

# $\kappa$ -Opioid Receptor Effects of Butorphanol in Rhesus Monkeys<sup>1</sup>

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## ABSTRACT

Butorphanol and nalbuphine have substantial affinity for  $\mu$  and  $\kappa$ -opioid receptor sites, yet their behavioral effects in monkeys are largely consistent with a  $\mu$  receptor mechanism of action. Using ethylketocyclazocine (EKC) discrimination and diuresis assays in rhesus monkeys (*Macaca mulatta*), the purpose of the current investigation was to characterize the in vivo  $\kappa$ -opioid activity of these compounds through the use of an insurmountable  $\mu$ -opioid receptor antagonist, clocinnamox. Alone, butorphanol (0.001–0.032 mg/kg i.m.) failed to generalize to EKC, and pretreatment with the competitive opioid receptor antagonist quadazocine (0.1 or 0.32 mg/kg i.m.) did not alter this generalization. At 24 h after clocinnamox (0.1 mg/kg i.m.) administration, butorphanol fully generalized to EKC, and this

generalization was maintained in two of three monkeys at 72 h. Parallel results were observed in diuresis: butorphanol alone and in the presence of quadazocine (1 mg/kg i.m.) did not alter urine output, and a marked diuretic effect was demonstrated 24 h to 2 weeks after clocinnamox administration. Clocinnamox did not alter the discriminative stimulus or diuretic effects of nalbuphine or of the  $\kappa$ -opioid receptor agonists EKC or U69593. These results are consistent with an in vivo agonist activity of butorphanol at  $\kappa$ -opioid receptors that can only be demonstrated when an insurmountable antagonist has substantially eliminated the dominant receptor population through which it exerts its action.

Butorphanol and nalbuphine are used clinically as analgesics with presumed decreased abuse liability due to their low efficacy at  $\mu$ -opioid receptor sites. Interestingly, these compounds have substantial affinity for, and effects through,  $\kappa$ -opioid receptor sites, results that are reasonably well documented in the rodent literature (Leander, 1983; Pick et al., 1992; Jaw et al., 1993) but not unequivocally documented in the primate and human literature. In monkeys, butorphanol and nalbuphine demonstrated respiratory-depressant, antinociceptive, discriminative stimulus and reinforcing properties consistent with their identification as agonists with intermediate efficacy at  $\mu$ -opioid receptors (Young et al., 1984; Gerak et al., 1994; Butelman et al., 1995; Zernig et al., 1997). Alternatively, butorphanol did not produce diuresis and generalized to nalbuphine in assays designed to more adequately reveal  $\kappa$ -opioid activity (Butelman et al., 1995; Gerak and France, 1996). Furthermore, the  $\mu$ -opioid receptor agonists fentanyl, morphine, and methadone, but not the  $\kappa$ -opioid receptor agonists ethylketocyclazocine methanesul-

fonate (EKC), U50488 [(*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolindinyl)-cyclohexyl]benzeneacetamide], enadoline, and spiradoline, generalized to the nalbuphine discriminative stimulus (Gerak and France, 1996).

In humans, butorphanol and nalbuphine produce complex effects. In methadone-maintained subjects, discriminating among the  $\mu$ -opioid receptor agonist hydromorphone, the opioid receptor antagonist naltrexone and saline, butorphanol, and nalbuphine engendered naltrexone responses, consistent with a  $\mu$  antagonist or low-efficacy  $\mu$  agonist activity (Preston et al., 1990). In postaddicted subjects, discriminating among hydromorphone, pentazocine and saline, butorphanol engendered pentazocine responses, perhaps consistent with a  $\kappa$  mechanism of action. However, subjects also identified these latter drugs as belonging to the barbiturate/benzodiazepine class (Preston et al., 1989), and the barbiturate secobarbital and the benzodiazepine receptor agonist lorazepam engendered partial pentazocine-appropriate responding in a subsequent study (Bickel et al., 1989). Although butorphanol, nalbuphine, and pentazocine share similar discriminative stimulus properties, the pharmacological specificity of these discriminative stimulus effects is not clear.

Behavioral results involving rodents have demonstrated predominantly  $\mu$ , but also  $\kappa$ , activity for butorphanol and

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**ABBREVIATIONS:** EKC, ethylketocyclazocine; FR, fixed-ratio; U50488, (*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolindinyl)-cyclohexyl]benzeneacetamide; U69593, (+)-(5 $\alpha$ ,7 $\alpha$ ,8 $\beta$ )-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamide.

nalbuphine. Antinociceptive effects of butorphanol and nalbuphine were reversed with naltrexone; subsequently, apparent  $pA_2$  values indicative of an interaction at the  $\mu$  receptor were determined (Walker et al., 1994; Garner et al., 1997). Additional evidence for  $\mu$  activity included the conditioning of a place preference by, and the self-administration of, butorphanol (Steinfelds et al., 1982; Mamoon et al., 1995); finally, nalbuphine engendered morphine lever selection in rats trained to discriminate morphine from saline (Walker et al., 1997). Support for  $\kappa$ -opioid activity included the demonstration of the diuretic effects of acute butorphanol (Leander 1983) and a withdrawal syndrome precipitated by the  $\kappa$ -opioid receptor antagonist norbinaltorphimine after chronic butorphanol (Feng et al., 1997), and antinociceptive effects of acute nalbuphine were reversed with norbinaltorphimine (Pick et al., 1992).

