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Discriminative-Stimulus Effects of Morphine in Combination With α- and β-Noradrenergic Agonists and Antagonists in Rats

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HUGHES, C. E., T. HABASH, L. A. DYKSTRA AND M. J. PICKER. *Discriminative-stimulus effects of morphine in combination with α- and β-noradrenergic agonists and antagonists in rats.* PHARMACOL BIOCHEM BEHAV **53**(4) 979–986, 1996.—Studies have shown that the noradrenergic system is involved in the analgesic effects of opioids and in the expression and development of physical signs of opioid withdrawal. The purpose of the present experiment was to determine if the noradrenergic system was involved in the discriminative effects of morphine in rats trained to discriminate 5.6 mg/kg morphine from saline under a fixed-ratio schedule of food presentation. A range of doses of morphine (0.3–10.0 mg/kg) produced dose-dependent increases in morphine-appropriate responding without substantial decreases in response rate. Several experiments were conducted to determine whether a number of noradrenergic agonists and antagonists 1) substitute for morphine or 2) alter the discriminative-stimulus effects of morphine when administered concurrently. The α_2 agonist clonidine (0.003–0.1 mg/kg), the α_1 antagonist prazosin (0.1–10.0 mg/kg), the α_2 antagonist yohimbine (0.1–10.0 mg/kg), the β_2 agonist salbutamol (0.03–10.0 mg/kg), and the β antagonist propranolol (1.0–10.0 mg/kg), neither substituted for morphine nor altered the discriminative-stimulus effects of morphine when administered in combination. These data suggest that the noradrenergic system is not involved in the discriminative-stimulus effects of 5.6 mg/kg morphine in rats.

Noradrenergic Clonidine Prazosin Yohimbine Propranolol Salbutamol Morphine
Drug discrimination Fixed-ratio schedule Rats

SEVERAL studies show that the noradrenergic and opioid systems interact in a complex manner. For example, opioids have been shown to inhibit noradrenergic activity in rat hippocampus (19), cortex (37), and in the locus coeruleus of rats (32) and cynomolgus monkeys (2). Morphine and fentanyl also tend to inhibit norepinephrine release from human neuroblastoma cells (3,14). Moreover, these interactions between noradrendergic and opioid systems are often reversible with opioid antagonists.

The noradrenergic and opioid systems also interact at the behavioral level. The α_2-noradrenergic agonists, clonidine and ST-91, enhance morphine's antinociceptive effects in rats and mice (4,24,27,38). In contrast, yohimbine, an α_2-noradrenergic antagonist, attenuates morphine's antinociceptive effects in rats (4,10). Also, rats tolerant to the antinociceptive effects of morphine are crosstolerant to the effects of ST-91 (34), norepinephrine (22), and clonidine (33). Moreover, rats tolerant to the antinociceptive effects of clonidine are crosstolerant to the effects of morphine (25).

The noradrenergic and opioid systems also are involved in the development and expression of opiate dependence. Clonidine attenuates some of the signs of morphine withdrawal in rats (7,9,15), hamsters (29), and rhesus monkeys (13), as well as the signs of methadone and morphine withdrawal in humans (5,8,12). Acute administration of yohimbine increases the physical effects of morphine withdrawal in rats (6), and repeated administration of yohimbine following a bolus dose of morphine significantly attenuates physical signs of mor-

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phine withdrawal in rats, suggesting that α_2 receptors are involved in the development of physical dependence upon opioids (10,35).

In order to characterize interactions between the opioid and noradrenergic systems further, the discriminative-stimulus effects of morphine alone and in combination with several noradrenergic agonists and antagonists were examined in the present experiment. If the noradrenergic system is involved in the expression of the discriminative-stimulus effects of morphine, then noradrenergic agonists might be expected to substitute for morphine. It has been demonstrated that clonidine, an α_2 agonist, substitutes for morphine under some conditions, but not other conditions (16,17,21). Further, noradrenergic agonists and antagonists might be expected to potentiate or antagonize the discriminative-stimulus effects of morphine when administered in combination with morphine.

In the present experiment, several noradrenergic agonists and antagonists that are selective for α- and β-receptor subtypes were examined. The noradrenergic compounds included the α_2 agonist clonidine, the α_1 antagonist prazosin, the α_2 antagonist yohimbine, the β_2 agonist salbutamol, and the β antagonist propranolol. First, the noradrenergic compounds were administered alone to determine if they would substitute for morphine. Second, to determine if any of the noradrenergic compounds would alter the discriminative-stimulus effects of morphine, selected doses of the noradrenergic compounds were administered concurrently with a range of doses of morphine.

METHODS

Subjects

Thirteen male experimentally naive Long-Evans hooded rats (Charles River, Wilmington, MA) were maintained at approximately 300-350 g through food obtained in the experimental session and restricted postsession feeding of Protein Rat Chow®. When not in experimental sessions, rats were housed individually in a colony room (12 L : 12 D cycle) with continuous access to water.

