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Discriminative Stimulus Effects of Nalbuphine in Nontreated and Morphine-Treated Pigeons

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WALKER, E. A., E. R. HAWKINS, M. J. TIANO, M. J. PICKER AND L. A. DYKSTRA. *Discriminative stimulus effects of nalbuphine in nontreated and morphine-treated pigeons.* PHARMACOL BIOCHEM BEHAV **64**(2) 445–448, 1999.—In the present study, the stimulus effects of the low efficacy agonist nalbuphine were examined under two conditions: nontreated and morphine treated. In the first experiment, five pigeons were trained to discriminate among 3.2 mg/kg morphine, 5.6 mg/kg nalbuphine, and saline. Nalbuphine produced nalbuphine-like responding. Low doses of morphine produced nalbuphine-like responding, whereas high doses produced morphine-like responding. Naltrexone produced saline-like responding and reversed the stimulus effects produced by the training doses of morphine and nalbuphine. Five different pigeons were treated daily with 10 mg/kg morphine (IM) and trained 6 h later to discriminate among 10 mg/kg morphine, 1.0 mg/kg nalbuphine and saline. In these pigeons, morphine produced morphine-like responding and nalbuphine produced nalbuphine-like responding. Morphine abstinence produced nalbuphine-like responding that was reversed by morphine. Additionally, naltrexone produced nalbuphine-like responding. These data suggest that the discrimination between morphine and nalbuphine in the nontreated and morphine-treated pigeons may be based on the relative efficacy differences between morphine, a higher efficacy μ-agonist, and nalbuphine a lower efficacy μ-agonist. © 1999 Elsevier Science Inc.

Nalbuphine Morphine Naltrexone Three-choice discrimination Withdrawal Efficacy Pigeons

THE opioid agonists morphine and nalbuphine share stimulus effects in a number of two-choice discriminations (2,10,12). Apparent pA_2 analyses with naltrexone indicate that these shared stimulus effects are mediated through common, presumably μ, opioid receptors (2,8). If the training dose of morphine is high, however, nalbuphine produces saline-like stimulus effects and blocks morphine's stimulus effects (12). Taken together, these data suggest that nalbuphine is a low efficacy agonist at the μ opioid receptor.

Three-choice discriminations have been established among two opioid agonists and saline to differentiate compounds that have multiple receptor selectivity yet share stimulus effects (11). Three-choice discriminations have also been established among high and low doses of morphine and saline to differentiate compounds according to relative efficacy (7). Finally, three-choice discriminations have been established among an agonist, an antagonist, and saline in subjects maintained chronically on an agonist to study the stimulus effects of withdrawal (1).

In the present experiment, three-choice discriminations were established between morphine, nalbuphine, and saline under two conditions. In the first discrimination, nontreated pigeons were trained to discriminate between 3.2 mg/kg morphine, 5.6 mg/kg nalbuphine, and saline. These training doses were selected for maximum cross-substitution between morphine and nalbuphine in two-choice discriminations (10,12). It is hypothesized that this discrimination will be based on differences in relative efficacy of morphine and nalbuphine as μ opioid agonists. In the second discrimination, morphine-treated pigeons were trained to discriminate among 10 mg/kg morphine, 1.0 mg/kg nalbuphine, and saline. These training doses were selected based on previous studies in pigeons treated daily with 10 mg/kg morphine in which higher training doses of morphine were used to establish the discrimination (1). It is hypothesized that the discriminative stimulus effects of morphine and nalbuphine in the morphine-treated pigeons will be based on differences in relative efficacy of morphine and nalbuphine. However, in these pigeons, the relative efficacy differences will be reflected as agonist and antagonist properties of morphine and nalbuphine, respectively.

METHOD

Subjects

Ten White Carneau pigeons maintained at approximately 85% of their free feeding weights (390–550 g) were used.

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Each pigeon was housed individually in a colony maintained on a 12 L:12 D cycle and had free access to grit and water. Five pigeons were injected 7 days a week with 10 mg/kg morphine, IM.