One explanation for the lack of reliable in vivo  $\kappa$ -like activity after butorphanol and nalbuphine administration is their relative affinities for, and efficacies at,  $\mu$  and  $\kappa$ -opioid receptors. Both compounds demonstrate substantial affinity for, and low efficacy at,  $\mu$  and  $\kappa$ -opioid receptors (Emmerson et al., 1996; Zhu et al., 1997), yet their relative affinities and efficacies marginally favor binding and activity at  $\mu$  over  $\kappa$  receptors, resulting in in vivo observations consistent with a  $\mu$  mechanism of action. In the present investigation,  $\mu$ -opioid receptor-mediated effects were blocked through the use of the insurmountable  $\mu$ -opioid receptor antagonist clocinnamox (Butelman et al., 1996; Zernig et al., 1996) in an attempt to demonstrate the  $\kappa$ -like activity of opioids with affinity for multiple receptors. The objective of present investigation was to characterize the effects of butorphanol and nalbuphine in the presence of clocinnamox in rhesus monkeys using two assays sensitive to  $\kappa$ -opioid receptor agonist activity: EKC discrimination and diuresis.

## Materials and Methods

### Subjects

Eight male and four female adult rhesus monkeys (*Macaca mulatta*) with complex experimental histories (including exposure to opioids and other behaviorally active drugs), weighing 6 to 11 kg, were individually housed in a vivarium maintained at  $21 \pm 1^\circ\text{C}$  with 40% to 60% humidity and with a 12-h light/dark cycle. Monkeys in discrimination experiments were maintained at 90% free-feeding weight. All monkeys were fed 10 to 25 biscuits (Purina Monkey Chow) and fresh fruit two or three times weekly. Water was available ad libitum, except during diuresis test sessions, which took place between 10:00 AM and 1:00 PM. [Animals used in these studies were maintained in accordance with the University of Michigan Committee on Animal Care and "Guidelines of the Committee on the Care and Use of Laboratory Animal Resources" (National Health Council, Department of Health, Education and Welfare, ISBN 0-309-05377-3, revised 1996).]

### Apparatus and Procedure

**EKC Discrimination.** All experiments used similar operant panels consisting of two primate response levers (model PRL-001; BRS-LVE, Laurel, MD), a panel of 7.5-W stimulus lights located above, and a food receptacle located between the levers, mounted on one wall of a testing chamber. The delivery of 300-mg banana-flavored food pellets (formula G/T; Noyes, Lancaster, NH) was controlled by an externally mounted food dispenser (model G5210; Gerbrands, Arlington, MA). A PC-compatible computer connected to an interface

controlled the scheduling of events and recorded data. A lever press defined a response ( $n = 4$ ; minimum 3/drug).

Monkeys were seated in primate restraint chairs and trained to press each of the two levers in the presence of light stimuli for food reinforcement. Initially, reinforcer delivery was made contingent on the completion of one lever press [fixed-ratio 1 (FR 1)], and this response requirement was gradually increased to 10 (FR 10). Subsequently, discrimination training was undertaken in which reinforcer delivery was made contingent on the selection of the lever paired with a drug stimulus, and the response requirement was increased to 30 (FR 30). For all monkeys, EKC (0.0032 mg/kg i.m.) was paired with right lever selection, and saline was paired with left lever selection. Training sessions consisted of multiple (two to five) 15-min cycles, with each cycle consisting of a 10-min time out followed by a 5-min response period. During time-out periods, saline or EKC was administered, light stimuli were extinguished, and responding had no consequences; during response periods, light stimuli were illuminated, and monkeys could obtain up to 10 pellets (reinforcers) through completion of the schedule of reinforcement on the drug-appropriate lever. If 10 reinforcers were obtained before the end of the response period, the stimulus lights were extinguished and responding had no consequences for the remainder of the response period (i.e., the response period was 5 min regardless of the number of reinforcers obtained). Multiple cycles were conducted to provide equal and numerous exposures to the saline and EKC discriminative stimuli and to mimic test sessions in which a cumulative-dosing (see below) procedure was used. Monkeys were trained five or six times per week and were not tested until more than 80% drug-appropriate responding was attained before the first reinforcer delivery and throughout the session; this criterion had to be maintained across four of five consecutive training sessions. In addition, no tests were conducted unless response rates were maintained within 20% across four of five consecutive sessions.

Test sessions were performed as described above, with multiple 15-min cycles (10-min time-out, 5-min response periods), and reinforcer delivery was made contingent on completion of the FR schedule regardless of the lever selected. Agonists were administered at the beginning of each cycle using a cumulative-dosing procedure (i.e., nominal dosing: 0.1, 0.32, 1, and 3.2 mg/kg; actual dosing: 0.1, 0.22, 0.68, and 2.2 mg/kg) and were tested no more than twice weekly. In antagonist experiments, quadazocine was administered 30 min before agonist administration and not more than once every 10 days. In clocinnamox experiments, agonists were tested at 1, 24, and 72 h and 1 and 2 weeks after single doses of clocinnamox were administered. All discrimination tests were performed in a minimum of duplicates.

**Diuresis.** Accumulated urine (measured in milliliters) was collected from pans in the monkey's home cage 3 h after drug administration. Test sessions with single doses of agonists were conducted two times per week (typically Tuesday and Friday), and saline sessions were conducted two times per week (typically Monday and Thursday). In antagonist experiments, quadazocine was administered 30 min before agonist administration. In clocinnamox experiments, agonists were tested at 1, 24, and 72 h and 1 and 2 weeks after single doses of clocinnamox were administered ( $n = 8$ ; minimum 4/drug).

