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Antagonism of Morphine-Induced Aversive Conditioning by Naloxone

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LEBLANC, A. E. AND H. CAPPELL. *Antagonism of morphine-induced aversive conditioning by naloxone.* PHARMAC. BIOCHEM. BEHAV. 3(2) 185–188, 1975. – In Experiment 1, 4 doses of morphine and 4 doses of naloxone were tested for their ability to induce a conditioned aversion to saccharin in rats. Morphine was much more potent than naloxone which had only weak effects at the highest dose (12.96 mg/kg). Based on the determinations of Experiment 1, doses of 0.096, 0.96, and 9.6 mg/kg were tested for their ability to antagonize conditioned aversions by 20 mg/kg of morphine in a second experiment. The highest dose of naloxone was an effective antagonist of morphine-induced aversion. The antagonism was incomplete, but this may have reflected the particular dose combinations that were employed. Although 12.96 mg/kg of naloxone induced only a weak conditioned aversion to saccharin in Experiment 1, 9.6 mg/kg had a substantial effect in Experiment 2. Thus naloxone was itself an agent of aversive conditioning at a dose which significantly antagonized the aversive effects of morphine. Because of the successful demonstration of antagonism, it was suggested that there may be common pharmacological mechanisms involved in both positive reinforcement and aversive conditioning by drugs of the opiate class.

Antagonism Morphine Naloxone Reinforcement Taste aversion

THERE have been numerous demonstrations that psychoactive drugs differing widely in pharmacological action may act as agents of gustatory avoidance conditioning [1–3]. Building upon these basic empirical findings, some investigators have applied the gustatory conditioning model to the study of theoretically significant issues in the study of drug dependence [12,14]. A particularly important application of the model has been in studies of the blockade of drug effects by chemical means. (The word blockade is used generically to signify interference with the action of one chemical by another. In the case of an antagonist such as naloxone, competition for receptor sites is involved with the net result that some actions of an opiate can be blocked; alpha-methyltyrosine functions by depletion of catecholamines, a qualitatively different mechanism, but the net result is nonetheless a blockade of some actions of amphetamine.) Here the results from operant studies of self-administration conflict with those involving gustatory conditioning. In the former case, pretreatment with alpha-methyltyrosine (AMT) blocked positive reinforcement by both morphine [6] and amphetamine [7] in rats. AMT is also known to attenuate the subjective experience of euphoria produced by amphetamine in man [10]. In contrast, AMT was ineffective in blocking conditioned aversion to saccharin by either morphine [5] or amphetamine [4]. This discrepancy could indicate that the pharmacological mechanism for positive reinforcement by these drugs differs substantially from those features of their pharmacological action that are punishing. If this is so, one would have to be even more than normally conservative drawing general inferences about the mechanisms underlying drug-directed behavior from studies of gustatory conditioning.

Naloxone is a drug that is known to antagonize a variety of behavioral effects produced by opiates [13,15]. Of course, the major interest in naloxone stems from its property as an antagonist to the positively reinforcing consequences of opiates. In man, this property has been clearly confirmed [8,9]. Naloxone has been shown to antagonize the action of codeine in monkeys in that stable responding for codeine ceased after pretreatment with naloxone [16]. However, as the authors observed, the cessation of responding could reflect either antagonism of the reinforcing effect of naloxone or an aversive consequence of the naloxone-codeine combination. Thus, direct antagonism of positive reinforcement has not been unequivocally demonstrated using an animal model of selfadministration of opiates.

In summary, the results of studies of AMT yielded conflicting results when gustatory conditioning and operant

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selfadministration models were compared; blockade has only been demonstrated where selfadministration is concerned. This discrepancy could indicate that different pharmacological mechanisms are involved in positive reinforcement and punishment by amphetamine and morphine. If analogous results were to be obtained with naloxone, the general argument for different mechanisms would be much stronger. On the other hand, antagonism of the punishing effect of morphine by naloxone would be evidence of commonality in the drug actions responsible for punishment and positive reinforcement.

EXPERIMENT 1: DOSE-RESPONSE DETERMINATIONS

Before determining whether naloxone can antagonize the punishing effect of morphine, it was first necessary to assess the punishing effect of naloxone alone. Although naloxone is generally believed to be largely free of agonistic actions, it is clearly not devoid of behavioral consequences [13]. If naloxone at effectively antagonistic dose levels could be shown to have punishing effects of its own, this might compromise an attempt to demonstrate its ability to antagonize punishment by morphine.

