Enadoline Discrimination in Squirrel Monkeys: Effects of Opioid Agonists and Antagonists

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ABSTRACT

Squirrel monkeys were trained to discriminate i.m. injections of the \( \kappa \)-opioid receptor agonist enadoline \((0.0017 \text{ mg/kg}) \) from saline in a two-lever drug-discrimination procedure. Enadoline produced a reliable discriminative stimulus that was reproduced by the \( \kappa \)-selective agonists PD 117302, U 50,488, GR 89686A, \((+-)\)-spirodoline, ICI 204448, and EMD 61753, and by the mixed-action \( \kappa/\mu \)-agonists bremazocine and ethylketocyclazocine. The discriminative stimulus effects of enadoline were not reproduced by the \( \mu \)-selective agonist morphine, the \( \delta \)-selective agonist BW373U86, the mixed-action opioids nalbuphine and nalorphine, or by the less active enantiomers of nor-BNI (3–10 mg/kg), and the mixed-action opioid nalbuphine \((0.3–30 \text{ mg/kg}) \) served to surmountably antagonize enadoline’s discriminative stimulus effects. The antagonist effects of nor-BNI were long-lasting and did not distinguish between drugs purported to act at different \( \kappa \)-receptor subtypes. The present results bolster the view that common discriminative stimulus effects of enadoline and other opioids are mediated by \( \kappa \)-agonist actions that are surmountantly antagonized by nor-BNI in a long-lasting manner. The enadoline-antagonist effects of nalbuphine support the idea that it acts with low efficacy at \( \kappa \)-opioid receptors.

Drug discrimination procedures have been used extensively to pharmacologically characterize opioids with \( \mu \)-receptor-mediated actions in rodent, avian, and primate species. \( \kappa \)-Opioid agonists have been studied less comprehensively, and the majority of studies has been conducted with mixed-action \( \kappa/\mu \)-agonists or selective \( \kappa \)-agonists in pigeons and rats. In those studies, other \( \kappa \)- but not \( \mu \)-agonists elicited responding on the lever associated with the training drug (Shearman and Herz, 1982; Picker and Dykstra, 1987, 1989; Holtzman et al., 1991; Picker et al., 1993; Picker, 1994a; Brandt and France, 1996). In contrast, responding on the drug-associated lever was produced only by \( \mu \)- but not \( \kappa \)-agonists when subjects were trained with \( \mu \)-agonists (Shearman and Herz, 1982; Holtzman et al., 1991; Comer et al., 1993, Picker et al., 1993). Results from limited studies in monkeys tend to agree with results obtained in rats and pigeons. In rhesus monkeys, for example, the discriminative stimulus effects of the \( \kappa/\mu \)-opioid EKC were reproduced by other \( \kappa \)-agonists, but not by the \( \mu \)-agonist alfen-tanil (Hein et al., 1981; Tang and Collins, 1985; Dykstra et al., 1987a; France et al., 1994). Taken as a whole, these data suggest that pharmacological specificity in the discriminative stimulus effects of \( \kappa \)-opioid drugs is conserved across species.

The availability of antagonists that selectively block different types of opioid receptors also has contributed to the pharmacological classification of opioids in drug discrimination studies. For instance, the selective \( \mu \)- and \( \delta \)-antagonists \( \beta \)-funaltrexamine (\( \beta \)-FNA; Portoghese et al., 1980) and naltrindole (Portoghese et al., 1988), respectively, have helped distinguish between discriminative stimulus effects mediated at these two types of opioid receptor (Dykstra et al., 1987b; Comer et al., 1993). Similarly, nor-binaltorphimine (nor-BNI; Portoghese et al., 1987), a selective and systemically active antagonist at \( \kappa \)-opioid receptors (Endoh et al., 1992; Horan et al., 1992), may be useful for the study of \( \kappa \)-mediated discriminative stimulus effects. In this regard, previous studies with nor-BNI have revealed that it has a slow onset and long duration of action (Jones and Holtzman, 1992; Butelman et al., 1993; Broadbear et al., 1994; Jewett and Woods, 1995).

ABBREVIATIONS: EKC, ethylketocyclazocine; \( \beta \)-FNA, \( \beta \)-funaltrexamine; nor-BNI, nor-binaltorphimine; FR, fixed ratio; TO, time-out.
**Materials and Methods**

**Subjects.** Five male squirrel monkeys (Saimiri sciureus), weighing 750 to 890 g were studied during daily experimental sessions. Between sessions, monkeys lived in individual home cages where they had unlimited access to water and received a nutritionally balanced diet consisting of Purina monkey chow, fresh fruit, and vegetables. All monkeys were experimentally naive at the beginning of these studies.

