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Differential antagonism of the rate-decreasing effects of κ-opioid receptor agonists by naltrexone and norbinaltorphimine [1]

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Abstract

Eight κ-opioid receptor agonists were examined for their effects in squirrel monkeys responding under a fixed interval 3-min schedule of stimulus termination. Six of these κ-opioid receptor agonists decreased dose-dependently the total number of responses and with an order of potency consistent with κ-opioid receptor interaction. Three of these κ-opioid receptor agonists, bremazocine, U69,593 {[(5*a*,7*a*,8*b*)-(+)-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl)] benzeneacetamide} and enadoline, were evaluated following pretreatment with 1.0 mg/kg of naltrexone or 3.0 mg/kg of norbinaltorphimine. The effects of the three agonists were antagonized significantly by naltrexone, but only those of bremazocine and U69,593 were antagonized significantly by norbinaltorphimine. Statistical analysis of the data averaged over six monkeys revealed that naltrexone was significantly more potent than norbinaltorphimine at antagonizing enadoline and U69,593, but naltrexone and norbinaltorphimine were equipotent at antagonizing bremazocine. Moreover, naltrexone was 8-fold more potent at antagonizing U69,593 and enadoline than at antagonizing bremazocine. These results suggest that under these conditions the effects of U69,593 and enadoline may be mediated, in part, by a different receptor population, perhaps a subtype of κ-opioid receptors, from the one that mediates the effects of bremazocine.

Keywords: κ-Opioid receptor; (Squirrel monkey); Fixed interval; Naltrexone; Nor-binaltorphimine

1. Introduction

Since the discovery of drugs that act selectively at κ-opioid receptors scientists have attempted to distinguish the mechanisms of action underlying the analgesic and unwanted side effects of these drugs. The hypothesis driving this line of investigation is that more selective κ-opioid receptor agonists can be developed that provide clinically useful analgesic effects without the unwanted side effects or abuse potential. To this end, functional differences between κ-opioid receptor agonists have been examined in various physiological and behavioral assays, suggesting the existence of subtypes of the κ-opioid receptor. For example, the κ-opioid receptor agonists U50,488 {[*trans*-(±)3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl) cyclohexyl] benzeneacetamide} and MR-2034 {[(−)-*a*-(l*r*, 5*r*,9*r*)-5,9-dimethyl-2-(1-tetrahydrofurfuryl-2′-hydro-6,7-benzomorphan)]}, but not ethylketocyclazocine and tifluadom, increase plasma levels of dihydroxyphenylacetic acid and homovanillic acid (Iyengar et al., 1986). In addition, whereas ethylketocyclazocine- and tifluadom-induced decreases in plasma levels of corticosterone and thyroid stimulating hormone are blocked with the opioid antagonist quadazocine, U50,488- and MR-2034-induced decreases are not. Furthermore, there are at least two studies in mice in which antinociceptive cross-tolerance between κ-opioid receptor agonists was not observed: U69,593 {[(5*a*,7*a*,8*b*)-(+)-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl)]benzene-acetamide} and bremazocine (Horan and Porreca, 1993), and U50,488 and naloxone benzoylhydrazone (Gistrak et al., 1989).

Traditionally, receptor-selective antagonists have been used to differentiate among receptors mediating the in vivo effects of various types of opioids (μ vs. κ vs. δ). The lack of antagonists selective for proposed subtypes of the κ-opioid receptor has been a major impediment to the differentiation and characterization of those receptors. Nonetheless, important behavioral distinctions among κ-opioid receptor agonists have been demonstrated using the

