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Effects of the Training Dose on Generalization of Morphine Stimulus to Clonidine

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KRIMMER, E. C., M. S. McGUIRE AND H. BARRY, III. *Effects of the training dose on generalization of morphine stimulus to clonidine*. PHARMACOL BIOCHEM BEHAV **20**(5) 669–673, 1984.—The relationship between the stimulus properties of morphine and clonidine was tested in rats trained to discriminate morphine sulfate (4, 2 or 1 mg/kg) from saline in a two-lever food-rewarded task. The response trained in the low dose group generalized to low doses of clonidine (0.125 to 0.5 mg/kg) whereas the response trained with the high dose of morphine generalized only to higher doses of clonidine (0.625 to 1.0 mg/kg). Naloxone blocked the generalization in the low dose group but only partially blocked it in the high dose group. Yohimbine blocked the generalization to clonidine in the high morphine dose group and reversed the response rate suppressant effect of clonidine in all groups.

Morphine Clonidine Naloxone Yohimbine Drug discrimination Rats

THE opioids are primarily used as analgesics but also produce other effects that tend to promote their recreational use and abuse. A probable basis for opioid abuse is a physiological drug effect that the user experiences by self-exposure to the drug condition.

The opioids have received considerable attention in the drug discrimination procedure [10]. In this paradigm animals are trained to make differential responses based entirely on the physiological drug effect at the time of testing. Thus the pharmacological effects of these drugs produce an internal stimulus that the animal uses as a cue for differential responding. The literature on the discriminative properties of drugs indicates a remarkable specificity of the discriminative effects of a class of drugs, such as opioids [2]. Animals trained to discriminate an opioid from saline will usually make the saline choice when tested with a non-opiate.

Analgesic properties have been reported for clonidine, a potent antihypertensive agent that decreases blood pressure and heart rate through activation of alpha-adrenergic receptors [18]. Morphine has similar cardiovascular effects in the spontaneous hypertensive rat. The cardiovascular effects of both clonidine and morphine are inhibited by naloxone, an opioid antagonist [3]. Farsang *et al.* reported that yohimbine, an alpha-adrenergic antagonist, blocks the cardiovascular effects of clonidine but not morphine in the spontaneous hypertensive rat [4]. These authors suggested that clonidine acts on central adrenergic receptors to release endogenous opioids. Clonidine has been used to eliminate the objective signs and subjective symptoms of opioid withdrawal in addicts and has been proposed for use in opioid detoxification programs [7,8].

The antinociceptive potency of clonidine is 17 times as great as that of morphine in mice [5]. These two drugs have been reported to potentiate the antinociceptive activity of each other [22]. Filibeck *et al.* reported, however, that in mice clonidine did not affect morphine analgesia measured by the hot plate test while it did decrease morphine induced running fits [6]. Malec *et al.* reported that clonidine did not affect the analgesic ED_{50} value of fentanyl and morphine but did intensify the catalepsy produced by these narcotics [15]. Daily administration of clonidine to rats for 7 days produced tolerance to the antinociceptive effects of both clonidine and morphine [19]. Naloxone does not antagonize the antinociceptive activity of clonidine [5,22].

Studies involving discriminative drug effects should provide effective tests for similarities of opioids and clonidine. Lal *et al.* reported that rats trained to discriminate morphine (probably 10 mg/kg) from saline chose the saline response in tests with clonidine doses as high as 2.5 mg/kg [12]. Miksic *et al.* reported that clonidine (0.08 to 0.64 mg/kg) failed to elicit the drug choice in rats trained to discriminate morphine (10 mg/kg) from saline [16]. Herling *et al.* reported that similarly trained pigeons predominantly made the saline choice when tested with clonidine [9]. Our present report is on three groups of rats trained with different morphine doses, lower than those previously reported.

METHOD

Subjects

Twenty-four male Wistar rats (Hilltop Lab Animals, Inc.) were housed individually with food and water continuously available in their cages, in a room with the environment controlled at approximately 21°C and a "light-dark" cycle of 12

hr duration for both conditions. After 7 days of laboratory acclimation, when the average body weight was 200 g, the food was removed from the cages and the animals were thereafter maintained on 12 g of food per day given approximately 1 hour after the usual testing time.

