ELSEVIER

European Journal of Pharmacology 426 (2001) 25–34

ejp

www.elsevier.com/locate/ejphar

Effects of κ-opioid receptor agonists on long-term cocaine use and dopamine neurotransmission

Stephanie L. Collins, Claudio D'Addario, Sari Izenwasser *

Department of Neurology, University of Miami School of Medicine, 1501 NW 9th Avenue (D4-5), Miami, FL 33136, USA

Received 5 February 2001; received in revised form 27 June 2001; accepted 3 July 2001

Abstract

κ-Opioid receptor agonists have been suggested as treatments for cocaine addiction based on studies showing that they block cocaine-related behaviors. To determine the effects of κ-opioid receptor agonists on long-term behavioral effects associated with cocaine and the neurochemical bases underlying these effects, rats were treated with the selective κ-opioid receptor agonist U-69593 ((+)(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1 oxaspiro[4.5]dec-8-yl]-benzeneacetamide) alone or in combination with cocaine and locomotor activity was measured daily. In addition, dopamine transporter and dopamine receptor densities were measured using autoradiographic techniques, and tyrosine hydroxylase was measured using immunoautoradiographic techniques. Treatment with U-69593 with or without cocaine decreased locomotor activity. When challenged with cocaine after a 5-day treatment period, the effects of cocaine were markedly reduced in rats initially treated with U-69593 compared to vehicle. When U-69593 was administered five times with 3-day intervals, it alone had no effect on locomotor activity but still reduced activity associated with a cocaine injection. After five daily injections, U-69593 decreased dopamine transporter and dopamine D_2 receptor densities and increased tyrosine hydroxylase levels. These changes were not seen after the 3-day interval regimen, even though cocaine-induced activity was greatly reduced. These findings show that the effects associated with daily U-69593 treatment are attenuated if the drug is administered with a greater interval, while maintaining a blockade of cocaine-induced activity. In addition, U-69593 can block cocaine-induced locomotor effects without major perturbation of the dopamine system. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Autoradiography; Cocaine; Dopamine; Immunoautoradiography; κ-Opioid receptor agonist; Locomotor activity; Sensitization

1. Introduction

It has been shown that treatment with a κ-opioid receptor agonist alters cocaine-related behaviors. For example, the κ-opioid receptor agonist U-69593 ((+)(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1 oxaspiro[4.5]dec-8-yl]-benzeneacetamide) or U50,488 (*trans*-(±)3,4-dichloro-*N*-methyl-*N*-[2-(pyrrolindinyl)-cyclohexyl)-benzeneacetamide) prevented enhancement of cocaine-induced place conditioning (Shippenberg et al., 1996) and U-69593 reduced cocaine-induced locomotor activity and blocked cocaine sensitization (Heidbreder et al., 1993). In addition, pretreatment with U-69593 or bremazocine alone or in combination with cocaine for 5 days decreased locomotor activity and reduced activity associated with a cocaine challenge 3 days later (Collins and Izenwasser, 2000a; Collins et al., in press). When activity levels were examined after a cocaine challenge in groups that had received U-69593 or bremazocine alone for only 1 day, activity levels were not significantly different from those previously given vehicle for 1 day (Collins et al., 2000a; Collins et al., in press), suggesting that multiple injections of κ-opioid receptor agonists are necessary to significantly affect locomotor activity related to cocaine administration other than acutely.

In cocaine self-administration studies, high doses of U50,488 blocked self-administration of high doses of cocaine (Kuzmin et al., 1997), while U50,488 and the κ-opioid receptor agonist spiradoline decreased cocaine self-administration in rats for up to 6 days (Glick et al., 1995). Responding maintained by low doses of self-administered cocaine was also reduced by U-69593, as were the priming effects of cocaine (Schenk et al., 1999). Furthermore, treatment with the κ-opioid receptor agonist enadoline, bremazocine, Mr2033, ethylketocyclazocine or U-69593 reduced cocaine self-administration in monkeys over a 10-day period, although all produced slight negative side

* Corresponding author. Tel.: +1-305-243-2032; fax: +1-305-243-3649.

E-mail address: s.izenwasser@miami.edu (S. Izenwasser).

0014-2999/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(01)01194-3

effects (i.e. decrease in food-maintained responding, emesis, and sedation) (Mello and Negus, 1998; Negus et al., 1997). This suggests that, although κ-opioid receptor agonists clearly have an effect on cocaine-induced behaviors, it is necessary to further study the interaction between κ-opioid receptor agonists and cocaine in an attempt to reduce the negative effects associated with κ-opioid receptor agonist treatment.

The mechanism by which κ-opioid receptor agonists block cocaine-induced behaviors is not known. It has been shown that κ-opioid receptor agonists inhibited dopamine release in vitro (Werling et al., 1988) and in vivo (Di Chiara and Imperato, 1988; Spanagel et al., 1990; Maisonneueve et al., 1994). In addition, repeated administration of U-69593 decreased dopamine transporter density (Collins et al., in press) and dopamine D_2 receptor density (Izenwasser et al., 1998) and function (Izenwasser et al., 1998; Acri et al., 2001). Together, these results suggest that κ-opioid receptor agonists may alter cocaine-induced behaviors through an interaction with dopaminergic systems.

One concern about a potential κ-opioid receptor agonist treatment, as mentioned above, is that it alone has depressant effects on both locomotor activity and dopamine release. To further understand the parameters of these effects, (1) the long-term effects of repeated κ-opioid receptor agonist treatment and (2) the effects of κ-opioid receptor agonist injections administered with a greater interval than once daily were studied. In addition, several measures of dopamine transmission were examined, including tyrosine hydroxylase levels, and dopamine transporter and dopamine receptor densities, to determine if κ-opioid receptor agonists administered in these different treatment schedules, alter dopamine neurotransmission. These studies will provide a better understanding of the relationship between regulation of markers of dopamine uptake and synthesis and the behavioral effects of cocaine after multiple κ-opioid receptor agonist treatment regimens.

2. Materials and methods

2.1. Treatments

Male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 300–350 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.

2.1.1. Experiment 1: Modification of locomotor activity and cocaine sensitization by U-69593

Sixteen rats were injected once daily for 5 days with a subcutaneous injection (s.c.) of the κ-opioid receptor agonist U-69593 (0.32 mg/kg) and 12 rats were injected with vehicle (20% dimethyl sulfoxide in sterile water) 15 min prior to locomotor activity testing (Table 1). Three days later, eight rats that had previously received U-69593 and eight rats that had been given vehicle were injected with an intraperitoneal (i.p.) injection of cocaine (10 mg/kg) daily for the next 5 days (days 8–12). The remaining 8 U-69593 rats and the remaining four vehicle rats were given saline injections immediately prior to the test session for the next 5 days (days 8–12). The 16 rats that had received U-69593 and then either cocaine or saline and the 8 rats that had received vehicle and then cocaine were injected with an i.p. injection of 10 mg/kg cocaine alone immediately prior to testing sessions 1 week later (day 19). The remaining four rats were injected with saline on day 19.