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non-selective opioid antagonists (antagonists that are capable of antagonizing μ- and/or δ- and/or κ-opioid receptor agonists) that are currently available. For example, in mice the antinociceptive effects of U69,593 are antagonized by the reported selective (De Costa et al., 1989) κ-opioid receptor antagonist (−)-UPHIT{1*S*,2*S*-*trans*-2-isothiocyanato-4,5-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl) cyclohexyl]benzeneacetamide}, but not by the non-selective (Dykstra et al., 1987; Pitts and Dykstra, 1994) opioid receptor antagonist quadazocine, whereas the antinociceptive effects of bremazocine are antagonized by quadazocine, but not by (−)-UPHIT (Horan et al., 1991, 1993). In these same studies, the antinociceptive effects of [D-Pen^2, D-Pen^5]enkephalin (δ-opioid receptor agonist) and Tyr-D-Ala-Gly-NMe-Gly-ol (μ-opioid receptor agonist) are antagonized by quadazocine at doses 3-fold less than the dose required to antagonize bremazocine. Additionally, in pigeons, lower doses of the non-selective opioid antagonists naltrexone and naloxone are required to antagonize the rate-decreasing and discriminative stimulus effects of bremazocine and enadoline than are required to antagonize these effects of U50,488 (Mattox et al., 1994; Picker, 1994). Furthermore, naltrexone differs in its ability to antagonize the rate-decreasing effects of bremazocine compared to U50,488 in rats (Pitts et al., 1996). The selective κ-opioid receptor antagonist norbinaltorphimine (Portoghese et al., 1987) antagonizes the antinociceptive effects of U69,593 and U50,488, but not those of bremazocine, enadoline, and ethylketocyclazocine in rhesus monkeys (Butelman et al., 1993). Similarly, the non-selective opioid antagonist naltrexone is much more potent at antagonizing the antinociceptive effects of U50,488 and U69,593 than at antagonizing the effects of bremazocine and enadoline in rhesus monkeys (Ko et al., 1998). Together, these reports suggest that non-selective antagonists might be useful in differentiating behavioral effects mediated by different receptor subtypes.

Along this line, some investigators have suggested that the differences in the pharmacological profiles of these antagonists against different κ-opioid receptor agonists represent activity at different subtypes of the κ-opioid receptor (Horan et al., 1993; Ko et al., 1998). Based on that assumption and in order to provide further evidence for κ-opioid receptor subtypes, the purpose of the current study was to compare the antagonism by naltrexone and norbinaltorphimine of the effects of various κ-opioid receptor agonists in squirrel monkeys responding under a fixed interval schedule of stimulus/shock termination. This stimulus termination procedure was chosen based on our observations that it is sensitive to the κ-opioid receptor-mediated rate-decreasing effects of κ-opioid receptor agonists (Jones and Holtzman, 1994). The κ-opioid receptor agonists that were examined, bremazocine (Romer et al., 1980), enadoline (Boyle et al., 1990), ethylketocyclazocine (Young and Stephens, 1984), nalorphine (Tang and Code, 1983), PD 117302 [(±)-*trans*-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzo[*b*]thiophene-4-acetamide] (Clark et al., 1989), spiradoline (VonVoigtlander and Lewis, 1988), U50,488 (VonVoigtlander et al., 1983), and U69,593 (Lahti et al., 1985), represent two chemical families and a range of relative affinities for κ-opioid receptors (see Nock et al., 1988a; France et al., 1994). In order to establish that κ-opioid receptors mediate the dependent measure in the present study, the rank order of potencies for the kappa agonists in this study were compared to the potency order of the same drugs in different procedures. Similar rank orders of potency across procedures is suggestive that the effects are mediated by the same population of receptors. In addition, antagonism of the effects of these κ-opioid receptor agonists by the selective κ-opioid receptor antagonist norbinaltorphimine should rule out non-κ-opioid receptors as the site of action for these effects. Finally, we compared the ability of the non-selective opioid receptor antagonist naltrexone to antagonize the effects of three of the κ-opioid receptor agonists. If these κ-opioid receptor agonists produce their effects through a homogenous receptor population, the potency of a given antagonist should not differ across agonists.

2. Materials and methods

2.1. Subjects

Six squirrel monkeys (*Saimiri sciureus*) with previous experience on a fixed interval stimulus termination schedule and previous exposure to various opioid drugs were used in the present study. All monkeys were drug-free for at least 1 year prior to this experiment. Monkeys were given food and water ad libitum in the home cages and received fresh fruit, peanuts, or a nutritional supplement daily. The facility for housing the animals was accredited by the American Association for Accreditation of Laboratory Animal Care and care of the animals conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus

The monkeys were restrained in a small primate chair that was placed inside a ventilated, sound-attenuating chamber provided with white noise during the experimental sessions. The chamber was illuminated by a house light that was mounted on the rear wall of the chamber. A shaved portion of the monkey's tail was secured beneath two brass electrodes and held in place by a Plexiglas stock; the electrodes were connected to a current generator (Med Associates, Fairfield, VT). One response lever was mounted on the front wall of the test chair. Experimental events were controlled and data were recorded with a microcomputer.

