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Buprenorphine as a Stimulus in Drug Discrimination Learning: An Assessment of Mu and Kappa Receptor Activity

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POURNAGHASH, S. AND A. L. RILEY. *Buprenorphine as a stimulus in drug discrimination learning: An assessment of mu and kappa receptor activity.* PHARMACOL BIOCHEM BEHAV 46(3) 593-604, 1993. – Using the conditioned taste aversion baseline of drug discrimination learning, different groups of animals were trained to discriminate either buprenorphine or morphine from distilled water. Specifically, animals were injected with buprenorphine or morphine prior to a saccharin-LiCl pairing and the drug vehicle prior to saccharin alone. By the fifth conditioning trial, animals differentially consumed saccharin on the basis of administration of the drug or its vehicle. In subsequent generalization tests, buprenorphine stimulus control generalized completely to the mu agonist morphine in four of the five subjects tested, while morphine stimulus control completely generalized to buprenorphine in two of five subjects and partially generalized in the remaining three. Buprenorphine failed to generalize to the relatively selective kappa antagonist MR2266 and the broad-based antagonist diprenorphine. Morphine also failed to generalize to MR2266, but did generalize to diprenorphine. That morphine and buprenorphine displayed some degree of cross-generalization suggests that these compounds share some stimulus property, presumably their agonist activity at the mu receptor, and that the mu activity of these compounds was used in the establishment of the discrimination, a conclusion supported by the fact that compounds with mu antagonist activity (e.g., naloxone, MR2266) blocked both buprenorphine and morphine stimulus control. That buprenorphine failed to generalize to compounds with kappa antagonist activity suggests that animals trained to discriminate buprenorphine from its vehicle do not use the kappa antagonist activity of the drug in the establishment of the discrimination. The basis for the differential ability of various receptor subtypes to mediate the discriminative properties of compounds with mixed receptor activity was discussed.

Drug discrimination learning Conditioned taste aversions Opiate antagonists Generalization

BUPRENORPHINE, an oripavine-derived narcotic with high affinity for both the mu and kappa subtypes of the opiate receptor (45,52), has been reported to substitute for morphine in animals trained to discriminate morphine from its vehicle in an operant drug discrimination design (11,12,40,46,57). That is, following the acquisition of a morphine vs. distilled water discrimination, animals display morphine-appropriate responding when buprenorphine is given in place of morphine. Similar generalization patterns have been reported when other mu agonists [e.g., codeine (17), fentanyl (21), etorphine (59), and hydromorphone (39)] were the training drugs. The fact that stimulus control produced by these mu agonists generalizes to buprenorphine is consistent with work in other behavioral designs demonstrating the mu agonist properties of buprenorphine [e.g., antinociception (5,10,23), production of Straub tail response and catalepsy (5), suppression of schedule-controlled behavior (21,32), and the development of cross-tolerance to morphine (5,32)].

Buprenorphine has also been reported to antagonize the diuretic (22,41) and schedule-controlled (23,31,32) effects of kappa agonists (e.g., bremazocine and U50,488). In such assessments, buprenorphine appears to have no kappa agonist activity [see also (32,47,59)], suggesting that buprenorphine is not only a partial mu agonist, but a pure kappa antagonist as well. Although buprenorphine has been reported to have kappa antagonist properties in a number of behavioral preparations, there have been no assessments of this activity of buprenorphine within drug discrimination learning; i.e., whether animals trained to discriminate buprenorphine from its vehicle would generalize this control to kappa antagonists. This was addressed in the present experiment, in which animals were trained to discriminate 0.56 mg/kg buprenorphine from distilled water within the taste aversion baseline of drug discrimination learning (24,27,29,43). Specifically, every fourth day buprenorphine was administered prior to a saccharin-LiCl pairing. On intervening days, the distilled water vehicle was administered prior to a nonpoisoned exposure to the same saccharin solution. Once animals had acquired the dis-

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crimination and were differentially consuming saccharin based on the presentation of the drug or its vehicle, animals were administered various doses of compounds with varying degrees of kappa antagonist activity; e.g., the broad-based antagonist diprenorphine and the relatively selective kappa antagonist MR2266, to assess the generalization of the stimulus properties of buprenorphine to these test compounds. Buprenorphine-trained animals were also administered morphine to determine whether the mu agonist properties of buprenorphine would be evident in animals for which buprenorphine (as opposed to another mu agonist) was the training drug. Finally, a number of compounds (e.g., naloxone, MR2266, U50,488) were administered concurrently with buprenorphine to assess their ability to block its stimulus properties. A second group of subjects was treated identically to those above except that morphine (and not buprenorphine) was the training stimulus. This group was run to determine the effects of the various drug manipulations on a mu agonist (i.e., morphine)-based discrimination.

METHOD

Subjects and Apparatus

The subjects were 24 experimentally naive, female rats of Long-Evans descent, approximately 120 days of age at the beginning of the experiment. The subjects were housed in individual wire-mesh cages and were maintained on a 12L : 12D cycle at an ambient temperature of 23°C for the duration of the experiment.

Drugs

Buprenorphine hydrochloride, diprenorphine hydrochloride, morphine sulfate, and U50,488 {trans-3,4-dichloro-*N*-methyl-*N*-[2-(*l*-pyrrolindinyl)cyclohxyl] benzeactamine methanesulfonate hydrate} were generously supplied by the National Institute on Drug Abuse. MR2266 [(−)-5,9-diethyl-2-(3-furylmethyl)-*s*-hydroxy-6,7-benzomorphan] was generously supplied by Boehringer Ingelheim, and naloxone hydrochloride was generously supplied by DuPont Pharmaceuticals, Inc. MR2266 was prepared as an emulsion in a vehicle of 4% Tween-80 in distilled water. All other drugs were prepared in distilled water. All drugs were injected in a volume of 1 ml/kg of body weight. Doses for all drugs are expressed in terms of the forms noted above.

