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Short communication

Continuous cocaine administration enhances μ- but not δ-opioid receptor-mediated inhibition of adenylyl cyclase activity in nucleus accumbens

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Abstract

Cocaine alters opioid receptor densities in rat brain. To investigate the functional consequences of such opioid receptor changes, adenylyl cyclase activity was measured in rat nucleus accumbens and caudate putamen following continuous cocaine administration (50 mg/kg/day, 7 days). In the nucleus accumbens chronic cocaine led to an increase in both the number of μ-opioid receptors and the maximal inhibition of adenylyl cyclase activity by DAMGO ([D-Ala^2,*N*-methyl-Phe^4,Glyol]enkephalin). There was no effect on inhibition of adenylyl cyclase activity by DPDPE ([D-Pen^2,D-Pen^5]enkephalin). There were no changes in the caudate putamen. Thus, continuous cocaine administration for 7 days results in a selective increase in μ-opioid receptor-mediated effector function in the nucleus accumbens.

Keywords: Cocaine; Opioid; Caudate; Nucleus accumbens

1. Introduction

There have been many studies examining the effects of chronic cocaine treatment on neurochemical function and the results have varied depending on length of treatment, dose of cocaine administered, treatment paradigm (i.e. repeated injections vs. continuous administration), and time since the last administration (e.g. Reith et al., 1987; Pettit et al., 1990; Pilotte et al., 1991; Baumann and Rothman, 1993). We have previously shown that there are differential changes in dopamine transporter function in the nucleus accumbens and caudate putamen following 7 days of chronic continuous cocaine administration (Izenwasser and Cox, 1992), and that these changes are different than those produced by daily cocaine injections (Izenwasser and Cox, 1990). These findings suggest that the schedule on which cocaine is chronically administered plays an important role in determining what neurochemical changes will occur.

Several studies have shown that repeated administration of cocaine leads to changes in opioid receptor densities in discrete brain regions of rats. Two weeks of either continuous administration of cocaine via subcutaneously implanted osmotic minipumps (Hammer, 1989) or daily injections in a 'binge-like' pattern (Unterwald et al., 1992) lead to increased μ-opioid receptor density in the nucleus accumbens. This is a mesolimbic terminal region that has been shown to be associated with cocaine-induced reinforcement. In contrast, continuous administration of cocaine produced no change in opioid receptor density in the caudate putamen (Hammer, 1989), while binge cocaine treatment increased μ-opioid receptors in the rostral caudate putamen (Unterwald et al., 1992).

Following the binge treatment, there was no change in the inhibition of adenylyl cyclase activity (a measure of receptor-mediated effector function) by DAMGO, a selective μ-opioid receptor agonist, in either of these

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brain regions, even though receptor numbers were increased (Unterwald et al., 1993). In contrast, in animals treated with naltrexone, μ-opioid receptor number was increased and there was a concomitant increase in the inhibition of adenylyl cyclase activity by DAMGO in both whole brain (Cote et al., 1993), and in nucleus accumbens and caudate putamen (unpublished data). Thus, it is possible for the inhibition of adenylyl cyclase via μ-opioid receptors to increase following treatment. The magnitude of the receptor change was much greater in the naltrexone-treated animals (receptor number was doubled) (Cote et al., 1993), than in the binge-cocaine-treated animals (approximately a 30% increase in receptors) (Unterwald et al., 1992), suggesting that the differences in these findings may be due to the magnitudes of the receptor changes.

It is unknown whether there are functional changes in opioid receptors after continuous, chronic administration of cocaine. In the present study, basal adenylyl cyclase activity and the inhibition of adenylyl cyclase by μ- and δ-opioid receptor agonists were measured in nucleus accumbens and caudate putamen of rats administered cocaine or saline continuously via subcutaneously implanted osmotic minipumps.

2. Materials and methods

2.1. Chemicals

Chemicals and reagents were obtained from the following sources: [^{3}H]DAMGO ([D-Ala^2,*N*-methyl-Phe^4,Glyol]enkephalin) from Amersham Corp. (Arlington Heights, IL, USA); [^{3}H]cAMP (adenosine 3′,5′-cyclic phosphate, ammonium salt; specific activity 31.4 Ci/mmol) from New England Nuclear (Boston, MA, USA); cocaine hydrochloride from Sigma Chemical Co. (St. Louis, MO, USA); DAMGO and DPDPE ([D-Pen^2,D-Pen^5]enkephalin) from Cambridge Research Biochemicals (Wilmington, DE, USA); naltrexone from Research Biochemicals (Natick, MA, USA); and morphine from the National Institute on Drug Abuse (Rockville, MD, USA).

