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Effects of Nalbuphine Alone and in Combination With Tripelennamine on Rewarding Brain Stimulation Thresholds in the Rat[1]

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UNTERWALD, E. M. AND C. KORNETSKY. *Effects of nalbuphine alone and in combination with tripelennamine on rewarding brain stimulation thresholds in the rat.* PHARMACOL BIOCHEM BEHAV 25(3) 629–632, 1986.—Reinforcing thresholds for rewarding brain stimulation to the medial forebrain bundle-lateral hypothalamus were determined in rats by means of a rate-free psychophysical method. Nalbuphine, a mixed agonist-antagonist opioid, alone caused a significant, but modest, dose-dependent lowering of the threshold for reinforcing stimulation. Concomitant administration of an ineffective dose of tripelennamine, an anithistamine, with nalbuphine potentiated the threshold lowering effect of nalbuphine. These results are similar to previous results obtained with tripelennamine and pentazocine suggesting that nalbuphine may have abuse potential if combined with tripelennamine.

Nalbuphine Tripelennamine Brain-stimulation reward Medial forebrain bundle-lateral hypothalamus
Mixed agonist-antagonist opioids Drug abuse Reward threshold

NALBUPHINE is a mixed agonist-antagonist opioid analgesic and is currently available in injectable form for clinical use for the treatment of pain. Both animal and human studies have documented nalbuphine's efficacy as a potent analgesic agent [8]. Furthermore, its abuse potential has been reported to be lower than that of morphine [5]. To further characterize its liability for abuse, we studied the effects of acute nalbuphine administration on the threshold for rewarding brain stimulation in the rat. Previous studies have demonstrated that many drugs of abuse, including morphine, cocaine, amphetamine, and phencyclidine, lower the threshold for brain-stimulation reward suggesting that this is a model of drug-induced euphoria and therefore predictive of abuse liability [6].

Many reports have documented that pentazocine, another agonist-antagonist opioid, is widly abused especially when combined with the antihistamine, tripelennamine [7,12]. Pentazocine and tripelennamine have each been shown to lower the reward threshold in a dose-dependent manner [14,15]. In addition, a low dose of tripelennamine (2.5 mg/kg), which is ineffective alone in lowering the reward threshold, will potentiate the lowering effect produced by pentazocine suggesting an increase in euphoria when these two agents are co-administered [14]. In order to determine if this interaction with tripelennamine occurs with other mixed agonist-antagonists, we studied the effects of concomitant administration of nalbuphine and tripelennamine in this same animal model.

METHOD

Bipolar stainless steel electrodes (0.13 mm in diameter and insulated except at the tips) were stereotaxically implanted bilaterally in the lateral hypothalamic region of the medial forebrain bundle (MFB-LH) of six male CDF rats (300 g, Charles River Laboratories). Surgical anesthesia was produced by systemic administration (0.3 mg/100 g of body weight) of Equi-Thesin® (a combination product containing pentobarbital, chloral hydrate, and magnesium sulfate). MFB-LH coordinates were 4.0 mm posterior to bregma, ±1.4 mm from the midline suture, and 8.5 mm ventral to the skull surface. The electrodes were placed through small burr holes in the skull and attached permanently to the surface with an acrylic platform. After surgery, animals received 60,000 units of penicillin (Bicillin®) IM and were given at least one week for post-operative recovery before behavioral

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testing was begun. Animals were maintained on a 12 hour light/dark cycle, housed in standard steel cages and had ad lib access to food and water.

During the initial phase of animal training each of the two electrodes was tested to determine the current intensity needed to produce appetitive behavior and to determine the presence or absence of motor artifact. The electrode which produced appetitive behavior at the lowest current intensity and had the least or no motor artifact was used in the subsequent study. Animals were trained and tested on a rate-independent threshold procedure in a plastic chamber (20×20×35 cm). A wheel manipulandum was located within one wall of the test chamber. Four equally spaced cams on one endplate of the wheel manipulandum operated a microswitch which resulted in the immediate delivery of a stimulation when the wheel was rotated one-quarter of a turn. A constant current stimulator (Sunrise Systems, Pembroke, MA) was used to deliver the biphasic symmetrical pulses. Each stimulus consisted of a 500 msec train with a pulse width of 0.2 msec and a delay of 0.2 msec between the positive and negative pulses at a frequency of 160 Hz.