Apparatus

Standard sound-attenuated and light-attenuated operant test chambers (Lehigh Valley Electronics) were used for all training and testing. Each chamber was equipped with a food cup located between two levers on one wall. Illumination was provided by a 7.5 W house light, which also signalled the start of all training and test sessions. A fan provided ventilation and also masked extraneous noises. Electromechanical and solid-state programming equipment located in a separate room controlled the experimental schedules and recorded lever presses.

Discrimination Training

Following 10 days of the deprivation diet, when the average body weight was 174 g, 2 magazine training sessions were conducted on separate days. In each session food pellets (P. J. Noyes, Lancaster, NH) were automatically delivered to the food cup at 3 min intervals for 120 min. Responses on either lever were also reinforced by food pellets.

The rats were divided into 3 subgroups, of 8 animals each, assigned to different doses of morphine (2, 4, or 8 mg/kg) used for discrimination training. Injections were SC, following which the animal was returned to its home cage until the beginning of the training session 30 min later.

The lever associated with the morphine condition was randomly assigned so that 4 animals of each subgroup were trained to press the left lever and 4 to press the right lever following morphine administration. Responding on the alternative lever was reinforced following saline to each animal.

After the 2 magazine training sessions, all animals were given 2 training sessions in the saline condition followed by 2 sessions in the drug condition. Responses on the reinforced lever were shaped during these four sessions given on successive days. Thereafter training conditions alternated so that both conditions occurred equally often in each block of 4 sessions but never more than twice in succession. Three training sessions were given per week (Monday, Wednesday, Friday), thus allowing at least 48 hours for recovery from drug effects before the next session.

The training dose of morphine disrupted lever pressing by all animals of the 8 mg/kg subgroup and 2 of the 8 animals in the 4 mg/kg subgroup. For these selected animals the training doses were therefore reduced to 2 mg/kg during sessions 6 through 8, subsequently increased to 4 mg/kg during sessions 9 and 10 and to the originally assigned dose levels beginning with session 11.

Reinforcement after a fixed interval of 5 sec (FI-5) was begun on session 9 and each session also started with an initial delay interval of 5 sec during which lever presses were recorded but reinforcements were not delivered. Both intervals were gradually increased to the final conditions by session 17. Thereafter half the sessions began with a 60 sec delay. All other sessions began with one of three shorter delays (1, 15, or 30 sec) that occurred with equal frequency and equally often with both drug conditions. A FI 10 sec schedule of reinforcement also began on session 17.

After 24 training sessions, tests with novel doses of morphine and novel onset times for the training doses were interspersed among additional training sessions. Starting with session 33, the training dose of each subgroup was reduced by 50 percent to 1, 2, and 4 mg/kg (0.75, 1.5, and 3.0 mg/kg morphine base) and 16 additional training sessions were given. Thereafter many tests with novel conditions were interspersed among further training sessions. These tests included various doses of either morphine or fentanyl with and without concomitant naloxone treatment.

The tests reported in the present paper began after training session 126 and include tests with various doses of morphine and of clonidine (0.0625–1.0 mg/kg, SC). Naloxone (0.0125–0.2 mg/kg) and yohimbine (0.125–2.5 mg/kg) were also tested by themselves and in combination with morphine or clonidine. The naloxone doses are given as the base whereas the doses for all other drugs are expressed in terms of their salt. During these tests all drugs were administered SC in a volune of 1.0 ml/kg. The interval from injection to the test was 30 min for morphine, 20 min for clonidine and yohimbine, and 10 min for naloxone.

During this test phase several animals died and others were omitted from the analysis because of poor performance during training sessions. The number of animals used in most of these tests was 4 in the low dose subgroup, 5 in the middle dose subgroup, and 5–7 in the high dose subgroup.

RESULTS

The animals of all three subgroups learned to respond differentially during the morphine and saline training conditions. Many novel drug conditions were tested in these animals prior to the series of experiments reported in the present paper. All comparisons were made using the paired t-test.

Percentage drug choice was determined by dividing the number of responses on the drug reinforced lever by the total number of responses on both levers during the initial 60 sec delay periods that preceded approximately 50 percent of the training sessions. The percentage drug choice for each morphine and saline training session was calculated individually for each animal. The average percentage choice differed reliably between the morphine and saline condition during training sessions 127 through 170 (Fig. 1A) for each subgroup, $t(3)=3.96$, $p<0.05$ for the low dose subgroup, $t(4)=6.31$, $p<0.01$ for the 2 mg/kg subgroup, and $t(6)=14.2$, $p<0.01$ for the high dose subgroup.

