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Interactions between the Discriminative Stimulus Effects of *Mu* and *Kappa* Opioid Agonists in the Squirrel Monkey[1]

S. STEVENS NEGUS,[2] MITCHELL J. PICKER and LINDA A. DYKSTRA[3]
Department of Psychology, University of North Carolina, Chapel Hill, North Carolina
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ABSTRACT

A series of *mu* and *kappa* opioid agonists with varying degrees of selectivity were evaluated for their agonist and antagonist effects in squirrel monkeys trained to discriminate either the selective *mu* agonist fentanyl or the selective *kappa* agonist U50,488 from water. In the fentanyl-trained monkeys, fentanyl, as well as the less selective *mu* agonists buprenorphine and (−)-metazocine, produced dose-dependent and complete substitution for the training stimulus. U50,488 produced neither agonist nor antagonist effects in the fentanyl-trained monkeys, but the less selective *kappa* agonists bremazocine and tifluadom generally produced either agonist or antagonist effects, depending on the monkey tested. In the U50,488-trained monkeys, U50,488, bremazocine and tifluadom all produced a dose-dependent and complete substitution for the training stimulus. Fentanyl produced neither agonist nor antagonist effects in the U50,488-trained monkeys, but buprenorphine and (−)-metazocine antagonized the discriminative stimulus effects of U50,488. The inability of the selective *mu* agonist fentanyl and the selective *kappa* agonist U50,488 to antagonize each other's discriminative stimulus effects suggests that the stimulus effects mediated by *mu* and *kappa* opioid receptors in squirrel monkeys do not interact with a common biologic substrate. Rather, these results suggest that the stimulus effects mediated by *mu* and *kappa* receptors function independently of one another. Interactions involving the less selective *mu* agonists buprenorphine and (−)-metazocine, or the less selective *kappa* agonists bremazocine and tifluadom, can be explained on the basis of the low receptor selectivity of these drugs.

Opioids produce their principal effects by binding to at least three different types of receptors, the *mu*, *kappa* and *delta* opioid receptors (Martin *et al.*, 1976; Lord *et al.*, 1977; Howard *et al.*, 1986; Mansour *et al.*, 1988). However, less is known about the degree to which these opioid receptor subtypes act independently in producing their respective effects. Some interdependency between opioid receptors has been suggested by studies demonstrating that opioids apparently selective for one opioid receptor subtype can interact with opioids selective for other opioid receptor subtypes (e.g., Leander, 1983a; Holaday *et al.*, 1985; Negus *et al.*, in press).

These opioid interactions appear to result from two general mechanisms. The first mechanism, a lack of receptor selectivity, is drug-dependent and is illustrated in figure 1a. Receptor selectivity is a measure of a drug's affinity for one type of receptor relative to its affinity for other types of receptors. Most opioids have a higher affinity for one opioid receptor subtype than for the other subtypes, and thereby display some receptor selectivity in their binding to *mu*, *kappa* or *delta* opioid receptors. However, none of the opioids is specific and a given dose of many opioid drugs will bind to more than one receptor subtype.

The issue of receptor selectivity is complicated by the additional variable of intrinsic efficacy. Intrinsic efficacy is a measure of a drug's ability to activate a receptor and initiate a biologic response, and a drug's intrinsic efficacy is theoretically assay-independent inasmuch as it is inferred from the drug's effects across a wide range of assays (Kenakin, 1987). Drugs that produce full-agonist effects across several assays are considered to have high efficacy, whereas drugs that generally produce antagonist effects are considered to have little or no efficacy. Intermediate efficacy drugs may act as full agonists in some assays, but in other assays they act either as antagonists or as partial agonists that produce submaximal effects when administered alone and partially reverse the effects of full agonists. Because a drug's intrinsic efficacy can vary across receptor subtypes, a given opioid may produce agonist effects *via* a receptor subtype at which it has high efficacy, and simultaneously produce antagonist effects *via* another receptor subtype at which it has little or no efficacy. Buprenorphine exemplifies this capacity of some opioids to bind to and interact with more than one receptor type (Cowan *et al.*, 1977; Wood *et*

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[2] Present address: Division of Preclinical Neuroscience and Endocrinology, Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Rd., La Jolla, CA 92037.
[3] Recipient of Research Scientist Award K05DA00033 from the National Institute on Drug Abuse.

ABBREVIATIONS: FR, fixed ratio; DAR, drug-appropriate responding; ARL, average response latency.

al., 1981; Leander, 1987; Negus et al., in press). Buprenorphine binds with high affinity to both *mu* and *kappa* receptors, but its intrinsic efficacy varies such that buprenorphine generally acts as an agonist at *mu* receptors, but as an antagonist at *kappa* receptors.

The second mechanism, a biologic interaction between opioids, is dependent on the biologic system in which drug effects are being evaluated and is illustrated in figure 1b. Specifically, this second mechanism describes those cases in which there is a biologic interaction between the effects mediated by different opioid receptors. It is known that, at a postreceptor level, many effects mediated by different opioid receptors can converge on a single experimental endpoint (Leander, 1983a; Mucha and Herz, 1985; Di Chiara and Imperato, 1988). Such convergence raises the possibility of physiologic potentiation or antagonism of effects mediated by different opioid receptor subtypes. For example, *kappa* agonists typically produce diuretic effects in rats, whereas *mu* agonists produce antidiuretic effects (Leander, 1983b; Huidobro-Toro and Parada, 1985). Moreover, the diuretic effects of *kappa* agonists such as U50,488, bremazocine and tifluadom can be blocked by *mu* agonists such as morphine, *l*-methadone and low doses of etorphine (Leander, 1983a; Leander, 1984; Richards and Sadee, 1985). The *kappa* antagonist effects of *mu* agonists in the rat urination procedure are probably not due to a blockade of *kappa* opioid receptors (Richards and Sadee, 1985). Rather, these data suggest that *kappa* agonists and *mu* agonists produce distinct but interacting effects on the experimental endpoint of urination.

Kappa and *mu* opioid agonists also produce distinct effects in the drug discrimination procedure, a procedure that has been used extensively to study the receptor-mediated effects of opioids (Holtzman, 1985). Both humans (Kumor et al., 1986; Pfeiffer et al., 1986) and laboratory animals (Teal and Holtzman, 1980; Picker and Dykstra, 1987; Picker et al., 1990) can discriminate *mu* and *kappa* opioid agonists from drug vehicle, and the discriminative stimulus effects produced by opioids selective for one receptor subtype typically differ from the discriminative stimulus effects produced by nonopioids or opioids selective for the other opioid receptor subtype. Furthermore, it has been suggested that the discriminative stimulus effects of *mu* and *kappa* opioid agonists not only differ, but are also diametrically opposed to one another in a manner not unlike the opposing effects of *mu* and *kappa* agonists on urination. In humans, for example, *mu* agonists produce subjective effects that have been described as euphoric, whereas *kappa* agonists produce subjective effects described as dysphoric (Kumor et al., 1986; Pfeiffer et al., 1986), and Pfeiffer et al. (1986) have suggested that *mu* and *kappa* opioids produce opposing effects on opioid systems affecting emotional and perceptual experience.