Apparatus

Five operant conditioning chambers were used. Each chamber contained three response keys that were 2.5 cm in diameter and located 23 cm from the bottom of the intelligence panel and centered approximately 12 cm apart. An opening located on the intelligence panel centered 8 cm above the floor of the chamber allowed access to a hopper filled with mixed grain when the hopper was raised. A 7-W white bulb illuminated the opening when the hopper was raised. A house light mounted 33 cm above the chamber floor provided ambient illumination. Each chamber was equipped with an exhaust fan and white noise. A microcomputer with software and interfacing (MED Associates, Georgia, VT) was used for scheduling of experimental events and data collection.

Discrimination Training and Testing

Pigeons were injected with morphine, saline, or nalbuphine and placed in the darkened operant chambers. The session for the morphine-treated pigeons was conducted 6 h after the daily morphine injection. Fifteen minutes later, the three response lights were illuminated and the pigeons were trained to respond on the right, center, and left key on a FR 1 schedule of food delivery (3-s access to mixed grain) for 15 min. Keypeck responses on the injection-inappropriate keys were counted but had no programmed consequences. Over several sessions, number of response required for food delivery was increased to 30 (FR 30). Morphine, saline, and nalbuphine training sessions were administered pseudorandomly with the restriction that a given training drug was not administered more than twice in a row. These training conditions stayed in effect until an individual pigeon met the following conditions for 9 out of 12 consecutive days: (a) the first 30 responses were made on the injection-appropriate key; (b) the percentage of responses emitted on the drug-appropriate key during the entire session was $\geqslant$80%; and (c) only one error (failure to fulfill conditions 1 or 2) for each training stimulus was allowed.

Test sessions were conducted using a multiple-trial, cumulative-dosing procedure with a 15-min pretreatment period and a 5-min ratio component (10). For repeated saline tests, five injections of saline were administered at the beginning of every trial. In the naltrexone reversal tests, the training dose of morphine or nalbuphine was administered to the nontreated pigeons and was followed by cumulative doses of naltrexone. In the morphine abstinence study, the daily 10 mg/kg morphine injection was replaced by saline and the pigeons were tested 6 h later.

Data Analysis

Drug discrimination data are presented as the mean percentage of drug-appropriate responses to total responses of all pigeons during the test session. Rate of responding is presented as the mean total number of responses divided by number of seconds during the session. SEM is used to express variance. Data from pigeons responding less than 30 times during a trial were included in the response rate figures but not the discrimination figures. The dose that produced 50% drug-appropriate responding was calculated by log-linear interpolation of the linear portion of the group dose–response curve. Morphine-like or nalbuphine-like responding refers to 80% or greater responding on the morphine or nalbuphine key, respectively.

Drugs

The following compounds were used: morphine sulfate (supplied by the National Institute on Drug Abuse, Rockville, MD), nalbuphine hydrochloride, and naltrexone hydrochloride (purchased from Research Biochemicals Inc., Natick, MA). All injections were IM, and all doses are expressed as the salt.

RESULTS

Acquisition

The morphine, nalbuphine, and saline discrimination in the nontreated pigeons was acquired in an average of 120 sessions, with a range of 83 to 150 sessions. The morphine, nalbuphine, and saline discrimination in morphine-treated pigeons was acquired in 38 sessions with a range of 14 to 120 sessions.

Substitution Tests

In the nontreated pigeons, low, intermediate, and high doses of morphine produced saline-like, nalbuphine-like, and morphine-like responding. ED_{50} values (95% C.L.) of 0.24 mg/kg (0.075–0.79) for nalbuphine-like responding and 1.9 mg/kg (0.73–5.2) for morphine-like responding were obtained (Fig. 1, top panels). Intermediate doses of morphine produced 90–100% nalbuphine-like responding in four out of five pigeons. A cumulative dose of 100 mg/kg morphine eliminated responding. Nalbuphine only produced dose-dependent nalbuphine-like responding with an ED_{50} value (95% C.L.) of 0.39 mg/kg (0.049–3.2). A cumulative dose of 100 mg/kg nalbuphine decreased response rates to approximately 0.2 responses/s. Five trials of repeated saline injections produced predominantly saline-like responding (a maximum of 20% nalbuphine-like responding and 16% morphine-like responding) (data not shown). Cumulative doses of naltrexone produced exclusive saline-like responding in four pigeons and 82% nalbuphine-like responding in one pigeon at a dose of 0.32 mg/kg (data not shown). In the naltrexone reversal experiments, naltrexone (0.1 mg/kg) reversed the stimulus effects of the training dose of nalbuphine or morphine to a similar extent (data not shown).