**Apparatus.** Experiments were conducted in ventilated, sound attenuated chambers in which white noise masked extraneous sounds. During sessions monkeys sat in a customized Plexiglas chair facing a panel through which two easily accessible levers were centered and mounted 15 cm apart (model 121-05, BRS/LVE, Beltsville, MD). Above the levers were pairs of red stimulus lights that could be illuminated to serve as visual stimuli. Depression of either lever with a minimum force of 0.25 N resulted in an audible click and was recorded as a response. The chair was also fitted with a small stock to secure a shaved portion of the monkey’s tail beneath brass electrode plates. Electrode paste ensured a low-resistance contact between the tail and the electrodes. Brief, low-intensity electric shock (3.0 mA, 200 ms) could be delivered through the electrodes to the tail.

**Drug Discrimination.** Each monkey was trained under a 10-response fixed-ratio (FR10) schedule of stimulus-shock termination to respond differentially on the left or right lever, depending on whether enadoline or saline was injected intramuscularly. Under this schedule, the completion of 10 consecutive responses on the injection-appropriate lever within 10 s turned off red stimulus lights, and initiated a 40-s time-out (TO) period. If 10 responses were not completed, a mild electric shock was delivered to the shaved portion of the tail every 10 s. If the response requirement was not met within 40 s (four shocks), the cycle ended automatically and the 40-s TO period began. Responding on the left lever was associated with injections of enadoline for monkeys s322, s205, and s220, whereas responding on the right lever was associated with injections of enadoline for monkeys s323 and s484. Initially, the training dose of enadoline was 0.003 mg/kg for all monkeys. Subsequently, initial dose-effect determinations revealed that 0.0017 mg/kg enadoline produced 100% responding on the drug-associated lever in all monkeys. Consequently, the training dose was lowered to 0.0017 mg/kg and was maintained at that level throughout the studies.

Training sessions consisted of a varying number (n = 1–4) of components. Each component was preceded by a 10-min TO period and ended after the completion of 10 FRs or 800 s, whichever occurred first. During most training sessions, saline was injected during the TO periods preceding all but the last component of the session, and drug was injected during the TO preceding the last component. Periodically saline was injected during all TOs to limit the association between the last component and the injection of enadoline. Training continued until criteria of ≥90% of all responses and completion of all 10 FRs on the injection-appropriate lever were achieved for five consecutive sessions.

Test sessions were conducted no more than twice weekly and only following training sessions during which all FRs in each component and ≥90% of all responses were completed on the injection-appropriate lever. Test sessions consisted of four components, each preceded by a 10-min TO period. During each component, the completion of 10 consecutive responses on either lever turned off the red stimulus lights and initiated the 40-s TO period. Except for enadoline, each drug in substitution experiments generally was studied in a group of four monkeys. The full dose-effect function for enadoline was determined in all five monkeys.

Drugs were studied using a cumulative dosing procedure that has been described previously (Spealman, 1985; Rosenzweig-Lipson and Bergman, 1990). Briefly, incremental doses of all drugs, except EKC and morphine, were injected at the beginning of the 10-min TO periods. The TO was shortened to 5 min for EKC, which has a short duration of action and lengthened to 20 min for morphine, which has a slow onset to action. For some drugs, data on five or more doses were obtained by studying overlapping dose ranges of cumulative doses in different test sessions. The drugs were studied up to doses that 1) substituted fully for enadoline or 2) reduced response rates to <0.2 responses/s. Studies involving pretreatment were completed in groups of three or four monkeys, and were conducted by administering injections of different doses of quazadocine or nalbuphine 10 min before the test session. Pretreatment doses of both drugs were selected on the basis of data from preliminary experiments. Pretreatment studies also were conducted with the selective μ-opioid antagonist β-FNA, and the selective κ-opioid antagonist nor-BNI (Portoghese et al., 1980, 1987). Previous studies have indicated that the antagonistic effects of these drugs are slow in onset and persist for a prolonged period of time (Butelman et al., 1993; Broadbear et al., 1994; Jewett and Woods, 1995). Therefore, test sessions were run on consecutive days, 60 min and 24 h following administration of β-FNA or nor-BNI. Studies with nor-BNI were the last to be conducted and were selected on the basis of data from preliminary experiments.
its effects were studied for up to 80 days by suspending training during this time and periodically reiterating the dose-related effects of enadoline or other κ-opioid agonists in individual subjects. Pretreatment doses of β-FNA and nor-BNI were selected on the basis of published data (see above).

