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Short communication

Chronic repeated cocaine administration increases dopamine D_1 receptor-mediated signal transduction

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Abstract

Alteration in dopamine D_1 receptor-mediated signal transduction following repeated cocaine administration was investigated. Male Fischer rats were administered saline or cocaine HCl (15 mg/kg, i.p.) three times daily at 1-h intervals for 1, 7, or 14 days. Stimulation of adenylyl cyclase activity by dopamine and the selective dopamine D_1 receptor agonist, (±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetra-hydro-1*H*-3-benzazepine hydrobromide (SKF 82958), was significantly greater in the nucleus accumbens and caudate putamen of animals injected with cocaine for 14 days compared with control animals, but was unchanged in animals administered cocaine for 1 or 7 days. These results suggest that dopamine D_1 receptor signal transduction in the nucleus accumbens and caudate putamen is enhanced following chronic repeated administration of cocaine.

Keywords: Adenylyl cyclase; Nucleus accumbens; Caudate putamen; SKF 82958; cAMP

1. Introduction

Cocaine is a widely abused psychomotor stimulant whose reinforcing effects are believed to be due to its ability to inhibit the re-uptake of dopamine. Cocaine administered to animals can induce locomotor activation and stereotypic behaviors which are mediated by an increase in dopaminergic transmission in the mesolimbic dopamine system (Kelley and Iversen, 1975; Kalivas et al., 1988). The degree of stimulation of locomotor and stereotypic behaviors induced by cocaine increases with repeated cocaine administration leading to behavioral sensitization (Downs and Eddy, 1932). Although the phenomenon of sensitization is well documented, the mechanisms underlying its development and expression have not been fully elucidated.

In addition to the behavioral changes produced by repeated cocaine administration, alterations in dopamine receptors have also been reported. We have found that binding to dopamine D_1 receptors was significantly increased in the olfactory tubercle, nucleus accumbens, ventral pallidum, and substantia nigra following chronic repeated injections of cocaine for 14 days (Unterwald et al., 1994). Likewise, Alburges et al. (1993) found increases in dopamine D_1 receptor binding in striatal and cortical membranes after twice daily injections of cocaine for 7, 14, or 21 days. The goal of the present study was to investigate the functional consequences of cocaine-induced increases in dopamine D_1 receptor binding. Adenylyl cyclase activity was used as a functional measure of dopamine D_1 receptor-mediated signal transduction. Activation of dopamine D_1 receptors has been shown to stimulate adenylyl cyclase activity via activation of G_s, the stimulatory guanine nucleotide binding protein (G protein; Stoof and Kebabian, 1981). In the present study, the ability of dopamine and a selective dopamine D_1 receptor full agonist to stimulate adenylyl cyclase activity in the nucleus accumbens and caudate putamen was measured in rats injected with cocaine or saline three times daily at 1-h intervals for 1, 7, or 14 days.

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2. Materials and methods

2.1. Chemicals

Chemicals and reagents were obtained from the following sources: [^{3}H]cAMP (adenosine 3′,5′-cyclic phosphate, ammonium salt; specific activity 31.4 Ci/mmol) from New England Nuclear (Boston, MA, USA); ATP, GTP, cAMP, theophylline, imidazole, dopamine, and EGTA from Sigma (St. Louis, MO, USA); (±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetra-hydro-1*H*-3-benzazepine hydrobromide (SKF 82958 HBr) from Research Biochemicals International (Natick, MA, USA).

2.2. Animals and drug administration

Sixty-day-old male Fischer rats (Charles River Laboratories, Kingston, NY, USA) were housed in a stress-minimized facility in single-animal cages with free access to food and water. Animals were maintained on a 12-h light/dark cycle (lights on at 9:00 a.m.) and were weighed daily at 9:00 a.m. After a one-week adjustment period in the facility, animals were injected intraperitoneally three times daily at 9:30, 10:30 and 11:30 a.m. with saline (1 ml/kg body weight) or cocaine HCl (15 mg/kg) dissolved in saline as previously described (Unterwald et al., 1994). Animals were injected with saline or cocaine for 1, 7, or 14 days. There were 6–8 animals in each experimental group at each time point.

