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BRIEF COMMUNICATION

Antagonism of Drug Discrimination Learning Within the Conditioned Taste Aversion Procedure

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STEVENSON, G. W., S. POURNAGHASH AND A. L. RILEY. *Antagonism of drug discrimination learning within the conditioned taste aversion procedure.* PHARMACOL BIOCHEM BEHAV **41**(1) 245–249, 1992.—Animals injected with morphine prior to the presentation of a saccharin-LiCl pairing and the morphine vehicle prior to saccharin alone rapidly acquired the drug discrimination, avoiding saccharin following the administration of morphine and consuming saccharin following its vehicle after only four conditioning trials. Once stimulus control was established, the opiate antagonist naloxone (1 mg/kg) was administered prior to morphine in a test of its ability to antagonize the morphine stimulus. Pretreatment times ranged from 10 to 180 min. Naloxone antagonized the stimulus properties of morphine for all subjects, although there were individual differences in the onset, duration (time course) and degree of antagonism. Together with the rapid acquisition typically reported in this design, the fact that antagonism was demonstrated in the present study suggests that the conditioned taste aversion procedure may be useful in the general assessment of drug discriminations.

Conditioned taste aversion Drug discrimination learning Morphine Naloxone Antagonism

RECENTLY, our lab and others have reported the rapid acquisition of drug discrimination learning within the conditioned taste aversion procedure (8, 13, 17, 21–24, 29, 30). For example, Mastropaolo et al. (23) reported that rats injected with 1.8 mg/kg phencyclidine (PCP) 10 minutes prior to a saccharin-LiCl pairing and the PCP vehicle prior to a nonpoisoned exposure to the same saccharin solution rapidly acquired the drug discrimination (after only three PCP-saccharin-LiCl pairings), avoiding saccharin consumption when it was preceded by an injection of PCP and consuming the same saccharin solution when it was preceded by the PCP vehicle. Similar rapid acquisition has been reported with alprazolam (8), fentanyl (13), morphine (22), naloxone (17), pentobarbital (29), and a range of serotonergic agonists (21).

Although this rapid acquisition within the taste aversion design suggests that this procedure may be useful in the general assessment of drug discrimination learning, there are a number of issues yet to be assessed. For example, to date there are no published accounts of antagonism within this baseline. As noted by Overton [(27); see also (15)], the more traditional drug discrimination procedure provides for an assessment of pharmacological antagonism. For example, once discriminative control has been established to a specific drug, putative antagonists can be given concurrent with the training drug to determine if the stimulus properties of the training drug can be blocked [see (3, 4, 7)]. Not only can putative antagonists be identified, but antagonists with known receptor activity can be used within this design to determine which specific receptors underlie the stimulus properties of a specific training drug (10).

The ability to utilize pharmacological antagonism within the drug discrimination design makes the basic procedure a useful tool, both for general pharmacological issues, e.g., identification of antagonists, and for specific issues related to drug discrimination learning, e.g., the receptor mediation of the cueing effect. To demonstrate this ability in the taste aversion baseline of drug discrimination learning, the present study investigated antagonism of stimulus control within this procedure using a drug combination for which antagonism is well characterized, i.e., morphine and naloxone [see (1, 12, 14, 19, 31, 34)]. Specifically, rats were trained to discriminate between the presence and absence of morphine. Once the discrimination was acquired and stable, the opiate antagonist naloxone was administered 10, 30, 60 and 180 min prior to morphine.

METHOD

Subjects and Apparatus

The subjects were 21 experimentally naive, Long-Evans female rats approximately 120 days of age at the beginning of the experiment. The subjects were housed in individual wire-mesh cages and were maintained on a 12-h light/12-h dark cycle and at an ambient temperature of 23°C for the duration of the experiment.

