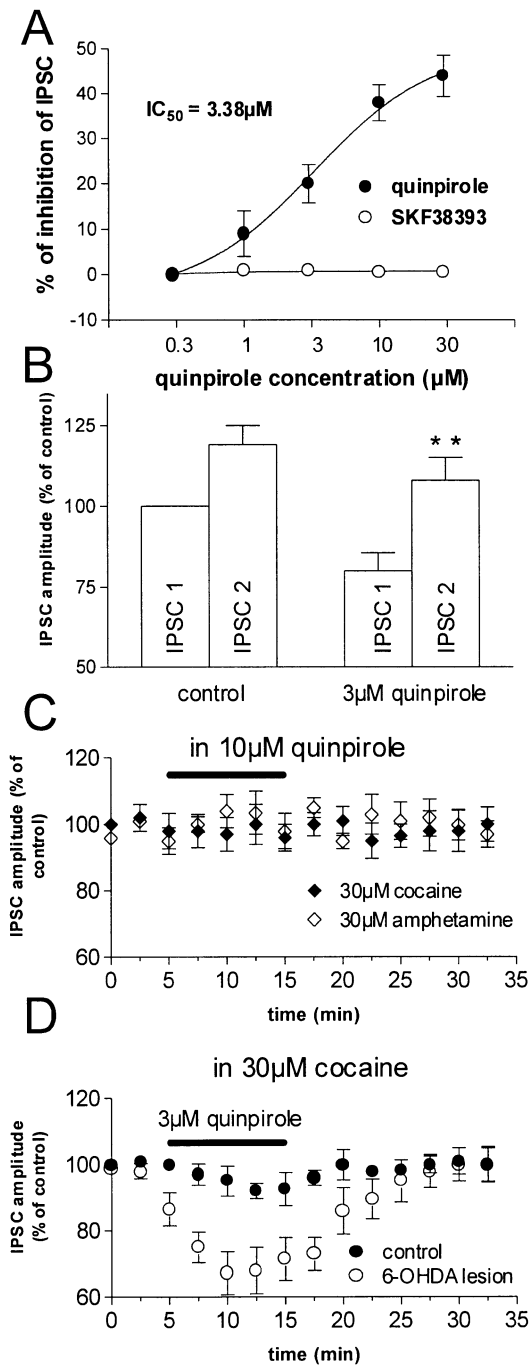

Figure 5. Stimulation of D2-like DA receptors mimics and occludes cocaine- and amphetamine-induced inhibition of striatal IPSCs. **(A)** Dose-response relationships of the effects of the D2-like DA receptor agonist quinpirole and of the D1-like DA receptor agonist SKF38393 on striatal IPSCs. **(B)** The histogram shows that quinpirole depresses striatal IPSCs by increasing paired pulse facilitation (**$p < 0.01$). **(C)** The graph shows the effects of both cocaine (filled diamonds) and amphetamine (open diamonds) on IPSCs evoked from striatal slices bathed in 10 μM quinpirole. **(D)** Quinpirole depresses striatal IPSCs recorded in the presence of 30 μM cocaine in 6-OHDA-treated rats (open circles) but not in control animals (filled circles).

agonist failed to further depress striatal IPSCs in control slices ($p > .05$ for each experimental condition). Conversely, quinpirole produced a robust inhibition of the synaptic currents evoked in DA-denervated striatal slices (Figure 5D) ($n = 7$; $p < .01$), which were insensitive to both psychostimulants (see above and Figure 4). These observations indicate that cocaine and amphetamine inhibit GABAergic transmission in the striatum through a mechanism shared by quinpirole, that is, the stimulation of DA D2-like receptors. Moreover, the evidence that quinpirole does inhibit GABAergic transmission in DA-denervated striatal slices demonstrates that in these slices the failure in affecting IPSCs by both cocaine and amphetamine is not caused by the disruption of the D2-like-receptor-dependent regulation of GABA release.