Apparatus

Experimental sessions were conducted in eight two-lever operant chambers. The levers were 6.0 cm apart on the front wall, 2.0 cm from either side wall, and 7.0 cm above the floor. Two red or white stimulus lights were located above the left and right levers, respectively. Two white house lights were located on the ceiling in the rear of the chamber. Responses on either lever with a force greater than 0.44 N operated a micro switch and were counted as responses. A pellet dispenser could deliver 45-mg Noyes pellets in a 4.5 × 4.5 cm receptacle located in the front wall 1.0 cm above the floor. A Sonalert® was located above the receptacle and was operated for 100 ms when a pellet was delivered. The chambers were located in a room with white noise continuously present. Contingencies were programmed and data were collected by MED-PC™ software (Georgia, VT) and a MED Associates interface located in a different room.

Procedure

Rats were trained to eat from the receptacle and then to press both response levers. During these sessions the stimulus lights located above either the right or left lever (alternated across sessions) and the house light were illuminated. Responses on the lever located below the illuminated lights were reinforced. The number of responses required for food presentation was increased gradually over several sessions until responding was maintained by a fixed-ratio 20 (FR20) schedule of food presentation on both levers. Then 3.0 mg/kg morphine or distilled water was injected IP 30 min prior to the start of the session; rats remained in their home cages during the 30 min. When 3.0 mg/kg morphine was administered prior to sessions, food presentation was contingent on completion of an FR20 on one lever; when distilled water was administered prior to sessions, food presentation was contingent on completion of an FR20 on the other lever. During these sessions the set of lights above each lever and the house light were illuminated. The type of injection that was administered was determined semirandomly, with the restrictions that neither morphine nor distilled water could be administered prior to three consecutive sessions and that there was an approximately equal number of each type of injection over a 30-session period. After at least 20 sessions, the dose of morphine administered prior to sessions was increased to 5.6 mg/kg, and remained as such for the remainder of the experiment. Sessions were 20 min in length and were run 5 days a week at approximately the same time of day.

Responding was considered under discriminative control of the type of injection when the mean percentage of responses that occurred on the injection-appropriate lever both before the first reinforcer delivery and across the session for 10 consecutive sessions was at least 80%. Once these criteria were met, testing was initiated. During these test sessions, both sets of stimulus lights and the house light were illuminated and completion of an FR20 on either lever was reinforced. Test sessions also were 20 min in length and occurred on Tuesdays and Fridays if at least 80% of the responses before the first reinforcer occurred on the injection-appropriate lever and response rates were within the control range on the immediately preceding Monday or Thursday, respectively. First, the morphine dose-effect curve was determined in all rats. Then, rats were divided into groups (n = 4-6). Doses of clonidine, prazosin, propranolol, salbutamol (albuterol), or yohimbine were administered IP 30 min prior to the beginning of test sessions alone and then in combination with a range of doses of morphine. All dose combinations of a specific noradrenergic compound and morphine were tested in an individual rat before another compound was tested. After a range of doses of prazosin was administered in combination with a range of doses of morphine, 10.0 mg/kg prazosin was administered alone. Two rats died a few days after this administration and, therefore, 10.0 mg/kg prazosin was not tested in combination with doses of morphine. One rat died a day after administration of 10.0 mg/kg propranolol in combination with 10.0 mg/kg morphine, and, therefore, propranolol could not be tested in combination with 0.3 mg/kg morphine in this rat.

Drugs

Clonidine HCl, propranolol HCl, salbutamol (albuterol) hemisulfate, yohimbine HCl (all from Research Biochemicals International, Natick, MA), prazosin HCl (Sigma Chemical Co., St. Louis, MO), and morphine sulfate (provided by the National Institute on Drug Abuse, Rockville, MD) were dissolved in distilled water. Doses are expressed in terms of the salt. Drugs were administered in a constant injection volume of 1.0 ml/kg b.wt. Because the largest concentration of prazosin possible was 3.0 mg/ml, multiple injections were given when larger doses were administered.

