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Reward and Reinforcement Produced by Drinking Water: Role of Opioids and Dopamine Receptor Subtypes

ANDERS ÅGMO,[1] IRITH FEDERMAN, VERONICA NAVARRO, MALENA PADUA AND GABRIELA VELAZQUEZ

Department of Psychology, Universidad Anáhuac, Apdo. Postal 10-844, Mexico City, 11000 Mexico, D.F.

ÅGMO, A., I. FERERMAN, V. NAVARRO, M. PADUA AND G. VELAZQUEZ. *Reward and reinforcement produced by drinking water: Role of opioids and dopamine receptor subtypes.* PHARMACOL BIOCHEM BEHAV 46(1) 183–194, 1993.—The conditioned place preference procedure was used to evaluate the reinforcing properties of drinking in water-deprived rats. Subjects were allowed to drink for 8 min and were then transferred to place preference cages. In Experiment 1, the effects of naloxone and pimozide on drinking-induced place preference were analyzed. Animals treated with naloxone, 16 mg/kg, before the conditioning sessions showed a place aversion instead of the place preference found in saline-treated animals. Naloxone also reduced drinking. It was proposed that naloxone induced a state of frustrative nonreward. Pimozide, 1 mg/kg, blocked place preference and somewhat reduced drinking. In Experiment 2, doses of 1 and 4 mg/kg naloxone were used. Both doses blocked place preference. A dose of 4 mg/kg had a marginal effect on drinking, while 1 mg/kg lacked effect on this behavior. Thus, naloxone may block the establishment of place preference without modifying drinking. The effects of the dopamine D_1 antagonist SCH23390 and the D_2 antagonist raclopride were studied in Experiment 3. SCH23390 blocked place preference and reduced drinking at doses of 0.25 and 0.125 mg/kg. A dose of 0.06 mg/kg did not affect drinking but inhibited place preference. Raclopride, 0.25 mg/kg, had the same effects as SCH23390 at the same dose while 0.125 mg/kg blocked place preference without affecting drinking. It appears that the effects of a D_1 and a D_2 antagonist are similar. Because the effects of these latter drugs also are similar to those obtained with naloxone, it is suggested that both dopamine and opioids are important for water-induced reinforcement. Possible interactions between these two neurotransmitter systems are discussed.

Drinking Reinforcement Reward Dopamine Opioids

WE previously analyzed the reinforcing effects of sexual behavior with the conditioned place preference procedure (1). Male rats were allowed to copulate until ejaculation and then immediately transferred to a place preference cage. After three pairings, a preference shift was obtained. Thus, the affective state produced by ejaculation could be associated with environmental cues. The opiate antagonist naloxone, administered before the conditioning sessions, blocked place preference without modifying sexual behavior. The dopamine antagonist pimozide was ineffective. It was proposed that the ejaculation-induced affective state is opioid dependent. This proposition coincides with several lines of evidence suggesting that opioids are released during sexual activity [discussed in (3,4,45)].

These observations posed some questions. Do natural reinforcers other than sex produce affective states that can be conditioned to environmental cues, and if this would be the case are opioids as important as they are for sexual reinforcement? Further, there is a large quantity of data suggesting that dopaminergic systems are important for drug-induced reinforcement and for sustaining intracranial self-stimulation [see (22,73) for reviews]. There is also evidence showing that haloperidol can block eating-induced place preference (61). It was therefore surprising to find that pimozide did not block sexual reinforcement. Is the lack of effect of pimozide a peculiarity to sex or could it be replicated with some other natural reinforcer?

In this context, it may be worthwhile to observe that sex and several other natural reinforcers have been found to release dopamine in the nucleus accumbens (26,27,38,46,49). This brain structure is generally believed to be an important site for the reinforcing effects of many drugs, among these the dopamine releasers amphetamine and cocaine (73) as well as endogenous and exogenous opioids (72). However, dopamine release in the accumbens has also been reported after several kinds of stressful events, such as footshock, aggressive encounters, or tail-pinch (21,37,60). It is, therefore, an open question whether dopaminergic activation is due to reward or increased arousal.

Opiate antagonists reduce drinking in both nondeprived and deprived rats (44,50,62). These effects appear to be of

[1] To whom requests for reprints should be addressed.

central origin (14,67,68). Behavioral analyses of the actions of naloxone on drinking patterns suggested that the main effect of the drug is to interfere with some aspect of the affective consequences of drinking (18,59). There is also evidence suggesting that opioid release is reduced during water deprivation in brain regions associated with reinforcement and that a short period (15 min) of drinking can activate opioid release in these regions (10). Moreover, drinking releases dopamine in the nucleus accumbens (75). These data suggest that drinking produces neurochemical events in the brain similar to those produced by sexual activity. The purpose with the present studies was to determine whether drinking and drinking-induced reward or reinforcement responded to dopamine and opioid antagonists in the same way as sexual behavior and sexual reinforcement have been reported to do.

GENERAL METHOD

SUBJECTS

Male Wistar rats (350–450 g) from a local colony were used in all experiments. They were housed under a reversed light/dark cycle (12 D : 12 L, light off 0900 h) at an ambient temperature of 22–23°C and given Purina rat pellets and tap water ad lib. During experiments, access to water was limited as described below.

APPARATUS

Drinkometer

Drinking was registered with a Coulbourn optical lickometer (Model S23-01) mounted to a standard operant chamber (Lafayette Instruments, Lafayette, IN, Model 80000). The ensemble was located in a sound-attenuating cage (LVE) lit with a 5-W houselight. The cage ventilator provided internal masking noise. The control equipment (BRS/LVE) was placed in an adjacent room.

Place Preference Cages

A detailed description has been given elsewhere (1). Briefly, three-compartment cages were used. One lateral compartment was painted white, with fresh wood shavings covering the floor. The opposite compartment was painted black, and the floor was of wood. Immediately before a subject was introduced into this compartment, the walls were wiped with a 2% (v/v) solution of glacial acetic acid in water. Place preference cages were located in the same room as the drinkometer. An external 60-dB white noise masked environmental sounds.

PROCEDURE

All subjects were habituated to the drinkometer at two sessions of 8 min each separated by 24 h. Twenty-four hours before the first habituation session, water was removed from subjects' home cages. After this session, when animals had been returned to the colony room, they were given access to water for 20 min. After the second habituation session, animals were allowed to drink freely for 24 h. Any subject that made fewer than 500 licks at any of the two habituation sessions was not included in the experiment. About 20% of subjects had to be eliminated.

Then, the place preference pretest was performed. Each animal was placed in the middle compartment of the place preference cage and allowed to move about the cage for 10 min. The time spent in each lateral compartment was recorded.

During place preference conditioning, all subjects were deprived of water for about 22 h before each session. Half the subjects were exposed to the putatively reinforcing event (usually drinking) and were then immediately transferred to their nonpreferred (reinforced) compartment in a place preference cage. The other half was directly placed in their preferred (nonreinforced) compartment. After 30 min, subjects were returned to their home cage. Once back in the colony room, water was made available for 20 min. At the next session, 24 h later, subjects that had been reinforced at the previous session were placed directly in their preferred compartment, while the others were exposed to the putatively reinforcing event and then transferred to their nonpreferred compartment.