In view of the potential for *mu* and *kappa* agonists to influence each other's effects in the drug discrimination procedure *via* either of the two mechanisms described above, we sought to determine the ability of agonists at one receptor subtype to antagonize the discriminative stimulus effects of agonists at the other receptor subtype. To this end, *mu* and *kappa* agonists of varying degrees of selectivity were tested alone and in combination with the training drug in squirrel monkeys trained to discriminate the *mu* agonist fentanyl (0.017 mg/kg) or the *kappa* agonist U50,488 (0.75 or 1.7 mg/kg) from water. In addition to fentanyl (Zimmerman et al., 1987; Dykstra and Massie, 1988), the *mu* agonists used in the study included (−)-metazocine (Villarreal, 1970; Slifer et al., 1986) and buprenorphine (Martin et al., 1976; Cowan et al., 1977; Dykstra, 1985). Of these, fentanyl has the highest selectivity in its affinity for *mu* receptors over *kappa* receptors (Chang and Cuatrecasas, 1981; Magnan et al., 1982), whereas (−)-metazocine is less selective (Magnan et al., 1982; Negus et al., 1989a; Berzetei-Gurski and Lowe, 1990) and buprenorphine is virtually nonselective (Wood et al., 1981; Leander, 1987; Negus et al., in press). The *kappa* agonists used in the study included U50,488 (VonVoightlander et al., 1983; Dykstra and Massie, 1988), tifluadom (Leander, 1984; Hayes and Kelly, 1985) and bremazocine (Romer et al., 1980; Corbett and Kosterlitz, 1986). Of these, U50,488 has the highest selectivity for *kappa* over *mu* receptors, whereas tifluadom is less selective and bremazocine is nonselective (James and Goldstein, 1984; Doty et al., 1989; Craft et al., 1989). We hypothesized that, if interactions were restricted to combinations of the training drugs with the less selective agonists, then the low receptor selectivity of these drugs would be sufficient to explain these interactions (see fig. 1a). However, if antagonist interactions extended to

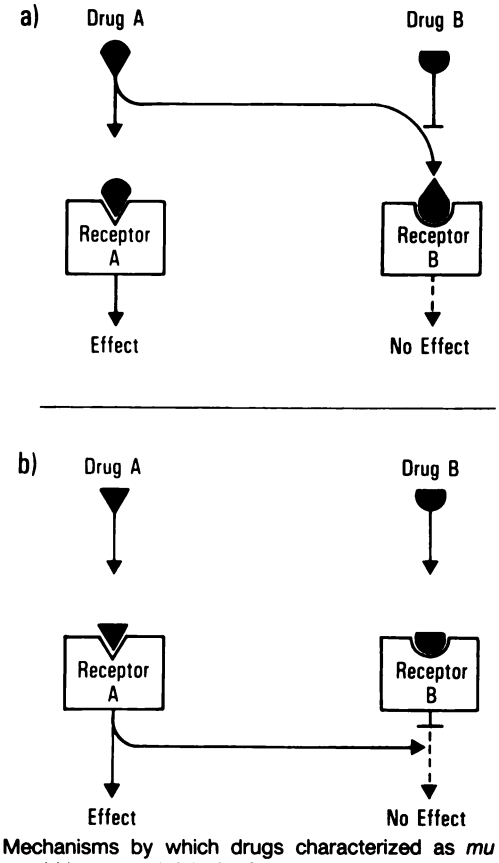

Fig. 1. Mechanisms by which drugs characterized as *mu* or *kappa* agonists could interact. a) A lack of receptor selectivity. A drug (Drug A) having low receptor selectivity could produce agonist effects by binding to one subtype of opioid receptor (Receptor A) at which it has high intrinsic efficacy, and simultaneously antagonize the effects of a second drug (Drug B) by binding to a second subtype of receptor (Receptor B) at which Drug A has low efficacy and Drug B has high efficacy. b) A biologic interaction. A drug (Drug A) could bind to one subtype of opioid receptor (Receptor A) at which it has high efficacy and activate the receptor to initiate a set of effects that, among other things, prevents the expression of effects produced by a second drug (Drug B) *via* a second type of opioid receptor (Receptor B).

combinations of the highly selective agonists fentanyl and U50,488 with each other, then this could be considered as evidence for a biologic interaction between *mu* and *kappa* agonists in the squirrel monkey drug discrimination procedure (see fig. 1b).

Methods

Subjects. Five adult male squirrel monkeys (*Saimiri sciureus*) were housed individually in a colony maintained on a 12-hr light-dark cycle and maintained at free-feeding weights. Four of the monkeys were experimentally naive at the start of the experiment, and one monkey (5-45) had had previous exposure to opioid agonists and antagonists. All monkeys had free access to water in the home cage and received 8 to 14 Purina Monkey Chows daily. Their diet was supplemented with fresh fruit. All housing facilities were accredited by the American Association for the Accreditation of Laboratory Animal Care.

Apparatus. Each monkey was held at the waist in the seated position in a primate chair, while its tail was held motionless by a small stock. Electric current was delivered *via* hinged brass electrodes that rested lightly on a shaved portion of the tail. Electrode paste was applied to the tail to ensure low resistance electrical contact. The electric shock was 120 V a.c., 60 Hz, delivered through an 18 kΩ dropping resistor. The shock intensity was adjusted to a final current of 3.0 mA by gating the current through a potentiometer. Two response levers were mounted side by side on the front wall facing the monkey. Each lever was located approximately 3.5 cm from the adjacent side wall. When either lever was pressed with a minimum force of 30 *g*, a response was recorded and an audible click produced. A Plexiglas barrier approximately 26 cm tall and 0.5 cm wide protruded 7 cm into the chamber between the two levers. The barrier was installed to discourage monkeys from pressing both levers simultaneously. A single red stimulus light was located 6 cm above each lever. The entire chair was enclosed in a ventilated, sound-attenuating chamber provided with continuous white masking noise and a house light. Programming and recording equipment were located in an adjacent room.

Behavioral procedure. A discrete-trial, shock-avoidance drug discrimination procedure, similar to that described by Schaefer and Holtzman (1977), was used. At the beginning of each session, the house light and stimulus lights were illuminated, and a 5-sec avoidance component was initiated. At the end of the 5-sec avoidance component, an escape component was initiated, during which the house light and stimulus lights remained on, and a series of 15 shocks (intensity: 3.0 mA; duration: 1 sec) was administered, with 1 sec between each shock. At the conclusion of the escape component, there was a 30-sec time-out. During the timeout, the house light and shock were turned off, but the stimulus lights remained on. At the end of the 30-sec time-out, a new trial was initiated with illumination of the house light and the beginning of a new 5-sec avoidance component. This sequence of discrete trials followed by 30-sec time-out periods continued until a maximum of 20 trials was completed.

During initial shaping of the lever press response, only one stimulus light was illuminated, and completion of a FR-1 response on the lever beneath that stimulus light during either the avoidance or escape component terminated the trial and initiated the 30-sec time-out period. Responses on the other lever were recorded but initially had no programmed consequences. Similarly, responses during the time-out period were recorded but had no programmed consequences. Once monkeys reliably terminated all 20 trials by responding on the appropriate lever during either the avoidance or escape component, drug discrimination training was initiated.

