Pharmacology Biochemistry & Behavior, Vol 10, pp 493–497 Printed in the U S A

Evidence Supporting Lack of Discriminative Stimulus Properties of a Combination of Naltrexone and Morphine[1]

T.U.C. JARBE, P LOMAN AND M.D.B. SWEDBERG

University of Uppsala, Department of Psychology, P.O. Box 227, S-751 Uppsala, Sweden

(Received 12 February 1978)

JARBE, T U C , P LOMAN AND M D B SWEDBERG *Evidence supporting lack of discriminative stimulus properties of a combination of naltrexone and morphine* PHARMAC BIOCHEM BEHAV 10(4) 493–497, 1979 —The aim of the present experiment was to study the potentially discriminable effects of combinations of morphine and naltrexone during long-term treatment Three groups of gerbils had to discriminate the effects of morphine (12 mg/kg) and those of either saline (4 ml/kg), naltrexone (2 mg/kg), or a combination of this dose of morphine plus naltrexone injected IP 60 min prior to the start of the discriminative training in a T-shaped maze. Rapid development of drug discriminative control of choice behavior (left or right turn in the maze) was evident in these 3 groups which is in marked contrast to the performance of gerbils trained with morphine-naltrexone combination vs saline or gerbils trained with naltrexone only vs saline Neither of these latter groups reached the criterion of performing 8 correct first-trial choices in 10 consecutive training sessions during the 60 training sessions allowed, while the 3 other groups began their criterion performance after only 7–8 training sessions Thus the discriminative properties of certain combinations of morphine and naltrexone are weak and therefore are not easily discriminable from the effects induced by saline

Drug discrimination Morphine Naltrexone Long-term treatment Gerbils

THE DRUG discrimination paradigm appears to be an interesting model to study the "subjective" response characteristics to drugs in laboratory animals. Drugs are studied with reference to their ability to act as discriminative stimuli, thereby guiding the choice behavior of animals [1, 2, 3, 11, 12, 18, 24, 25, 32] The procedure may be considered a counterpart of the use of experienced human subjects to indicate similarities or dissimilarities between drugs as assessed by various self-rating procedures [7, 10, 13, 22]

Dependence on narcotics presumably occur because of the pleasurable effects these drugs evoke as experienced by the drug taker Naltrexone, the N-cyclopropyl-methyl congener of naloxone, may be of value in drug detoxification programs because of its remarkable long duration of antagonism of the effects of opiates. The blockade would hinder the post-addict from experiencing euphoria in case of relapse [5, 9, 20, 21, 31]

Animals trained to discriminate the effects of opiate agonists and saline respond as if not being drugged when tested with combinations of naloxone or naltrexone and the opiates [4, 14, 16, 17, 18, 26, 27, 28, 29, 30, 33] That is animals perform the response associated with the saline condition and it is concluded that the antagonists blocked the stimulus or cue effects of the opiates. However, this does not necessarily mean that the effects of the drug combination is equal to the nondrug condition (saline) but only that the drug combination is not perceived as similar to the drugged (opiate) training condition.

It is generally found that animals trained to discriminate between a certain psychopharmacological compound and the vehicle condition will emit nondrug associated responses when tested by substitution with certain drugs of dissimilar pharmacological character This occurs in spite of the fact that the drugs used for the substitution tests may well serve a discriminative function when used as training drugs [6]. Thus in the usual drug discrimination procedure only the drug condition is defined. Therefore in the present study we examined whether or not drug discriminative control would be evident when gerbils explicitly were trained to discriminate a mixture of naltrexone and morphine from the vehicle condition. Such an approach may be useful for determining whether the combined effects of two drugs results in a condition similar to that produced by the vehicle An alternative conclusion is that the drug combination induced a new, "third" condition which is separable from the vehicle condition

METHOD

Animals

Our animals were adult male (60–80 g) mongolian gerbils (Meriones unguiculatus) which were maintained in pairs in

[1]A portion of the results was presented at the "Fifth Scandinavian Meeting on Physiology and Behavior," May 20–22, 1977, Helsinki, Finland

TABLE 1

SCHEMATIC PRESENTATION OF THE EXPERIMENTAL PLAN

Group	Symbol	Discrimination	Session-Treatments Before	After
1	●	Morph vs Saline n=20	a) Morph b) Saline	a) — b) Morph + Nax
2	◙	Morph vs Morph + Nax n=8	a) Morph b) Morph + Nax	a) — b) Saline
3	▲	Morph vs Nax n=8	a) Morph b) Nax	a) — b) Saline
4	✪	Morph + Nax vs Saline n=8	a) Morph + Nax b) Saline	a) — b) Morph
5	✲	Nax vs Saline n=4	a) Nax b) Saline	a) — b) Morph

