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Depletion of serotonin decreases the effects of the kappa-opioid receptor agonist U-69593 on cocaine-stimulated activity

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Abstract

Treatment with a kappa-opioid receptor agonist for 5 days decreases locomotor activity and reduces activity in response to a cocaine challenge 3 days later. In addition, chronic cocaine increases kappa-opioid receptor density, striatal dynorphin, and dynorphin gene expression in the striatum. The upregulation of kappa-opioid receptors after cocaine treatment occurs predominantly in brain regions that are highly innervated by serotonin. To determine if serotonin plays a role in the effects of kappa-opioid receptor agonists on cocaine-stimulated activity, parachloroamphetamine (PCA), which depleted serotonin by 53%–66%, or saline, was given prior to a five-day treatment with U-69593 or vehicle. Three days later each rat received a single injection of cocaine and locomotor activity was measured. Treatment with PCA had no effect on the ability of U-69593 alone to decrease locomotor activity. Thus, the behavioral effects of U-69593 alone were not dependent upon serotonin. In rats pretreated with saline, U-69593 treatment significantly blocked the locomotor-activating effects of cocaine. Following PCA pretreatment, however, there were no significant differences in locomotor activity in rats challenged with an injection of cocaine after treatment with U-69593 or vehicle. Thus, serotonin depletion prevented the long-lasting blockade of the locomotor-activating effects of cocaine subsequent to repeated administration of U-69593 but did not alter the effects of cocaine in rats that were treated with vehicle. Thus, the effects of PCA on U-69593 are not due to non-specific alterations in cocaine-induced locomotor activity. These findings suggest that serotonin plays an important role in mediating the effects of kappa-opioid receptor agonists on the behavioral response to cocaine.

Keywords: Cocaine; Kappa-opioid; Locomotor activity; PCA; Sensitization; Serotonin

1. Introduction

Treatment with the selective kappa-opioid receptor agonist U-69593 alters a range of behavioral and neurochemical effects of cocaine. For example, U-69593 reduces cocaine self-administration in rats (Schenk et al., 1999) and in monkeys (Mello and Negus, 1998; Nabeshima et al., 1992). U-69593 also blocks enhancement of cocaine-induced place conditioning (Shippenberg et al., 1996) and decreases acute locomotor effects associated with cocaine and cocaine sensitization (Collins et al., 2001a,b; Heidbreder et al., 1993). In addition, in the rat brain U-69593 prevents cocaine-induced phosphorylation of DARPP-32 at Thr^{34} (D'Addario et al., 2007a), which is considered to be an important mediator of the effects of drugs of abuse (Svenningsson et al., 2004). However, in humans it has been shown that activation of kappa-opioid receptors acutely may cause dysphoria and psychotomimesis (Pfeiffer et al., 1986) and this limits the clinical usefulness of these drugs. Research to develop kappa-opioid receptor agonists that do not have the dysphoric properties is ongoing (Hasebe et al., 2004; Park et al., 2006). A better understanding of the systems involved in mediating the long-term effects of kappa-opioid receptor agonists on cocaine-stimulated locomotor activity will aid in the development of compounds that may be able to bypass the dysphoric properties associated with the currently available kappa receptor agonists while preserving the ability to block the effects of cocaine.

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The mechanisms by which kappa-opioid receptor agonists alter cocaine-related effects are not clear. It has been shown previously that administration of U-69593 attenuated RTI-55-induced cocaine self-administration but not that of WIN 35,428 (Schenk et al., 2000). RTI-55 and cocaine are uptake inhibitors that inhibit uptake at the serotonin transporter with greater affinity than at the dopamine transporter (Boja et al., 1992), while WIN 35,428 exhibits greater selectivity for the dopamine transporter (Carroll et al., 1995). Because of this, Schenk and colleagues hypothesized that these data indicated an interaction between the kappa-opioid and serotonin systems in the brain.

Other studies have shown that there is an interaction between kappa-opioid receptors and serotonin. For example, both the full $5HT_{1A}$ receptor agonist 8-OH-DPAT and the partial $5HT_{1A}$ receptor agonist buspirone partially substitute for U-69593 in rats trained to discriminate U-69593 from saline (Powell et al., 1994). Depletion of serotonin by either parachloroamphetamine (PCA) or parachlorophenylalanine decreased U-50,488 analgesia (Nemmani and Mogil, 2003; Von Voigtlander et al., 1984). It also has been shown that depletion of serotonin by administration of PCA reduced prodynorphin mRNA by 40–60% in the hypothalamus, caudate putamen, nucleus accumbens and hippocampus, suggesting that serotonin plays a regulatory role in the tonic control of dynorphin message (Di Benedetto et al., 2004). In addition, the decrease in dynorphin message by chronic treatment with a kappa-opioid receptor agonist requires serotonin in the hippocampus (D'Addario et al., 2007b). It is not known, however, whether serotonin plays a role in mediating the long-term effects of kappa-opioid receptor agonists on cocaine-stimulated locomotor activity.