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Drugs

Morphine sulfate was generously supplied by NIDA. Naloxone hydrochloride was generously supplied by Du Pont Pharmaceuticals. Both drugs were dissolved in distilled water and injected intraperitoneally (IP) in a volume of 1.0 ml/kg. LiCl (Sigma Pharmaceuticals) was dissolved in distilled water and injected IP in a volume of 12.0 ml/kg.

Procedure

Phase I: Conditioning. Following water deprivation, all subjects were given 20-min access to water once a day for 45 consecutive days. Subjects were divided into two groups, matched on water consumption over Days 43–45. On Days 46–48 (saccharin habituation), a novel saccharin solution (0.1% w/v Sodium Saccharin, Fisher Purified) replaced water during the daily 20-min fluid-access period.

On Day 49, conditioning began. Subjects in Group L (n = 10) and Group W (n = 11) were injected with 5.6 mg/kg of morphine 15 min prior to 20-min access to saccharin. Immediately following saccharin access, subjects in Group L were injected with 1.8 mEq, 0.15 M LiCl (76.8 mg/kg), while subjects in Group W were given an equivolume injection of distilled water (i.e., the LiCl vehicle). On the following three recovery days, subjects in both groups were injected with distilled water 15 min prior to 20-min access to the same saccharin solution. No injections followed saccharin access on these days. This alternating procedure of conditioning and recovery was repeated until all animals received 15 complete cycles.

Phase II: Naloxone challenge. The procedure for this phase was identical to that of Phase I with the exception that on the second recovery day following each conditioning trial, animals were given an IP injection of 1 mg/kg of naloxone prior to the injection of morphine. The injection of naloxone was given either 10, 30, 60 or 180 min before morphine. Fifteen min following the injection of morphine, all subjects were given 20-min access to saccharin. No injections followed saccharin exposure on these test days. The pretreatment times were randomized across sessions. On a single test day, all subjects were given naloxone 10 min prior to an injection of distilled water and then 15 min later given access to saccharin. This procedure was run to test for the unconditioned effects of naloxone alone on saccharin consumption.

RESULTS

Statistical Analysis

All determinations of statistical significance are based on a Kruskal-Wallis one-way analysis of variance test. Statements of significance for the Kruskal-Wallis (H) is based on $p<0.05$, one-tailed. A single subject in Group L (Subject 4) died during the acquisition of the discrimination (i.e., Phase I). All statistical analyses for Group L during this phase are based on n = 9.

Phase I: Conditioning

Figure 1 presents the mean absolute saccharin consumption for Groups L and W throughout the conditioning/recovery cycles during this phase. There were no significant differences in saccharin consumption between groups during saccharin habituation or over the first three conditioning trials [all H's(1)<0.124]. On the fourth conditioning trial, subjects in Group L drank significantly less than subjects in Group W [H(1) = 8.99]. This difference was maintained for the remainder of conditioning. On the final conditioning trial of this phase, subjects in Groups L and W drank 3.0 and 12.6 ml, respectively. During recovery sessions, consumption for both groups remained high, approximating habituation levels.

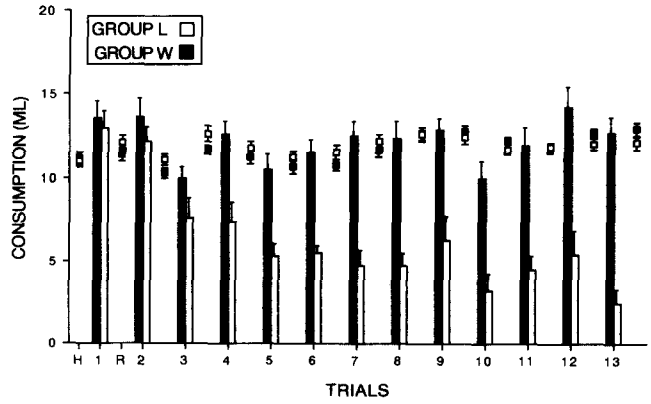

FIG. 1. The mean amount of saccharin consumed for subjects in Groups L and W over the repeated conditioning trials of Phase 1 (open and filled columns, respectively). The open and filled squares represent an average of saccharin consumption on the three days of saccharin habituation (H) and on the three recovery sessions (R) between each conditioning trial for subjects in Groups L and W, respectively. Bars represent S.E.M.