When all subjects had completed three reinforced and three nonreinforced sessions, the test was made. This was identical to the pretest. The interval between the last conditioning session and the test was 24 h, during which subjects were allowed to drink freely. A summary of the procedure is shown in Fig. 1.

During habituation as well as during conditioning, two parameters of drinking were registered: latency to the first lick and the number of licks during the session. Thus, all data reported refer to the number of licking responses and not to the actual amount of water drunk. Unpublished data from our laboratory show that there is a high correlation between the number of licks and the volume of water ingested after several drug treatments (saline, $r = 0.96$; SCH23390 0.25 mg/kg, $r = 0.90$; naloxone 16 mg/kg, $r = 0.94$). This makes it likely that ingestion of water (drinking) is associated with licking and that the reinforcing element in these studies indeed is drinking and not licking.

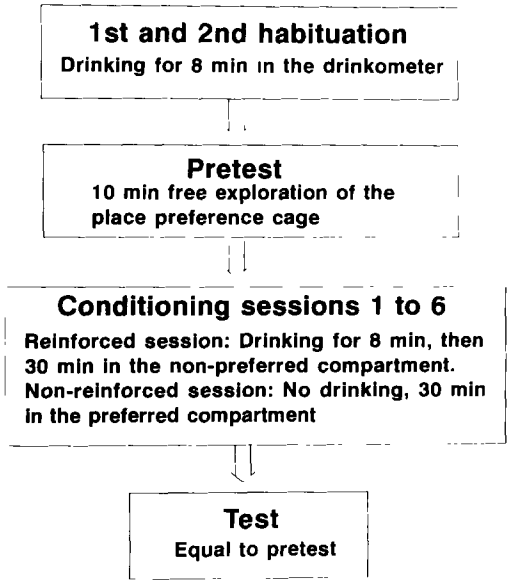

FIG. 1. Summary of the experimental procedure. At habituation and conditioning sessions, the latency to drink and the number of licks were recorded. At pretest and test, the time spent in each lateral compartment was registered. Drugs were administered before reinforced conditioning sessions and vehicle before nonreinforced sessions.

To consider a treatment-induced place preference, two criteria should be satisfied. First, the time in the reinforced compartment should increase between pretest and test. Second, the preference score [(time in reinforced compartment/(time in reinforced compartment + time in nonreinforced compartment)] should increase between pretest and test. It was considered important to use both criteria because each of them alone could indicate a false preference change. An increase in the time spent in the reinforced compartment could be a consequence of an unspecific reduction of the time spent in the middle, neutral, compartment. This, however, would not change the preference score. On the other hand, an increased preference score could be due to a reduction of the time spent in the nonreinforced compartment without any corresponding change in time spent in the reinforced compartment. It is not evident that this would represent place preference. Thus, the simultaneous use of the two criteria appears to be necessary if spurious effects are to be avoided.

STATISTICAL ANALYSIS

Drinking

The total number of licks at the two habituation sessions was compared across groups with a one-factor analysis of variance (ANOVA). Drinking during the conditioning sessions was analyzed with two-factor ANOVAs with repeated measures on one factor. The between-subjects factor was group and the within-subjects factor conditioning session. In case of significant interaction, tests for simple main effects of groups at each session were performed. The Tukey HSD test was used for *a posteriori* comparisons.

Place preference. Both parameters of place preference were evaluated with two-factor ANOVAs with repeated measures on one factor, the between-subjects factor being groups and the within-subjects factor pretest–test. After significant interaction, tests for simple main effects of pretest–test within each group were made.

All probabilities reported are two-tailed.

EXPERIMENT 1

In this experiment, it was determined whether drinking could induce an affective state of sufficient duration and intensity to become associated with environmental cues. Further, the effects of naloxone and pimozide on drinking and drinking-induced place preference were evaluated.

METHOD

Subjects and procedures were as described in the General Method section.

Drugs

Naloxone HCl (Rhone-Poulenc Pharma, Mexico City, Mexico) was dissolved in physiological saline and injected IP in a volume of 1 ml/kg b.wt. Pimozide (Janssen, Beerse, Belgium) was dissolved in a few drops of glacial acetic acid and then diluted with hot physiological saline. The solution was cooled to body temperature and pH adjusted to about 5.5 with 1 M NaOH before injection (IP, 5 ml/kg b.wt.). The larger injection volume was used to keep the pimozide concentration below the point of precipitation.

Design

Four groups of 10 animals each were used. The putatively reinforcing events were the following:

1. Eight-minute exposure to the drinkometer without access to water. These animals were deprived of water in the same way as the other groups, but the drinking spout was absent from the drinkometer during both habituation and conditioning.
2. Eight-minute drinking in the drinkometer. Saline (5 ml/kg b.wt.) was injected 7 min before drinking (reinforced sessions) and 15 min before the subject was placed in the place preference cage (nonreinforced sessions). In this way, the interval between injection and introduction in the place preference cage was always 15 min.
3. Eight-minute drinking. Naloxone, 16 mg/kg, was administered 7 min before the reinforced sessions and saline 15 min before nonreinforced sessions.
4. Eight-minute drinking. Pimozide, 1 mg/kg, was administered 52 min before drinking, and saline was given 60 min before nonreinforced sessions.

The doses of naloxone and pimozide were chosen to coincide with a previous study (1). Moreover, naloxone has a short half-life [15–20 min (40,65)], and it was considered important to assure blockade of opioid receptors during the entire session. Results in Experiment 2 showed that this reasoning was exaggerated, but it seems to be a valid initial assumption. The dose of pimozide has a rather strong inhibitory effect on ambulatory activity but is not sufficient to impair motor coordination (2).

Three additional groups of 10 rats each were used to evaluate the effects of the drugs in the absence of drinking. One group was given a saline injection 15 min before both "reinforced" and nonreinforced sessions. A second group received an injection of naloxone, 16 mg/kg, 15 min before being placed in the reinforced compartment and saline at the other sessions. A third group was injected with pimozide, 1 mg/kg, 60 min before reinforced sessions.

Because it was not practically possible to run all subjects in all groups simultaneously, a small number of animals were exposed to each reinforcing event or its control at each session. This was then repeated until 10 animals had completed conditioning with each reinforcing event.

RESULTS AND DISCUSSION

There was no group difference in drinking at habituation. The number of licks registered at the conditioning sessions is shown in Fig. 2A. ANOVA showed a significant effect of group, $F(2, 27) = 23.05$, $p < 0.001$, and the interaction group × session was also significant, $F(4, 54) = 4.01$, $p = 0.006$. There was no effect of session. Tests for simple main effects of groups at each session showed that the groups differed at all sessions ($p < 0.001$). *A posteriori* comparisons revealed that naloxone reduced drinking at conditioning sessions 2 and 3, while pimozide produced a reduction at sessions 1 and 2. At session 3, subjects treated with naloxone drank less than those treated with pimozide.

Neither naloxone nor pimozide had effects on lick latency ($p > 0.4$) (Fig. 2B).

