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Apomorphine-Induced Flavor-Drug Associations: A Dose-Response Analysis by the Taste Reactivity Test and the Conditioned Taste Avoidance Test

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PARKER, L. A. AND L. BROSSEAU. *Apomorphine-induced flavor-drug associations: A dose-response analysis by the taste reactivity test and the conditioned taste avoidance test.* PHARMACOL BIOCHEM BEHAV **35**(3) 583–587, 1990.—Apomorphine is a positively reinforcing drug at low to moderate doses, but appears to lose its reinforcing properties at higher doses. In Experiment 1, across a range of doses (0.5–15.0 mg/kg, intraperitoneally), apomorphine produced a CTA over 5 conditioning/testing trials which was not dose-dependent by a single bottle test. The rejection taste reactivity responses of chin rubbing and gaping, however, only occurred at the highest dose of apomorphine (15 mg/kg). In Experiment 2, a CTA test which was designed to more effectively discriminate among the different drug dose conditions indicated that the doses of 2.5, 7.5 and 15 mg/kg of apormophine produce CTAs of equivalent strength. Our results support the contention that CTAs produced by positively reinforcing drugs are not accompanied by a palatability shift.

Apomorphine Conditioned taste aversion Conditioned taste avoidance Taste reactivity test
Behavioral toxicology Ingestive behaviors Palatability Classical conditioning

APOMORPHINE is a classic emetic agent which appears to produce its emetic effect in dogs by activation of the area postrema (4). In rats, however, the area postrema does not appear to be involved in the establishment of apomorphine-induced conditioned taste avoidance (5,17). Furthermore, apomorphine serves as an effective reinforcer in a drug self-administration paradigm (1, 13, 18) and produces a conditioned place preference at doses (SC) ranging from 0.1–10 mg/kg (15,17); however, apomorphine produces a place aversion at the higher dose (IP) of 15 mg/kg (3). Therefore, it appears that apomorphine serves as a positive reinforcer in the place preference paradigm at low to moderate doses, but serves as an aversive stimulus at the higher dose of 15 mg/kg.

Parker (10) has recently suggested that CTAs produced by positively reinforcing drugs may qualitatively differ from those produced by nonreinforcing drugs. Although flavors paired with lithium elicited a rejection pattern of orofacial and somatic responding as assessed by the taste reactivity test (6), Parker (8,9) demonstrated that flavors paired with amphetamine did not elicit the rejection pattern of responding even though they were equally avoided in the CTA consummatory test. Further investigations validated this distinction by demonstrating that the reinforcing drugs of nicotine (11) and morphine (10) produce CTAs which are not accompanied by the rejection pattern of orofacial and somatic responses that accompanies CTAs produced by drugs which are ineffective reinforcers in the conditioned place preference or drug self-administration paradigms.

Smith and Parker (14) reported that apomorphine effectively established the rejection pattern of orofacial and somatic responding that is produced by nonreinforcing drugs such as lithium. The dose of apomorphine that Smith and Parker (14) employed was 15 mg/kg (IP) which also produces a place aversion (3). Since doses of apomorphine lower than 15 mg/kg (SC) have been shown to produce a place preference, lower doses may also produce CTAs over multiple conditioning trials that are not accompanied by the rejection pattern of orofacial and somatic responses. The following experiment assessed this possibility.

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EXPERIMENT 1

METHOD

Subjects

Forty-five male Sprague-Dawley rats weighing between 286–330 g on the first conditioning day served as subjects. The rats were maintained on ad lib access to food and water except as indicated. Their home-cage room was illuminated on a 12/12 lighting schedule and all procedures occurred in the light phase of the cycle.

Procedure

Surgery. One week after their arrival in the laboratory, the rats were surgically implanted with intraoral cannulae while anesthetized with sodium pentobarbital as described by Parker (7). A one week recovery period was given following surgery during which the cannulae were flushed with water every 2 days.

Conditioning/testing trials. The rats were given initial adaptation trials on 3 successive days prior to the conditioning trials. On each adaptation trial, each rat was transported into the room that contained the glass test chamber (22.5 × 26 × 20 cm). The room was illuminated by two 25-W light bulbs located 30 cm from either side of the cage. Each rat was placed individually into the test chamber and a 30-cm infusion hose was connected to the cannula through the ceiling of the chamber. A syringe was connected to the hose and placed into the holder of a Gage Infusion Pump. After 1 min, the pump delivered water through the cannula into the rat's mouth at the rate of 1 ml/min for 1 min. The rat was then returned to its home cage.

