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A Common Mechanism Mediates Long-Term Changes in Synaptic Transmission after Chronic Cocaine and Morphine

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Summary

The mesolimbic system is known to play a role in self-administration of opioids and psychostimulants. Although morphine and cocaine act by separate cellular mechanisms initially, the present study describes a common change in synaptic regulation of dopamine cells in the ventral tegmental area 1 week after termination of chronic treatment with either drug. Normally, D1 receptor activation augmented the amplitude of a γ-aminobutyric acid type B ($GABA_B$) inhibitory postsynaptic potential (IPSP), but in drug-experienced animals, D1 receptor activation caused an inhibition of the $GABA_B$ IPSP. The inhibition was blocked by adenosine A1 receptor antagonists and by agents that disrupted the metabolism of cAMP. This long-lasting dopamine–adenosine interaction may be one mechanism involved in dopamine-mediated craving and relapse to drug-seeking behaviors.

Introduction

One of the actions of drugs of abuse is to foster repeated drug experiences over an extended period of time. Considerable evidence suggests that opioids and psychostimulants act through the mesolimbic dopamine system to support repeated drug administration (Wise and Rompre, 1989). One long-term effect of chronic opioids or psychostimulants is the phenomenon of sensitization in which there is an increased sensitivity to drug for weeks or months after termination of initial treatment (Robinson and Becker, 1986; Robinson and Berridge, 1993). The development of sensitization is dependent on the mesolimbic dopamine system and can occur following injection of either opioids or psychostimulants directly into the ventral tegmental area (VTA) (Kalivas and Stewart, 1991; Kalivas and Duffy, 1993a, 1993b). Expression of sensitization requires injection of direct or indirect dopamine agonists into nucleus accumbens (Kalivas and Stewart, 1991; Kalivas and Duffy, 1993a, 1993b). After chronic cocaine, iontophoretic application of D1 agonists in vivo is more effective at producing inhibition of both spontaneous and glutamate-induced firing of neurons in the nucleus accumbens (Henry and White, 1991, 1995). In addition, transgenic animals lacking D1 receptors do not exhibit either an acute effect or sensitization to cocaine (Xu et al., 1994). These results indicate the importance of D1 receptors in psychostimulant and opioid action, but the cellular mechanism and other neuronal circuits that contribute to sensitization following chronic treatment have not been identified.

Axons of the γ-aminobutyric acid (GABA)-containing neurons in the nucleus accumbens project to the VTA and form inhibitory synapses on dopamine cells. Release of GABA from these terminals causes a $GABA_B$-mediated inhibitory postsynaptic potential (IPSP) recorded from the dopamine-containing neurons (Johnson and North, 1992). Activation of D1 receptors on these GABA-containing axon terminals augments the $GABA_B$-mediated IPSP (Cameron and Williams, 1993). In contrast, D1 antagonists inhibit the $GABA_B$-mediated IPSP. This observation suggests that D1 receptors can be tonically activated by endogenous dopamine and is consistent with neurochemical studies indicating that dopamine is tonically released both in vivo and in vitro (Geffen et al., 1976; Korf et al., 1976; Paden et al., 1976; Cheramy et al., 1981; Robertson et al., 1991). Thus, the D1 receptors in the VTA are ideally situated for study of the long-term effects of chronic drug treatment on D1 receptor activation. The purpose of this study is to determine the long-term effects of chronic drug treatment on the D1 regulation of $GABA_B$ IPSPs in dopamine cells of the VTA.

Results

Regulation of GABA Release by D1 Receptors Is Reversed after Chronic Cocaine

Dopamine (30–100 μM, in the absence of reuptake blockers) and the D1 agonists SKF82958 (1–10 μM) and SKF38393 (10 nM to 10 μM) produced an increase in the $GABA_B$ IPSP in dopamine cells of the VTA in slices from saline-treated control animals as was previously found in untreated animals (Figure 1). In contrast, all three agonists (SKF82958 85298, SKF38393, and dopamine) decreased the amplitude of the IPSP in slices from animals chronically injected with cocaine (10 mg/kg, once per day for 2 weeks) and tested 7–10 days after termination of cocaine treatment. The D1 receptor antagonists SCH23390 (1 μM, Figure 1) and *cis*-flupenthixol (10 μM, data not shown) decreased the IPSP in slices from saline-treated animals (*cis*-flupenthixol: $-26.4\% \pm 1.1\%$, $n = 4$), but increased the IPSP in slices from cocaine-treated animals (*cis*-flupenthixol: $28.9\% \pm 1.5\%$, $n = 4$).

Both D1 receptor activation and forskolin stimulate adenylyl cyclase (Stoof and Kebabian, 1981) and augment the $GABA_B$ IPSP (Cameron and Williams, 1993). Forskolin (1 and 10 μM) had the same effect as dopamine agonists in slices from both saline- and cocaine-treated animals (Figure 1). The IPSP amplitude was increased in slices from saline-treated animals and decreased in animals 7–10 days following the end of cocaine treatment. This observation indicates that the altered response to D1 receptor activation was mimicked by direct activation of adenylyl cyclase.

