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Chlordiazepoxide Enhances the Palatability of Lithium-, Amphetamine-, and Saline-Paired Saccharin Solution

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PARKER, L. A. *Chlordiazepoxide enhances the palatability of lithium-, amphetamine-, and saline-paired saccharin solution.* PHARMACOL BIOCHEM BEHAV **50**(3) 345-349, 1995.—The ability of chlordiazepoxide to modify taste reactions elicited by a saccharin solution that was paired on three occasions with amphetamine (1 or 3 mg/kg), lithium (0.3 or 1.2 mEq/kg), or saline solution was assessed using the taste reactivity test. Chlordiazepoxide enhanced positive ingestive reactions regardless of the conditional properties of the tastant and had no effect on aversive reactions. These results support previous reports that chlordiazepoxide directly modifies the palatability of tastants.

Chlordiazepoxide Taste reactivity Taste avoidance Taste aversion Amphetamine Lithium Saccharin Palatability Benzodiazepines Taste

WHEN a flavored solution is paired with lithium chloride, an emetic drug, the flavor becomes distasteful to rats [e.g., (8)]. Not only do rats avoid consuming the flavored solution, but they also display their distaste by emitting a sequence of aversive reactions including chin rubbing, gaping, and paw pushing during a forced exposure to the flavor in the taste reactivity (TR) test paradigm (9). On the other hand, when a flavored solution is paired with amphetamine, a drug that serves as a reward in other paradigms, the flavored solution does not become distasteful, although it is avoided (11).

The behavioral distinction between a flavor paired with lithium and a flavor paired with amphetamine suggests that rats learn a different kind of association based on the two drug agents. It has been suggested that flavor-lithium associations reflect a conditioned distaste, but flavor-amphetamine associations reflect a conditioned danger (15,16), because the pattern of taste reactions elicited by an amphetamine-paired flavor is more like that elicited by a shock-paired flavor than that elicited by a lithium-paired flavor (15).

If a flavor-amphetamine association is based on conditioned fear, then it is possible that pretreatment with an anxiolytic agent that modifies responding to shock-paired cues would also modify responding to an amphetamine-paired taste. Agents such as chlordiazepoxide (CDP) have been demonstrated to attenuate shock-associated responding [e.g., (17)]. In fact, Delamater and Treit (7) reported that CDP pretreatment selectively attenuated conditioned taste avoidance (CTA) produced by shock, but not CTA produced by lithium. They suggest that shock-based CTAs depend upon the action of the defensive system, and that the avoidance of the shock-paired flavor is the result of the flavor acquiring the capacity to signal danger [e.g., (15,16)]. On the other hand, lithium-based CTAs depend upon the action of the palatability system, and the avoidance of the lithium-paired flavored solution is the result of the flavor becoming conditionally distasteful [e.g., (16)]. The findings of Delamater and Treit (7), therefore, suggest that the anxiolytic effects of CDP pretreatment attenuated the avoidance of a dangerous stimulus (shock-paired flavor), but did not attenuate the avoidance of a distasteful stimulus (lithium-paired flavor).

If amphetamine-paired flavors, like shock-paired flavors, acquire the property of signalling danger, rather than becoming distasteful, then it is conceivable that CDP pretreatment will selectively modify the palatability of amphetamine-paired flavors, but not lithium-paired flavors, using the taste reactivity test paradigm (11,12). The following experiment was designed to assess the ability of CDP pretreatment to modify taste reactions elicited by a saccharin solution that was paired on three occasions with amphetamine (1 or 3 mg/kg), lithium (0.3 or 1.2 mEq/kg), or saline. The dose of CDP was 9 mg/kg, which was the dose employed by Delamater and Treit (7).

METHOD

Subjects

The subjects were 53 male Sprague-Dawley rats weighing 351-449 g on the first conditioning trial. They were maintained on ad lib rat chow and water, except as indicated. The rats were housed in individual stainless steel cages for 4 days prior to surgery.