Phase II: Naloxone Challenge

Figure 2 presents the amount of saccharin consumed for individual subjects in Group L following the distilled water vehicle, the naloxone/distilled water combination, morphine and the naloxone/morphine combination at various delay periods between the naloxone and morphine injections (i.e., 10, 30, 60 and 180 min). For consumption at any specific temporal interval to be included in the figure for any specific animal, that animal had to have discriminative control by morphine immediately prior to the antagonism test. Discriminative control was defined as an experimental subject (i.e., a subject in Group L) consuming no more than *50%* of the *mean* consumption of subjects in the control group (i.e., Group W) on the conditioning trial immediately preceding that specific test of antagonism. Such a criterion ensured that any test of antagonism was based on stable discriminative control. As a result of this criterion, data are not presented for the 10-min delay condition for two subjects (Subjects 10 and 17). For a third subject (Subject 19), that did not display discriminative control at any point in the testing phase, data are not presented for any of the delay conditions. To assess the general effects of naloxone and morphine on saccharin consumption, the mean amount of saccharin consumed by the control subjects for each of the aforementioned injection conditions is also presented in Fig. 2 (see bottom right panel).

As illustrated, naloxone antagonized the discriminative control by morphine in all subjects, i.e., consumption following the naloxone/morphine injections was greater than that following the injection of morphine alone. Although all subjects displayed complete antagonism, the onset of this antagonism varied among subjects. For example, at the 10-min delay condition, Subjects 1, 2 and 11 consumed saccharin at levels similar to the amount consumed following the naloxone/distilled water injections. Subjects 5 and 12 displayed only partial antagonism at this delay,