On the day following the third adaptation trial, the rats received their first conditioning trial. The procedure of the conditioning trials was identical to that of the adaptation trials except that the rats were intraorally infused with 0.5 M sucrose solution rather than water. Immediately following the sucrose infusion in the test chamber, the rats were returned to their home cage and presented with a bottle containing the 0.5 M sucrose solution. Fifteen minutes later, the bottles were removed and weighed. Immediately after the bottles were removed, the rats were injected (IP) with one of the following doses of apomorphine prepared in solution with physiological saline (1.25 mg/ml): 0.0 mg/kg (n = 9), 0.5 mg/kg (n = 9), 2.5 mg/kg (n = 9), 7.5 mg/kg (n = 9) or 15 mg/kg (n = 9). Each conditioning trial consisted of a taste reactivity test trial followed immediately by a taste avoidance test trial. The rats were given four conditioning trials and a final test trial each separated by 3–4 days. They were maintained on ad lib food and water throughout the trials.

During each conditioning/testing trial, the orofacial and somatic responses elicited by exposure to the sucrose solution were videotaped. A mirror, which hung at an angle below the test chamber, allowed viewing of the ventral surface of the rat. A Hitachi HV-62 videocamera was focused on the mirror to monitor the rat's responses during the intraoral infusion. The camera transmitted the image through a Panasonic videorecorder to an Electrohome 17-in. monitor.

The videotaped recordings were scored by a rater blind to the experimental conditions by means of an event recorder attached to an Apple IIe microcomputer. The orofacial and somatic responses which were measured have been previously described by Berridge and Grill (2). The rejection responses that we will report included the frequency of chin rubbing, gaping and paw treading. The ingestion response which was measured was duration of period devoted to tongue protrusions. Finally, the neutral response of frequency of passive drips is also reported. Additionally, rearing, limb flicking, face washing and paw licking were measured; however, these latter responses did not reveal evidence of conditioning and, thus, will not be discussed in the results.

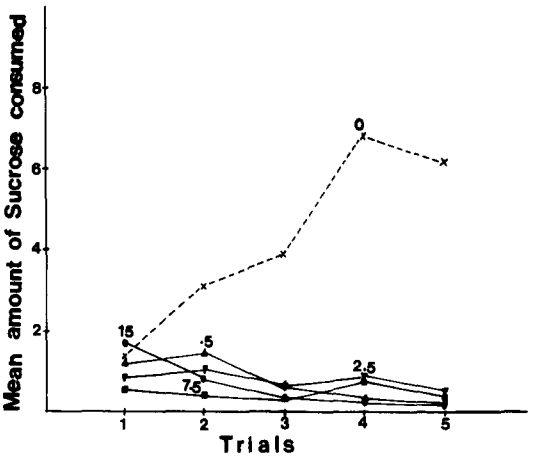

FIG. 1. Mean amount (ml) of sucrose solution consumed across conditioning/testing trials by the various groups in Experiment 1.

RESULTS

Figure 1 presents the mean amount consumed of 0.5 M sucrose solution for each of the groups on each conditioning/testing trial. A 5 × 5 Repeated Measures ANOVA revealed a significant effect of Dose, $F(4,40) = 27.8$, $p<0.01$, and a Dose × Trials interaction, $F(16,160) = 10.8$, $p<0.01$. Rats in Group 0.0 drank more sucrose than all other groups (p's<0.01). This difference first occurred on Test Day 2 (p's<0.05) and was maintained across all other test days (p's<0.01). Only on Test Day 2 did the Apomorphine groups differ from one another; rats in Group 0.5 drank significantly more sucrose than rats in Group 7.5 ($p<0.05$), but did not differ from rats in any other apomorphine group. On Test Days 3–5, rats in Group 0.0 drank significantly more sucrose than did rats in Groups 0.5, 2.5, 7.5 and 15 (p's<0.05), but none of the latter groups differed from one another.