Procedure

Phase I: Conditioning. Following 24 h of water deprivation, all subjects were given 20-min access to water once a day for 14 consecutive days. On days 15–17 (saccharin habituation), a novel saccharin solution (0.1% w/v sodium saccharin, Fisher Purified) replaced water during the daily 20-min fluid-access period. On day 17, all subjects were matched on saccharin consumption and assigned to one of four groups (groups BL, BW, ML, and MW; $n = 6$ per group). On day 18, subjects in groups BL and BW were given an intraperitoneal (IP) injection of 0.56 mg/kg of buprenorphine 30 min prior to saccharin access (11,23). Immediately following saccharin consumption, subjects in group BL were given an IP injection of 1.8 mEq, 0.15 M LiCl (76.8 mg/kg). Subjects in group BW were given an equivolume injection of the distilled water vehicle. Subjects in groups ML and MW were treated similarly except that 15 min prior to saccharin consumption they were given an IP injection of 5.6 mg/kg of morphine (38). On the following 3 days, all subjects were injected with distilled water 15 min prior to saccharin access. No injections were given following saccharin on these recovery days. This alternating procedure of conditioning/recovery was repeated until all experimental subjects were consuming less than *50%* of the *mean* of their respective control subjects following administration of the training drug (13 cycles).

Phase II: Generalization. The procedure in this phase was identical to that in phase I with the following exception. On the second recovery day following conditioning, subjects in groups BL and BW received one of a range of doses of morphine (0–18 mg/kg) and subjects in groups ML and MW received one of a range of doses of buprenorphine (0–1 mg/kg) prior to saccharin access. On subsequent probe sessions, all subjects were given a range of doses of diprenorphine (0–24 mg/kg) (48,49) or MR2266 (0–3.2 mg/kg) (53) 15 min prior to saccharin access. The subjects received the full range of doses of diprenorphine prior to testing with MR2266. For any individual drug, the doses were given in a mixed order with the order identical for all subjects. LiCl was not administered following any of these probes. Individual subjects in groups BL and ML were tested for generalization only if they had discriminative control by the training drug immediately prior to a generalization test; i.e., a subject in group BL or group ML consumed no more than 50% of the mean consumption of subjects in their respective control groups (groups BW and MW) on the conditioning trial immediately preceding that specific generalization session. Such a criterion ensured that the generalization function was based on stable discriminative control. During this phase, generalization was defined as consumption following the probe drug falling either at or below the mean (±SEM) consumption of saccharin following the training drug.

Phase III: Antagonism. The procedure in this phase was identical to that in phase I with the exception that on the second recovery session following conditioning in this phase all subjects were injected with one of three compounds prior to their respective training drug. Specifically, naloxone (1 mg/kg) (48), MR2266 (1.8 mg/kg) (53), or U50,488 (1 mg/kg) (33,38) was administered 15 min prior to buprenorphine for subjects in groups BL and BW and morphine for subjects in groups ML and MW. At their respective delays (i.e., 30 min for subjects in groups BL and BW; 15 min for subjects in groups ML and MW), all subjects were then given 20-min access to saccharin. Each of the above compounds was also administered prior to an injection of distilled water to assess their unconditioned effects on saccharin consumption in the absence of the training drug. For all subjects, the assessment of antagonism with naloxone was completed before MR2266 was tested. Similarly, the assessment with MR2266 was completed prior to testing U50,488. LiCl was not administered following any of these probe sessions. As above, individual subjects in groups BL and ML were tested in this phase only if they had discriminative control by the training drug immediately prior to the antagonism test; i.e., a subject in group BL or group ML consumed no more than 50% of the mean consumption of subjects in their respective control groups (groups BW and MW) on the conditioning trial immediately preceding that specific antagonism session.

If an individual subject displayed weight loss or obvious signs of distress during any phase of the conduct of the experiment, they were removed from training and testing, given supplemental water, and observed for recovery. Only when body weight and consumption were stable was the animal returned to the experimental procedures.

Statistical Analysis

All determinations of statistical significance during the acquisition of the drug discrimination are based on a Mann-Whitney *U*-test and the Wilcoxon matched-pairs signed-ranks test. The Mann-Whitney *U*-test was performed on all between-group comparisons of saccharin consumption. The Wilcoxon matched-pairs signed-ranks test was performed on all within-group comparisons of saccharin consumption. An analysis of variance (ANOVA) was performed on saccharin consumption over recovery sessions. Statements of significance are based on $p < 0.05$. Absolute probabilities are presented for all comparisons.

RESULTS

Phase I: Acquisition

Figure 1 illustrates the mean amount (±SEM) of saccharin consumption for subjects in groups BL and BW (top panel) and groups ML and MW (bottom panel) during saccharin habituation and over the repeated conditioning/recovery cycles in this phase. As illustrated, there were no significant differences in saccharin consumption between groups BL and BW and between groups ML and MW during saccharin habituation ($U = 151.5, 172.5, p = 0.741$, and $U = 162, 162, p = 1.00$, respectively). The mean consumption of saccharin averaged over the 3 days of saccharin habituation was 11.39 and 11.30 ml for subjects in groups BL and BW and 11.38 and 11.27 ml for subjects in groups ML and MW, respectively. On the initial conditioning trial, saccharin consumption for subjects in all groups decreased slightly, though nonsignificantly ($z = -0.135, p = 0.889$ and $z = -0.73, p = 0.459$ for groups BL and BW, and $z = -0.105, p = 0.912$ and $z = -1.67, p = 0.93$ for groups ML and MW, respectively), below that consumed during saccharin habituation. There were no significant differences between groups BL and BW ($U = 16.5, 19.5, p = 0.810$) or between groups ML and MW ($U = 12.5, 23.5, p = 0.373$) on the initial conditioning trial. By the fifth conditioning trial, subjects in group BL drank significantly less than subjects in group BW ($U = 5, 31, p = 0.036$), while subjects in group ML drank significantly less saccharin than subjects in group MW ($U = 0, 36,$