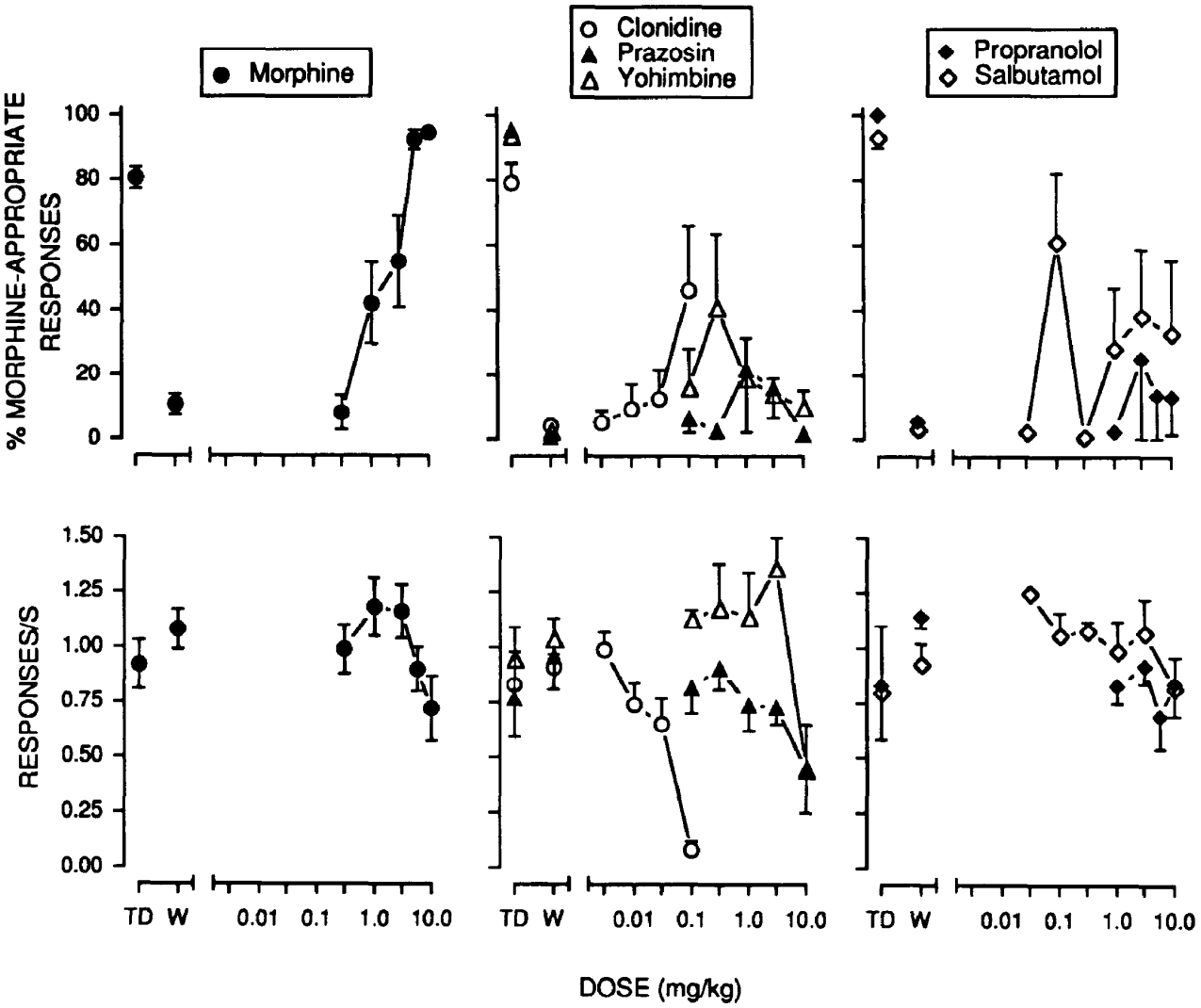

FIG. 1. Average dose-effect curves for morphine (filled circles), clonidine (open circles), prazosin (filled triangles), yohimbine (open triangles), propranolol (filled diamonds), and salbutamol (open diamonds). Abscissa: dose of drug expressed as mg/kg. Ordinate on upper panels: mean percentage of morphine-appropriate responses before the first reinforcer. Ordinate on lower panels: mean response rate (R/s) across the session. Symbols above TD and W represent mean percentage morphine-appropriate responses before the first reinforcer or responses/s from all training sessions in which 5.6 mg/kg morphine (TD) or distilled water (W) was administered, respectively, during the time period in which the dose-effect curves were determined. Each point represents an average of at least two determinations of morphine in 13 rats, of clonidine, prazosin, and salbutamol in 5 rats, of yohimbine in 6 rats, and of propranolol in 4 rats. Error bars indicate ± 1 SE.

Data Analysis

The percentage of responses on the injection-appropriate lever prior to the first reinforcer were calculated for individual rats by dividing the total number of responses on the injection-appropriate lever by the total number of responses prior to the first reinforcer. These data are expressed as percentage of morphine-appropriate responses. Overall response rates were calculated for individual rats by dividing the number of responses that occurred during the session by the time spent in the session and were expressed as responses per second (R/s). The dose of morphine or morphine in combination with a dose of a noradrenergic compound that occasioned 50% morphine-appropriate responding (i.e., the ED_{50} value) and 95% confidence intervals (CI) were calculated by log-linear interpolation using at least 2 points on the ascending portion of the dose-effect curve. Shifts in the dose-effect curves were considered significant if the 95% CIs did not overlap.

RESULTS

Figure 1 shows percentage of morphine-appropriate responding (upper panels) and responses/s (lower panels) as a function of dose of morphine. Morphine dose dependently increased the percentage of morphine-appropriate responding at doses that did not substantially decrease response rates. Figure 1 also shows the effects of the α_2 agonist clonidine, the α_1 antagonist prazosin, and the α_2 antagonist yohimbine. In

general, these drugs occasioned predominantly water-appropriate responding. That is, a large percentage of responses before the first reinforcer was on the lever associated with water injections. The highest percentage of morphine-appropriate responding (44.3%) was occasioned by 0.1 mg/kg clonidine; a dose that substantially decreased response rates in three rats and completely eliminated responding in the other two rats. Response rates also were dose dependently decreased by prazosin and yohimbine.

Figure 1 also shows the effects of the β_2 agonist salbutamol and the β antagonist propranolol. A dose of 0.1 mg/kg salbutamol occasioned a mean of 61.2% morphine-appropriate responding. Lower and higher doses occasioned predominately water-appropriate responding in all rats. Propranolol occasioned predominantly water-appropriate responding across the dose range tested. Response rates did not decrease across the dose range of salbutamol and propranolol tested; higher doses were not administered because of potential toxicity.