Thresholds were determined by a procedure involving the use of discrete trials systematically presented over a range of stimulus intensities. A trial began with the delivery of a non-contingent intracranial stimulus. A response of one-quarter wheel turn within 7.5 sec of this stimulus resulted in the delivery of a contingent stimulus, identical in all parameters to the non-contingent stimulus, and terminated the trial. Failure to respond had no scheduled consequences and the trial was terminated after 7.5 sec. The interval between trials varied around an average of 15 sec and responses made during the intertrial interval (error responses) resulted in a 15 sec delay before the start of the next trial.

Stimulus intensities were varied using a modification of the classical psychophysical method of limits. For further details on this procedure see Esposito and Kornetsky [2]. Stimuli were presented in alternating descending and ascending series with a step size of 5 or 10 μA (depending on the sensitivity of the individual animal) with 5 trials presented at each intensity level before the next lower or higher intensity was presented. Subjects completed 4 series (i.e., descending, ascending, descending, and ascending) prior to injection and 8 series post-injection. The duration of the pre-injection and the post-injection testing sessions was approximately 45 minutes and 90 minutes respectively. All experimental data were collected and stored by an on-line microcomputer. Each series' threshold value was defined in microamperes as the midpoint between the level at which the animal made 3 or more correct responses out of the 5 stimulus presentations (a plus score) and the level where less than 3 correct responses (a minus score) were made. The pre- and post-injection thresholds were defined as the respective series means.

Animals required approximately 6 one hour training sessions to learn the task and approximately 4 additional sessions for the establishment of a stable threshold level whereupon saline injections were begun. Animals were tested with saline injections for 5 days before drug administration was initiated. Also, saline days were interspersed with drug treatment days so that animals received drug only twice weekly.

Experiment I—Nalbuphine Alone

Six animals were injected subcutaneously with either nalbuphine hydrochloride dissolved in isotonic saline or isotonic saline control. All injections were in volumes of 1 ml/kg body weight and the post-injection testing session was begun 5 minutes after drug or saline administration. Doses of nalbuphine tested ranged from 0.32 mg/kg to 10.0 mg/kg and the sequence of doses was balanced between animals.

Experiment II—Nalbuphine Plus Tripelennamine

Four of the animals used in experiment I were subsequently used in experiment II. On drug test days, animals received 2.5 mg/kg tripelennamine hydrochloride dissolved in isotonic saline intraperitoneally followed immediately by a dose of nalbuphine ranging from 0.32 mg/kg to 10.0 mg/kg subcutaneously. The tripelennamine dose of 2.5 mg/kg was chosen because previous studies [14,15] have shown that this dose is typically ineffective in lowering reward threshold. In addition, this dose of tripelennamine has been shown to potentiate the effects of pentazocine [14]. On control days, isotonic saline was administered by both routes. Once again, all injections were in volumes of 1 ml/kg body weight and the post-injection testing session was begun 5 minutes after drug or saline administration.

Threshold values were calculated for both the pre-injection and the post-injection sessions, with the difference between the two scores taken as the dependent measure. These difference scores were transformed to standard scores (Z-scores) based on the mean and standard deviation of the difference scores for all saline days. A minimum of 20 control scores for each animal was used in determining each Z-score value. A Z-score of ± 2.0 or greater (95% confidence limits) was pre-selected as the level of significance.

Dose-response curves were generated for nalbuphine alone in experiment I. In experiment II, dose-response curves for the combination of nalbuphine and tripelennamine were determined and then compared to the curves obtained from nalbuphine alone.

Histology

Following testing, the animals were sacrificed with an overdose of anesthetic and perfused intracardially with saline followed by formalin. The brains were subsequently removed from the skull, fixed, embedded, and sliced at 40 μ. Mounted sections were stained with cresyl violet and luxol fast blue and examined under a light microscope to determine the placement of the electrode tips.