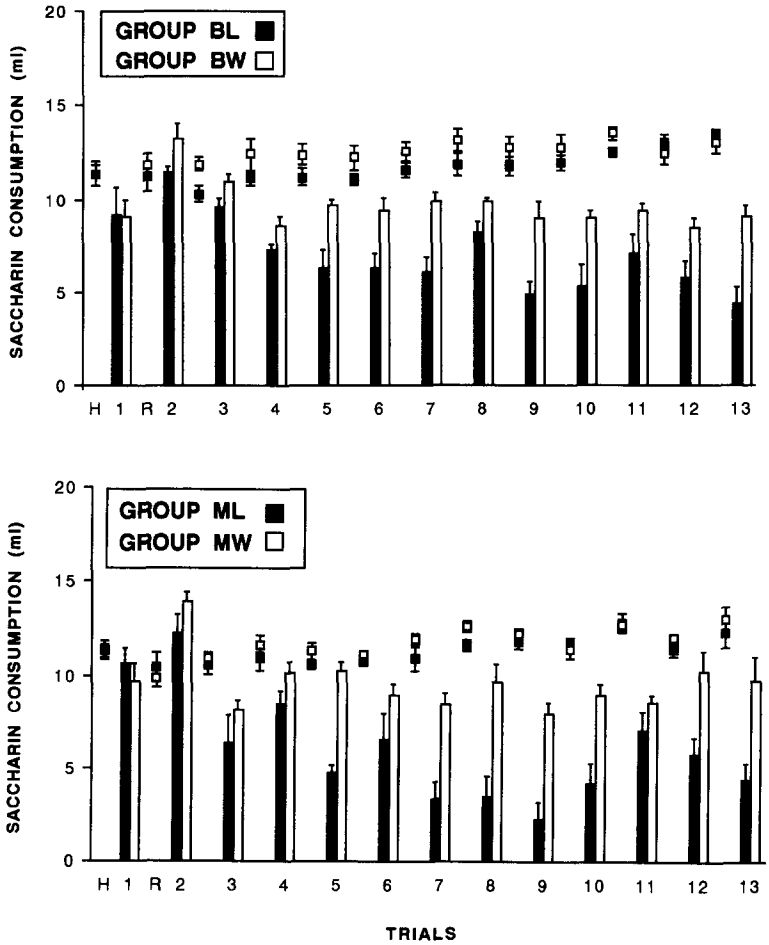

FIG. 1. The mean amount (± SEM) of saccharin consumption for subjects in Groups BL and BW (top panel) and Groups ML and MW (bottom panel) over the repeated conditioning trials (filled and open columns, respectively). The filled and open squares represent a mean (± SEM) of saccharin consumption on the three days of Saccharin Habituation (H) and on the three recovery sessions (R) between each conditioning trial.

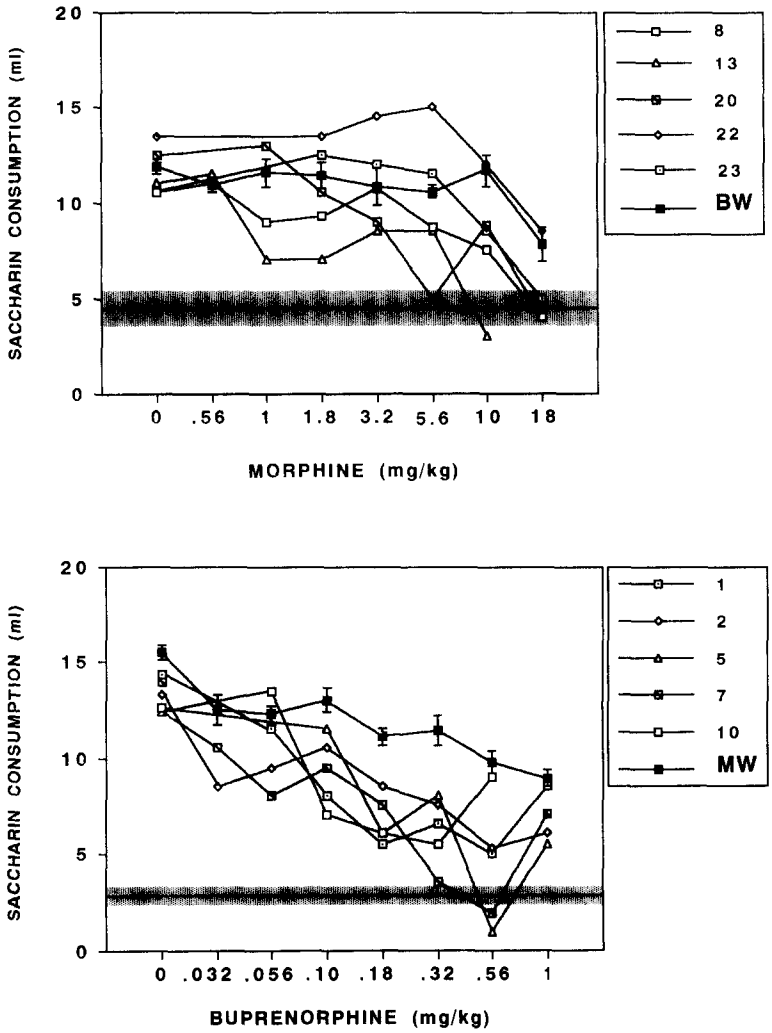

FIG. 2. The amount of saccharin consumption for individual subjects in group BL (top panel) and group ML (bottom panel) following 1/4 log doses of morphine and buprenorphine, respectively, during cross-generalization tests. The mean amount (±SEM) of saccharin consumption for subjects in groups BW and MW (i.e., vehicle-treated subjects) is indicated by the solid square in each figure. The mean amount of saccharin consumption following the training dose of buprenorphine (0.56 mg/kg) and morphine (5.6 mg/kg) for subjects in groups BL and ML, respectively, is indicated by the horizontal line in the center of the shaded area. The shaded area above and below this horizontal line illustrates ± SEM. The specific order in which given doses of morphine and buprenorphine were administered was 5.6, 3.2, 1.8, 10, 18, 1, and 0.56 mg/kg (morphine) and 0.56, 0.32, 0.18, 1, 0.1, 0.056, and 0.032 mg/kg (buprenorphine).

