Pharmacology Biochemistry and Behavior, Vol. 63, No. 3, pp. 481–488, 1999
© 1999 Elsevier Science Inc.
Printed in the USA. All rights reserved
0091-3057/99/$–see front matter

PII S0091-3057(99)00008-8

# Nalorphine's Ability to Substitute for Morphine in a Drug Discrimination Procedure is a Function of Training Dose

SHERI D. GRABUS, SCOTT T. SMURTHWAITE AND ANTHONY L. RILEY

*Psychopharmacology Laboratory, Department of Psychology, American University, Washington, DC 20016*

Received 28 August 1998; Revised 11 December 1998; Accepted 22 December 1998

GRABUS, S. D., S. T. SMURTHWAITE AND A. L. RILEY. *Nalorphine's ability to substitute for morphine in a drug discrimination procedure is a function of training dose.* PHARMACOL BIOCHEM BEHAV **63**(3) 481–488, 1999.—Rats trained to discriminate the mu agonists fentanyl or morphine from their respective vehicles generalize to the partial mu agonist nalorphine incompletely and inconsistently. Any number of factors may influence the generalization patterns obtained, one of which being the specific dose of the full opioid agonist used during training, a factor reported to influence generalization with other partial opioid agonists. To assess if training dose influences stimulus generalization to nalorphine and to support its role in the aforementioned variability across studies, in the present experiments rats were trained to discriminate either a low (5.6 mg/kg) or a high (10 mg/kg) dose of morphine from distilled water within the taste aversion baseline of drug discrimination learning. Subjects were then given a range of doses of morphine, nalorphine, methadone, or naloxone to assess the degree of substitution (if any) of these compounds for the training dose of morphine. For all subjects, morphine fully substituted for itself, and the opioid antagonist naloxone failed to substitute for the morphine cue. Rats generalized the morphine cue to nalorphine in subjects trained at the lower dose but not in subjects trained at the higher dose. Rats generalized the morphine cue to methadone in the latter group (the high dose group), indicating that the failure to generalize to nalorphine in this group was not a general inability of an opioid agonist to substitute for morphine. Naloxone blocked morphine stimulus control in all subjects and nalorphine control in the low-dose group for which nalorphine substituted for morphine, suggesting that morphine control (and the nalorphine substitution) was based on opioid activity. These results indicate that the substitution patterns of nalorphine in morphine-trained subjects are a function in part of the dose of morphine used in training and support the position that nalorphine is a partial opioid agonist with intermediate efficacy. © 1999 Elsevier Science Inc.

Training dose Morphine Nalorphine Drug discrimination learning Conditioned taste aversion

IN a recent discussion of the discriminative stimulus properties of nalorphine and other mixed action opioids (2,10,20, 22,24,34), Picker suggested that nalorphine produces a kappa (as opposed to mu)-like stimulus profile in rats [see (19,22)], a conclusion based on a variety of sources assessing the ability of nalorphine to substitute for mu [(3,7,9,10,17,41); however, see (4)] and kappa [(31,32); however, see (11)] opioid agonists within drug discrimination learning. Although nalorphine generally fails to substitute for mu agonists in rats, this failure may be more a function of training dose than the general absence of any similarity of discriminative effects between nalorphine and other mu agonists. Support for this position comes from the fact that in other assessments of mu agonists, training dose affects the substitution of partial agonists. For example, the partial mu opioid agonists meperidine and profadol substitute for a low, but not a high, dose of fentanyl in pigeons trained to discriminate two doses of fentanyl from saline (23). Similarly, the mu opioids nalbuphine (38,42) and cyclazocine (12) substitute for a low, but not a high, dose of morphine in pigeons trained to discriminate two doses of mor-

Requests for reprints should be addressed to Sheri D. Grabus, Psychopharmacology Laboratory, Department of Psychology, American University, 4400 Massachusetts Avenue, NW, Washington, DC 20016.

phine from saline. Consistent with the results described above with other partial mu agonists, nalorphine substitutes for a low, but not a high, dose of fentanyl in pigeons trained to discriminate two doses of fentanyl from vehicle within a two-key food-reinforced design. These results differ from those obtained with the full agonists morphine and LAAM, which completely substitute for both training doses of fentanyl (24). Training dose has also been shown to influence the substitution patterns obtained with a number of partial opioid agonists in rats trained to discriminate either a low or high dose of morphine from its vehicle. For example, in rats trained to discriminate two doses of morphine from its vehicle, the partial mu agonists (−)-pentazocine, (−)-metazocine, proxorphan, (−)-NANM, (−)-cyclazocine, and levallorphan completely substitute for morphine only in the low-dose group. Partial generalization to these partial agonists is demonstrated in those animals trained with the higher dose of morphine (20). In addition, the partial agonist nalbuphine substitutes for a low, but only partially for a high, dose of morphine in rats trained to discriminate two doses of morphine from saline (30). Assessments of the effects of training dose on nalorphine substitution have been limited to a single study [see (41)]. Conclusions from this study are difficult to make, however, given that nalorphine did not consistently substitute for either dose of morphine used during training. When comparing nalorphine's ability to substitute for morphine across different studies, however, nalorphine produces partial substitution in rats trained to discriminate low (9,10,29), but not high (7,17), doses of morphine from its vehicle, indicating that nalorphine substitution patterns in rats might vary with the training dose.

