Effects of Kappa Opioids on Cocaine Self-Administration by Rhesus Monkeys

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ABSTRACT

Kappa opioid agonists attenuate some neurochemical and behavioral effects of cocaine and are being considered as potential treatments for cocaine dependence. The present study examined the effects of two kappa opioid agonists, the benzomorphan ethylketocyclazocine (EKC) and the arylnitrile U50,488, on cocaine self-administration in rhesus monkeys. Monkeys responded for 0.032 mg/kg/injection cocaine (i.v.) and 1 g banana-flavored food pellets during alternating daily sessions of cocaine and food availability. Chronic treatment for 10 consecutive days with EKC (0.0032–0.032 mg/kg/hr) or U50,488 (0.032–0.1 mg/kg/hr) dose-dependently decreased self-administration of cocaine unit doses at the peak of the cocaine dose-effect curve (0.01 and 0.032 mg/kg/injection). These decreases in cocaine self-administration were often sustained throughout the 10 days of treatment. Doses of EKC and U50,488 that decreased cocaine self-administration usually decreased food-maintained responding as well. In addition, EKC and U50,488 often produced emesis and sedation during the first few days of treatment, although tolerance appeared to develop rapidly to these effects. In general, EKC produced fewer undesirable effects than U50,488 at doses that decreased cocaine self-administration. The kappa antagonist nor-binaltorphimine (3.2 mg/kg) did not affect responding maintained by cocaine or food. However, both norbinaltorphimine (3.2 mg/kg) and the opioid antagonist naloxone (1.0 mg/kg/hr) blocked the effects of EKC and U50,488. These results indicate that chronic administration of EKC and U50,588 produce a dose-dependent, kappa receptor-mediated and often sustained decrease in cocaine self-administration. However, these kappa agonists also produce undesirable behavioral effects that may complicate their use as treatments for cocaine dependence.

Cocaine abuse continues to be a major public health concern in the United States (National Institutes of Health, 1996). Consequently, one goal of preclinical research has been to characterize the neurobiological and pharmacological determinants of cocaine’s high abuse potential and to identify drugs that could be used in the treatment of cocaine abuse. For example, cocaine has been shown to block the reuptake of dopamine receptors and alter the neurochemical and behavioral effects of cocaine. The nucleus accumbens, for example, is a terminal field of mesolimbic dopamine, primarily the nucleus accumbens (Ritz et al., 1987; Koob and Bloom, 1988; Johanson and Fischman, 1989; Kuhar et al., 1991; Wolterton and Johnson, 1992). Dopamine receptor antagonists, which block the indirect dopamine agonist effects of cocaine, have been evaluated for their utility in treating cocaine abuse (see Mello and Negus, 1996, for review). However, dopamine antagonists also produce extrapyramidal motor effects and other unwanted effects that complicate their use in the treatment of cocaine dependence. In addition, the cocaine-antagonist effects of dopamine antagonists may not be sustained during chronic treatment (Kleven and Wolterton, 1990; Negus et al., 1996).

Compounds acting on other receptor systems may provide an alternative means of modifying the neurobiological and behavioral effects of cocaine by indirectly modulating the activity of dopaminergic systems. For example, a growing body of evidence suggests that agonists and antagonists at kappa opioid receptors may modulate the activity of dopaminergic neurons and alter the neurochemical and behavioral effects of cocaine. The nucleus accumbens contains high levels of both kappa opioid receptors (Mansour et al., 1987, 1988, 1994) and dynorphin (Hokfelt et al., 1984), an endogenous opioid peptide with high affinity for kappa receptors (Chavkin et al., 1982). In contrast to cocaine, kappa agonists have been shown to decrease striatal dopamine levels in rats (Di Chiara and Imperato, 1988; Donzanti et al., 1992; Spanagel et al., 1992; Devine et al., 1993). Kappa agonists also

ABBREVIATIONS: EKC, ethylketocyclazocine; FR, fixed ratio; nor-BNI, norbinaltorphimine; VR, variable ratio; ANOVA, analysis of variance.
attenuated cocaine-induced increases in dopamine levels in the nucleus accumbens (Maisonneuve et al., 1994) as well as cocaine-induced changes in the expression of immediate early onco genes such as c-fos and zif 268 (Steiner and Gerfen, 1995; Crawford et al., 1995). Cocaine also appears to have a reciprocal action on kappa opioidergic systems. Cocaine administration has been found to up-regulate kappa receptors (Hurd and Herkenham, 1993; Unterwald et al., 1994) and increase levels of both dynorphin and dynorphin mRNA (Hurd and Herkenham, 1993; Daunais and McGinty, 1995; Daunais et al., 1995; Hansen et al., 1995). This cocaine-induced stimulation of kappa opioidergic systems may function as a form of negative feedback that opposes and limits the direct effects of cocaine (e.g., Hyman and Nestler, 1996).

Kappa opioid agonists have also been found to attenuate many behavioral effects of cocaine. For example, the administration of kappa agonists in rodents has been reported to block or decrease cocaine-induced hyperactivity (Ukai et al., 1994; Crawford et al., 1995), sensitization to cocaine-induced hyperactivity and stereotypies (Heidbreder et al., 1995) and cocaine-induced place preferences (Suzuki et al., 1992; Crawford et al., 1995; Shippenberg et al., 1996). Kappa agonists also produced a surmountable antagonism of the discriminative stimulus effects of cocaine in squirrel monkeys (Spealman and Bergman, 1992, 1994). Taken together, these findings suggest that activation of kappa opioid receptors may functionally antagonize some effects of cocaine, possibly by inhibiting the release of dopamine from dopaminergic neurons.