2.3. Procedure

The monkeys were trained under a fixed interval 3-min schedule of stimulus termination with a 5-s limited hold. The houselight illuminated the chamber during the 3-min interval. After 3 min elapsed, the monkey had 5 s to press the lever in order to terminate the houselight and the impending electrical stimulus (tail shock). When a single response was made during the limited hold, the houselight was extinguished, the electrical stimulus was avoided, and a 60-s timeout period began during which the chamber was dark and lever presses had no programmed consequences. If the monkey failed to make a lever press within the 5-s limited hold, a 3- to 4-mA electrical stimulus of a 1-s duration was delivered every 5 s until either the monkey pressed the lever or five stimuli were delivered. After that a 60-s timeout period began during which lever responses had no programmed consequences. Each daily session consisted of 20 consecutive 3-min fixed interval components separated by 60-s timeout periods. Experimental sessions were conducted 5 days per week. Baseline sessions during which no injections were given were conducted on Mondays and Wednesdays. Control sessions that were preceded by a vehicle injection were conducted on Thursdays. Drug tests were conducted on Tuesdays and Fridays. Stable baseline performance was defined as 10 consecutive sessions of consistent rates and number of responding (within 20% of the mean for pretest control sessions) and fewer than four electrical stimuli received per session. An experimental session was terminated and data from that session was excluded from the analyses if a subject failed to complete any 3 of the 20 trials.

Once performance stabilized, dose–effect curves for bremazocine, enadoline, U69,593, PD 117302, spiradoline, ethylketocyclazocine, U50,488, and nalorphine were obtained. The performance on the day before a drug test session served as the baseline comparison for that test day. Once dose–effect curves were obtained for the κ-opioid receptor agonists alone, the dose–effect curves for bremazocine, U69,593 and enadoline were redetermined in the presence of 1.0 mg/kg of naltrexone or following 3.0 mg/kg of norbinaltorphimine. Drugs and drug doses were administered in a random sequence and were given intramuscularly 15 min before a session, with the exception of naltrexone and norbinaltorphimine when combined with the κ-opioid receptor agonists. For these test sessions, naltrexone was administered 5 min prior to the agonist dose (20 min total pretreat time). The κ-opioid receptor antagonist effects of norbinaltorphimine last up to 3 weeks in rhesus and squirrel monkeys (J. Bergman, personal communication; Butelman et al., 1993). In addition, pilot testing in our laboratory showed that norbinaltorphimine is equally effective as an antagonist against a single dose of a κ-opioid receptor agonist for up to 3 weeks. In the present study, effects of various doses of bremazocine, U69,593 and enadoline were redetermined in random order between 3 (normal time between test sessions) and 21 days after a dose of 3.0 mg/kg of norbinaltorphimine was administered. The random nature of testing κ-opioid receptor agonist doses following norbinaltorphimine allowed comparison of norbinaltorphimine's antagonist properties between doses and animals in order to determine whether these properties were changing over the 3 weeks. The doses of naltrexone and norbinaltorphimine were selected based on their ability to antagonize opioid agonist effects without themselves affecting rates of responding (Bergman and Warren, 1989; Butelman et al., 1993). All six monkeys required two norbinaltorphimine treatments to complete the agonist/antagonist combinations and the second injection of norbinaltorphimine was administered 3 weeks after the first injection.

2.4. Drugs

U69,593, U50,488, spiradoline mesylate and bremazocine hydrochloride were purchased from Research Biochemicals (Natick, MA). Nalorphine hydrochloride was a gift from Merck Sharpe and Dohme (West Point, PA), ethylketocyclazocine was a gift from Sterling-Winthrop Research Inst. (Rensselaer, NY) and enadoline and PD 117302 were gifts from Parke-Davis/Warner-Lambert (Ann Arbor, MI). Naltrexone hydrochloride was purchased from Sigma (St. Louis, MO) and norbinaltorphimine was obtained from the National Institute on Drug Abuse (Rockville, MD). All drugs were calculated as the base form of the drug, dissolved in distilled water and administered in a volume of 0.3 ml/kg.

2.5. Data analysis

For the effects of the κ-opioid receptor agonists alone, ED_{50} values with 95% confidence intervals (CI) were determined by log linear regression for each monkey and then were averaged across the SIX monkeys. ED_{50} values were calculated for the dose–effect curves of each κ-opioid receptor agonist alone and combined with 1.0 mg/kg naltrexone or following 3.0 mg/kg norbinaltorphimine, using the descending portion of the curves. A 50% effect was determined for each monkey as half the averaged total responses across control sessions.