RESULTS

The results from experiment I are summarized in Fig. 1 which illustrates the effects of nalbuphine on the threshold for reinforcing brain stimulation. Mean Z-scores from six animals are shown as a function of dose. A significant lowering of threshold was obtained for every animal with the optimal dose ranging from 1.25 mg/kg to 5.0 mg/kg. At no dose was the reward threshold ever raised.

Figure 2 depicts the effects of nalbuphine alone and in combination with 2.5 mg/kg tripelennamine on reward threshold. This dose of tripelennamine when administered alone did not produce a significant change in threshold for any animal. Concomitant administration of 0.32 mg/kg to 10.0 mg/kg nalbuphine and 2.5 mg/kg tripelennamine resulted in a greater decrease in the threshold for brain-stimulation reward than obtained with nalbuphine alone.

Histological verification of the electrode placement con-

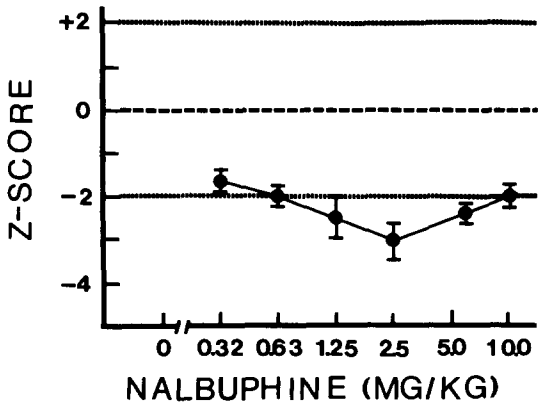

FIG. 1. Mean±SEM Z-score changes in reward threshold values from pre- to post-drug as a function of the dose of nalbuphine (n=6). The 95% confidence limits (Z-score of ±2.0) for all saline days are indicated by the horizontal dotted lines.

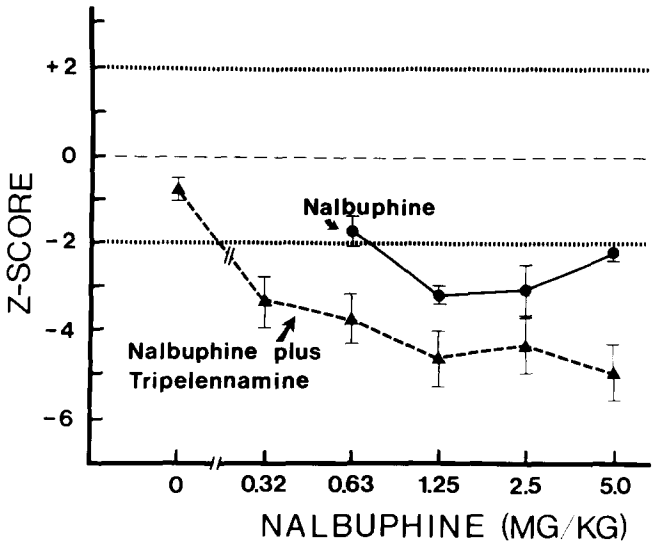

FIG. 2. Mean effect of nalbuphine alone and in combination with tripelennamine (2.5 mg/kg) on the reward threshold. Data are expressed as mean±S.E.M. for four of the subjects used in experiment II.

firmed that the electrodes were located within the medial forebrain bundle at the level of the lateral hypothalamus.

DISCUSSION

Reinforcing thresholds for self-stimulation behavior to the medial forebrain bundle were determined in rats by means of a rate-free psychophysical method. In experiment I, acute nalbuphine administration produced a dose-dependent lowering of the reward threshold. In experiment II, a small dose of tripelennamine (2.5 mg/kg) which was ineffective alone in lowering the reward threshold was administered concomitantly with various doses of nalbuphine. The lowering of threshold produced by this combination was of greater magnitude than that produced by nalbuphine alone at any dose. If a lowering of the threshold for rewarding brain stimulation is a model of drug induced euphoria, then these results suggest that these drugs may act synergistically in causing euphoria in man. The results of this study are similar to previous results obtained with pentazocine and tripelennamine [14] and thus suggest that the abuse liability of nalbuphine is comparable to that of pentazocine.