During drug discrimination training, both stimulus lights were illuminated, and an autotermination criteria was introduced such that failure by the monkey to terminate three consecutive trials automatically terminated the session. Monkeys in one group ($N = 3$) received i.m. injections of either distilled water or 0.017 mg/kg of fentanyl 15 min before the start of the session. A random sequence was used to determine which injection was administered, with the restriction that the same treatment was not given for more than two consecutive sessions and that the number of water and fentanyl injections was approximately equal. When water was administered, completion of an FR-1 response on one lever (water-appropriate lever) terminated the trial, whereas when fentanyl was administered, completion of an FR-1 response on the other lever (fentanyl-appropriate lever) terminated the trial. The left lever was designated as the fentanyl-appropriate lever for two of the monkeys, and the right lever was the fentanyl-appropriate lever for the third monkey. Beginning at this stage of training, each response on the inappropriate lever initiated a 3-sec changeover delay, during which responses on the injection-appropriate lever were recorded but had no programmed consequences (e.g., did not terminate the trial). Trials during which the first response was emitted on the injection-appropriate lever were designated as correct, whereas trials during which the first response was emitted on the injection-inappropriate lever, or during which no response was emitted, were designated as incorrect. These conditions remained in effect until the percentage of correct trials during a session was at or above a mean of 85% over 20 consecutive sessions.

All conditions were identical for a second group of monkeys ($N = 2$), except that U50,488 was used as the training drug. Both monkeys were initially trained to criterion to discriminate 0.75 mg/kg of U50,488 from water. However, one of the monkeys (7-14) subsequently lost the discrimination. As a result, the training dose of U50,488 for this monkey was raised to 1.7 mg/kg, and the monkey was retrained to criterion. Thus, for the remainder of the experiment, one monkey (7-9) discriminated 0.75 mg/kg of U50,488 from water, whereas the second monkey (7-14) discriminated 1.7 mg/kg of U50,488 from water. The left lever was designated as the U50,488-appropriate lever for both monkeys. It should be noted that attempts were made to train three other monkeys to discriminate U50,488 from water using these procedures, but these monkeys failed to reach criterion despite training for more than 100 sessions.

Substitution and antagonism tests. Once the discrimination criterion was met, substitution and antagonism tests were initiated. Test sessions differed from training sessions in three respects. First, during a test session, completion of an FR-1 response on either lever terminated the trial. Second, the escape component during a test session extended for a maximum of five shocks (rather than for a maximum of 15 shocks). Third, test sessions automatically terminated if a monkey failed to complete two (rather than three) consecutive trials. The second and third changes were implemented to protect monkeys from excessive exposure to shock during administration of sedative doses of the test drugs. Except for these differences, conditions during testing were identical to those described during training sessions. During all phases of testing, test sessions were conducted on Tuesdays and Fridays, whereas training sessions were continued on Mondays, Wednesdays and Thursdays.

During substitution tests, dose-effect relationships were determined for three *mu* agonists (fentanyl, buprenorphine and (−)-metazocine), three *kappa* agonists (U50,488, bremazocine and tifluadom), an opioid antagonist (naloxone) and a barbiturate (pentobarbital) in both groups of monkeys. A test drug was said to substitute for the training stimulus if some dose of the test drug produced at least 85% drug-appropriate responding. The dose-effect curves for the training drugs were determined twice, once at the beginning of the experiment and again at the end of the experiment.

During antagonism tests in the fentanyl-trained monkeys, the training dose of fentanyl was combined with selected doses of U50,488, bremazocine, tifluadom, naloxone or pentobarbital. In addition, the fentanyl dose-effect curve was redetermined in the presence of a high dose of U50,488 (0.56 mg/kg), bremazocine (0.01 or 0.03 mg/kg) or tifluadom (0.1 mg/kg). In the U50,488-trained monkeys, the training dose of U50,488 was combined with selected doses of fentanyl, buprenorphine, (−)-metazocine, naloxone or pentobarbital. The U50,488 dose-effect curve was also redetermined in the presence of a high dose of fentanyl (0.017 mg/kg), buprenorphine (0.03 mg/kg) or (−)-metazocine (3.0 or 5.6 mg/kg).

Dose-effect curves for all drugs in both groups of monkeys were probed up to doses that produced agonist effects (*e.g.*, substituted for the training drug), antagonist effects (*e.g.*, attenuated the discriminative stimulus effects of the training drug) or overt physiologic effects.

All drug doses were administered i.m. 15 min before the start of the session in a volume of 0.15 to 0.7 ml. When two drugs were administered in combination, the test drug was administered immediately before the training drug. All doses were administered in an irregular order that varied across monkeys.

Data analysis. The percent DAR was calculated as:

$$\frac{\text{no. drug-appropriate trials}}{20} \times 100$$

Dose-effect curves were then constructed by determining percentage of DAR as a function of drug dose. Data were used only from sessions in which all 20 trials were completed. The dose of a test drug required to increase DAR to 50% (ED_{50}) was determined where possible as follows. The linear portion of the dose-effect curve was defined as the line connecting the data points for doses producing an effect immediately above and immediately below 50% DAR. From these lines, the ED_{50} was determined by log-linear interpolation.

In addition, ARL was calculated as the average latency during a session between the beginning of each trial and the emission of the first response during that trial. Control latencies after administration of water were determined to be slightly more than 1.0 sec for all monkeys (see below) and, as the study progressed, it became apparent that increases in average response latency to values greater than 2.0 sec correlated with the emergence of overt physiologic effects such as sedation, tremors, rigidity (*mu* agonists) and/or salivation (*kappa* agonists). Thus, an increase in ARL to values greater than 2.0 sec was used as a criterion to identify the upper range of drug doses that could be probed safely.

Drugs. U50,488 methanesulfonate hydrate, (−)-metazocine fumarate, buprenorphine hydrochloride (all supplied by the National Institute on Drug Abuse), bremazocine methanesulfonate (Sandoz LT., Basel, Switzerland), fentanyl citrate (Janssen Pharmaceuticals, Beerse, Belgium) and naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO) were all dissolved in distilled water. *dl*-Tifluadom hydrochloride (Dr. M. Rance, Imperial Chemical Industries, Inc., Wilmslow, Cheshire, England) was dissolved in ethanol and propylene glycol in a 1:4 ratio, and pentobarbital (Sigma Chemical Co.) was dissolved in ethanol, propylene glycol and distilled water in a 1:2:7 ratio. Doses for all drugs are expressed in terms of the forms described above.

Results

Control performance. All three monkeys trained to discriminate 0.017 mg/kg of fentanyl from water reached criterion for testing and maintained their discrimination throughout the duration of the experiment. Table 1 shows the percentage of fentanyl-appropriate responding and ARLs for these monkeys on drug and water training days. Two monkeys trained to discriminate U50,488 (0.75 or 1.7 mg/kg) from water reached criterion for testing and maintained their discrimination throughout the duration of the experiment. The percentage of U50,488-appropriate responding and ARLs for these monkeys on drug and water training days are also shown in table 1.

Substitution tests with *mu* agonists in the fentanyl-trained monkeys. Figure 2 shows the discriminative stimulus effects of the *mu* agonists in the fentanyl-trained monkeys. Fentanyl (0.001–0.03 mg/kg), buprenorphine (0.001–1.0 mg/kg) and (−)-metazocine (0.1–10.0 mg/kg) substituted completely for the training dose of fentanyl in a dose-dependent manner in all three monkeys. Redetermination of the fentanyl dose-effect curves at the end of the study revealed little or no change in the sensitivity of individual monkeys to fentanyl during the course of the study. Table 2 shows the ED_{50} values for each of the *mu* agonists in individual monkeys. The relative potencies of the three *mu* agonists in producing fentanyl-like discriminative stimulus effects were fentanyl ≥ buprenorphine > (−)-metazocine. Thus, fentanyl and buprenorphine were roughly equipotent and at least 32 times more potent than (−)-metazocine.