The rank order of potency for the eight κ-opioid receptor agonists was determined by comparing the mean ED_{50} values, using a one-way analysis of variance (ANOVA) and Tukey–Kramer's test for post-hoc multiple comparisons. The mean ED_{50} values for the κ-opioid receptor agonists alone and combined with the antagonists were compared, using a one-way repeated measures ANOVA and a Dunnett multiple comparison test for post-hoc analysis. In addition, slopes ($\pm$95% CI) were calculated for all the individual dose–effect curves, using points on the descending portion of the curves where possible and averaged across the six monkeys. In instances where there is

Table 1
Mean ED_{50} values for each κ-opioid agonist with 95% confidence intervals listed in order of potency

Kappa agonist	ED_{50} (mg/kg)	95% CI
Bremazocine	0.01	(0.001–0.02)[a]
Enadoline	0.01	(0.003–0.02)[a]
Ethylketocyclazocine	0.05	(0.02–0.3)
U69,593	0.08	(0.02–0.2)
PD 117302	0.55	(0.2–0.9)
Spiradoline	0.64	(0.1–1.2)
U50,488	1.1	(0.2–2.0)
Nalorphine	> 56	[b]

[a]Significantly different from PD 117302, spiradoline and U50,488.
[b]Could not be determined.

no descending portion of a curve, data for that subject are not included in the analysis (e.g., S54-bremazocine + naltrexone; S59-U69,593 + naltrexone). The mean slopes were compared for parallelism using a repeated measures ANOVA and Tukey–Kramer's test for post-hoc multiple comparisons.

In addition, relative apparent pK_B values (±95% CI) were determined for 1.0 mg/kg of naltrexone in combination with bremazocine, enadoline and U69,593 by using the method of Tallerida et al. (1979), as modified by Negus et al. (1993), where

$$pK_B = -\log\left[B/(DR-1)\right]$$

''B'' represents the dose of the antagonist in moles per kilogram and ''DR'' the dose ratio obtained by dividing the ED_{50} value of the agonist plus antagonist by the ED_{50} value of the agonist alone. The pK_B values were compared using the Kruskal–Wallis non-parametric ANOVA and Dunn's multiple comparisons test. For all statistical analyses, a significant main effect was determined by a $P < 0.05$. It is understood that relative apparent pK_B values are a limited measure of antagonist affinity for a receptor; however, when combined with other data, these results can provide supportive evidence for differences in antagonist affinity.

3. Results

Non-drug experimental sessions were characterized by low responding early in the fixed interval followed by gradually increased responding as the 3-min interval elapsed, a pattern of responding commonly associated with fixed interval schedules. Total responses across the daily

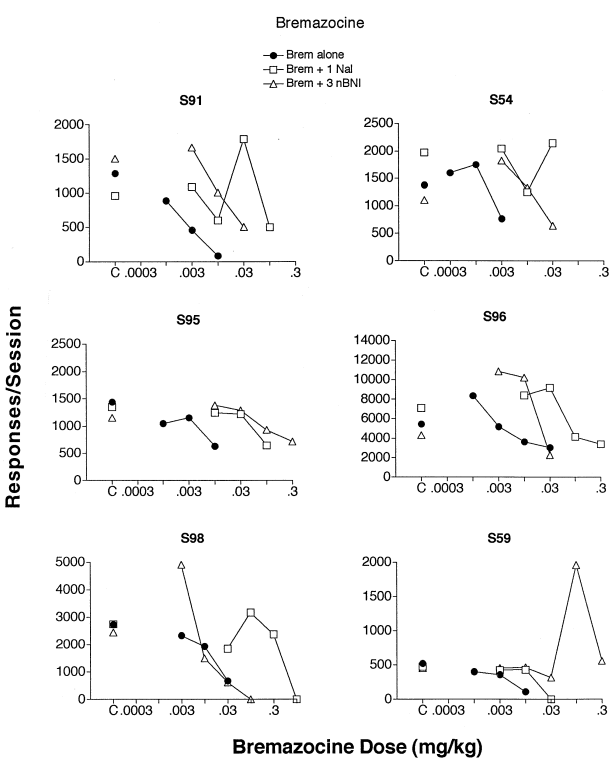

Fig. 1. The total number of responses following the administration of bremazocine alone (closed circles) and in the presence of 1.0 mg/kg of naltrexone (open squares) or following (by 3–21 days) the administration of 3.0 mg/kg of norbinaltorphimine (open triangles) in six monkeys. The points above C represent the mean total responses (±1 S.E.) following the administration of vehicle (closed circle), 1.0 mg/kg of naltrexone (open square) and 3.0 mg/kg of norbinaltorphimine (open triangle).