To the best of our knowledge, no other studies have been reported using nalbuphine and intracranial stimulation although its potential for abuse has been assessed using other measures. Direct addiction studies in animals have shown that nalbuphine will precipitate abstinence in morphine-dependent monkeys [16] and in addition abrupt withdrawal of drug from monkeys who had been receiving nalbuphine chronically resulted in a morphine-like abstinence syndrome. A study done with rats indicated that withdrawal from nalbuphine is less severe than withdrawal from morphine or pentazocine suggesting that nalbuphine produces a lower degree of physical dependence [13].

Shannon and Holtzman [9,10] have used the drug discrimination paradigm to study the subjective effects of several analgesic agents including nalbuphine. Rats trained to discriminate morphine from saline in a two-lever discrete trial avoidance paradigm were tested for generalization to nalbuphine. They found that the degree of generalization was related to the dose of morphine used in the training procedure. Rats trained with 1.75 mg/kg of morphine generalized completely to nalbuphine while those trained with 5.6 mg/kg of morphine showed only partial generalization to nalbuphine. This suggests that only some of the subjective effects of nalbuphine are similar to those of morphine and hence its abuse liability appears to be less than that of morphine.

Drug self-administration by various animal species has been used as a measure of a drug's reinforcing properties and is a predictor of its abuse potential [3]. Steinfels *et al.* [13] have used morphine post-addict rats to study nalbuphine self-administration. These animals had a history of self-administration of morphine but were not tolerant or physically dependent on morphine and therefore abstinence would not be precipitated by the mixed agonist-antagonist agents. These post-addict rats were given the opportunity to self-administer nalbuphine as well as morphine, pentazocine, and butorphanol. The patterns of mean daily number of self-injections during relapse were similar for nalbuphine, morphine, pentazocine, and butorphanol. The authors concluded that, in rats with a history of morphine addiction, the abuse potentials of nalbuphine, pentazocine, and butorphanol were similar to that of morphine. Other studies have shown nalbuphine to be self-administered by naive rats [1] and by monkeys [18].

The abuse liability of nalbuphine has also been studied in human subjects [5]. It was found that chronic administration of nalbuphine produced a greater degree of physical dependence than was produced by pentazocine. Similarly, abrupt withdrawal of nalbuphine was followed by an abstinence syndrome which was mild but significantly more intense than pentazocine abstinence and was accompanied by compulsive drug-seeking behavior. Single-dose studies suggested that nalbuphine may be less euphorigenic than pentazocine. On

the basis of these results, Jasinski and Mansky [5] concluded that nalbuphine possesses some properties which could lead to its abuse and that its abuse potential would probably be similar to that of pentazocine.

When evaluating nalbuphine's liability for abuse, it also must be kept in mind that nalbuphine will precipitate abstinence in morphine-dependent animals due to its antagonist activity. This opiate antagonistic activity has been reported to be greater than that seen with pentazocine [8] suggesting that self-administration of nalbuphine by a morphine addict would be dysphoric and hence, would not be abused by this population.

Results from the second part of the present study indicate that tripelennamine enhances nalbuphine's facilitation of brain-stimulation reward. Previous studies have shown that tripelennamine also potentiates the effects of pentazocine in this same procedure [14]. The mechanism underlying the interaction between tripelennamine and opioids is poorly understood. It has been suggested that tripelennamine might reduce the dysphoric psychotomimetic component of mixed agonist-antagonists which has been reported in humans at high doses [11]. It has been demonstrated that co-administration of sub-analgesic doses of tripelennamine with subanalgesic doses of nalbuphine causes a significant increase in antinociception in mice as measured by a modification of Haffner's tail clamp procedure [4]. Alternatively, this interaction may be mediated by a central histamine system. It has been shown in mice that H1 and H2 receptors in the brain may be involved in the development of morphine tolerance and physical dependence [17].