ANOVA of the preference score showed that there was no effect of group or pretest–test whereas the interaction was significant, $F(3, 36) = 12.73$, $p < 0.001$. When simple main effects of pretest–test were analyzed, no effect was found in the group that were exposed to the drinkometer without drinking. Drinking increased the preference score, $F(1, 36) = 22.09$, $p < 0.001$, while a reduction was observed in animals treated with naloxone before drinking, $F(1, 36) = 13.27$, $p = 0.001$. In animals treated with pimozide, the preference

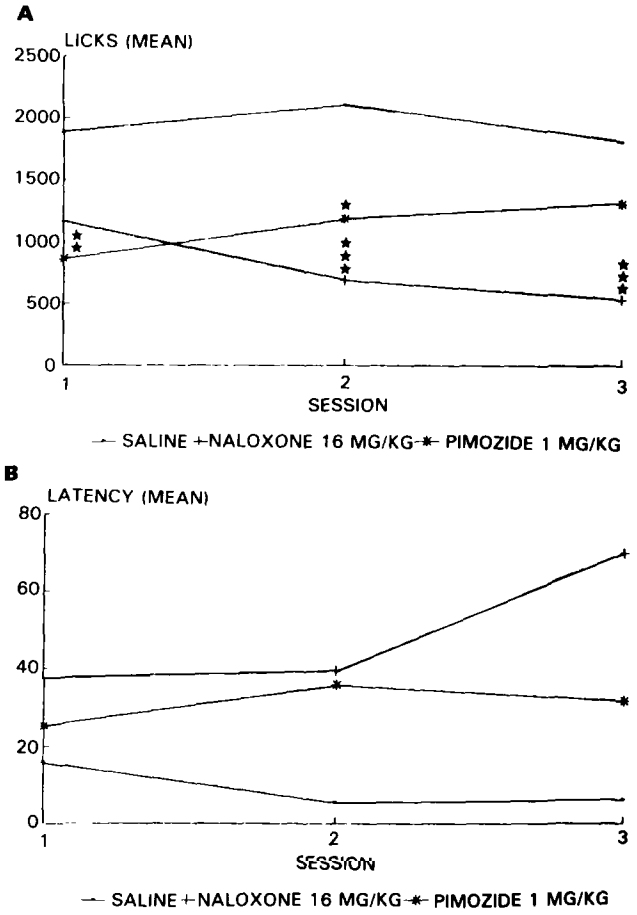

FIG. 2. Number of licks (A) and lick latency (B) in animals treated with saline, naloxone, or pimozide before drinking. Latency in seconds. *Different from saline, $p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

score increased between pretest and test, $F(1, 36) = 5.05$, $p = 0.03$ (Fig. 3A).

Analysis of the time spent in the reinforced compartment revealed a significant effect of group, $F(3, 36) = 3.02$, $p = 0.042$, as well as a significant interaction group × pretest-test, $F(3, 36) = 15.00$, $p < 0.001$. There was no overall difference between pretest and test. Tests for simple main effects of pretest-test within groups showed that the group exposed to the drinkometer without access to water reduced the time spent in the reinforced compartment, $F(1, 36) = 6.53$, $p = 0.015$. An opposite effect was obtained in the group that drank for 8 min, that is, the time in the reinforced compartment increased between pretest and test, $F(1, 36) = 18.69$, $p < 0.001$. Naloxone produced a drastic reduction of the time spent in the reinforced compartment, $F(1, 36) = 20.54$, $p < 0.001$. Pimozide inhibited the increase produced by drinking water, that is, no difference was obtained between pretest and test. Data are shown in Fig. 3B.

According to our criteria, exposure to the drinkometer without drinking had no reliable effect on place preference. Eight-minute drinking produced place preference. Naloxone not only blocked the reinforcing effect of drinking but also produced place aversion. Both the time spent in the reinforced compartment and the preference score were reduced between pretest and test. Pimozide blocked the reinforcing effect of drinking but did not produce place aversion.

Neither naloxone nor pimozide had any effect on place preference in the absence of drinking ($p > 0.1$). Data are shown in Figs. 4A and 4B.

Present data show that drinking indeed produces an affective state that can be associated with environmental cues. Because subjects drank before they were introduced into the place preference cage, it is not likely that the drinking responses per se became associated with the stimuli in that cage. Neither is it likely that the reinforcing stimulus (water) became associated with the place preference environment. It is suggested that the physiological changes (internal stimuli) produced by drinking function as the unconditioned stimulus. The unconditioned response would be a positive affective state. The conditioned stimuli would be the environmental cues present in the place preference compartment and the conditioned response some fraction of the affective state produced by drinking. That affective state would then cause approach to the conditioned stimuli. This latter proposal conforms to Mowrer's two-factor theory, where a conditioned

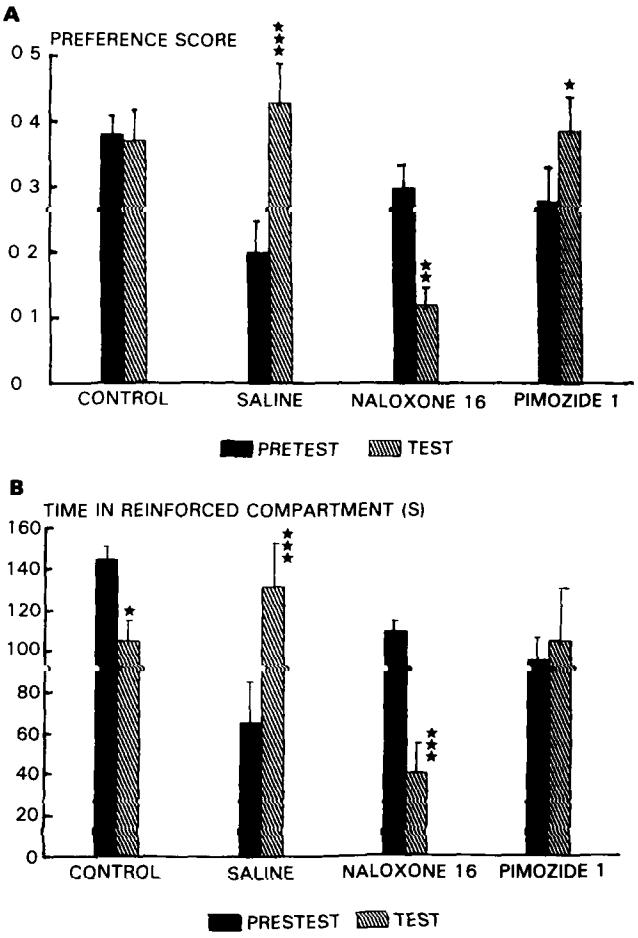

FIG. 3. Preference score (A) and time spent in the reinforced compartment (B) at pretest and test in animals exposed to the drinkometer without access to water (control) and in animals treated with saline, naloxone, or pimozide before being allowed to drink. Data are means ± SEM. *Different from pretest, $p < 0.05$; **$p < 0.01$; $p < 0.001$.

