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Long-term Cocaine Self-Administration Decreases Striatal Preproenkephalin mRNA in Rhesus Monkeys

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DAUNAIS, J. B., M. A. NADER AND L. J. PORRINO. *Long-term cocaine self-administration decreases striatal preproenkephalin mRNA in rhesus monkeys.* PHARMACOL BIOCHEM BEHAV **57**(3) 471–475, 1997.—The consequences of long-term exposure to cocaine on striatal preproenkephalin mRNA were assessed using quantitative in situ hybridization in monkeys that self-administered cocaine for approximately 2 years. Autoradiograms revealed high levels of preproenkephalin hybridization signal in both the caudate and putamen of the dorsal striatum, as well as in the shell and core subdivisions of the nucleus accumbens of drug-naive control monkeys. In general, there was a medial to lateral and ventral to dorsal gradient of preproenkephalin mRNA observed within the striatum of normal controls. Preproenkephalin mRNA was significantly reduced in widespread portions of both the dorsal and ventral striatum following chronic long-term cocaine self-administration when compared with levels in normal controls. These data confirm those observed in human drug abusers and suggest that long-term abuse of cocaine can result in significant alterations in the opioid regulation of striatal efferent neurons.

Preproenkephalin Primate Cocaine Self-administration Caudate Putamen Nucleus accumbens In situ hybridization

MANY of the behavioral properties of cocaine are mediated primarily via dopaminergic (DA) systems that project to the dorsal and ventral striatum. Cocaine binds to DA transporters and inhibits DA reuptake, an action that effectively increases synaptic DA levels and potentiates DA activity at pre- and postsynaptic receptors. How increased DAergic activity translates into further neurochemical and behavioral changes is unclear, although it has been hypothesized that regulation of postsynaptic gene expression may be involved (22). Indeed, the blockade of DA reuptake has profound effects on postsynaptic neurons intrinsic to the striatum, including those expressing mRNAs encoding the opioid peptide enkephalin (PPE). PPE mRNA colocalizes with γ-aminobutyric acid (GABA) in medium spiny neurons that constitute the striatal efferent pathway to the globus pallidus. Cocaine has been demonstrated to have differential effects on the level of expression of this peptide in rats (3,6,13,24) and humans (14,15).

To date, our knowledge of the genomic effects of cocaine on the opioid system has largely originated from either rodent or human studies. Although studies in rodents have been important in elucidating the molecular and cellular aspects of the opioid response to cocaine, the increased complexity of the anatomy and chemoarchitecture of the human brain makes it difficult to generalize rodent findings to results from postmortem human studies. Studies in postmortem tissue from human drug abusers can be difficult to interpret because of confounding factors of variable drug histories, comorbid psychiatric diseases, nutritional status, etc. Clearly, a systematic evaluation of many of the variables that could influence the interaction of cocaine with the endogenous opioids in a primate species would be beneficial.

The present study used a nonhuman primate model of cocaine abuse to investigate the consequences of long-term cocaine self-administration on PPE mRNA expression in the striatum. A fundamental reason for using this model, as opposed to a rodent model, is because the chemoarchitectural and neuroanatomical organization of the human is more closely homologous to that of the monkey than to that of the rodent.

METHOD

Subjects and Apparatus

Five adult male rhesus monkeys were used in the present study. Monkeys 9127 and 5526 had extensive histories of co-