Experimental plan showing the various groups and the corresponding discriminative conditions The treatments given IP (4 ml/kg) 60 min before sessions constituted the discriminative conditions for the T-maze training To equalize the amount drug given the various groups received an additional injection after the training Drug combinations were mixed shortly prior to use and were given in a single injection (4 ml/kg IP) Stock solutions of the respective drugs were not older than 48 hr Doses refer to the forms indicated i e , Morph = morphine HCl (12 mg/kg) and Nax = naltrexone HCl (2 mg/kg) Symbols are those used in Figs 1 and 2

macrolone cages under standard laboratory conditions (12 hrs light/dark cycle, temperature 20–22°C, humidity 50%) The gerbils had free access to pelleted food and tap water and once a week cabbage, hemp- and sunflower seeds were supplied.

Apparatus and Procedure

The apparatus (electrified T-shaped maze) and procedure have been described elsewhere in more detail [15] In brief, the animals had to escape aversive stimulation by running to the "correct" side alley in a T-shaped maze. Training sessions started 60 min after injection when the animal was dropped on the activated grid flood in the center alley of the maze The gerbil had to run in the maze until it reached the appropriate side alley. Escape through the left alley of the maze was required for 50% of the animals in a group when trained under one drug condition whereas the opposite alley was correct when trained under the other condition The reversed order was required for the remaining gerbils On a given training session escaping through the inappropriate alley was prevented by a barrier not visible to the animal when making the choice (left or right turn) The discriminative conditions (cf Table 1) usually alternated on successive sessions as did the required choice Gerbils were trained 1 session of 5 trials per day for 4 days a week (Wednesdays and weekends were drug free) On a given training session the exit was found on the same side of the maze at all trials Thus escaping the maze was contingent upon the prevailing training condition

Test sessions for assessing dose-generalization gradients of morphine in groups 1, 2 and 3 (cf Fig 2, see below) were similar to the regular training except that only 1 trial (instead of 5) was run and that escape was possible through either of the side arms of the maze. Two training sessions preceded a test Two drug-free days intervened between the 1st and 2nd test period Test trials were scheduled according to a cross-over design

RESULTS AND DISCUSSION

The results from the discriminative training are illustrated in Fig. 1 This figure shows that animals trained with morphine only as one of the two conditions learned and maintained the T-maze task during the 52 sessions allotted The performance of these 3 groups never fell below 80% correct first-trial choices after session block 5 The average number of sessions (± S E.M) until beginning a criterion of performing 8 correct first-trial choices in 10 consecutive training sessions were group 1. 8.8 (2.7), group 2· 7 8(1 6), and group 3 7.0(1 7). One gerbil in group 1 never met the criterion and was assigned a score of 52 One-way analysis of variance (ANOVA) suggested no significant effects ($p<0$ 05) meaning that the rapidity by which these 3 groups evidenced drug discriminative control was similar None of the gerbils in the two other groups met this criterion during the entire period of 60 training sessions Probably a more sensitive measure of differential responding because of less variability is the quotient between the number of correct versus the total number of first-trial choices The mean quotients (± S E M.) for the