Figures 2 and 3 show percentage of morphine-appropriate responding (upper panels) and response rate (lower panels) when various doses of the α- (Fig. 2) and β-noradrenergic compounds (Fig. 3) that did not substantially decrease response rates when given alone were tested in combination with morphine. In general, dose-effect curves for the percentage of morphine-appropriate responding were not affected by the concurrent administration of any of the noradrenergic compounds. There were a few exceptions to this general observation. First, when 0.03 mg/kg clonidine was combined with 0.3 mg/kg morphine, the mean percentage of morphine-appropriate responding was greater than that observed with morphine alone. It is important to note that response rates were decreased markedly by this clonidine/morphine combination. Second, when 0.3 and 3.0 mg/kg prazosin were combined with the training dose of morphine, less than 80% morphine-appropriate responding was occasioned. Again, it is important to note that response rates were decreased by these

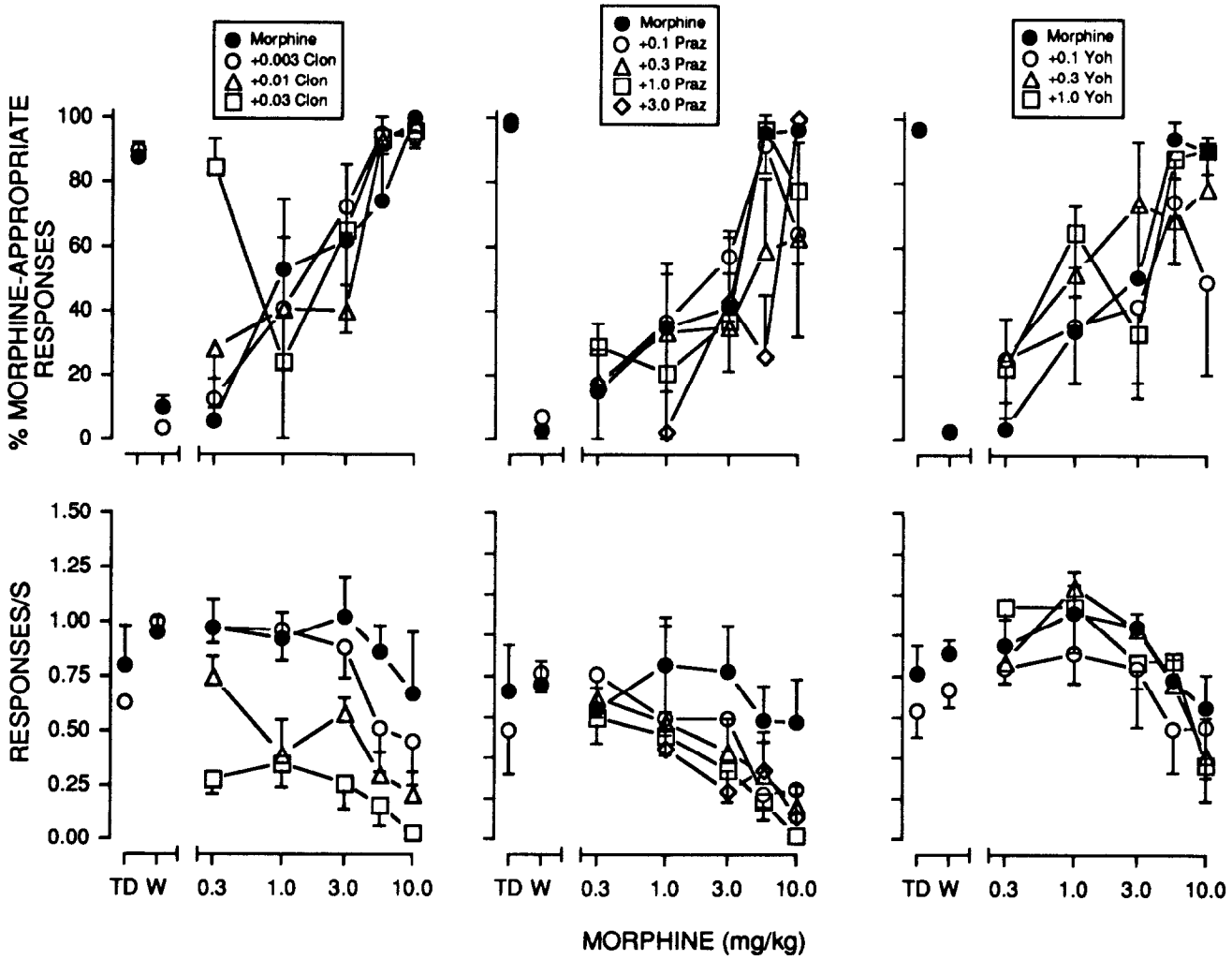

FIG. 2. Average dose-effect curves for morphine alone (filled circles) and morphine in combination with various doses of clonidine (left panel), prazosin (middle panel), and yohimbine (right panel). Each point represents one determination of a dose of morphine and a dose of clonidine or of prazosin in five rats or of yohimbine in six rats. Figure specifics are the same as in Fig. 1. Open circles above TD and W represent mean percentage morphine-appropriate responses before the first reinforcer or responses/s from all training sessions in which 5.6 mg/kg morphine (TD) or distilled water (W) was administered, respectively, during the time period in which all of the combination dose-effect curves were determined.