Although the degree to which nalorphine substitutes for morphine appears to be a function of training dose, this effect has not been demonstrated in rats trained and tested under similar conditions (as noted above the substitution patterns with nalorphine were based on between-study comparisons). To that end, in the present experiment rats were trained to discriminate either a low or a high dose of morphine within the conditioned taste aversion baseline of drug discrimination learning [see (16,25–28,33,36,37)] and then tested for the ability of nalorphine to substitute for the morphine training stimulus. Specifically, animals were injected with either 5.6 (Experiment 1) or 10 (Experiment 2) mg/kg morphine prior to a saccharin–LiCl pairing and the morphine vehicle prior to saccharin alone. Under these conditions, subjects learned to avoid the saccharin solution when it was preceded by morphine and to consume the same solution when it was preceded by the drug's vehicle. Following acquisition of the morphine vs. distilled water discrimination, subjects were administered various doses of nalorphine to assess its ability to substitute for morphine. Subsequently, naloxone was then administered to test the specificity of the opioid stimulus. Finally, naloxone was given in combination with morphine to demonstrate that the discriminative stimulus effects of morphine resulted from its interactions at the opiate receptor.

## METHOD

### *Subjects*

Subjects were 36 experimentally naive, female rats of Long–Evans descent, approximately 200–290 g at the start of the experiments. They were housed in individual wire-mesh cages and maintained on a 12 L:12 D cycle and at an ambient temperature of 23°C for the duration of the experiments. Rat chow (Prolab Rat, Mouse, Hamster 3000) was available ad lib. Fluid access was provided only during experimental sessions.

### *Drugs*

Morphine sulfate, methadone hydrochloride, nalorphine hydrochloride (all generously supplied by the National Institute on Drug Abuse), and naloxone hydrochloride (generously supplied by DuPont Pharmaceuticals) were dissolved in distilled water and injected intraperitoneally (IP) (1 ml/kg body weight). Lithium chloride (purchased from Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water and injected IP at a volume of 12 ml/kg body weight.

## EXPERIMENT 1 (LOW-DOSE GROUP)

### *Procedure*

*Phase I: acquisition.* At the outset of training, 18 subjects were given 20-min access to water for 23 consecutive days in their home cages until all subjects consistently drank levels greater than 10 ml. On days 24–26, a novel saccharin solution (0.1% w/v saccharin sodium salt, Sigma Chemical Co.) replaced water during the 20-min access period (saccharin habituation) and was preceded on the last day of saccharin habituation by an IP injection of distilled water (1 ml/kg).

On day 27, all subjects were given an IP injection of 5.6 mg/kg morphine 30 min prior to 20-min saccharin access. Immediately following saccharin access, subjects were ranked according to saccharin consumption (from lowest consumption to highest) and assigned to one of two groups (groups 5.6L and 5.6W, $n = 9$ per group). Subjects in group 5.6L were given an IP injection of 1.8 mEq/0.15 M LiCl (76.8 mg/kg), while subjects in group 5.6W were given an equivolume injection of the distilled water vehicle. On the following 3 days, all subjects were injected with distilled water (1 ml/kg) 30 min prior to saccharin access. No injections were given following saccharin access on these recovery days. The exceptions to this procedure were two subjects in group 5.6L, who did not acquire the morphine/distilled water discrimination by conditioning trial 28. For these subjects (and for two subjects in group 5.6W), morphine and distilled water were injected 15 min prior to saccharin access. Therefore, in this and all subsequent phases, four rats within this experiment received a 15-min morphine pretreatment period, whereas all remaining rats were given a 30-min morphine pretreatment period. This alternating procedure of one conditioning session (morphine–saccharin–LiCl or morphine–saccharin–distilled water) followed by three recovery sessions (distilled water–saccharin) was repeated until discriminative control had been established for all experimental subjects. Discriminative control for any specific subject in group 5.6L was defined as occurring when that subject consumed at least 50% less than the mean of group 5.6W on two consecutive conditioning trials. One subject within group 5.6L did not acquire the morphine/distilled water discrimination by conditioning trial 82, so this animal was removed from the experiment.

*Phase II: generalization.* The procedure followed in this phase was identical to that of Phase I with one exception. On the second day following conditioning (the second recovery day within phase I, but a probe day in this phase), subjects were administered one of a range of doses of morphine (1.8–18 mg/kg), nalorphine (1–18 mg/kg), or naloxone (3.2–18 mg/kg) 30 (morphine) or 15 (nalorphine and naloxone) min prior

to saccharin access. An individual subject in group 5.6L was tested only if its consumption was 50% or less than the mean of group 5.6W for the two preceding conditioning trials. The lowest dose tested of each drug was one at which subjects in group 5.6L demonstrated vehicle-appropriate responding (in which the mean amount of saccharin consumed by subjects in group 5.6L did not significantly differ from the mean amount consumed by group 5.6W). All drugs were tested up to the dose that either caused subjects in group 5.6L to consume amounts 50% or less than group 5.6W or that suppressed responding in all subjects within groups 5.6L and 5.6W. No injections followed these probe sessions. One subject within group 5.6L and one subject within group 5.6W died before the nalorphine generalization portion of this experiment could be completed. Two subjects within group 5.6L failed to complete the naloxone generalization portion of this experiment. Data for these animals are missing from a portion of this phase and from subsequent phases.

*Phase III: naloxone/morphine combination.* The procedure during this phase was identical to that of phase II except that on the second day following conditioning, a 3.2-mg/kg dose of naloxone (or its distilled water vehicle) was administered concurrent with morphine (10 mg/kg) 30 min prior to saccharin access. No injections followed these probe sessions.

*Phase IV: naloxone/nalorphine combination.* The procedure during this phase was identical to that of phase III except that on the second day following conditioning, one of three doses of naloxone (3.2, 5.6, or 10 mg/kg) or its distilled water vehicle was administered 15 min prior to nalorphine (10 mg/kg), which was administered 15 min prior to saccharin access. No injections followed these probe sessions.

### *Data Analysis*

Statements of statistical significance are based on the Mann–Whitney test, $p < 0.05$.