Figure 2 presents the taste reactivity test results for the various groups in the experiment. The upper half of the figure presents the mean frequency of each rejection response across conditioning/testing trials. Each of these responses was analyzed as a 5 × 5 Repeated Measures ANOVA. The analysis of the chin rub measure revealed a significant Dose effect, $F(4,40) = 4.9$, $p<0.002$, and Dose × Trials effect, $F(16,160) = 2.6$, $p<0.01$. Rats in Group 15 demonstrated more frequent chin rubbing than those in any other group on Trials 3, 4 and 5. No other groups significantly differed from one another in the frequency of chin rubs. The gape measure revealed a significant Dose effect, $F(4,40) = 2.6$, $p<0.05$, although subsequent Newman-Keuls tests revealed no significant differences among conditions. Since the strength of the rejection responses was expected to increase with conditioning trials, t-tests were conducted among groups for Trial 5. These tests revealed that on Trial 5, rats in Group 15 showed more gaping than those in any other group (p's<0.05), but no other group differences were significant. The data of the final rejection response of paw treading was analyzed, revealing a significant Dose × Trials effect, $F(16,160) = 1.97$, $p<0.025$. On Trial 5, rats in Group 15 showed more paw treading than those in all groups except Group 7.5 (p's<0.05). Rats in Group 7.5 showed more paw treading than those in Groups 0, 0.5 or 2.5

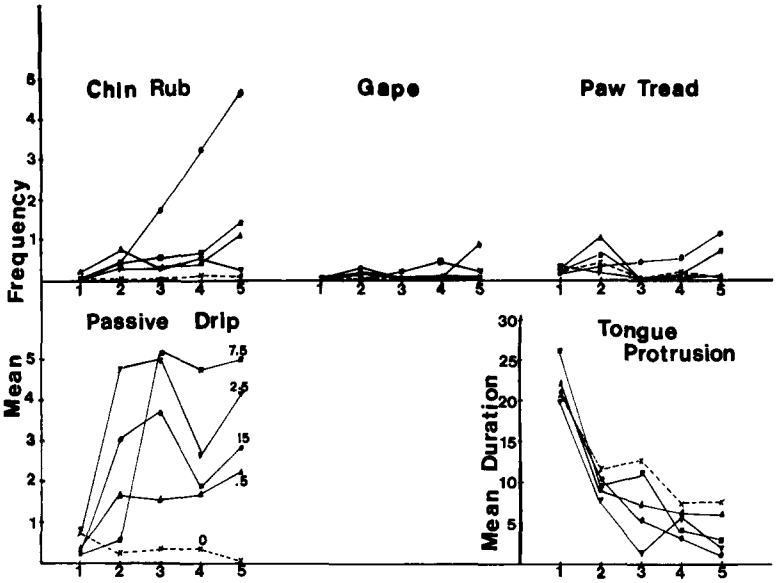

FIG. 2. Mean taste reactivity test responses (frequency or duration) of the various groups in Experiment 1. The legend is indicated in the passive dripping section of the figure.

(p's<0.05). No other effects were significant.

The mean frequency of passive drips is presented in the lower lefthand corner of Fig. 2. The 5×5 Repeated Measures ANOVA revealed a Dose effect, $F(4,40) = 3.0$, $p<0.05$. Rats in Groups 2.5 and 7.5 showed more passive dripping than those in Group 0.0 (p's<0.05). No other effects were significant.

The final taste reactivity measure in Fig. 2 is duration of test period spent showing tongue protrusions. The 5×5 Repeated Measures ANOVA revealed no significant differences among conditions. On the final test day, however, individual t-tests among conditions revealed that rats in Group 0.0 showed a significantly greater duration of tongue protrusion activity than those in Group 15 and Group 2.5 (p's<0.05), but did not differ from any other group.

DISCUSSION

Apomorphine effectively produced conditioned taste avoidance at each dose employed in the above experiment. In fact, we found little evidence that the strength of the avoidance response was differentially effected by the dose of apomorphine within the range of 0.5–15 mg/kg. This finding parallels that reported by Riley, Jacobs and Lolordo (12) who demonstrated that morphine-induced CTAs also do not appear to vary in strength according to the dose of morphine that is administered. Although our CTA test results must be interpreted cautiously, because of the potential problem of floor effects masking group differences, they may indicate that the CTA paradigm is a relatively insensitive measure of dose-response effects of drug agents. On the other hand, the taste reactivity test rejection pattern of responding did differentiate among doses of apomorphine, at least at the high end of the range of doses. A dose of 15 mg/kg of apomorphine was required in order to condition the rejection pattern of chin rubbing (after two conditioning trials) and gaping (after four conditioning trials). Paw treading was evident only after four conditioning trials with either 15 or 7.5 mg/kg of apomorphine. Since the taste reactivity test, but not the CTA test, demonstrated dose-response effects, it may be a more effective measure of flavor-drug associations across dosages.