$p = 0.036$). These differences were maintained over conditioning (though see trials 11 and 12 for group BL and trials 6 and 11 for group ML). On the final conditioning trial of this phase, subjects in groups BL and BW drank 4.41 and 9.08 ml and subjects in groups ML and MW drank 4.58 and 9.75 ml, respectively. On this trial, individual subjects in groups BL and ML drank less than 50% of the mean of their respective control groups. During recovery sessions, saccharin consumption for all groups remained high, approximating habituation levels. There were no significant differences between groups BL and BW, $F(1, 34) = 2.331$, $p = 0.136$, or between groups ML and MW, $F(1, 34) = 1.479$, $p = 0.2323$, during recovery.

Phase II: Generalization

Morphine and buprenorphine. Figure 2 illustrates absolute saccharin consumption for individual subjects trained with buprenorphine (i.e., group BL, top panel) and with morphine (i.e., group ML, bottom panel) following injections of morphine (0–18 mg/kg) and buprenorphine (0–1 mg/kg), respectively. For comparison, the mean amount (±SEM) of saccharin consumption following the training dose of buprenorphine or morphine for each experimental group is also included in the figures. Finally, the mean (± SEM) of saccharin consumption for control subjects (i.e., groups BW and MW) is presented to illustrate the unconditioned effects of the two

drugs on saccharin intake. A single subject in both groups BL and ML failed to maintain discriminative control during this phase (see above-mentioned criterion for testing). As such, generalization data were not collected for these two subjects. The data presented are for the remaining five subjects in each of the two experimental groups.

As illustrated in the top panel, for four of the five subjects in group BL, saccharin consumption following the higher doses of morphine—e.g., 10 mg/kg (subject #13) and 18 mg/kg (subjects #8, 20, and 23)—was similar to the levels consumed following the training dose of buprenorphine; i.e., buprenorphine stimulus control completely generalized to morphine. A single subject (subject #22) did not display the dose-related decreases noted above. This subject maintained saccharin consumption at or above control levels, displaying no generalization. Although control subjects also decreased saccharin consumption at the highest dose of morphine tested, no individual control subject drank at or below the mean (± SEM) consumption of saccharin by group BL following the training dose of buprenorphine. As illustrated in the bottom panel, for two of the five subjects in group ML (subjects #5 and 7) consumption following 0.56 mg/kg buprenorphine was below the level following the training dose of morphine; i.e., morphine stimulus control generalized to buprenorphine. For the remaining three subjects (subjects #1, 2, and 10), consumption decreased with increasing doses of buprenorphine; however, these subjects never fully generalized morphine control to buprenorphine (i.e., consumption only approached the

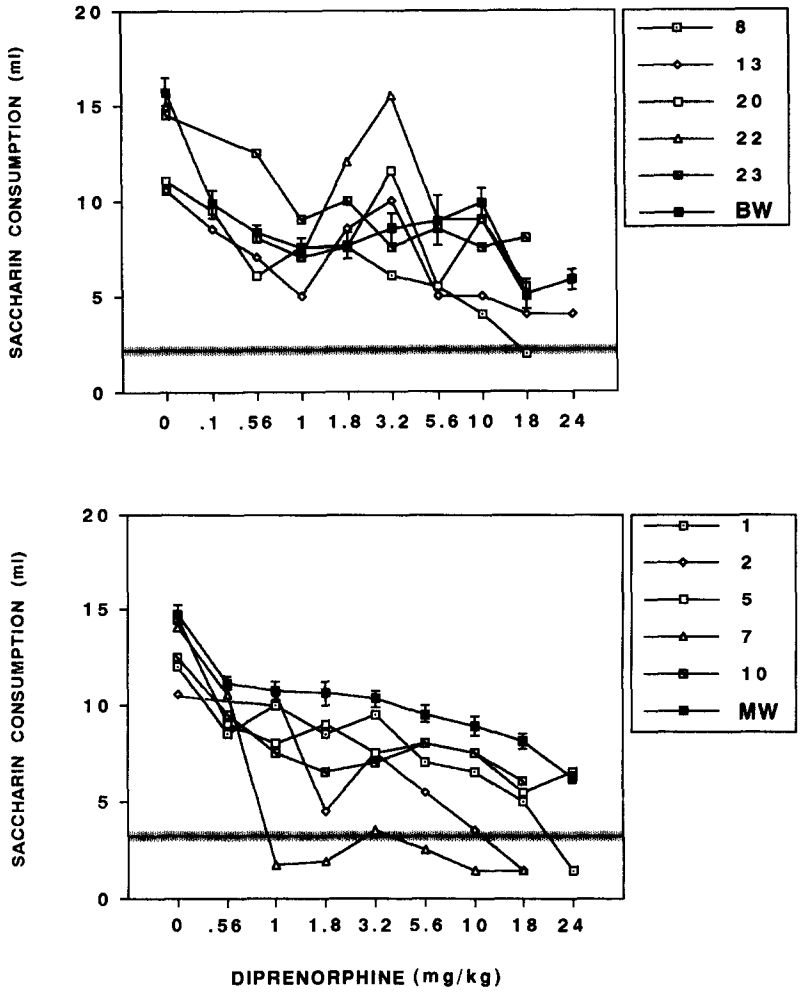

FIG. 3. The amount of saccharin consumption for individual subjects in group BL (top panel) and group ML (bottom panel) following 1/4 log doses of diprenorphine. The mean amount (± SEM) of saccharin consumption for subjects in groups BW and MW (i.e., vehicle-treated subjects) is indicated by the solid square in each figure. The mean amount of saccharin consumption following the training dose of buprenorphine (0.56 mg/kg) and morphine (5.6 mg/kg) for subjects in groups BL and ML, respectively, is indicated by the horizontal line in the center of the shaded area. The shaded area above and below this horizontal line illustrates ± SEM. The specific order in which given doses of diprenorphine were administered was 3.2, 5.6, 0.56, 10, 0.1, 1.8, 1, 18, and 24 mg/kg (groups BL and ML) and 3.2, 5.6, 1.8, 1, 0.56, 10, 18, and 24 mg/kg (groups ML and MW).

