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RAPID COMMUNICATION

Chin Rub CRs May Reflect Conditioned Sickness Elicited by a Lithium-Paired Sucrose Solution

LINDA A. PARKER AND KAREN B. MACLEOD

Department of Psychology, Wilfrid Laurier University

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PARKER, L. A. AND K. B. MACLEOD. *Chin rub CRs may reflect conditioned sickness elicited by a lithium-paired sucrose solution.* PHARMACOL BIOCHEM BEHAV **40**(4) 983–986, 1991.—Rats were given a single conditioning trial in which 20% sucrose solution was paired with an intraperitoneal (IP) injection of lithium chloride (127.2 mg/kg), d-amphetamine (3 mg/kg) or physiological saline. Thirty min before a subsequent 10-min taste reactivity (TR) test and a 1-h conditioned taste avoidance (CTA) test the rats were injected IP with either the antiemetic agent, trimethobenzamide (1 mg/kg) or with physiological saline solution. The lithium-paired, but not the amphetamine- or saline-paired, sucrose solution elicited the aversive TR responses of chin rubs, paw pushes and gapes. Trimethobenzamide suppressed the aversive TR response of chin rubs in the lithium-conditioned group, but not in a group given unconditionally aversive quinine solution. The CTA test was not sensitive to the antiemetic properties of trimethobenzamide, although the drug enhanced sucrose preference overall. The results suggest that chin rub responses may measure conditioned sickness.

Conditioned response Amphetamine Chin rub Rat Lithium Sucrose Conditioned taste aversion

A conditioned taste aversion (CTA) is produced when a flavored solution is paired with an emetic agent such as lithium chloride [e.g., (3)]. Garcia and his colleagues [e.g., (2)] argue that such a CTA is mediated by a conditioned sickness reaction [see also (6)]. That is, the conditioned stimulus (CS; the taste) having been paired with the unconditioned stimulus (US; the emetic agent, such as lithium) which produces the unconditioned response (UR; sickness) gains the associative capacity to elicit the conditioned response (CR; sickness). They argue that the CR elicited by the CS flavor reflects an hedonic shift displayed as disgust reactions which include gaping and chin rubbing. These responses have more recently been described by Grill and Norgren (5) as aversive taste reactivity (TR) responses. Aversive TR responses are elicited by unconditionally or conditionally aversive tasting solutions [e.g., (5)].

Aversive TR responses appear to be selectively elicited by flavors paired with nonreinforcing drugs [e.g., (8)]. Although rats avoid consuming flavored solutions paired with reinforcing drugs, such as amphetamine, they do not display aversive TR responses to these flavored solutions. In fact, even at a dose which produces a weaker CTA than that produced by amphetamine, lithium is more effective in establishing aversive TR responses than is amphetamine following pairings with sucrose (11).

If, as suggested by Garcia and colleagues [e.g., (2)], aversive TR responses reflect conditioned sickness, then lithium-based, but not amphetamine-based CTAs may be motivated by conditioned sickness. Furthermore, if a lithium-based CTA and aversive TR responses are motivated by conditioned sickness, then pretreatment with an antiemetic drug prior to a test for conditioning should reduce the strength of the conditioned sickness and, therefore, should reduce the strength of the aversive TR responses elicited by lithium-paired sucrose solution. Coil, Hankins, Jenden and Garcia (1) reported that pretreatment with a number of antiemetic agents attenuates a lithium-based CTA; however, others have failed to reproduce the effect (4,10). These studies employed the standard consummatory CTA test which only indirectly measures CRs elicited by the flavored solution. A more direct measure of the aversive CRs elicited by the tastant is the TR test (5). If aversive TR responses reflect conditioned sickness responses, then pretreatment with an antiemetic drug may attenuate the strength of these responses. The experiment below measured the ability of the antiemetic agent, trimethobenzamide, to modify the CRs elicited by lithium-paired and amphetamine-paired sucrose solution using both the TR and the CTA tests. The dose of trimethobenzamide (1 mg/kg) selected was based on the findings of Coil et al. (1).

METHOD

Subjects

Fifty-seven male Sprague-Dawley rats weighing between 231 and 295 grams on the conditioning day served as subjects. The

[1]Requests for reprints should be addressed to Linda Parker, Department of Psychology, Wilfrid Laurier University, Waterloo, Ontario N2L 3C5.

rats were maintained on ad lib rat chow and water except as described, and were housed in individual stainless steel cages in a room maintained on a 12:12 light:dark schedule.