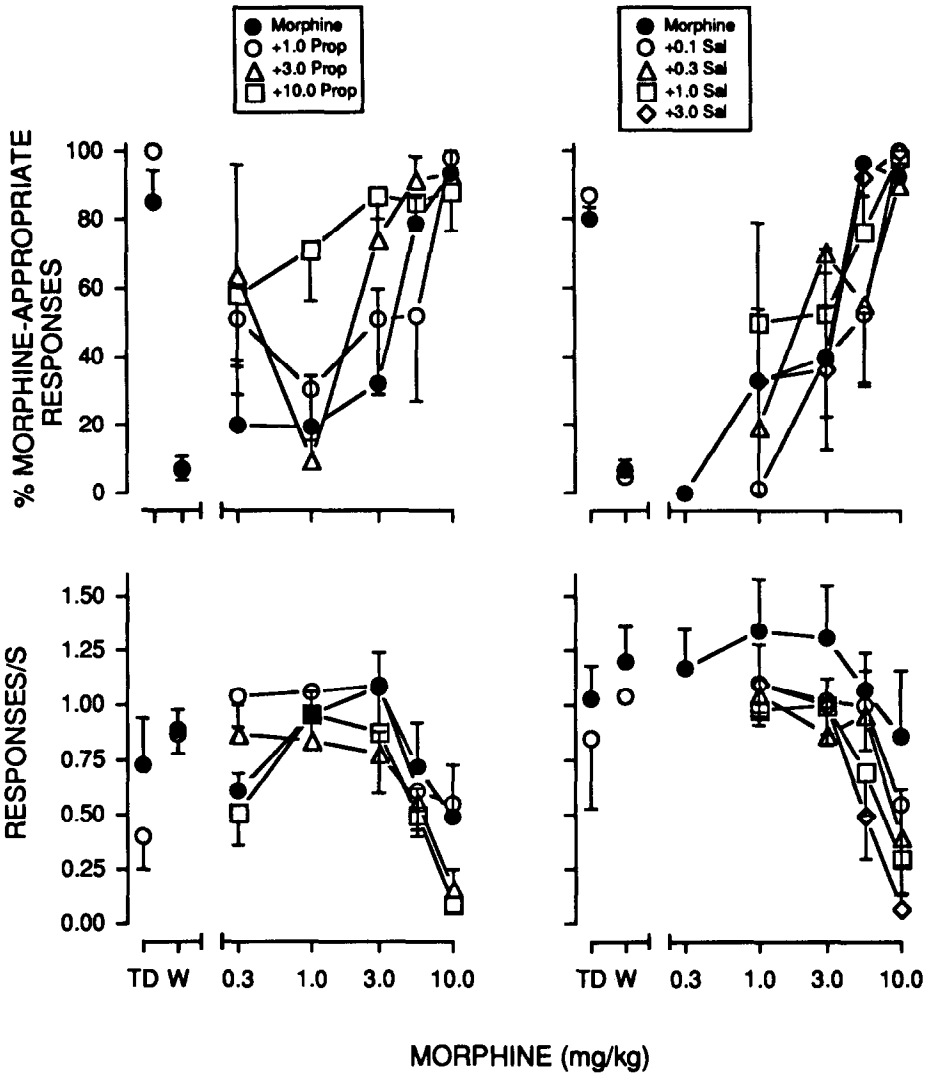

FIG. 3. Average dose-effect curves for morphine alone (filled circles) and morphine in combination with various doses of propranolol (left panel) and salbutamol (right panel). Each point represents one determination of a dose of morphine and a dose of propranolol in four rats (except the open symbols above 0.3 mg/kg morphine, which are means from three rats) or of salbutamol in five rats. Figure specifics are the same as in Fig. 1.

prazosin/morphine combinations. Third, when 0.3 mg/kg yohimbine was combined with doses of morphine lower than the training dose, morphine-appropriate responding was greater than that observed with morphine alone (Fig. 2). Fourth, when 10.0 mg/kg propranolol was combined with morphine, morphine-appropriate responding was greater than that observed with morphine alone (Fig. 3).

Although the above individual doses of the noradrenergic compounds changed the discriminative-stimulus effects of one to three doses of morphine, Table 1 shows that there were no significant alterations of the morphine dose-effect curves. That is, the 95% CIs for the ED_{50} values obtained when morphine was administered alone and in combination with the noradrenergic compounds overlapped in each case. Because of the nature of the dose-effect curve when 10.0 propranolol was combined with morphine, the ED_{50} value was estimated as less than 0.3 mg/kg morphine; 95% CIs could not be calculated.

DISCUSSION

The present experiment demonstrated that a dose of 5.6 mg/kg morphine can serve as a discriminative stimulus in rats. These data are consistent with previous studies that have examined the discriminative-stimulus effects of morphine in rats (27,40). In general, the α_1 antagonist prazosin, the α_2 antagonist yohimbine, the β_2 agonist salbutamol, and the β antagonist propranolol did not substitute for morphine. Taken together, these data suggest that noradrenergic agonists and antagonists do not share discriminative-stimulus effects with 5.6 mg/kg morphine.