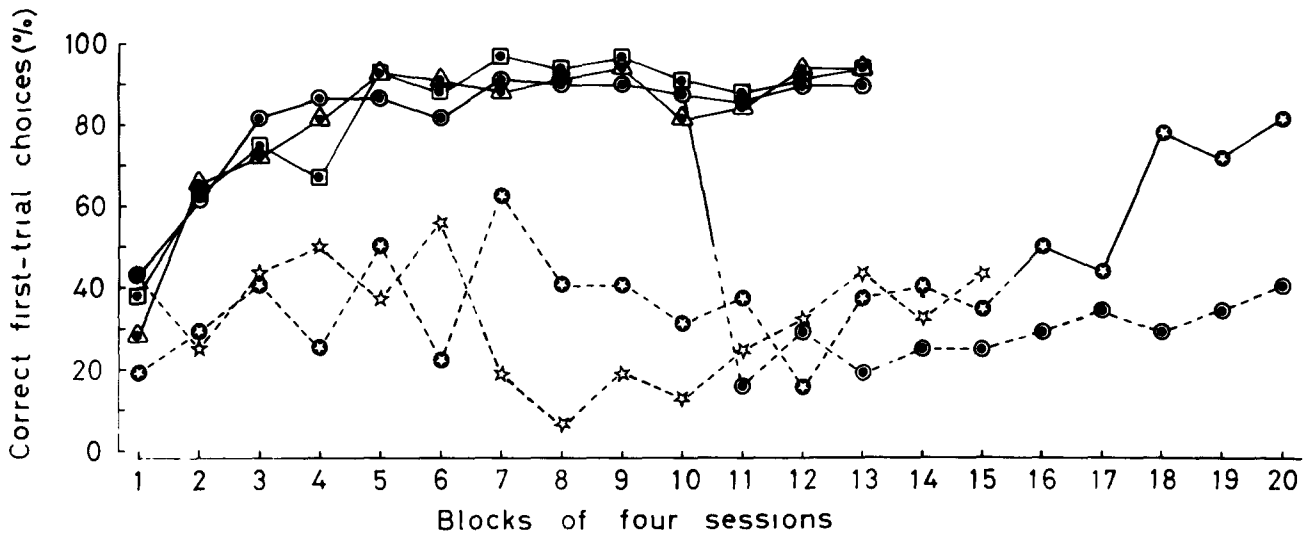

FIG 1 Acquisition of drug discriminations based upon the effects of morphine and/or naltrexone Ordinate, percent correct first-trial choices, abscissa, blocks of training sessions, each block composed of 2 sessions under each training condition Details about the various groups and treatments are found in Table 1 Eight gerbils from group 1 (morphine vs saline) were trained with morphine plus naltrexone rather than morphine singly after session block 10, and for group 4 (morphine plus naltrexone vs saline) naltrexone was withdrawn after session block 15 and training continued with morphine only for the next 20 sessions Injections were given IP 60 min prior to the sessions

initial 40 sessions were group 1 0 80(0 03), group 2. 0 80(0 02), group 3 0 78(0 04), group 4. 0 36(0 03), and group 5 0 32(0 04) ANOVA suggested that groups 4 and 5 did not differ significantly ($p>0$ 05) and that also this measure failed to separate groups 1, 2, and 3 ($p>0.05$) For these analyses group 1 was split into two groups (n = 8 and n = 12) ANOVA was also applied to the scores for all the 52 (all groups) and 60 (groups 4 and 5) training sessions and the results were similar to those based on the initial 40 training sessions

That the discriminative properties of the combination of morphine plus naltrexone are weak is further indicated by the performance of the gerbils that originally had learned the discrimination between morphine and saline but after session 40 were trained with naltrexone plus morphine versus the saline condition (cf Fig. 1). The animals did not regain a drug discrimination during 40 sessions, a period during which all these gerbils originally had met the criterion performance This portion of the study was run with the expectation that animals acquainted with drug discriminative responding would be more sensitive to the discriminative effects of drugs and hence be able to use drug effects of less magnitude as discriminative events. Such an increased sensitivity to drug discriminative effects has been described previously for rats trained with LSD [8] When naltrexone was withdrawn in group 4 after session 60 and training continued with morphine only there was a significant increase in the number of correct first-trial choices; 5 gerbils now reached the aforementioned criterion performance with the 20 sessions of training Therefore the lack of discrimination for the group retrained with the combination of morphine plus naltrexone (8 gerbils from group 1) cannot simply be attributed to possible development of tolerance to the stimulus effects of morphine Furthermore when groups 1, 2 and 3 were tested with novel doses of morphine (3 and 6 mg/kg) there were no marked differences in the resulting