EXPERIMENT 2

The CTA test of Experiment 1 indicated that the strength of sucrose avoidance was not a function of the dose of apomorphine administered in the nondeprived rats tested in a 15-min single bottle test. Since the rats were not water deprived during the CTA trials, they would not be motivated by thirst to consume the drug-paired solution; therefore, a floor effect may have masked group differences. Experiment 2 represented an attempt to verify the lack of a dose-response CTA produced by apomorphine in rats that were motivated to drink (24-hr water deprived) during conditioning and testing trials. The rats were given two sucrose-apomorphine conditioning trials, which was the number of trials sufficient to condition chin rub rejection responses in Group 15 in Experiment 1. Three days after the final conditioning trial, the rats were presented with sucrose solution in extinction and allowed to consume it for 24 hr. The resistance to extinction assessed at various intervals across the 24-hr period served as a measure of the CTA strength.

METHOD

Forty male Sprague-Dawley rats weighing between 202–235 g on the first conditioning day served as subjects. One week after arriving in the laboratory, the rats were deprived of water. On each of the next four days, the rats were presented with water in a graduated tube for 20 min a day. On the following day, while 24-hr water deprived, the rats were given their first conditioning trial.

On the conditioning trials, the rats were presented with 0.5 M sucrose solution in graduated drinking tubes for 20 min. Immediately upon removal of the tubes, the rats were injected (IP) with the appropriate solution (0.0, 0.5, 2.5, 7.5 or 15 mg/kg apomorphine HCl in solution with physiological saline at a concentration of 1.25 mg/ml). The rats received two identical conditioning trials, separated by two days during which water was presented in a graduated tube for 20 min a day. Two hr after the final conditioning trial, the rats each received water in a bottle for 18 hr in order

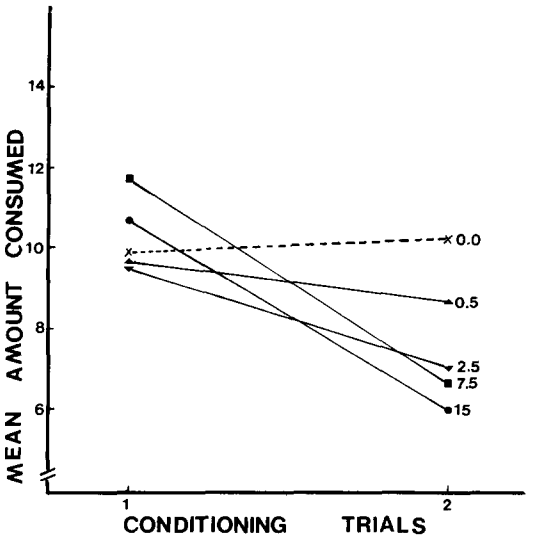

FIG. 3. Mean amount (ml) of sucrose solution consumed on each of two conditioning trials by the various groups in Experiment 2.

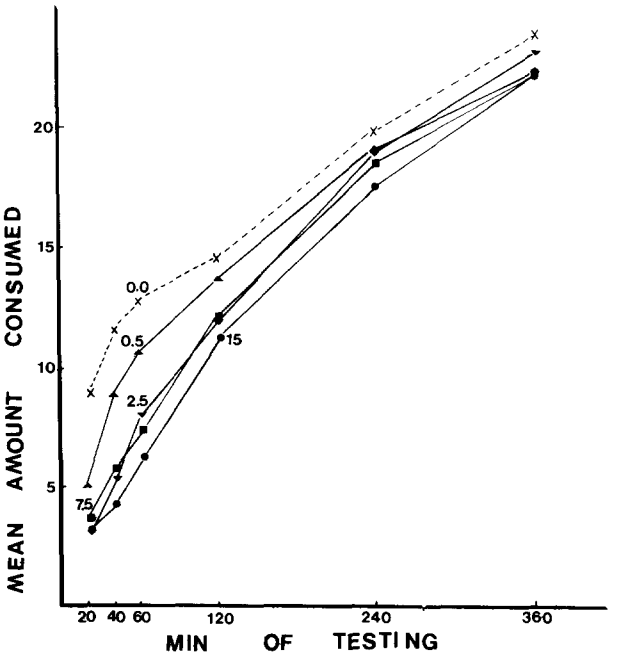

FIG. 4. Mean amount (ml) of sucrose solution consumed across the intervals of extinction testing in Experiment 2.

to replenish water deficits. On the following two days, the rats received 20 min of water a day in graduated tubes. The extinction test trial occurred on the following day. The rats were presented with 0.5 M sucrose solution in a graduated tube and the amount consumed at 20, 40, 60, 120, 240, 360 and 480 min and 24 hr was measured.