Regulation of GABA Release by D1 Receptors Is Reversed after Chronic Morphine

Modulations of the $GABA_B$ IPSP by D1 agonists (dopamine, SKF82958, and SKF38393), SCH23390, and forskolin in slices from animals that were treated with morphine (10 mg/kg, once per day for 1 week) tested 7–10

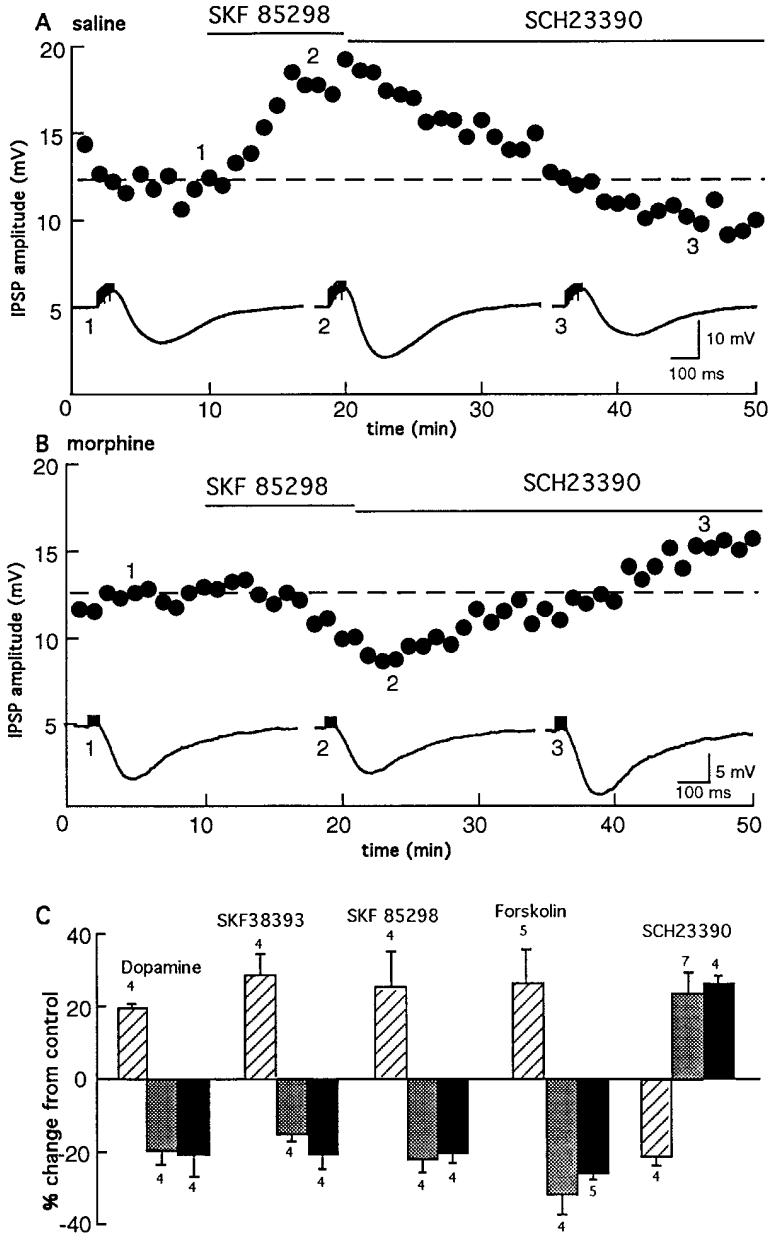

Figure 1. The D1 Receptor Augmentation of the $GABA_B$ IPSP Is Qualitatively Changed 7–10 Days after Termination of Chronic Cocaine or Morphine Treatment

(A) An experiment taken from a saline-treated animal, in which the D1 agonist SKF82958 (1 μM) increased the amplitude of the IPSP and the D1 antagonist SCH23390 (1 μM) decreased the IPSP. The amplitude of the $GABA_B$-mediated IPSP is plotted as a function of time. During the period indicated by the bars, the drugs in the indicated concentration were superfused. In the inset are IPSPs (average of three) taken from the periods indicated.

(B) A similar experiment as shown in (A), in a slice taken from a morphine-treated animal. In this experiment, SKF82958 depressed the IPSP and SCH23390 reversed this depression.

(C) Summarized data from similar experiments shown in (A) and (B). In this and other figures, the bars are the average change in IPSP amplitude using each cell as its own control. The number of cells for each experiment is indicated beside each bar. The hatched bars are from saline-injected animals tested 1 week after the last injection, stippled bars are from cocaine-treated animals tested 1 week after the last cocaine injection (cocaine), and the closed bars are from animals treated with morphine and tested 1 week after treatment (morphine). The effect of each of the dopamine agonists tested was an augmentation in slices from saline-treated animals and an inhibition in slices from drug-treated animals. The effect of forskolin (10 μM) was likewise affected in slices from drug-treated animals. The effect of the D1 antagonist was also the opposite in slices from saline- and drug-treated animals. The numbers indicate the number of cells tested, one cell per slice.

days after the last injection were almost identical to those from cocaine-treated animals (Figure 1). That is, dopamine, SKF82958, SKF38393, and forskolin decreased and SCH23390 increased the IPSP, just opposite to the response observed in the saline controls. This altered response to D1 receptor activation and forskolin was observed in 75 out of 77 animals tested.