Fentanyl and (−)-metazocine produced dose-dependent increases in ARL in all three monkeys (data not shown). A dose of 0.03 mg/kg of fentanyl increased ARL above 2.0 sec in all three monkeys, and prevented one monkey (7-13) from completing the session. (−)-Metazocine increased ARL above 2.0

TABLE 1

Percentage of drug-appropriate responding (%DAR) and average response latency (ARL; in seconds) on drug and water training days in individual monkeys trained to discriminate fentanyl (0.017 mg/kg) or U50,488 (0.75 mg/kg in monkey 7-9 or 1.7 mg/kg in monkey 7-14) from water

	Drug Training Days		Water Training Days	
	%DAR	ARL	%DAR	ARL
Fentanyl-trained monkeys				
7-13	99.8 ± 0.2	1.15 ± 0.02	6.7 ± 0.6	1.02 ± 0.02
7-12	99.7 ± 0.2	1.02 ± 0.02	0.9 ± 0.3	1.19 ± 0.02
5-45	99.8 ± 0.1	1.53 ± 0.06	4.4 ± 0.7	1.05 ± 0.02
U50,488-trained monkeys				
7-9	98.6 ± 0.1	0.9 ± 0.03	3.2 ± 0.5	1.16 ± 0.03
7-14	87.7 ± 1.4	1.82 ± 0.03	1.2 ± 0.1	1.15 ± 0.03

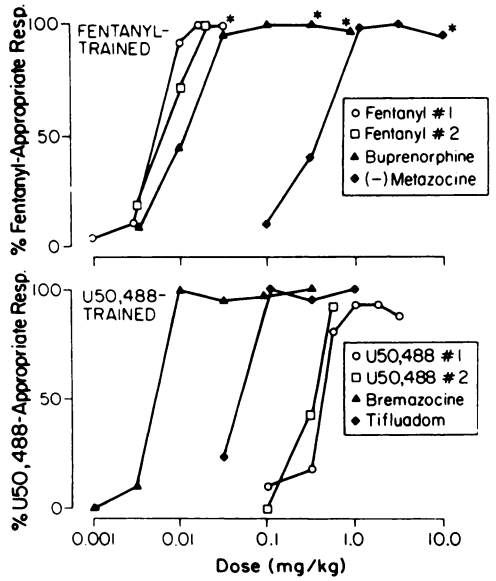

Fig. 2. Dose-effect determinations for *mu* opioid agonists in squirrel monkeys trained to discriminate fentanyl from water (top panel) or for *kappa* opioid agonists in squirrel monkeys trained to discriminate U50,488 from water (bottom panel). Abscissa: test drug dose in milligrams per kilogram, log scale. Ordinate: percentage of fentanyl-appropriate responding (top panel) or percentage of U50,488-appropriate responding (bottom panel). All points in the top panel represent the mean of a single determination in each of three monkeys except for points marked by *, which represent the mean of a single determination in two monkeys. All points in the bottom panel represent the mean of a single determination in each of two monkeys. For both the fentanyl and U50,488-trained monkeys, the training drug dose-effect curve no. 1 was determined at the beginning of the study, and the training drug dose-effect curve no. 2 was determined at the end of the study, approximately 1 year later.

TABLE 2

ED_{50} values in milligrams per kilogram for fentanyl alone (FENT), buprenorphine (BUP), (-)-metazocine (MTZ), fentanyl + 0.56 mg/kg U50,488 (FENT+U50), fentanyl + 0.01 or 0.03 mg/kg bremazocine (FENT+BREM), or fentanyl + .01 mg/kg tifluadom (FENT+TIFL) in monkeys trained to discriminate fentanyl from water.

Treatment	Monkey No.		
	7-13	7-12	5-45
FENT No. 1	0.006	0.006	0.005
FENT No. 2	0.012	0.005	0.004
BUP	0.015	0.017	0.005
MTZ	0.47	0.56	0.16
FENT + U50	0.012	0.005	0.004
FENT + BREM	—[a]	0.072	—[a]
FENT + TIFL	—[a]	0.023	—[a]

[a] ED_{50} could not be determined.

TABLE 3

ED_{50} values in milligrams per kilogram for U50,488 alone (U50), bremazocine (BREM), tifluadom (TIFL), U50,488 + 0.03 mg/kg of buprenorphine (U50 + BUP), U50,488 + 3.0 or 5.6 mg/kg of (−)-metazocine (U50 + MTZ) or U50,488 + 0.017 mg/kg fentanyl (U50 + FENT) in monkeys trained to discriminate U50,488 from water

Treatment	Monkey No.	
	7-9	7-14
U50 No. 1	0.39	0.42
U50 No. 2	0.22	0.41
BREM	0.005	0.005
TIFL	0.033	0.055
U50 + FENT	0.19	0.56
U50 + BUP	2.26	2.44
U50 + MTZ	0.87	1.00

sec in one monkey (5-45) at a dose of 3.0 mg/kg. In the other two monkeys, (−)-metazocine at doses up to 10.0 mg/kg had little effect on ARL, but a dose of 17.0 mg/kg prevented both monkeys from completing the session. Buprenorphine at a dose of 0.1 mg/kg increased ARL to 2.3 sec in one monkey (5-45), but buprenorphine at doses up to 1.0 mg/kg failed to increase ARL above 2.0 sec in the other two monkeys.

Substitution tests with *kappa* agonists in the U50,488-trained monkeys. Figure 2 also shows the discriminative stimulus effects of the *kappa* agonists in the U50,488-trained monkeys. U50,488 (0.1–3.0 mg/kg), bremazocine (0.001–0.3 mg/kg) and tifluadom (0.01–1.0 mg/kg) substituted completely for the training dose of U50,488 in a dose-dependent manner in both monkeys. Redetermination of the U50,488 dose-effect curves at the end of the study revealed little or no change in the sensitivity of individual monkeys to U50,488 during the course of the study. Table 3 shows the ED_{50} values for each of the *kappa* agonists in individual monkeys. The relative potencies of the three *kappa* agonists in producing U50,488-like discriminative stimulus effects were bremazocine > tifluadom > U50,488. Bremazocine was at least six times more potent than tifluadom and at least 44 times more potent than U50,488.

U50,488, bremazocine and tifluadom all produced dose-dependent increases in ARL in both monkeys (data not shown). ARL was increased above 2.0 sec by doses of 3.0 mg/kg of U50,488, 0.3 mg/kg of bremazocine and 1.0 mg/kg of tifluadom.