Figure 1A also shows that the percentage drug choice was dose related for morphine doses in all three subgroups. The ED_{50} values were 0.48 for the animals trained with 1 mg/kg, 0.57 with 2 mg/kg, and 1.41 with 4 mg/kg. Tests with double the training dose resulted in higher percentage drug choice by all subgroups.

Figure 1B shows the response rates for saline and various morphine doses, including the training dose for each subgroup. Morphine tended to increase the response rates of the two lower training dose subgroups (1 and 2 mg/kg) above their saline rates. The difference was significant in the 1 mg/kg subgroup for 0.25 mg/kg, $t(3)=9.94$, $p<0.01$, and for 0.5 mg/kg, $t(3)=5.68$, $p<0.05$.

The morphine training dose of 4 mg/kg depressed the response rates in comparison with the saline training condition, $t(6)=2.57$, $p<0.05$. Doses higher than the training dose tended to suppress response rates of all subgroups. The decrease became reliable for 8 mg/kg with the 4 mg/kg subgroup, $t(5)=3.73$, $p<0.05$.

The results of tests with various doses of clonidine HCl

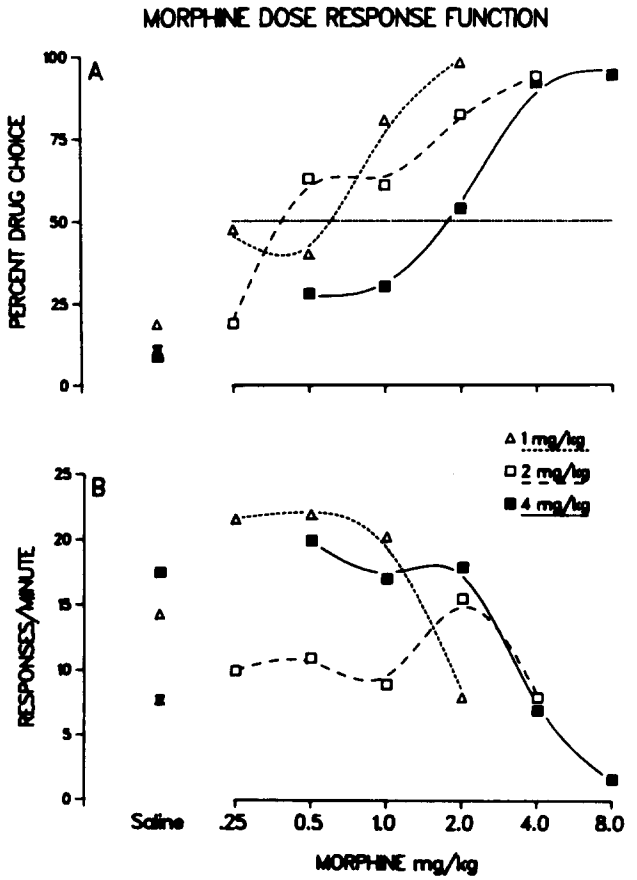

FIG. 1. Dose response curves for various doses of morphine sulfate tested in animals trained to discriminate morphine (1, 2, or 4 mg/kg) from saline. The top graph (A) portrays the percent of total responses made on the drug lever during 60 sec test sessions. The bottom graph (B) shows the average total response rate on both levers made in the same test period.