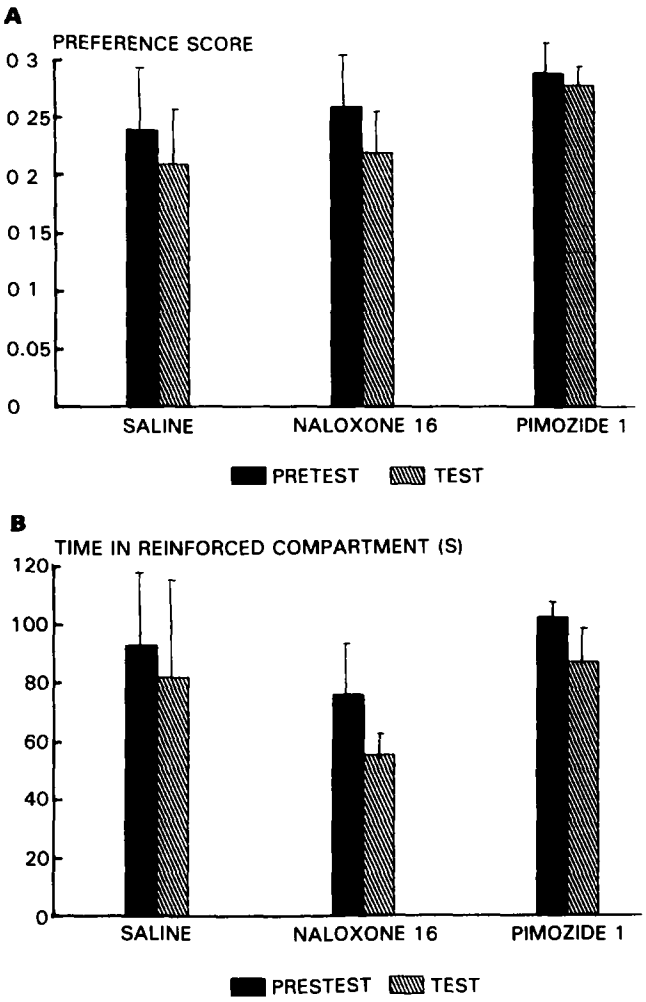

FIG. 4. Preference score (A) and time spent in the reinforced compartment (B) at pretest and test in animals given saline, naloxone, or pimozide. These animals were not exposed to the drinkometer. Data are means ± SEM.

emotional response may activate an instrumental response [(42); see also (51)].

According to this analysis, the establishment of place preference requires the activation of some internal stimuli leading to an affective state and the formation of associations between these internal stimuli and environmental cues. The activation of a positive affective state may be considered reward, while the establishment of associations may be called reinforcement (69). Behaviorally, reward would manifest itself as approach to or consumption of a stimulus, while reinforcement would make a previously neutral stimulus able to control behavior (learning). If any of these two processes fails, there will be no place preference.

Naloxone apparently reversed the affective state produced by drinking because a place aversion was obtained. The drug could not have inhibited the formation of associations. If this would have occurred, neither place preference nor place aversion would have been obtained. The mechanisms by which naloxone reverses the consequences of drinking from rewarding to aversive are not clear. It could be argued that naloxone may have affected some homeostatic mechanism involved in water balance because the drug reduced drinking. A diminished motivation to drink could have made drinking less rewarding. However, it is difficult to explain why reduced motivation should be associated with an aversive state.

The fact that naloxone reduced drinking is also indicative of a reduced reward value of water. It has been reported that a reduced reward value may be similar to nonreward (20). It is well known that nonreward may generate an aversive affective state (7). We tentatively suggest, therefore, that the place aversion produced by naloxone is an example of frustrative nonreward. Naloxone treatment also renders sexual activity (1) and sucrose drinking (23) aversive.

Some authors reported that naloxone by itself may cause place aversion (43,66). However, in the present study no such effect was found. This is in agreement with several other studies (12,47,48,56). It has been proposed that aversive properties of naloxone become evident only in unbiased place preference procedures and when SC administration is used (43).

Pimozide inhibited place preference and reduced water intake. This means that the drug may have interfered with either the affective state produced by drinking (reward) or with the formation of associations between that state and environmental cues (reinforcement). There is evidence showing that dopamine antagonists inhibit operant responding for water at doses lower than those required for reducing free water intake (24, 35). This could mean that learning or retrieval of previously learned associations are more easily disrupted by dopamine antagonists than reward. Further, in nondeprived rats antagonism of dopamine increases latency to drink, suggesting reduced motivation rather than reduced reward (17). It is also well known that dopamine antagonists have strong motor effects, and these could be responsible for the reduced drinking observed in the present experiment. However, these arguments should be regarded as tentative.

To proceed with the analysis of the role of opioids in drinking-induced reinforcement, it seemed important to reduce the naloxone dose until drinking was unaffected. This would allow for a distinction between effects on consummatory behavior and reinforcement. This was the purpose of Experiment 2.

EXPERIMENT 2

METHOD

Subjects and procedures were as described in the General Method section.

Design

Three groups of 6–11 rats each were used. All groups were allowed to drink for 8 min in the drinkometer. Group 1 was injected with saline 7 min before reinforced sessions and 15 min before nonreinforced sessions. Groups 2 and 3 were injected with naloxone, 1 and 4 mg/kg, respectively, 7 min before reinforced sessions and with saline 15 min before nonreinforced sessions.

RESULTS AND DISCUSSION

When drinking was analyzed by ANOVA, no difference was found between the groups neither at habituation nor during conditioning. There was a significant effect of session, $F(2, 42) = 06.68$, $p = 0.003$, and the interaction group × session was also significant, $F(4, 42) = 4.55$, $p = 0.004$.

However, tests for simple main effects of group at each session failed to reveal any difference ($p > 0.09$). Upon examination of the data (Fig. 5A), it can be seen that the group treated with naloxone drank somewhat more than the other groups at session 1 and somewhat less at sessions 2 and 3. This can probably explain the interaction group × session. Nevertheless, it must be concluded that no dose of naloxone reliably modified drinking.

With regard to latency to drink, the group effect as well as the interaction group × session were significant $F(2, 21) = 3.58$, $p = 0.046$, and $F(4, 42) = 2.78$, $p = 0.039$, respectively. There was no effect of session. Tests for simple main effects of groups at each session showed a difference only at session 3, $F(2, 21) = 3.49$, $p = 0.048$. *A posteriori* comparisons revealed that animals treated with naloxone, 4 mg/kg, had a longer latency to drink than those treated with saline. Data are shown in Fig. 5B.

Due to equipment failure at the preference test, data could not be obtained from one animal treated with naloxone 4 mg/kg. Analysis of the preference score showed effects of pretest-test, $F(1, 20) = 13.13$, $p = 0.002$, and an interaction group × pretest-test, $F(2, 20) = 4.78$, $p = 0.02$. The group effect was not significant. Tests for simple main effects of pretest-test revealed a significant effect in the group treated with saline before drinking, $F(1, 20) = 19.52$, $p < 0.001$. The preference score did not change in the groups given naloxone before drinking ($p > 0.25$) (Fig. 6A).