### Drugs

Butorphanol tartate (0.001–0.32 mg/kg; Bristol-Myers Squibb, Wallingford, CT), EKC (0.0001–0.32 mg/kg; Sanofi-Winthrop, Malvern, PA), fentanyl HCl (0.0001–0.01 mg/kg; National Institute on Drug Abuse, Bethesda, MD), and quadazocine methanesulfonate (0.1, 0.32, and 1 mg/kg; Sanofi-Winthrop, Malvern, PA) were dissolved in sterile water. Clocinnamox mesylate (0.1 mg/kg; J. W. Lewis, Bristol University, Bristol, UK), nalbuphine HCl (0.01–32 mg/kg; Dupont Merck, Wilmington, DE), and U69593 (+)-(5 $\alpha$ ,7 $\alpha$ ,8 $\beta$ )-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamide, 0.0001–0.1 mg/kg; Pharmacia-Upjohn, Kalamazoo, MI)

were dissolved in sterile water with a few drops of lactic acid. All drugs were administered i.m. in a volume of 1 ml/10 kg b.wt.

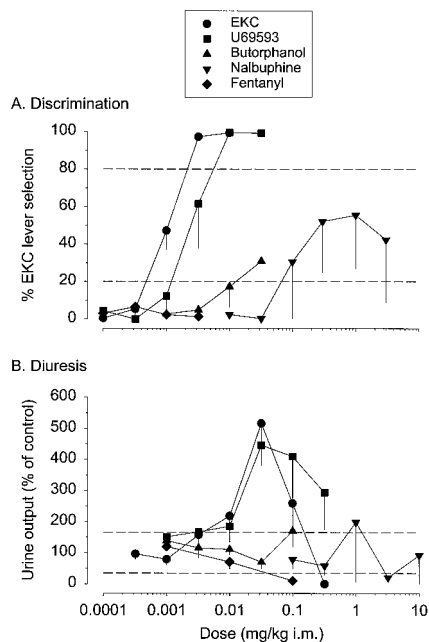
### Data Analysis

The percentage of EKC lever selection [(EKC lever presses/total lever presses)\*100] was determined for each subject; mean EKC lever selection below 20% and above 80% was designated as full saline and EKC generalization, respectively. EKC lever selection between 20% and 80% was designated as partial EKC generalization. Response rate and urine volume were converted to percentage of control [(drug response rate or urine volume/baseline response rate or urine volume)\*100] for each subject; mean agonist data deviating from baseline control by more than 2 S.D. were accepted as significantly different. In addition, ED<sub>50</sub> values (i.e., the dose at which a 50% effect was observed) and 95% confidence intervals for EKC lever selection and response rate were calculated from first order regression equations for each subject, and nonoverlapping confidence intervals were accepted as significant.

### Results

Figure 1 depicts the effects of various  $\mu$ - and  $\kappa$ -opioid receptor agonists in EKC discrimination (A) and diuresis (B) in rhesus monkeys. EKC training drug stimuli engendered drug-appropriate lever selection (saline, 1.6  $\pm$  1% EKC lever selection; EKC, 97.2  $\pm$  1.5% EKC lever selection; mean  $\pm$  S.E.M.) with a response rate of 1.9  $\pm$  0.1 responses/s, and baseline urine output was 52.1  $\pm$  6.0 ml. The  $\kappa$  receptor agonists EKC and U69593 produced and the  $\mu$  and nonselective  $\mu/\kappa$ -opioid receptor agonists fentanyl and butorphanol were without EKC-like discriminative stimulus and diuretic effects. The nonselective  $\mu/\kappa$ -opioid agonist nalbuphine produced variable between- and within-subject results.

**EKC.** Table 1 depicts estimations of the ED<sub>50</sub> value for the discriminative stimulus and rate-suppressive effects of EKC, as well as the other tested compounds, in rhesus monkeys.



**Fig. 1.** EKC discriminative stimulus (A) and diuretic (B) effects of  $\mu$ - and  $\kappa$ -opioid receptor agonists in rhesus monkeys. Each value represents the mean, and error bars represent 1 S.E.M. For discrimination, full EKC generalization is demonstrated above 80% EKC lever selection. For diuresis, horizontal dashed bars indicate 2 S.D. from control (saline administration) diuresis.

EKC generalized to the EKC discriminative stimulus, and full generalization was observed at the 0.0032 mg/kg training dose (Fig. 2A and Table 1). Quadazocine (0.32 and 1 mg/kg i.m.) dose-dependently decreased the discriminative stimulus effects of EKC; at the highest quadazocine dose tested, full generalization was observed at 0.1 mg/kg. Pretreatment with the insurmountable  $\mu$  antagonists clocinnamox (0.1 mg/kg i.m.) did not alter the discriminative stimulus effects of EKC at any of the time points evaluated.

EKC dose-dependently decreased response rate, and responding was abolished at 0.032 mg/kg (Table 1). Quadazocine dose-dependently decreased the rate-suppressive effects of EKC. Pretreatment with clocinnamox did not alter the rate-suppressive effects of EKC at any of the time points evaluated.

EKC produced biphasic effects on urine output, with a maximal diuretic effect ( $\sim$ 300% of control) demonstrated at 0.032 mg/kg i.m. (Fig. 2B). Quadazocine (1 mg/kg) reversed the diuretic effects of EKC. In the presence of clocinnamox, the diuretic effects of EKC were not altered at any of the time points evaluated (only data reflecting the diuretic effects of EKC in the presence of clocinnamox after 24 h are shown).

**U69593.** U69593 generalized to the EKC discriminative stimulus; full generalization was observed at 0.01 mg/kg (Fig. 1A and Table 1). Quadazocine (0.32 mg/kg) modestly decreased (N.S.) the discriminative stimulus effects of U69593; a greater antagonism was demonstrated with the highest dose of quadazocine (1 mg/kg) but was characterized in only one subject as this higher dose became rate suppressive as the experiment progressed. Pretreatment with clocinnamox did not alter the discriminative stimulus effects of U69593 at any of the time points evaluated.

U69593 dose-dependently decreased response rate; lever pressing was abolished at 0.1 mg/kg (Table 1). Pretreatment with quadazocine or clocinnamox did not alter the rate-suppressive effects of U69593.