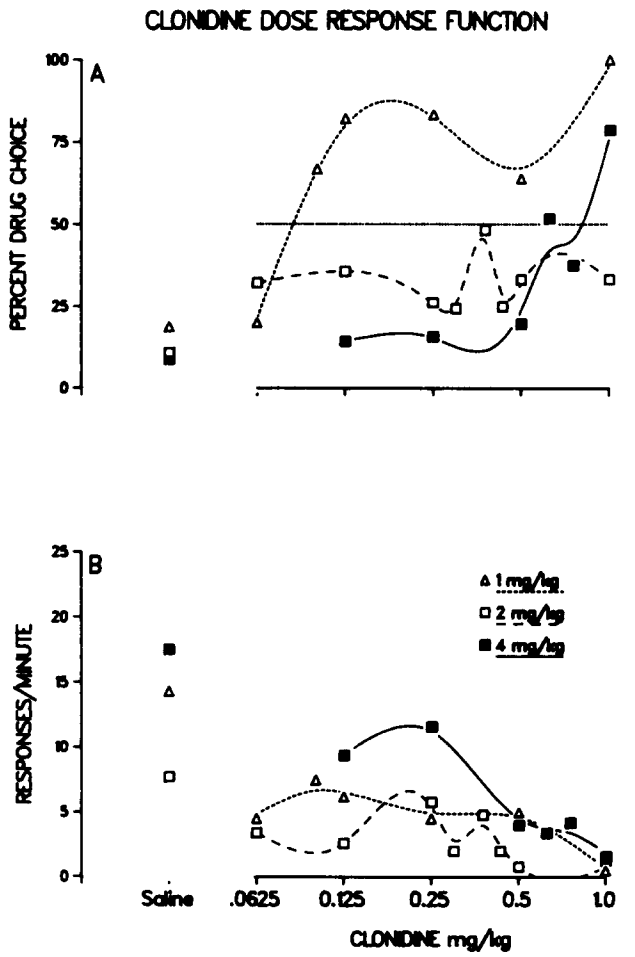

FIG. 2. Percentage drug lever choice (A) and total response rates (B), defined as in Fig. 1, in tests with various doses of clonidine.

are shown in Fig. 2. The animals trained with 4 mg/kg morphine made the saline choice after clonidine doses as high as 0.5 mg/kg. Higher clonidine doses increased the selection of the morphine lever (Fig. 2A) yielding an ED_{50} of 0.82 mg/kg. The difference from the saline condition was statistically significant for clonidine 1.0 mg/kg, $t(3)=3.66$, $p<0.05$. The low morphine subgroup (1 mg/kg) made the drug response after a clonidine dose as low as 0.125 mg/kg (ED_{50} 0.075 mg/kg). The difference from saline was statistically significant for 0.125 mg/kg clonidine, $t(2)=5.12$, $p<0.05$, and also in tests with higher doses. The middle dose subgroup (2 mg/kg) made predominantly the saline choice after all clonidine doses (0.0625 to 1.0 mg/kg).

Increasing clonidine doses had a generally dose related depressant effect on the response rates (Fig. 2B). Response rates were significantly below the saline level with clonidine doses as low as 0.125 mg/kg for the 1 mg/kg subgroup, $t(2)=4.38$, $p<0.05$, with clonidine doses as low as 0.0625 mg/kg for the 2 mg/kg subgroup, $t(2)=4.82$, $p<0.05$, and with a clonidine dose as low as 0.125 mg/kg for the 4 mg/kg subgroup, $t(6)=4.70$, $p<0.01$.

Various doses of naloxone were administered concomitantly with a selected dose of clonidine. In the low morphine subgroup 0.1 mg/kg naloxone with clonidine (0.25 mg/kg) resulted in 5% choice of the morphine lever compared to 83% with the same dose of clonidine alone. This antagonism of clonidine by naloxone was statistically significant, $t(3)=3.61$, $p<0.05$. Naloxone doses as high as 0.2 mg/kg, however, did not antagonize generalization to clonidine (1.0 mg/kg) in the high morphine subgroup. Naloxone did not reverse the rate suppressing effects of clonidine for any subgroup. The rate suppressing effects of the same doses of clonidine were reversed by the alpha-adrenergic blocker yohimbine at the dose of 2.5 mg/kg for the low morphine subgroup, $t(3)=3.96$, $p<0.05$, and at 2.0 mg/kg for the high morphine subgroup, $t(4)=2.78$, $p<0.05$. Yohimbine tended to antagonize the generalization to clonidine in all subgroups but the results were inconsistent.

DISCUSSION

Our results indicate that morphine and clonidine share some discriminative effects and that both compounds also have differential effects specific to their low and high doses. The response trained with the low dose of morphine generalized to relatively low doses of clonidine (0.125 to 0.5 mg/kg). The response trained with the high dose of morphine generalized only to very high doses (0.625 to 1.0 mg/kg) of clonidine.