## EXPERIMENT 2 (HIGH-DOSE GROUP)

### *Procedure*

*Phase I: acquisition.* The procedure followed during this phase was identical to that of phase I in Experiment 1 with the following exceptions. On the first conditioning day, 18 subjects were given an IP injection of 10 mg/kg morphine 10 min prior to 20-min saccharin access. Immediately following saccharin access, subjects were ranked according to saccharin consumption (from lowest consumption to highest) and assigned to one of two groups (groups 10L and 10W, $n = 9$ per group). Subjects in group 10L were given an IP injection of 1.8 mEq/0.15 M LiCl (76.8 mg/kg), while subjects in group 10W were given an equivolume injection of the distilled water vehicle. On the following three days, all subjects were injected with distilled water (1 ml/kg) 10 min prior to saccharin access. No injections were given following saccharin access on these recovery days. This alternating procedure of one conditioning session (morphine–saccharin–LiCl or morphine–saccharin–distilled water) followed by three recovery sessions (distilled water–saccharin) was repeated until conditioning trial 14 (day 55). Because subjects in both groups showed decreases in consumption when injected with morphine 10 min prior to saccharin access, the time period between morphine administration and saccharin access was increased to 30 min on this conditioning trial to eliminate any unconditioned suppressant effects of morphine. All injections in this phase subsequent to this conditioning trial were administered 30 min prior to saccharin access. This alternating procedure of one conditioning session (morphine–saccharin–LiCl or morphine–saccharin–distilled water) followed by three recovery sessions (distilled water–saccharin) was then repeated until discriminative control had been established for all experimental subjects.

*Phase II: generalization.* The procedure followed during this phase was the same as that of phase II of Experiment 1 with the following exceptions. On the second day following conditioning, subjects were administered one of a range of doses of morphine (1.8–10 mg/kg), nalorphine (3.2–32 mg/kg), methadone (3.2–10 mg/kg), or naloxone (1–18 mg/kg) 30 (morphine and methadone) or 15 (nalorphine and naloxone) min prior to saccharin access.

*Phase III: naloxone/morphine combination.* The procedure during this phase was identical to that of phase III in Experiment 1. Specifically, a 3.2-mg/kg dose of naloxone (or its distilled water vehicle) was administered concurrent with morphine (10 mg/kg) 30 min prior to saccharin access. One animal in group 10W died prior to this phase, so data for this subject are excluded.

### *Data Analysis*

Statements of statistical significance are based on the Mann–Whitney test, $p < 0.05$.

## RESULTS

Although subjects in groups 5.6L and 5.6W were tested separately from subjects in groups 10L and 10W, the results are grouped according to experimental phase to compare the effects of training dose on specific drug substitution patterns.

### *Phase I: Acquisition*

Experimental subjects within group 5.6L acquired the morphine discrimination within 13 conditioning trials. Experimental subjects within group 10L acquired the morphine discrimination within seven trials from the point when morphine administration was moved to the terminal period of 30 min prior to saccharin access.

### *Phase II: Generalization*

Figure 1 presents the mean amounts (±SEM) of saccharin consumed for subjects in groups 5.6L and 5.6W (Fig. 1A) and groups 10L and 10W (Fig. 1B) following various doses of morphine. As illustrated, subjects in groups 5.6L and 10L, but not in groups 5.6W and 10W, decreased saccharin consumption as the dose of morphine increased. At lower doses of morphine (1.8 mg/kg for subjects in group 5.6L and 1.8 and 3.2 mg/kg for subjects in group 10L), consumption by subjects in groups 5.6L and 10L did not significantly differ from that of their respective controls in groups 5.6W and 10W ($p$s = 0.630, 0.185, and 0.216, respectively). However, beginning at 3.2 (group 5.6L) and 5.6 (group 10L) mg/kg morphine, differences in consumption began to appear; at these doses subjects in groups 5.6L and 10L drank significantly less than their respective controls in groups 5.6W and 10W ($p$s = 0.003 and 0.015, respectively). At the highest dose tested (18 mg/kg morphine), subjects in both groups 5.6L and 5.6W decreased saccharin consumption. The mean amounts of saccharin consumed did not significantly differ between the two groups ($p = 0.085$), indicating that morphine had a general suppressant effect within this preparation at higher doses.

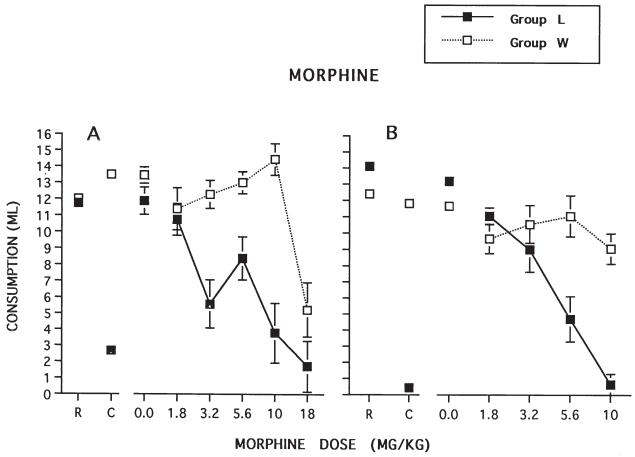


FIG. 1. Mean amounts (±SEM) of saccharin consumed for subjects in groups 5.6L and 5.6W (A) ($n$ = 8 and 9, respectively) and groups 10L and 10W (B) ($n$ = 9 per group) following various doses of morphine. Mean amounts (±SEM) of saccharin consumed on recovery (R) and conditioning (C) days for each group are noted at the left of the graphs. In some cases, the SEM falls within the range of the data point.