Effects of the Genetic Disruption of DA D2 Receptors on Cocaine-, Amphetamine-, and Quinpirole-induced Depression of Striatal GABAergic Transmission

Since the available pharmacological tools do not allow discriminating among the various DA receptors included in the D2-like receptor family, we measured the effects of both cocaine and amphetamine on striatal GABAergic IPSCs recorded from transgenic mice selectively lacking D2 receptors. As already reported (Calabresi et al. 1997), the intrinsic and synaptic properties of striatal neurons recorded from these mice were indistinguishable from those of their wild-type counterparts and from rat striatal neurons (not shown). In these mice, however, both cocaine (30 μM) ($n = 4$; $p > .05$) and amphetamine (30 μM) ($n = 4$; $p > .05$) failed to affect striatal IPSCs (Figures 6A and 6B), implying DA D2 receptors in the pharmacological action of these drugs within the striatum. As expected, neither did 10 μM quinpirole produce significant effects on striatal GABAA-mediated transmission in D2 lacking mice (n = 3; $p > 0.05$), whereas cocaine, amphetamine and quinpirole normally inhibited striatal IPSCs in control mice (Figure 6C) ($n = 4$ and $p < .01$ for each experimental condition).

DISCUSSION

The present results demonstrate that both cocaine and amphetamine depress striatal GABAergic transmission by favoring DA release from nigrostriatal nerve terminals which, in turn, acts by stimulating D2 DA receptors. Glutamate-mediated corticostriatal synaptic transmission is conversely unaffected by these addictive drugs. The enhancement of paired pulse facilitation, together with the lack of any postsynaptic action of these compounds on IPSC reversal potential, holding current

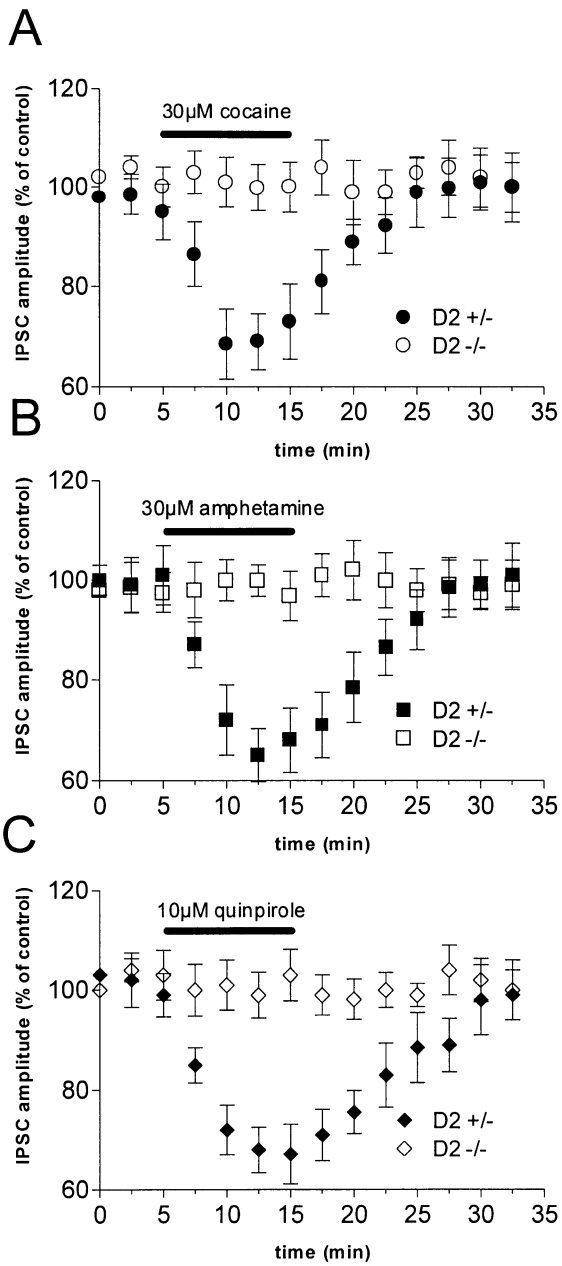

Figure 6. Effects of the genetic disruption of D2 DA receptors on cocaine-, amphetamine-, and quinpirole-induced inhibition of striatal IPSCs. **(A)** Cocaine depresses striatal IPSCs in wild-type but not in transgenic mice lacking D2 receptors. **(B)** Amphetamine depresses striatal IPSCs in wild-type but not in transgenic mice lacking D2 receptors. **(C)** Quinpirole depresses striatal IPSCs in wild-type but not in transgenic mice lacking D2 receptors.