In the present study, clonidine showed partial substitution

TABLE 1

ED_{50} VALUES (95% CI) FOR PERCENTAGE OF MORPHINE-APPROPRIATE RESPONDING WHEN MORPHINE WAS ADMINISTERED ALONE AND IN COMBINATION WITH DOSES OF THE NORADRENERGIC COMPOUNDS

Morphine + 0.0 Clonidine	1.49 (0.76-2.91)
+ 0.003 Clonidine	1.32 (0.79-2.21)
+ 0.01 Clonidine	1.44 (0.53-3.91)
+ 0.03 Clonidine	*
Morphine + 0.0 Prazosin	1.71 (0.93-3.15)
+ 0.1 Prazosin	1.72 (0.65-4.56)
+ 0.3 Prazosin	4.17 (0.86-20.29)
+ 1.0 Prazosin	2.75 (1.54-4.93)
+ 3.0 Prazosin	5.07 (2.12-12.14)
Morphine + 0.0 Yohimbine	1.85 (1.13-3.04)
+ 0.1 Yohimbine	3.06 (0.84-11.15)
+ 0.3 Yohimbine	1.31 (0.50-3.43)
+ 1.0 Yohimbine	1.61 (0.76-3.44)
Morphine + 0.0 Propranolol	2.47 (1.45-4.22)
+ 1.0 Propranolol	2.70 (1.10-6.62)
+ 3.0 Propranolol	2.22 (1.61-3.05)
+ 10.0 Propranolol	<0.3†
Morphine + 0.0 Salbutamol	2.03 (1.18-3.50)
+ 0.1 Salbutamol	3.92 (2.54-6.02)
+ 0.3 Salbutamol	2.56 (1.16-5.66)
+ 1.0 Salbutamol	1.47 (0.31-6.93)
+ 3.0 Salbutamol	1.94 (0.82-4.59)

*Could not be determined.
†Estimated.

for morphine; however, substitution only occurred at the highest dose (0.1 mg/kg) tested. This dose of clonidine substantially decreased response rates in three rats and completely eliminated responding in the other two rats tested. These substitution data are consistent with those of Lal et al. (17) and Miksic et al. (21), who showed that clonidine did not substitute for morphine in rats trained to discriminate 10.0 mg/kg morphine from saline. These data contrast, however, with those of Krimmer et al. (16), who showed that clonidine substituted for the discriminative stimulus of 1.0 or 4.0 mg/kg morphine in rats. Interestingly, the degree of substitution in the Krimmer et al. study depended on the training dose. In rats trained to discriminate 1.0 mg/kg morphine from saline (the low training dose), all but the lowest dose of clonidine (.0625 mg/kg) occasioned greater than 60% morphine-appropriate responding. In rats trained to discriminate 4.0 mg/kg morphine from saline (the high training dose), only the highest dose of clonidine (1.0 mg/kg) occasioned greater than 50% morphine-appropriate responding.

Taken together, these data suggest either that a noradrenergic component of the discriminative-stimulus effects of morphine is apparent at very low training doses of morphine or, alternatively, that the low training dose of morphine produces discriminative effects that are less selective than those of a high training dose. The latter explanation is in keeping with previous reports that *d*-amphetamine, ketamine, phencyclidine, and various kappa opioids substitute for the stimulus effects of low training doses of morphine, whereas the same doses of these compounds do not substitute for high training doses of morphine (26,30,39).

In the present experiment, the various noradrenergic agonists and antagonists did not shift the morphine dose-effect curve significantly. One exception was the 10.0 mg/kg dose of propranolol that did not substitute for morphine, but shifted the morphine dose-effect curve for morphine-appropriate responding to the left. Unfortunately, higher doses of propranolol could not be tested because of toxicity. Propranolol has affinity for both 5-HT_{1a} and 5-HT_{1b} receptors (20,23) and antagonizes the discriminative-stimulus effects produced by 5-HT_{1a} agonists (1,36). Moreover, Powell et al. (27) showed that 8-OH-DPAT, a 5-HT_{1a} agonist, attenuated the discriminative-stimulus effects of morphine in rats. Therefore, it is possible that the potentiation of morphine's discriminative-stimulus effects by 10.0 mg/kg propranolol reflects its 5-HT_{1a} antagonistic action. Thus, it is not completely clear as to whether the effects of propranolol observed in the present investigation were mediated by noradrenergic or 5-HT activity.

The discriminative-stimulus effects of morphine also were altered by selected doses of the other noradrenergic compounds. For example, a combination of 0.03 mg/kg clonidine and a dose of morphine, that occasioned less than 10% morphine-appropriate responding when administered alone, occasioned greater than 80% morphine-appropriate responding. Similarly, combinations of 0.3 mg/kg yohimbine and doses of morphine lower than the training dose occasioned higher percentages of morphine-appropriate responding than the doses of morphine when administered alone. In contrast, combinations of 0.3 and 3.0 mg/kg prazosin with the training dose of morphine occasioned less than 80% morphine-appropriate responding. Although, these data may suggest that the noradrendergic system is involved in the discriminative-stimulus effects of morphine, in general, these effects were not dose dependent and usually only occurred at dose combinations that markedly reduced response rate. In addition, ED_{50} values for the dose-effect curves when the noradrenergic compounds were combined with morphine were not significantly different from the ED_{50} values when morphine was administered alone.