The response trained with the intermediate morphine dose (2 mg/kg) generalized only partially to clonidine.

A possible explanation for these effects of clonidine is that the low dose subgroup learned to make a discriminative response on the basis of one portion of the total morphine stimulus complex, different from that component of the morphine stimulus used by animals trained with the highest morphine dose. Ambiguous results for clonidine found with the middle dose subgroup (2 mg/kg) further suggest that those animals used a morphine stimulus complex sharing some properties of both the low and high morphine doses.

Shannon and Holtzman [21] reported differential generalization by rats trained to discriminate morphine 1.75 mg/kg and 5.6 mg/kg from saline. Both morphine training doses generalized to the analgesic narcotic antagonists pentazocine and profadol. The low morphine dose generalized to nalbuphine and the non-opioid d-amphetamine while the high morphine dose only partially generalized to nalbuphine. Differential effects of low and high doses of clonidine are consistent with the suggestion that clonidine, an adrenergic agonist, stimulates pre-synaptic alpha-adrenergic receptor sites preferentially at lower doses and additionally acts at postsynaptic sites at higher doses [11,14].

Differential effects of low and high doses of both morphine and clonidine were also indicated by our results after morphine or clonidine were administered concomitantly with various doses of naloxone. Naloxone antagonized the discriminative effects of morphine in agreement with the findings by others [10]. Naloxone also antagonized generalization to clonidine in the subgroup trained with the low morphine dose but only partially antagonized generalization in the subgroup trained with the high morphine dose. The results for the middle morphine dose subgroup were inconsistent. Naloxone, however, failed to reverse the rate suppressing effects of clonidine in any subgroup.

Concomitant treatment with yohimbine and clonidine gave evidence that yohimbine blocked the generalization to clonidine in the high morphine dose subgroup and partially antagonized the generalization to clonidine in both the middle and low morphine dose subgroups. Yohimbine, however, clearly reversed the disruptive effects of clonidine as evidenced by the increased response rates in all subgroups when yohimbine and clonidine were administered together.

Ortmann *et al.* used similar clonidine treatment (0.1 mg/kg, IP) to depress rearing and ambulation activity in rats and reversed these effects by concomitant treatment with yohimbine (0.3 to 3 mg/kg) [17]. The authors suggested that the adrenoreceptor mediating the depressant effect of clonidine is of the alpha-type. Evidence for adrenergic involvement in the effects of clonidine was reported by Bennett and Lal, who trained rats to discriminate a low dose of clonidine (0.04 mg/kg) from the saline control condition [1]. There remains some disagreement, however, as to whether morphine mediates some of its effects by direct action on presynaptic alpha-adrenergic receptors [20].

Lal *et al.* [12], Lal and Shearman [13], and Herling *et al.* [9] reported that morphine failed to generalize to clonidine. Several differences from their procedures may account for our successful generalization. These prior studies used higher training doses and we found the subgroup trained with the lowest morphine dose was the most sensitive to the effects of clonidine. Lal and colleagues used the intraperitoneal route of administration. Our use of the SC route is preferable for morphine and probably enhanced the sensitivity of the animals to the drug effect [10]. It is possible that the effects of morphine and clonidine differ appreciably in pigeons, the species used by Herling *et al.* [9].

The comparison of three training doses of morphine in this study provides new information about the discriminative properties of morphine in rats. The results for the 1 mg/kg dose (0.75 mg/kg base) subgroup indicated that the rat is capable of discriminating a morphine dose lower than that previously reported, without a need for extensive dose titration. The ED_{50} of 0.39 mg/kg (39% of the training dose) also shows that the use of low doses enables the animals to develop a high degree of sensitivity to the discriminative effects of morphine. The ED_{50} values for the other subgroups are also appreciably below 50% of their training doses (28% for the 2 mg/kg subgroup and 36% for the 4 mg/kg subgroup).

The low training dose subgroup (1 mg/kg) was very sensitive to the disruptive effects of morphine, indicated by a marked suppression of response rates in tests with 2 mg/kg for this subgroup but not for the other subgroups. The highest training dose of 4 mg/kg severely suppressed the response rate below the saline response rate. These findings further indicate that training with doses lower than previously reported is not only successful but desirable in order to minimize impairment of performance.

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