Substitution and antagonism tests with *kappa* agonists in the fentanyl-trained monkeys. Figure 3 shows the discriminative stimulus effects of the *kappa* agonists U50,488, bremazocine and tifluadom either alone or in combination with the training dose in the fentanyl-trained monkeys. U50,488

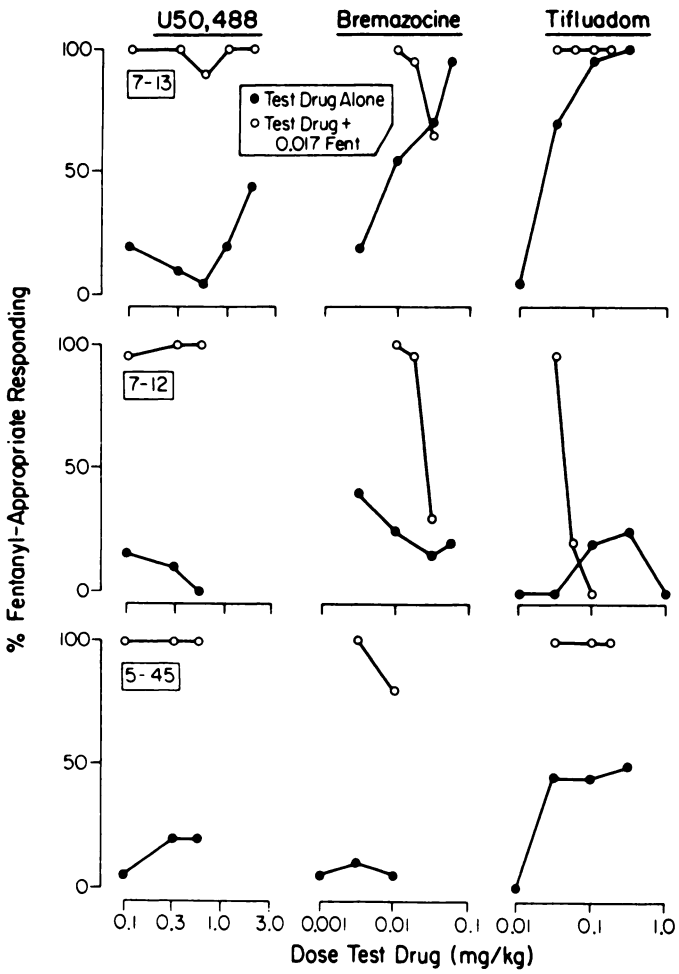

Fig. 3. Dose-effect determination for U50,488, bremazocine and tifluadom either alone or in combination with the training dose of fentanyl in individual squirrel monkeys trained to discriminate fentanyl from water. Abscissa: test drug dose in milligrams per kilogram, log scale. Ordinate: percentage of fentanyl-appropriate responding. All points represent the effects of a single determination in each monkey.

(0.1–1.7 mg/kg) neither substituted for fentanyl nor antagonized the discriminative stimulus effects of the training dose of fentanyl in any of the monkeys. Bremazocine and tifluadom, in contrast, produced both agonist and antagonist effects in the fentanyl-trained monkeys. Bremazocine (0.001–0.056 mg/kg) substituted completely for fentanyl in monkey 7-13, and attenuated the discriminative stimulus effects of the training dose of fentanyl to various degrees in all three monkeys. Tifluadom (0.01–0.3 mg/kg) substituted completely for fentanyl in monkey 7-13, and produced partial agonist effects that plateaued at 45 to 50% fentanyl-appropriate responding in monkey 5-45. Tifluadom did not substitute for fentanyl in monkey 7-12, but completely antagonized the discriminative stimulus effects of the training dose of fentanyl in this monkey.

When administered alone, all three *kappa* agonists produced dose-dependent increases in ARL (data not shown), with each of the *kappa* agonists being more potent in producing latency-increasing effects in the fentanyl-trained monkeys than in the U50,488-trained monkeys. U50,488 increased ARL above 2.0 sec in individual monkeys at doses ranging from 0.56 mg/kg (7-12, 5-45) to 1.7 mg/kg (7-13). Bremazocine increased ARL above 2.0 sec at doses of 0.01 mg/kg (5-45) and 0.56 mg/kg (7-

12, 7-13). Tifluadom increased ARL above 2.0 sec at doses of 0.3 mg/kg (5-45, 7-13) and 1.0 mg/kg (7-12).

Figure 4 shows the effects of redetermining individual fentanyl dose-effect curves after pretreatment with a high dose of each of the *kappa* agonists, and table 2 shows the ED_{50} values for these redeterminations of the fentanyl dose-effect curves. Pretreatment with 0.56 mg/kg of U50,488 did not affect the fentanyl dose-effect relationship in any of the three monkeys. For each monkey, the ED_{50} for fentanyl in combination with 0.56 mg/kg of U50,488 was within the range of the ED_{50} values for fentanyl alone.

Pretreatment with bremazocine produced a less consistent profile of effects. In two monkeys, pretreatment with bremazocine (0.03 mg/kg in monkey 7-13; 0.01 mg/kg in monkey 5-45) increased fentanyl-appropriate responding produced by low doses of fentanyl, but decreased fentanyl-appropriate responding produced by higher doses of fentanyl. Thus, the top half of the fentanyl dose-effect curve was shifted slightly to the right in both monkeys. In monkey 7-12, pretreatment with 0.03 mg/kg bremazocine shifted the entire fentanyl dose-effect curve to the right and produced a more than 10-fold increase in the fentanyl ED_{50}.

Tifluadom also produced a mixed profile of effects across monkeys. In two monkeys (7-13, 5-45), pretreatment with 0.1 mg/kg of tifluadom increased fentanyl-appropriate responding produced by low doses of fentanyl and had no effect on fentanyl-appropriate responding produced by higher doses of fentanyl. In monkey 7-12, on the other hand, pretreatment with 0.1 mg/kg of tifluadom shifted the entire fentanyl dose-effect curve approximately 4-fold to the right.

Substitution and antagonism tests with *mu* agonists in the U50,488-trained monkeys. Figure 5 shows the discriminative stimulus effects of the *mu* agonists fentanyl, buprenorphine and (−)-metazocine either alone or in combination with the training dose in the U50,488-trained monkeys. Fentanyl (0.003–0.03 mg/kg) neither substituted for U50,488 nor antagonized the discriminative stimulus effects of the training dose of U50,488 in either of the monkeys. Buprenorphine and (−)-metazocine, in contrast, produced primarily antagonist effects in the U50,488-trained monkeys. Buprenorphine (0.003–0.3 mg/kg) did not substitute for U50,488 in either of the two monkeys, but produced a dose-dependent and complete antagonism of the discriminative stimulus effects of the training dose of U50,488 in both monkeys. (−)-Metazocine (1.0–10.0 mg/kg) also failed to substitute for U50,488 in either monkey while producing a dose-dependent and a complete antagonism of the discriminative stimulus effects of the training dose of U50,488 in one monkey (7-9). A dose of 5.6 mg/kg of (−)-metazocine attenuated the discriminative stimulus effects of U50,488 in monkey 7-14, reducing DAR induced by the training dose of U50,488 to 75%. A dose of 10.0 mg/kg of (−)-metazocine