Although these studies suggest that kappa opioids modify some effects of cocaine that may contribute to its abuse, a direct examination of kappa opioid effects on the reinforcing effects of cocaine in drug self-administration procedures has only begun. Glick and co-workers (1995) recently reported that after acute administration, kappa agonists were slightly more potent in decreasing the self-administration of an intermediate unit dose of cocaine (0.4 mg/kg/injection) than in decreasing water-maintained responding in rats. In addition, the kappa antagonist nor-BNI (Portoghese et al., 1987) had no effect on cocaine self-administration but blocked the effects of the kappa agonists on cocaine self-administration (Glick et al., 1995). However, the effects of kappa agonists and antagonists on cocaine self-administration in primates are unknown, and there is some evidence which suggests that species may be an important determinant of the effects of kappa opioids (cf. Broadbear et al., 1994; Butelman et al., 1993a). In addition, pharmacotherapies for drug dependence are usually administered chronically, and the effects of chronic treatment with kappa opioids on cocaine self-administration have not been examined in any species.

Consequently, the purpose of the present study was to evaluate the effects of chronic treatment with kappa opioids on the reinforcing effects of cocaine in rhesus monkeys trained to self-administer cocaine. Two kappa agonists, the arylacetamide U50,488 (VonVoigtlander et al., 1983) and the benzomorphan EKC (Martin et al., 1976), were selected for evaluation in this study. Both U50,488 and EKC produce qualitatively similar behavioral effects mediated by kappa opioid receptors in rhesus monkeys (e.g., Dykstra et al., 1987a,b; Gmerek et al., 1987; France et al., 1994). However, EKC may also produce agonist effects at mu opioid receptors (Gmerek et al., 1987; Butelman et al., 1993b). In addition, evidence from studies conducted both in vitro (Su, 1985; Zukin et al., 1988; Nock et al., 1990; Rothman et al., 1990) and in vivo (Horan et al., 1991; Butelman et al., 1993a, 1995) suggests that kappa receptor subtypes may exist, and the effects of U50,488 and EKC may be mediated by different kappa receptor subtypes in rhesus monkeys (Butelman et al., 1993a). The effects of the kappa antagonist nor-BNI (Portoghese et al., 1987; Butelman et al., 1993a) and the opioid antagonist naloxone on the behavioral effects of EKC and U50,488 were also examined.

Methods

Subjects

Two female (075F and CH701) and seven male (152F, 89B058, 89B084, 900E, 944E, 90B134 and 606.5) adult rhesus monkeys were studied. Monkey 90B134 was experimentally naive at the beginning of the study. All other monkeys had an extensive experimental history involving the evaluation of dopaminergic and/or opioid compounds on cocaine self-administration. Monkeys weighed 4.4 to 10.9 kg and were fed a diet of multiple vitamins, fresh fruit and vegetables (2 pieces per day), and Lab Diet Jumbo Monkey biscuits (3–5 per day; PMI Feeds, Inc., St. Louis, MO). This diet was sufficient to maintain constant body weights (±10% of mean body weight) in these adult monkeys. In addition, monkeys could earn 1-g banana pellets (P. J. Noyes Co., Lancaster, NH) during daily operant sessions (see below). Water was continuously available. A 12-hr light-dark cycle was in effect (lights on from 7 A.M. to 7 P.M.).

Animal maintenance and research were conducted in accordance with the guidelines provided by the NIH Committee on Laboratory Animal Resources. The facility was licensed by the United States Department of Agriculture, and protocols were approved by the Institutional Animal Care and Use Committee. The health of the monkeys was monitored periodically by consulting veterinarians. Monkeys had visual, auditory and olfactory contact with other monkeys throughout the study. Operant procedures provided an opportunity for environmental manipulation and enrichment (Line et al., 1989).

Surgical Procedure

Double-lumen Silicone rubber catheters (inside diameter, 0.7 mm; outside diameter, 2.0 mm) were implanted in the jugular or femoral vein and exited in the midscapular region. All surgical procedures were performed under aseptic conditions. Monkeys were initially sedated with ketamine (5 mg/kg s.c.), and anesthesia was induced with thiopental (10 mg/kg i.v.). In addition, monkeys were treated with 0.05 mg/kg atropine to reduce salivation. After insertion of a tracheal tube, anesthesia was maintained with halothane (1–1.5% in oxygen). After surgery, aspirin or acetaminophen (80–160 mg/day p.o.) was administered for 3 days. An antibiotic, Procaine Penicillin G (300,000 U/day i.m.), was administered every day for 5 days. The i.v. catheter was protected by a tether system consisting of a custom-fitted nylon vest connected to a flexible stainless steel cable and fluid swivel (Lomir Biomedical, Malone, NY). This flexible tether system permitted monkeys to move freely. Catheter patency was periodically evaluated by i.v. administration of either ketamine (5 mg/kg) or the short-acting barbiturate methohexitol (3 mg/kg). The catheter was considered to be patent if i.v. administration of ketamine or methohexitol produced a loss of muscle tone within 10 sec.

Apparatus

Each monkey was housed individually in a well-ventilated stainless steel chamber (64 x 64 x 79 cm). The home cages of all monkeys were modified to include an operant panel (28 x 28 cm) mounted on the front wall. Three square translucent response keys (6.4 x 6.4 cm) were arranged 2.54 cm apart in a horizontal row 3.2 cm from the top
of the operant panel. Each key could be transilluminated by red, green or white stimulus lights (Superbright LEDs). In addition, three circular translucent panels (1.9 cm in diameter) were located in a vertical column below the center response key and could be transilluminated by red, green or white stimulus lights (Superbright LEDs). The operant panel also supported an externally mounted pellet dispenser (Gerbrands, model G5210) that delivered 1-g fruit-flavored food pellets (Precision Primate Pellets Formula I/L Banana Flavor, P. J. Noyes Co., Lancaster, NH) to a food receptive mounted on the cage above the operant response panel. In addition, two syringe pumps (model B5P-IE, Braintree Scientific, Braintree, MA; or model 980210, Harvard Apparatus, South Natick, MA) were mounted above each cage for delivery of saline or drug solutions through the two lumen of the intravascular catheters. Operation of the operant panels and data collection were accomplished with Apple IIGS computers located in a separate room.