Procedure

On the fourth day after arriving in the laboratory, the rats were surgically implanted with intraoral cannulae, as previously described (10), under sodium pentobarbital anesthesia. After 3 weeks of recovery, the rats were placed on a 24-h water deprivation schedule during which they had 15 min per day access to water from a graduated tube. They received water for 15 min per day for each of 4 days before receiving conditioning trials.

On the conditioning trials, the rats were presented with a graduated cylinder containing 0.1% saccharin solution for 15 min. Immediately after consuming the saccharin solution, all rats were injected IP with the appropriate solution, which was one of: 0.3 mEq/kg lithium chloride [2 ml/kg of 0.15 M LiCl (n = 10)], 1.2 mEq/kg lithium chloride [8 ml/kg of 0.15 M LiCl (n = 10)], 1 mg/kg *d*-amphetamine [2 ml/kg of 0.5 mg/ml (n = 11)], 3 mg/kg *d*-amphetamine [6 ml/kg of 0.5 mg/ml (n = 11)], or physiological saline [8 ml/kg (n = 11)]. The rats received three such conditioning trials, each separated by a recovery day on which they were presented 15 min of water during the drinking period. If a rat drank less than 2 ml on any conditioning trial, a saccharin-filled syringe was inserted into its mouth and 2 ml of saccharin solution was washed over its tongue over a 1-min period. After the final conditioning trial, the rats' water bottles were returned and they were maintained on ad lib food and water for the duration of the experiment.

Three days after the final conditioning trial, the rats were adapted to the taste reactivity test procedure on each of two trials that were separated by 24 h. On each trial, the rat was transported into the testing room and placed into the test chamber (22 × 26 × 20 cm). A 15-cm infusion hose was connected to a Gage Infusion Pump. After remaining in the chamber for 1 min, the rat received an infusion of water through its cannula for 2 min at the rate of 1 ml/min. The rat was then returned to its home cage and the test chamber was cleaned.

Twenty-four hours later, the rats received the first of two taste reactivity test trials that were separated by 24 h. One test occurred after pretreatment with chlordiazepoxide and the other test occurred after pretreatment with saline solution. The order of the trials was counterbalanced among the groups. On each test trial, the rats were injected IP with either 9 mg/kg of chlordiazepoxide [prepared with saline (4.5 mg/ml)] or saline solution 30 min prior to placement in the taste reactivity chamber. On these trials, the rats were treated identically as in the adaptation trials except that they were introrally infused with the 0.1% saccharin solution with which they had been conditioned. The rats' orofacial and somatic responses were videotaped during the infusions.

The videotapes were later scored by a rater blind to experimental conditions with the use of an event recorder program, the Observer (Noldus, NL), on an IBM computer. The behaviors that were measured have been previously described [e.g., (1)]. These behaviors included aversive responses, ingestive responses, neutral/mildly aversive passive dripping, and activity responses. The aversive responses included the frequency of chin rubbing (mouth in direct contact with the floor or a wall and projecting the body forward), gaping (large amplitude, rapid opening of the mandible with concomitant retraction of the corners of the mouth), and paw treading (sequential extension of one forelimb forward against the floor while the other forelimb is being retracted). These aversive responses were combined to produce a composite aversive score. The ingestive responses included tongue protrusions (rapid movement of the tongue in a forward and lateral direction), paw licking (licking the forelimb paws while they are held close to the mouth), and mouth movements (low amplitude movements of the mandible). The number of seconds that the rats displayed each of these reactions within the 2-min period was scored and these scores were combined to produce a composite ingestion score. The neutral/mildly aversive response of frequency of passive dripping (number of drips that fall from the rats mouth when the rat is not actively ejecting the solution by a rejection response) was also measured. Finally, as an assessment of general activity level, the frequency of bouts of vertical movements (rearing with forepaws off the floor) and horizontal movements (movement with forepaws on the floor of the cage) were measured and combined.