METHOD

Animals

Ninety male Wistar rats, each weighing approximately 300 g at the beginning of the experiment, were used. All were housed individually with free access to food and water prior to the introduction of experimental treatments.

Procedure

Adaptation to restricted drinking. For 6 days prior to conditioning trials each animal was maintained on a schedule of limited access to water. At the same time each day, access to water was permitted for a 15 min period in a test cage equipped with a 100 ml drinking tube. Food was continuously available in the home cages, to which animals were returned immediately after drinking trials.

Conditioning trials. Conditioning trials began 24 hr following the sixth day of adapation to the restricted schedule of drinking. For 15 min each animal was permitted access to a 0.1% solution of sodium saccharin in the test cage. Five min later, equal numbers of animals (n = 10 per group, randomly assigned) received intraperitoneal injections as follows: morphine sulphate, 1.0 (M_1), 3.0 (M_2), 9.0 (M_3), or 27.0 mg/kg (M_4); naloxone hydrochloride, 0.48 (N_1), 1.44 (N_2), 4.32 (N_3), or 12.96 mg/kg (N_4). Concentrations were adjusted to provide an injection volume of 1 ml/100 g. The doses of morphine and naloxone were selected such that their molar concentrations were equivalent at corresponding points in the range of 4 doses. Each drug solution was prepared in a vehicle of physiological saline. A control group (S) received injections of the vehicle only after drinking saccharin. There were 6 conditioning trials spaced at intervals of 72 hr. Each animal received the same drug treatment on each trial. On the 2 days intervening between conditioning trials, the animals continued to have access to water in the test cage for a 15 min period.

RESULTS AND DISCUSSION

The dose-response curves for the 6 conditioning trials are illustrated in Fig. 1. Because of the great degree of overlap among data points, the results for morphine and naloxone are displayed in separate panels. A single analysis of variance, however, incorporated the data from all 9 of the groups. There was a significant effect of treatments, $F(8,81) = 17.99$, $p<0.001$, trials, $F(5,405) = 21.65$, $p<0.001$, and a significant treatments x trials interaction, $F(40,405) = 9.95$, $p<0.001$. For a more detailed analysis of the data, individual comparisons were made among means [11]. A criterion of $p<0.01$ was required for rejection of the null hypothesis. Although saccharin consumption was somewhat lower among rats that received naloxone rather than saline following saccharin, only one of all possible comparisons resulted in a significance difference. On the sixth trial at the highest dose of naloxone, the animals consumed significantly less saccharin than saline controls. In the case of morphine, doses of 9.0 and 27.0 mg/kg had a significant punishing effect from the third trial onward in comparison to saline controls. When contrasted to its molar equivalent of naloxone (4.32 mg/kg), a dose of 9.0 mg/kg produced significant punishment from the third trial onward. A similar difference obtained comparing the highest dose of naloxone to the highest dose of morphine. To summarize the major outcomes of Experiment 1: (1) Naloxone was relatively devoid of punishing effects in the dose range employed. (2) Morphine was punishing at the two highest dose levels. (3) At equivalent molar concentrations, morphine was a more potent punishing agent than naloxone.

EXPERIMENT 2: ANTAGONISM OF THE PUNISHING EFFECT OF MORPHINE BY NALOXONE

The dose-response data of Experiment 1 provided a rationale for selecting doses of naloxone in order to examine its properties as an antagonist in the gustatory conditioning paradigm. A range was selected such that the highest dose of naloxone was somewhat below the highest dose used in Experiment 1. This minimized the likelihood that unintentional punishment by naloxone would be confounded with the punishing effects of morphine. Each dose of naloxone was tested for its ability to antagonize punishment by a single dose of morphine. The latter dose was selected to be one which would certainly be punishing to rats not pretreated with naloxone.

METHOD

Animals

Ninety-six male Wistar rats, each weighing approximately 300 g at the beginning of the experiment, were used. All were housed individually with free access to food and water prior to the introduction of experimental treatments.

Procedure

Adaptation to restricted drinking. The adaptation schedule was identical to that used in Experiment 1.