2.2. Treatments

Male Sprague-Dawley rats (250–300 g, Taconic, Germantown, NY, USA) were kept on a 12 h light/dark cycle with food and water available ad libitum. Animals were anesthetized with halothane and Alzet osmotic minipumps model 2001 (Alza Corp., Palo Alto, CA, USA) delivering approximately 1 μl/h for 7 days were implanted s.c. between the scapulae. The pumps contained either saline (0.9% sodium chloride; 24 μl/day), or a concentration of drug resulting in delivery of approximately 50 mg/kg/day of cocaine, expressed as free base. The minipumps were filled and soaked in saline at 37°C for at least 4 h prior to surgery, so as to reach a constant pumping volume before being implanted into the animals. The dose of drug delivered was determined by the pumping rate and average body weight of the animals in each experiment which was comprised of four rats in each treatment group.

2.3. Binding assays

2.3.1. Membrane preparation for binding assays

Seven days after minipump implantation, the rats were anesthetized and the pumps were removed. Twenty-four hours later the rats were killed by decapitation, brains were rapidly removed and the rostral caudate putamen and/or nucleus accumbens were dissected on ice. Dissected tissue was kept in ice-cold saline until dissections were complete. The tissues were homogenized in 50 mM Tris HCl, pH 7.4 at 4°C, at a volume of 25 mg/ml tissue wet weight and centrifuged for 15 min at $30000 \times g$ at 4°C. The pellets were resuspended in Tris buffer and recentrifuged. Each pellet was resuspended in Tris buffer and left to incubate in a shaker bath for 30 min at 37°C to allow for dissociation of endogenous ligand from the receptors. The pellets were then centrifuged again and resuspended in a final volume of 25 mg/ml Tris buffer.

2.3.2. [^{3}H]DAMGO binding assay

Fresh tissue homogenate was used in all experiments, using methods described previously (Unterwald et al., 1992). Twelve-point [^{3}H]DAMGO saturation curves were performed over a concentration range of 0.1 nM to 15 nM [^{3}H]DAMGO for caudate putamen tissue. Because of the small size of the nucleus accumbens, each curve contained only five points. Nonspecific binding was determined as binding in the presence of 10 μM levorphanol. Protein values were determined using a modification of the Lowry procedure.

2.4. Adenylyl cyclase assays

Membranes were prepared and adenylyl cyclase activity was determined using a [^{3}H]cAMP protein binding assay, as described previously (Cote et al., 1993; Izenwasser et al., 1993).

2.5. Statistical analysis

Saturation data were analyzed by the use of the nonlinear least squares curve-fitting computer program LIGAND. Data from replicate experiments were modeled together to produce a set of parameter estimates and the associated standard errors of these estimates.

Differences across treatment groups were analyzed using analysis of variance (ANOVA) and were considered to be significant when $P \leq 0.05$. The amount of cAMP formed as a function of concentration of agonist was analyzed using ANOVA and linear regression techniques. From this analysis, IC_{50} values and their 95% confidence limits were derived from data using the linear portion of the concentration-effect curves. Differences among maximal effects were assessed by unpaired t-tests.

3. Results

[^{3}H]DAMGO bound with an affinity of approximately 1 nM in both caudate putamen and nucleus accumbens tissues (Table 1). The density of [^{3}H]DAMGO binding sites in the caudate putamen was twice that in the nucleus accumbens in saline control animals. Following cocaine treatment, the affinity of [^{3}H]DAMGO was unchanged in both brain regions. In nucleus accumbens, there was a 23% increase in the number of [^{3}H]DAMGO binding sites following cocaine treatment; however, there was no significant change in binding site density in the caudate putamen (Table 1).

Basal adenylyl cyclase activity was not significantly changed in either the nucleus accumbens or caudate putamen in cocaine-treated animals as compared to saline controls. There was a 1.5-fold increase in the inhibition of adenylyl cyclase activity in the nucleus accumbens by the μ-opioid receptor agonist DAMGO after continuous treatment with cocaine (Fig. 1A). Furthermore, in this brain region, there was a 3.5-fold decrease in the IC_{50} for DAMGO inhibition of adenylyl cyclase (Table 1). Analysis of variance showed that the inhibition curve for DAMGO was significantly shifted following cocaine treatment as compared to saline pretreatment in the nucleus accumbens ($F(1,69) = 11.7$, $P \leq 0.001$). There were no significant effects of cocaine treatment on inhibition of adenylyl cyclase activity by DPDPE in either brain region. Further, there was no change in the inhibition of adenylyl cyclase activity by DAMGO in the caudate putamen ($F(1,48) = 0.36$, $P \geq 0.05$; Table 1). Unlike the changes