Adenosine Mediates D1 Inhibition of GABA Release after Chronic Drug Treatment

The interaction between D1 receptor activation and adenosine was investigated because an increased production of cyclic AMP (cAMP) would be expected with both D1 receptor activation and forskolin (Stoof and Kebabian, 1981), and it is known that cAMP can be metabolized to adenosine (Barber and Butsher, 1981; Lazarena et al., 1985; Egawa et al., 1988; Rosenberg and Dichter, 1989; Rosenberg et al., 1994). Adenosine acting at A1 receptors inhibits transmitter release in many areas of the CNS (Dunwiddie, 1985; Mitchell et al., 1993; Dunwiddie and Diao, 1994; Manzoni et al., 1994; Gereau and Conn, 1994) including the $GABA_B$ IPSP in the VTA (Wu et al., 1995). Thus, the activation of A1 receptors could be mediated via an increase in adenosine subsequent to the rise in cAMP following D1 receptor activation. To test this hypothesis, we treated slices with the adenosine (A1) receptor antagonists 8-cylcopentyltheophylline (8CPT, 1 μM) or 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX, 1 μM) for 5–10 min (Figure 2). This treatment changed the action of dopamine from an inhibition to an augmentation in slices from drug-treated animals (Figure 2). In the presence of 8CPT (1

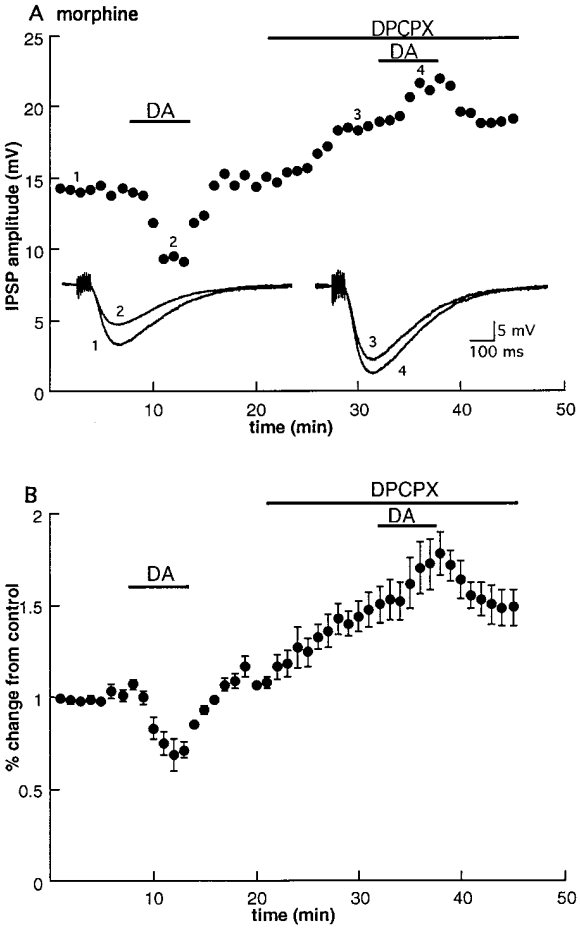

Figure 2. The Decrease of the $GABA_B$ IPSP Caused by Dopamine after Chronic Drug Treatment Was Reversed by the A1 Receptor Antagonist DPCPX

(A) Experiment taken from a chronic morphine-treated animal. Dopamine (30 μM) produced a decrease of the IPSP. Superfusion with DPCPX (1 μM) augmented the IPSP, and after DPCPX, dopamine (30 μM) now further augmented the IPSP. In the inset are shown the average of three IPSPs taken during the periods indicated by the numbers.

(B) Normalized and averaged data, from chronic cocaine (n = 2) and morphine (n = 2) treated animals, showing the reversal of the dopamine effect after DPCPX. The percent change from control was determined by averaging seven control IPSPs and dividing each IPSP by this mean value.

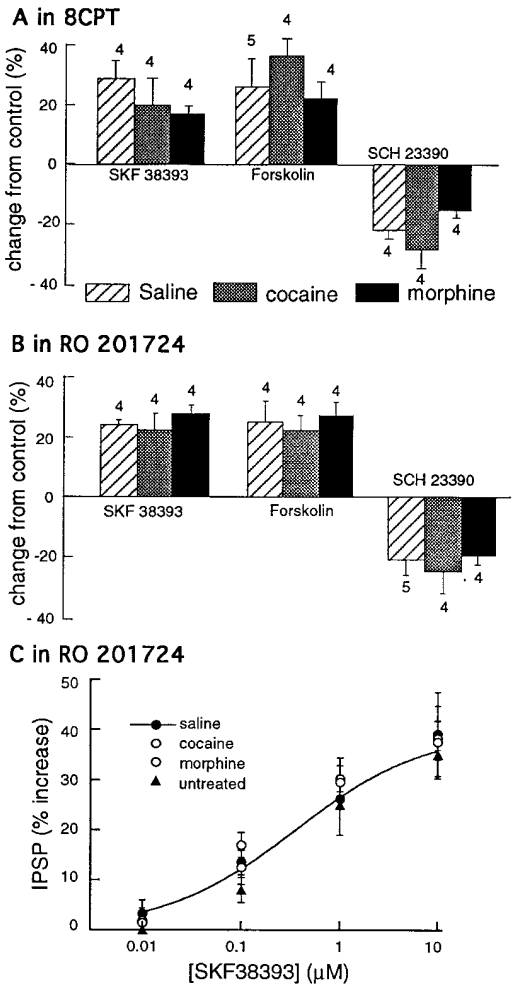

Figure 3. The Effects of Chronic Drug Treatment on the D1 Regulation of the $GABA_B$ IPSP Are Blocked by the Adenosine Receptor Antagonist 8CPT and the Phosphodiesterase Inhibitor RO201724

(A) After blockade of A1 adenosine receptors with 8CPT (1 μM), SKF38393 (1 μM), and forskolin (10 μM) caused an augmentation in the IPSP in slices from all treatment groups. Similarly, the augmentation of the IPSP caused by SCH23390 (1 μM) in drug-treated animals was reversed to an inhibition by 8CPT.