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caine self-administration (20,21), and three other monkeys served as cocaine-naive controls. The monkeys weighed between 8.0 and 10 kg under free-feeding conditions. Body weights of the cocaine-experienced monkeys were maintained at 90–95% of their free-feeding weight. Each of the cocaine-experienced monkeys was surgically prepared with an indwelling intravenous catheter into a major vein (internal or external jugular, femoral, or brachial). Both monkeys were individually housed in sound-attenuating cubicles (91 × 91 × 91 cm; Plas Labs, Lansing, MI, USA); the front wall of each cubicle was Plexiglas to allow the monkey visual access to its surroundings. During experiments, the front wall was covered with a drape. Each cubicle was equipped with two response levers (BRS/LVE, PRL-001, Beltsville, MD, USA) and a peristaltic infusion pump (7531-10, Cole-Parmer Co., Chicago, IL, USA) for delivering drug injections at a rate of approximately 1 ml/10 s. Each monkey was fitted with a stainles-steel restraint harness and spring arm (Restorations Unlimited, Chicago, IL, USA) that attached to the rear of the cubicle. During daily 4-h experimental sessions, cocaine (0.01–0.3 mg/kg/injection) was available for self-administration. Lifetime cocaine intakes for monkeys 9127 and 5526 were 576.14 mg/kg and 588 mg/kg, respectively, delivered over a period of 626–679 days. Although the two self-administering monkeys were also used for subsequent [^{14}C]2-deoxyglucose (2DG) analysis, their only behavioral and pharmacological history was with cocaine self-administration. On the final day of drug exposure, they self-administered 1.0 mg/kg cocaine in a bolus injection, followed 45 min later by 2DG, at which time the animals were euthanized by an overdose of IV sodium pentobarbital (100 mg/kg). Animal maintenance and research were conducted in accordance with guidelines of the NIH Office of Protection from Research Risks. The protocol for this experiment was reviewed and approved by the Wake Forest University Animal Care and Use Committee.

In Situ Hybridization Histochemistry

Brains were harvested, blocked, and quick frozen at −40°C in isopentane and stored at −80°C until they were cut. Coronal sections (20 μm) were cut through the anterior striatum of each brain and thaw-mounted onto gelatin/chrom alum-coated slides. The sections were fixed in 4% paraformaldehyde followed by pretreatment with 0.25% acetic anhydride/0.1 M triethanolamine HCl to neutralize charges in the tissue and reduce nonspecific binding. The sections were then defatted in ethanol and chloroform, dried, and hybridized using a synthetic cDNA oligodeoxynucleotide probe (synthesized in the DNA Synthesis Core Laboratory at Bowman Gray School of Medicine) of 48 base pairs complementary to bases 130–145 of human PPE (5). This probe was labeled at the 3′ end using alpha-[^{35}S]-deoxyadenosine triphosphate (>1000 Ci/mmol; New England Nuclear) and terminal deoxynucleotidyl transferase (Boehringer Mannheim) and was extracted with phenol/chloroform/isoamyl alcohol and 4 M NaCl/100% ethanol. The probe was then added to a hybridization cocktail containing 50% formamide, 4 × SSC, 500 μg/ml sheared single-stranded DNA, 250 μg/ml yeast tRNA, 1× Denhardt's solution, and 10% dextran sulfate. A volume of 250 μl/section containing 1 × 10^6 cpm of labeled probe was added to each section, followed by hybridization for 20 h at 37°C in a humid environment.

Following hybridization, the slides were washed under stringent conditions as previously described (25). After drying, experimental slides and slides with ^{14}C standards were apposed to Amersham β-Max film and developed after 1 week of exposure. Because of the numerous stringent washes following hybridization and the short exposure time necessary for visualization of the PPE hybridization signal, interference from ^{14}C from the 2DG procedure did not constitute a problem during quantitative assessment of the hybridization signal.

Image Analysis

Hybridization signals were analyzed as described previously (7). After correcting for nonuniform illumination and subtracting film background by density slicing, the ^{14}C standards (American Radiolabeled Chemicals, Inc., St. Louis, MO, USA) were measured and calibrated to ^{35}S equivalences; a standard curve was generated by plotting transmittance values against known dpm/mg, using a third-degree polynomial equation as previously described (25). This standard curve was then used to calibrate each film. Quantitative changes were expressed as: a) the number of labeled pixels per area, b) the mean density in tissue (dpm/mg), and c) the integrated density (ID). ID is the product of area × mean density and is used to estimate the number of mRNA copies expressed over the area measured.