Training Procedure

This report is one of a series of studies designed to evaluate the effects of potential treatment medications on cocaine self-administration in rhesus monkeys, and the experimental procedures have been described previously (Mello et al., 1989, 1990, 1992, 1993a,b; Lukas et al., 1995; Negus et al., 1995a, 1996). After initial shaping of key-pressing behavior for food reinforcement, responding was maintained on a VR schedule that was gradually increased to a VR 16. After a stable performance for food developed on a VR 16 schedule, behavior was maintained on a second-order schedule that consisted of two components, a VR and FR. After completion of a variable number of responses that averaged 16, a red light originally associated with food delivery was illuminated for 1 sec (VR [16:S]). The FR component was gradually increased from 1 to 4 until the terminal FR 4 (VR 16:S) second-order schedule was reached. Under this terminal schedule, monkeys had to complete 4 VR components, and an average of 64 responses (range, 53–78) was required to earn each food pellet. There were four food sessions during each 24-hr period beginning at 11 A.M., 3 P.M., 7 P.M. and 6 A.M. the next morning. Each age of 64 responses (range, 53–78) was required to earn each food schedule, monkeys had to complete 4 VR components, and an average of 64 responses (range, 53–78) was required to earn each food pellet. There were four food sessions during each 24-hr period beginning at 11 A.M., 3 P.M., 7 P.M. and 6 A.M. the next morning. Each session lasted for 1 hr or until a maximum of 25 food pellets had been delivered, whichever occurred first. Once food-maintained responding was stable, intravenous double-lumen catheters were implanted as described above. After recovery from surgery, key-pressing behavior for cocaine reinforcement (0.032 mg/kg/injection) was shaped under a series of increasing variable ratios identical with those used during training for food reinforcement. The conditions of food and cocaine availability each were associated with different colored stimulus lights projected on the center response key of the operant response panel. The two side keys were not transilluminated during these studies, and responding on these keys had no scheduled consequences. During food sessions, the center key was transilluminated with a red stimulus light, whereas during cocaine sessions, the center key was transilluminated with a green stimulus light. Completion of each VR component of the second-order schedule was followed by a 10-sec time-out period, during which the stimulus light illuminating the center response key was turned off for 10 sec and responding had no scheduled consequences. In addition, the appropriate colored stimulus light (red for food, green for injections) was illuminated for 1 sec below the center response key. Room lights were extinguished during all food and drug sessions.

A maintenance dose of 0.032 mg/kg/injection cocaine was used throughout the study. The cocaine was delivered through one lumen of the double-lumen catheter. The final second-order schedule response requirement was identical for food and drug sessions (FR 4 [VR 16:S]). There were four cocaine sessions during each 24-hr period beginning at 12 noon, 4 P.M., 8 P.M. and 7 A.M. the next morning (i.e., 1 hr after the beginning of the food sessions). Each cocaine session lasted 1 hr or until a maximum of 20 injections had been delivered, whichever occurred first.

Saline was delivered through the second lumen of the double lumen catheter. From 9:30 to 10:20 A.M. every morning, saline was delivered as a daily pretreatment at a rate of 0.1 ml/min for a total of 5.0 ml. For the remaining 23 hr of each experimental day, 0.1 ml saline was delivered every 20 min for a total of 6.9 ml.

The dependent variables were the number of food pellets and the number of cocaine injections delivered per session and each day. Monkeys were trained until their behavior met the following criteria for stable food- and cocaine-maintained responding under the terminal FR4 (VR16:S) schedule: 1) three consecutive days during which the number of drug injections on each day differed by no more than 20% from the mean number of drug injections per day and there was no upward or downward trend; and 2) during the same three consecutive days, the mean number of both drug injections per day and food pellets per day was greater than 50.

Test Procedures

Cocaine dose-effect curve determinations. Once behavior met the criteria for high, stable levels of responding for cocaine and food, cocaine dose-effect curves were determined. Either saline or different unit doses of cocaine (0.001, 0.0032, 0.01 and 0.1 mg/kg/injection) were substituted for the cocaine maintenance dose (0.032 mg/kg/injection). These cocaine doses were substituted in an irregular order across monkeys. Saline continued to be administered through the second lumen of the double-lumen catheter as described above. Each substitution condition remained in effect for at least 5 consecutive days and until one of the following three criteria had been met: 1) 3 consecutive days during which the number of drug injections delivered on each day differed by no more than 20% from the mean number of drug injections per day, and there were no upward or downward trends; 2) 3 consecutive days during which the number of drug injections per day on each day was 20 or less; or 3) the substitution treatment had been in effect for 10 days. At the conclusion of each substitution dose-condition, the maintenance dose of 0.032 mg/kg/injection cocaine was reinstated for at least 2 days and until responding for cocaine and food returned to base-line levels.

Kappa agonist treatment. Once cocaine dose-effect curves were determined, the effects of saline, U50,488 and EKC on the self-administration of unit doses of cocaine at the peak of the cocaine dose-effect curve (0.01 and 0.032 mg/kg/injection) were evaluated for 10 consecutive days. U50,488 (0.032–0.1 mg/kg/hr) was substituted for a saline infusion and administered through the second lumen of the double-lumen catheter from 9:30 to 10:20 A.M. every morning, until responding for cocaine and food returned to base-line levels.

Kappa antagonist treatment. The effects of 3.2 mg/kg nor-BNI were evaluated on behavior maintained by food and cocaine in a group of three monkeys (89B058, 89B084, 900E and 90B134). The dose of nor-BNI and the time-course parameters of these experiments were based on a previous study with nor-BNI in an assay of thermal antinociception in rhesus monkeys (Butelman et al., 1993a). In that experiment, a dose of 3.2 mg/kg nor-BNI shifted the dose-effect curves for the kappa agonists U50,488 and U69,593 0.5 to 1 log unit to the right for 21 days. The long duration of nor-BNI’s effects has also been reported in rats and mice (Jones and Holtzman, 1992;
Each experiment with nor-BNI lasted a total of 30 days. On the morning of day 1, 3.2 mg/kg nor-BNI (i.v.) was slowly infused through the second lumen of the double-lumen catheter between 9:30 and 10:20 A.M. For the remaining 29 days of the experiment, monkeys received only i.v. saline injections through the second lumen of the double-lumen catheter.