(B) Similar observations as shown in (A) were made after treatment of the slices with RO201724 (200 μM) for 10 min. In RO201724, the response to SKF38393 (1 μM), forskolin (10 μM), and SCH23390 (1 μM) were the same in all groups of animals. Forskolin (1 μM) had a similar effect in slices from all three treatment groups (saline, 28% ± 8%, n = 4; cocaine, 21% ± 5%, n = 4; morphine, 28% ± 6%, n = 4).

(C) Shows concentration response curves to the D1 agonist SKF38393 in slices from saline- (closed circles), cocaine-, and morphine-treated animals (open circles). Data from untreated animals (closed triangles) were taken from a previous publication that did not include RO201724 in the superfusion solution (Cameron and Williams, 1993). The line is a least squares fit to the data from the saline-treated animals. There was no significant difference between the results obtained between any of the groups of animals. Each data point is obtained from at least four cells from four different animals.

μM), SKF38393 (1 μM) and forskolin (10 μM) also augmented the IPSP (Figure 3A). In addition, the augmentation of the IPSP induced by SCH23390 (1 μM) in slices from drug-treated animals was also reversed to an inhibition in the presence of 8CPT (1 μM, Figure 3A). Identical results were obtained after treatment of slices with RO201724 (200 μM, 10 min, Figure 3B), an inhibitor of cAMP-dependent phosphodiesterase (Beavo and Reifsynder, 1990).

The D1 receptor- and forskolin-mediated augmentation of the IPSP was determined under conditions in which adenosine tone had been reduced with RO201724. The concentration response curve to SKF38393 in slices from drug-treated animal groups was not different from either saline-treated controls (Figure 3C) or from untreated animals (Cameron and Williams, 1993). Thus, chronic drug treatment did not seem to affect the sensitivity of D1 receptors to activation by

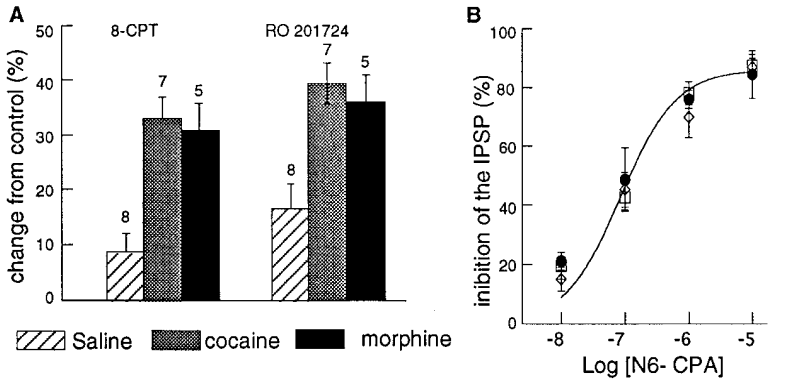

Figure 4. Adenosine Tone Is Greater in Slices from Drug-Treated Animals

(A) The adenosine antagonist 8CPT and the phosphodiesterase inhibitor RO201724 both increased $GABA_B$ IPSP to a significantly greater extent in slices from drug-treated animals than saline-treated controls (ANOVA, $p < 0.001$). The amplitude of the IPSP was measured 10 min after superfusion of 8CPT (1 μM) and RO201724 (200 μM).

(B) Concentration response curves to the A1 receptor agonist N6-CPA in slices from saline- (closed circles), cocaine- (open diamonds), and morphine-treated (open squares) animals. The line is a least squares fit to the data obtained from the saline-treated animals. There was no significant difference between any of the groups. Each point is data obtained from at least four cells from four different animals. All data was collected in the presence of RO201724 (200 μM) to reduced the levels of endogenous adenosine.

agonist. In addition, the augmentation of the IPSP by forskolin (1 and 10 μM) was not changed (Figure 3B).

Adenosine Tone Is Augmented after Chronic Drug Treatment

The $GABA_B$ IPSP itself was augmented by both 8CPT and RO201724, an effect that was significantly larger in slices from drug-treated animals (Figure 4A). An increase in the sensitivity of adenosine receptors was not responsible for the augmented response to 8CPT and RO201724 in drug-treated animals because the inhibition of the IPSP caused by an exogenously applied agonist was not changed (Figure 4B). The A1 receptor agonist N6-cyclopentyladenosine (N6-CPA) was tested in the presence of RO201724 (200 μM) to reduce the level of endogenous adenosine. There was no effect of this agonist on the membrane potential. The EC_{50} for inhibition of the IPSP was 63 ± 12 nM in saline-treated animals and was not significantly different in either drug treatment group (Figure 4B). The sensitivity to N6-CPA was very similar to that reported in rat VTA (Wu et al., 1995). Thus, the altered response to D1 agonists and forskolin did not result from a change in the sensitivity of A1 receptors.