Analysis of Drug Effects. Data from drug discrimination experiments were analyzed for individual monkeys by computing the percent-age of responses on the enadoline-associated lever in each FR component, i.e., the number of responses on the enadoline lever was divided by the total number of responses on both levers, provided that the rate of responding in that component was ≥0.2 responses/s. When response rates were <0.2 responses/s, data were recorded but not analyzed further. A drug was considered to have substituted fully for the training dose of enadoline in individual monkeys when responding on the enadoline-associated lever was ≥90% and response rates were ≥0.2 responses/s in all monkeys. When effects were consistent across monkeys, data are expressed as percentage of responding on the enadoline-associa-
ted lever averaged for the group of monkeys (mean ± S.E.M.). Additionally, response rates were calculated for each session component by dividing the total number of responses made on either lever by the total time during which stimulus lights were illuminated. Response rates are expressed as responses per second averaged for the group of monkeys (mean ± S.E.M.). For drugs that substituted for enadoline alone or in the presence of an antagonist, estimated ED$_{50}$ values were calculated by log-linear interpolation along the ascending portion of individual dose-effect functions and averaged for the group of monkeys. Average estimated ED$_{50}$ values were used to 1) establish potency relationships among drugs that substituted for enadoline, and 2) examine antagonist of the discriminative stimulus effects of enadoline by dose-ratio analysis. For dose-ratio analysis, dose ratios were calculated for enadoline in combination with quadazocine, nalbuphine, and nor-BNI by dividing the estimated ED$_{50}$ for enadoline in the presence of each dose of antagonist by the estimated ED$_{50}$ for enadoline alone. For quadazocine and nalbuphine, at least three doses of each drug were used in combination with enadoline, and dose-ratio analyses were used to calculate pA$_2$ values. For this analysis, the log of the dose-ratio minus 1 was plotted as a function of the negative log of the antagonist dose in moles per kilogram. These points were used to derive regression lines for agonist/antagonist interactions; the apparent pA$_2$ value was defined as the point where the regression line intersected the abscissa (i.e., where the dose-ratio equals 2). Despite some inter subject variability, the confidence interval of the slope of each calculated regression line included unity; consequently, slopes were not constrained to −1 for further analysis. All apparent pA$_2$ values were performed using the Pharmacological Calculation System, version 3, based on the procedures of Tallarida et al. (1979).

Drugs. All drugs were administered intramuscularly into the thigh or calf muscle of the seated monkey. All compounds were dissolved in sterile 0.9% saline. Drugs were obtained from the following sources: enadoline, its (±)-enantiomer PD 129829, and PD 117,302 (Parke-Davis Pharmaceuticals, Cambridge, UK and Ann Arbor, MI); GR 89686A (Glaxo Research Inc., Research Triangle Park, NC); U 50,488, the enantiomers of spiradoline, U 63640 (1R,4R)-spiradoline (Upjohn, Kalamazoo, MI); bremazocine (Sandoz, Basel, Switzerland); ethylketocyclazocine and quadazocine (Sterling-Winthrop, Rensselaer, NY); BW 373U86 (Burroughs Welcome, Research Triangle Park, NC); IC 204448 (Zeneca Pharmaceuticals, Wilmington, DE); morphine (Sigma Chemical Co., St. Louis, MO); nalorphine (National Institute on Drug Abuse, Rockville, MD); EMD 60400 (E. Merck, Darmstadt, Germany); nalbuphine (DuPont, Wilmington, DE); butorphanol (Bristol-Meyers, Wallingford, CT); β-funaltrexamine; and nor-binal-
torphimine (Research Biochemicals International, Natick, MA).

Results

Initial Training. Monkeys met testing criteria over an average of 66 trials (range 24–124) following the initiation of discrimination training with 0.003 mg/kg enadoline. Injection of this dose of enadoline consistently elevated response rates above those associated with injections of saline. For the group of monkeys, response rates averaged 2.32 ± 0.02 responses/s following injections of enadoline and 1.79 ± 0.01 responses/s following injections of saline.

Effects of Enadoline. Following training, test sessions began with determination of the effects of enadoline. Administration of graded doses of enadoline (0.0001–0.01 mg/kg) before sequential components of the sessions produced dose-related increases in responding on the enadoline-associated lever, and full substitution (≥90%) was observed at doses of 0.0017 and 0.003 mg/kg enadoline. The training dose was therefore lowered to 0.0017 mg/kg for all monkeys and the effects of enadoline alone were periodically redetermined throughout the course of the present studies. Over the course of redeterminations, full substitution eventually was observed following administration of the cumulative dose of 0.001 mg/kg in all monkeys. Nevertheless, the training dose was kept at 0.0017 mg/kg to maintain consistency in the experimental protocol throughout substitution and antagonism studies. At doses of enadoline that produced full substitution (0.001–0.003 mg/kg), rates of responding continued to be elevated compared with saline control values. Administration of yet higher doses of enadoline (0.01 and 0.03 mg/kg) resulted in a slight decrease in the level of enadoline-associated responding and reduced response rates to an average of approximately 0.3 responses/s for the group of monkeys (Fig. 1).