These studies were done to determine the role of serotonin in the non-acute attenuation of cocaine-stimulated locomotor activity by U-69593. The effect of serotonin depletion by PCA administered 3 days before the treatment with U-69593 on cocaine-stimulated locomotor activity was examined. In a separate study, the effects of U-69593 on serotonin transporter binding were measured to determine whether treatment with a kappa-opioid receptor agonist alters the serotonin system.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 175–200 g were housed two per cage in a temperature and humidity-controlled environment under a 12 h light/dark cycle. Food and water were available ad libitum. All rat procedures were conducted in an AAALAC approved facility under an approved rat care and use protocol following the guidelines established for humane care and use of rats by the University of Miami IACUC.

2.2. Chemicals

Chemicals and reagents were obtained from the following sources: U-69593 ((+)-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide) from Research Biochemicals Inc. (Natick, MA). PCA (DL-*p*-chloroamphetamine hydrochloride) from Sigma Chemical Co. (St. Louis, MO). Cocaine hydrochloride from the National Institute on Drug Abuse (Rockville, MD). [^{3}H]Citalopram (approximately 85 Ci/mmol) from Amersham Corp. (Arlington Heights, IL).

2.2.1. Experiment 1: Effect of PCA pretreatment on U-69593 regulation of cocaine-stimulated locomotor activity

Rats were injected with 7.5 mg/kg of PCA (s.c.) or saline on day 1. Beginning 3 days later, the rats were injected subcutaneously daily for the next 5 days with 0.32 mg/kg U-69593 (saline/U-69593 $n=8$; PCA/U-69593 $n=14$) or vehicle (20% dimethyl sulfoxide/80% sterile water; used to dissolve U-69593) (saline/vehicle $n=8$; PCA/vehicle $n=14$) and 15 min later locomotor activity was examined (days 4–8). Three days later (day 11) rats were injected with 10 mg/kg cocaine (i.p.) and locomotor activity was measured for 60 min. The doses and treatment regimens were chosen based on our previous studies (Collins et al., 2001a,b; D'Addario et al., 2007b) and those from other laboratories (e.g. Acri et al., 2001; Chefer et al., 2000; Heidbreder et al., 1993; Thompson et al., 2000) showing that this dose produces an optimal decrease in cocaine-stimulated locomotor activity. PCA is a neurotoxin that depletes serotonin by acting as a substrate for the serotonin transporter and releasing serotonin from axon terminals by a non-exocytotic mechanism, and by inhibiting tryptophan hydroxylase (Sprague et al., 1996). To avoid potential confounds from the immediate release of serotonin by the neurotoxin, treatment with U-69593 or vehicle began 3 days after PCA administration.

A separate group of rats was injected subcutaneously with 7.5 mg/kg PCA ($n=6$) or saline ($n=7$) and killed by decapitation 3 days later (to match the start of the behavioral experiment) for determination of serotonin levels. Tissues were dissected rapidly and individual brain regions (striatum, frontal cortex, and hippocampus) were weighed and stored frozen in individual vials at −70 °C until assayed for serotonin.

2.2.2. Experiment 2: Effects of U-69593 treatment on serotonin transporter binding

Male Sprague–Dawley rats were injected subcutaneously daily for 5 days with 0.08 or 0.32 mg/kg U-69593 ($n=4$ per group) or vehicle (20% dimethyl sulfoxide in sterile water; $n=4$) and 3 days later killed by decapitation, their brains were quickly removed and frozen in isopentane at −35 °C, then stored at −70 °C prior to slicing. Slices (20 μm) from the caudate putamen and nucleus accumbens, olfactory tubercle, endopiriform and claustrum, and lateral septal nucleus were thaw-mounted on gelatin/chromate-coated slides and stored at −70 °C prior to assay.