2.3. Membrane preparation

Thirty minutes after the last injection, animals were exposed to CO_2 for 15 s and subsequently killed by decapitation. Their brains were rapidly removed and nucleus accumbens and caudate putamen were separately dissected on ice. Crude membranes were prepared by homogenizing the tissue in 25 ml of ice-cold 20 mM Tris HCl, 2 mM EGTA, 1 mM $MgCl_2$ and 250 mM sucrose, pH 7.4, followed by centrifugation at $30\,000 \times g$ for 15 min at 4°C. The pellets were resuspended in 25 ml of fresh buffer and centrifuged again for 15 min. The resulting pellets were homogenized in 30 volumes of ice-cold buffer containing 10 mM imidazole and 2 mM EDTA, pH 7.4, and stored at −70°C in 10% glycerol until assayed for adenylyl cyclase activity.

2.4. Determination of adenylyl cyclase activity

Adenylyl cyclase activity was measured as described previously (Izenwasser and Katz, 1993). Tissue homogenate (10 μl) was incubated in 10 mM imidazole (pH 7.4), 10 mM theophylline, 6 mM $MgSO_4$, 0.6 mM EGTA, 1.5 mM ATP and 0.01 mM GTP, in the absence or presence of 1 nM–10 μM of dopamine or SKF 82958 (final reaction volume 60 μl) in triplicate for 5 min at 30°C. Determinations of the stimulatory activities of dopamine and SKF 82958 were made using the same tissue membrane preparations in parallel assays conducted simultaneously. Adenylyl cyclase activity was terminated by placing the tubes into boiling water for 2 min. The amount of cAMP formed was determined by a [^{3}H]cAMP binding protein assay. [^{3}H]cAMP (final concentration 4 nM) in citrate-phosphate buffer, pH 4.0, followed by binding protein prepared from bovine adrenal glands were added to each sample. Additional samples were prepared without tissue containing known amounts of cAMP and served as standards for quantification. The binding reaction was allowed to reach equilibrium by incubation for 90 min at 4°C. The assay was terminated by the addition of charcoal and centrifugation to separate the free cAMP from that bound to the binding protein. Aliquots of the supernatants were assayed for radioactivity by liquid scintillation spectrometry using CytoScint Scintillation Fluid (ICN Biomedicals, CA, USA). Radioactivity was converted to pmol of cAMP by comparison to the curve derived from the standards. Protein concentrations were determined using the Lowry procedure (Lowry et al., 1951). Concentration curves were evaluated by two-way analysis of variance (ANOVA) with repeated measures. Maximal stimulation values were evaluated by an unpaired two-tailed *t*-test. Differences between treatment groups were regarded as significant if $P < 0.05$.

3. Results

3.1. Nucleus accumbens

Both dopamine and the selective dopamine D_1 receptor full agonist SKF 82958 (10^{-8}–10^{-4} M) produced a concentration-dependent increase in cAMP production in the nucleus accumbens. One day of cocaine injections had no effect on the ability of dopamine or SKF 82958 to stimulate adenylyl cyclase activity (data not shown). Similar results were found following 7 days of cocaine administration; there were no significant differences in the ability of dopamine ($F(1,41) = 0.09$; $P = 0.44$) or SKF 82958 ($F(1,33) = 3.98$; $P = 0.054$) to stimulate adenylyl cyclase activity between animals injected with saline or cocaine for 7 days (Fig. 1, top panels). However, following 14 days of cocaine administration, the stimulation of cAMP production by dopamine ($F(1,64) = 25.73$; $P < 0.0001$) or by SKF 82958 ($F(1,61) = 29.71$; $P < 0.0001$) was significantly greater in the nucleus accumbens of animals injected with cocaine for 14 days than in saline-injected control animals (Fig. 1, bottom panels). Dopamine produced a maximum stimulation of cAMP production of 165 ± 5% over basal levels in the nucleus accumbens of saline-injected animals and 187 ± 9% in the nucleus accumbens of the chronic cocaine-injected animals ($P <$ 0.05). In control saline-injected animals, SKF 82958 pro-