U69593 produced biphasic effects on urine output, with a maximal diuretic effect ( $\sim$ 450% of control) demonstrated at 0.032 mg/kg i.m. (Fig. 1B). Quadazocine (1 mg/kg) reversed the diuretic effects of U69593 (data not shown). U69593 was not tested in the presence of clocinnamox.

**Butorphanol.** Butorphanol failed to generalize to the EKC discriminative stimulus when administered alone and in the presence of quadazocine (0.1 and 0.32 mg/kg); a maximal 30% EKC lever selection was observed in one monkey at 0.032 mg/kg (Fig. 3A and Table 1). At 24 h after the administration of clocinnamox, butorphanol (0.1 mg/kg) fully generalized to the EKC discriminative stimulus in all monkeys, and butorphanol continued to engender EKC lever selection at 72 h in two of three monkeys with a reduced potency. Preclocinnamox responding returned at 1 week (i.e., determination of a butorphanol dose-effect curve revealed saline responding through doses at which behavior was suppressed).

Butorphanol dose-dependently decreased response rate; responding was abolished at 0.1 mg/kg (Table 1). Quadazocine dose-dependently decreased the rate-suppressive effects of butorphanol (N.S.); at the highest dose tested (0.32 mg/kg), butorphanol completely suppressed responding at 1 mg/kg. At 24 h after the administration of clocinnamox, a 20-fold rightward shift in the rate-suppressive effects of butorphanol was demonstrated; this antagonistic effect of clocinnamox

TABLE 1  
Discriminative stimulus and rate-suppressive effects of opioid agonists

Drug	EKC Discrimination		Rate Suppression	
	ED <sub>50</sub>	95% CI	ED <sub>50</sub>	95% CI
			<i>mg/kg</i>	
EKC	0.001	0.001–0.002	0.010	0.006–0.016
+ Quadazocine (0.32 mg/kg)	0.006	0.005–0.006 <sup>a</sup>	0.026	0.011–0.059
+ Quadazocine (1 mg/kg)	0.060	0.057–0.064 <sup>a</sup>	0.034	0.013–0.086 <sup>a</sup>
+ Clocinnamox (0.1 mg/kg; 24-h PT)	0.002	0.001–0.005	0.008	0.001–0.060
+ Clocinnamox (0.1 mg/kg; 72-h PT)	0.001	0.001–0.009	0.006	0.001–0.024
+ Clocinnamox (0.1 mg/kg; 1-wk PT)	0.001	0.001–0.004	0.005	0.003–0.007
U69593	0.002	0.001–0.007	0.022	0.018–0.028
+ Quadazocine (0.32 mg/kg)	0.004	0.001–0.010	0.042	0.008–0.022
+ Quadazocine (1 mg/kg)	0.013 <sup>b</sup>		0.023 <sup>b</sup>	
+ Clocinnamox (0.1 mg/kg; 24-h PT)	0.002	0.001–0.013	0.013	0.003–0.050
+ Clocinnamox (0.1 mg/kg; 72-h PT)	0.002	0.002–0.002	0.009	0.001–0.190
+ Clocinnamox (0.1 mg/kg; 1-wk PT)	0.002	0.001–0.011	0.012	0.003–0.049
Butorphanol			0.012	0.004–0.040
+ Quadazocine (0.1 mg/kg)			0.075	0.006–0.923
+ Quadazocine (0.32 mg/kg)			0.132	0.038–0.464
+ Clocinnamox (0.1 mg/kg; 24-h PT)	0.031	0.013–0.072 <sup>a</sup>	0.242	0.053–1.103 <sup>a</sup>
+ Clocinnamox (0.1 mg/kg; 72-h PT)	0.099	0.099–0.100 <sup>a</sup>	0.076	0.005–1.145
+ Clocinnamox (0.1 mg/kg; 1-wk PT)			0.021	0.002–0.177
Nalbuphine	0.181	0.002–21.640 <sup>c</sup>	0.697	0.178–2.735
+ Quadazocine (0.1 mg/kg)	<sup>a</sup>		0.329	0.002–494.172
+ Quadazocine (0.32 mg/kg)	<sup>a</sup>		0.104	0.006–1.908
+ Clocinnamox (0.1 mg/kg; 24-h PT)	0.056 <sup>b</sup>		1.320	0.592–2.937
+ Clocinnamox (0.1 mg/kg; 72-h PT)	<sup>a</sup>		1.756	0.434–7.103
+ Clocinnamox (0.1 mg/kg; 1-wk PT)	<sup>a</sup>		0.141	0.013–1.540

No value indicates ED<sub>50</sub> was inestimable. PT, pretreatment time.

<sup>a</sup> Significant difference ( $p < .05$ ) from agonist treatment alone.

<sup>b</sup> Determination for one subject.

<sup>c</sup> Determination for two subjects.

diminished at 72 h and returned to preclostinamox responding within 1 week.

Butorphanol failed to alter baseline urine output alone or in the presence of quadazocine (Fig. 3B). At 24 h after the administration of clostinamox, butorphanol dose-dependently increased urine output to ~300% of baseline at 0.1 mg/kg. The diuretic effects of butorphanol were maximal at 72 h (at doses above 0.01 mg/kg), but they were not consistently dose related; reliable and diminishing diuretic effects were demonstrated 1 and 2 weeks after clostinamox administration (data not shown).