2.3. Locomotor activity

Rats were placed in clear acrylic chambers (16×16 in.) inside Digiscan activity monitors (Omnitech Electronics, Columbus, OH) that were equipped with infrared light sensitive

detectors mounted 2.5 cm apart along two perpendicular walls. Mounted along the opposing walls were infrared light beams that were directed at the detectors. One count of horizontal activity was registered each time the subject interrupted a beam. Animals were maintained on a 12 h light/dark schedule with lights on at 7 a.m. and off at 7 p.m. All behavioral testing was done during the light schedule between 9 a.m. and 4 p.m. with each group tested at the same hour each day and the groups randomized over the course of the day. Data were analyzed by ANOVA and followed by post hoc analysis using Fisher's Protected Least Significant Difference (PLSD) when warranted. Significant interactions were followed by tests for simple pretreatment/drug effects. *P* values less than 0.05 were considered significant for all tests.

2.4. Quantitative autoradiography

For the citalopram binding assay, sections were thawed to room temperature and incubated for 120 min with 2 nM [^{3}H] citalopram (85 Ci/mmol) in binding buffer (50 mM TRIS, 120 mM NaCl, 5 mM KCl). Sections were washed 4 times in ice-cold buffer, dipped in ice-cold deionized water, and dried with a stream of cool dry air. Slides and standards (^{3}H-labeled microscales, Amersham Corp., Arlington Heights, IL) were apposed to radiosensitive film for 1 week at −20 °C. Nonspecific binding was defined by the presence of 20 μM fluoxetine.

Films were developed in Kodak GBX developer and fixative, and autoradiograms were analyzed using a Macintosh-based image analysis system (NIH, Image 1.60 software). Brain images were quantified using curves generated from the labeled standards. Data were analyzed by Analysis of Variance and Fisher's Protected Least Significant Difference.

2.5. Quantification of serotonin in the rat brain

Individual brain regions (striatum, frontal cortex, and hippocampus) of rats were dissected, weighed and stored frozen in individual vials at −70 °C until assayed for serotonin. The assay of serotonin in each brain region was carried out using HPLC equipped with a CoulArray, 16-channel electrochemical detector system (Model 5600, ESA, Chelmsford, MA), a dual programmable solvent delivery module (Model 582), an autosampler and injector (ESA Model 540) and CoulArray for Windows Application Software. Extraction of serotonin was carried out by modification of a previously described method (Kennett et al., 1985). Briefly, brain tissue was homogenized in cold 0.1 M perchloric acid using a disposable pestle and minihomogenizer. The homogenate was spiked with the internal standard (IS) *n*-methyl-5-hydroxytryptamine (NM), centrifuged at 13,000 rpm at 4 °C. The supernatant was filtered through 0.2 μm filter and an aliquot was injected into the HPLC-ECD system.

Forty microliters of the filtered extract was injected into 5 μ C_{18} reverse phase HPLC column. Separation of serotonin was carried out at a potential of 250 mV. Sensitivity was set at 0–15 nA in order to achieve the optimum response for variable concentrations of serotonin in different tissues. The compounds were eluted at a flow rate of 1.0 ml/min of mobile phase containing 0.1 M sodium acetate buffer, 0.5 mM sodium octyl sulphate, 0.15 mM disodium-EDTA, 1 mM dibutylamine and 7.5% methanol, and pH was set at 4.5. The peak of serotonin was identified by its retention time, determined during calibration with known concentrations of standard solutions of serotonin. In test tissues, recovery of serotonin standards added to the brain tissue homogenates was 94.63±3.56% and 106.64±5.9% and coefficient of variance (% CV) was 3.51% and 4.81% respectively in two separate tissues. Results were calculated using the peak area ratio method as described earlier (Samuel et al., 1990), and are expressed as pg/mg tissue.

Data were analyzed by one-way Analysis of Variance (ANOVA) for each brain region. *P* values less than 0.05 were considered significant for all tests.

3. Results

3.1. Experiment 1: Effect of PCA on the regulation of cocaine-induced locomotor activity by U-69593

Three days after 7.5 mg/kg PCA or saline injections, serotonin levels in the striatum, hippocampus and prefrontal cortex were measured to ensure that serotonin was decreased. Serotonin levels were decreased significantly by 53% in the striatum ($F[1,11]=7.537$, $P<0.05$; Fig. 1); 56.5% in the hippocampus ($F[1,11]=4.99$, $P<0.05$; Fig. 1) and 66.2% in the prefrontal cortex ($F[1,11]=7.758$, $P<0.05$; Fig. 1) after PCA pretreatment.