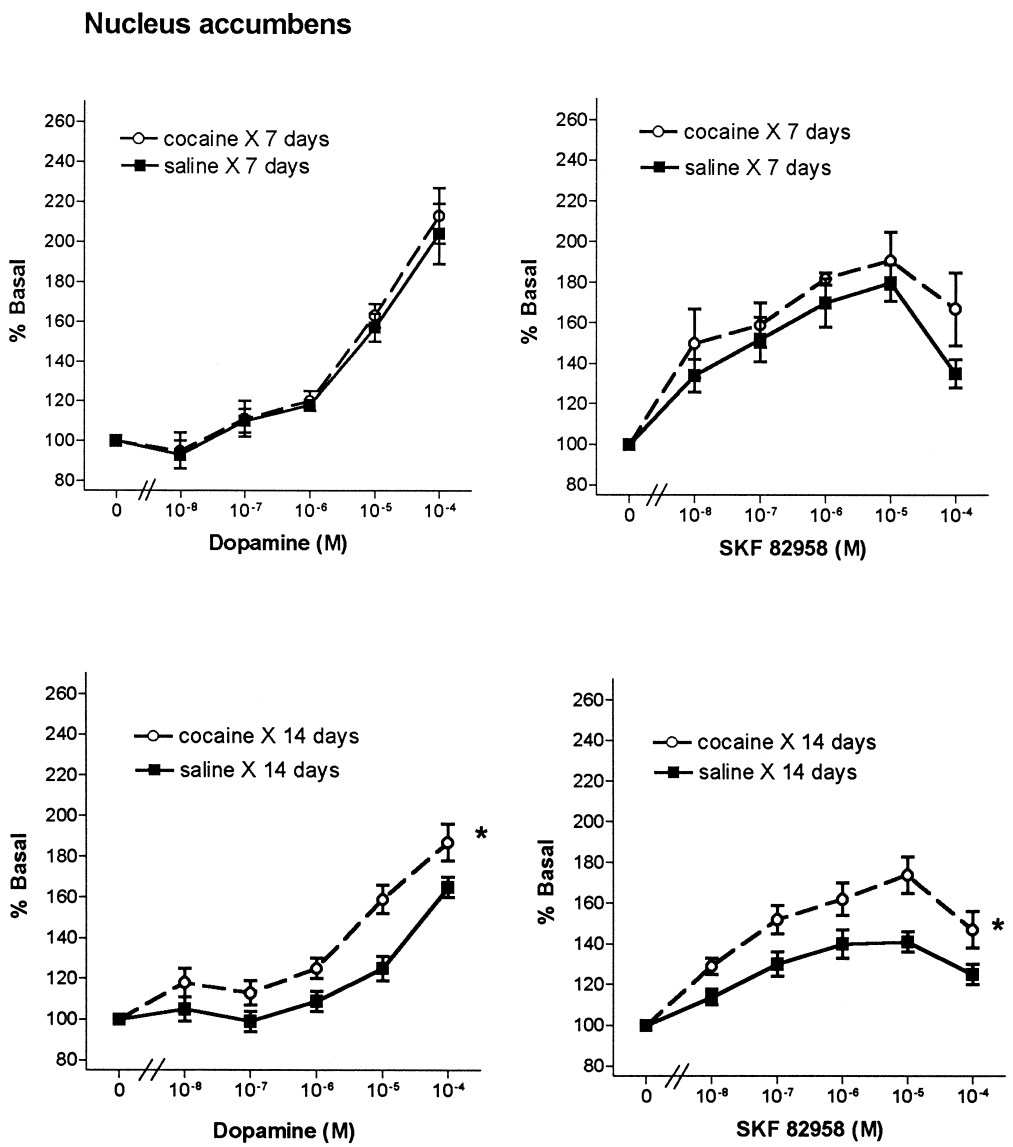

Fig. 1. Stimulation of adenylyl cyclase activity by dopamine (left) and by the selective dopamine D_1 receptor full agonist SKF 82958 (right) in the nucleus accumbens of animals administered saline or cocaine, 15 mg/kg, 3 times daily for 7 (top) or 14 (bottom) days. Data are expressed as percent of basal activity and are shown as mean ± S.E.M. from 6–8 experiments, each performed in triplicate. The stimulation curves for dopamine ($F(1,64) = 25.73$, $P < 0.001$) and SKF 82958 ($F(1,61) = 29.71$, $P < 0.0001$) are significantly different in the animals injected with cocaine for 14 days than in the saline-injected control animals.

duced a maximum stimulation of cAMP accumulation of 141 ± 5% compared to 174 ± 9% in the animals injected with cocaine for 14 days ($P < 0.01$).

3.2. Caudate putamen

Both dopamine and SKF 82958 (10^{-8}–10^{-4} M) produced a concentration-dependent increase in cAMP production in the caudate putamen. The ability of dopamine or SKF 82958 to stimulate adenylyl cyclase activity was not significantly altered after one day of cocaine injections (data not shown). Likewise, there were no significant differences in the ability of dopamine ($F(1,49) = 3.36$; $P = 0.07$) or SKF 82958 ($F(1,49) = 2.25$; $P = 0.14$) to stimulate adenylyl cyclase activity between animals injected with saline or cocaine for 7 days (Fig. 2, top panels). However, stimulation of cAMP production by dopamine ($F(1,64) = 19.04$; $P < 0.0001$) and by SKF 82958 ($F(1,63) = 31.47$; $P < 0.0001$) was significantly greater in the caudate putamen of animals injected with cocaine for 14 days than in control animals injected with saline (Fig. 2, bottom panels). Dopamine produced a maximum stimulation of cAMP production of 203 ± 9% over basal levels in the caudate putamen of control animals as compared with 256 ± 13% in the caudate putamen of the chronic cocaine-injected animals ($P < 0.005$). SKF 82958 produced a maximum stimulation of cAMP accumulation of 193 ± 7% over basal in control animals compared with