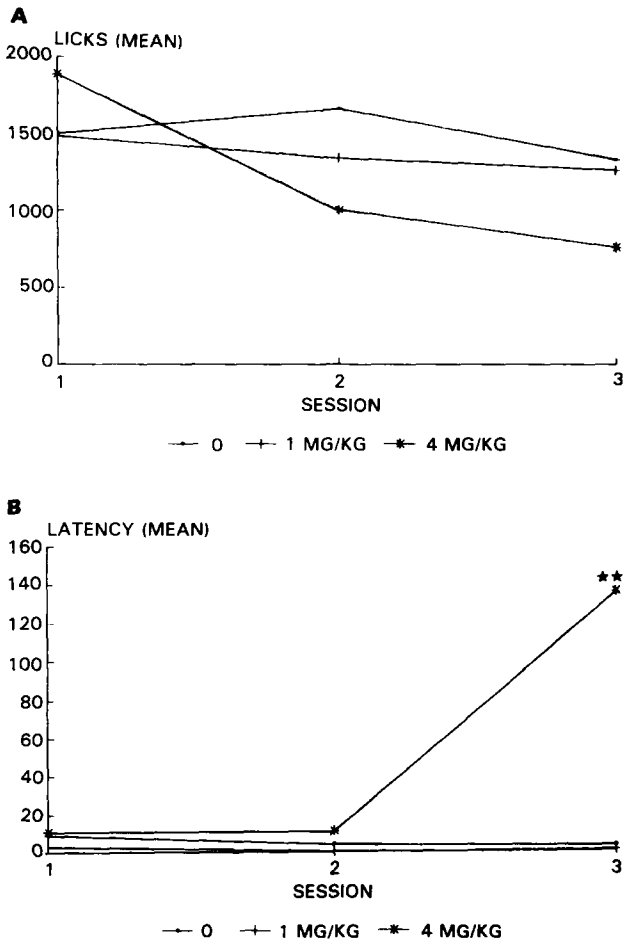

FIG. 5. Number of licks (A) and lick latency (B) in animals treated with saline or naloxone, 1 or 4 mg/kg, before drinking. For further details, see Fig. 1.

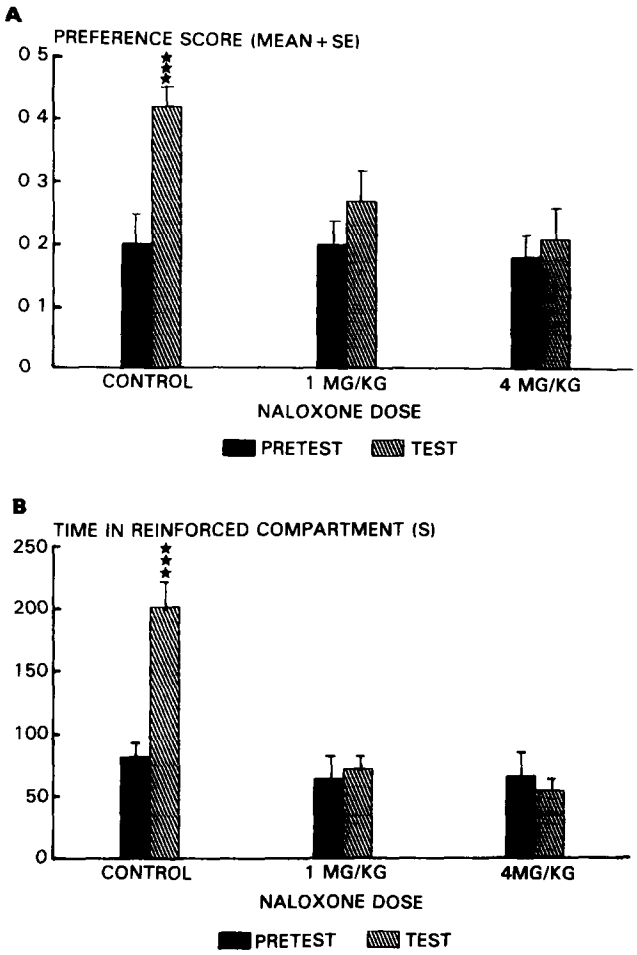

FIG. 6. Preference score (A) and time spent in the reinforced compartment (B) at pretest and test in animals treated with saline or naloxone, 1 or 4 mg/kg, before drinking. For further details, see Fig. 2.

ANOVA of the time spent in the reinforced compartment demonstrated effects of group, $F(1, 20) = 12.82$, $p < 0.001$, pretest-test, $F(1, 20) = 12.26$, $p = 0.002$, and an interaction group × pretest-test, $F(1, 20) = 14.99$, $p < 0.001$. When tests of simple main effects of pretest-test within each group were performed, a significance was obtained in the group given saline before drinking, $F(1, 20) = 38.93$, $p < 0.001$. No effect was found in the groups treated with naloxone before drinking ($p > 0.45$) (Fig. 6B).

Summarizing, these data confirm that 8 min of drinking can produce conditioned place preference. The effects of drinking are blocked by naloxone at doses of 1 and 4 mg/kg. The lower dose had no effect on drinking while the dose of 4 mg/kg increased latency to drink at session 3. It appears, then, that a low dose of naloxone may inhibit place preference (reinforcement) while leaving consummatory behavior (reward) unaffected. This conclusion is somewhat different from the one proposed in Experiment 1, where a large dose of nal-

oxone was found to have strong effects on drinking and produce place aversion.

There is much published evidence showing that naloxone reduces water intake, even after low doses (see the introductory section). That reduction has been related to diminished reward value of drinking (59). There may be at least two reasons why the 1-mg/kg dose did not affect drinking in the present experiment. First, it has been reported that naloxone has no effect during the first few minutes of drinking. The larger the dose, the shorter the time until naloxone displays its inhibitory effect (59). Because we used a short test, it is reasonable that only a large dose was effective. Second, the effects of naloxone appears to depend upon deprivation in the way that inhibition becomes larger as deprivation becomes less intense (6).

It appears, then, that although naloxone under certain circumstances interferes with the rewarding effects of drinking (consumption) this effect is not necessarily related to naloxone's capacity to inhibit drinking-induced place preference.

The effect observed after treatment with pimozide in Experiment 1 was ambiguous. In the following experiment, an effort was made to elucidate the role of dopamine in drinking-induced place preference.

EXPERIMENT 3

There are at least two dopamine receptors within the CNS [see (55) for a review]. The dopamine D_1 as well as the D_2 receptors have been reported to be important for reinforcement induced by drugs of abuse, among these amphetamine and cocaine (13,29,74). Brain self-stimulation and operant responding for food or water are disrupted after administration of either D_1 or D_2 antagonists (8). Although there are a considerable number of studies dedicated to determine the effects of specific D_1 and D_2 agonists on place preference conditioning [(28,71) and references therein], there is, to our knowledge, no study where the effects of specific dopamine receptor antagonists on place preference conditioning produced by a natural reinforcer have been evaluated. Thus, rather than using additional doses of pimozide we decided to analyze the effects of the specific D_1 antagonist SCH23390 (30) and the specific D_2 antagonist raclopride (32) on drinking-induced conditioned place preference and on water intake.

METHOD

Subjects and procedures were the same as described in the General Method section.

Drugs

Raclopride tartrate (Astra Alab, Södertälje, Sweden) was dissolved in physiological saline while SCH23390 hydrogen maleate (Schering-Plough Corp., Bloomfield, NJ) was suspended in saline containing one drop of Tween-80. Both drugs were injected IP in a volume of 1 ml/kg.

