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Characterization of the Conditioned Taste Aversion Produced by 7-OH-DPAT in Rats

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BEVINS, R. A., T. A. DELZER AND M. T. BARDO. *Characterization of the conditioned taste aversion produced by 7-OH-DPAT in rats.* PHARMACOL BIOCHEM BEHAV 53(3) 695-699, 1996.—Using the conditioned taste aversion preparation, we described the dose-effect curve for the reputed dopamine D_3 agonist (±)7-OH-DPAT (0, 0.001, 0.01, 0.1, 1.0, or 10 mg/kg). Rats received a 0.1% saccharin taste paired on repeated occasions with one of the 7-OH-DPAT doses. The 0.1, 1.0, and 10.0 mg/kg doses of 7-OH-DPAT produced a significant conditioned saccharin aversion. This aversion was evident regardless of whether saccharin intake of 7-OH-DPAT-treated rats was compared to their own water consumption or to saccharin intake by saline-treated rats.

7-Hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin 7-OH-DPAT Saccharin taste aversion Dopamine
D_3 Receptor D_2 Receptor Reward Amphetamine Cocaine Sigma receptor

ANIMALS that have had an emetic experience following ingestion of a novel food or fluid will avoid that item upon subsequent exposure. This phenomenon has come to be termed conditioned taste aversion (CTA). In the laboratory, taste aversion is typically studied by allowing a rat brief access to a novel taste solution (e.g., saccharin) and then administering an illness-inducing agent such as lithium chloride (LiCl). On the next experience with this solution, previously poisoned rats consume less of the fluid relative to their own baseline intake or relative to nonpoisoned control rats.

Much of the work examining the behavioral and neural mechanisms of CTA have employed peripheral emetics like LiCl (30,31). However, drugs of abuse such as amphetamine and cocaine that primarily act on central dopamine systems also induce taste aversions (5,6,9,11). Indeed, central dopamine systems appear to play an important part in the acquisition of taste aversion (12,20,28). For instance, conditioned taste aversions have been reported with the direct dopamine agonist apomorphine, the dopamine D_1-receptor agonist SKF 38393, and the D_2-receptor agonist quinpirole (2,16,27). Furthermore, acquisition of amphetamine-induced taste aversion is attenuated by dopamine D_1- and D_2-like antagonists (14,19).

Although D_1 and D_2 receptors have been implicated in CTA, recent molecular cloning research has identified a dopamine D_3 receptor subtype (24). With the finding that D_3 receptors were more concentrated in the nucleus accumbens than the striatum (24), so came the interest in the behavioral function of this binding site. The putative dopamine D_3-selective agonist 7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) has provided researchers an initial tool for evaluating the role of the D_3 receptor in preparations like self administration, place conditioning, drug discrimination, and drug-induced behaviors (1,4,8,21,22,25). The aversive properties of 7-OH-DPAT as assessed in the taste aversion paradigm has yet to be described. Although most substances administered at a high dose will produce a taste aversion, an aversion acquired using a relatively low dose of 7-OH-DPAT would suggest a role for the dopamine D_3 receptor subtype in dopamine-agonist induced CTAs.

METHOD

Animals

Forty-two male Sprague-Dawley rats from Harlan Industries (Indianapolis, IN) were housed individually in standard hanging wire-mesh cages. Food was continuously available in the home cage, but water access was restricted as described later. The colony was on a 12 L : 12 D cycle; all work was performed during the light cycle.

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Apparatus and Drugs

For each experiment, all fluids were presented in a rack of hanging cages (24.5 × 17.5 × 17.5 cm high) similar to the home cages. A 100-ml graduated drinking tube was mounted on the front of each cage. This tube allowed fluid intake to be measured to the nearest milliliter. The taste cue was a 0.1% sodium saccharin solution (w/v).

(±)7-OH-DPAT (Research Biochemicals International, Natick, MA) was dissolved in saline (0.9% NaCl). The salt form of the drug was used to calculate dosages and all injections were subcutaneous (SC).

Procedure

Two different experiments were conducted. In one experiment, 24 rats (n = 6 per group; 206-252 g) served to examine the ability of low doses of 7-OH-DPAT (0, 0.001, 0.01, or 0.1 mg/kg) to induce a saccharin aversion. The second experiment used 18 rats (n = 4 to 5 per group; 288-354 g) to examine higher doses of 7-OH-DPAT (0, 0.1, 1.0, or 10.0 mg/kg).

The day before each experiment started, each rat's water was removed from the home cage. All subsequent fluids were given in the rack of cages with the graduated drinking tubes. On each of the first 3 days, rats received 15-min access to water. The first conditioning trial was on day 4. Conditioning was conducted every other day as follows: rats received 15-min access to the 0.1% saccharin solution followed immediately by a 1 ml/kg injection of the appropriate drug. In the two experiments, all rats were given three saccharin conditioning trials. This training was extended by three trials (six trials total) for the low-dose 7-OH-DPAT experiment (see later). Intervening between each conditioning trial was a 15-min water access day.