Beginning 3 days after injection of PCA or saline, rats were injected once daily for 5 days (days 4–8 of the experiment) with either 0.32 mg/kg U-69593 or vehicle and locomotor activity was measured daily for 60 min beginning 15 min after injection. Over the five-day treatment period, U-69593 significantly decreased locomotor activity compared to rats injected with vehicle ($F[1,200]=37.963$, $P\leq 0.0001$; Fig. 2) regardless of pretreatment with PCA or saline. Thus, PCA did not alter the locomotor-decreasing effect of U-69593. Similarly, pretreatment with PCA did not significantly change baseline locomotor

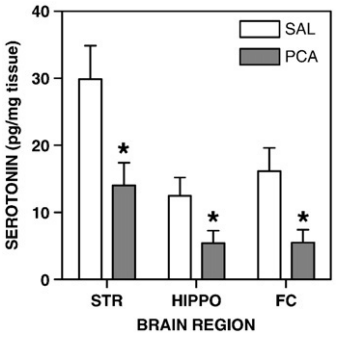

Fig. 1. PCA ($n=6$) decreased the serotonin levels in the striatum (STR), hippocampus (HIPPO) and prefrontal cortex (FC) compared to rats pretreated with saline ($n=7$). Serotonin levels were measured 3 days after sc injections of 7.5 mg/kg PCA or saline (SAL). Data are expressed as mean±SEM 5HT (pg/mg) levels in the brain regions examined. * — Indicates a significant difference from saline, $P\leq 0.05$.

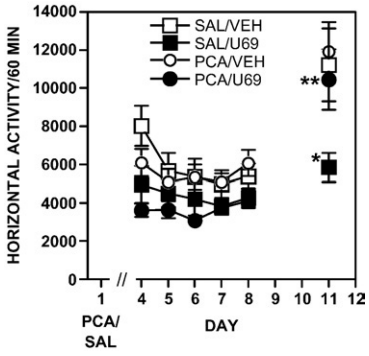

Fig. 2. After pretreatment with saline, repeated treatment with U-69593 significantly decreased the locomotor-activating effects of cocaine. In contrast, U-69593 did not block cocaine-stimulated activity in rats pretreated with PCA. All rats were treated with PCA (7.5 mg/kg, sc) or saline (SAL) on day 1. On days 4–8 each rat was treated with either 0.32 mg/kg U-69593 (U69) (SAL/U69 $n=8$; PCA/U69 $n=14$) or vehicle (VEH) (SAL/VEH $n=8$; PCA/VEH $n=14$) and locomotor activity was measured for 60 min each day. Three days later (day 11), each rat was injected with cocaine (10 mg/kg, ip). After pretreatment with PCA, the ability of U-69593 to block the stimulant effects of cocaine is no longer evident. Values are mean±SEM. * — Indicates a significant difference from SAL/VEH group, $P<0.05$. ** — Indicates a significant difference from SAL/U69 group, $P<0.05$.

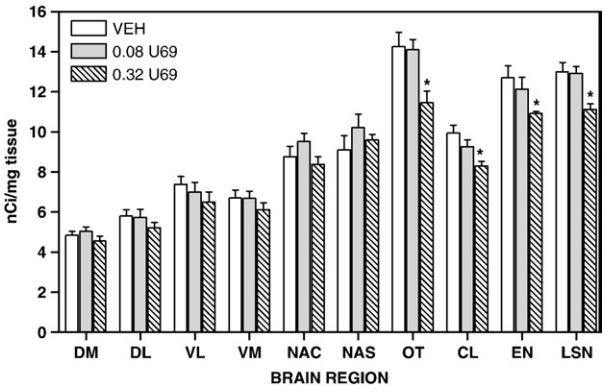

Fig. 3. [^{3}H] Citalopram binding is significantly lower in the endopiriform, claustrum and lateral septal nucleus in rats treated with U-69593 (U69, 0.32 mg/kg; $n=4$) compared to rats treated with vehicle (VEH, $n=4$). Data are expressed as mean±SEM SERT (nCi/mg) levels in the brain regions examined. Serotonin transporter density was measured in four quadrants of the caudate putamen (DM: dorsomedial, DL: dorsolateral, VL: ventrolateral, VM: ventromedial), the nucleus accumbens core (NAC) and shell (NAS), the olfactory tubercle (OT), the claustrum (CL), the endopiriform nucleus (EN), and the lateral septal nucleus (LSN). * — Indicates a significant difference from vehicle, $P\leq0.05$.

activity, compared to pretreatment with saline in vehicle-treated rats. None of the groups exhibited stereotypy, as measured by observer ratings halfway through the test sessions.