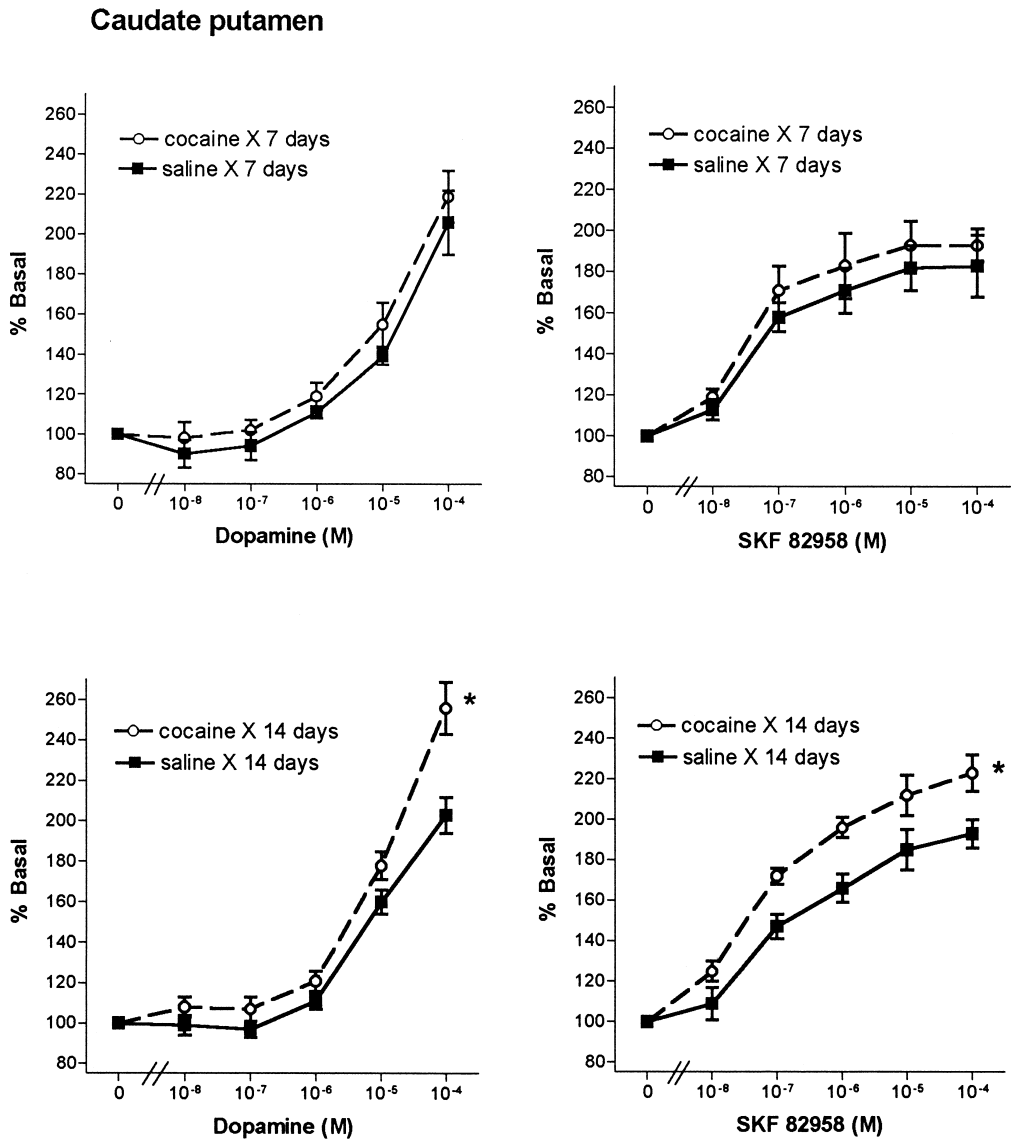

Fig. 2. Stimulation of adenylyl cyclase activity by dopamine (left) and by the selective dopamine D_1 receptor full agonist SKF 82958 (right) in the caudate putamen of animals administered saline or cocaine, 15 mg/kg, 3 times daily for 7 (top) or 14 (bottom) days. Data are expressed as percent of basal activity and are shown as mean ± S.E.M. from 6–8 experiments, each performed in triplicate. The stimulation curves for dopamine ($F(1,64) = 19.04$, $P < 0.0001$) and SKF 82958 ($F(1,63) = 31.47$, $P < 0.0001$) are significantly different in the animals injected with cocaine for 14 days than in the saline-injected control animals.