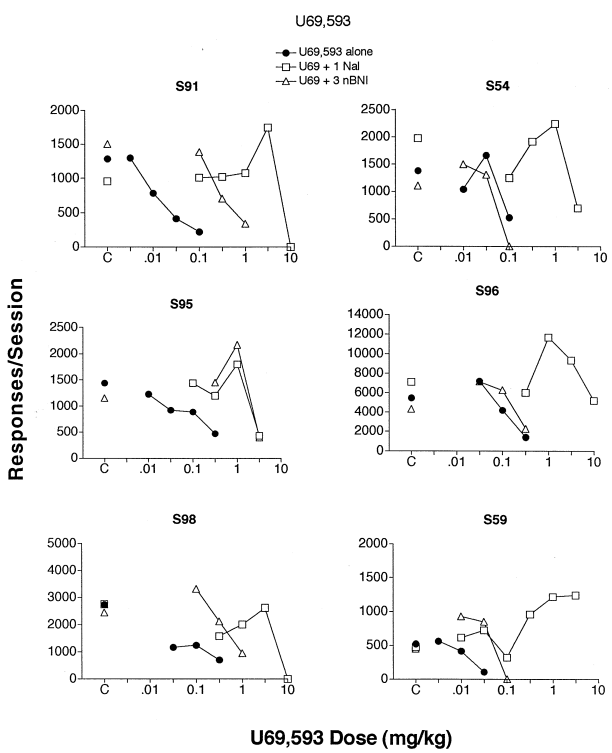

Fig. 2. The total number of responses following the administration of U69,593 alone (closed circles) and in the presence of 1.0 mg/kg of naltrexone (open squares) or following (by 3–21 days) the administration of 3.0 mg/kg of norbinaltorphimine (open triangles) in six monkeys. The points above C represent the mean total responses (±1 S.E.) following the administration of vehicle (closed circle), 1.0 mg/kg of naltrexone (open square) and 3.0 mg/kg of norbinaltorphimine (open triangle).

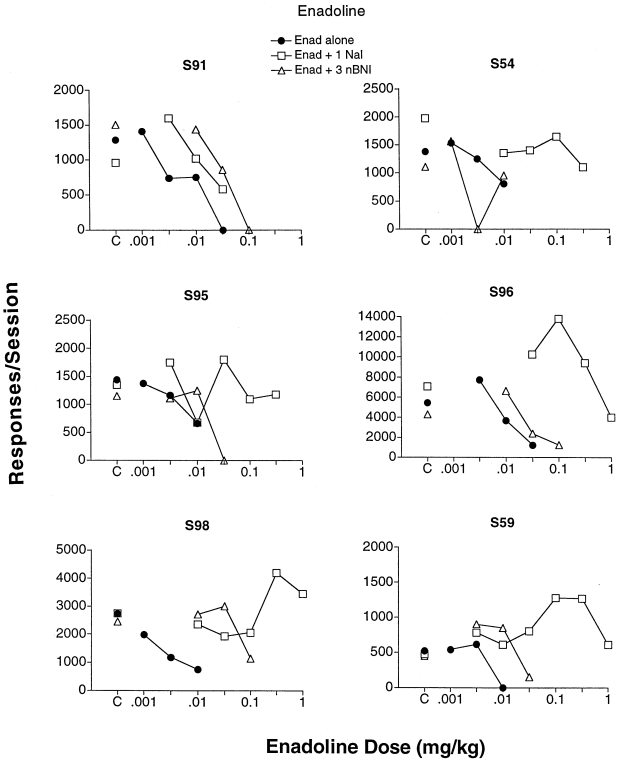

Fig. 3. The total number of responses following the administration of enadoline alone (closed circles) and in the presence of 1.0 mg/kg of naltrexone (open squares) or following (by 3–21 days) the administration of 3.0 mg/kg of norbinaltorphimine (open triangles) in six monkeys. The points above C represent the mean total responses (±1 S.E.) following the administration of vehicle (closed circle), 1.0 mg/kg of naltrexone (open square) and 3.0 mg/kg of norbinaltorphimine (open triangle).

session (twenty 3-min fixed intervals) ranged from an average of 552 (±151) to an average of 5522(±1758) and response rate ranged from an average of 9.4(±2.5) to an average of 92.2(±29.8) responses per min following control injections in individual monkeys. Given the large variability across individual performances under control conditions, each monkey served as its own control. All monkeys learned to terminate the stimulus-paired houselight within the 5 s limited hold and, therefore, seldom received tail shock under control conditions.