Procedure

One week after their arrival in the laboratory, the rats were implanted with intraoral cannulae as previously described (7).

After each rat had at least 3 days to recover from surgery, the rats were trained to consume their daily water supply in 20 min for two days. On the following day, 44 rats received a conditioning trial and 15 rats received 20 minutes access to water. During the conditioning trial, each rat was presented with a tube of 20% sucrose solution for 20 minutes in its home cage and the amount consumed was measured. Immediately following sucrose consumption, the rat was injected intraperitoneally (IP) with 127.2 mg/kg of 0.15 M lithium chloride (L; n=14), 3.0 mg/kg of d-amphetamine sulfate (A; n=16), or physiological saline solution (S; n=14). All rats had water bottles returned to their cages at the end of the conditioning trial and were maintained on ad lib water for the remainder of the experiment.

On the following two days all rats were adapted to the TR test procedure. During the adaptation trials, a rat was transported into the room that contained the 22.5 by 26 by 20 cm glass TR test chamber. The room was illuminated by three 100-W light bulbs, one focused on either side of the chamber and one directed towards a mirror located beneath the chamber at a angle to facilitate viewing orofacial and somatic responses of the rat. The rat was placed into the test chamber and a 30-cm infusion hose was then connected to the cannula through the ceiling of the chamber. A syringe was connected to the hose and placed into the holder of an Infusion Pump (Harvard Apparatus, Model 22). After 60 s, the pump delivered water through the tube into the rat's mouth at the rate of 1 ml/min for 2 min. The rat was then removed from the test chamber, had its cannula flushed with water, and was returned to its home cage.

Two to four days after the adaptation trial, the rats received the TR test. Twenty minutes before the TR test, the rats were given an IP injection of 1 mg/kg of trimethobenzamide (T) or physiological saline (S). The conditioned groups that received 20% sucrose during the TR test were as follows: TL (n=7), TA (n=8), TS (n=7), SL (n=7), SA (n=8), and SS (n=7). Additionally, the 15 rats that did not receive a conditioning trial with sucrose were injected with trimethobenzamide (TQ, n=8) or saline (SQ, n=7) 20 minutes before receiving a TR test with 0.05% quinine solution in order to determine the effect of trimethobenzamide on TR responding elicited by the unconditionally aversive quinine solution. During the TR test, the rat was placed in the test chamber and one minute later was intraorally infused with 20% sucrose solution (n=44) or 0.05% quinine solution (n=15) rather than water at a rate of 1 ml/minute over a 10-minute period. The rats' orofacial and somatic responses were videotaped during the TR test by means of a Panasonic videocamera focused on the mirror beneath the chamber; these videotaped records were later scored by a rater blind to the experimental conditions by means of The Observer event recording program (Noldus, The Netherlands) on an IBM microcomputer.

Immediately after the TR test, each rat was presented with two graduated tubes, one containing the same solution received during the TR test (either 20% sucrose solution or 0.05% quinine solution), and the other containing water for 1 h. The spouts of the bottles were located within 3 cm of one another with the water always being presented on the right side. The amounts consumed were converted to sucrose or quinine preference ratios (PRs) on the basis of the following formula: The amount of sucrose or quinine solution consumed divided by the total of the amount of sucrose or quinine solution and the amount of water consumed.

The behaviors that were later scored by the rater from the videotapes of the TR test included the following. The frequency of the aversive TR responses of chin rubbing (CR: mouth or chin in direct contact with floor or wall of chamber and body projected forward), gaping (G: rapid large amplitude opening of the mandible with retraction of corners of mouth), and paw pushing (PP: sequential extension of one forelimb against the floor or wall of the chamber while the other forepaw was being retracted). The duration of the ingestive TR responses of tongue protrusions (TP: extensions of the tongue out of the mouth), paw licking (PL: licking the flavored solution from the forepaws) and mouth movements (MM: movement of the lower mandible without opening the mouth). These scores were combined to produce a total ingestive response score. Finally, to ensure that changes in TR responding were not merely a function of modified generalized activity, the following activity measures were also scored from the videotapes: Duration of rearing (both front forepaws lifted off the floor, whether placed against wall or not) and horizontal locomotion (horizontal movement of the rat's forepaws along the floor of the chamber). These scores were combined to produce a total activity score (ACT).