Design

The effects of SCH23390 were analyzed in four groups of 7–11 animals each. All groups were allowed to drink for 8 min in the drinkometer at reinforced sessions. Group 1 was given saline 22 min before reinforced sessions and 30 min before nonreinforced sessions. In group 2, saline was replaced with SCH23390, 0.06 mg/kg, before reinforced sessions. In group 3, the SCH23390 dose was 0.125 mg/kg, and in group 4 the dose was 0.25 mg/kg.

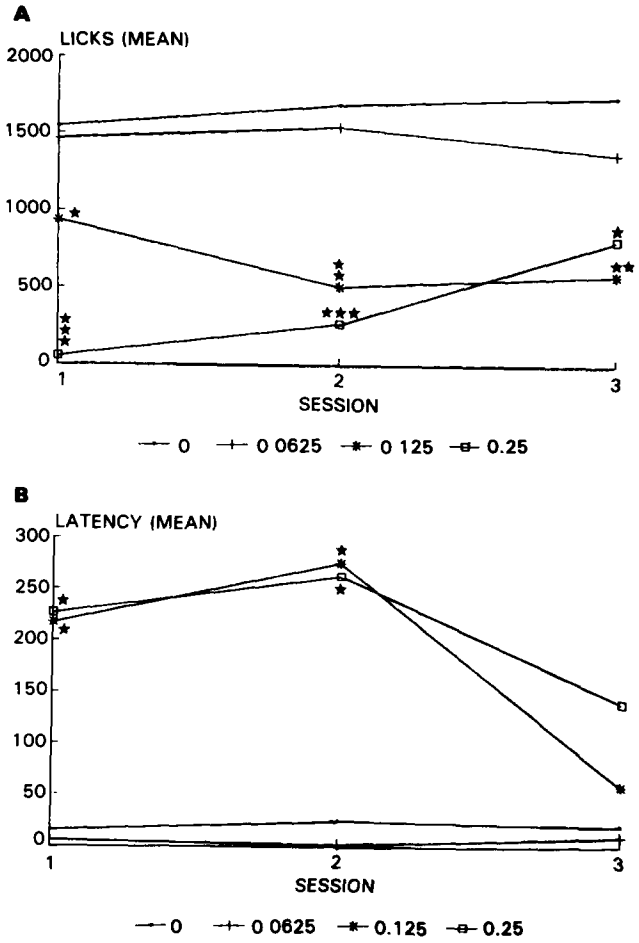

FIG. 7. Number of licks (A) and lick latency (B) in animals treated given saline or varying doses of SCH23390 before drinking. Doses are in mg/kg. For further details, see Fig. 1.

Three groups of 7–11 rats each were used to evaluate the effects of raclopride. The intervals between injection and conditioning were the same as those used with SCH23390, and the doses of raclopride were 0 (saline), 0.125, and 0.25 mg/kg. The largest dose of these drugs reduces ambulatory activity, an indication of functional dopamine receptor blockade, but does not produce motor incoordination (Ågmo and Vizcarra, unpublished observations).

Finally, the effect of the dopamine antagonists on place preference in the absence of drinking was studied in three groups of seven to eight animals each. The putatively reinforcing events were injections of saline, SCH23390, and raclopride, the latter two at a dose of 0.25 mg/kg.

RESULTS AND DISCUSSION

In the experiment with SCH23390, ANOVA of drinking showed a significant effect of group, $F(3, 30) = 21.55$, $p < 0.001$. Neither session nor the interaction group × session reached significance ($p > 0.07$). *A posteriori* comparisons revealed that the doses of 0.25 and 0.125 mg/kg reduced drinking at all sessions. The group treated with SCH23390, 0.06 mg/kg, did not differ from the group given saline. Data are shown in Fig. 7A.

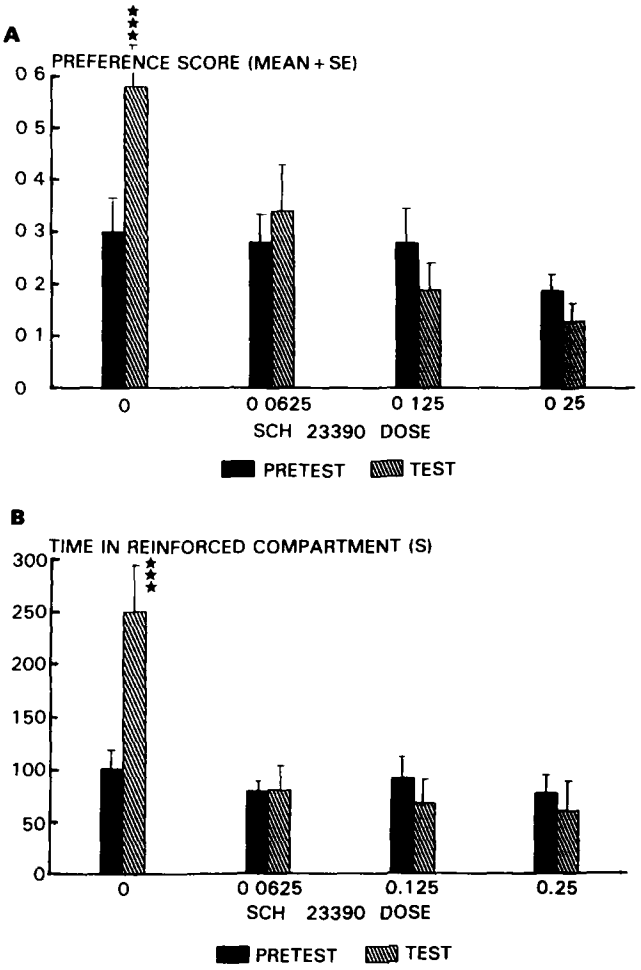

FIG. 8. Preference score (A) and time spent in the reinforced compartment (B) at pretest and test in animals treated with saline or varying doses (in mg/kg) of SCH23390 before drinking. For further details, see Fig. 2.

ANOVA of the latency to drink revealed an effect of group, $F(3, 32) = 11.42$, $p < 0.001$. There was no effect of session and the interaction group × session was also nonsignificant ($p > 0.1$). *A posteriori* comparisons demonstrated that the groups treated with SCH23390 0.125 and 0.25 mg/kg had longer latencies than saline at sessions 1 and 2. At session 3, there was no group difference (Fig. 7B).

With regard to the preference score, ANOVA showed an effect of group, $F(1, 32) = 6.92$, $p = 0.001$, and of the interaction group × pretest-test, $F(3, 32) = 5.74$, $p = 0.003$. Tests of simple main effects of pretest-test within each group revealed a significance only in the group treated with saline, $F(1, 32) = 17.58$, $p < 0.001$ (all other $p > 0.28$). Data are found in Fig. 8A.

Almost identical effects were obtained when the time spent in the reinforced compartment was analyzed. Groups and the interaction group × pretest-test were significant, $F(1, 32) = 9.78$, $p < 0.001$, and $F(3, 32) = 6.74$, $p = 0.001$, respectively, while no effect was found of the variable pretest-test ($p > 0.1$). Again, only the group treated with saline showed a difference between pretest and test, $F(1, 32) = 24.03$, $p < 0.001$ (all other $p > 0.5$) (Fig. 8B).