Figure 2 presents the mean amounts (±SEM) of saccharin consumed by subjects in groups 5.6L and 5.6W (Fig. 2A) and groups 10L and 10W (Fig. 2B) following various doses of nalorphine. As shown, subjects in group 5.6L, but not in group 5.6W, decreased saccharin consumption as the dose of nalorphine increased. At the lowest dose of nalorphine (1.0 mg/kg), consumption by subjects in group 5.6L did not significantly differ from that of group 5.6W ($p$ = 0.115). However, at the 1.8-mg/kg dose of nalorphine, differences in consumption began to appear. That is, at this dose (and at higher nalorphine doses), subjects in group 5.6L consumed significantly less than subjects in group 5.6W (all $p$s < 0.046). Although individual subjects in group 5.6L decreased saccharin consumption as the dose of nalorphine increased, these subjects differed in the dose of nalorphine that produced the lowest amount of saccharin consumption. Further, two subjects lost stimulus control with nalorphine at the highest doses tested (10 and/or 18 mg/kg; individual data not shown). Consumption for subjects in group 10L following nalorphine did not significantly differ from the mean amount consumed by group 10W at any dose (all $p$s > 0.3314).

Because nalorphine failed to substitute for morphine stimulus control in group 10L, various doses of methadone were administered to groups 10L and 10W to ensure that this lack of substitutution was not due to a general inability of another opioid agonist to substitute for the morphine cue. As illustrated in Fig. 3, subjects in group 10L generalized morphine stimulus control to methadone, decreasing saccharin consumption as the dose of methadone increased. Levels of consumption differed significantly from those of group 10W at 7.5 and 8.7 mg/kg ($p$s = 0.033 and 0.008, respectively). At the highest dose tested (10 mg/kg methadone), subjects in both groups 10L and 10W decreased saccharin consumption. The mean amounts of saccharin consumed did not significantly differ between the two groups at this dose ($p$ = 0.278), indicating that methadone had a general suppressant effect within this preparation at higher doses.

Figure 4 presents the mean amounts (±SEM) of saccharin consumed by subjects in groups 5.6L and 5.6W (Fig. 4A) and in groups 10L and 10W (Fig. 4B) following various doses of naloxone. As shown, subjects in groups 5.6L and 10L failed to generalize morphine stimulus control to naloxone. Consumption for subjects in groups 5.6L and 10L following naloxone did not significantly differ from the mean amounts consumed by groups 5.6W and 10W (all $p$s > 0.301). The exception to this was at the 1.0 mg/kg dose of naloxone, with subjects in group 10L consuming a mean amount that was significantly greater than the mean amount consumed by subjects in group 10W ($p$ = 0.034). Subjects in all groups showed slight decreases in saccharin consumption as the dose of naloxone increased.

*Phase III: Naloxone/Morphine Combination*

Figure 5 illustrates the mean amounts (±SEM) of saccharin consumed by subjects in groups 5.6L and 5.6W (Fig. 5A) and in groups 10L and 10W (Fig. 5B) when naloxone was administered in combination with morphine. For subjects in groups 5.6L and 10L, morphine in combination with distilled

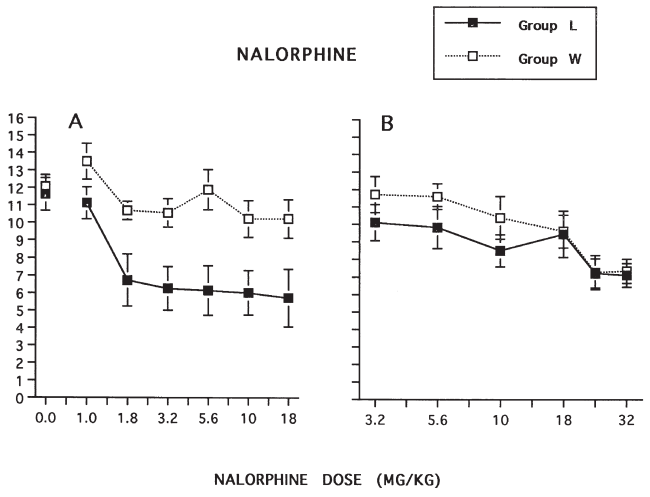


FIG. 2. Mean amounts (±SEM) of saccharin consumed for subjects in groups 5.6L and 5.6W (A) ($n$ = 7–8 and 8–9, respectively) and groups 10L and 10W (B) ($n$ = 9 per group) following various doses of nalorphine.

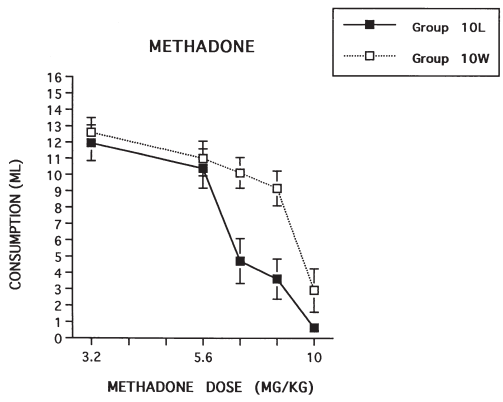


FIG. 3. Mean amounts (±SEM) of saccharin consumed for subjects in Group 10L and 10W ($n$ = 9 per group) following various doses of methadone. At 10 mg/kg methadone (group 10L), the SEM falls within the range of the data point.