Test for antagonism. The test for antagonism was introduced 24 hr following the last adaptation trial. Equal numbers of rats (n = 12 per group) were randomly assigned to 1 of 8 experimental conditions. As in Experiment 1, a conditioning trial involved exposure to a 0.1% solution of saccharin for 15 min. Five min after exposure to saccharin

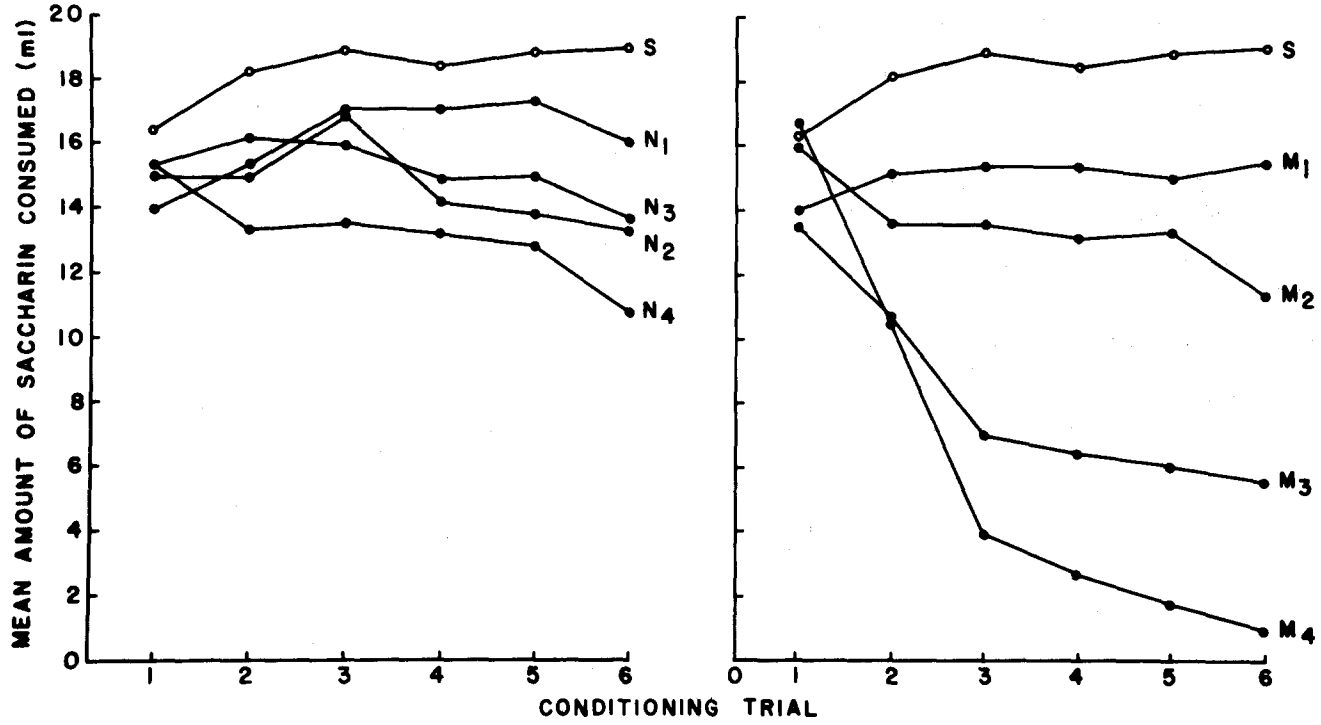

FIG. 1. Dose-response curves of conditioned aversion by naloxone and morphine (S = saline, N = naloxone, M = morphine). Subscripts refer to drug doses, which are given in the Methods section of Experiment 1. To avoid confusion in the figure, standard errors have been omitted. Tables giving the means and standard errors for all data points are available from the second author.

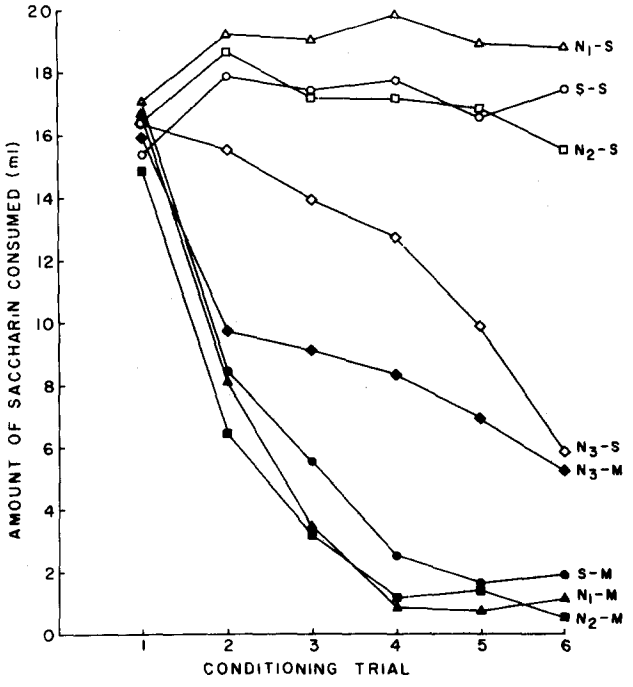

FIG. 2. Effects of various doses of naloxone on conditioned aversion by 20 mg/kg of morphine (S = saline, N = naloxone, M = morphine). Subscripts refer to drug doses, which are given in the Methods section of Experiment 2. To avoid confusion in the figure, standard errors have been omitted. Tables giving the means and standard errors for all data points are available from the second author.