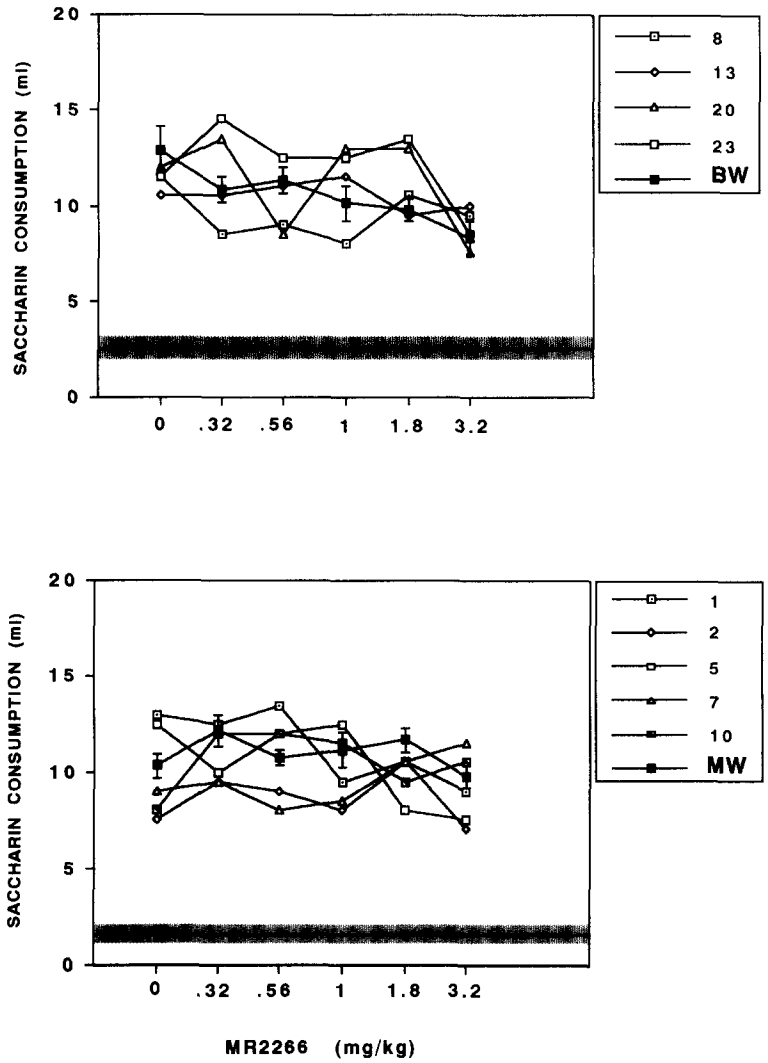

FIG. 4. The amount of saccharin consumption for individual subjects in group BL (top panel) and group ML (bottom panel) following 1/4 log doses of MR2266. The mean amount (±SEM) of saccharin consumption for subjects in groups BW and MW (i.e., vehicle-treated subjects) is indicated by the solid square in each figure. The mean amount of saccharin consumption following the training dose of buprenorphine (0.56 mg/kg) and morphine (5.6 mg/kg) for subjects in groups BL and ML, respectively, is indicated by the horizontal line in the center of the shaded area. The shaded area above and below this horizontal line illustrates ± SEM. The specific order in which given doses of diprenorphine were administered was 1, 1.8, 3.2, 0.56, and 0.32 mg/kg for all groups.

level consumed following the training drug). It is of interest to note that for all subjects in group ML, saccharin consumption following the higher doses of buprenorphine increased and was at or near the level consumed following buprenorphine in the control subjects (group MW). Although subjects in group MW also decreased saccharin consumption with increasing doses of buprenorphine, this decrease was not as dramatic as that seen in group ML. No control subject approximated the level consumed by subjects in group ML following the training dose of morphine.

Diprenorphine. Figure 3 presents the same measures as Fig. 2 during generalization tests with various doses of diprenorphine (0–24 mg/kg). As above, two subjects (one from both groups BL and ML) failed to maintain discriminative control during testing with diprenorphine. The data presented are for the remaining five subjects in each experimental group. As illustrated in the top panel, only a single subject in group BL generalized buprenorphine control to diprenorphine (see subject #8). Consumption for the remaining subjects did not approach that following the training dose of buprenorphine and showed no consistent differences from the mean consumption for subjects in group BW. On the other hand, three of the subjects in group ML (subjects #1, 2, and 7) reduced consumption following diprenorphine below the level following the training dose of morphine. Although for the remaining two subjects generalization was weak and partial (see subjects

#5 and #10), with only a single exception (see subject #5 at 24 mg/kg diprenorphine) consumption for these subjects was consistently lower than that of the control subjects.

MR2266. Figure 4 presents the same measures as Fig. 2 during generalization tests with various doses of MR2266 (0–3.2 mg/kg). Two subjects (one from both groups BL and ML) again failed to maintain discriminative control during testing, and the data presented are for the remaining five subjects in each experimental group. The top and bottom panels illustrate saccharin consumption for individual subjects in groups BL and ML, respectively. As illustrated, at no dose of MR2266 did consumption by subjects in either Groups BL or ML ap-