RESULTS

Figure 1 presents the mean amount of saccharin solution consumed during each of the three conditioning trials by the various groups. A 5 × 3 repeated-measures ANOVA revealed a significant group effect, $F(4, 48) = 17.8$, $p < 0.01$, trials effect, $F(2, 96) = 52.6$, $p < 0.01$, and group × trials interaction, $F(8, 96) = 17.1$, $p < 0.01$. Subsequent single-factor ANOVAs assessed the group effect on each conditioning trial. On Trials 2 and 3, the group effect was significant, $Fs(4, 48) > 17.2$, $ps < 0.01$. Newman-Keuls pairwise comparison tests revealed that on Trial 2, Groups 1.2 mEq/kg lithium and 3 mg/kg amphetamine drank less saccharin solution than all groups ($ps < 0.05$) and Group saline drank more saccharin than all groups ($ps < 0.05$). On Trial 3, Groups 1.2 mEq/kg lithium and 3 mg/kg amphetamine drank less saccharin than all other groups ($ps < 0.05$) and Group saline drank more saccharin than all other groups ($ps < 0.05$). No other groups differed significantly.

The mean frequency or duration of the taste reactivity responses displayed during the infusion of saccharin solution during the test phase is presented in Figs. 2 and 3. Figure 2

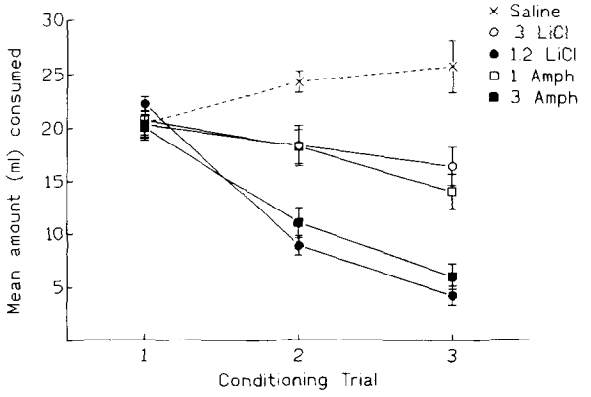

FIG. 1. Mean amount of saccharin solution consumed by the various groups on each conditioning trial.

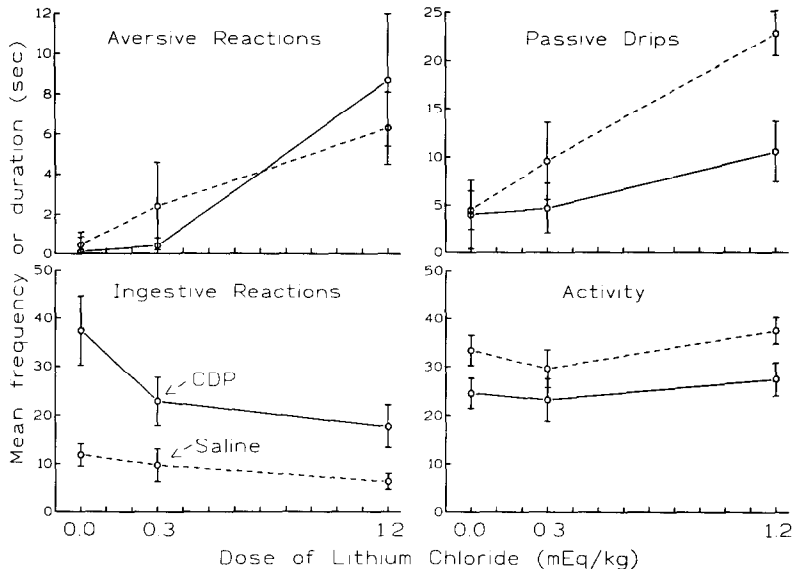

FIG. 2. Mean frequency or duration of each category of taste reaction elicited by 0.1% saccharin solution previously paired with one of 0.0, 0.3, or 1.2 mEq/kg of 0.15 M LiCl following pretreatment with CDP or saline.

presents the data for the rats conditioned with lithium and Fig. 3 presents the data for the rats conditioned with amphetamine. The rats conditioned with saline are included in both figures and in both sets of analyses. For both sets of analyses, the frequency or duration of each reaction was analyzed by a 3 × 2 mixed-factors ANOVA with the between-groups factor of dose of drug and the within-groups factor of pretreatment condition.