2.1.2. Experiment 2: Modification of cocaine-induced locomotor activity and cocaine sensitization by κ-opioid receptor agonists

Eight rats were injected once daily for 5 days with a s.c. injection of U-69593, the κ-opioid receptor agonist bremazocine (0.32 mg/kg), or vehicle (total of 24 rats) 15 min prior to an injection of 10 mg/kg cocaine (i.p.), while four rats were given vehicle alone, immediately after which the locomotor activity testing began (Table 1). All rats that had received a pretreatment in combination with cocaine were injected with a 10 mg/kg cocaine challenge on both day 8 and day 15, immediately prior to locomotor activity testing. The remaining four rats were injected with saline on day 8 and day 15.

2.1.3. Experiment 3: Modification of cocaine-induced locomotor activity after five daily injections of U-69593 administered at 3-day intervals

To determine whether the behavioral effects associated with U-69593 treatment alone could be diminished if the treatment paradigm was altered, U-69593 (0.32 mg/kg; 15 min before locomotor activity testing began) or vehicle

Table 1
Behavioral treatment protocols

Experiment 1	Days 1–5	Days 8–12	Day 19
Group 1	vehicle	saline	saline
Group 2	vehicle	cocaine	cocaine
Group 3	U-69593	cocaine	cocaine
Group 4	U-69593	saline	cocaine
Experiment 2	**Days 1–5**	**Day 8**	**Day 15**
Group 5	vehicle	saline	saline
Group 6	vehicle + cocaine	cocaine	cocaine
Group 7	U-69593 + cocaine	cocaine	cocaine
Group 8	bremazocine + cocaine	cocaine	cocaine
Experiment 3	**Days 1–15**	**Day 18**	
Group 9	U-69593 every third day	saline	
Group 10	U-69593 every third day	cocaine	
Group 11	Vehicle every third day	cocaine	

was administered every 3 days over a 15-day period (total of 20 rats) (Table 1). Vehicle injections were given to all rats on the days between the U-69593 or vehicle test days. On day 18 (3 days after the last injection), a 10-mg/kg cocaine challenge (i.p.) was given to the vehicle group ($N = 4$) and one of the U-69593 groups ($N = 8$), while the other U-69593 group received a saline injection ($N = 8$).

2.1.4. Experiment 4: Modification of tyrosine hydroxylase levels after 5-day treatment with κ-opioid receptor agonists

For the immunoautoradiography assay, rats were treated for 5 days (once daily) with either 0.08 or 0.32 mg/kg U-69593 or bremazocine, or vehicle (the same protocol as in Experiment 1) and killed 3 days later ($N = 4$/group). Their brains were quickly removed and frozen in isopentane on dry ice, then stored at −70 °C. Slices (20 μm) from the caudate putamen/nucleus accumbens and the substantia nigra/ventral tegmental area were thaw-mounted on gelatin/chromate-coated slides and stored at −70 °C prior to assay.

2.1.5. Experiment 5: Modification of dopamine transporter and dopamine receptor density after various treatments with U-69593

For the autoradiography assays, rats ($N = 4$/group) were administered one of the following: 0.32 mg/kg U-69593 (s.c.) or vehicle; according to the same protocol as in Experiment 1 or Experiment 2 and killed 3 days later, or Experiment 3 and killed the following day by decapitation. Their brains were quickly removed and frozen in isopentane on dry ice, then stored at −70 °C. Slices (20 μm) from the caudate putamen/nucleus accumbens and the substantia nigra/ventral tegmental area were thaw-mounted on gelatin/chromate-coated slides and stored at −70 °C prior to assay.

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) and bremazocine from Research Biochemicals (Natick, MA). Cocaine hydrochloride from the National Institute on Drug Abuse (Rockville, MD). [^{125}I]RTI-121 (approximately 2200 Ci/mmol), [^{3}H]SCH 23390 (*R*-(−)-8-chloro-2,3,4, 5-tetrahydro-3,1-methyl-5-phenyl-11-3-benzyoepine-7-ol; approximately 75.5 Ci/mmol), and [^{125}I]sheep × mouse IgG (immunoglobulin G; approximately 30 μCi/l) from New England Nuclear (Boston, MA). [^{125}I]iodosulpiride (approximately 2200 Ci/mmol) from Amersham (Arlington Heights, IL). Monoclonal Anti-Tyrosine Hydroxylase from Sigma (St. Louis, MO).

2.3. Locomotor activity testing

Locomotor activity was measured for 1 h daily, as previously described (Izenwasser et al., 1999; Kunko et al., 1998). 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. Each test session was 60 min in duration. Animals were maintained on a 12-h light/dark schedule with lights on at 7 AM and off at 7 PM. All behavioral testing was done during the light schedule between 9 AM and 4 PM with each group tested at the same hour each day.

Locomotor activity data were analyzed by two-way Analysis of Variance (ANOVA) with repeated measures, for each drug. Significant treatment effects were followed by post hoc analyses with Fisher's Protected Least Significant Difference (PLSD). Significant treatment by time interactions were followed by tests for simple main effects and Fisher's PLSD. For the challenge data, a one-way Analysis of Variance, with drug as the independent variable was used. P values less than 0.05 were considered significant.

2.4. Tyrosine hydroxylase immunoautoradiography

Slide-mounted tissue sections were brought to −20 °C and fixed in 6% paraformaldehyde, 20% ethanol, 20% ethylene glycol, 10% glycerol, 0.32 M sucrose in phosphate buffered saline (PBS) for 1 h at −20 °C. The fix was rinsed off in PBS (0.3% tween) in three successive rinses totaling 40 min. The sections were then incubated for 2 h in PBS (0.3% tween) containing 3% bovine serum albumin + 3% goat serum + 0.05% NaN_3 at room temperature to block. The slides were then rinsed in fresh PBS (0.3% tween) for 10 min. Primary antibody against tyrosine hydroxylase (1:10000, Monoclonal Anti-Tyrosine Hydroxylase, Sigma) in PBS (0.05% tween) + 1.5% goat serum + 0.05% NaN_3 was applied overnight at approximately 2 °C. The slides were then rinsed in three successive washes in fresh PBS (0.3% tween) for a total of 45 min. The sections were incubated for 1 h at room temperature in secondary antibody (^{125}I -sheep × mouse IgG, 30 μCi/l, NEN) containing 1% bovine serum albumin + 5% goat serum + 0.05% NaN_3. The tissue was then rinsed with two 30-min washes in fresh PBS (0.3% tween). The slides were dipped in distilled water and dried. They were then apposed to Kodak Biomax film, along with [^{125}I] standards (Amersham). Control experiments omitting the primary antibody were run on adjacent sections for estimates of non-specific binding of the secondary antibody.