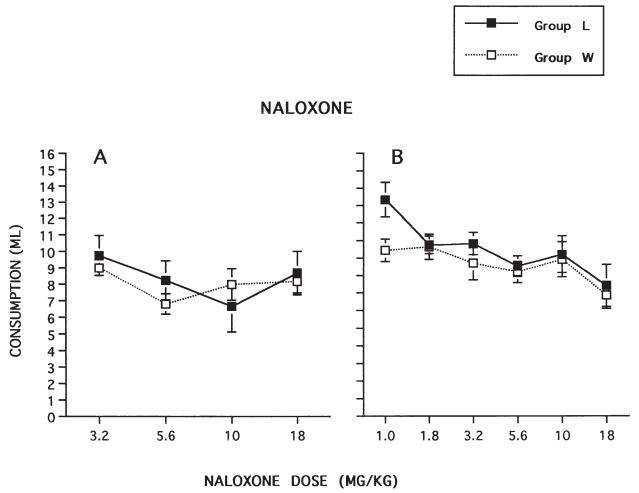


FIG. 4. Mean amounts (±SEM) of saccharin consumed for subjects in groups 5.6L and 5.6W (A) ($n$ = 5–6 and 8–9, respectively) and groups 10L and 10W (B) ($n$ = 9 per group) following various doses of naloxone.

water (the naloxone vehicle) decreased saccharin consumption to mean amounts (1.5 and 0 ml, respectively, ±SEM) that were significantly less than the mean amounts (14.8 and 8.1 ml, respectively, ±SEM) consumed by subjects in groups 5.6W and 10W ($p$s = 0.003 and 0.0005, respectively). When 3.2 mg/kg naloxone was given in combination with this dose of morphine, subjects in groups 5.6L and 10L consumed mean amounts (7.6 and 7.2 ml, respectively, ±SEM) that did not significantly differ from the mean amounts (7.9 and 5.2 ml, respectively, ±SEM) consumed by subjects in groups 5.6W and 10W ($p$s = 0.714 and 0.229, respectively). Naloxone at this dose blocked morphine's stimulus control for subjects in both groups 5.6L and 10L.

Because subjects in group 5.6L, but not group 10L, generalized morphine stimulus control to nalorphine, naloxone was given in combination with nalorphine for subjects in group 5.6L to examine the ability of naloxone to block nalorphine's stimulus control. As illustrated in Fig. 6, subjects in group 5.6L drank amounts that were not significantly different than the mean amount consumed by group 5.6W when nalorphine was given in combination with the naloxone vehicle ($p$ = 0.086). This lack of significance was due to one animal within group 5.6L that consumed a high amount of saccharin (9 ml) following this combination. However, at the 3.2-mg/kg dose of naloxone (combined with 10 mg/kg nalorphine), subjects in group 5.6L consumed a mean amount that was significantly less than the mean amount consumed by subjects in group 5.6W ($p$ = 0.003). In contrast, at the higher doses (5.6 and 10 mg/kg), naloxone blocked nalorphine's stimulus control. Consumption did not significantly differ between subjects in groups 5.6L and 5.6W at these doses ($p$s = 0.296 and 0.315, respectively). Subjects differed in the dose of naloxone that fully blocked nalorphine's stimulus control. That is, naloxone at 5.6 mg/kg completely blocked nalorphine control for one subject, whereas 10 mg/kg naloxone failed to block nalorphine's stimulus properties for this animal. For three other animals, 5.6 mg/kg naloxone partially blocked nalorphine control, while 10 mg/kg naloxone fully antagonized nalorphine's stimulus effects. In contrast, naloxone only partially blocked nalorphine control for another animal.

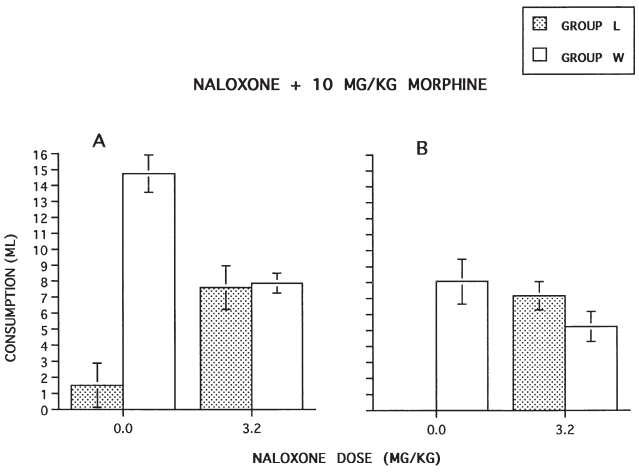


FIG. 5. Mean amounts (±SEM) of saccharin consumed for subjects in groups 5.6L and 5.6W (A) ($n$ = 5 and 8, respectively) and groups 10L and 10W (B) ($n$ = 9 and 8, respectively) following either the distilled water vehicle or 3.2 mg/kg naloxone in combination with 10 mg/kg morphine. For the distilled water and morphine combination (group 10L) , the SEM falls within the range of the data point.

## DISCUSSION

Although nalorphine substitutes inconsistently for the mu agonists fentanyl [(3); although see (4)] and morphine (7,9,10, 17,29,41) in rats, this may be more a function of the training dose than the general absence of any similarity in their subjective effects. For example, nalorphine substitutes for a low, but not a high, dose of the mu agonist fentanyl in pigeons trained to discriminate two doses of fentanyl from vehicle (24). Similarly, other partial agonists substitute for a low, but not a high, dose of the mu agonist morphine in rats trained to discriminate various doses of morphine from vehicle (20,30). Thus, regardless of the species examined, partial agonist substitution appears to depend upon the training dose utilized.