The following agents were tested to investigate further the source of the endogenous adenosine: probenecid (100 μM), which among other actions is an inhibitor of cAMP transport (Rosenberg et al., 1994; Beavo and Reifsnyder, 1990), and adenosine deaminase (0.4 U/ml), the enzyme that converts adenosine to inosine (Haas and Greene, 1988; Ragazzi et al., 1991; Linden, 1989). Neither treatment had a consistent effect on the membrane potential, although both increased the amplitude of the IPSP. The increase in IPSP was significantly larger in slices from drug-treated animals than from the saline controls (Figures 5A). After treatment of the slices with either probenecid or adenosine deaminase, the response to SKF38393, SCH23390, and forskolin in slices from cocaine- and saline-treated animals were identical (Figures 5B and 5C). Thus, experiments with RO201724, probenecid, 8CPT, and adenosine deaminase all suggest that the basal level of extracellular adenosine was increased in slices from drug-treated animals and that this increased adenosine tone may result from metabolism of cAMP.

The link between the increased adenosine tone, cAMP, and D1 receptors was further investigated by testing the effect of RO201724 after blockade of D1 receptors with SCH23390 (1 μM). In SCH23390, RO201724 (200 μM) had no significant effect on the IPSP (1.2% ± 0.6%, n = 4 cocaine-treated animals; data not shown). That is, reduction of cAMP formation by a D1 antagonist also blocked the augmentation of the IPSP by disruption of cAMP metabolism. This experiment suggests that tonic activation of D1 receptors maintained a level of cAMP that was metabolized to adenosine. This cAMP-dependent pool of adenosine may account for greater adenosine tone in drug-treated animals.

To investigate further the suggestion that metabolism of cAMP to adenosine resulted in the augmented tone, the inhibition of the IPSP by superfused cAMP was determined in the three groups of animals (saline, morphine, and cocaine treated). Superfusion of cAMP (0.01–1 mM) caused a concentration-dependent inhibition of the IPSP that was significantly larger in slices from drug-treated animals (Figure 6). Binding studies indicate that cAMP has an extremely low affinity for adenosine receptors. cAMP (100 μM) caused only a 26% displacement of [^{3}H]N^6-phenylisopropyladenosine binding to a rat brain membrane preparation (Schwabe and Trost, 1980). These results suggest that the metabolism cAMP to adenosine may be augmented in drug-treated animals.

Discussion

The primary observation made here was that activation of D1 dopamine receptors has the opposite effect in drug-treated animals compared with controls. The mechanism mediating this change involves the activation of A1 adenosine receptors. The fact that D1 receptor activation augments GABA synaptic potentials in control and inhibits them after chronic drug treatment is a dramatic functional change. D1 receptors play a necessary role in the development and maintenance of long-term behaviors induced by psychostimulants and opioids (Robinson and Becker, 1986; Robinson and Berridge, 1993; Kalivas and Stewart, 1991; Kalivas and Duffy, 1993a, 1993b). The molecular mechanisms that underlie

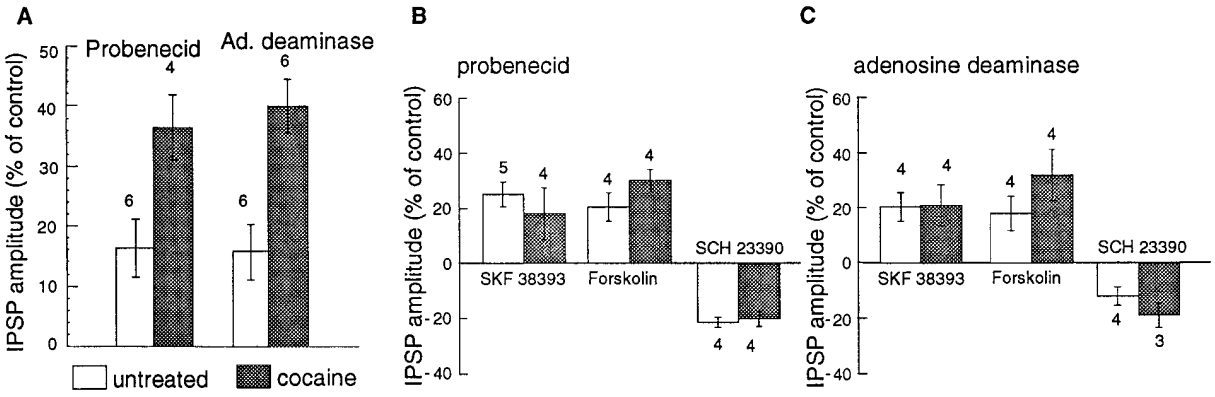

Figure 5. Probenecid and Adenosine Deaminase Had the Same Effect as the Adenosine Receptor Antagonist and Phosphodiesterase Inhibitor on the IPSP and on the Regulation of the IPSP by Dopamine

(A) Summary of experiments that show that probenecid (100 μM) and adenosine deaminase (0.4 U/ml) augmented the IPSP in slices from cocaine-treated animals to a significantly greater extent than untreated animals (ANOVA, $p < 0.001$). The amplitude of the IPSP was measured 15 min after the onset of superfusion of each of these agents.

(B) In the presence of probenecid (100 μM), the regulation of the IPSP by SKF38393 (1 μM), forskolin (10 μM), and SCH23390 (1 μM) was the same in slices from cocaine-treated animals as untreated controls.

(C) A similar experiment as shown in (B), in the presence of adenosine deaminase (0.4 U/ml).