In conclusion, the results of this study demonstrate that nalbuphine alone causes a significant, but modest, lowering of the threshold for rewarding intracranial stimulation to the medial forebrain bundle-lateral hypothalamus in the rat. Concomitant administration of tripelennamine resulted in a potentiation of this lowering effect. These results suggest that the abuse potential of nalbuphine alone is modest and similar to that of pentazocine but that this potential is increased upon co-administration of tripelennamine. Results from other studies using different techniques are in agreement with these results in that they also predict nalbuphine's abuse potential to be lower than that of morphine and similar to that of pentazocine.

REFERENCES

1. Collins, R. J., J. R. Weeks, M. M. Cooper, P. I. Good and R. R. Russell. Prediction of abuse liability of drugs using IV self-administration by rats. *Psychopharmacology (Berlin)* **82:** 6–13, 1984.
2. Esposito, R. and C. Kornetsky. Morphine lowering of self-stimulation thresholds: Lack of tolerance with long-term administration. *Science* **195:** 189–191, 1977.
3. Griffiths, R. R. and R. L. Balster. Opioids: Similarity between evaluations of subjective effects and animal self-administration results. *Clin Pharmacol Ther* **25:** 611–617, 1979.
4. Hui, F. W., C. J. Sun, E. C. Tocus and J. P. Honig. The effects of tripelennamine alone and in combination with opiates to produce antinociception in mice. *Life Sci* **32:** 1531–1538, 1983.
5. Jasinski, D. R. and P. A. Mansky. Evaluation of nalbuphine for abuse potential. *Clin Pharmacol Ther* **13:** 78–90, 1971.
6. Kornetsky, C., R. U. Esposito, S. McLean and J. O. Jacobson. Intracranial self-stimulation thresholds: A model for the hedonic effects of drugs of abuse. *Arch Gen Psychiatry* **38:** 289–292, 1979.
7. Lahmeyer, H. W. and R. G. Steingold. Pentazocine and tripelennamine: A drug abuse epidemic? *Int J Addict* **15:** 1219–1232, 1980.
8. Schmidt, W. K. The pharmacology of nalbuphine: Profile of an agonist-antagonist analgesic. Reported to the Committee on Problems of Drug Dependence—Agonist-Antagonist Analgesics, February 17, 1983.
9. Shannon, H. E. and S. G. Holtzman. Further evaluation of the discriminative effects of morphine in the rat. *J Pharmacol Exp Ther* **201:** 55–66, 1977.
10. Shannon, H. E. and S. G. Holtzman. Morphine training dose: A determinant of stimulus generalization to narcotic antagonists in the rat. *Psychopharmacology (Berlin)* **61:** 239–244, 1979.
11. Shannon, H. E. and T. Su. Effects of the combination of tripelennamine and pentazocine at the behavioral and molecular levels. *Pharmacol Biochem Behav* **17:** 789–795, 1985.
12. Showalter, C. V. T's and Blues. Abuse of pentozocine and tripelennamine. *JAMA* **244:** 1224–1225, 1980.
13. Steinfels, G. F., G. A. Young and N. Khazan. Self-administration of nalbuphine, butorphanol and pentazocine by morphine post-addict rats. *Pharmacol Biochem Behav* **16:** 167–171, 1982.
14. Unterwald, E. M. and C. Kornetsky. Effects of concomitant pentazocine and tripelennamine on brain-stimulation reward. *Pharmacol Biochem Behav* **21:** 961–964, 1984.
15. Unterwald, E. M., L. T. Kucharski, J. E. G. Williams and C. Kornetsky. Tripelennamine: Enhancement of brain-stimulation reward. *Life Sci* **34:** 149–153, 1984.
16. Villarreal, J. E. and M. H. Seevers. Evaluation of new compounds for morphine-like physical dependence in the rhesus monkey. Report of 33rd Meeting, Committee on Problems of Drug Dependence, National Research Council, National Academy of Sciences, Washington, DC, 1971.
17. Wong, C. L. and M. B. Roberts. The possible role of brain histamine and H1 and H2 receptors in the development of morphine tolerance and physical dependence in mice. *Agents Actions* **5:** 476–483, 1975.
18. Woods, J. H. Narcotic-reinforced responding: A rapid screening procedure. Reported to the Committee on Problems of Drug Dependence, 1977, pp. 420–437.