and membrane conductance, all point to a presynaptic site for this action. Accordingly, although DA receptors are abundantly expressed postsynaptically on striatal neurons (Surmeier et al. 1996), their activation is known to modulate membrane conductances operating in voltage ranges far from the values chosen in this study to evoke synaptic events (−80 mV and −40 mV) (Schiffmann et al. 1995; Surmeier et al. 1995; Lin et al. 1996; Hernandez-Lopez et al. 2000). This observation, therefore, further confirms that the postsynaptic effects of DA receptor activation cannot be responsible for the observed modulation of striatal IPSCs.

In a recent in vivo study, cocaine was found to exert a preferential local-anaesthetic-like action on striatal neurons (Kiyatkin and Rebec 2000), suggesting that this kind of effect might in principle have occurred in our experimental condition. The quiescent behavior of spiny neurons in vitro, however, prevented the observation of cocaine effects on voltage dependent-sodium channels. Accordingly, the effects of cocaine described here were fully blocked by DA antagonists, DA-denervation and D2 receptor genetic disruption, demonstrating that they were not caused by DA-independent actions.

Anatomo-functional Characteristics of Synaptic Inputs to Striatal Neurons

Glutamate-mediated excitatory synaptic inputs and GABA-dependent inhibitory potentials govern striatal neuron firing activity. GABAergic medium spiny projection neurons, which account for about 95% of the whole neuronal population of the striatum, in fact, are silent at rest and fire action potentials only when depolarized by glutamate released from corticostriatal and thalamostriatal terminals (Calabresi et al. 1996; Wilson and Kawaguchi 1996; Stern et al. 1998). GABAergic inputs to these neurons, conversely, are essentially intrinsic and tend to counter the excitatory action of glutamate (Kita 1996; Plentz and Kitai 1998). Accordingly, in vivo, blockade of ongoing GABAergic inhibition of medium spiny neurons significantly elevates basal activity (Nisenbaum and Berger 1992), supporting the idea that psychostimulants, by inhibiting GABAergic inhibition, would substantially enhance the ability of cortical activity to evoke spiking in medium spiny neurons. Recurrent collaterals of projection neurons and GABAergic interneurons, such as fast-spiking and parvalbumin-immunoreactive cells contact striatal spiny neurons in their somato-dendritic region (Wilson and Groves 1980; Yung et al. 1996; Bennet and Bolam 1994; Plentz and Kitai 1998; Koos and Tepper 1999) and express D2 DA receptors (Lenz et al. 1994; Delle Donne et al. 1997; Aizman et al. 2000). It is therefore conceivable that terminals from all these different cell types would also possess D2 DA receptor and be sensitive to a similar extent to the pharmacological action of psychostimulants. Recent electrophysiological evidence, however, strengthened the idea that among the various GABAergic inputs to spiny neurons, those originating from fast-spiking interneurons exert the predominant inhibitory control on the functional activity of these cells. Although it has been proposed that recurrent col-

laterals of spiny neurons provide an important feedback inhibition in the striatum (Wilson and Groves 1980; Beiser and Houk 1998; Wickens et al. 1995), functional studies showed that mutual inhibitory interaction between medium spiny neurons is conversely very weak or non-existent (Jaeger et al. 1994). By contrast, action potentials evoked in a fast-spiking interneuron have been found capable to produce GABA-mediated synaptic events in nearby medium spiny neurons, dramatically impacting spike generation and timing in these cells (Plentz and Kitai 1998; Koos and Tepper 1999). These observations, therefore, suggest that the GABAergic nerve terminals stimulated in our study to produce IPSCs were likely those of striatal fast-spiking interneurons, the transmitter release of which appeared to be modulated by cocaine and amphetamine via DA receptors.