Of the eight κ-opioid receptor agonists evaluated, 6 produced significant main effects in reducing the total responses under the fixed interval schedule compared to control performance levels. Significant ($P < 0.05$) decreases in total responses were observed with bremazocine ($F(3,15) = 5.5$), ethylketocyclazocine ($F(3,12) = 3.8$), enadoline ($F(3,15) = 4.1$), U69,593 ($F(3,15) = 3.3$), U50,488 ($F(3,15) = 4.9$), and spiradoline ($F(3,15) = 3.3$). The effects of PD 117302 fell just short of statistical significance ($F(3,12) = 3.3$; $P = 0.058$). Nalorphine was without effect within the dose range tested (10–56 mg/kg); higher doses of these drugs were not tested due to their adverse effects that were of questionable safety to the monkeys (e.g., emesis, heavy salivation, decreased respiration, and muscle tremors). Mean ED_{50} values for all κ-opioid receptor agonists are listed in Table 1 in order of potency (bremazocine = enadoline > ethylketocyclazocine > U69,593 > PD 117302 > spiradoline > U50,488 > nalorphine).

Three of the κ-opioid receptor agonists, bremazocine, U69,593, and enadoline, were chosen to be evaluated in combination with 1.0 mg/kg of naltrexone and 3.0 mg/kg of norbinaltorphimine based on their different chemical structures and previous suggestive evidence that their effects are not mediated by a homogenous population of κ-opioid receptors. With three exceptions, both naltrexone and norbinaltorphimine shifted the dose–effect curves for bremazocine (Fig. 1) and U69,593 (Fig. 2) to the right. The dose–effect curves for enadoline were shifted to the right and upward by naltrexone in five of the six monkeys, but were only shifted by norbinaltorphimine in three of the six monkeys (Fig. 3). Statistical comparisons of the mean slopes of the dose–effect curves for agonist alone to agonist plus naltrexone or norbinaltorphimine indicated that the slopes did not differ significantly indicative of competitive antagonism. Although there was a significant main effect for the comparison of U69,593 alone and combined with either antagonist ($F = 4.7$; $P = 0.045$), post hoc analysis revealed no significant group differences between the slopes. Due to the large overlap in 95% CI, slopes of dose–effect curves among individual monkeys were not significantly different from each other with the one exception (S59–U69,593 + naltexone) where there was no descending limb on the curve. Comparison of the mean ED_{50} values indicate that naltrexone shifted the curves for enadoline and U69,593 to a greater degree than did norbinaltorphimine (Table 2). The bremazocine curve was not shifted differently by naltrexone and norbinaltor-

Table 2
Individual and mean ED_{50} values in mg/kg (±95% CI) for bremazocine, U69,593 and enadoline alone and following pretreatment with 1.0 mg/kg of naltrexone or 3.0 mg/kg of norbinaltorphimine in individual monkeys

	Bremazocine	U69,593	Enadoline
	0.01 (0.002–0.025)	0.08 (0.025–0.16)	0.01 (0.004–0.02)
+1 NTX	0.16 (0.016–0.32)[a]	4.2 (2.5–7.9)[a]	0.89 (0.1–0.63)[a]
+3 norbinal-torphimine	0.25 (0.01–0.35)[a]	0.67 (0.07–1.6)[a,b]	0.04 (0.012–0.071)[b]

[a]Significantly different from agonist alone ($p < 0.05$).
[b]Significantly different from agonist +1 naltrexone ($p < 0.05$).

Table 3
Relative apparent pK_B values (±95% CI) for 1.0 mg/kg of naltrexone when combined with bremazocine, U69,593 and enadoline in individual monkeys

	Bremazocine	U69,593	Enadoline
S54	6.73	7.10	7.35
S59	5.71	8.36	7.84
S91	6.82	7.88	5.57
S95	6.47	6.88	7.58
S96	6.58	7.22	7.34
S98	6.95	7.22	8.06
Mean	6.54	7.44[a]	7.29[a]
95% CI	(6.17–6.92)	(6.97–7.92)	(6.61–8.01)

[a] Significantly different from bremazocine ($p < 0.05$).

phimine except in monkeys S98 and S59 where naltrexone and norbinaltorphimine had the opposite effects.