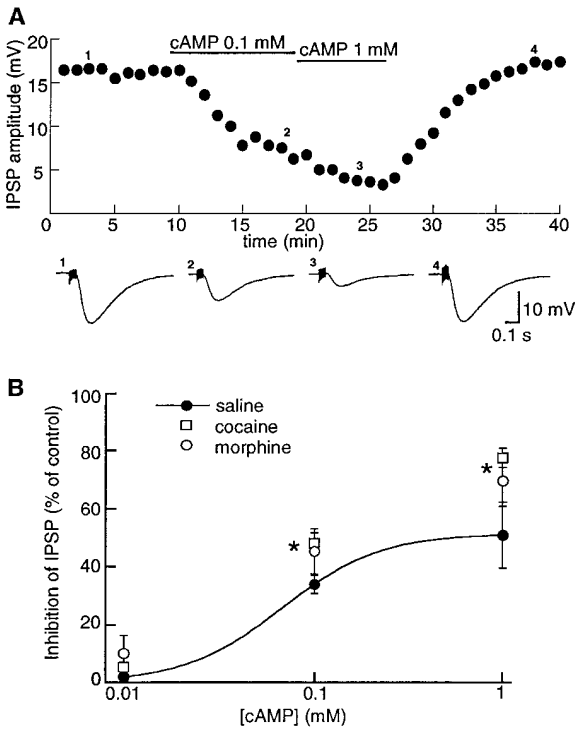

Figure 6. Exogenous Application of cAMP Depresses the IPSP

(A) Plot of the IPSP amplitude as a function of time. During the period indicated by the bar, cAMP was superfused in the indicated concentration. Traces below the plot are selected IPSPs taken during the period indicated by the numbers.

(B) Concentration response curves for cAMP in slices from saline (closed circles), cocaine (open squares), and morphine (open circles) treated animals. The line is a least squares fit to the data obtained from the saline-treated animals. Each point is the average of at least four experiments taken from four different animals. The asterisks indicate the concentrations at which cAMP had a significantly larger effect in both drug treatment groups.

the persistent behavioral changes have centered on the dopamine system. Dopamine receptors, adenylyl cyclase, cAMP-dependent kinase, and substrates for cAMP-dependent kinase including transcription factors have all been targets for these studies (reviewed by Woolverton and Johnson, 1992; Nestler, 1992, 1993, 1994). Any one of these mechanisms may be required to initiate the cascade of events leading to the augmentation of adenosine tone found in the present study. The fact that drugs with very different initial actions (cocaine and morphine) result in the same long-term action suggests that multiple synaptic mechanisms or pathways (or both) may be involved.

In slices taken from animals tested 1 day after the last cocaine injection, the effects of D1 agonists/antagonists and forskolin were not significantly different from the saline-treated animals (data not shown) and the augmented adenosine tone developed during the week following termination of drug treatment. The period immediately following drug treatment is complicated by several phenomena including recovery from tolerance and acute withdrawal (Henry et al., 1989). The delay in the development of the altered adenosine tone after termination of cocaine treatment could result from the synthesis or transport (or both) of key molecules from the afferent neurons in the nucleus accumbens to the VTA. The persistence of augmented adenosine tone also suggests a gene regulation mechanism.

Regulation of Endogenous Adenosine

In the present study, an altered metabolism of cAMP rather than up-regulation of dopamine receptors or adenylyl cyclase appears to result from chronic drug treatment (Figure 6). After blockade of adenosine receptors, there was no difference in the D1 receptor– or forskolin-mediated augmentation of GABA release between slices from control and drug-treated animals. Although D1 receptor–driven adenylyl cyclase activity was required, the augmented level of adenosine resulted from a

change subsequent to the adenylyl cyclase. The regulation of extracellular adenosine content is dependent on the balance between mechanisms that increase (transport and metabolism of adenosine nucleotides) and decrease (reuptake and metabolism) adenosine levels.

In the CA1 region of hippocampus, endogenous adenosine inhibits the glutamate-mediated excitatory postsynaptic potential (EPSP) (Dunwiddie and Diao, 1994). The basal concentration of endogenous adenosine was estimated to be 140–200 nM, which approached the EC_{50} for adenosine (600–760 nM; Dunwiddie and Diao, 1994). Thus, endogenous adenosine is in a functionally important range. In the hippocampus, blockade of the adenosine receptor with 8CPT augmented the EPSP by 20% (Dunwiddie and Diao, 1994). In the present study, the augmentation of the $GABA_B$ IPSP caused by 8CPT was smaller in slices from control animals (10%), but substantially larger in both cocaine- and morphine-treated animals (32%). Since the EC_{50} of the stable agonist N6-CPA and the augmentation of the synaptic potentials by antagonists were comparable in the hippocampus and the VTA, the resting concentration of endogenous adenosine may be similar. In drug-treated animals, the resting levels of adenosine would be expected to be slightly higher that the 200 nM determined by Dunwiddie and Diao (1994). Based on their results (Dunwiddie and Diao, 1994), a concentration response curve was constructed with an EC_{50} of 620 nM and a slope factor of 1. This curve was used to estimate the increase in adenosine required to mediate an inhibition after forskolin or D1 receptor activation in the drug-treated animals. The resting concentration of adenosine was calculated to be 300 nM, which is the concentration required to cause a 32% inhibition. In drug-treated animals, D1 receptor activation caused a 20% inhibition of the IPSP (Figure 3C). After blockade of adenosine receptors D1 agonists caused a 20% increase in the amplitude of the IPSP (Figure 3A). Taken together, the rise in adenosine concentration would be expected to reach 72% of the peak inhibition (32% + 20% + 20%), which would require a concentration of about 1.6 μM.