In the morphine-treated pigeons, morphine only produced dose-dependent morphine-like responding with an ED_{50} value (95% C.L.) of 2.1 mg/kg (0.57–7.5) and nalbuphine only produced dose-dependent nalbuphine-like responding with an ED_{50} value (95% C.L.) of 0.48 mg/kg (0.025–9.4) (Fig. 1, bottom panels). Five trials of repeated saline injections produced exclusive saline-like responding (data not shown). Naltrexone produced nalbuphine-like responding with an ED_{50} (95% C.L.) of 0.0033 mg/kg (0.0012–0.0089) (Fig. 2, top panels). Additionally, morphine abstinence produced 80% nalbuphine-like responding that was reversed by a cumulative dose of 10 mg/kg morphine (Fig. 2, bottom panels).

DISCUSSION

In the present series of experiments, two three-choice discriminations were established among morphine, nalbuphine, and saline. In the nontreated pigeons, it appears the discrimi-

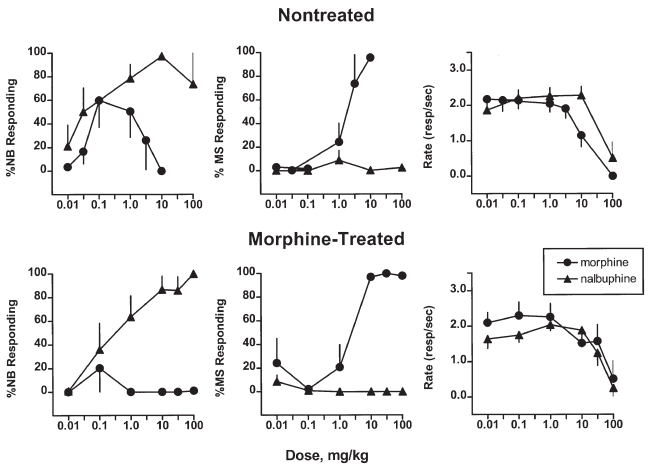

FIG. 1. Effects of the training stimuli, morphine (●) and nalbuphine (▲). In nontreated pigeons (upper panels), nalbuphine produced only nalbuphine-like responding. Low doses of morphine produced some nalbuphine-like responding and high doses of morphine produced morphine-like responding. In morphine-treated pigeons (lower panels), nalbuphine produced only nalbuphine-like responding and morphine produced only morphine-like responding. Ordinate: % of total responses made on the nalbuphine key (left panels) or morphine key (center panels). % of responses made on the saline key is not shown, but is the difference of 100% and the sum of the total responses made on the morphine and nalbuphine key. Ordinate (right panels): Response rate measured as total responses made on all keys divided by time in secconds. Data from pigeons making less than 30 responses were included in the rate figures but not the discrimination figures. Abscissa: cumulative doses of drug, in mg/kg. $n = 7$; vertical bars represent ± SEM.

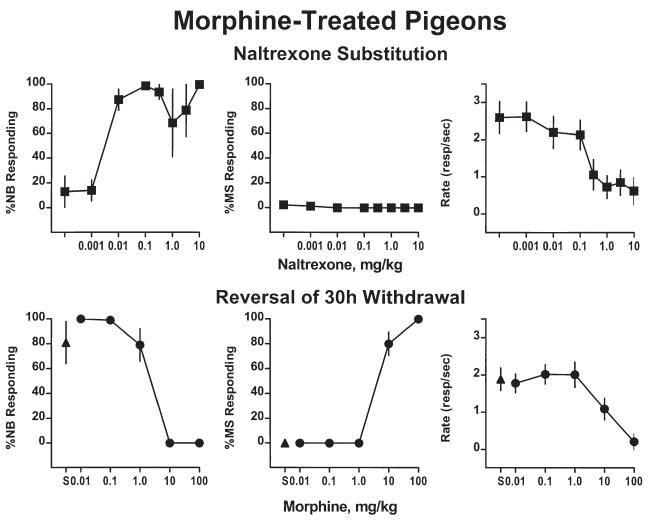

FIG. 2. In morphine-treated pigeons, opioid antagonist naltrexone (■) produced nalbuphine-like responding and no morphine-like responding (upper panels). Reversal of nalbuphine-like responding produced by substitution of the morning morphine injection with saline (lower panels). When 30-h abstinent pigeons are tested 6 h later, saline (▲) produced nalbuphine-like responding that was reversed by cumulative doses of morphine (●). $n = 5$–7. Other details as in Fig. 1.