For the rats conditioned with lithium, the analyses revealed a significant dose effect for the categories of aversive reactions, $F(2, 28) = 3.6$, $p < 0.05$, passive drips, $F(2, 28) = 6.3$, $p < 0.01$, and ingestive reactions, $F(2, 28) = 3.6$, $p < 0.05$. Subsequent pairwise Newman-Keuls comparison tests revealed that the rats conditioned with 1.2 mEq/kg of lithium displayed more aversive reactions and passive drips than any other group ($ps < 0.05$), and the rats conditioned with 1.2 mEq/kg of lithium displayed less ingestive responding than the rats conditioned with saline. The 3 × 2 ANOVAs also revealed a significant effect of pretreatment condition for passive dripping, $F(1, 28) = 10.8$, $p < 0.01$, ingestive reactions,

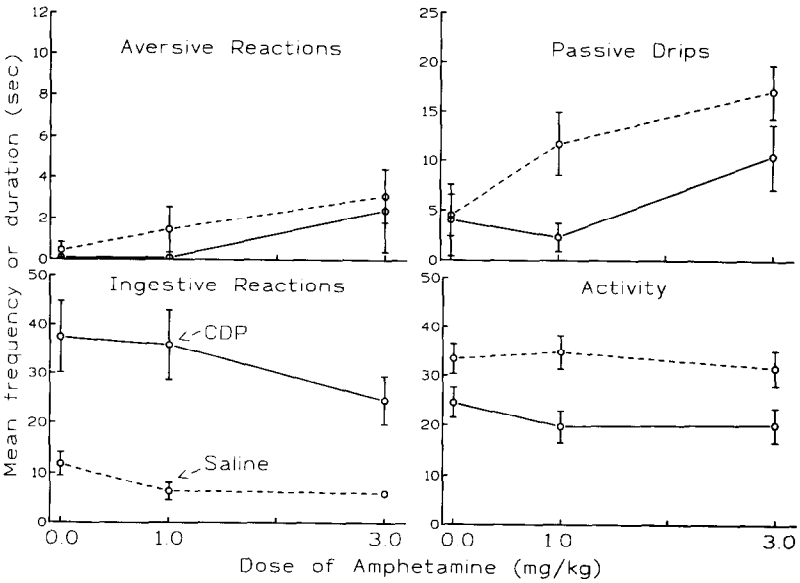

FIG. 3. Mean frequency or duration of each category of taste reaction elicited by 0.1% saccharin solution previously paired with one of 0.0, 1, or 3 mg/kg of *d*-amphetamine following pretreatment with CDP or saline.

$F(1, 28) = 25.6, p < 0.01$, and activity $F(1,18) = 10.8, p < 0.01$. Chlordiazepoxide pretreatment suppressed passive drips, enhanced ingestive reactions, and suppressed activity, regardless of the dose of lithium. Finally, the 3 × 2 ANOVAs revealed a significant dose × pretreatment condition interaction for the reaction of passive dripping only, $F(1, 28) = 3.7$, $p < 0.05$; only the group conditioned with 1.2 mEq/kg of lithium displayed less passive dripping after chlordiazepoxide pretreatment than after saline pretreatment ($p < 0.01$).