At the largest doses, the D_1 antagonist reduced drinking, increased latency to drink, and inhibited place preference. However, the dose of 0.06 mg/kg had no effect on drinking, yet place preference was inhibited. Thus, consummatory behavior (reward) was unaffected by this dose. Nevertheless, no association between the consequences of drinking and environmental cues took place. It appears, then, that reinforcement was blocked.

The effects obtained after treatment with raclopride were similar to those found with SCH23390. Drinking was affected [group, $F(2, 22) = 8.36$, $p = 0.002$; other $p > 0.3$] by the drug. *A posteriori* comparisons showed that the 0.25-mg/kg dose reduced drinking at sessions 2 and 3 but not at session 1. Latency to drink was also modified [group, $F(2, 22) = 3.98$, $p = 0.034$; other $p > 0.3$]. The group given raclopride 0.25 mg/kg had longer latency than saline treated animals at sessions 2 and 3. See Figs. 9A and 9B.

The preference score turned out to differ between pretest and test, $F(1, 22) = 9.67$, $p = 0.005$, and the interaction group × pretest-test was also significant, $F(2, 22) = 5.35$, $p = 0.013$. Tests for simple main effects of pretest-test showed a difference only in the group treated with saline, $F(1, 22) = 16.63$, $p < 0.001$ (all other $p > 0.07$).

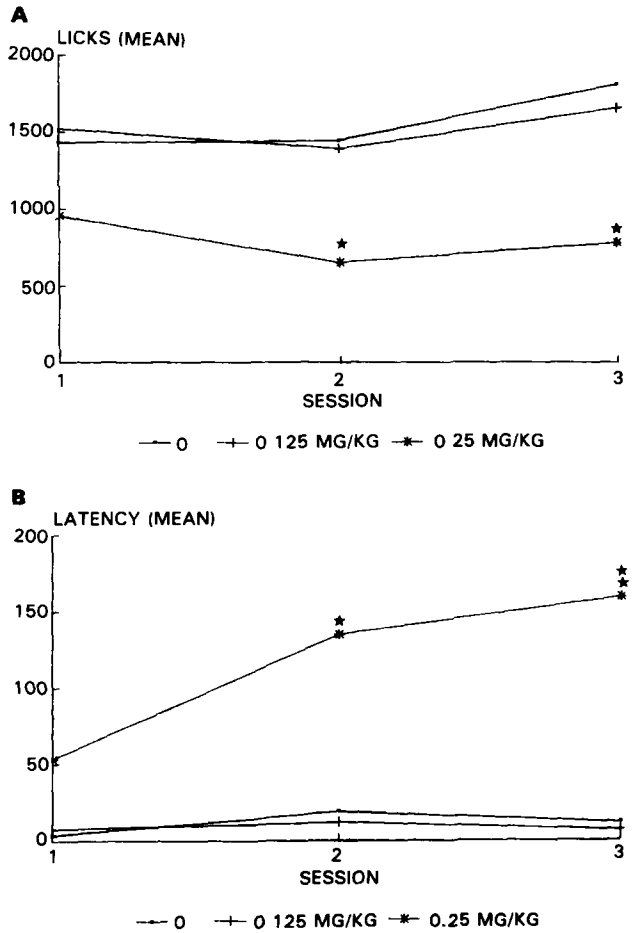

FIG. 9. Number of licks (A) and lick latency (B) in subjects treated with saline or raclopride, 0.125 and 0.25 mg/kg, before drinking. For further details, see Fig. 1.

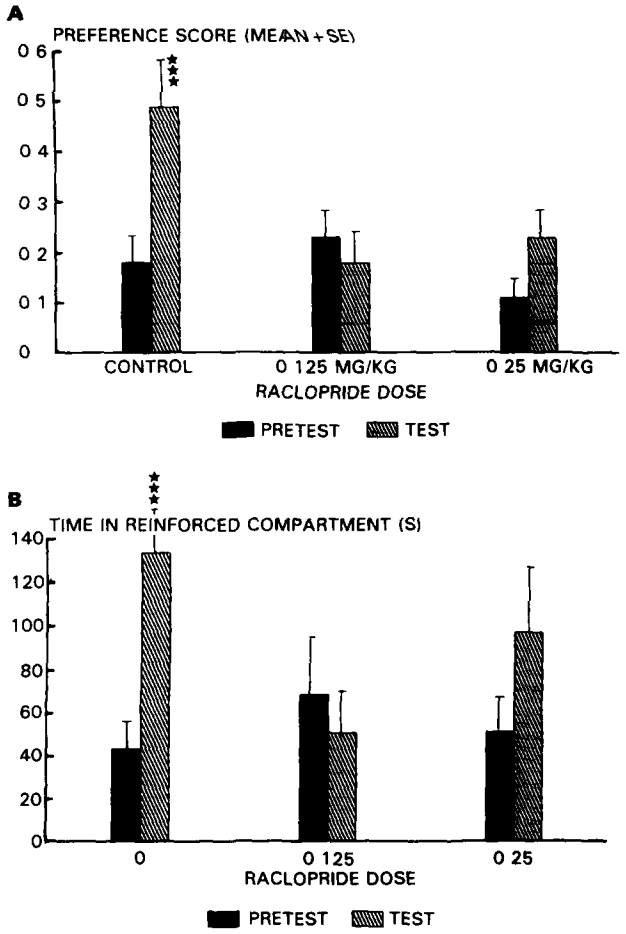

FIG. 10. Preference score (A) and time spent in the reinforced compartment (B) at pretest and test in rats treated with saline or raclopride, 0.125 or 0.25 mg/kg, before drinking. For further details, see Fig. 2.

ANOVA of the time spent in the reinforced compartment showed an effect of pretest-test, $F(1, 22) = 6.67$, $p = 0.018$, and of the interaction group × pretest-test, $F(2, 22) = 3.63$, $p = 0.043$. The only group that differed between pretest and test was the one given saline, $F(1, 22) = 10.02$, $p = 0.004$ (other $p > 0.1$). Data are shown in Figs. 10A and 10B.

Just as occurred with SCH23390, a large dose of raclopride inhibited drinking, increased latency to drink, and blocked place preference. The dose of 0.125 mg/kg did not affect drinking but effectively inhibited place preference. It appears that the actions of a D_1 and a D_2 dopamine antagonist are almost identical.

No effect was found when the drugs were administered in the absence of drinking ($p > 0.5$). Data are shown in Fig. 11.

These results show that large doses of dopamine antagonists reduce drinking. This reduction was generally (but not always) associated with increased latency to drink. Similar effects of SCH23390 and raclopride on drinking have been reported previously (17,36). The mechanisms behind the reduced drinking are not clear. Motor deficiencies caused by the dopamine antagonists may be crucial, but there are many alternative explanations. Among those are impairment of activational aspects of motivated behavior (52,53).

What is more interesting is the fact that dopamine antagonists block place preference at doses that do not affect drinking. This constitutes clear evidence for a role of dopamine in water-induced reinforcement. Interestingly, it has repeatedly been suggested that dopamine is involved in acquisition of and responding to secondary reinforcers (9,15,63,64). In the conditioned place preference procedure, the environmental cues in the reinforced compartment would be secondary reinforcers. It is, then, not surprising that dopamine antagonists inhibit water-induced place preference.