2.5. Quantitative autoradiography

For the dopamine transporter binding assay, sections were thawed to room temperature and incubated for 60 min with 0.07 nM [^{125}I]RTI-121 in binding buffer (137

mM NaCl, 2.7 mM KCl, 10.14 mM Na_2HPO_4, 1.76 mM KH_2PO_4 and 10 mM NaI). Sections were then washed twice in ice-cold buffer, dipped in ice-cold deionized water, and dried with a stream of cool dry air. Slides and standards (^{125}I-labeled microscales, Amersham) were apposed to radiosensitive film for 2 days at 4 °C. Nonspecific binding was defined by the presence of 100 μM cocaine HCl.

For the dopamine D_1 receptor binding assay, sections were thawed to room temperature and incubated for 30 min with 1 nM [^{3}H]SCH 23390 in binding buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$) and 1 μM mianserin (to prevent binding of the ligand to 5-HT_2 receptors), as previously described (Tella et al., 1996). Sections were then washed twice 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) were apposed to radiosensitive film for 7 days at 4 °C. Nonspecific binding was defined by the presence of 10 μM R(+) SCH 23390.

For the dopamine D_2 receptor binding assay, sections were thawed to room temperature and incubated for 30 min with 0.1 nM [^{125}I]iodosulpiride in binding buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$) as previously described (Tella et al., 1996). Sections were then washed twice in ice-cold buffer, dipped in ice-cold deionized water, and dried with a stream of cool dry air. Slides and standards (^{125}I-labeled microscales, Amersham) were apposed to radiosensitive film for 2 days at 4 °C. Nonspecific binding was defined by the presence of 1 μM domperidone.

Films for the autoradiography and immunoautoradiography studies 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.

3. Results

3.1. Locomotor activity

3.1.1. Experiment 1: Modification of locomotor activity and cocaine sensitization by U-69593

3.1.1.1. Pretreatment phase (days 1–5). As shown previously (Collins and Izenwasser, 2000a; Collins et al., in press), U-69593 significantly decreased activity on each of the test days compared to vehicle ($P \leq 0.05$) (Fig. 1).

3.1.1.2. Sensitization phase (days 8–12). Cocaine (group 2), compared to saline (group 1), increased activity in rats treated with vehicle during the pretreatment phase ($P \leq 0.05$; Fig. 1). Although it appears that activity increased in group 2 over the 5 days in the sensitization phase, an overall ANOVA comparing activity over these 5 days (days 8–12) was not significant, showing that group 2 did not show sensitization to cocaine at this time. Rats treated with U-69593 then cocaine (group 3) had significantly lower activity levels than group 2 ($P \leq 0.05$) and there was no significant difference in response to cocaine or saline in the groups treated with U-69593 (group 3 or 4, respectively). In addition, the response to cocaine or saline for rats initially given U-69593 (group 3 or 4) did not change over the 5-day treatment period (i.e. no sensitization appeared to occur).

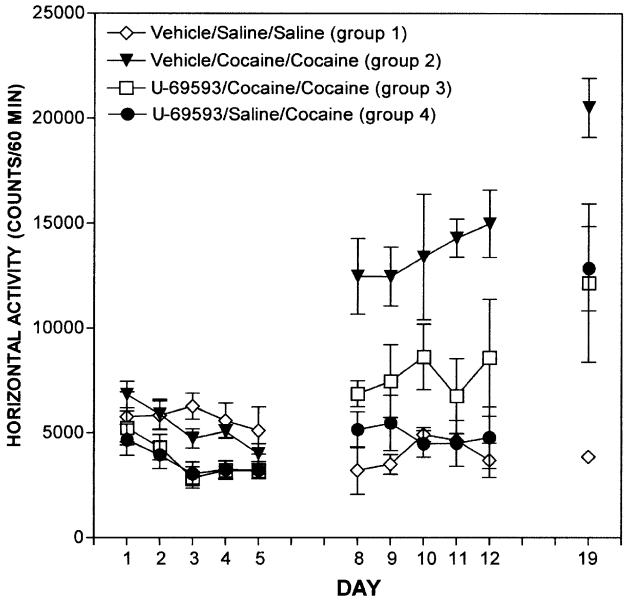

Fig. 1. U-69593 (0.32 mg/kg) or vehicle was administered alone for 5 days (1–5) and locomotor activity was examined daily. On day 8 through day 12, 10 mg/kg cocaine was given to one of the U-69593 groups as well as one group treated with vehicle. The other U-69593 group and the other vehicle group received saline. On day 19, a 10-mg/kg cocaine challenge was given to the vehicle/cocaine group, the U-69593/cocaine group and the U-69593/saline group. The vehicle/saline group received a saline injection on day 19. The response to cocaine in the group that was pretreated with U-69593 was significantly lower than the group pretreated with vehicle ($P \leq 0.05$). Further, the U-69593 group challenged with cocaine was not significantly different from the U-69593 group given saline. On day 19, both groups that had U-69593 were significantly higher than the vehicle/saline group ($P \leq 0.05$); however, they were not significantly different from each other and were significantly lower than the vehicle/cocaine group ($P \leq 0.05$). Values shown are the means ± S.E.M. for eight rats treated with U-69593/group and four rats treated with vehicle/group.

3.1.1.3. Cocaine challenge (day 19). Two weeks after the pretreatment period (1 week after the sensitization period), all rats were given a cocaine or saline challenge. Rats that had been treated with vehicle initially then treated with cocaine for 5 days (group 2) showed a significantly higher level of activity ($P \leq 0.05$) after this cocaine challenge (i.e. sensitization) than they did upon their first exposure to

cocaine on day 8 (Fig. 1). Group 2 also had a significantly greater response to a cocaine challenge than group 3 (pretreated with U-69593 then cocaine) or group 4 (pretreated with U-69593 then saline). Both groups of rats that had initially been treated with U-69593 (group 3 or 4) had significantly higher levels of activity in response to a cocaine challenge compared to rats initially treated with vehicle then saline and challenged with saline on day 19 ($P \leq 0.05$; group 1) showing that there was an acute effect of cocaine 2 weeks after the U-69593 treatment ended. The U-69593 groups showed similar levels of activity in response to cocaine. Thus, while cocaine still had an acute effect, sensitization to this cocaine challenge did not appear to have occurred in the group that was initially treated with U-69593 then treated with cocaine for 5 days (group 3; Fig. 1).

3.1.2. Experiment 2: Modification of cocaine-induced locomotor activity and cocaine sensitization by κ-opioid receptor agonists

3.1.2.1. Pretreatment phase (days 1–5). Pretreatment with U-69593 (group 7) or bremazocine (group 8) in combination with cocaine significantly decreased cocaine-induced locomotor activity compared to rats pretreated with vehicle in combination with cocaine (group 6) over a 5-day period ($P \leq 0.05$; Fig. 2).