Time course studies showed that responding was maintained exclusively on the enadoline-associated lever for approximately 50 min following injection of the training dose of 0.0017 mg/kg (Fig. 2). After 70 min, the level of enadoline-associated responding decreased to an average of approximately 80% for the group of monkeys. Testing at 90-min postinjection revealed approximately 40% responding on the enadoline-associated lever and, after 110 min, monkeys responded exclusively on the saline-associated lever.

Effects of Selective Opioid Agonists. Administration of the κ-selective opioids PD 117302 (0.03–0.3 mg/kg), U 50488H (0.03–0.3 mg/kg), GR 89686A (0.0003–0.003 mg/kg), and (−)-spiradoline (0.01–0.1 mg/kg) produced dose-related increases in responding and ≥90% responding on the enadoline-associated lever in all monkeys. Except for (−)-spiradoline, full substitution for enadoline by these opioids was produced at doses that, except for elevated response rates, did not markedly disrupt fixed-ratio performance. For (−)-spiradoline, full substitution was produced at doses that disrupted the fixed-ratio pattern of responding and reduced response rates to approximately 1.0 response/s for the group of monkeys (Fig. 3). Like κ-selective opioid agonists, the mixed-action κ/μ-opioids bremazocine (0.0003–0.003 mg/kg) and EKC (0.003–0.03 mg/kg) substituted fully for enadoline at doses that did not greatly affect response rates. In addition, two κ-selective opioids that have been reported to penetrate the brain poorly, EMD 61753 and IC 204448 (Shaw et al., 1989; Barber et al., 1994), produced dose-dependent increases in enadoline-associated responding; the highest cumulative doses, 1.0 and 3.0 mg/kg, respectively, fully substituted for the training drug in all monkeys. As with the other κ-agonists, the doses of EMD 61753 and IC 204448 that
produced enadoline-lever responding did not markedly disrupt fixed-ratio performance (Fig. 3).

For all drugs that substituted fully for the training dose of enadoline in these experiments, comparison of the dose estimated to produce 50% responding on the enadoline-associated lever (ED50) revealed the following potency relationship:

enadoline > GR 89686A > bremazocine > EKC > (-)-spiroadoline > PD 117302 > U 50488H > EMD 61753 > ICI 204448 (Table 1). With few exceptions (EMD 61753 and ICI 204448, which penetrate the brain poorly, and EKC), the rank order of potency with which \( \kappa \)-agonists reproduced the discriminative stimulus effects of enadoline appears to correlate well with their potency for eliciting other \( \kappa \)-mediated effects, e.g., reduction of electrically evoked contractions in rabbit vas deferens \( r = 0.87, F(1,5) = 15.6, p = 0.01; \) Table 1).

Unlike selective \( \kappa \)-opioid agonists, the \( \mu \)-opioid agonist morphine (0.3–5.6 mg/kg) and the \( \delta \)-opioid agonist BW 373U86 (0.1–0.3 mg/kg) did not increase responding on the enadoline-associated lever and, at the highest doses, markedly decreased response rates. As well, enadoline-appropriate responding was not observed following cumulative doses of the (+)-enantiomer of enadoline PD 129829 (0.1–1.0 mg/
iorally relevant doses were studied. Agonists, elevated rates of responding, indicating that behav-

or of both drugs, like other

did not effectively substitute for enadoline, the highest doses

phine (data not shown). Although nalbuphine and nalorphine

lever following cumulative doses as high as 30 mg/kg nalbu-

phine was found to substitute for enadoline but again, rede-

ed no longer observed. Subsequently, 17.8 mg/kg nalbu-

doses, however, responding on the enadoline-associated lever

respectively. Upon redetermination of the effects of the same

monkeys trained to discriminate enadoline from saline. Other details as in Fig. 3.

ED$_{50}$ for the discriminative stimulus effects of enadoline. A

Schild plot of the antagonist effects of quadazocine revealed an apparent pA$_2$ of 6.92 ± 0.49 for the group of monkeys (slope = $-0.87 ± 0.34$). In addition to antagonizing the discriminative stimulus effects of enadoline, quadazocine also antagonized the rate-decreasing effects of the highest doses of enadoline (0.01 and 0.03 mg/kg). However, the an-
tagonism of these effects was not fully characterized to avoid untoward effects that might accompany administration of yet higher doses of enadoline (>0.1 mg/kg).