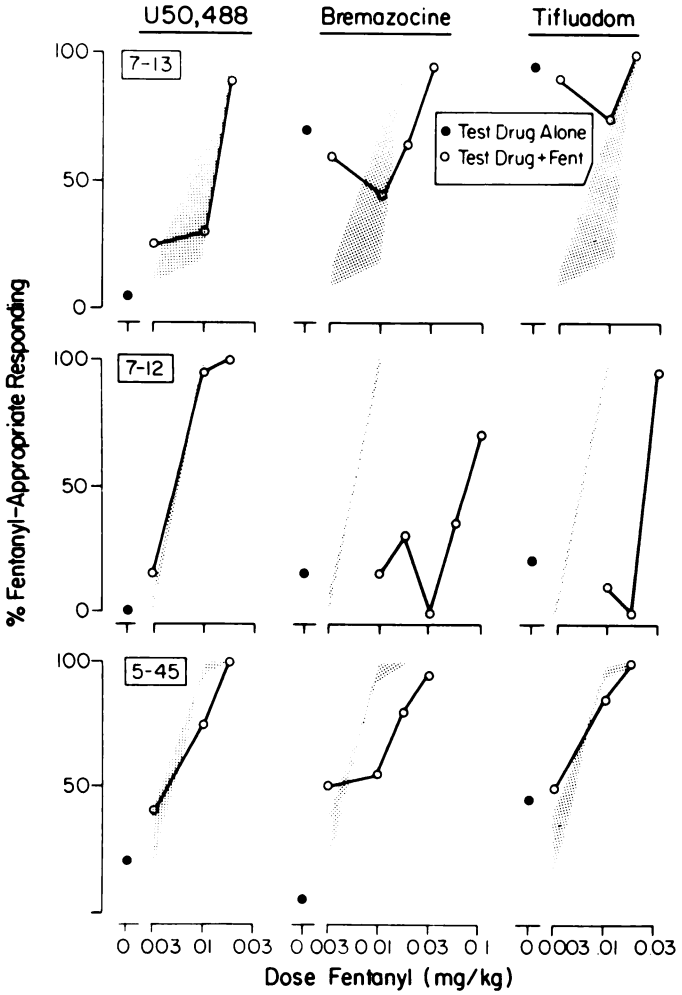

Fig. 4. Redeterminations of the fentanyl dose-effect curve in the presence of a high dose of U50,488 (0.56 mg/kg), bremazocine (0.01 mg/kg in monkey 5-45; 0.03 mg/kg in monkeys 7-13 and 7-12) or tifluadom (0.1 mg/kg) in individual monkeys trained to discriminate fentanyl from water. Abscissa: fentanyl dose in milligrams per kilogram, log scale. Ordinate: percentage of fentanyl-appropriate responding. The shaded regions in each graph show the range of values for two determinations of the fentanyl dose-effect curve, one obtained at the beginning of the study and one obtained at the end of the study. All points represent the effects of a single determination in each monkey.

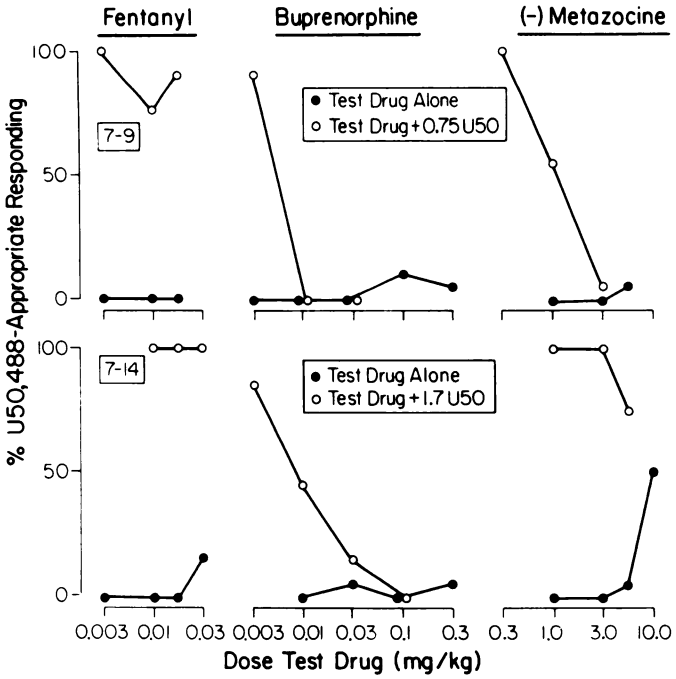

Fig. 5. Dose-effect determination for fentanyl, buprenorphine and (−)-metazocine either alone or in combination with the training dose of U50,488 in individual squirrel monkeys trained to discriminate U50,488 from water. Abscissa: test drug dose in milligrams per kilogram, log scale. Ordinate: percentage of U50,488-appropriate responding. All points represent the effects of a single determination in each monkey.

appeared to produce a further reduction in the discriminative stimulus effects of U50,488, but the monkey did not complete the session.

Fentanyl and (−)-metazocine produced dose-dependent increases in ARL at doses roughly equivalent to those increasing ARL in the fentanyl-trained monkeys (data not shown). With fentanyl, the lowest doses to increase ARL above 2.0 sec (0.03 mg/kg in 7-9; 0.56 mg/kg in 7-14) also prevented the monkeys from completing the session. With (−)-metazocine, a dose of 10.0 mg/kg increased ARL above 2.0 sec in both monkeys, and prevented one monkey (7-9) from completing the session. No dose of buprenorphine up to 1.0 mg/kg increased ARL above 2.0 sec in either monkey.

Figure 6 shows the effects of redetermining individual U50,488 dose-effect curves after pretreatment with a high dose of each of the *mu* agonists. Table 3 shows the ED_{50} values for these redeterminations of the U50,488 dose-effect curves. Pretreatment with 0.017 mg/kg of fentanyl produced only minor changes in sensitivity to U50,488, with the U50,488 dose-effect curve shifting slightly to the left in monkey 7-9, and slightly to the right in monkey 7-14.

Pretreatment with either buprenorphine or (−)-metazocine, in contrast, produced rightward shifts in the U50,488 dose-effect curves. Pretreatment with 0.03 mg/kg of buprenorphine produced a 5- to 10-fold rightward shift in the U50,488 dose-effect curves in both monkeys, whereas pretreatment with (−)-metazocine (3.0 mg/kg in monkey 7-9; 5.6 mg/kg in monkey 7-14) shifted the U50,488 dose-effect curve more than 2-fold to the right in both monkeys.

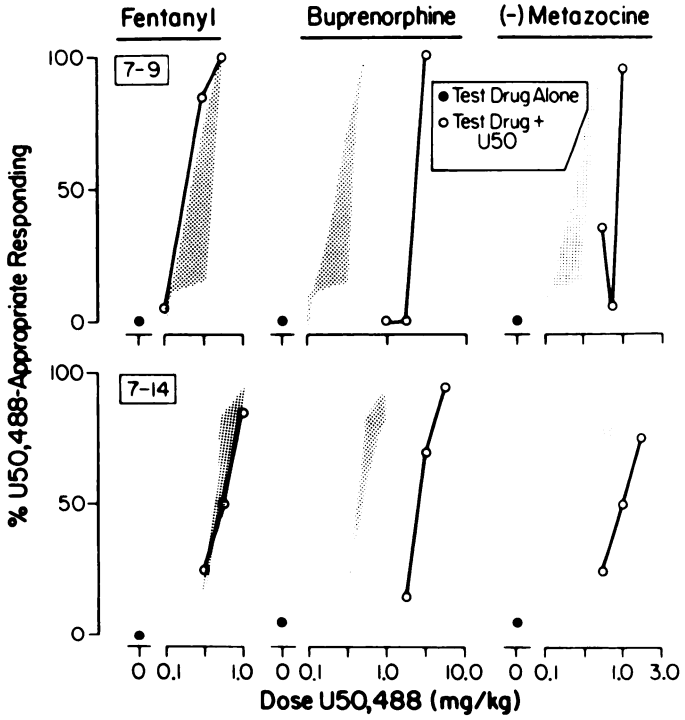

Fig. 6. Redeterminations of the U50,488 dose-effect curve in the presence of a high dose of fentanyl (0.017 mg/kg), buprenorphine (0.03 mg/kg) or (−)-metazocine (3.0 mg/kg in monkey 7-9; 5.6 mg/kg in monkey 7-14) in individual monkeys trained to discriminate U50,488 from water. Abscissa: U50,488 dose in milligrams per kilogram, log scale. Ordinate: percentage of U50,488-appropriate responding. The shaded regions in each graph show the range of values for two determinations of the U50,488 dose-effect curve, one obtained at the beginning of the study and one obtained at the end of the study. All points represent the effects of a single determination in each monkey.