Hybridization signals were assessed in various oval and circular fields in various regions of the dorsal and ventral striatum. Measurements were taken from the anterior striatum at the level of the anterior nucleus accumbens (NAc), which lacks clear shell/core delineation, and from caudal striatum, where both core and shell are present. The areas measured include the dorsal and ventral caudate, dorsal and ventral putamen, and NAc core and shell (where present).

Statistical Analysis

Statistical significance between control and cocaine self-administration groups was determined with a Student's *t*-test for independent samples.

RESULTS

Autoradiograms revealed high basal levels of specific hybridization to PPE mRNA in the rostral striatum of normal control animals (Fig. 1A). In the rostral caudate and putamen, PPE hybridization signal displayed a prominent medial to lateral gradient. This pattern was also evident in more caudal sections. Another striking feature of the PPE hybridization pattern was the ventral to dorsal gradient, which was similar to the enkephalin immunoreactivity pattern described by Haber and Elde (10). Dense PPE hybridization signal was also evident in the core and shell of the NAc. No differentiation of these regions was observable on the basis of the pattern of PPE mRNA. Additionally, clusters of PPE hybridization signal were evident within both the caudate and the putamen. This pattern resembles the patchy organization described by Haber and Lu (11). The present sections, however, were not dipped in autoradiographic emulsion, and a formal determination was not made as to the compartmental organization of the striatum.

It was determined that both area and mean density contributed equally to the ID. Quantitative changes, therefore, are expressed as the ID, which is used to estimate the number of mRNA copies expressed over the area measured. In the rostral striatum, chronic self-administration of cocaine significantly reduced PPE mRNA in all areas measured, including the caudate, putamen, and NAc (Figs. 1B, 2A). The hybridization signal was decreased to 16% of contral values in the

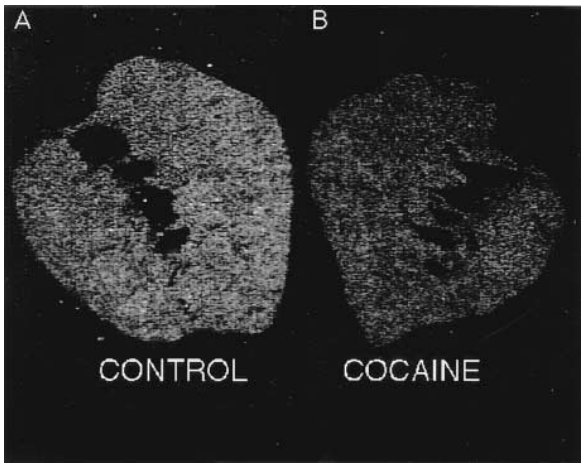

FIG. 1. Autoradiogram displaying preproenkephalin mRNA in naive control (A) and cocaine-treated (B) caudate and putamen of rhesus monkeys. Coronal sections taken through the dorsal and ventral striatum at the level of the rostral nucleus accumbens. Note the medial to lateral and ventral to dorsal gradients of preproenkephalin hybridization signal illustrated in the control brain (A).