The lack of systematic alterations in morphine's discriminative-stimulus effects in the present experiment as well as in previous studies (17,21) is in contrast to the interactions observed between the noradrenergic and opioid systems when their antinociceptive effects are examined (4,10,24,28,38). These differences may be related to particular brain regions and/or pathways involved in the behavioral effects. Indeed, when morphine is injected into the prabrachial nucleus or the ventral tegmental area, it dose dependently substitutes for systemically administered morphine in rats (11,18,31), whereas, injections of morphine in the periaqueductal gray, believed to be involved in the antinociceptive effects of morphine, do not substitute for systemically administered morphine (30).

In summary, the α_2 agonist clonidine, the α_1 antagonist prazosin, the α_2 antagonist yohimbine, the β_2 agonist salbutamol, and the β antagonist propranolol did not substitute for morphine in rats trained to discriminate 5.6 mg/kg morphine from saline. Doses of these noradrenergic compounds also did not alter significantly the discriminative-stimulus effects of morphine. Taken together, these data suggest that the discriminative-stimulus effects of 5.6 mg/kg morphine in rats do not involve a noradrenergic component.

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REFERENCES

1. Arnt, J. Characterization of the discriminative stimulus properties induced by 5-HT_1 and 5-HT_2 agonists in rats. Pharmacol. Toxicol. 64:165–172; 1989.
2. Aston-Jones, G.; Rajkowski, J.; Kubiak, P.; Akaoka, H. Acute morphine induces oscillatory discharge of noradrenergic locus coeruleus neurons in the waking monkey. Neurosci. Lett. 140: 219–224; 1992.
3. Atcheson, R.; Rowbotham, D. J.; Lambert, D. G. Fentanyl inhibits the release of [^{3}H]noradrenaline from SH-SY5Y human neuroblastoma cells. Br. J. Anaesth. 72:98–103; 1994.
4. Browning, D. L.; Livingston, A.; Morris, B. Interactions of drugs active at opiate receptors and drugs active at α_2-receptors on various test systems. Br. J. Pharmacol. 77:487–491; 1982.
5. Charney, D. S.; Riordan, C. E.; Kleber, H. D.; Murburg, M.; Braverman, P.; Sternberg, D. E.; Heninger, G. R.; Redmond, E. Clonidine and naltrexone: A safe, effective, and rapid treatment of abrupt withdrawal from methadone therapy. Arch. Gen. Psychiatry 39:1327–1332; 1982.
6. Dwoskin, L. P.; Neal, B. S.; Sparber, S. B. Yohimbine exacerbates and clonidine attenuates acute morphine withdrawal in rats. Eur. J. Pharmacol. 90:269–273; 1983.
7. Fielding, S.; Wikler, J.; Hynes, M.; Szewczak, M.; Novick, W. J.; Lal, H. A comparison of clonidine with morphine for antinociceptive and antiwithdrawal actions. J. Pharmacol. Exp. Ther. 207:899–905; 1978.
8. Gold, M. S.; Redmond, D. E., Jr.; Kleber, H. D. Clonidine blocks acute opiate-withdrawal symptoms. Lancet 16:599–602; 1978.
9. Holtzman, S. G. Discriminative stimulus effects of morphine withdrawal in the dependent rat: Suppression by opiate and nonopiate drugs. J. Pharmacol. Exp. Ther. 233:80–86; 1985.
10. Iglesias, V.; Alguacil, L. F.; Alamo, C.; Cuenca, E. Effects of yohimbine on morphine analgesia and physical dependence in the rat. Eur. J. Pharmacol. 211:35–38; 1992.
11. Jaeger, T. V.; Van der Kooy, D. Morphine acts in the parabrachial nucleus, a pontine viscerosensory relay, to produce discriminative stimulus effects. Psychopharmacology (Berlin) 110:76; 1993.
12. Jasinski, D. R.; Johnson, R. E.; Kocker, T. R. Clonidine in morphine withdrawal. Differential effects on signs and symptoms. Arch. Gen. Psychiatry 42:1063–1066; 1985.
13. Katz, J. L. Effects of clonidine and morphine on opioid withdrawal in rhesus monkeys. Psychopharmacology (Berlin) 88:392–397; 1986.
14. Keren, O.; Garty, M.; Sarne, Y. Dual regulation by opioids of [^{3}H]-norepinephrine release in the human neuroblastoma cell-line SK-N-SH. Brain Res. 646:319–323; 1994.
15. Kosten, T. A. Clonidine attenuates conditioned aversion produced by naloxone-precipitated opiate withdrawal. Eur. J. Pharmacol. 254:59–63; 1994.
16. Krimmer, E. C.; McGuire, M. S.; Barry, H., III. Effects of training dose on generalization of morphine stimulus to clonidine. Pharmacol. Biochem. Behav. 20:669–673; 1984.