As with quadazocine, pretreatment doses of nalbuphine (0.3–30 mg/kg) produced consistent dose-dependent rightward shifts in the discriminative stimulus effects of enadoline in all monkeys (Fig. 6). Averaged for the group of mon-
knots, the lowest dose of nalbuphine (0.3 mg/kg) produced an

approximately 3-fold increase in the estimated ED$_{50}$ for the discriminative stimulus effects of enadoline. Higher doses of nalbuphine, 3.0 and 30 mg/kg, produced correspondingly greater rightward shifts in the dose-effect function for enado-

line, with approximately 30- and 90-fold increases, respec-
tively, in estimated ED$_{50}$ values. Schild analysis of the an-
tagonist effects of nalbuphine revealed an apparent pA$_2$

value of 5.86 ± 0.46 for the group of monkeys (mean slope = $-0.77 ± 0.25$). In addition to antagonizing the discriminative stimulus effects of enadoline, nalbuphine also produced dose-
dependent rightward shifts in the rate decreasing effects of

kg) or (+)-spiradoline (0.3–3.0 mg/kg). These enantiomers

were tested to doses up to 1000-fold greater than doses of

enadoline or (+)-spiradoline that substituted for the training
dose of enadoline. At the highest doses, rates of responding

were not disrupted by injection of either drug (data not shown).

Effects of Mixed-Action $\kappa/\mu$-Opioids. In contrast to

EKC and bremazocine, nalbuphine (0.1–30 mg/kg) and na-

lorphine (0.03–30 mg/kg), which also act at $\mu$- and $\kappa$-recep-
tors (Leander, 1983; Schmidt et al., 1985) did not engender
dose-related responding on the enadoline-associated lever
(Fig. 4). In one monkey (s322), administration of nalbuphine

or nalorphine initially produced full enadoline-associated re-
sponding following cumulative doses of 3.0 or 0.3 mg/kg,
respectively. Upon redetermination of the effects of the same
doses, however, responding on the enadoline-associated lever
was no longer observed. Subsequently, 17.8 mg/kg nalbu-

phine was found to substitute for enadoline but again, rede-

termination of the dose-effect function showed that respond-
ing was confined almost exclusively to the saline-associated lever following cumulative doses as high as 30 mg/kg nalbu-

phine (data not shown). Although nalbuphine and nalorphine
did not effectively substitute for enadoline, the highest doses

of both drugs, like other $\kappa$-selective and mixed-action $\kappa/\mu$

agonists, elevated rates of responding, indicating that behav-
orally relevant doses were studied.

Antagonism Studies. Pretreatment with the nonselective

opioid antagonist quadazocine (0.03–30 mg/kg), but not the

$\mu$-selective antagonist $\beta$-FNA (data not shown), attenu-

ated the discriminative stimulus and the rate-decreasing

effects of enadoline in all monkeys. Attenuation of the dis-

criminative stimulus effects of enadoline by quadazocine was

categorized by rightward shifts of the dose-effect function,

indicative of surmountable antagonism (Fig. 5). The degree

to which the enadoline-discrimination function was displaced

rightward varied as a function of antagonist dose: lower

doses of quadazocine (0.03 and 0.3 mg/kg) produced a 2- and

4-fold increase, respectively, in the estimated ED$_{50}$ for the

group of monkeys, whereas the highest dose of quadazocine

(3.0 mg/kg) produced over a 30-fold increase in the estimated

TABLE 1

Doses of $\kappa$-opioid agonists estimated to produce 50% enadoline lever responding, their relative potencies in the present experiments, and their relative potencies in studies of electrically evoked contractions in rabbit vas deferens.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Enadoline Discrimination</th>
<th>Relative Potency</th>
<th>Relative Potency to Reduce Electrically Evoked Contraction in Rabbit Vas Deferens $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enadoline</td>
<td>$0.0006 \pm 0.0001$</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>GR 89696A</td>
<td>$0.0006 \pm 0.0001$</td>
<td>1.0</td>
<td>0.03$^b$</td>
</tr>
<tr>
<td>Bremazocine</td>
<td>$0.0018 \pm 0.0002$</td>
<td>4.4</td>
<td>3.1</td>
</tr>
<tr>
<td>EKC</td>
<td>$0.008 \pm 0.003$</td>
<td>13.3</td>
<td>6.8</td>
</tr>
<tr>
<td>(+)-Spiro-</td>
<td>$0.03 \pm 0.012$</td>
<td>50</td>
<td>8.7</td>
</tr>
<tr>
<td>Spiradoline</td>
<td>$0.07 \pm 0.014$</td>
<td>115</td>
<td>17.6</td>
</tr>
<tr>
<td>PD 117,302</td>
<td>$0.13 \pm 0.02$</td>
<td>217</td>
<td>67</td>
</tr>
<tr>
<td>U 50,488</td>
<td>$0.18 \pm 0.03$</td>
<td>300</td>
<td>16.5</td>
</tr>
<tr>
<td>EMD 61753</td>
<td>$1.22 \pm 0.52$</td>
<td>2003</td>
<td>4.5$^c$</td>
</tr>
</tbody>
</table>

$^a$ Relative potency for each drug calculated by dividing its IC$_{50}$ (nM) by the IC$_{50}$ (nM) of enadoline. Data taken from Hunter et al. (1990).

$^b$ Barber et al. (1994).

$^c$ Hayes et al. (1990).