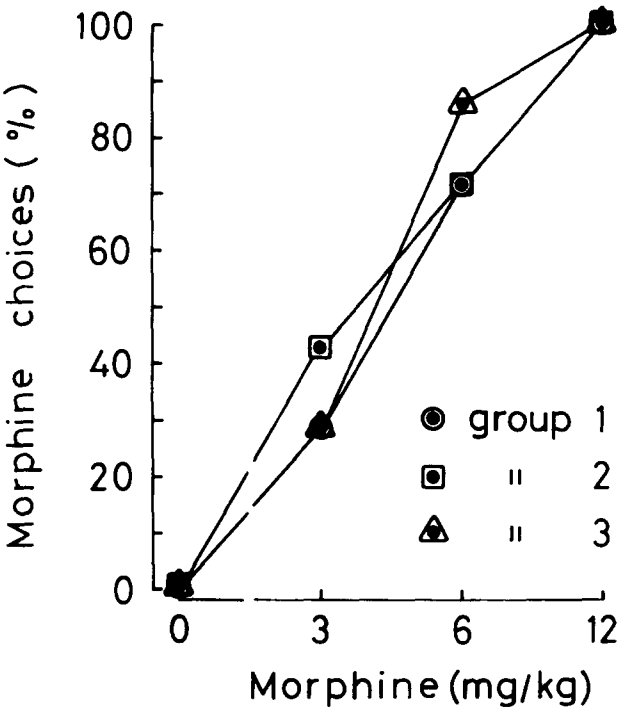

FIG 2 Dose-generalization gradients for morphine Ordinate, precent choices into morphine "correct" arm of the maze, abscissa, doses of morphine Data points for novel, morphine doses (3 and 6 mg/kg) are based on 7 observations each while the 2 other data points represent average percentage first-trial choices into morphine-associated side of the maze during 4 training sessions (14 observations for each training condition) The number of gerbils per group=7 The results from 1 gerbil in each group were excluded because of incorrect first-trial choices during training The training conditions are described in Table 1

dose-effect lines (cf. Fig 2). The calculated ED_{50} values, i.e., the dose yielding 50% drug responses, were similar (4.4, 3.7, and 4.0 mg/kg respectively) and potency ratio determinations suggested no significant differences ($p>0.05$) for the dose-effect lines [19].

Taken together the data suggest that a nondiscriminable dose of naltrexone not only blocks the stimulus properties of morphine acutely but also hinders a drug discrimination with a discriminable dose of morphine to develop during a long period of training. Thus the combination of naltrexone plus morphine induces effects more similar to a nondrug condition and therefore is not easily discriminable from saline under the present experimental conditions.

Apart from the obvious possibility of studying discriminative effects during long-term treatment of opiate agonists and antagonists, the experimental protocol described should be a useful complement also in other areas of research For example Meyer and colleagues [23] found that rats with an extended history of 'voluntary' drinking of opioid (etonitazene) solutions continued to drink significant amounts of these solutions in spite of being pretreated with naloxone. The present results would suggest that the most likely explanation relates to secondary reinforcement properties of stimuli present during the acquisition of the etonitazene preferance (i e., the pairing of the taste of the drug solution and alleviation of possible drug-withdrawal effects) rather than state-dependency effects or lack of blockade by naloxone [23]

ACKNOWLEDGEMENTS

We thank G Ohlin for expert technical assistance and I Dureman, G Krook, L Terenius and B Åman for their help with the drugs used and J D Leander and D E McMillan for helpful comments on an earlier draft of this manuscript Thanks are also due to P Hansen and U Wallin for typing the manuscripts Naltrexone was generously supplied by Endo Labs (New York) This study was supported by grants from the Swedish Council for Social Science Research (Dnr 251/77) and the Faculty of Social Sciences, University of Uppsala