**Nalbuphine.** Nalbuphine produced variable results in each of the dependent measures. Nalbuphine fully generalized to the EKC discriminative stimulus in two of three monkeys, and EKC lever selection was abolished in the presence of quadazocine (0.1 and 0.32 mg/kg; Fig. 4A and Table 1). Nalbuphine produced different effects in each of the monkeys after clostinamox administration. At 24 h, 1) nalbuphine generalized to the EKC discriminative stimulus in one monkey that had previously demonstrated a nalbuphine-EKC substitution, 2) nalbuphine produced 50% EKC lever selection in a second monkey that had previously demonstrated a nalbuphine-EKC substitution, and 3) nalbuphine engendered saline responding in a third monkey that had not previously demonstrated a nalbuphine-EKC substitution. Nalbuphine engendered predominantly saline responding at 72 h and 1 week after clostinamox administration in all monkeys.

Nalbuphine dose-dependently decreased response rate; responding was abolished at 10 mg/kg (Table 1). Intersubject and intrasubject variability precluded meaningful analysis of the quadazocine and clostinamox pretreatments.

Nalbuphine also produced variable and not dose-related

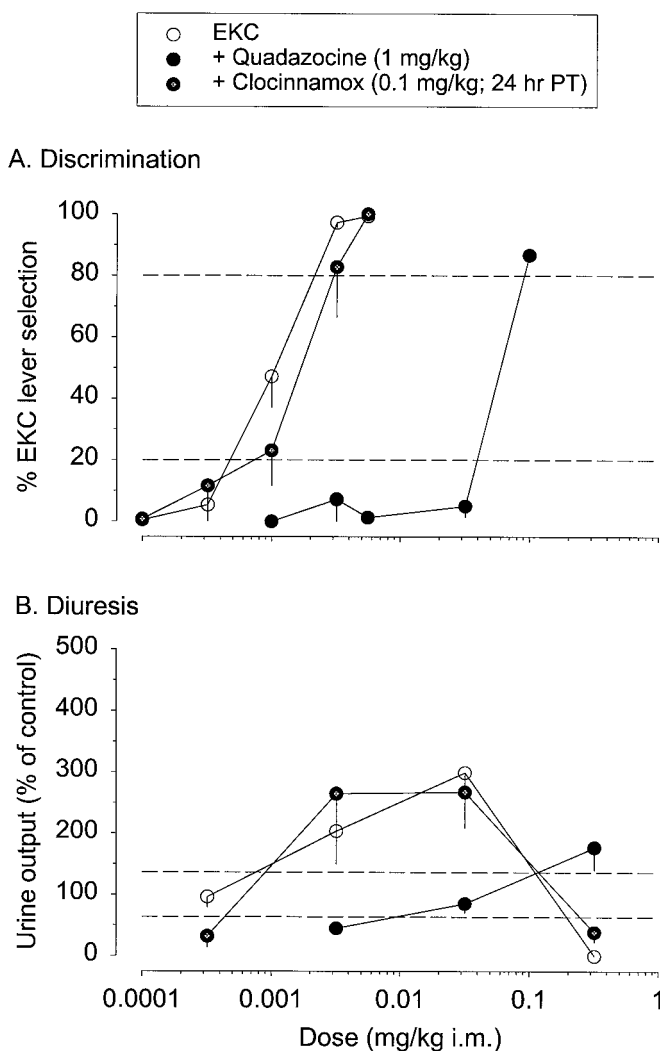
diuretic effects; urine output was increased to 200% of baseline at 1 mg/kg (Fig. 4B). At 24 h after clostinamox administration, nalbuphine increased urine output to ~350% of baseline at 0.1 and 3.2 mg/kg. Nalbuphine did not alter baseline diuresis 72 h through 2 weeks after clostinamox administration (data not shown). Quadazocine pretreatment was not performed.

**Fentanyl.** Fentanyl failed to produce EKC lever selection (Fig. 1A) and dose-dependently decreased response rate (data not shown) and urine output (Fig. 1B).

## Discussion

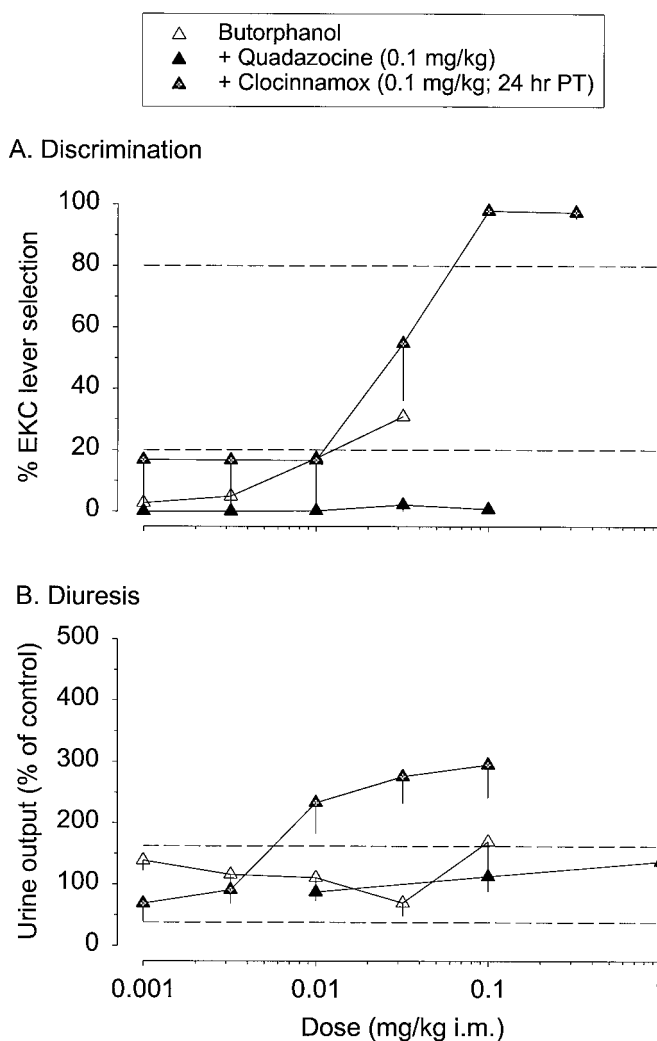
The primary objective of the present study was to characterize the  $\kappa$ -like opioid receptor effects of the nonselective  $\mu/\kappa$ -opioid agonists butorphanol and nalbuphine. When administered alone or in the presence of the competitive opioid antagonist quadazocine, butorphanol and nalbuphine did not produce (or did not produce consistent) EKC discriminative stimulus or diuretic effects. Conversely, butorphanol generalized to the EKC discriminative stimulus and produced diuresis after the administration of the insurmountable  $\mu$ -opioid receptor antagonist clostinamox, effects that are consistent with  $\kappa$ -opioid receptor activity. Nalbuphine continued to produce inconsistent results in the presence of clostinamox. Taken together, these results demonstrate substantial  $\kappa$ -like opioid activity of butorphanol and demonstrate the use of insurmountable antagonists such as clostinamox in the *in vivo* investigation of nonselective receptor agonists.