RESULTS

Figure 1 presents the mean number of the aversive TR responses of chin rubs, paw pushes and gapes displayed by the various groups during the TR test. The solid bars represent the rats pretreated with trimethobenzamide and the speckled bars represent the rats pretreated with saline during the test trial. The left-hand section of the figure depicts the conditioned TR responses elicited by sucrose that had previously been paired with lithium, amphetamine or saline and the right-hand section of the figure depicts the unconditioned TR responses elicited by quinine solution. The data for the sucrose conditioned TR responses was analyzed as a 2 by 3 ANOVA for the factors of pretreatment condition and US drug condition for each of the aversive TR responses. Each ANOVA revealed a significant effect of US drug condition, F's(2,38)>6.1, p's<0.01; subsequent Newman-Keuls tests revealed that for each of the conditioned aversive TR responses, the lithium US group displayed more responding than did the amphetamine or saline US groups (p's<0.05). Furthermore, for the TR response of chin rubbing only, there was a significant pretreatment by US drug condition interaction, $F(2,38)=4.4$, $p<0.025$; subsequent Newman-Keuls analysis revealed that group SL showed more chin rubbing than any other group (p's<0.05). Group TL did not differ from any other group. A 2 by 3 ANOVA for the total activity score revealed no significant effects.

The data on the right-hand side of Fig. 1 depicts the unconditioned aversive TR responses elicited by quinine solution. The mean frequency of responding elicited by trimethobenzamide- and saline-pretreated groups were compared with t-tests for each aversive TR response. None of these analyses were significant.

Although trimethobenzamide attenuated conditioned chin rub responding elicited by lithium-paired sucrose solution, it did not attenuate unconditioned chin rub responding elicited by quinine solution. A t-test between the pretreatment conditions for the total activity score revealed no significant effect.

Figure 2 presents the mean number of seconds that the rats in the various groups spent displaying the ingestive responding of tongue protrusions, paw licking and mouth movements. A 2 by 3 ANOVA revealed no significant US drug, pretreatment or

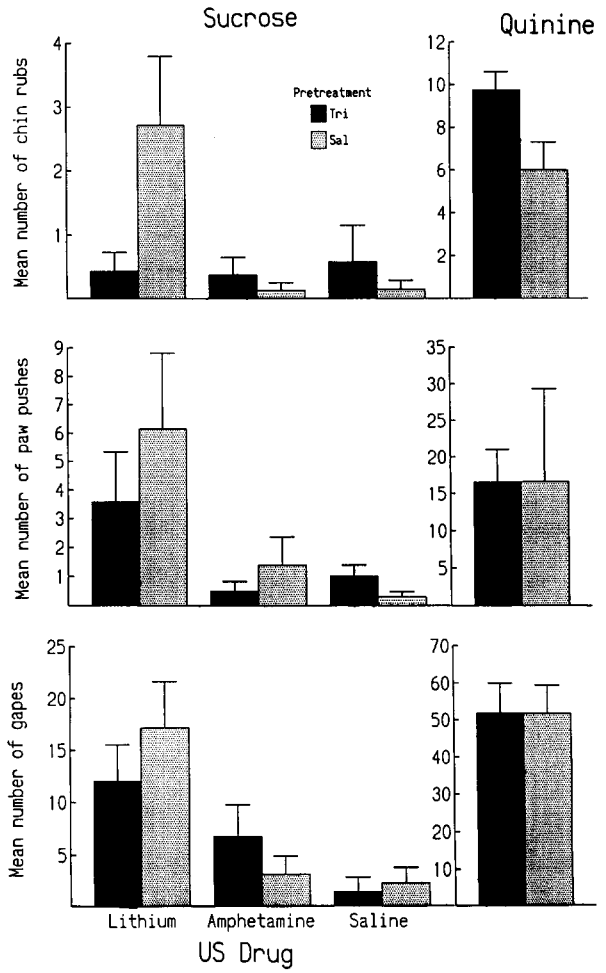

FIG. 1. Mean number of the aversive TR responses of chin rubs, paw pushes and gapes elicited by sucrose previously paired with lithium, amphetamine or saline (left-hand side) or by unconditionally aversive quinine solution (right-hand side). The solid bars represent the trimethobenzamide-pretreated group and the speckled bars represent the saline-pretreated group.