3.1.2.2. First challenge (day 8). Three days after the end of the pretreatment phase, the rats pretreated with vehicle + cocaine (group 6) were sensitized to a challenge injection of cocaine (compare responses on day 8 to day 1). Rats pretreated with bremazocine + cocaine and challenged with cocaine (group 8) had a small increase in activity compared to rats pretreated with vehicle then administered saline ($P \leq 0.05$; group 5) showing a small acute effect of cocaine, but they were clearly not sensitized. In fact, their activity was still below the normal initial response to cocaine (see group 6, day 1). Rats pretreated with U-69593 + cocaine and challenged with cocaine (group 7) had no response to cocaine, and were not significantly different from rats pretreated with vehicle and administered saline (group 5). Both groups pretreated with a κ-opioid receptor agonist + cocaine (group 7 or 8) exhibited significantly less locomotor activity in response to a cocaine challenge than rats previously pretreated with vehicle + cocaine then challenged with cocaine (group 6), ($P \leq 0.05$; Fig. 2).

3.1.2.3. Second challenge (day 15). Ten days after the last pretreatment (1 week after the first challenge), all three groups challenged with cocaine had significantly higher locomotor activity than rats challenged with saline ($P \leq 0.05$, group 5). However, rats pretreated with either κ-opioid receptor agonist + cocaine (group 7 or 8) again did not appear to be sensitized to this cocaine challenge injection, shown by significantly lower activity than those initially treated with vehicle + cocaine then challenged with cocaine ($P \leq 0.05$; Fig. 2). The lack of sensitization is also shown by a lack of a significant difference between the responses to cocaine in these two groups compared to the response to cocaine by group 6 on day 1 (first exposure to cocaine). The response to cocaine was not significantly altered between days 8 and 15 in any of the groups.

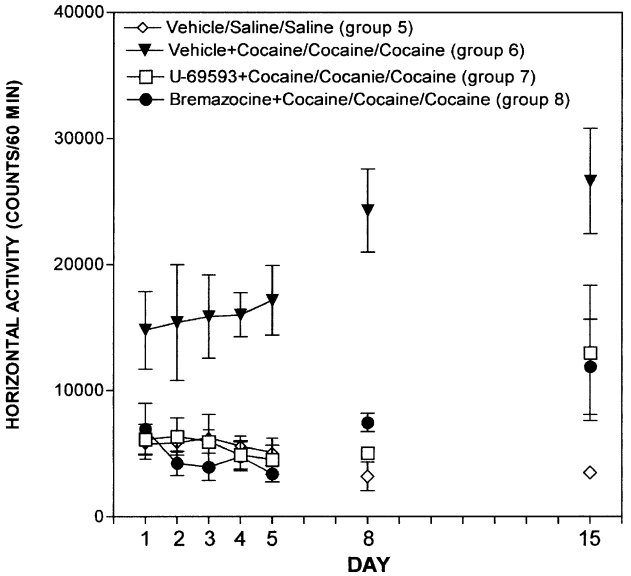

Fig. 2. U-69593 (0.32 mg/kg), bremazocine (0.32 mg/kg), or vehicle in combination with 10 mg/kg cocaine or vehicle alone was administered for 5 days and locomotor activity was examined daily. A 10-mg/kg cocaine challenge injection was administered on day 8 and day 15 to all groups previously given a pretreatment in combination with cocaine and locomotor activity was examined. The remaining vehicle group received a saline injection on day 8 and day 15. The groups that were treated with one of the κ-opioid receptor agonists + cocaine were not significantly different from the vehicle alone group and exhibited significantly lower locomotor activity than the group that received vehicle + cocaine ($P \leq 0.05$). Although the responses to the cocaine challenge 3 and 10 days after the end of the pretreatment period were higher in the κ-opioid receptor agonist + cocaine pretreated groups than the vehicle alone group ($P \leq 0.05$), they were significantly lower compared to the group that had been pretreated with vehicle + cocaine ($P \leq 0.05$). Values shown are the means ± S.E.M. for eight rats/pretreatment in combination with cocaine group and four rats/vehicle alone group.

3.1.3. Experiment 3: Modification of cocaine-induced locomotor activity after five daily injections of U-69593 administered at 3-day intervals

There were no significant differences between the vehicle group and either U-69593 group, or between test days and saline days, during the initial 15-day treatment period (Fig. 3A). There was a significant increase in activity in the vehicle group (group 11) in response to a cocaine challenge injection 3 days later on day 18 ($P \leq 0.05$). While cocaine did appear to produce a small increase in activity in the U-69593 pretreated rats (group 10) when compared to saline (group 9), the activity levels were significantly lower than the vehicle pretreated group that

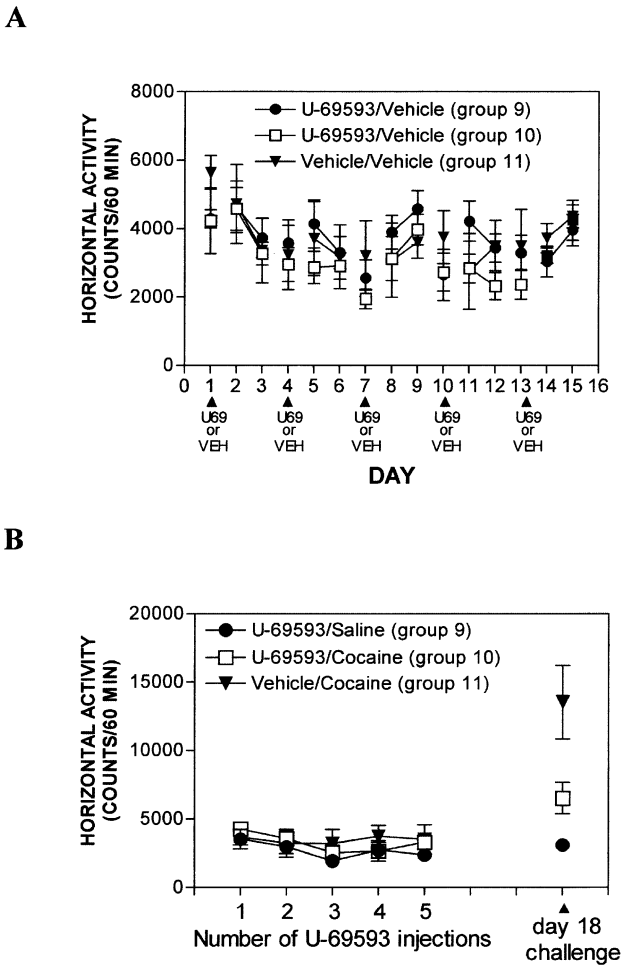

Fig. 3. (A) U-69593 (0.32 mg/kg) or vehicle was administered every third day for 15 days and vehicle was given on the interim days. Locomotor activity was measured daily. There were no significant differences between any of the treatments on any of the 15 days. Values shown are the means ± S.E.M. for eight animals. (B) U-69593 (0.32 mg/kg) or vehicle was administered every third day for 15 days, totaling five U-69593 or vehicle injections (data are from Fig. 3A). On day 18, 10 mg/kg cocaine was given to the vehicle group and one of the U-69593 groups, while the other U-69593 group received saline. There were no significant differences between any of the groups during the initial five treatment injections. Although cocaine significantly increased activity over saline in U-69593 pretreated rats, the effect was greatly diminished compared to vehicle pretreated rats ($P \leq 0.05$). Values shown are the means ± S.E.M. for eight rats.

received a cocaine challenge (group 11), ($P \leq 0.05$, Fig. 3B).