each rat received 2 intraperitoneal injections in rapid succession. There were 4 levels of treatment with naloxone: saline control (S), 0.096 mg/kg (N_1), 0.96 mg/kg (N_2) or 9.6 mg/kg (N_3). Within each of these treatment levels, equal numbers of rats received an injection of saline (S) or 20 mg/kg of morphine (M). Injections were administered on opposite sides of the peritoneal cavity. All drug solutions were prepared in physiological saline with concentrations adjusted to deliver a fluid volume of 1 ml/100 g for each injection. There were 6 conditoning trials in all, spaced at 72 hr intervals. An animal received the same combination of injections throughout the experiment. Injections were dispensed with on the final conditioning trial. On the days intervening conditioning trials, the animals were maintained on the schedule of restricted access to water.

RESULTS AND DISCUSSION

The mean quantities of saccharin consumed are illustrated in Fig. 2. An analysis of variance revealed that all main effects and interactions, with the exception of a nonsignificant main effect of the antagonist, attained significance at the 0.005 level or beyond. Subsequent to the analysis of variance, significant treatment effects of interest were analysed in detail by individual comparisons of trial means [11], using a criterion of $p<0.01$ for rejection of the null hypothesis. Several effects were apparent. First, the highest dose of naloxone had a significant punishing effect, as the animals receiving this dose in combination with saline (N_3S) drank significantly less saccharin than controls receiving only saline (SS) from the third trial onward. At the lower doses (Groups N_1S and N_2S), naloxone had no

punishing effect. Thus in comparison to Experiment 1, naloxone had a more pronounced punishing effect, even at a somewhat lower dose. Since the procedures applied to the comparable groups in the two experiments were so similar, the discrepancy probably reflects interexperimental variability.

Despite the fact that the naloxone dose had a more substantial punishing effect than expected, a test of antagonism was not obviated, since controls were provided. The appropriate test of antagonism involved contrasts of the saccharin consumption of rats receiving only morphine after saccharin (SM) to those that received combinations of naloxone and morphine (N_1M, N_2M, and N_3M). At the two lowest doses of naloxone, there was no evidence of antagonism. However, at the highest dose (Group N_3M), the saccharin consumption of naloxone-treated animals significantly exceeded that of controls on Trials 3, 4, and 5. The difference on Trial 6 dropped to a marginal level of significance ($p<0.05$). The diminution of the difference by the sixth trial is not surprising since the cumulative punishing effect of morphine was not totally antagonized by naloxone; this was indicated by the fact that on Trials 3, 4, and 5, rats that received a combination of the highest dose of naloxone and morphine (N_3M), drank significantly less saccharin ($p<0.01$) than those that received only the naloxone (N_3S). The saccharin consumption of Group N_3M was thus subject to three major influences: the punishing effect of naloxone alone, the punishing effect of morphine alone, and the incomplete antagonism of morphine's effect by naloxone.

GENERAL DISCUSSION

Although its effect was somewhat complicated by its own punishing action, naloxone clearly antagonized the punishing consequence of morphine to a significant extent. The antagonism was not complete; however, it can be reasonably assumed that different combinations of doses of naloxone and morphine would have yielded different orders of magnitude of antagonism. Thus while parametric questions remain unanswered, there can be little doubt that the gustatory conditioning paradigm is not one in which antagonism is intrinsically impossible to demonstrate. Clearly, the behavioral mechanisms involved in demonstrating punishment and positive reinforcement by morphine are quite different; however, the comparable response of both behavioral mechanisms to antagonism by naloxone enhances the likelihood of common pharmacological mechanisms in punishment and positive reinforcement. The discrepancy in the blocking actions of AMT in the conditioned aversion and positive reinforcement paradigms may reflect something peculiar about this drug rather than a general discrepancy in the pharmacological mechanisms underlying punishment and positive reinforcement by psychoactive drugs. Further elaboration of the reasons for this discrepancy and its generality across drugs is obviously required. In the interim, it seems safe to conclude that studies of conditioned aversion by drugs may contribute to an elucidation of the operation of pharmacological mechanisms in maintaining drug-directed behavior.

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