In nonhuman primates, butorphanol has been characterized as a low-efficacy  $\mu$ -opioid receptor agonist (Butelman et al., 1995; Liguori et al., 1996), consistent with *in vitro* evi-



**Fig. 2.** EKC discriminative stimulus (A) and diuretic (B) effects of EKC. Each value represents the mean, and error bars represent 1 S.E.M. For discrimination, full EKC generalization is demonstrated above 80% EKC lever selection. For diuresis, horizontal dashed bars indicate 2 S.D. from control (saline administration) diuresis.

dence of its affinity for, and low efficacy at,  $\mu$ -opioid receptors ( $K_{i\mu} = 0.5$  nM; 12% maximal effect compared with fentanyl; Emmerson et al., 1996; Butelman et al., 1998). These behavioral effects are demonstrated despite the fact that butorphanol has equal affinity for, and low efficacy at,  $\kappa$ -opioid receptors ( $K_{i\kappa} = 0.68$  nM; 27% maximal effect compared with the full  $\kappa$  agonist U50488; Butelman et al., 1998; Remmers et al., 1999). When administered alone or in the presence of the competitive opioid antagonist quadazocine, butorphanol produced few discriminative stimulus or diuretic effects that were consistent with  $\kappa$ -opioid receptor activity. Nevertheless, after blocking the potential  $\mu$ -opioid receptor-mediated effects with clocinnamox,  $\kappa$ -like opioid effects of butorphanol were demonstrated. These  $\kappa$ -opioid effects were dose and time related, with peak discriminative stimulus and diuretic effects observed at 24 and 72 h after clocinnamox administration, respectively. Additionally, the diuretic effects of butorphanol in the presence of clocinnamox were less than those observed with the traditional and full  $\kappa$ -opioid receptor agonist U69593; these former effects are consistent with the

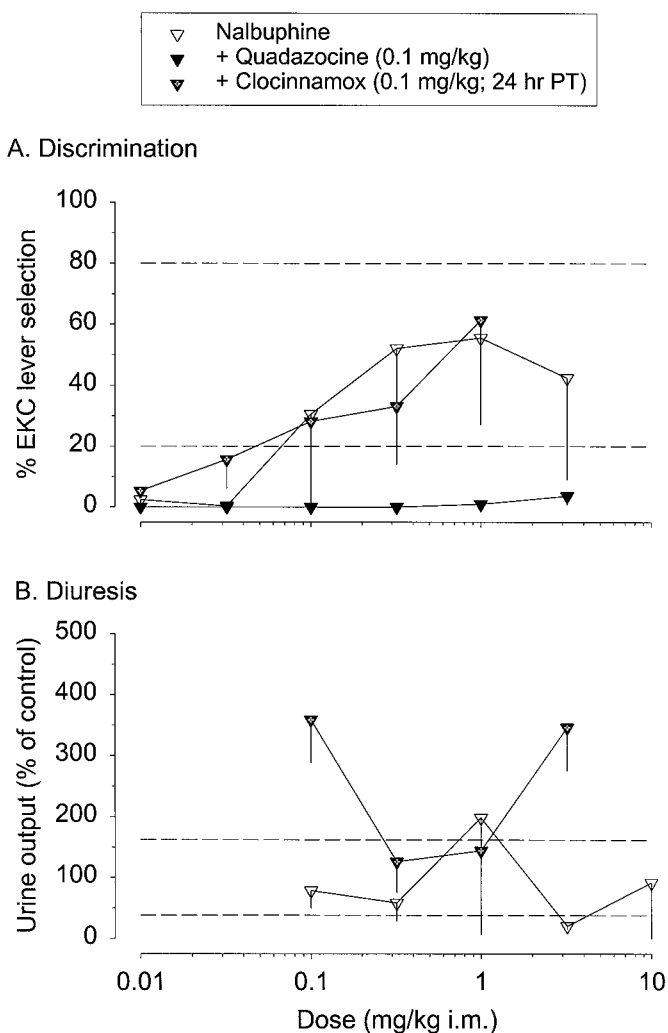


**Fig. 3.** EKC discriminative stimulus (A) and diuretic (B) effects of butorphanol. Each value represents the mean, and error bars represent 1 S.E.M. For discrimination, full generalization is demonstrated above 80% EKC lever selection. For diuresis, horizontal dashed bars indicate 2 S.D. from control (saline administration) diuresis.

identification of butorphanol as a partial  $\kappa$ -opioid receptor agonist (Leander, 1983; Remmers et al., 1999).