Although shown to be an important determinant of nalorphine substitution in pigeons, there are no studies in rats

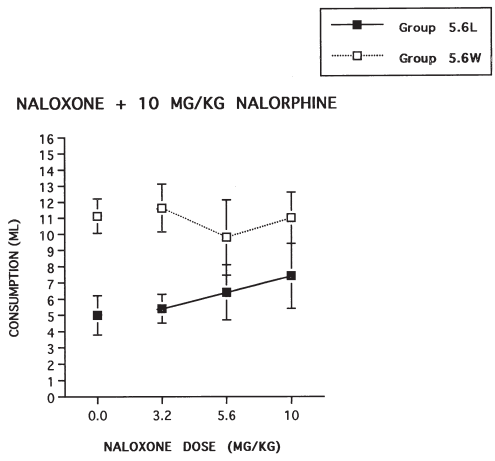


FIG. 6. Mean amounts (±SEM) of saccharin consumed for subjects in groups 5.6L ($n$ = 5) and 5.6W ($n$ = 5–8) following the distilled water vehicle or various doses of naloxone (3.2–10 mg/kg) in combination with 10 mg/kg nalorphine.

demonstrating that nalorphine substitution is also dependent upon training dose. This possibility was tested in the present experiment. As described, nalorphine substitution appeared to be dependent on the training dose of morphine. Specifically, nalorphine substituted for morphine in the low-dose group (group 5.6L) but failed to substitute for morphine in the high-dose group (group 10L). Although subjects generalized morphine to nalorphine, it appeared that this generalization was only partial. That is, for subjects trained at the 5.6-mg/kg dose of morphine, nalorphine did not reduce mean levels of consumption to that consumed following the training dose of morphine. As described, on conditioning days with the 5.6-mg/kg training dose of morphine, subjects in group 5.6L consumed a mean of 2.5 ml, whereas they consumed a mean of 5.7 ml following the highest dose of nalorphine tested on probe days. Further, although subjects decreased consumption when administered nalorphine, mean consumption did not appear to vary at the intermediate and higher doses of nalorphine. This partial generalization may reflect the possibility that nalorphine's subjective effects were mediated by activity at both mu and kappa receptor subtypes (as opposed to only the mu activity of morphine), an effect that would likely increase with increasing doses of nalorphine. It is interesting in this context that for several subjects, morphine failed to generalize to the highest doses of nalorphine tested.

Although the substitution of nalorphine for morphine appeared only partial, it may be that nalorphine fully substituted for morphine (at the 5.6-mg/kg training dose). In this case, the apparent partial substitution may reflect the fact that individual animals greatly differed in the dose of nalorphine that substituted for the morphine training stimulus. That is, for some animals nalorphine substituted for morphine at intermediate nalorphine doses, whereas other animals demonstrated substitution for morphine at higher nalorphine doses. For the animals in which higher doses of nalorphine substituted for morphine, nalorphine only partially substituted for morphine at the intermediate doses described above. Further, as described previously, two subjects completely lost stimulus control at the highest doses of nalorphine tested. Therefore, although all animals in group 5.6L did generalize morphine stimulus control to nalorphine, the partial generalization obtained following nalorphine administration for the group (see Fig. 2) reflected an averaging of data from individual animals for which nalorphine at a particular dose completely substituted, partially substituted, and completely failed to substitute for morphine [for a similar analysis of individual subject variability in buprenorphine substitution for morphine, see (25)].

That nalorphine substituted for the low (but not the high) training dose of morphine is unlikely due to the absence of selectivity that has been reported to occur with low training doses (21) [note that naloxone failed to substitute for morphine at the low training dose; see also (11,12,14,38)]. Instead, the differential substitution patterns are consistent with the position that nalorphine is a partial opioid agonist [see (2,8,15,35) for reviews of nalorphine's partial agonist activity]. Partial opioid agonists possess opioid activity, yet are not as efficacious as full agonists in their behavioral and physiological effects (2,15). Therefore, if a high dose of a full agonist is used within drug discrimination learning, partial agonists may not possess sufficient efficacy to mimic the discriminative effects of the training compound [see (5)]. However, by lowering the training dose of the fully efficacious compound, the agonist effects of the partial agonists may be revealed (20,24,30,38). That is, at lower doses a fully efficacious compound may produce an effect that is comparable to the effects produced by partial agonists. Consistent with this, it has been shown that two doses of morphine (38,42) may be discriminated from each other; therefore, different doses of the same training drug appear to produce different subjective effects.

The discussion thus far has focused on the relative efficacy of various opioids in relation to their production of discriminative stimulus effects. Such a discussion assumes that the effects of these various opioids are receptor mediated. The evidence that the stimulus properties of these compounds are mediated at the opiate receptor is that methadone, another opioid agonist with opiate receptor activity, substituted for morphine, and that the discriminative stimulus effects of both morphine and nalorphine were blocked by the opiate receptor antagonist naloxone. Although the stimulus effects of morphine and nalorphine appear opiate receptor mediated, it is not known at which specific opiate receptor subtype these effects are produced. Morphine is generally described as a mu opioid agonist (8,11,20,34,39,43); however, it binds to and has activity at other opiate subtypes, for example, delta and kappa (1,6,18,40). Accordingly, it is possible that the discriminative effects of morphine were mediated by activity at these other subytpes and not by its activity at the mu receptor. Although possible, it should be noted that morphine binding at subtypes other than mu is generally expressed at high doses, doses substantially higher than that which result in binding at the mu subtype (1,6,18,40). Furthermore, although morphine binds to kappa receptors at higher doses, it does not appear that this binding results in kappa-like subjective effects. For example, in animals trained to discriminate intermediate doses of morphine and U50488H (a kappa agonist) from saline within a three-choice procedure, high doses of morphine and U50488H did not substitute for one another (14). In the present study, if morphine's discriminative effects were based on activity at multiple opiate receptor subtypes, it might be expected that rats would generalize the higher training dose of morphine (10 mg/kg) to nalorphine, especially given nalorphine's broad-based binding to both mu and kappa subtypes (13,39). That only subjects trained at the relatively low dose of morphine (5.6 mg/kg) generalized morphine control to nalorphine is thus consistent with the position that morphine's mu activity mediated its discriminative effects. Further, the relatively selective mu receptor antagonist naloxone blocked the discriminative effects of both morphine and nalorphine. As noted, however, this antagonism appeared greater and occurred at lower doses for morphine. For example, although naloxone (at 3.2 mg/kg) completely antagonized morphine stimulus control (at 10 mg/kg), this dose was ineffective against 10 mg/kg nalorphine. As described, although 5.6 mg/kg naloxone fully antagonized nalorphine control for a single subject (for which a higher dose of naloxone was ineffective), for three other subjects 5.6 mg/kg produced only partial antagonism. Higher doses of naloxone (10 and 18 mg/kg) fully antagonized nalorphine control in these subjects. For another animal, naloxone produced only partial antagonism at any dose tested. Although direct binding comparisons of morphine and nalorphine at the mu opiate receptor subtype generally suggest comparable binding affinities (13), in at least two studies the relative affinity of nalorphine was higher than that for morphine [see (39)]. Such a difference would account for the differential effect of naloxone on morphine's and nalorphine's discriminative effects. On the other hand, such differential effects of naloxone on morphine and nalorphine might also suggest that nalorphine's binding at other opiate receptor subtypes (e.g., kappa) contributed (along with mu) to its discriminative effects.