Four different unit doses of cocaine on the ascending limb and at the peak of the cocaine dose-effect curve (0.001, 0.0032, 0.01 and 0.032 mg/kg/injection) were studied. On days 1 to 10, one test unit dose of cocaine was available for self-administration. On day 11, the maintenance dose of cocaine (0.032 mg/kg/injection) was made available for 1 day. On days 12 to 21, a second test unit dose of cocaine was evaluated. On days 22 to 30, the maintenance dose of cocaine was again reinstated. Thus, two different unit doses of cocaine were evaluated on days 1 to 21 after nor-BNI administration, and no additional treatment was given during days 22 to 30. A second dose of nor-BNI (3.2 mg/kg) was then administered at least 31 days after the initial treatment, and two more unit doses of cocaine were evaluated for a total of four unit doses of cocaine. Cocaine doses were examined in an irregular order except that 0.01 and 0.032 mg/kg/injection cocaine were evaluated on days 1 to 10, and 0.001 and 0.0032 mg/kg/injection cocaine were evaluated on days 12 to 21, after nor-BNI administration. The effects of 3.2 mg/kg nor-BNI on each unit dose of cocaine were compared with the effects of 10 consecutive injections of saline treatment on the same unit doses of cocaine.

**Treatment with opioid antagonists + kappa agonists.** To evaluate the degree to which the effects of EKC and U50,488 were mediated by kappa opioid receptors, the effects of the opioid antagonist naloxone (1.0 mg/kg/hr) and nor-BNI (3.2 mg/kg) on EKC- and U50,488-induced suppression of cocaine self-administration were examined in four monkeys (CH701, 89B058, 89B084 and 90B134). In experiments with naloxone, a dose of 1.0 mg/kg/hr naloxone was administered by repeated infusion in combination with either 0.032 mg/kg/hr EKC or 0.1 mg/kg/hr U50,488 for 10 consecutive days during which the unit dose of cocaine was 0.01 mg/kg/injection. In experiments with nor-BNI, a dose of 3.2 mg/kg nor-BNI was administered between 9:30 and 10:20 A.M. on day 1 of a 30-day experiment. On days 1 to 10, one of the kappa agonists (either 0.032 mg/kg/hr EKC or 0.1 mg/kg/hr U50,488) was administered by repeated infusion, and the unit dose of cocaine was 0.01 mg/kg/injection. On day 11, saline was substituted for the kappa agonist as the solution being administered by repeated infusion, and the unit dose of cocaine was changed to 0.032 mg/kg/injection. On days 12 to 21, the other kappa agonist was administered by repeated infusion, and the unit dose of cocaine was 0.032 mg/kg/injection. On days 22 to 30, maintenance conditions were reinstated (i.e., 0.032 mg/kg/injection cocaine and repeated infusion of saline). The order of presentation of the kappa agonists after nor-BNI treatment was counter-balanced across monkeys.

**Data analysis.** The total number of injections or food pellets delivered per day and per session were determined. Data for the cocaine dose-effect curve are expressed as the mean and S.E.M. of the last 3 days of availability of saline and each unit dose of cocaine. Data from experiments with kappa opioids are shown in their entirety.

The effects of kappa opioids on the numbers of injections/day and food pellets/day were evaluated by a two-factor ANOVA, with kappa opioid dose and treatment day as the two factors. The antagonist effects of naloxone and nor-BNI were also evaluated by a two-factor ANOVA, with treatment (saline, EKC or U50 alone, naloxone + EKC or U50, and nor-BNI + EKC or U50) and treatment day as the two factors. The criterion for a significant ANOVA was set a priori at P < .05. In the event of a significant ANOVA, contrasts with Huynh-Feldt corrections for degrees of freedom were used to compare within-group means (Morrison, 1990).

**Drug preparation.** Nor-BNI dihydrochloride was synthesized in the laboratory of Dr. P. S. Portoghese for these collaborative studies. We are grateful to the Sanofi-Winthrop Company (Toulouse, France) for providing the EKC. U50,488 was kindly donated by the Upjohn Co. (Kalamazoo, MI) or purchased from Research Biochemicals International (Natick, MA). Cocaine hydrochloride was obtained in crystalline form from the National Institute on Drug Abuse, and purity was certified to be greater than 98%. All drugs were dissolved in sterile saline or sterile water, and were filter-sterilized with a 0.22-μ Millipore filter. Drugs were stored in pyrogen-free vials. Cocaine, EKC and U50,488 were delivered i.v. in a volume of 0.1 ml/injection, whereas nor-BNI pretreatments were administered in a series of 50 0.1-ml injections (total volume, 5.0 ml) between 9:30 and 10:20 A.M. Fresh solutions of nor-BNI were prepared for each experiment.

**Results**

**Control cocaine dose-effect curves.** Figure 1 shows the effects of manipulating the unit dose of cocaine on the number of injections/day and food pellets/day. Unit doses of 0.01 and 0.032 mg/kg/injection cocaine maintained the highest numbers of injections/day. Unit doses of cocaine up to 0.032 mg/kg/injection had little effect on the number of food pellets/day; however, food-maintained responding often decreased during availability of 0.1 mg/kg/injection cocaine.

**Effects of kappa agonists on responding maintained by cocaine and food.** Figures 2 and 3 show the effects of saline, EKC (0.0032-0.032 mg/kg/hr) and U50,488 (0.032-0.1 mg/kg/hr) on the numbers of cocaine injections/day and food pellets/day when the unit dose of cocaine was 0.01 mg/kg/injection (top panels) or 0.032 mg/kg/injection (bottom panels). During treatment with saline, monkeys usually obtained the maximum number of injections/day and food pellets/day when 0.01 or 0.032 mg/kg/injection cocaine was available. EKC produced a dose-dependent decrease in the number of 0.01 mg/kg/injection cocaine injections/day (fig. 2, top panel). Post hoc analysis revealed that the number of injections/day during treatment with both 0.01 mg/kg/hr EKC (46.6 ± 12.4 injections/day) and 0.032 mg/kg/hr EKC (38.9 ± 7.5 injections/day) was significantly lower than the number of injections/day during continuous saline infusion (78.0 ± 1.2 injections/day). Treatment with 0.032 mg/kg/hr
EKC also produced a significant decrease in the self-administration of 0.032 mg/kg/injection cocaine (fig. 2, bottom panel). When 0.032 mg/kg/injection cocaine was available during saline treatment, monkeys self-administered 76.9 ± 0.7 injections/day, whereas monkeys self-administered an average of only 36.9 ± 6.2 injections/day when 0.032 mg/kg/injection cocaine was available during saline treatment. 