nation is based on a quantitative difference of relative efficacy between the low efficacy agonist nalbuphine and the higher efficacy agonist morphine. This hypothesis is supported by the observation that low doses of morphine were saline-like, intermediate doses of morphine were nalbuphine-like, and high doses of morphine were morphine-like. Furthermore, nalbuphine produced nalbuphine-like responding only. These data are very similar to data collected in pigeons trained to discriminate among a low dose of morphine, a high dose of morphine, and saline (7). In that study, low doses of morphine were saline-like, intermediate doses of morphine produced low dose morphine-like responding, and high doses of morphine produced high-dose morphine-like responding. Also, in that study, nalbuphine only produced low-dose morphine-like responding. In the present experiments, however, instead of a dose continuum, the morphine, nalbuphine, and saline discrimination may be based on an efficacy continuum.

It is unlikely that the morphine, nalbuphine, and saline discrimination in nontreated or morphine-treated pigeons is based on differences in receptor selectivity because the same dose of naltrexone reversed the stimulus effects of morphine in both groups of pigeons and nalbuphine in the nontreated pigeons. In the morphine-treated pigeons, naltrexone substituted for nalbuphine. Future experiments with high and low efficacy μ agonists, as well as κ and δ opioid agonists will further delineate the receptor selectivity of these discriminations.

The differences between the nontreated and morphine-treated pigeons could also be due to the different training doses of morphine and nalbuphine used in the two groups. Clearly, small changes in morphine training doses can alter the potency and effectiveness of a number of opioid agonists in two-choice discriminations (12). However, because large changes in the training dose of low-efficacy agonists fail to alter agonist substitution patterns significantly (5,10), altering the nalbuphine training dose probably would not appreciably change the pattern of substitution observed in the present experiment. Furthermore, the lower training doses of 3.2 mg/kg nalbuphine was not discriminated from saline accurately in the nontreated pigeons in the present study (E. A. Walker, unpublished observations). A final consideration is that the difference between training doses of morphine and nalbuphine might be as important, or more important, than is the absolute value of each of the training drugs.

Although all pigeons eventually learned the discrimination, clearly the morphine, nalbuphine, and saline discrimination was acquired more quickly in the morphine-treated pigeons. Perhaps the differences among the stimulus effects of an agonist, antagonist, and saline in morphine-treated pigeons are greater than among the stimulus effects of agonists of different efficacies in nontreated pigeons. In the morphine-treated pigeons, morphine did not produce nalbuphine-like responding, indicating that morphine does not share any stimulus effects with nalbuphine in this three-choice discrimination. However, in the nontreated pigeons, morphine clearly shares stimulus effects with nalbuphine, probably making the discrimination more difficult.

The discriminative stimulus effects of naltrexone in morphine-dependent subjects appears to be based on the capacity of naltrexone to precipitate withdrawal, because withholding the daily morphine injection produces naltrexone-like stimulus effects (1,3). In the morphine-treated pigeons in the present experiments, withholding the daily morphine injection produced nalbuphine-like stimulus effects that were reversed by morphine. Additionally, cumulative doses of naltrexone produced nalbuphine-like responding. Nalbuphine

produces a range of antagonist effects in morphine-treated subjects based on the magnitude of dependence and the assay used to measure effects. For example, in morphine-dependent subjects, nalbuphine precipitates withdrawal (4,6), blocks the effects of morphine (9) and produces considerable naltrexone-like stimulus effects (1). Despite the similarity between nalbuphine and naltrexone in morphine-treated subjects, the fact that nalbuphine has some intrinsic efficacy may suggest that the stimulus effects of nalbuphine could be a weaker withdrawal cue than the stimulus effects of naltrexone. This hypothesis can be tested by comparing the substitution patterns of high-, intermediate-, and low-efficacy μ agonists in morphine-treated pigeons trained to discriminate among morphine, nalbuphine, and saline to the data collected in morphine-treated pigeons trained to discriminate among morphine, naltrexone, and saline.

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