3.2. Tyrosine hydroxylase immunoautoradiography

3.2.1. Experiment 4: Modification of tyrosine hydroxylase levels after 5-day treatment with κ-opioid receptor agonists

There was a significant increase in tyrosine hydroxylase levels in the ventrolateral portion of the caudate putamen and the core of the nucleus accumbens after treatment with the lower dose of 0.08 mg/kg U-69593 ($P \leq 0.05$), while tyrosine hydroxylase levels were significantly increased overall in the caudate putamen and nucleus accumbens after treatment with the higher dose of 0.32 mg/kg U-

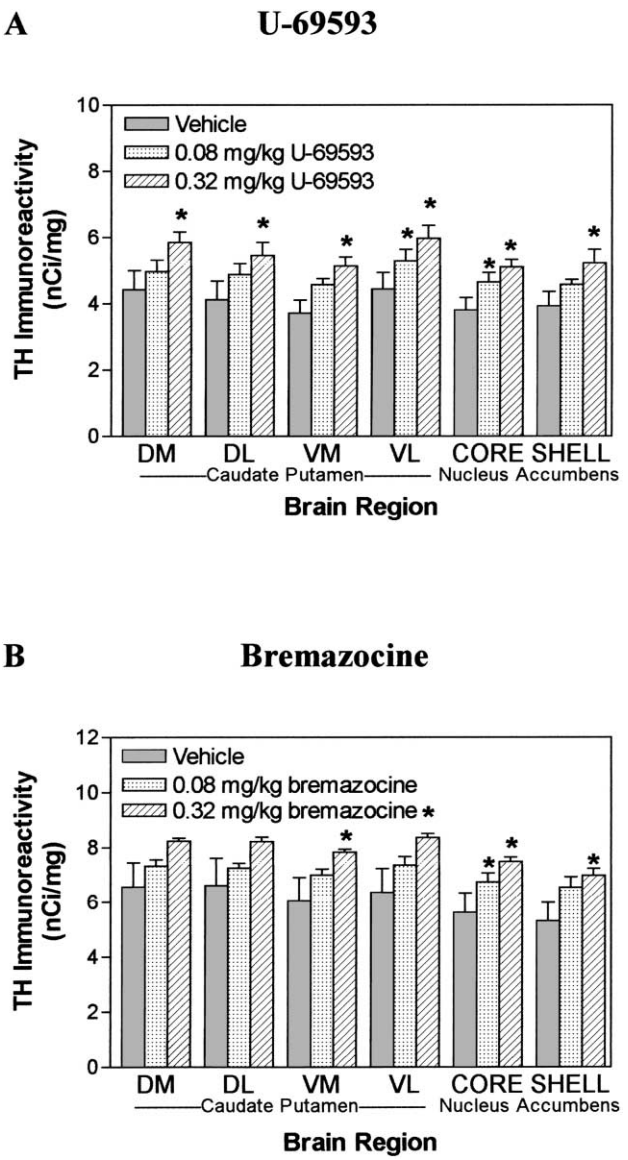

Fig. 4. (A) Rats were treated with U-69593 (0.08 or 0.32 mg/kg) or vehicle for 5 days, killed 3 days later, and tyrosine hydroxylase levels were measured. The group that was treated with 0.08 mg/kg U-69593 had significantly higher tyrosine hydroxylase levels in the ventrolateral part of the caudate putamen and the nucleus accumbens core compared to vehicle ($P \leq 0.05$). Those treated with 0.32 mg/kg U-69593 had significantly higher tyrosine hydroxylase levels throughout the caudate putamen and the nucleus accumbens compared to vehicle ($P \leq 0.05$). Values shown are the means ± S.E.M. for four rats. (B) Rats were treated with bremazocine (0.08 or 0.32 mg/kg) or vehicle for 5 days, killed 3 days later, and tyrosine hydroxylase levels were measured. The group that was treated with 0.08 mg/kg bremazocine had significantly higher tyrosine hydroxylase levels in the nucleus accumbens core and those treated with 0.32 mg/kg U-69593 had significantly higher tyrosine hydroxylase levels in the ventral part of the caudate putamen and in the nucleus accumbens core and shell compared to vehicle ($P \leq 0.05$). Values shown are the means ± S.E.M. for four rats.

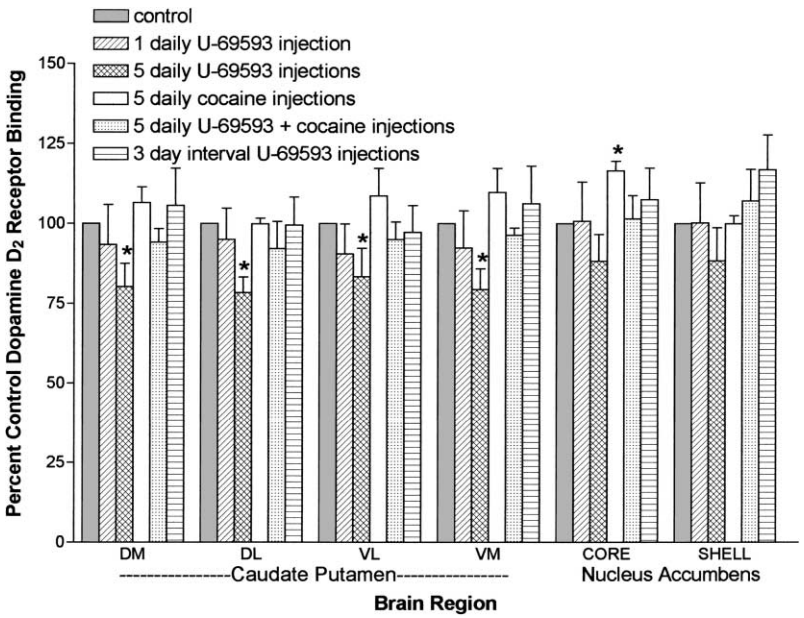

Fig. 5. Densitometric analysis of [^{125}I] iodosulpiride binding was conducted in the caudate putamen and nucleus accumbens of rats treated with one of the following: U-69593 (0.32 mg/kg) once daily for 1 or 5 days, cocaine (10 mg/kg) once daily for 5 days, U-69593 (0.32 mg/kg) + cocaine (10 mg/kg) once daily for 5 days and killed 3 days later, or five daily injections of U-69593 (0.32 mg/kg) administered at 3-day intervals and killed 1 day later. The density of dopamine D_2 receptors was significantly increased in the nucleus accumbens core of rats that received cocaine once daily for 5 days and significantly decreased in the caudate putamen in rats treated with U-69593 once daily for 5 days, as compared to vehicle. Values shown are the means ± SEM for four animals per group. Abbreviations: DM, dorsomedial; DL, dorsolateral; VM, ventromedial; VL, ventrolateral. $^{*}P < 0.05$ compared to vehicle.