Fig. 2. Effects of EKC on the number of injections/day and food pellets/day when the unit dose of cocaine was 0.01 mg/kg/injection (inj) (top) or 0.032 mg/kg/inj (bottom). Abscissa, dose of EKC administered chronically in mg/kg/hr; left ordinates, number injections/day; right ordinates, number food pellets/day. Bars above “Sal” show control data obtained during chronic treatment with saline. Each bar shows mean data for 10 days of treatment in each of four monkeys (top panels) or three monkeys (bottom panels). *Significantly different from saline control treatment (P < .05).
Cocaine was available during treatment with 0.032 mg/kg/hr EKC. In addition to decreasing the number of cocaine injections/day, EKC also decreased the mean number of food pellets/day. However, EKC decreased the mean number of pellets/day less than the mean number of injections/day, and the effects of EKC on food-maintained responding did not attain statistical significance. Moreover, changes in food-maintained responding did not always increase with increasing doses of EKC.

U50,488 also produced a dose-dependent decrease in the number of 0.01 mg/kg/injection cocaine injections/day (fig. 3). Post hoc analysis revealed that the number of cocaine injections/day during treatment with 0.1 mg/kg/hr U50,488 (46.5 ± 1.7 injections/day) was significantly lower than the number of injections/day during continuous saline infusion (73.2 ± 4.2 injections/day). U50,488 also tended to produce a dose-dependent decrease in the number of food pellets/day during 0.01 mg/kg/injection cocaine availability. For example, during saline treatment, monkeys earned an average of 99.4 ± 0.4 pellets/day, but during treatment with 0.1 mg/kg/hr U50,488, the average number of food pellets/day was 60.3 ± 17.8. However, the effects of U50,488 on food-maintained responding did not attain statistical significance. Treatment with 0.1 mg/kg/hr U50,488 also produced a significant decrease in the self-administration of 0.032 mg/kg/injection cocaine from 76.7 ± 0.5 injections/day during saline treatment to 46.0 ± 7.9 injections/day during treatment with 0.1 mg/kg/hr U50,488. The mean number of food pellets/day decreased from 90.2 ± 5.1 during saline treatment to 68.2 ± 16.9 during treatment with 0.1 mg/kg/hr U50,488, but this effect did not attain statistical significance.

**Kappa agonist effects in individual monkeys.** Figure 4 shows the effects of the highest doses of EKC (0.032 mg/kg/hr) and U50,488 (0.1 mg/kg/hr) on responding maintained by 0.01 mg/kg/injection cocaine and food in individual monkeys during each of the 10 days of treatment. The effects of both EKC and U50,488 varied across time and across monkeys. Treatment with 0.032 mg/kg/hr EKC produced a sustained decrease in 0.01 mg/kg/injection cocaine self-administration in monkeys 89B084 and 944E, while producing little or no change in the number of food pellets/day. However, the same dose of EKC produced more variable changes in 0.01 mg/kg/injection cocaine self-administration in monkeys 89B058 and 900E across time, and decreases in the number of injections/day were usually accompanied by decreases in the number of food pellets/day in these monkeys. Treatment with 0.1 mg/kg/hr U50,488 produced highly variable changes in 0.01 mg/kg/injection cocaine self-administration across time in all four monkeys. Moreover, although the number of cocaine injections/day was usually decreased more than the number of food pellets/day in monkeys 900E and 89B058, the reverse was often found in monkeys 89B084 and 90B134. Indeed, in monkey 90B134, treatment with 0.1 mg/kg/hr U50,488 almost eliminated food-maintained responding and had inconsistent effects on cocaine self-administration. Because U50,488 was so much more effective in decreasing food-maintained responding than cocaine-maintained responding in monkey 90B134, we subsequently examined the

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**Fig. 4.** Effects of 0.032 mg/kg/hr EKC (top) or 0.01 mg/kg/hr U50,488 (bottom) on the number of injections/day and food pellets/day in individual monkeys. Abscissae, consecutive days of treatment with EKC or U50,488; left ordinates, number injections/day (filled circles); right ordinates, number food pellets/day (open circles). Each panel shows data from a single monkey, and the monkey’s identification number is shown above each panel. Points above “S” show mean control data obtained during 10 days of treatment with saline.
effects of EKC in this monkey. Chronic treatment with 0.032 mg/kg/hr EKC decreased the number of 0.01 mg/kg/injection cocaine injections approximately 60% (from 60.9 ± 2.6 injections/day to 24.1 ± 4.3 injections/day), whereas the number of food pellets/day was decreased by only 24% (from 98.8 ± 0.7 pellets/day to 74.7 pellets/day). Thus, in contrast to the effects of U50,488, EKC produced a moderately selective decrease in cocaine self-administration even in monkey 90B134 (data not shown).

**Kappa agonist effects on diurnal patterns of responding for cocaine and food.** In previous studies, we observed that there was a difference in sensitivity to changes in the unit dose of cocaine as a function of the time of the session (Negus et al., 1995b). Accordingly, we examined patterns of cocaine- and food-maintained responding during saline and kappa opioid treatment as a function of time of session. Analysis of the diurnal patterns of cocaine self-administration revealed that both EKC and U50,488 decreased cocaine self-administration more during the 8 to 9 P.M. and 7 to 8 A.M. drug sessions than during the noon to 1 P.M. and 4 to 5 P.M. sessions. Figure 5 shows the effects of the highest doses of EKC (0.032 mg/kg/hr) and U50,488 (0.1 mg/kg/hr) on the numbers of 0.01 mg/kg/injection cocaine injections and food pellets delivered during each of the four daily sessions. During saline treatment, monkeys usually obtained the maximum number of cocaine injections and food pellets available during each of the four sessions, and there were no statistically significant differences across sessions in the number of injections/session or pellets/session. Treatment with 0.032 mg/kg/hr EKC significantly decreased the number of injections/session during each of the four sessions while producing only minimal and nonsignificant decreases in the numbers of food pellets/session. Moreover, during EKC treatment, the number of injections/session during the 8 to 9 P.M. and 7 to 8 A.M. sessions was significantly lower than the number of injections/session during the noon to 1 P.M. and 4 to 5 P.M. sessions.