Relative potency for each drug calculated by dividing its IC$_{50}$ (nM) by the IC$_{50}$ (nM) of enadoline. Data taken from Hunter et al. (1990).

$^d$ Hunter et al. (1990).

$^e$ Barber et al. (1994).

$^f$ Schild analysis of the antagonist effects of quadazocine revealed an apparent pA$_2$ of 6.92 ± 0.49 for the group of monkeys (slope = $-0.87 ± 0.34$). In addition to antagonizing the discriminative stimulus effects of enadoline, quadazocine also antagonized the rate-decreasing effects of the highest doses of enadoline (0.01 and 0.03 mg/kg). However, the an-
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dependent rightward shifts in the rate decreasing effects of

**Fig. 4.** Effect of the mixed-action opioids nalorphine and nalbuphine in monkeys trained to discriminate enadoline from saline. Other details as in Fig. 3.
enadoline. Comparisons of ED$_{50}$ values alone and following pretreatment indicated that 0.3 mg/kg nalbuphine had little, if any, antagonist action, whereas 3.0 mg/kg nalbuphine produced an approximately 4-fold increase in average ED$_{50}$ values. Pretreatment with 30 mg/kg nalbuphine further antagonized the effects of high doses of enadoline on rates of responding, and, following the highest cumulative dose of enadoline (0.1 mg/kg) in the presence of this dose of nalbuphine, averaged response rates remained above 50% of control values.

Nor-BNI, a selective and reportedly long-acting $\kappa$-selective receptor blocker in behavioral studies (Portoghese et al., 1987; Butelman et al., 1993; Jewett and Woods, 1995) also antagonized the effects of enadoline in the present experiments. Dose-dependent rightward shifts in the behavioral effects of enadoline were evident in all monkeys following treatment with 3.0 mg/kg and, subsequently, 10.0 mg/kg, of nor-BNI. The effects of nor-BNI were relatively slow to onset and long-lived. Whereas neither substitution for enadoline nor antagonism were observed within 60 min following i.m. administration of 3.0 mg/kg nor-BNI, antagonism was apparent 24 h later and persisted for more than 20 days. Six days following treatment, the dose-effect function for enadoline was shifted rightward by an average of more than 0.5 log unit (0.25–0.75 log units in individual monkeys; Fig. 7). The effects of 3.0 mg/kg nor-BNI diminished thereafter but, on average, had not completely dissipated by day 14 (Fig. 8). The third monkey, discriminative control of performance by enadoline no longer was fully evident by the 9th day following treatment with 10.0 mg/kg nor-BNI and, in subsequent sessions, cumulative doses of enadoline up to 0.1 mg/kg failed to engender appreciable responding on the enadoline-associated lever. However, even in this subject, evidence of antagonism persisted through day 48 following treatment with 10.0 mg/kg nor-BNI, as doses of enadoline up to 0.01 mg/kg did not markedly decrease responding (data not shown). In the three monkeys for which full dose-effect data still were obtained, the averaged ED$_{50}$ value for enadoline discrimination after 10 weeks continued to be 0.75 log units higher than before treatment with nor-BNI.

In additional experiments, the enadoline-like discriminative stimulus effects of U 50,488, bremazocine, and EKC were also antagonized by nor-BNI. Based on data obtained in three monkeys studied 18 to 25 days after pretreatment with 10.0 mg/kg nor-BNI, the dose-effect function for each drug was shifted rightward and average ED$_{50}$ values were increased by approximately 10- to 20-fold (Table 2).

**Discussion**

**Effects of Enadoline Alone.** The present results, indicating that the $\kappa$-selective opioid agonist enadoline can serve as a reliable discriminative stimulus in squirrel monkeys, are consistent with the findings of Brandt and France (1996) in enadoline-trained pigeons and extend them to a primate
species. The discriminative stimulus effects of enadoline occurred at doses that moderately increased fixed-ratio response rates maintained under a schedule of stimulus-shock termination above values associated with vehicle injection. These observations are in keeping with earlier findings that \( \kappa \)-opioids may increase response rates under schedules of stimulus-shock termination at doses that typically decrease responding maintained by food presentation (Bergman and Warren, 1989).