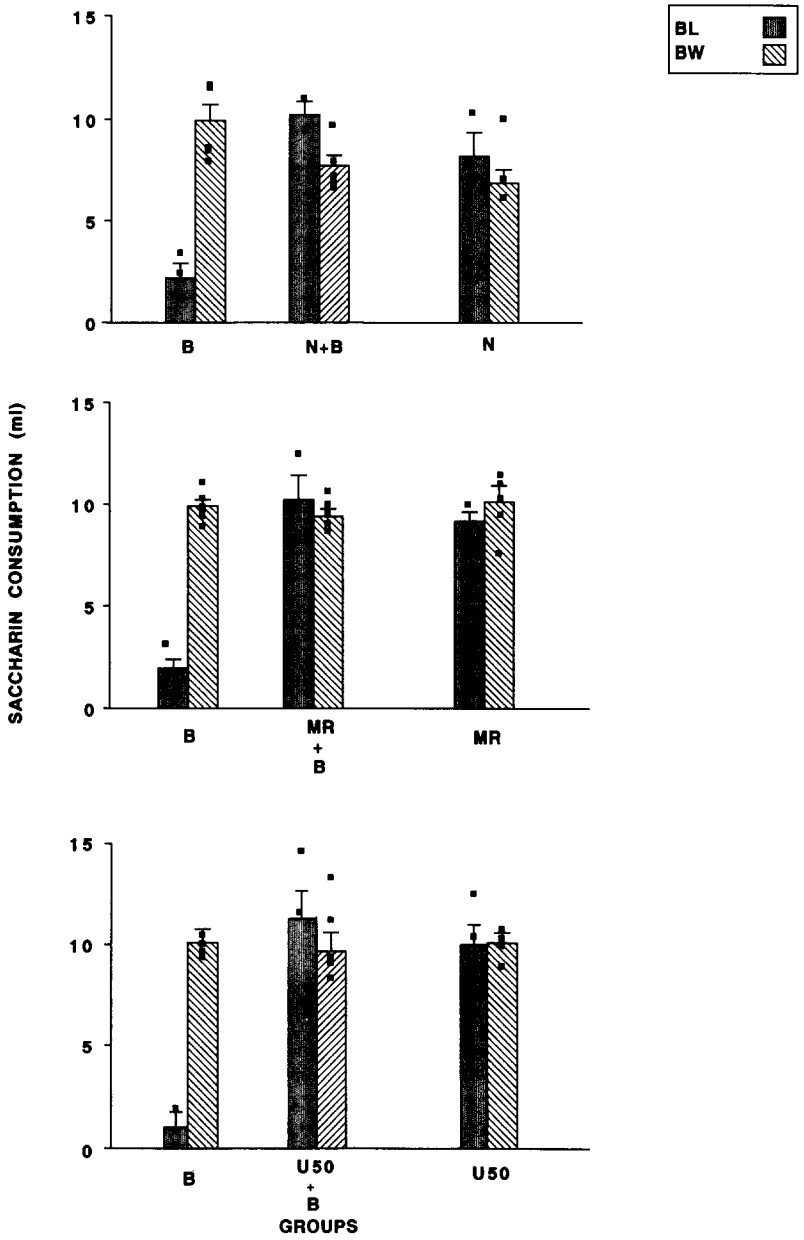

FIG. 5. The mean amount (±SEM) of saccharin consumption for subjects in groups BL (shaded bars) and BW (striped bars) following buprenorphine (B), naloxone (N), and the naloxone/buprenorphine combination (N + B) (top panel). The mean amount (±SEM) of saccharin consumption for subjects in groups BL (shaded bars) and BW (striped bars) following buprenorphine (B), MR2266 (MR), and the MR2266/buprenorphine combination (MR + B) (middle panel). The mean amount (±SEM) of saccharin consumption for subjects in groups BL (shaded bars) and BW (striped bars) following buprenorphine (B), U50,488 (U50), and the U50,488/buprenorphine combination (U50 + B) (bottom panel). The closed squares on each bar represent saccharin consumption for individual subjects in these assessments.

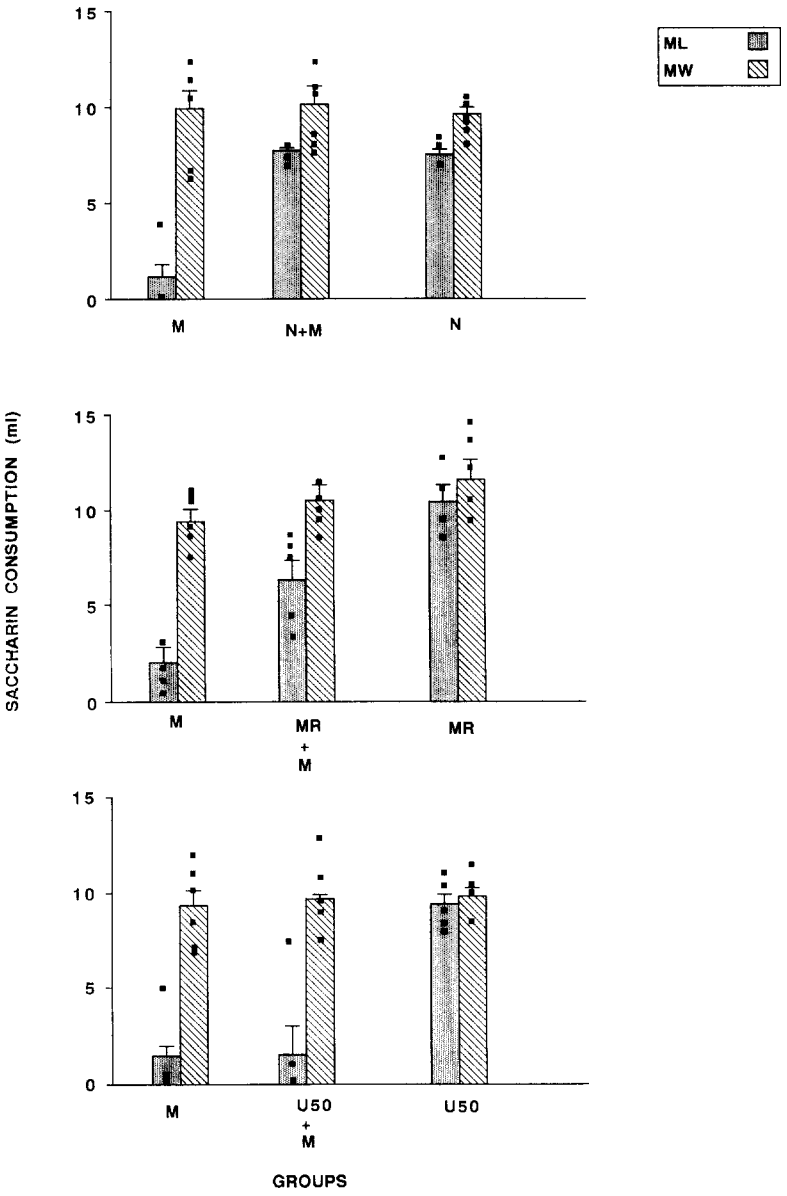

FIG. 6. The mean amount (± SEM) of saccharin consumption for subjects in groups ML (shaded bars) and MW (striped bars) following morphine (M), naloxone (N), and the naloxone/morphine combination (N + M) (top panel). The mean amount (± SEM) of saccharin consumption for subjects in groups ML (shaded bars) and MW (striped bars) following morphine (M), MR2266 (MR), and the MR2266/morphine combination (MR + M) (middle panel). The mean amount (± SEM) of saccharin consumption for subjects in groups ML (shaded bars) and MW (striped bars) following morphine (M), U50,488 (U50), and the U50,488/morphine combination (U50 + M) (bottom panel). The closed squares on each bar represent saccharin consumption for individual subjects in these assessments.

proach the level consumed following their respective training drugs. Further, there was no consistent difference between experimental and control groups when MR2266 was administered for either training condition.