Figure 3 presents the mean frequency or duration of each category of reaction elicited by saccharin solution paired with 0.0, 1.0, or 3.0 mg/kg of amphetamine. The 3 × 2 mixed-factor ANOVAs revealed a significant pretreatment effect on ingestive reactions, $F(1, 30) = 45.1, p < 0.01$, passive dripping, $F(1, 30) = 8.3, p < 0.01$, and activity, $F(1, 30) = 30.3$, $p < 0.01$. Chlordiazepoxide pretreatment nonselectively enhanced ingestive reactions and suppressed passive dripping and activity elicited by an infusion of saccharin solution. Additionally, the dose effect was significant for the reaction of passive dripping, $F(2, 30) = 4.6$, $p < 0.025$; subsequent Newman-Keuls tests revealed that Group saline demonstrated fewer passive drips than did Group 3.0 amphetamine ($p < 0.05$). No other effects were significant.

DISCUSSION

Chlordiazepoxide nonselectively enhanced the palatability of saccharin solution that had been previously paired on three occasions with lithium, amphetamine, or saline solution. The enhancement of palatability was apparent by the increased proportion of the test period that the rats spent displaying ingestive reactions (a measure of positive palatability) and the decreased frequency of passive drips (a measure of mildly aversive, neutral palatability) displayed after CDP pretreatment.

Although a floor effect in the display of aversive reactions in all other groups precluded the demonstration of CDP-induced suppression of aversive reactions, CDP did not attenuate the frequency of aversive reactions displayed in the group of rats conditioned with the highest dose of lithium (1.2 mEq/kg). CDP did not appear to modify aversive reactions in the group that displayed aversive reactions. The selective enhancement of ingestive reactions produced by CDP provides some support for Berridge and Grill's (1) two-dimensional model of palatability, which contends that the hedonic and aversive properties of tastants are processed independently of one another. An alternative unidimensional model of palatability processing has also been recently proposed (3).

Benzodiazepines have been reported to enhance feeding [see (5)]. Cooper (4) has suggested that this effect is the result of the enhancement of the positive palatability of foods by benzodiazepine pretreatment. In two-choice saccharin preference tests, a number of benzodiazepine agonists have been shown to selectively enhance the intake of a preferred saccharin solution relative to that of water (5,6). Furthermore, using the taste reactivity test, Berridge and Treit (2) demonstrated that the benzodiazepine agonist, chlordiazepoxide (CDP), enhanced ingestive taste reactivity patterns during an intraoral infusion of a variety of flavored solutions without modifying the aversive reactions elicited by those tastants. This CDP-induced enhancement of positive palatability was also effectively blocked by pretreatment with benzodiazepine antagonists (17).

CDP has also been reported to nonspecifically enhance rats' preference for saccharin solution in a two-bottle taste avoidance test, regardless of whether it had been previously paired on three occasions with lithium, amphetamine, or saline solution (13). Therefore, the results of the present experiment suggest that the effect of CDP on the intake of conditionally avoided flavors is a function of enhancement of palatability (2) rather than a function of its anxiolytic properties (7). Furthermore, they suggest that CDP pretreatment effects do not effectively distinguish between CTAs based on "danger" and CTAs based on "distaste" (16), because CDP pretreatment modified both lithium-induced palatability shifts and taste avoidance (13).

Even though the strength of the taste avoidance produced by the high dose of lithium and amphetamine was equivalent on the third conditioning trial, lithium- but not amphetamine-paired saccharin elicited aversive reactions [e.g., (11)]. This suggests that the mechanism responsible for lithium-induced CTA is different from that responsible for an amphetamine-induced CTA. However, the results of the present experiment suggest that CDP pretreatment did not selectively modify the palatability of a lithium-paired, amphetamine-paired, and saline-paired saccharin solution; instead, it nonselectively enhanced the hedonic properties of saccharin solution regardless of its conditional properties. On the other hand, it has been reported (14) that pharmacological pretreatment with the antiemetic agent, trimethobenzamide, selectively attenuated the chin rub reaction of the aversive pattern elicited by lithium-paired saccharin without modifying the taste reactions elicited by amphetamine-paired saccharin. This suggests that a lithium-paired flavor, but not an amphetamine-paired flavor, may elicit a conditioned sickness reaction. However, the mechanism responsible for amphetamine-induced taste avoidance remains elusive.

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