This is probably an overestimate of the increased adenosine. First, it assumes that the increase in endogenous adenosine caused by forskolin or dopamine receptors is similar to superfusing exogenous adenosine. Since the D1 receptors are on the GABA-releasing terminals, the local concentration of adenosine could be substantially higher than elsewhere in the slice. Second, this estimate also assumes that there is no interaction between the augmentation induced by forskolin and D1 receptors with the adenosine-mediated inhibition. Finally, the maximum augmentation of the IPSP induced by D1 receptor activation is about 36% (Cameron and Williams, 1993), an effect that is small compared with the inhibition caused by A1 receptor activation ($>$80%).

It appears that cAMP is the source for the increased extracellular adenosine. We suggest that D1 receptors localized on GABA terminals from nucleus accumbens activate adenylyl cyclase to increase cAMP (Figure 7). The metabolism of cAMP to adenosine could occur within the GABA terminal or in the extracellular space and is a matter of speculation. About ten times more cAMP than adenosine was found extracellularly after stimulation of cortical cultures with β-adrenergic receptor agonists (Rosenberg et al., 1994). In that study, the increase in extracellular cAMP was also blocked with probenecid, an agent known to inhibit cAMP efflux. The same concentration of probenecid was effective in the present study and suggests that cAMP is transported extracellularly before metabolism. The transport mechanism itself has not been characterized, although potential candidates include a nucleotide transporter, the multidrug resistance transporter, and even adenylyl cyclase itself (Krupinski et al., 1989; Rosenberg et al., 1994). An augmented efficiency of transport would be one potential mechanism to increase extracellular adenosine.

Another potential mechanism supported in part by experiments with superfusion of cAMP is to increase adenosine levels by increased metabolism of cAMP. Ecto-5′-nucleotidase is an enzyme that mediates the final step in the extracellular metabolism to adenosine. This key membrane-bound enzyme has been found on cholinergic nerve terminals (Cunha et al., 1992), on the mossy fibers of hippocampus (Zimmermann et al., 1993), and on the glomerular and mitral synapses within the olfactory bulb (Schoen and Kreutzberg, 1995). It has been suggested that this enzyme was only present near synapses during development or at synapses that are actively turning over (Schoen and Kreutzberg, 1995). An up-regulation of this enzyme on the GABA terminals after cocaine withdrawal could provide the anatomical substrate for a localized increase in adenosine tone.

Adenosine–Dopamine Interactions Disinhibit Dopamine Cells

Previous studies found that chronic psychostimulant treatment did not change D1 receptor binding in the striatum, nucleus accumbens, or VTA (Peris et al., 1990), and variable effects on the activity of adenylyl cyclase in the nucleus accumbens and striatum have been reported (Terwilliger et al., 1991; Mayfield et al., 1992; Roseboom et al., 1990). This study supports evidence for the lack of any change in the regulation of D1 receptors or adenylyl cyclase after chronic cocaine treatment. The results of the present study are entirely consistent with in vivo studies measuring dopamine in nucleus accumbens and the VTA (Kalivas and Duffy, 1993a, 1993b). In microdialysis studies, dopamine release in the VTA was not different between control and cocaine withdrawn animals, whereas in nucleus accumbens, both the basal and dopamine release in response to cocaine injection were augmented in withdrawn animals (Kalivas and Duffy, 1993a, 1993b). The present study suggests that in cocaine- and morphine-treated animals, inhibition of GABA release through increased adenosine tone could disinhibit dopamine cells. Disinhibition of dopamine cells would be expected to increase dopamine release in projection areas such as the nucleus accumbens.

In contrast with the disinhibition predicted by the present results, the number of spontaneously active VTA cells found in anesthetized animals was decreased 10–14 days after withdrawal from cocaine treatment (Ackerman and White, 1992). Comparisons of the observations made in vivo and in vitro is not possible, since

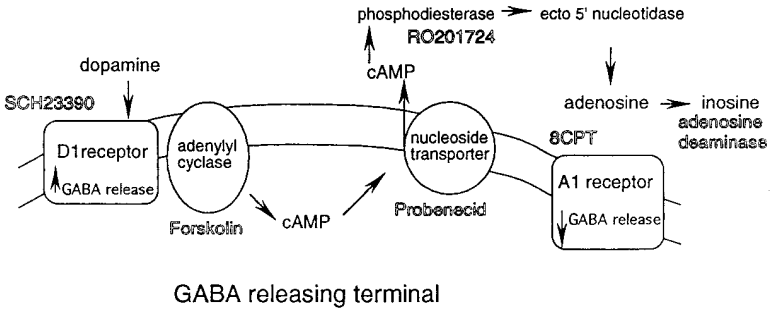

Figure 7. Schematic Figure Illustrating the Proposed Mechanism for Increased A1 Receptor Activation Following Chronic Drug Treatment

D1 receptor activation increases the formation of cAMP, which is a substrate for the nucleotide transporter. Once in the extracellular space, cAMP is metabolized to adenosine and acts on A1 adenosine receptors to inhibit GABA release. Probenecid was used to block the nucleoside transporter. RO201724 was used to inhibit phosphodiesterase. Adenosine deaminase was used to metabolize endogenous adenosine to inosine. The A1 receptor antagonist 8CPT was used to block the adenosine receptor. Although each of these agents have potential nonselective actions, all produced the same effect on the D1 receptor–mediated inhibition of the $GABA_B$ IPSP.

the afferent inputs were stimulated electrically in vitro and since it is not clear what factors regulate the spontaneous activity of dopamine cells in anesthetized animals. The GABA inputs to VTA arise from the nucleus accumbens where the activity in anesthetized animals is generally low. Under these conditions, $GABA_B$-mediated synaptic regulation of activity in the VTA would be limited.