Behavior sustained by secondary reinforcement is disrupted by lower doses of dopamine antagonists than consummatory behaviors (54). This difference in sensitivity to dopamine antagonism may explain the fact that the effects of place preference were obtained with doses lower than those required to modify drinking. It is important to note that the above arguments support the idea that reinforcement (learning) is more closely related to dopamine than reward (consumption). The larger doses of dopamine antagonists required to interfere with consumption may indicate that nonspecific effects, like those mentioned above, may be the ultimate cause of reduced consumption.

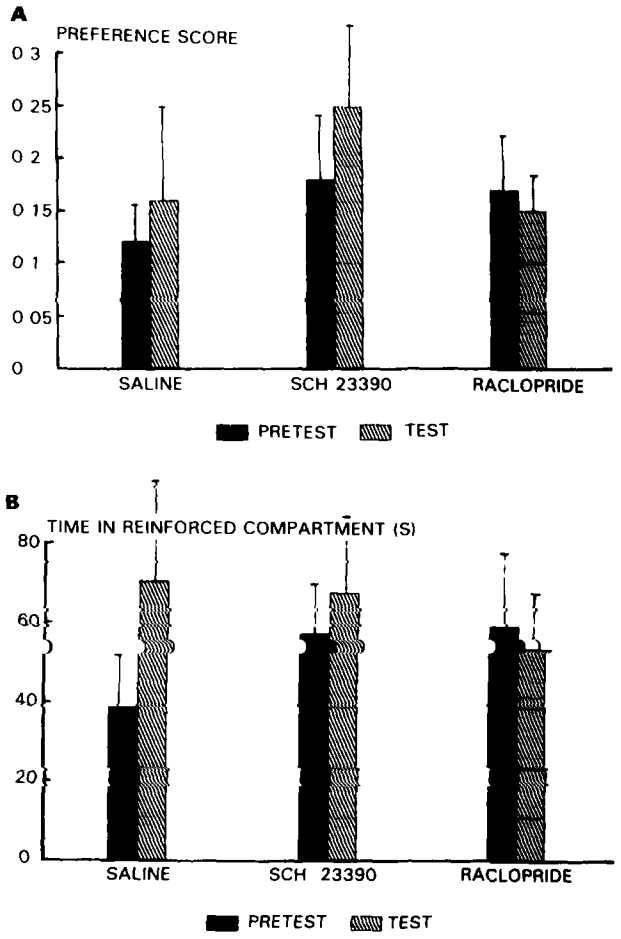

FIG. 11. Preference score (A) and time spent in the reinforced compartment (B) at pretest and test in animals given saline, SCH23390, 0.25 mg/kg, or raclopride, 0.25 mg/kg, in the absence of drinking.

Some studies have shown that SCH23390 may have aversive properties of its own (57,58). Others found D_1 agonists to be aversive in the conditioned place preference procedure (28,71). There are also reports where SCH23390 is unable to modify place preference (28,33). Present results, showing no effect of SCH23390 on place preference when the drug was administered alone, coincide with the latter studies. At present, there is no explanation available for these contradictory findings. We know of no previous data concerning the effects of raclopride on place preference. It seems, however, unlikely that aversive properties of the dopamine antagonists could be responsible for the inhibition of water-induced reinforcement.

GENERAL DISCUSSION

Antagonism of opioids or dopamine has similar effects on drinking and drinking-induced place preference. Large doses impair both drinking and place preference while lower doses have effect only on place preference. The exception to this was the place aversion obtained after treatment with naloxone, 16 mg/kg, before drinking. We proposed, in Experiment 1, that this may be due to frustrative nonreward. The fact that lower doses of naloxone did not produce place aversion coincides with the moderate or no effects observed on drinking behavior. These latter doses, therefore, seem to have only marginal effects on reward, and there would be no reason to expect them to induce a state of frustrative nonreward. If the above hypothesis is correct, then naloxone is able to block reinforcement produced by positive affect but not that produced by negative affect. This intriguing hypothesis could easily be tested.

A weakness in the above arguments is the magnitude of the naloxone dose required to reduce reward. For example, 1 mg/kg naloxone is sufficient to inhibit the reinforcing effects of 10 mg/kg morphine, a large dose, in the place preference procedure (5). It is possible that the effect obtained with naloxone, 16 mg/kg, is purely pharmacological and hence of limited physiological relevance.

Large doses of dopamine antagonists also reduced drinking. However, they did not produce place aversion. As pointed out, there may be several factors other than reduced reward responsible for diminished drinking behavior.

The actions of the lowest dose of naloxone and the dopamine antagonists were almost identical, suggesting a common mechanism of action. Naloxone does not bind to dopamine receptors (16), and there is no evidence that raclopride or SCH23390 bind to opioid receptors, excluding a common pharmacological action. There are however, a large number of studies reporting interactions between opioids and dopamine. Morphine enhances firing rate of dopaminergic neurons in the ventral tegmental area (39), and infusion of morphine at this site stimulates dopamine release in the nucleus accumbens (34). There is also evidence that dopamine antagonists block reinforcing effects of opioids [reviewed in (72)]. It was therefore proposed that reinforcement produced by opioids ultimately depends upon dopaminergic activation (11). However, naloxone blocks the reinforcing properties of amphetamine (66) and cocaine (19). Further, chronic exposure to cocaine produces an enhancement of naloxone binding in the nucleus accumbens and ventral tegmental area (25). These latter data may suggest that dopamine-induced reward depends upon opioid receptors. It appears that the interactions between opioids and dopamine are more complex than once believed. Nevertheless, because several kinds of reinforcements seem to be blocked equally well by dopamine and opioid antagonists it is not strange that the same occurs with a natural reinforcer.

It has been reported that dopamine release in the nucleus accumbens in response to sexual stimuli is blocked by systemic naloxone (41). Moreover, footshock-induced dopamine release is inhibited by naloxone infused into the ventral tegmental area (31). These observations suggest that dopamine release in response to reinforcing and aversive stimuli depends upon activation of opioidergic systems. However, it is not known whether dopamine antagonists can modify opioid release. Until this has been evaluated, it is not possible to speculate about the exact nature of dopamine-opioid interactions with regard to reinforcement.

Water-induced reinforcement is different from sexual reinforcement in the way in which the latter is not affected by dopamine antagonism. Interestingly, both drinking and sex release dopamine in the nucleus accumbens (see the introductory section) yet only drinking-induced reinforcement appears to be related to dopamine. Further, sucrose and saccharin drinking are about equally effective in promoting accumbens dopamine release [Ågmo and Gómez, unpublished data; (27)]. Sucrose drinking produces place preference while saccharin drinking does not (70). These observations suggest that dopamine release in the accumbens is not always a determinant of reinforcement. Perhaps brain structures outside the nucleus accumbens are important for some kinds of reinforcements or transmitters other than dopamine are critically involved. It could be argued that there may be important differences between brain mechanisms activated by different kinds of natural reinforcers. A more extensive range of reinforcing events needs to be studied to confirm this hypothesis.

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