Table 3 shows the pK_B values for naltrexone combined with bremazocine, enadoline, and U69,593. The mean pK_B value for bremazocine was significantly lower than the mean pK_B values for enadoline and U69,593 (bremazocine vs. enadoline, $P < 0.05$ and bremazocine vs. U69,593, $P < 0.05$). Specifically, naltrexone had the same relative potency for antagonizing enadoline and U69,593, but was approximately 8-fold less potent for antagonizing bremazocine.

4. Discussion

Six of the eight κ-opioid receptor agonists investigated produced dose-dependent decreases in the total number of responses by squirrel monkeys responding under a fixed interval 3-min schedule of stimulus termination. The order of potency in producing these effects was bremazocine = enadoline > ethylketocyclazocine > U69,593 > PD 117302 > spiradoline > U50,488 > nalorphine. The present results correlate to a high degree (Spearman Rank correlation coefficient, $r_s = 0.95–1.0$; $P < 0.05$) with the orders of potency reported in other in vivo preparations that presumably measure interactions with κ-opioid receptors, including ethylketocyclazocine discrimination, naltrexone discrimination, and tail withdrawal in rhesus monkeys (France et al., 1994) and schedule-controlled responding (Bergman and Warren, 1989), shock titration and urine output (Craft and Dykstra, 1992; Pitts and Dykstra, 1994) in squirrel monkeys. Moreover, the effects of most of these kappa agonists in monkeys are antagonized by the selective κ-opioid receptor antagonist norbinaltorphimine as shown in the present study and at least one other study (Butelman et al., 1993), but are not antagonized by the selective μ-opioid receptor antagonist β-funaltrexamine (Dykstra et al., 1987; Pitts and Dykstra, 1994). These results indicate that the effects of the κ-opioid receptor agonists in the present study are likely mediated by κ-opioid receptors.

The order of potency of κ-opioid receptor agonists in vivo differs substantially from their in vitro binding affinities for κ-opioid receptors in rhesus monkeys (Spearman Rank Correlation coefficient, $r_s = 0.54$), although all of the agonists evaluated in the binding study showed high affinity for the κ binding site labeled by [^{3}H]U69,593 (France et al., 1994). The existence of multiple κ-opioid receptor subtypes might explain this discrepancy. If there are multiple κ-opioid receptors in primates, investigations of the binding affinities of various κ-opioid receptor agonists should use more than one ligand to label the binding sites. For example, one recent binding study in rhesus monkeys shows that there is a 3-fold greater affinity of [^{3}H]U69,593 for receptors labelled with [^{3}H]bremazocine than for receptors labelled with [^{3}H]U69,593 (Ko et al., 1998). In rodents, non-benzomorphan κ-opioid receptor agonists, such as U69,593 and U50,488, have much higher affinity for binding sites labeled with [^{3}H]U69,593 than for sites labeled with [^{3}H]ethylketocyclazocine, whereas benzomorphan κ-opioid receptor agonists, such as bremazocine and ethylketocyclazocine, have equally high affinity for both sites (Nock et al., 1990). The fact that norbinaltorphimine readily blocked the in vivo actions of bremazocine in the present study as well as in other studies (Horan et al., 1991; Broadbear et al., 1994; Jewett and Woods, 1995) suggests that these actions of bremazocine are, in fact, mediated via κ-opioid receptors. Other in vitro binding and autoradiographic data support the existence of at least two κ-opioid receptor subtypes for which various κ-opioid receptor agonists and antagonists show different affinities (Nock et al., 1988a,b; Zukin et al., 1988; Rothman et al., 1990; Tiberi and Magnan, 1990). Thus, differences between in vitro affinity and in vivo potency could be due to differences in the receptor populations that are being targeted under these two conditions. Moreover, the intrinsic efficacy and bioavailability of these drugs in vivo might contribute to the differences between in vivo potency and in vitro affinity.