There is substantial evidence that dopamine is spontaneously released from neurons in the VTA and substantia nigra both in slices and in vivo, although the mechanism for spontaneous release in each preparation is not completely understood (Geffen et al., 1976; Korf et al., 1976; Paden et al., 1976; Cheramy et al., 1981; Robertson et al., 1991). It appears that there is no correlation between the rate of spontaneous activity and release of endogenous dopamine in the VTA. Although amphetamine-induced dopamine release has been shown to inhibit both dopamine cell activity by activation of D2 receptors (Mercuri et al., 1989) and GABA cell activity in the substantia nigra zona reticulata by activation of D1 receptors (Timmerman and Abercrombie, 1995), dopamine-mediated synaptic inhibition evoked by electrical stimulation is rare or has failed altogether (Johnson and North, 1992: D. L. Cameron and J. T. W., unpublished data). As such, if disinhibition of dopamine cells occurs, there would no increase in an opposing D2-mediated inhibition caused by an augmented release of dopamine. In fact, there was no change in the dopamine levels after cocaine withdrawal as measured by dialysis experiments (Kalivas and Duffy, 1993b).

Sensitization to psychostimulants is a complex phenomenon involving interactions between several CNS areas (Robinson and Becker, 1986; Robinson and Berridge, 1993). This study suggests that neurochemical mechanisms that may be unrelated to the initial action of cocaine or opioids on the dopamine system, such as the augmentation of adenosine tone, can result in a persistent change in the synaptic regulation of dopamine cell activity.

Experimental Procedures

Recordings of membrane potential are made from dopamine neurons in horizontal slices of guinea pig midbrain. This method has been described previously (Cameron and Williams, 1994). In brief, guinea pigs (300–400 g) were anesthetized with halothane and killed. The midbrain was sliced (300 μm) in the horizontal plane using a vibratome. Slices (up to three) containing the VTA were stored before being placed in the recording chamber and superfused (1.5 ml/min) with warmed (35°C) Krebs/bicarbonate buffer containing the following: 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 11 mM glucose, 21.4 mM $NaHCO_3$ saturated with 95% O_2 and 5% CO_2. Recordings were made with KCl (2 M) filled glass microelectrodes (20–50 MΩ) using standard techniques. Identification of dopamine cells was made based on the physiological properties, including the presence of a regular spontaneous firing activity, a large H current, and the $GABA_B$-mediated IPSP (previously described by Johnson and North, 1992). Bipolar tungsten-stimulating electrodes were placed mediocaudal and rostral to the VTA. To evoke synaptic potentials, neurons were maintained at a membrane potential of −60 to −65 mV by injecting hyperpolarizing current (10–20 pA) and a train of stimuli (500 μs at 70 Hz for 143 ms, i.e., 10 stimuli) ranging from 0.5–1.5 mA were delivered at 60 s intervals using a constant current stimulation unit.

Drugs were applied in known concentrations to the superfusion medium. In experiments examining the $GABA_B$ synaptic potential, the superfusion medium contained 2-amino-5-phosphonopentanoic acid (AP5, 100 μM), 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline (CNQX, 10 μM), picrotoxin (100 μM), and eticlopride (100 nM) to block fast NMDA, AMPA, $GABA_A$, and dopamine D2 mediated synaptic potentials, respectively. There was no effect of this solution on the firing rate or membrane potential of the dopamine cells used in the present study. The same observation has been reported using a similar mixture of antagonists in rat VTA (Johnson and North, 1992). IPSPs were blocked by the $GABA_B$ receptor antagonist CGP35348 (100 μM). The stimulus intensity was adjusted such that IPSPs recorded in all groups of animals had the same range of amplitudes, so that all drug induced effects are presented as a percentage change from control and can be compared directly. Experiments with SKF38393, SKF82958, and SCH23390 required separate groups of slices, since the washout time of these drugs was too slow to allow repeated administrations. Cocaine, AP5, and picrotoxin were obtained from the Sigma Chemical Company. CNQX and eticlopride were obtained from Research Biochemicals, International. Results in the text and figures are presented as the mean ± SEM. The percent changes presented in all bar graphs are determined as follows: (IPSP amplitude in the presence of drug)/(IPSP amplitude in control)*100–100. The average of four IPSPs was taken in control just before adding the drug and again after the effects of the drug had reached steady state (5–20 min). A p value of less than 0.05 was taken as indicating statistical significance.

Animals were given once daily intraperitoneal injections of saline (n = 16), cocaine (10 mg/kg) for 2 weeks or morphine (10 mg/kg) for 1 week. Some cocaine-treated animals (n = 4) were tested the first day after the last injection and called the chronic cocaine group. Most experiments were carried out 7–10 days after the last drug injection (cocaine, n = 33; morphine, n = 18).

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