**\( \kappa \)-Agonist Substitution for Enadoline.** The discriminative stimulus effects of enadoline were reproduced by other \( \kappa \)-selective opioid agonists and mixed-action \( \kappa/\mu \)-opioid agonists but not by \( \mu \)- and \( \delta \)-selective agonists. These results generally agree with findings in monkeys trained to discriminate the mixed-action \( \kappa/\mu \)-agonist EKC (Hein et al., 1981; Young and Stephens, 1984; Dykstra et al., 1987a; France et al., 1994) and suggest commonality in their mechanism of action. Furthermore, the discriminative stimulus effects of enadoline were not reproduced by the (+)-isomers of enadoline or spiradoline in the present studies, indicating the stereoselectivity of the interaction of enadoline with the \( \kappa \)-receptors mediating its effects. The relative potency with which the \( \kappa \)-agonists used in the present study reproduced enadoline’s discriminative stimulus effects are in close agreement with the relative potencies of these compounds in other behavioral studies (Dykstra et al., 1987a,b; Broadbear et al., 1994; Smith and Picker, 1995; Brandt and France, 1996), and correlate well with their ability to reduce electrically induced contractions in rabbit vas deferens in vitro (Hayes et al., 1990; Hunter et al., 1990; Barber et al., 1994). This finding suggests that the intrinsic efficacy of the compounds at \( \kappa \)-receptors influences their ability to produce the behavioral effects observed in the present studies.

Interestingly, the discriminative stimulus effects of enadoline also were reproduced by EMD 61743 and ICI 204448, two \( \kappa \)-selective agonists that are reported to penetrate the brain to a limited extent (Shaw et al., 1989; Barber et al., 1994). On the basis of their reported \( \kappa \)-opioid affinities, the doses administered in the present studies ensured that concentrations sufficient to reproduce enadoline’s discriminative stimulus penetrated the central nervous system and likely were considerably higher than those required to produce peripheral \( \kappa \)-receptor-mediated actions.

**Effects of Nalorphine and Nalbuphine.** Both mixed-action opioids nalorphine and nalbuphine have affinity for \( \kappa \)- and \( \mu \)-receptors and, depending on the species and conditions of the experiment, may produce agonist effects through \( \kappa \)- or \( \mu \)-actions (Leander, 1983; Schmidt et al., 1985; France et al., 1994; Gerak et al., 1994). On the basis of their reported \( \kappa \)-opioid affinities, the doses administered in the present studies ensured that concentrations sufficient to reproduce enadoline’s discriminative stimulus penetrated the central nervous system and likely were considerably higher than those required to produce peripheral \( \kappa \)-receptor-mediated actions.

**TABLE 2**

<table>
<thead>
<tr>
<th>Drug</th>
<th>( \text{ED}_{50} ) for Enadoline-Discrimination (mean \pm 95% CI)</th>
<th>Drug Alone</th>
<th>Drug after 10 mg/kg nor-BNI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enadoline</td>
<td>0.0004 (0.0002–0.0009)</td>
<td>0.005 (0.003–0.009)</td>
<td></td>
</tr>
<tr>
<td>U 50,488</td>
<td>0.11 (0.08–0.13)</td>
<td>0.71 (0.11–4.76)</td>
<td></td>
</tr>
<tr>
<td>Bremaezocine</td>
<td>0.00173 (0.00172–0.00174)</td>
<td>0.02 (0.016–0.029)</td>
<td></td>
</tr>
<tr>
<td>EKC</td>
<td>0.011 (0.010–0.011)</td>
<td>0.02 (0.004–0.1)</td>
<td></td>
</tr>
</tbody>
</table>

### References
- France et al., 1994.
- Dykstra et al., 1987a.
- France et al., 1994.
- Brandt and France, 1996.
- Shaw et al., 1989.
- Hunter et al., 1990.
- Barber et al., 1994.
- Hayes et al., 1990.
- Brandt and France, 1996.
- Leander, 1983.
- Schmidt et al., 1985.
- France et al., 1994.
- Gerak et al., 1994.
and Stephens, 1984; France et al., 1994). Consistent with μ-receptor mediation of its effects, μ-opioid agonists, but not enadoline and other κ-opioid agonists, have been found to fully reproduce the effects of nalbuphine in nalbuphine-trained monkeys (Gerak and France, 1996). In the present study, neither nalbuphine nor nalorphine consistently reproduced the discriminative stimulus effects of enadoline. These findings in monkeys are consistent with those of Brandt and France (1996), showing that neither nalorphine nor nalbuphine reproduced the discriminative stimulus effects of enadoline in pigeons. Previous studies have provided evidence that nalorphine may serve as a low-efficacy κ-agonist (Lean-der, 1983; France et al., 1994) and its ability to substitute for EKC but not enadoline may reflect a lower efficacy requirement for substitution in EKC-trained subjects than in enadoline-trained subjects. However, EKC, like nalorphine, is a mixed-action opioid and, possibly, a μ-mediated aspect of its discriminative stimulus effects may increase the likelihood of substitution by nalorphine. Unlike nalorphine, nalbuphine typically does not mimic the discriminative stimulus effects of EKC. In conjunction with the lack of substitution for enadoline by nalbuphine, its ability to surmountably antagonize the effects of enadoline (see below) encourages the view that nalbuphine has relatively low efficacy as a κ-agonist.