Treatment with 0.1 mg/kg/hr U50,488 did not significantly alter 0.01 mg/kg/injection cocaine self-administration during the noon to 1 P.M. and 4 to 5 P.M. sessions, but U50,488 did significantly decrease the number of injections/session during the 8 to 9 P.M. and 7 to 8 A.M. sessions. There was also a tendency for U50,488 to decrease the mean number of food pellets/session; however this effect did not attain statistical significance during any session. In contrast to the effects of U50,488 on cocaine self-administration, decreases in the numbers of pellets/session were similar across all four daily sessions.

**Emetic and sedative effects of EKC and U50,488.** Both EKC and U50,488 were tested up to doses that caused emesis in some monkeys. Infusion of 0.032 mg/kg/hr EKC caused emesis in 1 of 4 monkeys tested, and infusion of 0.1 mg/kg/hr U50,488 produced emesis in 3 of 4 monkeys. These emetic effects always occurred on the first or second day of treat-

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**Fig. 5.** Effects of chronic treatment with 0.032 mg/kg/hr EKC (top) or 0.1 mg/kg/hr U50,488 (bottom) on the number of 0.01 mg/kg/injection cocaine injections and food pellets delivered during each of the four daily drug sessions and four daily food sessions. Abscissae, time of each daily drug or food session; left ordinates, number of injections/session (maximum, 20); right ordinates, number of food pellets/session (maximum, 25). Each point shows mean data from four monkeys. Open symbols show data obtained during chronic saline treatment.
Kappa antagonist effects on cocaine self-administration. Figure 6 shows the effects of the selective kappa antagonist nor-BNI (3.2 mg/kg) on the self-administration of cocaine (0.001–0.032 mg/kg/injection) at doses on the ascending limb and peak of the cocaine dose-effect curve in a group of three monkeys. Nor-BNI treatment had no effect on the number of injections/day at any of the cocaine unit doses tested. Nor-BNI also had no effect on the number of food pellets/day when these different unit doses of cocaine were available (data not shown).

Effects of naloxone and nor-BNI on kappa agonist-induced suppression of cocaine- and food-maintained responding. Figure 7 shows the numbers of 0.01 mg/kg injection cocaine injections/day and food pellets/day in a group of four monkeys during treatment with saline, EKC (0.032 mg/kg/hr) or U50,488 (0.1 mg/kg/hr) alone, EKC or U50,488 in combination with chronic naloxone (1.0 mg/kg/hr) and EKC or U50,488 after pretreatment with nor-BNI (3.2 mg/kg). This group of monkeys usually obtained the maximum number of 0.01 mg/kg/injection cocaine injections/day and pellets/day during treatment with saline, and both EKC (0.032 mg/kg/hr) and U50,488 (0.1 mg/kg/hr) produced a significant decrease in the number of cocaine injections/day. In addition, treatment with U50,488 (0.1 mg/kg/hr) produced a significant decrease in the number of pellets/day in this group of monkeys. EKC also decreased the mean number of pellets/day, but this effect was not statistically significant. Concurrent treatment with 1.0 mg/kg/hr naloxone completely antagonized the effects of EKC on cocaine self-administration. Pretreatment with 3.2 mg/kg nor-BNI attenuated the effects of EKC on cocaine self-administration, but the number of injections/day was still significantly less than during saline treatment. Both naloxone and nor-BNI completely antagonized the effects of U50,488 on responding maintained by both cocaine and food.

Discussion

The purpose of this study was to evaluate the potential utility of kappa opioids for the treatment of cocaine dependence. We recently reviewed the use of preclinical drug self-administration procedures to evaluate medications that might be useful in treating drug dependence, and we described a profile of effects that might be produced by a promising medication (Mello and Negus, 1996). First, a promising medication should decrease self-administration behavior maintained by a wide range of unit doses of the self-administered drug, and these decreases in drug self-administration should be sustained during chronic administration of the medication. Second, a promising medication should produce selective decreases in drug-maintained responding while producing a relatively mild array of other undesirable side effects. The results of the present study are discussed in terms of these criteria for preclinical assessment of the efficacy and safety of potential treatment medications.

Effects of EKC and U50,488 on cocaine self-administration. Chronic infusion of the kappa opioid agonists EKC (0.0032–0.032 mg/kg/hr) and U50,488 (0.032–0.1 mg/kg/hr) produced dose-dependent decreases in the self-administration of 0.01 mg/kg/injection cocaine, a unit dose of cocaine located at the peak of the cocaine dose-effect curve. Doses of EKC (0.032 mg/kg/hr) and U50,488 (0.1 mg/kg/hr) that decreased self-administration of 0.01 mg/kg/injection cocaine also decreased self-administration of a higher unit dose of 0.032 mg/kg/injection cocaine. Finally, cocaine self-administration was often decreased as much or more at the end of each 10-day treatment period as at its beginning. Thus, EKC and U50,488 produced dose-dependent and often sustained decreases in the self-administration of two different unit doses of cocaine spanning a range of 0.5 log units. The present results obtained with chronic administration of EKC and U50,488 in rhesus monkeys confirm and extend the findings of a previous study that examined the effects of acute kappa agonist treatment on cocaine self-administration in rats (Glick et al., 1995). In that study, pretreatment with the kappa agonists U50,488 and spiradoline produced dose-dependent decreases in responding maintained by a single unit dose of cocaine (0.4 mg/kg/injection).