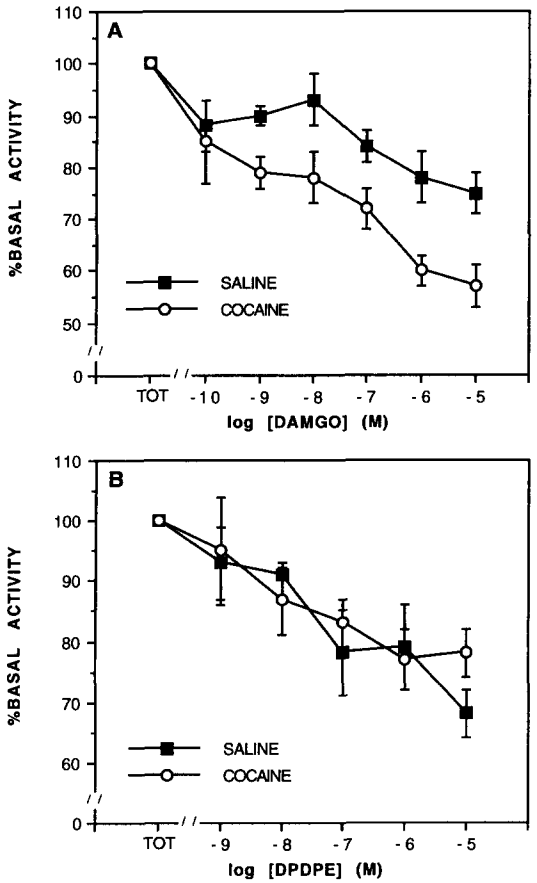

Fig. 1. Inhibition of adenylyl cyclase activity by (A) the selective μ-opioid receptor agonist DAMGO and (B) the selective δ-opioid receptor agonist DPDPE in nucleus accumbens from animals continuously administered saline (DAMGO, $n = 9$; DPDPE, $n = 5$) or cocaine (50 mg/kg/day; DAMGO, $n = 7$; DPDPE, $n = 7$) for 7 days. Each data point represents mean ± S.E.M. from n individual experiments, each performed in triplicate. Analysis of variance showed that the inhibition curve for DAMGO was significantly shifted following cocaine treatment as compared to saline pretreatment in the nucleus accumbens ($F(1,69) = 11.7$, $P \leq 0.001$). The basal level of adenylyl cyclase activity was 35.4 ± 4.29 pmol/mg protein/min and was not significantly changed following treatment with cocaine. There were no significant effects of cocaine treatment on inhibition of adenylyl cyclase activity by DPDPE in either brain region.

Table 1
K_D (nM) and B_{max} (fmol/mg protein) values for [^{3}H]DAMGO binding in nucleus accumbens and caudate putamen following treatment with cocaine or saline

Treatment	Binding		Adenylyl cyclase			
	K_D	B_{max}	DAMGO		DPDPE	
			IC_{50}	Max. inhib.	IC_{50}	Max. inhib.
Nucleus accumbens						
Saline	1.17 ± 0.37	79 ± 8	9.1 ± 1.0	25 ± 4	41 ± 38	32 ± 4
Cocaine	1.08 ± 0.45	97 ± 10 [a]	2.8 ± 2.4 [a]	43 ± 4 [a]	69 ± 12	22 ± 4
Caudate putamen						
Saline	1.07 ± 0.25	164 ± 22	39 ± 169	39 ± 4	53 ± 38	35 ± 7
Cocaine	1.22 ± 0.46	190 ± 40	21 ± 13	42 ± 9	74 ± 77	30 ± 10

Each value is comprised of data from 10 individual experiments, each performed in triplicate. IC_{50} values (nM) and maximal inhibition of adenylyl cyclase activity produced by 10 μM DAMGO or DPDPE (% basal activity) in nucleus accumbens and caudate putamen. Each value is comprised of data from at least 5 independent experiments, each performed in triplicate. [a] $P \leq 0.05$, compared to saline.

seen with DAMGO, the effect of the δ-opioid receptor agonist DPDPE on adenylyl cyclase activity did not change in either nucleus accumbens (Fig. 1B) or caudate putamen (Table 1) following cocaine treatment.

4. Discussion

Chronic, continuous administration of cocaine for 7 days produced a significant increase in μ-opioid receptor number in the nucleus accumbens, with no change in the caudate putamen. These receptor changes are similar in magnitude to those seen following 14 days of injections of 45 mg/kg/day administered in a binge-like pattern (i.e. three injections daily at 1 h intervals) and assessed using autoradiographic techniques (Unterwald et al., 1994). In the present study, this increased receptor number was accompanied by a concomitant increase in inhibition of adenylyl cyclase activity by DAMGO. However, in the intermittently treated animals there was no change in regulation of adenylyl cyclase by DAMGO (Unterwald et al., 1993). These findings suggest that an increase in the number of μ-opioid receptors is not always accompanied by an increase in μ-opioid receptor-regulated second messenger function. Further, these data show, once again, that the paradigm by which cocaine is administered can greatly influence the results.