Despite the lack of substitution for enadoline, the highest doses of nalbuphine and nalorphine (30 mg/kg) produced elevations in response rate comparable with the rate-increasing effects observed with intermediate doses of enadoline. As noted above, κ-opioid agonists previously have been reported to produce increases in response rate under schedules of stimulus-shock termination in squirrel monkeys and the rate-increasing effects of κ-agonists such as nalbuphine and nalorphine may result from similar mechanisms of action. However, previous studies have shown that the rate-increasing effects of κ-agonists are not antagonized by high doses of the nonselective opioid receptor blocker naltrexone, suggesting that these effects either are the product of nonopioid actions or are mediated through naltrexone-insensitive κ-opioid mechanisms (Bergman and Warren, 1989).

Antagonism of Enadoline Discrimination. In the present study, the nonselective opioid receptor blocker quazadocine, the mixed-action agonist nalbuphine, and the κ-selective antagonist nor-BNI generally produced dose-dependent rightward shifts in the dose-effect function for enadoline discrimination and, as well, attenuated the rate-decreasing effects of high doses of enadoline. In contrast, a high dose of β-FNA, which previously has been shown to antagonize the behavioral effects of μ-agonists (Picker and Dykstra, 1987b; Dykstra et al., 1989), was without antagonistic effect. These data indicate that the discriminative stimulus effects of enadoline, which recently was reported to have only approximately 50-fold selectivity in affinity for κ-compared with μ-receptors in monkey brain (France et al., 1994), may be wholly ascribed to its κ-opioid actions.

The effects of nor-BNI were remarkably persistent (>80 days), consistent with its previous characterization as a long-lasting κ-opioid antagonist (Portoghese et al., 1987; Butelman et al., 1993; Jewett and Woods, 1995). Although control experiments were not conducted to ensure that the discriminative stimulus effects of enadoline were intact over the time training was suspended, several observations suggest this was indeed the case. First, loss of stimulus control most often leads to a loss of predictable dose response in discrimination, such as occurred in one monkey. Second, dose-related and predictable data during test sessions with enadoline were generated consistently in the three monkeys that were studied for >80 sessions despite the absence of explicit training sessions. In a sense, test sessions in which surmountable antagonism to enadoline was observed served as training sessions for these subjects. Third, antagonism of the rate-disruptive effects of enadoline was evident throughout the testing period, supporting the idea that the persistent rightward shift in the dose-effect function for enadoline discrimination revealed a true antagonism.

Previously, nor-BNI has been shown to antagonize antinoceptive effects of U 50,488 but not those of enadoline, bremazocine, or EKC in tail-withdrawal studies in rhesus monkeys (Butelman et al., 1993, 1998). These findings provided support in monkeys for the view that the existence of different subtypes of κ-opioid receptors in radioligand binding experiments might have functional consequences (Zukin et al., 1988; Clark et al., 1989; Butelman et al., 1998). This view has been strengthened by findings in monkeys that the κ-opioid peptide dynorphin may differentially antagonize the antinoceptive effects of κ agonists in a manner comparable with that observed in experiments with nor-BNI and, additionally from findings that pA2 values for antagonism by naltrexone differ for these effects of EKC and U 50,488, on the one hand, and bremazocine and enadoline, on the other (Butelman et al., 1995; Ko et al., 1998). In the present experiments, however, no clear difference was observed in the ability of nor-BNI to surmountably antagonize the discriminative stimulus effects of these different κ-opioids in enadoline-trained monkeys. Although limited in scope, these data do not provide further evidence for the functional importance of κ-receptor subtypes but, rather, are consistent with the view that the discriminative stimulus effects of κ-opioids in enadoline-trained subjects are not preferentially mediated through a single subtype of κ-opioid receptor.

Despite some variability in the average slope of the Schild plot regression, the pA2 values of 6.9 ± 0.5 for antagonism of enadoline discrimination by quazadocine and 5.9 ± 0.4 for antagonism by nalbuphine are comparable with pA2 values obtained with quazadocine and nalbuphine in other studies of behavioral effects of enadoline in monkeys and pigeons, respectively (Pitts and Dykstra, 1994; Brandt and France, 1996) and in other studies of the antagonism of behavioral effects of other κ-agonists in squirrel and rhesus monkeys (Bertalmio and Woods, 1987; Dykstra and Massie, 1988; Dykstra, 1990). Although nor-BNI appeared to surmountably antagonize the behavioral effects of enadoline, its long-lasting antagonist effects suggest that its actions are not the result of simple competition at a single receptor site, precluding the evaluation of its effects by pKp analysis. The mechanism by which nor-BNI exerts its actions is not yet understood but likely differs from mechanisms that underlie the effects of other long-acting antagonists such as β-FNA or clocinamox, which presumably act by reducing the μ-opioid receptor population. However, their effects dissipate as μ-opioid receptors are restored over the course of days, whereas the effects of nor-BNI in this and other studies appear to persist over the course of weeks (Jewett and Woods, 1995).
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References


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