Comparison with Other Electrophysiological Studies

Cocaine has been shown to cause a strong inhibition of striatal neurons in vivo (Qiao et al. 1990; White et al. 1993; Kiyatkin and Rebec 2000). This inhibitory effect was highly dependent on the activity state of the neuron (being generally undetectable in slow-firing cells and maximal in fast-firing neurons), and was attributed, at least in part, to a DA-independent local-anesthetic-like interaction with sodium transport (Kiyatkin and Rebec 2000). Taken together with our in vitro data, these observations indicate that cocaine, by preventing synaptic inhibition through a DA-dependent mechanism, preferentially enhances the excitability of spiny neurons weakly activated, whereas, through a partially DA-independent action, it preferentially inhibits firing activity when striatal neurons are massively stimulated. This dual action of cocaine might force striatal neurons to fire constantly action potentials within a narrow intermediate interval rate, altering profoundly the physiological activity of these cells, normally oscillating between a very quiescent "down" state and a very active "up" state (Calabresi et al. 1996; Wilson and Kawaguchi 1996; Stern et al. 1998).

A recent in vitro study reported that neither DA nor amphetamine depressed GABAergic synaptic transmission in the dorsal striatum (Nicola and Malenka 1998). Although we have no conclusive explanation for the discrepancy existing between this study and our report, it is possible that different experimental conditions might account for these contrasting results. We can speculate that the two studies differ for the location of the stimulating electrodes producing GABAergic responses. This different arrangement of the electrodes might activate heterogeneous GABAergic terminals showing different sensitivity to D2 DA receptor agonists.

Conflicting results also exist on the possible role of D2-like receptors in the modulation of excitatory transmission in the dorsal striatum. Some laboratories, in fact, reported that DA D2-like receptor activation depressed excitatory synaptic transmission in this brain area, either presynaptically (Hsu et al. 1995; Cepeda et al. 2001) or postsynaptically (Cepeda et al. 1992; Levine et al. 1996). In contrast, our group and others found that, at least under control conditions, activation of D2-like receptors caused significant changes neither of glutamate-mediated synaptic potentials (Calabresi et al. 1988; Calabresi et al. 1992; Calabresi et al. 1993; Nicola and Malenka 1998; Pisani et al. 2000; present study) nor of exogenous applied AMPA and glutamate (Calabresi et al. 1995). A D2-like-receptor-dependent attenuation of excitatory transmission was conversely observed only in slices taken from animals exposed to treatments intended to upregulate D2 receptors, as obtained following chronic administration of reserpine (Calabresi et al. 1988) and haloperidol (Calabresi et al. 1992) and 6-OHDA-induced nigrostriatal denervation (Calabresi et al. 1993). Although reconciling all these experimental data is not simple, the heterogeneous distribution of active D2-like receptors on corticostriatal terminals might partially account for these conflicting results. Accordingly, a recent study reported that the presynaptic D2-like-receptor-dependent inhibition of spontaneous excitatory potentials induced in the striatum by 4-aminopyridine was observed only in a subset of striatal neurons (Flores-Hernàndez et al. 1997).

Functional Implications

Current models of the basal ganglia propose that the striatum is an important component of sensorimotor, cognitive, and limbic circuits, as also suggested by the anatomical evidence that this brain area receives inputs not only from the motor cortex but also from sensory, cingulated, and association neocortical areas (Albin et al. 1989; Graybiel et al. 1994). These non-motor connections account, at least in part, for the involvement of the striatum in memory formation, mnemonic guidance of behavior and rewarding processes (Whishaw et al. 1987; Saint-Cyr et al. 1988; Knowlton et al. 1996; Graybiel 1998; Apicella et al. 1991). Interestingly, most recent investigations into the molecular neurobiology of addiction have emphasized the view that addictive drugs lead to the compulsive nature of drug abuse and to the persistent tendency to relapse by engaging a set of molecular mechanisms normally involved in striatally based associative learning and reward. These processes critically depend on the integrity of the DAergic signal in this brain area, since disturbance of striatal DA system severely interferes with these physiological abilities (see Berke and Hyman 2000 for a comprehensive review of these concepts).

We propose, in conclusion, that the DA-dependent

modulation of striatal inhibitory synaptic transmission by cocaine and amphetamine might play a role in the development of those drug-taking behaviors critical for addiction.

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