As discussed in the introduction, it has been proposed that kappa agonists may functionally antagonize some effects of cocaine by binding to kappa receptors on mesolimbic dopaminergic neurons and inhibiting dopamine release. The diurnal patterns of cocaine self-administration in the presence of kappa agonists provide some support for this proposition. Specifically, both kappa agonist treatment (present study) and decreases in the unit dose of cocaine (Negus et al., 1995b) decreased cocaine self-administration primarily during the 8 to 9 P.M. and 7 to 8 A.M. drug sessions. This similarity in diurnal patterns of cocaine self-administration produced by kappa agonist treatment and by decreasing the unit dose of cocaine suggests that EKC and U50,488 may have produced at least a partial antagonism of the reinforcing effects of cocaine.
cocaine. However, other nonspecific behavioral effects of EKC and U50,488 (e.g., sedation, see below) may also have contributed to the observed decreases in cocaine self-administration. Moreover, it is important to note that the effects of EKC and U50,488 on cocaine self-administration differ from the effects of the dopamine receptor antagonist flupenthixol, which was studied under similar experimental conditions (Negus et al., 1996). Flupenthixol also produced dose-dependent decreases in the self-administration of 0.01 mg/kg/injection cocaine, but the effects of flupenthixol were not sustained during 10-day treatment periods, and doses of flupenthixol that decreased self-administration of 0.01 mg/kg/injection cocaine did not alter self-administration of a higher unit dose of 0.032 mg/kg/injection cocaine (Negus et al., 1996). Decreases in cocaine self-administration produced by the dopamine D₁ receptor-selective antagonist SCH23390 also diminished during chronic administration (Kleven and Woolverton, 1990). Thus, relative to dopamine receptor antagonists, kappa agonists produce a more sustained decrease in cocaine self-administration across a broader range of cocaine doses.

**Other effects of EKC and U50,488.** The selectivity of kappa agonist effects on cocaine self-administration was assessed with concurrent measures of food-maintained responding. In general, doses of EKC and U50,488 that decreased cocaine self-administration also tended to decrease food-maintained responding in most monkeys. However, the relative effects of these kappa agonists on responding maintained by cocaine and food varied across monkeys and across treatments. The most selective decreases in cocaine self-administration were produced by EKC. For example, in one monkey (89B084), chronic treatment with EKC (0.032 mg/kg/hr) dramatically decreased 0.01 mg/kg/injection cocaine self-administration while producing little or no effect on food-maintained responding. Chronic treatment with U50,488 (0.1 mg/kg/hr), in contrast, often produced greater decreases in food-maintained responding than in cocaine-maintained responding in this monkey. In another monkey (90B134), EKC (0.032 mg/kg/hr) and U50,488 (0.1 mg/kg/hr) decreased both cocaine and food-maintained responding. However, EKC produced greater decreases in the number of cocaine injections/day than in the number of food pellets/day, whereas U50,488 nearly eliminated food-maintained responding while producing smaller and less consistent decreases in cocaine-maintained responding.

In a previous study conducted in rats, acute pretreatment with either U50,488 or spiradoline decreased water-maintained responding; however, doses of these kappa agonists that significantly decreased water-maintained responding were two to five times higher than doses that decreased cocaine-maintained responding (Glick et al., 1995). Taken together with the present findings, these results suggest that kappa agonists decrease responding maintained by other reinforcers at doses similar to or only slightly higher than those that decrease cocaine-maintained responding.

Doses of EKC and U50,488 that decreased cocaine self-administration also produced sedation and emesis in some monkeys during the first few days of treatment. However, EKC produced emesis in only one of four monkeys, whereas U50,488 produced emesis in three of four monkeys tested. Moreover, emesis was never observed after the second day of treatment with either EKC or U50,488, and the severity of sedation also decreased over time. These results suggest that tolerance developed to the emetic and sedative effects of EKC and U50,488. A previous study also reported that during...
chronic administration, tolerance developed to the unconditioned behavioral effects of U50,488 and the benzomorphans kappa agonist MR2033 in rhesus monkeys (Gmerek et al., 1987). Overall, EKC produced fewer unwanted side effects than U50,488 at doses that decreased cocaine self-administration.

In addition to the side effects described above, the acute administration of kappa selective opioid agonists to humans has been reported to produce subjective effects that have been described as “dysphoric” and “psychotomimetic.” (Pfeiffer et al., 1986; Kumor et al., 1986; Rimoy et al., 1991; Reece et al., 1994). Although, these subjective effects might impair compliance and complicate the use of kappa agonists as treatments for cocaine dependence, the impact of these subjective effects on the clinical use of kappa opioids remains to be determined. It is possible that the subjective effects of kappa agonists may present a relatively minor barrier to their clinical use. For example, the diuretic effects of the kappa agonist enadoline (CI-977) were recently evaluated in humans, and although enadoline produced dose-dependent increases in some subjective effects that might be considered dysphoric (e.g., dizziness, fatigue, emotional lability, abnormal thinking), subjects did not withdraw from the study (Reece et al., 1994). Furthermore, medications used in the treatment of drug abuse would be administered chronically, and tolerance may develop to the subjective effects as well as to other unwanted effects of kappa agonists. For example, the opioid cyclazocine produces dysphoric subjective effects that may be mediated by kappa receptors in humans, and tolerance developed to these dysphoric effects when gradually increasing doses of cyclazocine were administered chronically for 2 to 4 weeks (Martin et al., 1965, 1966).