Data Analyses

Saccharin and water intake were first analyzed using repeated measure analyses of variance (ANOVAs) with dose as one factor and trial as the repeated measure. Given a significant trial by dose interaction, Student-Newman-Keuls tests were employed to determine the source of the interaction. Statistical significance for all analyses was declared at a two-tailed probability of 0.05.

RESULTS

The top panel of Fig. 1 shows the mean saccharin intake across the three conditioning trials from the low-dose 7-OH-DPAT experiment. The repeated-measure ANOVA found a main effect of dose, $F(3, 20) = 3.61$, and a significant trial × dose interaction, $F(6, 40) = 5.35$. The main effect of trial was not significant, $F(2, 40) = 1.92$. Subsequent contrasts revealed that the 0.1 mg/kg group consumed significantly less saccharin than the control group and the 0.001 mg/kg group after a single administration of 7-OH-DPAT (saccharin trial 2). The 0.01 mg/kg group did not differ from any of the groups on saccharin trial 2. On saccharin trial 3, the 0.1 mg/kg group drank less saccharin than all other groups. No other differences among the groups were significant. Because of a trend for aversion at the lower doses, we conducted three more conditioning trials (data not shown). On the sixth trial, the 0.001 mg/kg group (mean = 14.5 ml) did not differ from the saline control group (mean = 16.17 ml). However, saccharin intake was significantly lower than the saline group for the 0.01 and 0.1 mg/kg groups (mean = 12.83 and 3.17 ml, respectively).

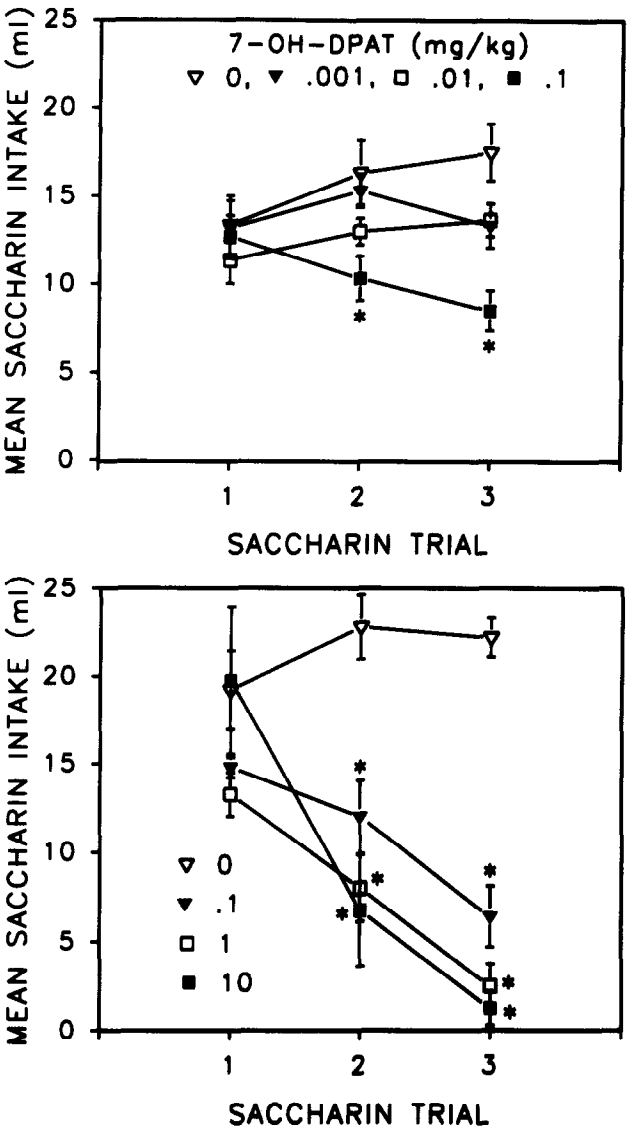

FIG. 1. The top graph shows the mean saccharin consumption across the three saccharin conditioning trials for each group in the low-dose 7-OH-DPAT experiment. The bottom graph shows the corresponding data from the high-dose 7-OH-DPAT experiment. The asterisk (*) denotes significantly less consumption than the saline control ($p < 0.05$).

The bottom panel of Fig. 1 shows the consumption data from the high-dose 7-OH-DPAT experiment. The main effect of dose, $F(3, 14) = 14.2$, the main effect of trial, $F(2, 28) = 39.01$, and the interaction, $F(6, 28) = 11.64$, were significant. All doses produced a taste aversion relative to the saline control after one conditioning trial. On saccharin trial 3, all dose groups again consumed less saccharin than the control group. Also, the 10 mg/kg group drank less saccharin than the 0.1 mg/kg group. No other differences among groups at any trial were significant.