Substitution and antagonism tests with naloxone and pentobarbital. Figure 7 shows the discriminative stimulus effects of naloxone and pentobarbital either alone or in combination with the training drug in the fentanyl- and U50,488-trained monkeys. Neither naloxone (0.01–1.0 mg/kg) nor pentobarbital (1.0–5.6 mg/kg) substituted for the training stimulus in any of the monkeys. In addition, pentobarbital had no effect on the discriminative stimulus effects of either fentanyl or U50,488, but naloxone produced a dose-dependent antagonism of both fentanyl and U50,488. Naloxone was 10- to 30-fold more potent as an antagonist of fentanyl than as an antagonist of U50,488.

The dose-effect curves for pentobarbital alone and pentobarbital in combination with the training dose of either U50,488 or fentanyl were probed up to doses that increased ARL above 2.0 sec in each monkey (data not shown). The range of naloxone doses probed in the present experiment had no effect on ARL.

Discussion

In the present study, a series of *mu* agonists and *kappa* agonists, as well as the opioid antagonist naloxone and the nonopioid barbiturate pentobarbital, were tested alone and in combination with the training drug in squirrel monkeys trained to discriminate either the selective *mu* agonist fentanyl or the selective *kappa* agonist U50,488 from water. Once established, the discrimination between either fentanyl or U50,488 and water was accurate and, insofar as the present experiment covered a period of more than 1 year, maintained over a long period of time. Thus, this study demonstrates that squirrel monkeys, like other species (Colpaert, 1978; Picker and Dykstra, 1987; Picker and Dykstra, 1989; Picker *et al.*, 1990), can be trained to discriminate either fentanyl or U50,488 from vehicle.

In the fentanyl-trained monkeys, only the *mu* agonists fentanyl, buprenorphine and (−)-metazocine substituted completely for the training stimulus in all three monkeys. Thus, all three drugs acted as full *mu* agonists in the present study. The potency order of the *mu* agonists in producing fentanyl-appropriate responding was fentanyl ≥ buprenorphine > (−)-metazocine. This potency order agrees with the potency order of

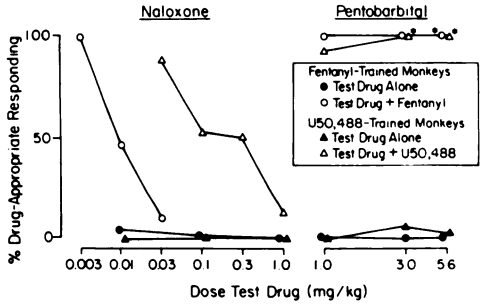

Fig. 7. Dose-effect determination for naloxone and pentobarbital either alone or in combination with the training dose of the training drug in squirrel monkeys trained to discriminate either fentanyl or U50,488 from water. Abscissa: test drug dose in milligrams per kilogram, log scale. Ordinate: percentage of drug-appropriate responding. Points marked by * show data for only one monkey. All other points for the fentanyl-trained monkeys represent the mean of a single determination in each of three monkeys, whereas all other points for the U50,488-trained monkeys represent the mean of a single determination in each of two monkeys.

these compounds in other assays and in other species (Young *et al.*, 1981; Young *et al.*, 1984; Slifer *et al.*, 1986; Negus and Dykstra, 1988).

In addition, the full *mu* agonist effects of fentanyl, buprenorphine and (−)-metazocine in the present study agree with other studies demonstrating that these compounds act as full *mu* agonists in drug discrimination procedures using a *mu* agonist as the training drug (Picker and Dykstra, 1989; Negus *et al.*, 1989a). However, these results contrast with the partial agonist and antagonist effects of buprenorphine and (−)-metazocine in other assays of *mu* agonist activity. For example, both buprenorphine and (−)-metazocine have been reported to produce little if any dependence after chronic administration in monkeys, but to precipitate withdrawal in monkeys dependent on morphine (Ager *et al.*, 1969; Villarreal, 1970; Cowan *et al.*, 1977; Dum *et al.*, 1981). These assay-dependent effects suggest that buprenorphine and (−)-metazocine have an intrinsic efficacy at *mu* opioid receptors that is intermediate between low efficacy ligands such as naloxone and high efficacy ligands such as fentanyl (see Kenakin, 1987, for a discussion of the determination of relative efficacies). Furthermore, the ability of buprenorphine and (−)-metazocine to produce full-agonist effects in the present study suggests that the squirrel monkey drug discrimination procedure is highly sensitive to the agonist effects of *mu* receptor ligands.

The selective *kappa* agonist U50,488 neither substituted for nor antagonized the discriminative stimulus effects of fentanyl in the fentanyl-trained monkeys. This lack of agonist or antagonist effects by U50,488 was probably not a result of inadequate dosages inasmuch as U50,488 was probed up to doses that increased ARL above 2.0 sec in the fentanyl-trained monkeys and that produced clear discriminative stimulus effects in the U50,488-trained monkeys. These results agree with the demonstration that U50,488 does not modify the discriminative stimulus effects of morphine in rats (Negus *et al.*, in press), and they suggest that effects mediated by *kappa* opioid receptors do not interact with the discriminative stimulus effects mediated by *mu* opioid receptors.

The less selective *kappa* agonists tifluadom and bremazocine produced both agonist and antagonist effects in the fentanyl-trained monkeys. Because U50,488 produced neither agonist nor antagonist effects in these monkeys, it is unlikely that the effects of tifluadom or bremazocine were mediated by *kappa* opioid receptors. Rather, these results can be explained on the basis of the similar affinities of these nonselective drugs for both *mu* and *kappa* receptors. Tifluadom has been demonstrated to produce both *mu* antagonist and weak *mu* agonist effects (Jackson and Sewell, 1984; Ureta *et al.*, 1987; Sheldon *et al.*, 1987; Picker and Dykstra, 1989), suggesting that it has low to intermediate intrinsic efficacy at *mu* receptors. In the present study, its profile of agonist and antagonist effects varied considerably between monkeys. Tifluadom acted as a full agonist in one monkey (7-13), a partial agonist in a second monkey (5-45) and an antagonist in the third monkey (7-12). However, this differential sensitivity to the effects of tifluadom was consistent within each monkey: in those monkeys in which tifluadom acted as an agonist it had no antagonist effects, whereas in the monkey in which tifluadom lacked agonist effects it acted as an antagonist. Bremazocine produced qualitatively similar results, with bremazocine's agonist effects being most pronounced in monkey 7-13, and the antagonist effects being most pronounced in monkey 7-12. The demonstration of *mu* agonist effects by bremazocine in the present study contrasts with other studies indicating that bremazocine acts purely as an antagonist at *mu* receptors (Corbett and Kosterlitz, 1986; Craft *et al.*, 1989). However, Hayes and Birch (1988) reported that bremazocine's analgesic effects in the mouse abdominal constriction test could be partially reversed by the selective *mu* antagonist β-funaltrexamine, suggesting that bremazocine may produce some agonist effects at the *mu* receptor. The present results further support the contention that bremazocine may have liminal efficacy at *mu* opioid receptors that can be detected only in sensitive assays such as the drug discrimination procedure.