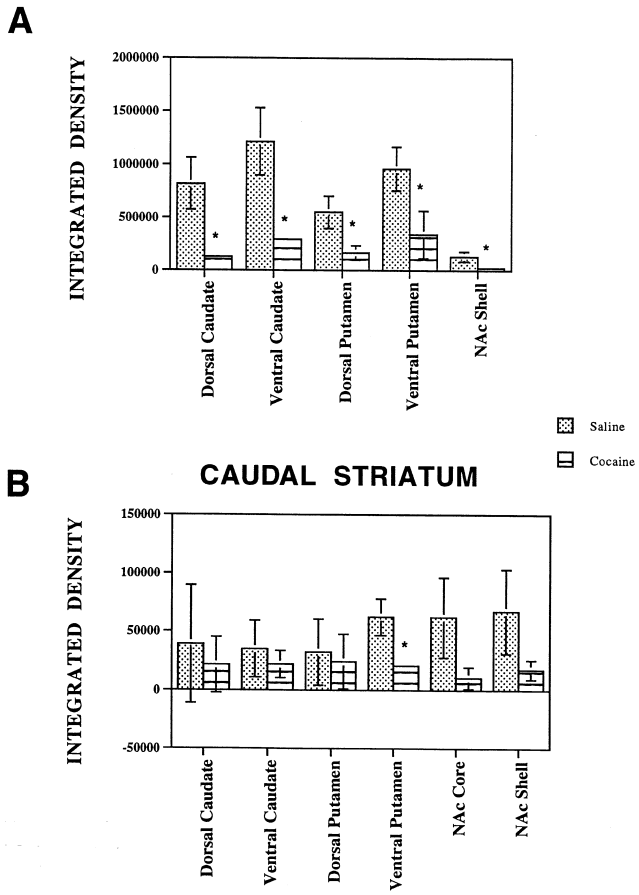

FIG. 2. Quantitative analysis of changes in integrated density for preproenkephalin mRNA in rostral (A) and caudal (B) caudate, putamen, and nucleus accumbens of rhesus monkeys following chronic cocaine self-administration. $*p < 0.05$ vs. naive control.

dorsal portions of the caudate, 24% of control values in the ventral caudate, and 30% and 35% of control values in the dorsal and ventral putamen, respectively. The largest decreases were observed in the nucleus accumbens, where the PPE mRNA hybridization signal was reduced by 83% compared with control values. In general, cocaine self-administration appeared to have greater effects on PPE hybridization signal more medially within the striatum, following the medial to lateral gradient described above.

There was a trend for chronic cocaine self-administration to decrease PPE mRNA in all areas measured in the more caudal regions as well. The changes detected in the caudal caudate, putamen, and NAc, however, were not as great as those observed in the rostral striatum. Due to the large variability between control animals and the small number of animals examined, significant changes between controls and chronically cocaine-exposed animals were detected only in the ventral putamen (33% of control) in the caudal sections (Fig. 2B). Although each control animal displayed a similar pattern of hybridization signal, the intensity of signal was lower in one of these animals. In contrast, both cocaine-treated animals exhibited similar patterns and intensity of signal. Nonetheless, visual assessment of the autoradiograms indicated that in caudal sections, the overall greatest decrease in PPE hybridization signal occurred in the NAc of cocaine-exposed monkeys.

Finally, the clustering of hybridization signal described above did not appear to be altered by cocaine treatment. The clustering was as evident in cocaine-exposed monkeys as it was in cocaine-naive controls.

DISCUSSION

The present study demonstrated that chronic cocaine self-administration decreased the expression of PPE mRNA in both the dorsal and ventral striatum of rhesus monkeys. This is the first study, to our knowledge, that has investigated the genomic effects of cocaine on the opioid system in adult nonhuman primates. These data agree with previously reported findings that demonstrated cocaine-induced decreases in PPE mRNA in the caudate and putamen of human cocaine addicts (14) and extend those data by demonstrating that the decreases in PPE mRNA that result from chronic cocaine self-administration are widespread within the striatum. Reduced expression of PPE mRNA occurred in all portions of the striatum in rostal to caudal and dorsal to ventral gradients. Furthermore, because cocaine was the only drug self-administered by the monkeys in the present study, it is possible to attribute any changes directly to the actions of cocaine rather than to an interaction among a combination of drugs. Studies in human addicts are complicated by problems such as comorbid psychiatric conditions, variable self-reported drug intake, and differing lifestyles that make the interpretation of data extremely difficult at times. The present study eliminated many of these confounds, controlling drug histories and environmental variables, thus permitting the reduction in PPE mRNA signal to be associated with the self-administration of cocaine rather than other variables.

The present data contrast with recent findings that cocaine administered chronically (3 mg/kg/injection $\times$ 4 injections/day for 42–52 days) to pregnant rhesus monkeys *augmented* PPE mRNA in fetal monkey striatum (4). The organization of striatal enkephalins undergoes signficant changes during development until it is finally established in its adult pattern post-

natally. Because the role of enkephalins is likely to be quite different during development than it is in the mature adult, it is not surprising that the effects of cocaine were disparate between the two times studied.