US drug by pretreatment effects for the groups tested with the drug-paired sucrose solution. Furthermore, the pretreatment condition did not effect ingestive TR responding elicited by quinine solution.

Figure 3 presents the mean sucrose preference ratios displayed during the two-bottle CTA consumption test for each of the groups. A 2 by 3 mixed factor ANOVA for the conditioned groups sucrose preference ratios revealed only a significant effect of pretreatment condition, $F(1,38)=5.4$, $p<0.025$; the rats pretreated with trimethobenzamide demonstrated an overall enhanced preference for sucrose solution regardless of the US drug condition. No other effects were significant. Presumably the 10-min TR sucrose exposure attenuated the strength of the subsequent CTA. A *t*-test revealed that the rats' preference for quinine solution was not affected by trimethobenzamide pretreatment.

DISCUSSION

Although both conditionally aversive lithium-paired sucrose solution and unconditionally aversive quinine solution elicited

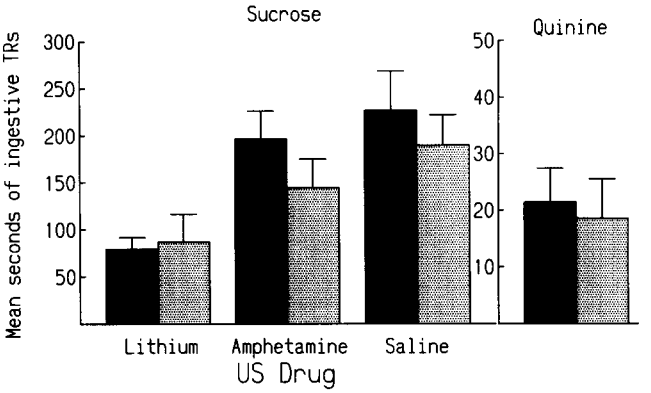

FIG. 2. Mean number of seconds that the rats in the various groups displayed ingestive TR responding. The solid bars represent the trimethobenzamide-pretreated group and the speckled bars represent the saline-pretreated group.

chin rub TR responses, pretreatment with the antiemetic agent trimethobenzamide eliminated only the conditioned chin rub responses elicited by the lithium-paired sucrose solution. This was not simply a function of nonspecific suppression of activity, since trimethobenzamide did not affect vertical and horizontal activity during the TR test.

Since chin rub responding was selectively elicited by the emetic agent, lithium chloride, and was specifically suppressed in the lithium-conditioned group by the antiemetic agent, trimethobenzamide, these responses may reflect conditioned sickness as initially suggested by Garcia and colleagues (2). The chin rub responding elicited by unconditionally aversive quinine solution, on the other hand, does not appear to be mediated by a sickness reaction, since trimethobenzamide pretreatment did not modify the frequency of such responding.

The antiemetic agent, trimethobenzamide, not only suppressed chin rub CRs elicited by a lithium-paired flavor, but also nonspecifically enhanced preference for sucrose in the consumption test. Regardless of the conditioned properties of the sucrose solution, trimethobenzamide enhanced sucrose preference, but did not affect the ingestive TR responding elicited by sucrose solution. A similar nonspecific enhancement of sucrose preference regardless of its conditioned properties is evident in chlordiazepoxide pretreated rats (9). Whether this effect is related to the antiemetic properties of trimethobenzamide is presently unclear.

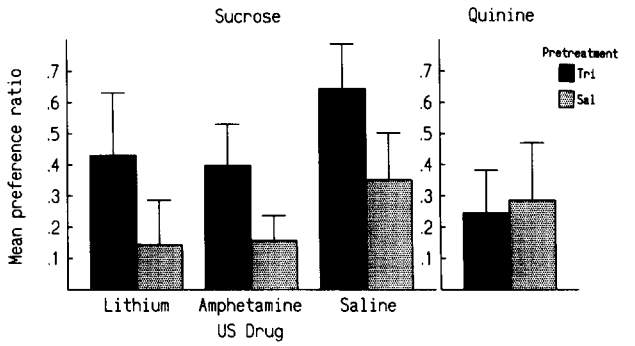

FIG. 3. Mean sucrose and quinine preference ratios for the various groups which were pretreated with either trimethobenzamide (solid bars) or saline (speckled bars) during the CTA test.

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