Phase III: Antagonism

The top panel of Fig. 5 illustrates the mean amount (± SEM) of saccharin consumption for subjects in groups BL and BW following buprenorphine (B), naloxone (N), and the naloxone/buprenorphine combination (N + B). Data for individual subjects in each condition are noted by points on the bars. Three subjects from group BL failed to maintain discriminative control during this phase of testing, and the data presented are for the remaining three subjects in this experimental group. As illustrated, subjects in group BL drank less saccharin than subjects in group BW following the training dose of buprenorphine. When naloxone (1 mg/kg)

was given in combination with buprenorphine, subjects in group BL increased consumption of saccharin above the level consumed following buprenorphine alone; i.e., naloxone antagonized the discriminative control of buprenorphine. This antagonism was complete in that consumption by subjects in group BL following the combination was similar to (or greater than) the amount consumed by the control subjects given the same drug combination. Antagonism of discriminative control was also evident when MR2266 (0.56 mg/kg) and U50,488 (1 mg/kg) were given in combination with buprenorphine (see Fig. 5, middle and bottom panels, respectively). As illustrated, subjects in group BL increased consumption when given the drug combinations above the amount consumed when given buprenorphine alone. Further, these subjects drank at levels comparable to those consumed by subjects in group BW, again indicating that the antagonism was complete for both MR2266 and U50,488.

The top panel of Fig. 6 illustrates the mean amount (± SEM) of saccharin consumption for subjects in groups ML and MW following morphine (M), naloxone (N), and the naloxone/morphine combination (N + M). As above, data for individual subjects in each condition are noted by points on the bars. Again, two subjects from group ML failed to maintain discriminative control during this phase of testing, and the data presented are for the remaining four subjects in this experimental group. As with buprenorphine, naloxone antagonized the stimulus effects of morphine; i.e., saccharin consumption following the combination was greater than that following morphine alone and comparable to the amount consumed by subjects in group MW following the same drug combination. The effects of MR2266 on stimulus control by morphine were mixed (see Fig. 6, middle panel), with two subjects drinking at levels only slightly higher than that following morphine alone, i.e., minimal antagonism, and three subjects drinking at levels similar to or only slightly less than controls (near complete antagonism). Finally, only a single animal in group ML displayed any antagonism of the morphine stimulus by U50,488 (this subject drank slightly less than the lowest amount consumed by control subjects). The remaining four animals in group ML continued to avoid saccharin consumption when injected with the combination of U50,488 and morphine; i.e., there was no evidence of antagonism for these subjects (see Fig. 6, bottom panel).

DISCUSSION

As described, animals injected with buprenorphine prior to a pairing of saccharin and LiCl acquired the drug discrimination, consuming less saccharin following buprenorphine than following the buprenorphine vehicle. Although buprenorphine has been reported to produce discriminative effects sufficient to support drug discrimination learning [as evidenced by the fact that animals trained on a different opiate drug generalize this control to buprenorphine; see (11,12, 40,46,57,58)], the present study represents the first demonstration that discriminative control can be established to buprenorphine. The rate of acquisition of the buprenorphine discrimination, as well as the degree of control it produced, are quite similar to effects reported with other opiate (19,27,28,48, 49,51) and nonopiate (8,15,18,24,25,29,30,42,56) compounds within the taste aversion baseline of drug discrimination learning.

As described, all animals trained in the present experiment to discriminate morphine from its vehicle displayed some degree of morphine stimulus control to buprenorphine. Although subjects initially decreased saccharin consumption as the dose of buprenorphine increased (generalizing morphine control), at 0.56 and 1 mg/kg they increased consumption of saccharin (i.e., subjects displayed vehicle-appropriate responding). Such biphasic dose–response functions with buprenorphine have been reported by others in a variety of behavioral preparations [(2,5,16); though see (22,50)], including drug discrimination learning [(32); though see (46,57,58)]. Also, the majority of animals trained to discriminate buprenorphine from its vehicle displayed some degree of generalization of buprenorphine stimulus control to morphine. Although a single animal drank at control levels throughout testing, four of the five animals following the administration of the higher doses of morphine drank at (or below) the level consumed following the training dose of buprenorphine. Given the common activity of morphine and buprenorphine at the mu receptor, the ability of these two compounds to display cross-generalization is likely based on their shared activity at this receptor. As such, buprenorphine's mu receptor activity appears able to establish discriminative control.

Although buprenorphine's activity at the mu receptor may be able to support drug discrimination learning, there has been no assessment of whether its kappa antagonist activity can do so as well. To test this, in the present experiment several opiates with kappa antagonist activity were given in place of buprenorphine in buprenorphine-trained animals. As described, neither the broad-based opiate antagonist diprenorphine (4,26) nor the relatively selective kappa antagonist MR2266 (60) produced buprenorphine-appropriate responding; i.e., buprenorphine stimulus control did not generalize to either compound. Thus, these generalization patterns do not support the position that kappa antagonist activity mediates (or contributes to) the drug discrimination with buprenorphine. In animals trained to discriminate morphine from distilled water, the kappa antagonist MR2266 also failed to substitute for morphine. Interestingly, diprenorphine did produce morphine-appropriate behavior. Given that diprenorphine is generally described as an antagonist at mu, delta, and kappa receptors (4,26), it is somewhat surprising that it would substitute for the mu agonist morphine. Although typically described as an antagonist, diprenorphine has also been reported to have opiate agonist effects in several different preparations (9,20). It is possible that this receptor activity is the basis for the generalization between morphine and diprenorphine. If the generalization was mu mediated, it might be expected that buprenorphine stimulus control would have generalized to diprenorphine as well, given the aforementioned generalization between buprenorphine and morphine. Thus, the basis for the morphine/diprenorphine generalization remains unknown.