Given the  $\kappa$ -like opioid receptor effects of butorphanol reported above and the similar effects of nalbuphine and butorphanol in antinociception, discrimination, and respiration assays, the discriminative stimulus and diuresis results obtained for nalbuphine in the presence of clocinnamox might be considered discouraging. Nalbuphine produced EKC discriminative stimulus effects in two of three monkeys, but the generalization was not improved after the administration of clocinnamox, and it is likely that EKC lever selection was due to other common stimulus properties of nalbuphine and EKC (i.e., sedation, dysphoria). In humans, complex and equivocal behavioral effects of nalbuphine have been reported, including its characterization as a low-efficacy  $\mu$ -opioid receptor agonist (or  $\mu$  antagonist) and a  $\kappa$ -opioid receptor agonist, as well as the identification of nalbuphine as a barbiturate/benzodiazepine-like agent (Preston et al., 1990).

Furthermore, nalbuphine alone and in the presence of clocinnamox did not produce reliable diuretic effects in the



**Fig. 4.** EKC discriminative stimulus (A) and diuretic (B) effects of nalbuphine. Each value represents the mean, and error bars represent 1 S.E.M. For discrimination, full generalization is demonstrated above 80% EKC lever selection. For diuresis, horizontal dashed bars indicate 2 S.D. from control (saline administration) diuresis.

present experiment: neither dose- or time-related effects were demonstrated. Interestingly, although the affinities for nalbuphine are similar for  $\mu$ - and  $\kappa$ -opioid receptors ( $K_{i\mu} = 1.4$  nM;  $K_{i\kappa} = 8.5$  nM; Butelman et al., 1998), the reported efficacies are at variance and dependent on the cell line on which the receptor is expressed. In  $C_6$  cells, the relative efficacy of nalbuphine is 22% ( $EC_{50} = 5$ –8 nM) of that seen with U50488 (Remmers et al., 1999). In contrast, nalbuphine shows almost no efficacy in Chinese hamster ovary cells expressing the  $\kappa$  receptor. Indeed, although a 38% maximal effect compared with U50488 was reported (i.e., the former being designated as a low-efficacy  $\kappa$  agonist; Zhu et al., 1997), this estimate was obtained at very high nalbuphine concentrations (30  $\mu$ M) and is likely to be a nonspecific effect. Taken together, the failure to demonstrate unequivocal EKC lever selection and diuretic effects agrees with the finding at  $\kappa$  cloned opioid receptors expressed in Chinese hamster ovary cells and thus are likely due to the minimal efficacy of nalbuphine at  $\kappa$ -opioid receptor sites.

In vitro, EKC has substantial affinity for  $\mu$ - and  $\kappa$ -opioid receptors ( $K_{i\mu} = 1.7$  nM;  $K_{i\kappa} = 1.2$  nM; Butelman et al., 1998)

and high-efficacy at  $\kappa$ -opioid receptors (100% maximal stimulation compared with U50488; Remmers et al., 1999). These in vitro findings parallel in vivo demonstrations of respiratory depression through  $\mu$ -opioid receptor sites (Butelman et al., 1993) and discriminative and diuretic effects through  $\kappa$ -opioid receptor sites (Dykstra et al., 1987). Although the  $\kappa$ -opioid receptor activity in EKC discrimination and diuresis is well documented, it remained possible that these EKC effects were compromised due to its activity at the  $\mu$ -opioid receptor: decreased potency or efficacy might be revealed through a leftward shift in the dose-effect function (discrimination and diuresis) or a larger maximal effect (diuresis) in the presence of cloccinnamox. Because cloccinnamox pretreatment failed to alter the discriminative stimulus and diuretic effects of EKC, there was no evidence for  $\mu$ -opioid involvement in these effects.

Although  $\kappa$ -like opioid discriminative stimulus and diuretic effects were demonstrated in the presence of the insurmountable antagonist cloccinnamox, it is puzzling why the use of the competitive antagonist quadazocine, at doses that targeted  $\mu$  (0.1 mg/kg) and some of the  $\kappa$  (0.32 and 1 mg/kg) population of opioid receptors (Negus et al., 1993), did not also eliminate the  $\mu$  receptor population through which butorphanol exerted its action. This failure to demonstrate  $\kappa$ -like opioid receptor activity was not due to an insufficient quadazocine dose as the potency of butorphanol to suppress responding was decreased. Alternatively, it is likely that quadazocine was attenuating (i.e., shifting to the right) the  $\mu$ - and  $\kappa$ -opioid effects of butorphanol, whereas cloccinnamox was selectively abolishing the  $\mu$ -opioid effects of butorphanol.

In conclusion, previous primate research characterized butorphanol and nalbuphine as low-efficacy  $\mu$ -opioid receptor agonists, and the present investigation demonstrated an in vivo  $\kappa$ -like opioid activity of butorphanol in two assays sensitive to  $\kappa$ -opioid receptor activity: EKC discrimination and diuresis. Furthermore, these results support the use of insurmountable antagonists such as cloccinnamox as tools to investigate the pharmacology of nonselective opioid agonists.