69593 ($P \leq 0.05$; Fig. 4A). Additionally, after treatment with the lower dose of 0.08 mg/kg bremazocine tyrosine hydroxylase levels were significantly increased in the nucleus accumbens core ($P \leq 0.05$) and there was a significant increase in tyrosine hydroxylase levels in the ventral portion of the caudate putamen and the core and shell of

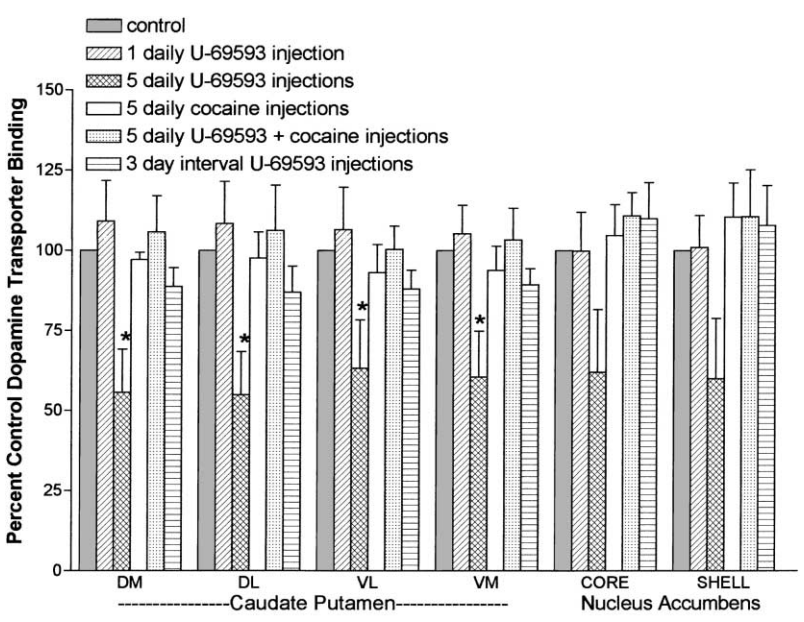

Fig. 6. Densitometric analysis of [^{125}I] RTI-121 binding was conducted in the caudate putamen and nucleus accumbens of rats treated with one of the following: U-69593 (0.32 mg/kg) once daily for 1 or 5 days, cocaine (10 mg/kg) once daily for 5 days, U-69593 (0.32 mg/kg) + cocaine (10 mg/kg) once daily for 5 days and killed 3 days later, or five daily injections of U-69593 (0.32 mg/kg) administered at 3-day intervals and killed 1 day later. The density of dopamine transporters was significantly decreased in the caudate putamen of rats that received U-69593 once daily for 5 days, as compared to vehicle. Values shown are the means ± SEM for four animals per group. Abbreviations: DM, dorsomedial; DL, dorsolateral; VM, ventromedial; VL, ventrolateral. $^{*}P < 0.05$ compared to vehicle.

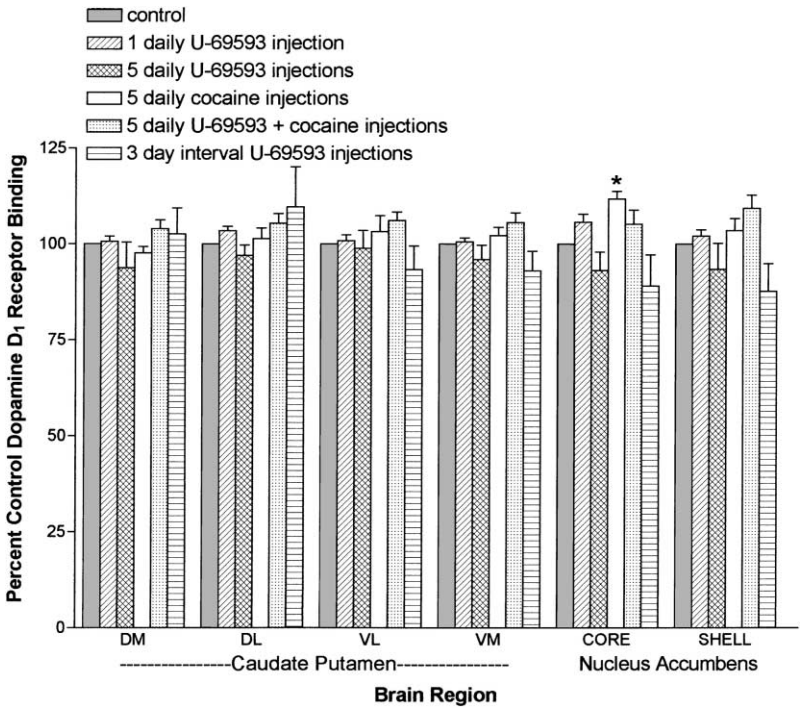

Fig. 7. Densitometric analysis of [^{3}H] SCH 23390 binding was conducted in the caudate putamen and nucleus accumbens of rats treated with one of the following: U-69593 (0.32 mg/kg) once daily for 1 or 5 days, cocaine (10 mg/kg) once daily for 5 days, U-69593 (0.32 mg/kg) + cocaine (10 mg/kg) once daily for 5 days and killed 3 days later, or five daily injections of U-69593 (0.32 mg/kg) administered at 3-day intervals and killed 1 day later. The density of dopamine D_1 receptors was significantly increased in the nucleus accumbens core of rats that received cocaine once daily for 5 days, as compared to vehicle. Values shown are the means ± SEM for four animals per group. Abbreviations: DM, dorsomedial; DL, dorsolateral; VM, ventromedial; VL, ventrolateral. $^*P < 0.05$ compared to vehicle.

the nucleus accumbens after treatment with the higher dose of 0.32 mg/kg bremazocine ($P \leq 0.05$; Fig 4B).

3.3. Quantitative autoradiography

3.3.1. Experiment 5: Modification of dopamine transporter and dopamine receptor densities after treatment with u-69593

Previously, it was shown that after treatment with U-69593 for 5 days, there were significant decreases in dopamine transporter density (Collins et al., in press). It was also shown that dopamine receptor D_2 densities were decreased following a 3-day treatment with U-69593, while dopamine D_1 receptors were unchanged (Izenwasser et al., 1998). The current study furthered those findings by showing that a 5-day treatment also leads to a decrease in dopamine D_2 receptors ($P \leq 0.05$; Fig. 5). In contrast, when U-69593 was given in combination with cocaine for 5 days, there were no changes in dopamine D_2 receptor (Fig. 5), dopamine transporter (Fig. 6), or dopamine D_1 receptor (Fig. 7) densities when compared to vehicle. Treatment with cocaine alone for 5 days produced significant increases in dopamine D_1 receptor (Fig. 7) and D_2 receptor (Fig. 5) densities in the nucleus accumbens core compared to vehicle ($P \leq 0.05$) and co-administration of U-69593 had no effect on this. Furthermore, there were no significant changes in dopamine transporter (Fig. 6), dopamine D_1 receptor (Fig. 7), or dopamine D_2 receptor (Fig. 5) densities after the one daily injection or 3-day interval treatment with U-69593 when compared to vehicle.