Mechanisms of action of EKC and U50,488. The receptor mechanisms that mediated the effects of EKC and U50,488 were examined by evaluating the ability of the opioid antagonists naloxone and nor-BNI to antagonize EKC and U50,488. Although the effects of naloxone alone were not examined in this study, previous studies have reported that naloxone does not alter cocaine self-administration by rhesus monkeys (Woods and Schuster, 1971, Killian et al., 1978). Similarly, nor-BNI alone had no effect on cocaine self-administration (see below). However, the effects of both EKC and U50,488 were blocked or attenuated by concurrent treatment with naloxone and by pretreatment with nor-BNI. Although naloxone has a slightly higher affinity for mu receptors than for kappa receptors (e.g., Emmerson et al., 1994), this difference is small, and acute pretreatment with naloxone doses of 0.1 mg/kg and higher is sufficient to antagonize the effects of both mu and kappa opioid agonists in rhesus monkeys (France et al., 1990; Davis et al., 1992). In the present study, monkeys were treated chronically with 1.0 mg/kg/hr naloxone for a period of 23 hr/day. Thus, monkeys received a total dose of 23 mg/kg naloxone each day. This dose should have been sufficient to block mu, kappa and possibly delta opioid receptors. Consequently, the demonstration that 1.0 mg/kg/hr naloxone blocked the effects of EKC and U50,488 suggests that these effects were mediated by opioid receptors. The type of opioid receptor mediating the effects of EKC and U50,488 cannot be inferred from the results of these experiments with naloxone. However, the finding that a kappa-selective dose of nor-BNI also attenuated the effects of EKC and blocked the effects of U50,488 suggests that suppression of cocaine- and food-maintained responding by EKC and U50,488 were mediated, at least in part, by kappa opioid receptors. Nor-BNI also blocked the effects of kappa agonists on cocaine self-administration in rats (Glick et al., 1995).

Nor-BNI was more effective in blocking the effects of U50,488 on cocaine self-administration than in blocking the effects of EKC. Specifically, pretreatment with 3.2 mg/kg nor-BNI completely blocked the effects of U50,488 on cocaine self-administration. In contrast, the effects of EKC were attenuated by nor-BNI pretreatment, but EKC still significantly decreased the number of cocaine injections/day. These results agree with several previous studies in finding that nor-BNI is more effective as an antagonist of U50,488 than of EKC (Takemori et al., 1988; Broadbent et al., 1994; Butelman et al., 1993a). For example, in rhesus monkeys 3.2 mg/kg nor-BNI produced a surmountable and long-acting antagonism of the antinociceptive effects of U50,488, but did not alter the antinociceptive effects of EKC (Butelman et al., 1993a). There are at least two possible explanations for the different sensitivities of EKC and U50,488 to antagonism by nor-BNI (see Butelman et al., 1993a, for discussion). First, kappa opioid receptor subtypes have been proposed (Su, 1985; Zukin et al., 1988; Nock et al., 1990; Rothman et al., 1990), and U50,488 and EKC may produce their effects by acting at different kappa receptor subtypes. Second, EKC may produce at least some of its effects by acting at non-kappa opioid receptors, and indeed, several studies suggest that EKC produces agonist effects at mu as well as kappa receptors (Gmerek et al., 1987; Butelman et al., 1993b). The observation that naloxone completely blocked the effects of EKC in the present study could be consistent with either of these possibilities.

Effects of nor-BNI on cocaine self-administration. The selective kappa opioid antagonist nor-BNI (3.2 mg/kg) had no effect on cocaine self-administration maintained by a wide range of unit doses (0.001–0.032 mg/kg/injection). The inability of nor-BNI to alter cocaine self-administration probably did not result from inadequate doses, because previous studies have found that a single dose of 3.2 mg/kg nor-BNI produced 0.5 to 1 log unit rightward shifts in the dose-effect curves for U50,488-induced antinociception in rhesus monkeys for up to 21 days (Butelman et al., 1993a). Moreover, in the present study, 3.2 mg/kg nor-BNI antagonized the effects of U50,488 and EKC on cocaine- and food-maintained responding. Our findings in rhesus monkeys agree with a previous study reporting that nor-BNI did not alter self-administration of 0.4 mg/kg/injection cocaine by rats (Glick et al., 1995).

It has been hypothesized that cocaine administration may activate endogenous kappa opioidergic systems (Hurd and Herkenham, 1993; Unterwald et al., 1994; Daunais et al., 1995; Hanson et al., 1995), and that these kappa opioidergic systems may function as a negative feedback loop to oppose and limit the direct effects of cocaine (Hyman and Nestler, 1996). This hypothesis predicts that administration of a kappa opioid antagonist such as nor-BNI should disinhibit dopaminergic neurons, enhance the reinforcing effects of cocaine and shift the cocaine dose-effect curve to the left. However, we did not observe significant changes in cocaine self-administration after kappa antagonist administration. Consequently, these results suggest that endogenous kappa opioidergic systems were not activated by cocaine self-admini-
istration under the conditions studied here. It is possible that the total intake of cocaine permitted in these studies was not sufficient to activate endogenous kappa opioidergic systems. Drug self-administration behavior was usually maintained with a unit dose of 0.032 mg/kg/injection cocaine, and monkeys could self-administer a maximum of 80 injections/day. Consequently, the maximum dose of cocaine that could be self-administered on any 1 day was 2.56 mg/kg. Similarly, in the study by Glick et al. (1995), rats usually self-administered approximately 2 mg/kg/day cocaine. However, much higher doses appear to be necessary to elicit changes in kappa opioidergic systems. For example, chronic administration of 30 mg/kg/day cocaine increased striatal preprodynorphin immunoreactivity in rats; however, chronic administration of 10 or 20 mg/kg/day cocaine had no effect on striatal preprodynorphin immunoreactivity (Daunais and McGinty, 1994).

Summary. In summary, EKC and U50,488 produced dose-dependent and often sustained decreases in the self-administration of two different unit doses of cocaine. The kappa antagonist nor-BNI had no effect on cocaine self-administration; however, both nor-BNI and the opioid antagonist naloxone blocked the effects of EKC and U50,488, which suggests that the effects of EKC and U50,488 were mediated by kappa opioid receptors. Doses of kappa agonists that decreased cocaine self-administration also often produced undesirable behavioral effects, including decreases in rates of food-maintained responding, emesis, and sedation. At doses that decreased cocaine self-administration, these untoward effects were less severe for EKC than for U50,488 (e.g., EKC produced smaller decreases in food-maintained responding and less frequent emesis than U50,488). The extent to which similar undesirable effects may limit the clinical utility of kappa agonists for the treatment of cocaine dependence remains to be determined.

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References


Kappa Opioids and Cocaine