Given that buprenorphine's stimulus control appeared to be based on its mu agonist activity, the ability of the mu antagonist naloxone to block buprenorphine's control was assessed. As noted, naloxone completely blocked the stimulus properties of buprenorphine [see also (11,46)]. The ability of the mu antagonist naloxone to block buprenorphine's effects is again consistent with the position that buprenorphine's stimulus control was based on its mu agonist activity. MR2266 also antagonized buprenorphine stimulus control with the degree of antagonism comparable to that produced by naloxone. MR2266 only partially antagonized morphine's stimulus control, with subjects drinking at levels intermediate to that consumed following morphine alone and that consumed by control subjects receiving the morphine/naloxone combination. Although MR2266 is a relatively selective kappa antagonist, it binds (26,55) and has antagonist activity at the mu receptor as

well (1,44,53). That MR2266 failed to substitute for buprenorphine, but blocked its effects instead, again suggests that buprenorphine's stimulus control was based on its mu agonist activity.

As described, U50,488 completely blocked the stimulus effects of buprenorphine, while having only a marginal effect on that of morphine (a single animal displayed antagonism). The fact that a kappa agonist blocked the effects of buprenorphine suggests that the discriminative properties of buprenorphine may have been mediated to some degree by its kappa antagonist activity. Accordingly, the stimulus properties of buprenorphine may have been based on a combination of mu and kappa activity. Although possible, it would be difficult to account for the fact that there was no apparent generalization between buprenorphine and other kappa antagonists, an effect that would be expected if buprenorphine's stimulus control was in any part mediated by its kappa antagonist activity. It could be argued that generalization tests are less sensitive than antagonism test in detecting the stimulus properties of a compound; however, the basis for such a difference is not immediately clear. Several other possibilities exist for U50, 488's antagonism of buprenorphine's stimulus control. For example, Craft and Dykstra (6,7) have recently reported that in monkeys a variety of kappa agonists (including U50,488) may have antagonist activity at the mu receptor. Accordingly, the antagonism of buprenorphine by U50,488 could still be consistent with the aforementioned conclusion that buprenorphine's stimulus control was mu based. Secondly, given that U50,488 has been reported to support drug discrimination learning when used as the training drug (33,38), it is possible that its own stimulus properties overshadowed those of buprenorphine or combined with them in such a way to change the stimulus properties available at the time of the test [(32); see also (13,14,34,35)]. Under such a condition, buprenorphine's stimulus control would be affected in a way that would be difficult to distinguish from that due to a pharmacological interaction (i.e., receptor competition).

If buprenorphine's stimulus effects are mediated primarily at the mu receptor, it remains to be determined why its kappa activity does not support such learning. Several possibilities exist. One possibility is that discriminative control cannot be established by kappa antagonist activity. This possibility does not assume anything unique about buprenorphine, but simply notes that for some as yet undetermined reason kappa antagonist activity does not produce a stimulus effect detectable by the rat. Interestingly, in unpublished data from our lab utilizing the same taste aversion baseline, the relatively selective kappa antagonist MR2266 was unable to establish discriminative control even at high doses and with repeated training. One might argue that it is not that the kappa antagonist properties of buprenorphine cannot support drug discrimination learning, but that opiate antagonists in general are relatively weak in establishing such control. Although it has been very difficult to establish discriminative control with the opiate antagonists in more traditional assessments of drug discrimination learning unless extremely high doses are used or extensive training is given (3,36), such learning has been reported within the taste aversion baseline for both naloxone (19,48) and diprenorphine (49). That these antagonists with relatively high mu opiate antagonist activity can serve as discriminative stimuli suggests that selective kappa antagonist receptor activity (and not opiate antagonism in general) may be insufficient as a discriminative stimulus.

A second account of the failure to demonstrate kappa discriminative control with buprenorphine is related to the fact that the different receptor activity of compounds with multiple receptor effects are not equally salient. For example, Negus et al. (32) have recently reported that in opiate-naive rats buprenorphine (like morphine and the kappa agonist bremazocine) suppressed schedule-controlled responding (FR30). When buprenorphine was given in combination with bremazocine, there was no effect on the bremazocine-induced suppression of responding; i.e., there was no evidence of the kappa antagonist property of buprenorphine. After the subjects were made tolerant to morphine (as evidenced by the fact that neither morphine nor buprenorphine affected responding when given alone), buprenorphine now antagonized bremazocine's effect; i.e., buprenorphine acted as a kappa antagonist. Negus et al. concluded from these data that the kappa antagonist effects of buprenorphine were masked by its mu agonist's effects in opiate-naive animals. Only when animals were made tolerant to these latter effects could one see the kappa antagonist properties of buprenorphine. Thus, the different receptor activity had different saliencies, dependent upon the presence or absence of an opiate history (37). In relation to the present failure of the kappa properties of buprenorphine to establish discriminative control, it is possible that during the acquisition of the drug discrimination the mu properties of buprenorphine overshadowed its kappa properties and prevented these latter properties from establishing control. Although morphine-tolerant rats display cross-tolerance to buprenorphine within the drug discrimination procedure [see (57,58)], there have been no assessments of whether these subjects trained on buprenorphine and made tolerant to morphine now generalize buprenorphine control to kappa antagonists or are unaffected by mu antagonists.

Independent of the basis for the failure of kappa activity to contribute to the acquisition of discriminative control with buprenorphine, it is clear that the mu and kappa activities of buprenorphine do not contribute equally to its stimulus effects. Future work may determine whether such differential control reflects an inability of specific receptor activity to establish control or an overshadowing of specific receptor activity that, in the absence of the masking stimulus, could be an effective drug stimulus.

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