One problem with measuring the effects of a drug that decreases activity is that the vehicle-treated animals tend to go to sleep during the session, making it difficult to observe a decrease in activity. As in our previous studies (Collins et al., 2001a,b), we purposely used non-habituated rats in these studies because this allowed us to observe a decrease in activity in response to the kappa-opioid receptor agonist. When the vehicle rats are habituated, they exhibit very little activity over the entire session, and it is difficult to see a decrease in response to a drug. Because of this, there is a general pattern of activity levels being higher on the first day of treatment than on subsequent days in the vehicle-treated rats.

Three days after the end of the kappa-opioid treatment period (day 11), the rats were injected with cocaine (10 mg/kg) and locomotor activity was measured for 60 min. There was a significant difference in the level of locomotor activity in response to the cocaine challenge ($F[1,40]=4.417$, $P<0.04$; Fig. 2). The rats pretreated with saline and treated for 5 days with U-69593 had significantly lower level of locomotor activity in response to the cocaine challenge compared to saline/vehicle group ($P<0.05$). After PCA pretreatment, the response to the cocaine challenge in the group treated with vehicle was not significantly different from the vehicle-treated group that had been pretreated with saline. Thus, a 50–60% decrease in serotonin did not alter the effects of cocaine on this challenge day, subsequent to 5 days of vehicle treatment. PCA pretreatment, however, did block the attenuation of the effects of cocaine by U-69593 in that the group pretreated with saline and treated with U-69593 exhibited significantly lower levels of locomotor activity in response to cocaine compared to the group pretreated with PCA and treated with U-69593 ($P<0.05$). There was no significant difference between the effects of cocaine on locomotor activity after pretreatment with PCA and treatment with U-69593, compared to rats in the PCA/vehicle group.

3.2. Experiment 2: Effect of U-69593 on serotonin transporter density

Three days after the last injection of U-69593, serotonin transporter densities were measured. There was no significant change in transporter binding in any of the four quadrants of the caudate putamen, or in the nucleus accumbens core or shell after treatment with U-69593 (Fig. 3). In contrast, there were significant differences in serotonin transporter densities in the olfactory tubercle ($F[2,8]=6.968$, $P=0.018$), claustrum ($F[2,8]=6.657$, $P=0.020$), endopiriform ($F[2,8]=4.890$, $P=0.041$), and the lateral septal nucleus ($F[2,8]=7.742$, $P=0.014$). Post hoc testing showed that binding densities were significantly decreased in these brain regions after treatment with 0.32 mg/kg U-69593, compared to vehicle (Fig. 3). In contrast, there were no significant alterations in binding in any of the brain regions examined after treatment with 0.08 mg/kg U-69593.

4. Discussion

As in the present study, it has been shown previously that repeated administration of the kappa-opioid receptor agonist U-69593 produces significant long-lasting reductions in cocaine-stimulated locomotor activity (Collins et al., 2001a; Collins et al., 2002a; Heidbreder et al., 1993; Heidbreder and Shippenberg, 1994). This has been shown previously not to be a conditioned response to the pairing of U-69593 with the locomotor chamber (Collins et al., 2001b). Similarly, in the present experiment, the group treated with PCA showed

decreases in activity in response to U-69593 treatment, but did not show decreased activity in response to a cocaine challenge 3 days after the end of the treatment. Thus, the present study shows that depletion of serotonin blocks the long-term effect of U-69593 on cocaine-stimulated locomotor activity.

It has been shown by a number of different laboratories that the dose of PCA used in this study (7.5 mg/kg) depleted serotonin within 1–2 h of administration, and that serotonin remained depleted for over 30 days (e.g. (Fuller, 1992; Sanders-Bush et al., 1975; Vorhees et al., 1975). In the present study the serotonin levels at the beginning of treatment with U-69593 (day 4) were decreased by 56.5%, 53.1% and 66.2% in the hippocampus, striatum and prefrontal cortex, respectively, in animals pretreated with PCA compared to saline.