223 ± 9% in the animals injected with cocaine for 14 days ($P < 0.05$).

4. Discussion

The results of the present study demonstrate that the sensitivity of dopamine D_1 receptors is altered by chronic repeated administration of cocaine. Dopamine D_1 receptor-stimulated adenylyl cyclase activity was significantly enhanced in the nucleus accumbens and caudate putamen of animals injected with cocaine for 14 days. The enhancement of dopamine D_1 receptor signal transduction was not found after acute (1 day) or subacute (7 days) cocaine administration. In a previous study, this same regimen of cocaine administration produced behavioral sensitization and increased binding to dopamine D_1 receptors in the nucleus accumbens, but not in the caudate putamen (Unterwald et al., 1994). Therefore, it appears that changes in dopamine D_1 receptor-mediated signal transduction can occur in the presence (as in the nucleus accumbens) or absence (as in the caudate putamen) of changes in dopamine D_1 receptor number. In addition, behavioral sensitization and dopamine D_1 receptor upregulation were present after 14 days of cocaine administration, but not after 1 or 7 days (Unterwald et al., 1994) which is

a similar time course to the present findings of enhanced dopamine D_1 receptor-mediated adenylyl cyclase activity after 14 days of cocaine, but not after 1 or 7 days.

Our findings of enhanced dopamine D_1 receptor sensitivity is in agreement with the electrophysiological data of Henry and White (1991) who demonstrated that there are increased inhibitory responses of neurons in the nucleus accumbens to D_1 receptor agonists following 14 days of twice daily cocaine injections and Higashi et al. (1989) who showed enhanced dopamine D_1 receptor supersensitivity in the nucleus accumbens after repeated methamphetamine administration. The alterations in dopamine D_1 receptor sensitivity following chronic exposure to cocaine may be due to enhanced dopamine D_1 receptor stimulation by endogenous dopamine secondary to cocaine's inhibition of dopamine uptake. This is supported by studies showing that repeated administration of the selective dopamine D_1 receptor agonist, SKF 38393, also enhanced the sensitivity of nucleus accumbens and caudate putamen neurons to dopamine D_1 receptor agonists (Henry and White, 1991; White et al., 1990). The increased neuronal response to dopamine D_1 receptor agonists may be mediated by increases in dopamine D_1 receptor-stimulated cAMP production as demonstrated here.

Several aspects of signal transduction pathways have been investigated following chronic cocaine administration. Twice-daily injections of cocaine for 14 days produced an increase in forskolin-stimulated adenylyl cyclase activity and cyclic AMP-dependent protein kinase activity (Terwilliger et al., 1991) and reductions in the levels of $G_{i\alpha}$ and $G_{o\alpha}$ (Nestler et al., 1990) in the nucleus accumbens one day later. The decrease in inhibitory G proteins following cocaine administration could facilitate the activation of adenylyl cyclase by dopamine D_1 receptors. Striplin and Kalivas (1993) found no changes in G protein levels following 5 daily cocaine injections when measured 1 h after the last injection, but found significant reductions in $G_{i1\alpha}$ in the nucleus accumbens 14 days following the last of 5 daily cocaine injections. Neither study found changes in the levels of G_s (Nestler et al., 1990; Striplin and Kalivas, 1993). Similar to our findings of no significant changes in adenylyl cyclase activity immediately following 7 days of cocaine administration, Mayfield et al. (1992) found no significant alterations in dopamine-stimulated adenylyl cyclase activity in the nucleus accumbens or striatum following a 7-day abstinence period from 6 daily cocaine injections.

Results from the present study demonstrate an enhanced ability of dopamine D_1 receptors to stimulate the production of cAMP in the nucleus accumbens and caudate putamen of rats administered cocaine three times daily at 1-h intervals for 14 days. Our previous studies have demonstrated that this regimen of cocaine administration results in behavioral sensitization and produces an upregulation of dopamine D_1 receptors in the nucleus accumbens (Unterwald et al., 1994). The increase in dopamine D_1 receptor-mediated signal transduction may play a role in the development of sensitization to cocaine.

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