In the U50,488-trained monkeys, only the *kappa* agonists U50,488, bremazocine and tifluadom substituted completely for the training stimulus. The potency order of the *kappa* agonists in producing U50,488-appropriate responding was bremazocine > tifluadom > U50,488. This potency order agrees with the relative potencies of these drugs in other assays (Leander, 1985; Hayes *et al.*, 1987; Dykstra and Massie, 1988; Negus *et al.*, in press). In addition, U50,488, bremazocine and tifluadom all acted as full *kappa* agonists in the present experiment. This is consistent with both other drug discrimination studies and with studies using other procedures, that have characterized U50,488, bremazocine and tifluadom as high-efficacy *kappa* agonists (Leander, 1985; Hayes *et al.*, 1987; Picker and Dykstra, 1989; Negus *et al.*, in press).

The selective *mu* agonist fentanyl produced neither agonist nor antagonist effects in either of the U50,488-trained monkeys. This lack of effect by fentanyl probably does not reflect inadequate dosages because fentanyl was probed up to doses that increased ARL above 2.0 sec in the U50,488-trained monkeys, and that produced clear discriminative stimulus effects in the fentanyl-trained monkeys. These results contrast with the ability of both fentanyl and morphine to attenuate the discriminative stimulus effects of U50,488 in the rat (Negus *et al.*, in press). In that study, Negus *et al.* concluded that, in the rat, *mu* agonists appear to be capable of perceptually masking the discriminative stimulus effects of U50,488, and that this masking presumably reflects a biologic interaction between the discriminative stimulus effects mediated by *mu* and *kappa* receptors. Interestingly, this example of perceptual masking in the rat was not reciprocated in that U50,488 did not attenuate the discriminative stimulus effects of morphine. In the present experiment, the lack of an effect by fentanyl in the U50,488-trained monkeys suggests that, in squirrel monkeys, the effects mediated by *mu* opioid receptors do not interact with the discriminative stimulus effects mediated by *kappa* receptors.

The less selective *mu* agonists buprenorphine and (−)-metazocine both produced primarily antagonist effects in the U50,488-trained monkeys. Buprenorphine has been demonstrated to have low intrinsic efficacy at *kappa* receptors such that it functions exclusively as a *kappa* antagonist (Richards and Sadee, 1985; Leander, 1987; Negus and Dykstra, 1988; Negus *et al.*, 1989b), and the present results agree with this characterization of buprenorphine as an antagonist at *kappa* receptors. To our knowledge, the relative binding affinity of (−)-metazocine for *mu* and *kappa* opioid receptors has not been explored; however, binding studies with racemic metazocine have concluded that metazocine does bind with moderate affinity to *kappa* receptors (Magnan *et al.*, 1982). In addition, previous studies have demonstrated that (−)-metazocine can act as a *kappa* antagonist both *in vitro* (Berzetei-Gurski and

Loew, 1990) and *in vivo* (Negus *et al.*, 1989a). The present results concur with this characterization of (−)-metazocine as a low-efficacy *kappa* ligand.

Naloxone did not substitute for the training stimulus in either the fentanyl- or the U50,488-trained monkeys. However, naloxone did produce a dose-dependent antagonism of the training dose of the training drug in both groups of monkeys, indicating that the discriminative stimulus effects of both fentanyl and U50,488 were mediated by opioid receptors. Furthermore, naloxone was 10 to 30 times more potent in antagonizing the effects of fentanyl than in antagonizing the effects of U50,488. This agrees with the approximately 10-fold selectivity of naloxone for *mu vs. kappa* receptors (Wood *et al.*, 1981; Magnan *et al.*, 1982) and further supports the contention that the discriminative stimulus effects of fentanyl are mediated by *mu* receptors, whereas the effects of U50,488 are mediated by *kappa* receptors.

Pentobarbital, even at doses up to those increasing ARL above 2.0 sec, produced neither agonist nor antagonist effects in either group of monkeys. Pentobarbital has been demonstrated to produce marked discriminative stimulus effects that are distinct from the discriminative stimulus effects of opioids (Barry, 1974; Herling *et al.*, 1980). The present results extend the data base, indicating that the discriminative stimulus effects of pentobarbital differ from those of the opioids and additionally suggest that the stimulus effects produced by pentobarbital do not interact with the stimulus effects mediated by either *mu* or *kappa* opioid receptors.

The effects of all eight drugs tested were qualitatively similar in both of the U50,488-trained monkeys despite the more than 2-fold difference in the training doses used (0.75 mg/kg in monkey no. 7-9 and 1.7 mg/kg in monkey no. 7-14). In general, the *kappa* agonists were slightly more potent in the monkey trained with the lower training dose of U50,488. This agrees with previous studies demonstrating that, as the training dose increases, the potency of test drugs in substituting for the training drug decreases (Shannon and Holtzman, 1979; Picker *et al.*, 1990). Drugs with *kappa* antagonist effects [buprenorphine, (−)-metazocine and nalaxone] also tended to be more potent in blocking the discriminative stimulus effects of the lower training dose in monkey 7-9 than the higher training dose in monkey 7-14. This probably reflects differential potency of naloxone in reversing the effects of low *vs.* high doses of U50,488 rather than differential sensitivity of the two monkeys to *kappa* antagonist effects. For example, although buprenorphine was approximately 10 times more potent in reversing the discriminative stimulus effects of 0.75 mg/kg of U50,488 in monkey 7-9 than in reversing the discriminative stimulus effects of 1.7 mg/kg of U50,488 in monkey 7-14, redetermination of the U50,488 dose-effect curves after pretreatment with 0.03 mg/kg of buprenorphine resulted in equivalent 6- to 7-fold rightward shifts in both monkeys.

In conclusion, this study presents evidence that some drugs characterized as either *mu* agonists or *kappa* agonists can produce interacting effects in the squirrel monkey drug discrimination procedure. However, these interactions appear to rely on drug-dependent mechanisms (see fig. 1a) because they are observed only with combinations involving drugs with low selectivity for *mu* and *kappa* receptors. The inability of the highly selective agonists fentanyl and U50,488 to antagonize each other's discriminative stimulus effects argues against a biologic interaction between the discriminative stimulus effects mediated by *mu* and *kappa* receptors (fig. 1b). Rather, these results suggest that *mu* and *kappa* receptors mediate independent discriminative stimulus effects in the squirrel monkey.

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Send reprint requests to: Dr. Steve Negus, Division of Preclinical Neuroscience and Endocrinology, Dept. of Basic and Clinical Research, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Rd., La Jolla, CA 92037.