The position that the ability of nalorphine to substitute for morphine is dependent upon training dose does not eliminate other factors (such as species differences) as possible influences on the patterns of morphine generalization. It is possible that nalorphine may have fully substituted for low and high doses of a kappa compound had it been used as the training drug, a finding that would suggest that in rats nalorphine has greater efficacy at the kappa than mu opiate receptor [see (19,22)]. Independent of such a finding, it remains clear that the training dose of morphine may be an important factor in the ability of the partial agonist nalorphine to substitute for morphine.

ACKNOWLEDGEMENTS

This research was supported in part by a grant from the Mellon Foundation to Anthony L. Riley. The authors would like to thank C. Meisch for his contributions to this project.

## REFERENCES

1. Clark, M. J.; Carter, B. D.; Medzihradsky, F.: Selectivity of ligand binding to opioid receptors in brain membranes from the rat, monkey and guinea pig. Eur. J. Pharmacol. 148:343–351; 1988.
2. Colpaert, F. C.: Drug discrimination: Behavioral, pharmacological, and molecular mechanisms of discriminative drug effects. In: Goldberg, S. R.; Stolerman, I. P., eds. Behavioral analysis of drug dependence. New York: Academic Press, Inc.; 1986:161–193.
3. Colpaert, F. C.; Janssen, P. A. J.: Agonist and antagonist effects of prototype opiate drugs in rats discriminating fentanyl from saline: Characteristics of partial generalization. J. Pharmacol. Exp. Ther. 230:193–199; 1984.
4. Colpaert, F. C.; Niemegeers, C. J. E.; Janssen, P. A. J.: On the ability of narcotic antagonists to produce the narcotic cue. J. Pharmacol. Exp. Ther. 197:180–187; 1976.
5. Comer, S. D.; France, C. P.; Woods, J. H.: Training dose: Influences in opioid drug discrimination. Drug discrimination: Applications to drug abuse research. NIDA Mongr. Series 116:145–161; 1991.
6. Emmerson, P. J.; Liu, M.-R.; Woods, J. H.; Medzihradsky, F.: Binding affinity and selectivity of opioids at mu, delta and kappa receptors in monkey brain membranes. J. Pharmacol. Exp. Ther. 271:1630–1637; 1994.
7. Gianutsos, G.; Lal, H.: Selective interaction of drugs with a discriminable stimulus associated with narcotic action. Life Sci. 19:91–98; 1976.
8. Gilbert, P. E.; Martin, W. R.: The effects of morphine- and nalorphine-like drugs in the nondependent, morphine-dependent and cyclazocine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 198:66–82; 1976.
9. Hirschhorn, I. D.; Rosecrans, J. A.: Generalization of morphine and lysergic acid diethylamide (LSD) stimulus properties to narcotic analgesics. Psychopharmacology (Berlin) 47:65–69; 1976.
10. Holtzman, S. G.: Drug discrimination studies. Drug Alcohol Depend. 14:263–282; 1985.
11. Holtzman, S. G.; Cook, L.; Steinfels, G. F.: Discriminative stimulus effects of spiradoline, a kappa-opioid agonist. Psychopharmacology (Berlin) 105:447–452; 1991.
12. Koek, W.; Woods, J. H.: Partial generalization in pigeons trained to discriminate morphine from saline: Applications of receptor theory. Drug Dev. Res. 16:169–181; 1989.
13. Magnan, J.; Paterson, S. J.; Tavani, A.; Kosterlitz, H. W.: The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. Naunyn-Schmiedeberg's Arch. Pharmacol. 319:197–205; 1982.
14. Makhay, M. M.; Young, A. M.; Poling, A.: Establishing morphine and U-50,488H as discriminative stimuli in a three-choice assay with pigeons. Exp. Clin. Psychopharmacol. 6:3–9; 1998.
15. Martin, W. R.: Opioid antagonists. Pharmacol. Rev. 19:463–521; 1967.
16. Mastropaolo, J. P.; Moskowitz, K. H.; Dacanay, R. J.; Riley, A. L.: Conditioned taste aversions as a behavioral baseline for drug discrimination learning: An assessment with phencyclidine. Pharmacol. Biochem. Behav. 32:1–8; 1989.
17. Overton, D. A.; Batta, S. K.: Investigation of narcotics and antitussives using drug discrimination techniques. J. Pharmacol. Exp. Ther. 211:401–408; 1979.
18. Pasternak, G. W.; Wood, P. J.: Minireview: Multiple mu opiate receptors. Life Sci. 38:1889–1898; 1986.
19. Picker, M. J.: Kappa agonist and antagonist properties of mixed action opioids in a pigeon drug discrimination procedure. J. Pharmacol. Exp. Ther. 268:1190–1198; 1994.
20. Picker, M. J.; Craft, R. M.; Negus, S. S.; Powell, K. R.; Mattox, S. R.; Jones, S. R.; Hargrove, B. K.; Dykstra, L. A.: Intermediate efficacy mu opioids: Examination of their morphine-like stimulus effects and response rate-decreasing effects in morphine-tolerant rats. J. Pharmacol. Exp. Ther. 263:668–681; 1992.
21. Picker, M. J.; Doty, P.; Negus, S. S.; Mattox, S. R.; Dykstra, L. A.: Discriminative stimulus properties of U50,488 and morphine: Effects of training dose on stimulus substitution patterns produced by mu and kappa opioid agonists. J. Pharmacol. Exp. Ther. 254:13–22; 1990.
22. Picker, M. J.; Dykstra, L. A.: Discriminative stimulus effects of mu and kappa opioids in the pigeon: Analysis of the effects of full and partial mu and kappa agonists. J. Pharmacol. Exp. Ther. 249:557–566; 1989.
23. Picker, M. J.; Smith, M. A.; Morgan, D.: Assessment of the relative intrinsic efficacy of profadol and meperidine in a pigeon drug discrimination procedure: Relevance to partial substitution patterns. Behav. Pharmacol. 5:61–70; 1994.
24. Picker, M. J.; Yarbrough, J.; Hughes, C. E.; Smith, M. A.; Morgan, D.; Dykstra, L. A.: Agonist and antagonist effects of mixed action opioids in the pigeon drug discrimination procedure: Influence of training dose, intrinsic efficacy and interanimal differences. J. Pharmacol. Exp. Ther. 266:756–767; 1993.
25. Pournaghash, S.; Riley, A. L.: Buprenorphine as a stimulus in drug discrimination learning: An assessment of mu and kappa receptor activity. Pharmacol. Biochem. Behav. 46:593–604; 1993.
26. Riley, A. L.: Use of drug discrimination learning in behavioral toxicology: Classification and characterization of toxins. In Chang, L. W.; Slikker, W., Jr., eds. Neurotoxicology: Approaches and methods. New York: Academic Press, Inc.; 1995:309–321.
27. Riley, A. L.: Drug discrimination learning: Assessment of opioid receptor pharmacology. In: Bouten, M. E.; Fanselow, M. S., eds. Learning, motivation, and cognition: The functional behaviorism of Robert C. Bolles. Washington, DC: American Psychological Association; 1997:225–254.
28. Riley, A. L.; Kautz, M. A.; Geter, B.; Smurthwaite, S. T.; Pournaghash, S.; Melton, P. M.; Ferrari, C. M.: A demonstration of the graded nature of the generalization function of drug discrimination learning within the conditioned taste aversion procedure. Behav. Pharmacol. 2:323–334; 1991.
29. Shannon, H. E.; Holtzman, S. G.: Further evaluation of the discriminative effects of morphine in the rat. J. Pharmacol. Exp. Ther. 201:55–66; 1977.
30. Shannon, H. E.; Holtzman, S. G.: Morphine training dose: A determinant of stimulus generalization to narcotic antagonists in the rat. Psychopharmacology (Berlin) 61:239–244; 1979.
31. Shearman, G. T.; Herz, A.: Discriminative stimulus properties of narcotic and non-narcotic drugs in rats trained to discriminate opiate κ-receptor agonists. Psychopharmacology (Berlin) 78:63–66; 1982.
32. Smith, M. A.; Picker, M. J.: Examination of the kappa agonist and antagonist properties of opioids in the rat drug discrimination procedure: Influence of training dose and intrinsic efficacy. Behav. Pharmacol. 6:703–717; 1995.