4. Discussion

The results of these experiments show that κ-opioid receptor agonists can block the acute locomotor effects of cocaine as well as the development of sensitization to cocaine for at least 2 weeks after the last κ-opioid receptor agonist injection was administered. The decrease in cocaine-induced behavior by κ-opioid receptor agonists is supported by other studies. U-69593 reduced locomotor effects associated with cocaine at various time points within 5 days of initial treatment (Heidbreder et al., 1993; Vanderschuren et al., 2000; Collins et al., in press) and reduced reinstatement of cocaine self-administration (Schenk et al., 1999). In addition, U50,488 blocked self-administration of cocaine (Glick et al., 1995; Kuzmin et al., 1997).

It has been suggested previously that the effect of κ-opioid receptor agonist treatment on the behavioral effects of cocaine may be due to an interaction with the dopamine system. For example, it has been shown that dopamine release (Di Chiara and Imperato, 1988; Spanagel et al., 1990; Maisonneueve et al., 1994), dopamine trans-

porter density (Collins et al., in press) and dopamine D_2 receptor density (Izenwasser et al., 1998) are decreased after treatment with κ-opioid receptor agonists. Furthermore, in the current study, increased levels of tyrosine hydroxylase were observed in rats treated with U-69593 or bremazocine. The decrease in dopamine transporter density and the resultant initial increase of extracellular dopamine (Heidbreder et al., 1998) would potentially cause a depletion of dopamine. To compensate for dopamine depletion, it is possible that levels of tyrosine hydroxylase would be elevated leading to increased dopamine synthesis. Thus, there are significant alterations in the dopamine system following treatment with κ-opioid receptor agonists. It is not entirely clear, however, which of these changes are primarily related to the treatment and which are compensatory reactions to the treatment effects.

A concern with κ-opioid receptor agonists is that repeated κ-opioid receptor agonist treatment alone reduced food-maintained responding (Negus et al., 1997) and locomotor activity (Collins et al., in press). The current studies show that the reduction in locomotor activity after administration of U-69593 alone could be eliminated if the inter-injection interval is increased. Furthermore, after this extended inter-injection interval, locomotor activity associated with a cocaine challenge is still significantly reduced, comparable to when κ-opioid receptor agonists are given daily. This is important from a treatment perspective because a less intensive treatment regimen may reduce the negative effects associated with a daily treatment regimen. It is especially interesting to note that dopamine transporter and dopamine receptor levels were unchanged after the 3-day interval treatment regimen. The lack of effect on these dopaminergic markers at the same time when there is a significant reduction in cocaine-induced behaviors shows that it is not necessary for the dopamine transporter or dopamine receptors to be altered in order to see reduced activity of cocaine. This suggests that a mechanism other than alterations in the dopamine transporter or dopamine receptors may be responsible for the reduction in cocaine's behavioral effects following treatment with a κ-opioid receptor agonist. Thus, even though a daily treatment with a κ-opioid receptor agonist produces multiple effects on the dopamine system, these changes may not be solely responsible for the changes in cocaine-induced behaviors. Therefore, it may be necessary to look at other systems to determine the underlying cause of the reduction in the behavioral effects of cocaine.

There is evidence that the interaction between κ-opioid receptor agonists and cocaine may be regulated by serotonin. For example, it has been shown that U-69593 attenuated RTI-55-induced cocaine self-administration but not that of WIN 35,428 (Schenk et al., 2000). This was interesting because both RTI-55 and cocaine inhibit uptake at the serotonin transporter with similar affinities to the dopamine transporter (Boja et al., 1992a,b; Eshleman et al., 1999), whereas WIN 35,428 binds only to the dopamine transporter, suggesting that the κ-opioid receptor agonist may be interacting with the serotonin system rather than the dopamine system in decreasing cocaine self-administration. Furthermore, although it has been suggested repeatedly that the reinforcing effects of cocaine are due primarily to an interaction with the dopamine transporter (Ritz et al., 1987; Kuhar et al., 1991; Volkow et al., 1997), dopamine transporter knockout mice have been shown to self-administer cocaine (Rocha et al., 1998), suggesting that cocaine retains its reinforcing properties without interacting with the dopamine transporter. In addition, injection of the $5\text{-}HT_{1A}$ receptor antagonist WAY 100635 reduced cocaine-produced reinstatement of extinguished drug-taking behavior (Schenk, 2000). Cocaine-induced locomotor activity has also been shown to be modulated by serotonin. For example, administration of the $5\text{-}HT_3$ receptor antagonist ondansetron, the serotonin-specific reuptake inhibitor fluoxetine, or the $5\text{-}HT_{1A}$ receptor antagonist WAY 100635 attenuated cocaine-induced locomotor activity (Carey et al., 2000; Herges and Taylor, 2000).

There appear to be reciprocal effects between serotonin and κ-opioid receptors. κ-Opioid receptors, measured by binding of [^{3}H] U-69593, are upregulated after intermittent (Unterwald et al., 1994) or continuous infusion (Collins et al., 2000b) of cocaine. This upregulation was found in serotonin-rich areas of the brain, such as the endopiriform nucleus, claustrum, medial portion of the caudate putamen, and the nucleus accumbens shell, but was not observed after treatment with the selective dopamine uptake inhibitors GBR 12909 or RTI-117 (Collins et al., 2000b). The lack of effect of selective dopamine uptake inhibitors on [^{3}H] U-69593 binding, in contrast to the upregulation seen following cocaine treatment, further demonstrates that the interaction between cocaine and κ-opioid receptors may be regulated by serotonin, or another non-dopaminergic system.

Overall, these data provide further evidence that the diminishment of cocaine's behavioral effects by κ-opioid receptor agonists is fairly long-lasting. Thus, these drugs may prove to be effective in the treatment of long-term cocaine abuse. Further, it may be possible, with the proper dosing regimen, to reduce the effects that this treatment alone has on behavior. The current results also add to the growing literature suggesting the possibility that the effects of κ-opioid receptor agonists on cocaine-related behaviors may be due to an interaction with systems other than dopaminergic, possibly in combination with dopaminergic effects, and that it is possible to block the behavioral effects of cocaine without major perturbation of the dopamine system.

Acknowledgements

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 11960.

References

Acri, J.B., Thompson, A.C., Shippenberg, T., 2001. Modulation of pre- and postsynaptic dopamine D2 receptor function by the selective kappa-opioid receptor agonist U69593. Synapse 39, 343–350.

Boja, J.W., Cline, E.J., Carroll, F.I., Lewin, A.H., Phillip, A., Cannals, R., Wong, D., Scheffel, U., Kuhar, M.J., 1992a. High potency cocaine analogs: neurochemical, imaging and behavioral studies. Ann. N. Y. Acad. Sci. 654, 282–291.

Boja, J.W., Mitchell, W.M., Patel, A., Kopajtic, T.A., Carroll, F.I., Lewin, A.H., Abraham, P., Kuhar, M.J., 1992b. High-affinity binding of [125I]RTI-55 to dopamine and serotonin transporters in rat brain. Synapse 12, 27–36.