It has previously been reported that continuous administration for 14 days of a low dose of cocaine (10 mg/kg/day) produced an increase in opioid receptors in the nucleus accumbens ranging from 60–200% over saline, depending on which part of the accumbens was measured (Hammer, 1989). The magnitude of those changes was considerably larger than those seen in either the present study, or following repeated cocaine injections (Unterwald et al., 1992). There were several differences between these studies that could account for these discrepancies. (1) The dose of cocaine administered was significantly lower in the Hammer (1989) study. There is evidence to suggest that dopamine D_1 and D_2 receptors may play differential roles in the effects of cocaine depending on dose. For example, dopamine D_1 receptor agonists appeared to be equally effective substitutes for cocaine in animals trained to discriminate either a high or low dose of cocaine from saline. In contrast, D_2 receptors appear to be involved in the mediation of the discriminative stimulus properties of only high doses of cocaine (Terry et al., 1994). Thus, different doses of cocaine may lead to preferential activation of different subpopulations of dopamine receptors and thus of different neurons, which could lead to differential regulation of μ-receptor function. (2) Receptors were assessed using autoradiographic techniques to measure [^{3}H]naloxone binding in the Hammer (1989) study. As discussed by Unterwald et al. (1992), naloxone binds to δ- and κ-opioid receptors, although with lower affinity than μ-opioid receptors. In an effort to use a concentration of [^{3}H]naloxone that is selective for μ-opioid receptors, a non-saturating concentration of [^{3}H]naloxone was used. Therefore, it is possible that these changes represent in part a shift in the K_D of naloxone, as opposed to a change in B_{max} of μ-opioid receptors. Further study is needed in order to better understand the nature of these discrepancies.

The present findings are consistent with changes observed in μ- and δ-opioid receptor mRNAs following continuous cocaine infusion. After 3 days of treatment, μ-opioid receptor mRNA was significantly increased in the nucleus accumbens, with no change in the caudate putamen. There were no changes in δ-opioid receptor mRNA in either brain region, as is reported here for receptor binding (Azaryan et al., 1996).

The findings in this study suggest that the change in opioid receptor number in the nucleus accumbens appears to be of approximately the same magnitude if a similar dose of cocaine is administered intermittently or via continuous infusion (compare the present findings with those of Unterwald et al., 1992). The increased inhibition of adenylyl cyclase activity in nucleus accumbens from animals continuously infused with cocaine, but not in the injected animals (Unterwald et al., 1993) suggests that this effect is not due merely to the magnitude of change in receptor number. It may be that continuous infusion of drug produces changes more rapidly than intermittent injections, and that if the daily injections were continued for a longer period of time, increased activity might be observed. This idea is consistent with the findings that morphine tolerance occurs more rapidly when injections are spaced only a few hours apart, than when the injections are greater than 12 h apart (although tolerance does occur in both cases) (Goldstein and Sheehan, 1969).

It is interesting that cocaine produced significant changes in the nucleus accumbens, a mesolimbic dopamine terminal region, but not in the caudate putamen, a region densely populated with dopamine terminals projecting from the substantia nigra. Presumably, cocaine is equally active at inhibiting dopamine uptake in both of these brain regions. In fact, we have previously shown that there are no differences between the inhibition of dopamine uptake by cocaine in nucleus accumbens and in caudate putamen in vitro (Izenwasser and Cox, 1990). However, it does seem that the nucleus accumbens responds more easily to change following chronic cocaine administration, than does the caudate putamen. Daily injections of cocaine produced a decrease in total dopamine uptake in the nucleus accumbens with no change in the caudate putamen (Izenwasser and Cox, 1990). Following the current con-

tinuous administration paradigm, however, tolerance to the inhibition of dopamine uptake by cocaine was seen in both the nucleus accumbens and caudate putamen (Izenwasser et al., 1993), with no changes in total dopamine uptake in either region. Thus, it is unlikely that the changes in μ-opioid receptor number and function in the nucleus accumbens are due directly to differential alterations in extracellular dopamine levels in these two regions.

The present findings suggest that the relationship between dopamine and opioid receptors is different in these regions, although the mechanism by which cocaine produces these effects is still unclear. It does seem, however, that the effects of chronic cocaine on μ-opioid receptors are similar to those seen following chronic opioid receptor antagonist treatment (i.e. an increase in receptor number and increased inhibition of adenylyl cyclase activity). Since cocaine does not bind to opioid receptors, this effect is likely to be via an indirect action (e.g. release of an agent that binds to opioid receptors or a heterologous regulation of receptors regulating common transduction systems). Further studies in our laboratories are aimed at elucidating the mechanism by which cocaine produces these effects.

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