33. Smurthwaite, S. T.; Kautz, M. A.; Geter, B.; Riley, A. L.: Naloxone as a stimulus in drug discrimination learning: Generalization to other opiate antagonists. Pharmacol. Biochem. Behav. 41:43–47; 1991.
34. Smurthwaite, S. T.; Riley, A. L.: Nalorphine as a stimulus in drug discrimination learning: Assessment of the role of μ- and κ-receptor subtypes. Pharmacol. Biochem. Behav. 48:635–642; 1994.
35. Smurthwaite, S. T.; Riley, A. L.: Animals trained to discriminate morphine from naloxone generalize morphine (but not naloxone) to nalorphine. CPDD 153:94; 1995.
36. Sobel, B.-F. X.; Wetherington, C. L.; Riley, A. L.: The contribution of within-session averaging of drug- and vehicle-appropriate responding to the graded dose–response function in drug discrimination learning. Behav. Pharmacol. 6:348–358; 1995.
37. Stevenson, G. W.; Pournaghash, S.; Riley, A. L.: Antagonism of drug discrimination learning within the conditioned taste aversion procedure. Pharmacol. Biochem. Behav. 41:245–249; 1991.
38. Vanecek, S. A.; Young, A. M.: Pharmacological characterization of an operant discrimination among two doses of morphine and saline in pigeons. Behav. Pharmacol. 6:669–681; 1995.
39. Wood, P. L.: Multiple opiate receptors: Support for unique mu, delta and kappa sites. Neuropharmacology 21:487–497; 1982.
40. Wood, P. L.: Opioid receptor affinities of kappa agonists, agonist/antagonists and antagonists *in vitro* and *in vivo.* Prog. Neuropsychopharmacol. Biol. Psychiatry 7:657–662; 1983.
41. Young, A. M.; Masaki, M. A.; Geula, C.: Discriminative stimulus effects of morphine: Effects of training dose on agonist and antagonist effects of mu opioids. J. Pharmacol. Exp. Ther. 261:246–257; 1992.
42. Young, A. M.; Walton, M. A.; Perkins, A. N.: Characteristics of a discrimination among two doses of morphine and saline in pigeons. Drug. Dev. Res. 16:163–168; 1989.
43. Zimmerman, D. M.; Leander, J. D.: Selective opioid receptor agonists and antagonists: Research tools and potential therapeutic agents. J. Med. Chem. 33:895–902; 1990.