RESULTS

The mean amount (ml) of sucrose solution consumed during each conditioning trial of Experiment 2 is presented in Fig. 3. Single-factor ANOVAs for each trial revealed that the groups did not differ on Trial 1, but on Trial 2, there was a significant Groups effect, $F(4,35) = 6.4$, $p<0.01$. As measured by subsequent Newman-Keuls tests, on Trial 2, Group 0.0 drank more sucrose than all other groups (p's<0.05) and Group 0.5 drank more sucrose than Groups 2.5, 7.5, or 15 mg/kg (p's<0.05). Groups 2.5, 7.5 and 15 did not significantly differ from one another.

Figure 4 presents the results of the extinction testing phase of the experiment. The mean cumulative amount (ml) of sucrose solution consumed by each group at each interval is depicted in the figure. Single-factor ANOVAs revealed a significant Groups effect at intervals 20–60 min of testing, F's$(4,35)>2.7$, p's<0.05, but not at any other interval of testing including the 24-hr interval which is not depicted in Fig. 4. Subsequent Newman-Keuls tests at the 20-min interval revealed that Group 0.0 drank more sucrose solution than all other groups (p's<0.05). Additionally, Group 0.5 drank more sucrose than Groups 15 or 2.5 (p's<0.05), but did not differ significantly from Group 7.5. At the 40-min interval, Group 0.0 drank more than all other groups (p's<0.05) and Group 0.5 drank more than Groups 15, 7.5 or 2.5 (p's<0.05). Finally, at the 60-min interval, Group 0.0 drank more than all other groups ($p<0.05$) and Group 0.5 drank more than Group 15 ($p<0.05$), but did not differ from Group 2.5 or 7.5. Groups 15, 7.5 and 2.5 did not significantly differ in their sucrose intake at any interval of extinction testing.

DISCUSSION

In a procedure designed to more effectively determine differences in the strength of a CTA produced by various doses of apomorphine, doses of 2.5, 7.5 and 15 mg/kg did not produce CTAs of different strengths after one conditioning trial as measured by 15-min intake in 24-hr deprived rats or after two conditioning trials as measured by resistance to extinction with repeated assessments taken across 24 hr of testing.

GENERAL DISCUSSION

Although doses of 2.5, 7.5 and 15 mg/kg of apomorphine did not differ in their ability to establish a CTA after two conditioning trials in Experiment 2, they did differ in their ability to condition taste reactivity rejection responses after two conditioning trials in Experiment 1. The rejection responses were only evident during the conditioning/testing trials in Group 15 mg/kg; none of the remaining apomorphine conditioned groups showed rejection responses even though the strength of the CTA produced by the three highest doses of apomorphine was equivalent.

At a dosage of 15 mg/kg (IP), apomorphine produces a conditioned place aversion (3) and rejection responses (14). At doses between 0.25–10 mg/kg (SC), apomorphine has been shown to produce a place preference (17), and at doses between 0.5–7.5 mg/kg (IP) apomorphine produces a CTA, but does not produce rejection responses. Our results thus suggest that only doses of apomorphine which are higher than those which serve as positive reinforcers in rats are capable of conditioning rejection taste reactivity responses. Although doses within the range that serve as positive reinforcers in the conditioned place preference paradigm [e.g., (15,16)] produced strong CTAs over five conditioning/testing trials, they did not produce rejection taste reactivity responses. These results are consistent with those reported for the reinforcing drugs of amphetamine (8,9), nicotine (11) and morphine (10). It is conceivable that high doses of each drug that serves as a positive reinforcer includes toxic properties which may preclude the demonstration of a conditioned place preference. In fact, in the self-administration paradigm, investigators often report that the animals regulate their schedule of responding in accor-

dance with the dose of the drug agent [e.g., (19)]. Although caution must be applied in generalizing our results to drugs other than apomorphine, the taste reactivity test may serve as an additional tool for assessing the abuse potential of pharmacological agents. It may also serve as a test of behavioral toxicity of agents by determining the dose at which a drug agent is no longer positively reinforcing.

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