The cocaine-induced decreases in PPE mRNA in monkey striatum observed in the present study conflict with findings in rodents. Depending on the paradigm or time point utilized, cocaine either did not alter or increased the expression of enkephalin immunoreactivity or PPE mRNA in rats. For instance, repeated exposures to cocaine did not alter enkephalin immunoreactivity (24) or PPE mRNA levels (3,7,8,12) in rat striatum. Indeed, in one study, rats self-administered cocaine for up to 6 weeks, but PPE mRNA remained unaltered (8). Although the rodent study utilized the same sacrifice time (1 h after the last cocaine injection), the duration of self-administration was far less: 6 weeks as compared with 97 weeks in the present study. Thus, the consequences of long-term, repeated exposure to cocaine, and not time point of sacrifice, may be critical in altering PPE gene expression in chronic studies with rodents. Survival time does, however, appear to play a role in gene expression following acute exposures to cocaine: e.g., the expression of PPE mRNA was augmented in rat striatum 2 h (13) or 5 h (6) but not 1 h (7) following a single injection of cocaine. Finally, it is important to consider species differences as a source of the discrepancies between the present findings and those in rodents. The distribution and physiology of opioid peptides in monkeys, a relatively unexplored area of investigation (19), may be sufficiently different from those in rodents to account for the differences.

Decreased PPE gene expression in the striatum of cocaine-exposed monkeys, as observed in the present study, may play a role in the regulation of normal voluntary movement. Efferent projections from the striatum to the external segment of the globus pallidus colocalize enkephalin with GABA, and abnormal functioning of this pathway is thought to constitute an underlying mechanism of both hypo- and hyperkinetic movement disorders (18). Enkephalin has been shown to decrease potassium-evoked GABA release in the globus pallidus (18), sustaining the hypothesis that enkephalin in the striatopallidal pathway attenuates GABAergic inhibition of the globus pallidus (1). Additional evidence supporting a role for enkephalin in locomotion comes from the demonstration that injection of enkephalin analogs into the globus pallidus caused dose-dependent increases in locomotor activity (16). Enkephalins appear to modulate behavioral activity via DA-dependent and -independent mechanisms as well. Microinjection of enkephalin analogs into the ventral tegmental area elicited increased locomotor activity that was blocked by neuroleptic administration (2), but injection of enkephalin analogs into the NAc, the terminal field of midbrain DA projections, elicited dose-dependent increases in activity that were not blocked by neuroleptic administration or ablation of the mesolimbic DA projection (17). Alterations in enkephalinergic neurons, as reported in the present study, therefore may lead to disruptions in neuronal processing in the pallidum, which may in turn result in the production of abnormal motor behavior.

An additional role of enkephalins in the mediation of cognition and behavior has been suggested by decreased levels of enkephalin in the substantia nigra ipsilateral to a striatopallidal infarction in patients exhibiting cognitive and other behavioral abnormalities (23). Enkephalin analogs are self-administered into the NAC (9). Moreover, it was recently proposed that altered striatal opioid peptide integrity might be one mechanism underlying the states of dysphoria and craving that ultimately result from a state of addiction (14). This proposal was based on findings that preproenkephalin and preprodynorphin mRNAs were altered in the brains of cocaine addicts. Further investigation is necessary to determine the role of alterations in PPE mRNA in cognitive or motor processing in cocaine self-administering monkeys.

In summary, chronic cocaine self-administration resulted in decreased preproenkephalin mRNA in the caudate, putamen, and NAc of rhesus monkeys. These findings agree with existing data on the effects of cocaine on enkephalin in human cocaine addicts but contrast with findings in rats, underscoring the need for a more appropriate model to bridge the gap between rodents and humans. The advantages of using a non-human primate model of cocaine self-administration include control of environment, drug access, and nutrition in a species that displays close neuroanatomical and chemoarchitectural homology to humans.

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