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#### References

- Bickel WK, Bigelow GE, Preston KL and Liebson IA (1989) Opioid drug discrimination in humans: Stability, specificity and relation to self-reported drug effect. *J Pharmacol Exp Ther* **251**:1053–1063.
- Butelman ER, France CP and Woods JH (1993) Apparent  $pA_2$  analysis on the respiratory depressant effects of alfentanil, etonitazene, ethylketocyclazocine (EKC) and Mr2033 in rhesus monkeys. *J Pharmacol Exp Ther* **264**:145–151.
- Butelman ER, Ko M-C, Sobczyk-Kojiro K, Mosberg HI, van Bommel B, Zernig G and Woods JH (1998)  $\kappa$ -opioid receptor binding populations in rhesus monkey brain: Relationship to an assay of thermal antinociception. *J Pharmacol Exp Ther* **285**:595–601.
- Butelman ER, Negus SS, Lewis JW and Woods JH (1996) Cloccinnamox antagonism of opioid suppression of schedule-controlled responding in rhesus monkeys. *Psychopharmacology* **123**:320–324.
- Butelman ER, Winger G, Zernig G and Woods JH (1995) Butorphanol: Characterization of agonist and antagonist effects in rhesus monkeys. *J Pharmacol Exp Ther* **272**:845–853.
- Dykstra LA, Gmerek DE, Winger G and Woods JH (1987)  $\kappa$ -opioids in rhesus monkeys: I. Diuresis, sedation, analgesia and discriminative stimulus effects. *J Pharmacol Exp Ther* **242**:413–420.
- Emmerson PJ, Clark MJ, Mansour A, Akil H, Woods JH and Medzihradsky F (1996) Characterization of opioid agonist efficacy in a  $C_6$  glioma cell line expressing the  $\mu$  opioid receptor. *J Pharmacol Exp Ther* **278**:1121–1127.
- Feng YZ, Rockhold RW and Ho IK (1997) Nor-binaltorphimine precipitates withdrawal and excitatory amino acid release in the locus ceruleus of butorphanol—but not morphine-dependent rats. *J Pharmacol Exp Ther* **283**:932–938.
- Garner HR, Burke TF, Lawhorn CD, Stoner JM and Wessinger WD (1997) Butor-

- phanol-mediated antinociception in mice: Partial agonist effects and  $\mu$  receptor involvement. *J Pharmacol Exp Ther* **282**:1253–1261.
- Gerak LR, Butelman ER, Woods JH and France CP (1994) Antinociceptive and respiratory effects of nalbuphine in rhesus monkeys. *J Pharmacol Exp Ther* **271**:993–999.
- Gerak LR and France CP (1996) Discriminative stimulus effects of nalbuphine in rhesus monkeys. *J Pharmacol Exp Ther* **276**:523–531.
- Jaw SP, Makimura M, Hoskins B and Ho IK (1993) Effects of nor-binaltorphimine on butorphanol dependence. *Eur J Pharmacol* **239**:133–140.
- Leander JD (1983) Evidence that nalorphine, butorphanol and oxilorphan are partial agonists at a kappa-opioid receptor. *Eur J Pharmacol* **86**:467–470.
- Liguori A, Morse WH, Bergman J (1996) Respiratory effects of opioid full and partial agonists in rhesus monkeys. *J Pharmacol Exp Ther* **277**:462–472.
- Mamoon AM, Barnes AM, Ho IK and Hoskins B (1995) Comparative rewarding properties of morphine and butorphanol. *Brain Res Bull* **38**:507–511.
- Negus SS, Burke TF, Medzihradsky F and Woods JH (1993) Effects of opioid agonists selective for  $\mu$ ,  $\kappa$  and  $\delta$  opioid receptors on schedule-controlled responding in rhesus monkeys: Antagonism by quadazocine. *J Pharmacol Exp Ther* **267**:896–903.
- Pick CG, Paul D and Pasternak GW (1992) Nalbuphine, a mixed  $\kappa_1$  and  $\kappa_3$  analgesic in mice. *J Pharmacol Exp Ther* **262**:1044–1050.
- Preston KL, Bigelow GE, Bickel WK and Liebson IA (1989) Drug discrimination in human postaddicts: Agonist-antagonist opioids. *J Pharmacol Exp Ther* **250**:184–196.
- Preston KL, Bigelow GE and Liebson IA (1990) Discrimination of butorphanol and nalbuphine in opioid-dependent humans. *Pharmacol Biochem Behav* **37**:511–522.
- Remmers AE, Clark MJ, Mansour A, Akil H, Woods JH and Medzihradsky F (1999) Opioid efficacy in a C<sub>6</sub> glioma cell line stably expressing the human  $\kappa$  opioid receptor. *J Pharmacol Exp Ther* **288**:827–833.
- Steinfelds GF, Young GA and Khazan N (1982) Self-administration of nalbuphine, butorphanol and pentazocine by morphine post-addict rats. *Pharmacol Biochem Behav* **16**:167–171.
- Walker EA, Makhay MM, House JD and Young AM (1994) In vivo apparent pA<sub>2</sub> analysis for naltrexone antagonism of discriminative stimulus and analgesic effects of opiate agonists in rats. *J Pharmacol Exp Ther* **271**:959–968.
- Walker EA, Richardson TM and Young AM (1997) Tolerance and cross-tolerance to morphine-like stimulus effects of  $\mu$  opioids in rats. *Psychopharmacology* **133**:17–28.
- Young AM, Stephens KR, Hein DW and Woods JH (1984) Reinforcing and discriminative stimulus properties of mixed agonist-antagonist opioids. *J Pharmacol Exp Ther* **229**:118–126.
- Zernig G, Burke T, Lewis JW and Woods JH (1996) Mechanism of clocinamox blockade of opioid receptors: Evidence from *in vitro* and *ex vivo* binding and behavioral assays. *J Pharmacol Exp Ther* **279**:23–31.
- Zernig G, Lewis JW and Woods JH (1997) Clocinamox inhibits the intravenous self-administration of opioid agonists in rhesus monkeys: Comparison with effects on opioid agonist-mediated nociception. *Psychopharmacology* **129**:233–242.
- Zhu J, Luo L-Y, Li J-G, Chen C and Liu-Chen L-Y (1997) Activation of the cloned human  $\kappa$  opioid receptor by agonists enhances [<sup>35</sup>S]GTP $\gamma$ S binding to membranes: Determination of potencies and efficacies of ligands. *J Pharmacol Exp Ther* **282**:676–684.

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