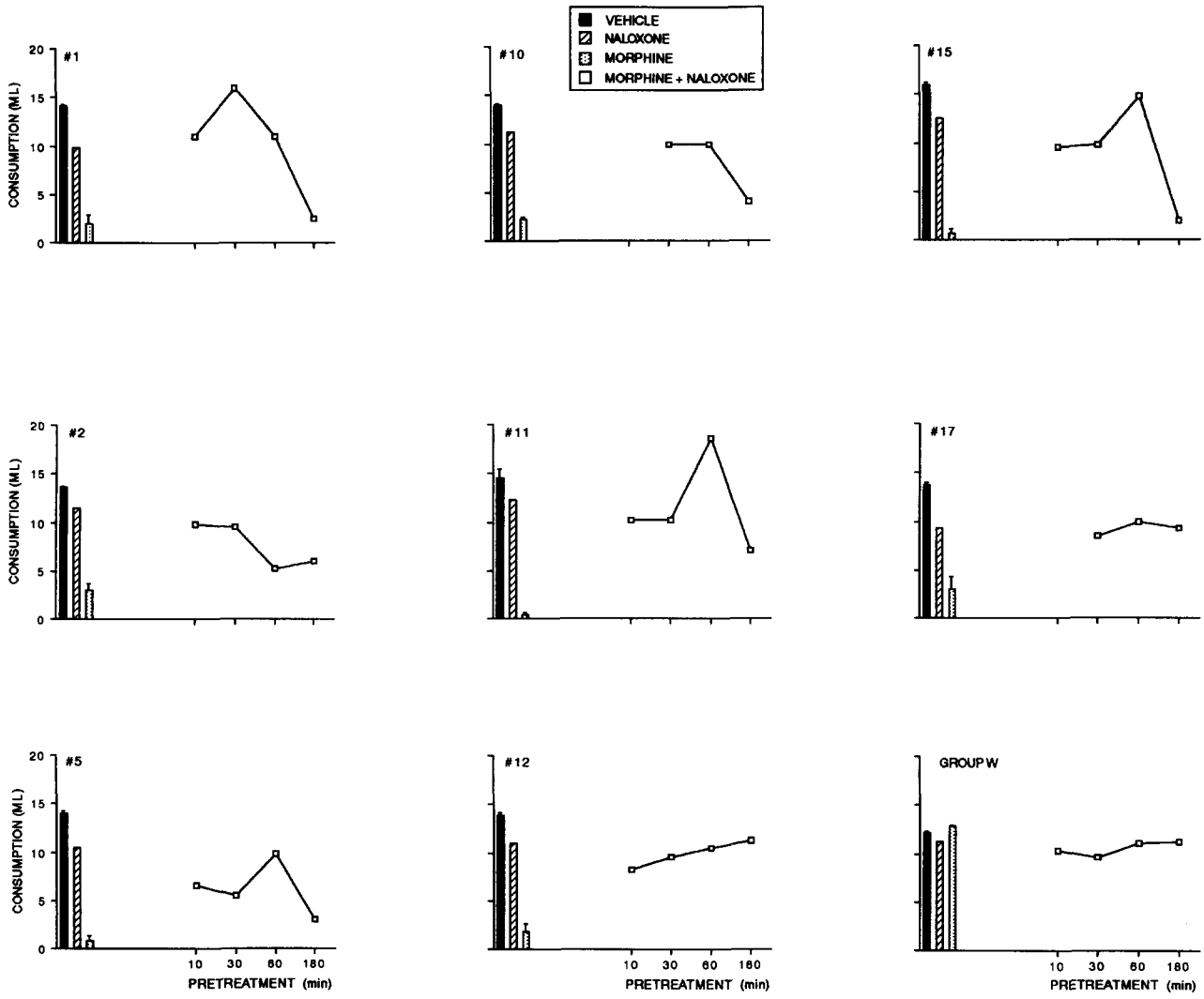

FIG. 2. The absolute amount of saccharin consumed for individual subjects in Group L following the distilled water vehicle (filled column), the naloxone/distilled water combination (hatched column), morphine (stippled column) and the naloxone/morphine combination (open squares) with naloxone pretreatment intervals of 10, 30, 60 and 180 min. Bars represent S.E.M. The mean amount consumed following these same injections for Group W is presented in the lower right panel.

i.e., consumption following the naloxone/morphine injections was intermediate to that following naloxone + distilled water and following morphine. Each of these subjects, however, displayed complete antagonism when naloxone was given 60 min prior to morphine. Subjects 10 and 17 drank at control levels at the 30-min delay condition. No data were available at the 10-min condition for these subjects.

The duration of the naloxone antagonism also varied among subjects. For example, when naloxone was administered 180 min prior to morphine, Subjects 1, 5 and 15 drank at levels similar to that following morphine alone, i.e., naloxone was without effect at this delay condition. Subjects 2, 10 and 11 displayed intermediate consumption at the 180-min delay. Finally, Subjects 12 and 17 drank at control levels when naloxone was given 180 min before morphine administration, i.e., complete antagonism. Control subjects displayed a high level of consumption under each of the injection conditions and at each delay condition in the naloxone/morphine combination.

DISCUSSION

The present study examined whether antagonism of a drug discriminative stimulus could be demonstrated within the conditioned taste aversion procedure. Specifically, following the establishment of a morphine/distilled water discrimination, animals were injected with the opiate antagonist naloxone in an assessment of its ability to block the morphine stimulus (1, 12, 14, 19, 31, 34). As reported, naloxone completely antagonized the morphine stimulus in this design. That naloxone antagonized the discriminative effects of morphine within this procedure is consistent with other work in drug discrimination learning assessing the interaction of these two compounds. For example, the discriminative effects of moderate doses of morphine (3.0–6.0 mg/kg) are antagonized by low doses of naloxone (0.03–1.0 mg/kg)

in rats, pigeons, and squirrel monkeys when assessed within more traditional operant procedures (12, 33, 34).