It could be argued that the 7-OH-DPAT-induced saccharin aversion just described may reflect a general aversion to the water bottle and not to the saccharin CS. If so, then consumption on water days should have a pattern similar to that seen

for saccharin even though only tap water was present in the bottles. We found no evidence to support this suggestion. For the low- and high-dose 7-OH-DPAT experiments, there were no significant differences in water intake on each day that preceded a saccharin trial, Fs $\leq$ 1.39.

It is of interest to determine for each group whether saccharin consumption reflects a preference or an aversion relative to water intake. To do so, we compared consumption of saccharin on trial 3 for each group relative to its water intake on the preceding day. These analyses provided a within-subject test for the preference/avoidance of saccharin relative to water. The top half of Fig. 2 shows the water (open bars) and saccharin intake (hatched bars) for each group in the low-dose

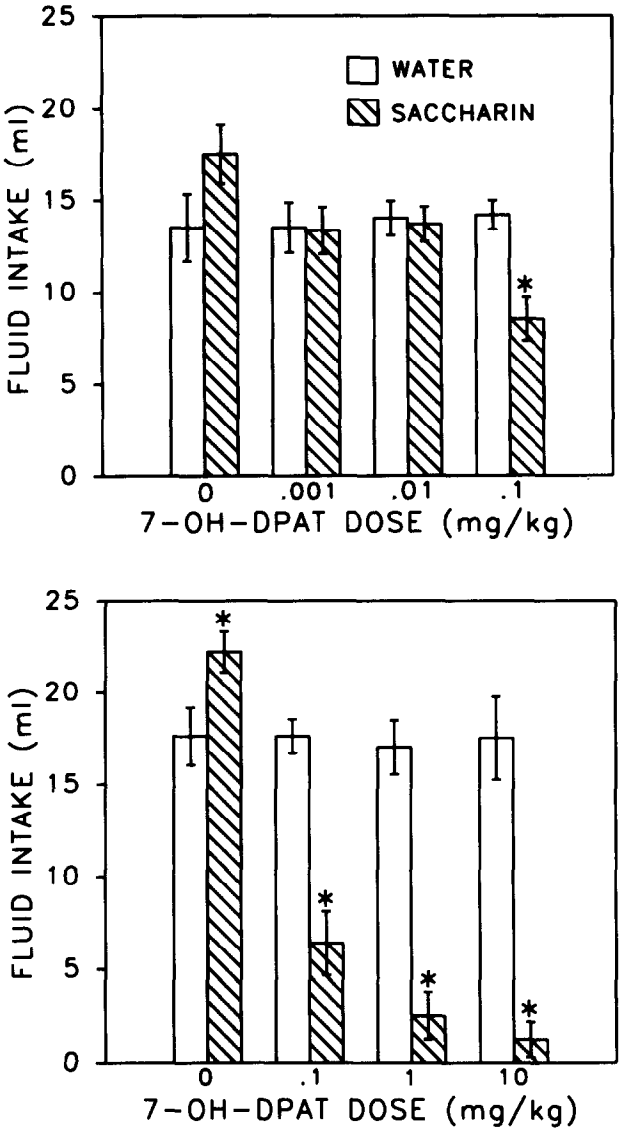

FIG. 2. The top graph shows for each group in the low-dose 7-OH-DPAT experiment the mean saccharin intake on the third conditioning trial (hatched bars) vs. its water intake on the immediately preceding water day (empty bars). The bottom graph shows the corresponding data from the high-dose 7-OH-DPAT experiment. The asterisk (*) denotes a significant difference between water and saccharin consumption ($p < 0.05$).

7-OH-DPAT experiment. Although the saline control showed a tendency for a saccharin preference, this difference in consumption was not significant, $t(10) = 1.65$, $p < 0.131$. On saccharin trial 3, only the 0.1 mg/kg group consumed less of the saccharin than the water, $t(10) = 4.06$. This result is consistent with the between-groups analyses of saccharin consumption described earlier. A similar analysis on saccharin trial 6 (data not shown) revealed a saccharin preference for the saline control and the 0.001 mg/kg group, ts(10) > 2.94. Not surprisingly, the 0.1 mg/kg group continued to drink less saccharin than water, $t(10) = 7.33$. Water vs. saccharin consumption did not differ for the 0.01 mg/kg group. This latter result indicates that the between-groups comparison suggesting an aversion in the 0.01 mg/kg group on saccharin trial 6 was the result of an increase in saccharin intake by the saline control group.