REFERENCES

1 Barry, H , III Classification of drugs according to their discriminable effects in rats Fedn Proc **33**: 1814–1824, 1974
2 Colpaert, F C Narcotic cue and narcotic state *Life Sci* **20**: 1097–1108, 1977
3 Colpaert, F C , C J E Niemegeers and P A J Janssen Theoretical and methodological considerations on drug discrimination learning. *Psychopharmacologia* **46**: 169–177, 1976
4 Colpaert, F C , C J E. Niemegeers and P A J Janssen Fentanyl and apomorphine asymmetrical generalization of discriminative stimulus properties *Neuropharmacology* **15**: 541–545, 1976
5 Fink, M Narcotic antagonists in opiate dependence *Science* **169**: 1005–1006, 1970
6 Frey, L -R G and J C Winter Current trends in the study of drugs as discriminative stimuli In *Drug Discrimination and State Dependent Learning*, edited by D Chute, B T Ho, and D W Richards New York Academic Press, 1978
7 Gotestam, K and L -M Gunne Subjective effects of two anorexigenic agents, fenfluramine and AN448, in amphetamine-dependent subjects *Br J Addict* **67**: 39–44, 1972
8 Greenberg, I , D M Kuhn and J B Appel Behaviorally induced sensitivity to the discriminable properties of LSD *Psychopharmacologia* **43**: 229–232, 1975
9 Gritz, E R , S. M Shiffman, M E Jarvik, J Schesinger and V C Charuvastra Naltrexone physiological and psychological effects of single doses *Clin Pharmac Ther* **19**: 773–776, 1976
10 Haertzen, C A Subjective effects of narcotic antagonists In *Narcotic Antagonists*, edited by M C Braude, L S Harris, E L May, J P Smith and J E Villarreal New York Raven Press, 1974, pp 383–398
11 Harris, R T and R L Balster An analysis of psychological dependence In *Advances in Mental Science II Drug Dependence*, edited by R T Harris, W N McIsaac and C R. Shuster, Jr Austin Univ Texas Press, 1970, pp 214–226
12 Harris, R T and R L Balster An analysis of the function of drugs in the stimulus control of operant behavior In *Stimulus Properties of Drugs*, edited by T Thompson and R Pickens New York Appleton-Century-Crofts, 1972, pp 111–132
13 Hill, H E , C A Haertzen, A B Wolbach and E J Miner The Addiction Research Center Inventory Standardization of scales which evaluate subjective effects of morphine, amphetamine, pentobarbital, alcohol, LSD-25, pyrahexyl and chlorpromazine *Psychopharmacologia* **4**: 167–183, 1963
14 Hirschhorn, I D and J A Rosecrans A comparison of the stimulus effects of morphine and lysergic acid diethylamide (LSD) *Pharmac Biochem Behav* **2**: 361–366, 1974
15 Jarbe, T U C Methaqualone-discrimination in gerbils. interactions with bemegride and imidazoline (DH-524) *Med Biol* **54**: 364–368, 1976
16 Jarbe, T U C Discriminative effects of morphine in the pigeon, Pharmac Biochem Behav **9**: 411–416, 1978
17 Jarbe, T U C and C Rollenhagen Morphine as a discriminative cue in gerbils drug generalization and antagonism *Psychopharmacology* **58**: 271–275, 1978
18 Lal, H , G Gianutsos and S Miksic Discriminable stimuli produced by analgesics In *Discriminative Stimulus Properties of Drugs*, edited by H Lal New York Plenum Press, 1977, pp 23–45
19 Litchfield, J T and F Wilcoxon A simplified method of evaluating dose-effect experiments *J Pharmac exp Ther* **96**: 99–113, 1949
20 Martin, W. R The basis and possible utility of the use of opiate antagonists in the ambulatory treatment of the addict *J Nerv Ment Dis* **46**: 367–377, 1968
21 Martin, W R Realistic goals for antagonist therapy *Am J Drug Alcohol Abuse* **2**: 353–356, 1975
22 McClace, T K and W R Martin Subjective and physiologic effects of morphine, pentobarbital, and meprobamate *Clin Pharmac Ther* **20**: 192–198, 1976
23 Meyer, R K , R Marcus, G Carnathan and J Cochin Narcotic blockade, length of addiction, and persistence of etonitazene consumption in rats *Psychopharmacology* **47**: 273–279, 1976
24 Overton, D A Discriminative control of behavior by drug states In *Stimulus Properties of Drugs*, edited by T Thompson and R Pickens New York Appleton-Century-Crofts, 1972, pp 87–110
25 Overton, D A and S K Batta Relationship between abuse liability of drugs and their degree of discriminability in the rat In *Predicting Dependence Liability of Stimulant and Depressant Drugs*, edited by T Thompson and K R Unna Baltimore-London-Tokyo University Park Press, 1977, pp 125–135
26 Rosecrans, J A , M H Goodloe, G J Bennett and I D Hirschhorn Morphine as a discriminative cue effects of amine depletors and naloxone *Eur J Pharmac* **21**: 252–256, 1973
27 Schaefer, G J and S G Holtzman Discriminative effects of morphine in the squirrel monkey *J Pharmac exp Ther* **201**: 67–75, 1977

28 Shannon, H E and S G Holtzman Evaluation of the discriminative effects of morphine in the rat *J Pharmac exp Ther* **198:** 54–65, 1976a
29 Shannon, H E and S G Holtzman. Blockade of the discriminative effects of morphine in the rat by naltrexone and naloxone *Psychopharmacology* **50:** 119–124, 1976b
30 Shannon, H E and S G Holtzman Further evaluation of the discriminative effects of morphine in the rat *J Pharmac exp Ther* **201:** 55–66, 1977
31 Wikler, A Opioid antagonists and deconditioning in addiction treatment In *Drug Dependence Treatment and Treatment Evaluation,* edited by H Bostrom, T Larsson and N Ljungstedt Stockholm Almgvist and Wiksell International, 1975, pp 157–184
32 Winter, J C The hallucinogens as discriminative stimuli *Fedn Proc* **33:** 1825–1832, 1974
33 Winter, J C The stimulus properties of morphine and ethanol *Psychopharmacologia* **44:** 209–214, 1975