As noted, for all subjects naloxone completely antagonized the stimulus properties of morphine, although the point at which this effect was evident varied among the individual subjects. Interestingly, for several subjects consumption following the combined injections of naloxone and morphine was greater than that when naloxone alone was given prior to saccharin access. When such an increase was evident, it occurred when naloxone was administered either 30 or 60 min prior to morphine (see Subjects 1 and 11). One possible account for this increase in consumption above the naloxone baseline is that at the 10 and 30 min delays the adipsogenic effects of naloxone unconditionally suppressed consumption (2, 5, 6, 32). Although the discriminative effects of morphine may have been blocked by naloxone at these temporal intervals, consumption was still suppressed by the general effects of naloxone on fluid consumption (compare consumption following naloxone + distilled water with that following the distilled water injection alone, i.e., recovery). As the interval between the naloxone injection and saccharin access increased (to 30 and 60 min), these unconditioned effects weakened and the antagonism of the morphine stimulus appeared more pronounced. Although possible, this explanation remains speculation in that the only time naloxone was given alone was 10 min prior to saccharin access. That is, there was no assessment of the time course of the adipsogenic effects of naloxone in the present study.

The effects of naloxone were clearly reduced when it was administered 180 min prior to the morphine injection. At the 180-min condition, six of the eight subjects drank saccharin at levels approximating the amounts consumed following morphine alone. The remaining two subjects drank at control levels, i.e., there was no diminution of antagonism. This time course is similar to that previously reported in more traditional assessments of naloxone's effects on morphine's stimulus properties, i.e., strong antagonism within the first hour following naloxone administration with recovery of morphine stimulus control between 2 and 4 hours postnaloxone (1,34).

That opiate antagonism occurs within the taste aversion design is interesting in light of an earlier report from our lab utilizing the aversion procedure which failed to demonstrate such antagonism when ethanol was used as the training drug (18). Specifically, animals were trained to discriminate ethanol from distilled water in a procedure similar to that described in the present study. Once the discrimination was acquired, the benzodiazepine inverse agonist Ro 15-4513, a compound widely reported to antagonize a range of ethanol's effects [see (20,36)], was administered to subjects 15 min prior to the administration of ethanol in a test of its ability to block the ethanol cue. At no dose was the stimulus control of ethanol affected by Ro 15-4513. Given the current demonstration of the antagonism of stimulus control within the taste aversion procedure, this failure to antagonize the stimulus properties of ethanol by Ro 15-4513 is unlikely due to the insensitivity of the aversion design to antagonism. Instead, the absence of antagonism may be more a function of the failure of Ro 15-4513 to block the stimulus properties of ethanol [(11,12); though see (28)], a conclusion consistent with other recent reports on the failure of Ro 15-4513 to block specific effects of ethanol (9, 16, 25, 26).

Although antagonism can be demonstrated in the aversion design, it remains unknown if other effects of the opiate antagonists in more traditional assessments of drug discrimination learning can be reproduced within this procedure, e.g., are the effects of naloxone reversed with increases in the dose of morphine (i.e., competitive antagonism), does the antagonism vary with the dose of the antagonist (i.e., dose-response relationships), is the antagonism selective for the opiates and within the opiates is the antagonism dependent upon the specific opiate examined (i.e., class and receptor specificity). Further, because assessments of drug discrimination learning with taste aversions have been limited to demonstrations of stimulus control and the generalization of that control to other compounds, it is not known how findings generally reported in more traditional assessments of discrimination learning will compare to those produced in the aversion design (e.g., the range of stimuli that support drug discrimination learning, the degree of generalization) or if specific procedures (e.g., two-drug discriminations, conditional discriminations) and issues (e.g., can animals differentiate different compounds that act at different receptor subtypes) can be addressed within this baseline. The present demonstration of antagonism adds to the similarity of effects already established between the aversion design and more traditional assessments and suggests that the aversion procedure may be useful in the general assessment of drug discrimination learning.

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