Carey, R., Damianopoulos, E., DePalma, G., 2000. The 5-HT(1A) antagonist WAY 100635 can block the low-dose locomotor stimulant effects of cocaine. Brain Res. 862, 242–246.

Collins, S.L., Gerdes, R.M., D'Addario, C., Izenwasser, S., 2000. Kappa-opioid agonists decrease dopamine transporter density and cocaine-stimulated locomotor activity. Beh. Pharmacol., in press.

Collins, S.L., D'Addario, C., Izenwasser, S., 2000a. Kappa opioid agonists alter cocaine-induced locomotor activity and cocaine sensitization. Drug. Alc. Dep. 60, 540.

Collins, S.L., Kunko, P.M., Ladenheim, B., Cadet, J.L., Carroll, F.I., Izenwasser, S., 2000b. Chronic cocaine increases kappa-opioid receptor density: lack of effect by selective dopamine uptake inhibitors. Soc. Neurosci. Abstr. 26, 794.

Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. U. S. A. 85, 5274–5278.

Eshleman, A.J., Carmolli, M., Cumbay, M., Martens, C.R., Neve, K.A., Janowsky, A., 1999. Characteristics of drug interactions with recombinant biogenic amine transporters expressed in the same cell type. J. Pharmacol. Exp. Ther. 289, 877–885.

Glick, S., Maisonneuve, I., Raucci, J., Archer, S., 1995. Kappa opioid inhibition of morphine and cocaine self-administration in rats. Brain Res. 681, 147–152.

Heidbreder, C., Goldberg, S., Shippenberg, T., 1993. The kappa-opioid receptor agonist U-69593 attenuates cocaine-induced behavioral sensitization in the rat. Brain Res. 616, 335–338.

Heidbreder, C., Schenk, S., Partridge, B., Shippenberg, T., 1998. Increased resposiveness of mesolimbic and mesostriatal dopamine neurons to cocaine following repeated administration of a selective κ-opioid receptor agonist. Synapse 30, 255–262.

Herges, S., Taylor, D.A., 2000. Involvement of 5-HT(3) receptors in the nucleus accumbens in the potentiation of cocaine-induced behaviours in the rat. Br. J. Pharmacol. 131, 1294–1302.

Izenwasser, S., Acri, J., Kunko, P., Shippenberg, T., 1998. Repeated treatment with the selective kappa opioid agonist U-69593 produces a marked depletion of dopamine D_2 receptors. Synapse 30, 275–283.

Izenwasser, S., French, D., Carroll, F., Kunko, P., 1999. Continuous infusion of selective dopamine uptake inhibitors or cocaine produces time-dependent changes in rat locomotor activity. Behav. Brain Res. 99, 201–208.

Kuhar, M.J., Ritz, M.C., Boja, J.W., 1991. The dopamine hypothesis of the reinforcing properties of cocaine. Trends Neurosci. 14, 299–302.

Kunko, P., French, D., Izenwasser, S., 1998. Alterations in locomotor activity during chronic cocaine administration: effect on dopamine receptors and interaction with opioids. J. Pharmacol. Exp. Ther. 285, 277–284.

Kuzmin, A.V., Semenova, S., Gerrits, M.A., Zvartau, E.E., Van Ree, J.M., 1997. Kappa-opioid receptor agonist U50,488H modulates cocaine and morphine self-administration in drug-naive rats and mice. Eur. J. Pharmacol. 321, 265–271.

Maisonneueve, I., Archer, S., Glick, S., 1994. U50,488, a κ opioid receptor agonist, attenuates cocaine-induced increases in extracellular dopamine in the nucleus accumbens of rats. Neurosci. Lett. 181, 57–60.

Mello, N.K., Negus, S.S., 1998. Effects of kappa opioid agonists on cocaine- and food-maintained responding by rhesus monkeys. J. Pharmacol. Exp. Ther. 286, 812–824.

Negus, S.S., Mello, N.K., Portoghese, P.S., Lin, C.E., 1997. Effects of kappa opioids on cocaine self-administration by rhesus monkeys. J. Pharmacol. Exp. Ther. 282, 44–55.

Ritz, M.C., Lamb, R.J., Goldberg, S.R., Kuhar, M.J., 1987. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237, 1219–1223.

Rocha, B.A., Fumagalli, F., Gainetdinov, R.R., Jones, S.R., Ator, R., Giros, B., Miller, G.W., Caron, M.G., 1998. Cocaine self-administration in dopamine-transporter knockout mice. Nat. Neurosci. 1, 132–137.

Schenk, S., 2000. Effects of the serotonin 5-HT(2) antagonist, ritanserin, and the serotonin 5-HT(1A) antagonist, WAY 100635, on cocaine-seeking in rats. Pharmacol., Biochem. Behav. 67, 363–369.

Schenk, S., Partridge, B., Shippenberg, T., 1999. U69593, a kappa-opioid agonist, decreases cocaine self-administration and decreases cocaine-produced drug-seeking. Psychopharmacology 144, 339–346.

Schenk, S., Partridge, B., Shippenberg, T.S., 2000. Reinstatement of extinguished drug-taking behavior in rats: effect of the kappa-opioid receptor agonist, U69593. Psychopharmacology 151, 85–90.

Shippenberg, T.S., LeFevour, A., Heidbreder, C., 1996. κ-opioid agonists prevent sensitization to the conditioned rewarding effects of cocaine. J. Pharmacol. Exp. Ther. 276, 545–554.

Spanagel, R., Herz, A., Shippenberg, T., 1990. The effects of opioid peptides on dopamine release in the nucleus accumbens: an in vivo microdialysis study. J. Neurochem. 55, 1734–1740.

Tella, S.R., Ladenheim, B., Andrews, A.M., Goldberg, S.R., Cadet, J.L., 1996. Differential reinforcing effects of cocaine and GBR-12909: biochemical evidence for divergent neuroadaptive changes in the mesolimbic dopaminergic system. J. Neurosci. 16, 7416–7427.

Unterwald, E.M., Rubenfeld, J.M., Kreek, M.J., 1994. Repeated cocaine administration upregulates kappa and mu, but not delta, opioid receptors. NeuroReport 5, 1613–1616.

Vanderschuren, L., Schoffelmeer, A., Wardeh, G., De Vries, T., 2000. Dissociable effects of the κ-opioid receptor agonists bremazocine, U69593, and U50488H on locomotor activity and long-term behavioral sensitization induced by amphetamine and cocaine. Psychopharmacology 150, 35–44.

Volkow, N.D., Wang, G.J., Fischman, M.W., Foltin, R.W., Fowler, J.S., Abumrad, N.N., Vitkun, S., Logan, J., Gatley, S.J., Pappas, N., Hitzemann, R., Shea, C.E., 1997. Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature 386, 827–830.

Werling, L.L., Frattali, A., Portoghese, P.S., Takemori, A.E., Cox, B.M., 1988. Kappa receptor regulation of dopamine release from striatum and cortex of rats and guinea pigs. J. Pharmacol. Exp. Ther. 246, 282–286.