Three days after pretreatment with PCA, locomotor activity was decreased in response to daily injections of U-69593, similar to what was seen in rats that were not pretreated (Collins et al., 2001a) or that were pretreated with saline. Thus, serotonin depletion did not alter the behavioral effects of U-69593, suggesting that the direct interaction between the kappa-opioid receptor agonist and kappa-opioid receptors was not markedly changed by pretreatment with PCA. Although serotonin seems to be important in regulating the tonic levels of prodynorphin mRNA (Di Benedetto et al., 2004), there do not appear to be marked effects on the kappa-opioid receptor. This is in contrast to the acute reduction in analgesia produced by a kappa-opioid receptor agonist that has been reported subsequent to depletion of serotonin (Nemmani and Mogil, 2003; Von Voigtlander et al., 1984). It is not clear why kappa-opioid receptor agonist regulation of analgesia but not of locomotor activity would be altered by serotonin depletion, but it is likely that distinct brain regions, which could be differentially affected by either U-69593 or PCA treatment, mediate these two behavioral effects. In fact, it was reported recently that kappa-opioids differentially regulated dopamine neurons projecting to the prefrontal cortex versus the nucleus accumbens (Margolis et al., 2006), and we have shown previously that repeated treatment with U-69593 produces either increases, decreases, or no change in prodynorphin mRNA depending upon which brain region is studied (Collins et al., 2002a).

In response to an injection of cocaine 3 days after the final U-69593 or vehicle injection, locomotor activity was stimulated in both U-69593-pretreated and vehicle-pretreated rats exposed to PCA, and the levels of activity in the two groups were not significantly different from one another or from rats pretreated with saline followed by 5 days of vehicle. Thus, the ability of a kappa-opioid receptor agonist to block the behavioral effects of cocaine on a long-term basis is markedly diminished subsequent to serotonin depletion. This suggests that serotonin plays an important role in mediating the interaction between the kappa-opioid system and cocaine. In fact, interactions between serotonin and the kappa-opioid system have been shown in previous studies where PCA pretreatment followed by repeated U-69593 administration increased prodynorphin mRNA in the hippocampus, while PCA alone or chronic U-69593 treatment decreased prodynorphin mRNA in this region (D'Addario et al., 2007b).

These data also show that, in addition to serotonin regulating the effects of a kappa-opioid receptor agonist, treatment with a kappa-opioid receptor agonist alters the serotonin system. Treatment with U-69593 decreased serotonin transporter binding in the claustrum, endopiriform nucleus, olfactory tubercle and lateral septal nucleus. It is interesting to note that while treatment with cocaine increased kappa-opioid receptors in the nucleus accumbens (Collins et al., 2002b; Unterwald et al., 1994), treatment with the selective dopamine uptake inhibitors RTI-117 or GBR 12909 had no effect on [^{3}H]U-69593 binding in any brain region measured (Collins et al., 2002b). The highest levels of kappa-opioid receptor binding following chronic cocaine administration were observed in the endopiriform nucleus and claustrum (Collins et al., 2002b). These two brain regions are not highly enervated by dopaminergic cells but have high levels of serotonin receptors (Battaglia et al., 1991; Pompeiano et al., 1994). Similarly, GBR 12909 treatment did not significantly alter prodynorphin mRNA in the caudate putamen (Romualdi et al., 2001) while prodynorphin mRNA was increased in this brain region after treatment with cocaine, regardless of whether the cocaine was administered systemically (Adams et al., 2003; Hurd and Herkenham, 1995; Mathieu-Kia and Besson, 1998; Romualdi et al., 2001; Spangler et al., 1996; Turchan et al., 1998), intracerebroventricularly (Romualdi et al., 1996), or intravenously self-administered (Daunais et al., 1993). In addition, it has been found that it was not necessary for dopamine transporters or dopamine receptors in the caudate putamen or nucleus accumbens to be altered in order to see reduced activity of cocaine observed after five intermittent injections of U-69593 administered every third day (Collins et al., 2001a). This suggests that the interaction between cocaine and the kappa-opioid system, even in the highly dopaminergic caudate putamen likely is not mediated solely by dopaminergic mechanisms.

In summary, treatment with cocaine produces significant increases in kappa-opioid receptor binding in serotonin-rich brain regions, and repeated administration of a kappa-opioid receptor agonist produces significant decreases in serotonin transporter density in the claustrum and the endopiriform nucleus. These findings, combined with the greatly diminished effect of U-69593 on cocaine-stimulated activity after depletion of serotonin, suggest that the serotonin system plays an important role in mediating the interaction between the kappa-opioid system and cocaine. A better understanding of this interaction may lead to additional treatments that will produce long-lasting diminishment of the effects of cocaine without the side effects of the currently available kappa-opioid receptor agonists.

Acknowledgments

The animals used in this study were maintained and the studies were conducted in accordance with the guidelines of the Guide for Care and Use of Laboratory Animals, National Research Council, Department of Health, Education and Welfare, NIH Publication 85-23, revised 1985. This work was supported by NIDA grant DA 17343.

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