The bottom half of Fig. 2 shows the data from the high-dose 7-OH-DPAT experiment. The saline control showed a saccharin preference on the third trial (i.e., greater saccharin intake than water intake), $t(8) = 2.39$. All doses of 7-OH-DPAT induced a decrease in saccharin intake relative to water, ts > 5.72. Thus, regardless of whether a between-groups or within-subject analysis was conducted, we found reliable conditioned taste aversion with the 0.1, 1.0, and 10 mg/kg doses of 7-OH-DPAT.

DISCUSSION

The results of the present report clearly establish that 7-OH-DPAT can produce a conditioned saccharin aversion. Whereas past work has shown a role for dopamine D_1 and D_2 receptors in amphetamine-induced CTA, the present work suggests that D_3 receptors may also play a role. It has been argued that low doses of 7-OH-DPAT primarily activate the dopamine D_3 receptor, while higher doses act on D_3 and other D_2-like receptors (8). If so, then the moderate aversion conditioned by 0.1 mg/kg 7-OH-DPAT may be due to D_3 stimulation, while the more robust aversion at higher doses may be mediated by D_3 and other D_2-like receptors (D_2 and D_4). Unfortunately, understanding the relative contribution of D_3 receptors to the aversion conditioned by 7-OH-DPAT and other dopamine agonists will have to wait until highly selective D_3 antagonists become available.

Yamamoto and colleagues (30,31) have recently described the neural pathways believed to mediate taste aversion induced by LiCl. Visceral and gustatory input traveling via the nucleus of the solitary tract converge in the pontine parabrachial nucleus. Some of the input into the parabrachial nucleus arises from the area postrema. The pontine parabrachial nucleus is believed to send this converging information to medial thalamic nuclei and then to the basolateral nucleus of the amygdala. Separate visceral and gustatory information is also sent to these areas as well as to the gustatory cortex.

Unfortunately, the neuroanatomical pathways in dopamine agonist-induced CTAs are not as well worked out. A recent study using c-Fos immunohistochemistry found that relative to saline controls, amphetamine activated the area postrema, the nucleus of the solitary tract, and the pontine parabrachial nucleus (26). This activation however was not as pronounced as that of LiCl. The nucleus of the solitary tract and parabrachial nucleus may be required for dopamine agonist-induced CTA, but the area postrema does not seem to be necessary. Although bilateral microinjections of amphetamine into the area postrema will induce a taste aversion (7), lesioning the area postrema does not prevent the acquisition of apo-

morphine or amphetamine taste aversion [(3,27); but see (13)]. Similar to LiCl-induced aversion, lesioning of the amygdala attenuates the development of saccharin aversion (15). To our knowledge, the role of different thalamic nuclei has not been evaluated using a dopamine agonist. D_3 receptors, however, have been localized in some areas of the thalamus (24). The above discussion suggests that D_3-induced CTA may involve, in part, neural substrates that overlap those that mediate LiCl-induced aversions. However, the role of D_3 receptors, if any, in modulating these CTA structures is unknown.

Studies examining the functional role of D_3 receptors in behavior have concentrated on drug reward and drug-induced locomotor behavior (4,8,21,25). These functional studies were designed in response to autoradiography work indicating a heavy concentration of D_3 receptors in forebrain structures that subserve these functions (i.e., nucleus accumbens, striatum, and olfactory tubercles). The relatively high density of D_3 receptors in the nucleus accumbens makes it a possible site of D_3 stimulation by low doses of 7-OH-DPAT (8,24). However, bilateral microinjections of amphetamine into the nucleus accumbens do not induce a taste aversion (7). Moreover, bilateral 6-hydroxydopamine lesions of the nucleus accumbens do not weaken the taste aversion induced by the dopamine agonist apomorphine (27). These results suggest that dopamine terminals in the nucleus accumbens are not part of the critical circuitry for D_3 agonist-induced taste aversions.

The likelihood that D_3 receptor activation is exclusively involved in the acquisition of 7-OH-DPAT taste aversion is decreased by recent work assessing the selectivity of 7-OH-DPAT. Early studies reported a 100-fold selectivity of 7-OH-DPAT for the D_3 receptor over the D_2 receptor (18). More recent work has reported, at best, only a sevenfold selectivity for the D_3 receptor (10). If this latter study provides a better estimate of binding in vivo, then it seems unlikely that the current conditioned aversion, at any dose, would be due exclusively to D_3 activation.

Finally, recent work indicates that 7-OH-DPAT binds with high affinity to the σ receptor site (23,29). Wallace and Booze (29) reported a 4 : 1 ratio of D_3 to σ binding sites in the core of the nucleus accumbens and a 1 : 1 ratio in the shell of the nucleus accumbens. These authors argued that the effects of 7-OH-DPAT may be the result of an interaction between σ and D_3 receptors. Thus, we cannot rule out the possibility that the 7-OH-DPAT–induced taste aversion reported here may be the result of activating σ receptors, especially because selective σ ligands have been shown to induce taste aversions (17).

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