17. Lal, H.; Gianutsos, G.; Miksic, S. Discriminable stimuli produced by analgesics. In: Lal, H., ed. Discriminative stimulus properties of drugs. Advances in behavioral biology. Vol. 22. New York: Plenum Press; 1977:23–45.
18. Locke, K. W.; Holtzman, S. G. Characterization of the discriminative stimulus effects of centrally administered morphine in the rat. Psychopharmacology (Berlin) 87:1–6; 1985.
19. Matsumoto, M.; Yoshioka, M.; Togashi, H.; Hirokami, M.; Tochihara, M.; Ikeda, T.; Smith, C. B.; Saito, H. mu-Opioid receptors modulate noradrenaline release from the rat hippocampus as measured by brain microdialysis. Brain Res. 636:1–8; 1994.
20. Middlemess, D. N.; Blakeborough, L.; Leather, S. R. Direct evidence for an interaction of β-adrenergic blockers with the 5-HT receptor. Nature 267:289–291; 1977.
21. Miksic, S.; Shearman, G.; Lal, H. Generalization study with some narcotic and nonnarcotic drugs in rats trained for morphine-saline discrimination. Psychopharmacology (Berlin) 60: 103–104; 1978.
22. Milne, B.; Cervenko, F. W.; Jhamandas, K.; Loomis, C.; Sutak, M. Analgesia and tolerance to intrathecal morphine and norepinephrine infusion via implanted mini-osmotic pumps in the rat. Pain 22:165–172; 1985.
23. Nahorski, S. R.; Willcocks, A. L. Interaction of β-adrenoceptor antagonists with 5-hydroxytryptamine receptor subtypes in rat cerebral cortex. Br. J. Pharmacol. 78:107P; 1983.
24. Ossipov, M. H.; Harris, S.; Lloyd, P.; Messineo, E. An isobolographic analysis of the antinociceptive effect of systemically and intrathecally administered combinations of clonidine and opiates. J. Pharmacol. Exp. Ther. 255:1107–1116; 1990.
25. Paalzow, G. Development of tolerance to the analgesic effects of clonidine in rats cross-tolerant to morphine. Naunyn Schmiedebergs Arch. Pharmacol. 304:1–4; 1978.
26. 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.
27. Powell, K. R.; Picker, M. J.; Dykstra, L. A. Serotonin involvement in the discriminative stimulus effects of mu and kappa opioids in rats. Behav. Pharmacol. 5: 255–264; 1994.
28. Roerig, S. C.; Lei, S.; Kitto, K.; Hylden, J. K. L.; Wilcox, G. L. Spinal interactions between opioid and noradrenergic agonists in mice: Multiplicativity involves delta and alpha$_2$ receptors. J. Pharmacol. Exp. Ther. 262:365–262; 1992.
29. Schnur, P.; Espinoza, M.; Flores, R.; Ortiz, S.; Vallejos, S.; Wainwright, M. Blocking naloxone-precipitated withdrawal in rats and hamsters. Pharmacol. Biochem. Behav. 43:1093–1098; 1992.
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. Shoaib, M.; Spanagel, R. Mesolimbic sites mediate the discriminative stimulus effects of morphine. Eur. J. Pharmacol. 252:69–75; 1994.
32. Sklair-Tavron, L.; Nestler, E. J.; Segal, M. Locus coeruleus (LC)—Target interaction and cAMP in control of LC development. Brain Res. Bull. 35:397–401; 1994.
33. Solomon, R. E.; Gebhart, G. F. Intrathecal morphine and clonidine: Antinociceptive tolerance and cross-tolerance and effects on blood pressure. J. Pharmacol. Exp. Ther. 245:444–454; 1988.
34. Stevens, C. W.; Monasky, M. S.; Yaksh, T. L. Spinal infusion of opiate and alpha$_2$ agonists in rats: Tolerance and cross-tolerance studies. J. Pharmacol. Exp. Ther. 244:63–70; 1988.
35. Taylor, J. R.; Lewis, V. O.; Elsworth, J. D.; Pivirotto, P.; Roth, R. H.; Redmond, D. E., Jr. Yohimbine co-treatment during chronic morphine administration attenuates naloxone-precipitated withdrawal without diminishing tail-flick analgesia in rats. Psychopharmacology (Berlin) 103:407–414; 1991.
36. Tricklebank, M. D.; Neill, J.; Kidd, E. J.; Fozard, J. R. Mediation of the discriminative stimulus properties of 8-OH-DPAT by the putative 5-HT_{1a} receptor. Eur. J. Pharmacol. 133:47–56; 1987.

37. Werling, L. A.; Brown, S. R.; Cox, B. M. Opioid receptor regulation of the release of norepinephrine in brain. Neuropharmacology 26:987–996; 1987.
38. Wilcox, G. L.; Carlsson, K. H.; Jochim, A.; Jurna, I. Mutual potentiation of antinociceptive effects of morphine and clonidine on motor and sensory responses in rat spinal cord. Brain. Res. 405:84–93; 1987.
39. 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.
40. Young, A. M.; Sannerud, C. A.; Steigerwald, E. S.; Doty, M. D.; Lipinski, W. J.; Tetrick, L. E. Tolerance to morphine stimulus control: Role of morphine maintenance dose. Psychopharmacology (Berlin) 102:59–67; 1990.