The effects of bremazocine were antagonized significantly and to a similar degree by naltrexone and norbinaltorphimine, whereas the effects of enadoline and U69,593 were antagonized to a significantly greater degree by naltrexone than by norbinaltorphimine. Given that naltrexone shifted the dose–effect curves for both U69,593 and enadoline to a greater degree than did norbinaltorphimine, it is possible that non-κ-opioid receptors are mediating these effects of enadoline and U69,593. If non-κ-opioid receptors mediate the rate-decreasing effects of U69,593 and enadoline, one would predict no antagonism or only partial antagonism, represented by a downward, but no rightward shift in the dose–effect curves, of the effects of these two drugs by norbinaltorphimine. In the monkeys where norbinaltorphimine antagonized U69,593 and enadoline, the curves were shifted in a parallel manner with no indication of partial antagonism. It is also unlikely that norbinaltorphimine is acting as an antagonist at μ- or

δ-opioid receptors, for which it has very low affinity compared to the κ-opioid receptor (Portoghese et al., 1987; Takemori et al., 1988). Furthermore, norbinaltorphimine is ineffective as an antagonist against selective μ- and δ-opioid receptor agonists (Butelman et al., 1993; Broadbear et al., 1994). Therefore, a non-κ-opioid receptor mechanism for the effects of enadoline and U69,593 cannot fully explain their effects in the present study.

The apparent pK_B values for interactions between naltrexone and three κ-opioid receptor agonists revealed that naltrexone was approximately 8-fold less potent at antagonizing bremazocine than at antagonizing either enadoline or U69,593. Theoretically, the value of the apparent pK_B should be the same as that of the apparent pA_2 as a measure of the affinity of the antagonist for the receptor mediating the effects of the agonist (Tallerida et al., 1979). Whereas pA_2 values are determined from the agonist dose–effect curve combined with three or more doses of the antagonist, pK_B values are determined from the agonist dose–effect curve combined with a single dose of the antagonist. Therefore, the apparent pK_B analysis is less robust than the apparent pA_2 analysis; however, using the pK_B analysis can be valuable in situations where testing the agonist dose–effect curve with multiple doses of the antagonist is not feasible as in the present study where two of the six monkeys died and a third monkey was diagnosed with liver failure before further testing could be completed with other doses of the antagonists. Nonetheless, these data are supported by a recent study in rhesus monkeys showing that naltrexone has different affinities for the receptor population labeled with [^{3}H]U69,593 and the receptor population labeled with [^{3}H]bremazocine (Ko et al., 1998). In that study, the measure of affinity correlated with the in vivo pA_2 measures of affinity of naltrexone against the κ-opioid receptor agonists U69,593 and bremazocine. The pK_B values in the present study for U69,593 (7.44) and bremazocine (6.54) are very similar to the pA_2 values in the study by Ko and colleagues for U69,593 (7.73) and bremazocine (6.92). This similarity between pK_B and pA_2 values between studies provides more credence to the accuracy of the pK_B values as a measure of in vivo affinity in the present study.

The present results are consistent with those of other in vivo studies that show differences in the abilities of opioid antagonists to antagonize κ-opioid receptor agonists in non-primates (Clark et al., 1989; Horan et al., 1991, 1993; Horan and Porreca, 1993; France et al., 1994; Mattox et al., 1994; Picker, 1994; Pitts et al., 1996); however, the present results provide some contrasts with a study by Butelman et al. (1993) in rhesus monkeys. Butelman et al. (1993) reported that a dose of 3.2 mg/kg of norbinaltorphimine antagonized the antinociceptive effects of U69,593 and U50,488, but not the effects of enadoline or bremazocine. In the present study, U69,593 and bremazocine, but not enadoline, were antagonized by a dose of 3.0 mg/kg of norbinaltorphimine. It is possible that the rate-decreasing effects of these κ-opioid receptor agonists are mediated by different receptor populations than are their antinociceptive effects; however, more studies are required to determine why such differences in the pharmacological profile of a κ-opioid receptor agonist like enadoline occur across different assays.

In summary, the results of the present study suggest that the rate-decreasing effects of various κ-opioid receptor agonists in squirrel monkeys responding under a fixed interval 3-min schedule of stimulus termination are mediated via κ-opioid receptors based on their rank order of potency and the antagonism of their effects by the selective κ-opioid receptor antagonist norbinaltorphimine. Naltrexone and norbinaltorphimine showed significant differences in antagonizing U69,593 and enadoline, but not bremazocine. These results provide further supportive evidence that the effects of different κ-opioid receptor agonists might not be mediated by a homogenous population of receptors. Further investigation to corroborate the existence of κ-opioid receptor subtypes could provide beneficial information about the feasibility of distinguishing the analgesic and unwanted side effects of κ-opioid receptor agonists for clinical pain management.

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