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(–)-Hydroxycitrate and Conditioned Aversions

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PANKSEPP, J., A. POLLACK, R. B. MEEKER AND A. C. SULLIVAN. *(–)-Hydroxycitrate and conditioned aversions.* PHARMAC. BIOCHEM. BEHAV. 6(6) 683–687, 1977. – The capacity of various salts of (–)-hydroxycitrate to produce conditioned rejection of a 0.25% saccharin solution was evaluated. The ethylenediamine salt of (–)-hydroxycitrate produced strong conditioned rejection of saccharin under both deprivation and nondeprivation conditions, but this effect was less than produced by equimolar doses of lithium chloride. The sodium salt of hydroxycitrate produced no conditioned rejection of saccharin in water deprived rats but did so in nondeprived animals. In these experiments, food intake was reduced by (–)-hydroxycitrate only during the first hour following administration of the drug. The magnitude of appetite rejection did not correspond to the degree of conditioned rejection, lending support to the conclusion that the food intake reduction was not merely a consequence of aversive effects of the drug.

Hydroxycitrate Ethylenediamine Conditioned aversions Appetite reduction Food intake

(–)-HYDROXYCITRATE reduces food intake, weight gain, and body lipid levels in both lean and obese rats and mice [8, 9, 10]. This compound was initially found to be a potent competitive inhibitor of ATP citrate lyase (E.C.4.1.3.8), which catalyzes the extramitochondrial cleavage of citrate to acetyl CoA and oxaloacetate [11]. As expected from this activity, subsequent studies demonstrated a marked suppression of fatty acid and cholesterol synthesis in vivo in rats by (–)-hydroxycitrate [2, 6, 7]. The reduction in food consumption produced by (–)-hydroxycitrate occurred concomitantly with an alteration in metabolic flux of dietary nutrients. Carbohydrates and their metabolites were diverted from lipid synthesis and simultaneously significant increases in hepatic glycogen synthesis and levels were observed [9].

Because it reduces both lipid synthesis and appetite, (–)-hydroxycitrate may prove to be a useful agent for the management of obesity. However, before accepting the appetite reduction as a primary therapeutic property of the drug, it must be determined whether the drug produces nonspecific effects such as illness or gastrointestinal distress. To test for such effects, in the following experiments we evaluated the capacity of various salts of (–)-hydroxycitrate to produce conditioned rejection of novel foodstuffs. Comparisons were made with lithium chloride, an agent commonly used to produce temporary gastric distress in conditioned gustatory aversion experiments [1, 3, 4].

GENERAL METHOD

All rats used in these experiments were individually housed in 14.5 × 7 × 9 cm high wire mesh cages with free access to Wayne powdered laboratory chow except as indicated. Food intakes and body weights were measured to 0.1 and 1.0 g, respectively. In all tests of conditioned gustatory rejection, a novel 0.25% sodium saccharin solution odourized with 0.1% orange extract (Durkee) was presented to animals in graduated drinking tubes for one-hr periods, and intakes were measured to 1.0 ml. Immediately following the drinking period, separate groups of animals were intubated with (–)-hydroxycitrate salts, lithium chloride, or a control solution. Intragastric tubing was accomplished with a 9.5 cm, 16 ga stainless steel feeding needle (Popper), and animals were habituated to the procedure at least twice before testing. Throughout each experiment, temperature remained stable at 22 ± 2°C, and lighting was maintained on a 12–12 hr light-dark cycle. Testing, unless otherwise indicated, occurred during the last third of the light cycle.

EXPERIMENT 1

Method

Thirty-two female Sprague-Dawley albino rats weighing 243–301 g were divided into four equal groups. Prior to the first test day, all animals were water deprived for 23 hr,

whereupon they were given access to the odourized saccharin solution for one hour. Immediately following the drinking period, animals in the four groups were tubed with the following four solutions at a dose of 2.63 mmoles/kg: (1) sodium chloride (2) lithium chloride (3) (−)-hydroxycitrate ethylenediamine, or (4) (−)-hydroxycitrate sodium. All solutions were mixed 2.63 mmoles/10 cc. This procedure was continued for three successive days followed by three days of extinction, during which the drinking periods were not followed by injections. To maintain water balance in animals which exhibited conditioned rejection of the saccharin during acquisition, rats which drank 10 ml less than the average control (NaCl) intake were tubed with 10 ml of water three hours following the daily drinking period. This supplementation was discontinued during the extinction phase.

Results

Average daily fluid intakes are summarized in Fig. 1. Both LiCl and the ethylenediamine salt of (−)-hydroxycitrate produced strong conditioned rejection of the saccharin (t's>5.6, $df = 14$, p's<0.001, for all comparisons of these groups with control animals). The only difference between LiCl and (−)-hydroxycitrate ethylenediamine was during the first test following the initial training session when intake of the LiCl group was reliably lower than the ethylenediamine group ($t = 2.49$, $df = 14$, $p<0.01$). The sodium salt of (−)-hydroxycitrate produced no reliable suppression of saccharin intake during the course of testing.

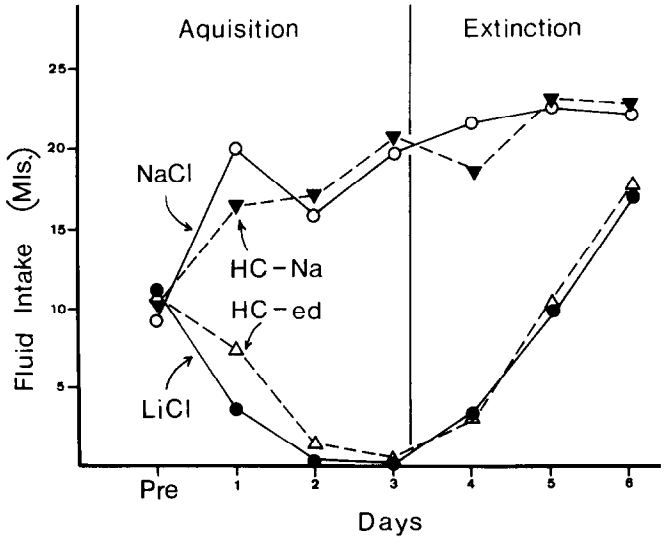

FIG. 1. One-hr saccharin intake for Experiment 1. Hc-Na: Hydroxycitrate sodium; Hc-ed: Hydroxycitrate ethylenediamine.

EXPERIMENT 2

The observation that the ethylenediamine salt of (−)-hydroxycitrate produces strong conditioned rejection of saccharin while an identical dose of the sodium salt does not suggest that ethylenediamine rather than the (−)-hydroxycitrate may be the cause of the effect. Still, it cannot be concluded that (−)-hydroxycitrate does not produce any conditioned aversion, since the lack of effect with the sodium salt in Experiment 1 may have been due to insensitivity of the testing procedures. Namely, the high level of thirst may have masked an effect which might be apparent under lower drive conditions. In the following experiment, sensitivity was increased by foregoing the use of water deprivation to sustain fluid intake. Also, conditioning was done every 12 hr so that the effects of (−)-hydroxycitrate on diurnal food intakes could be assessed.

Method

Forty female Long-Evans rats weighing approximately 300 g were divided into four equal groups. Body weights and food intakes were monitored at 12-hr intervals (at transitions of the lighting cycles) for four baseline days. During the conditioning phase, all animals were given access to the odourized saccharin solution for one hr just before the transition of the lighting cycle, followed promptly by intragastric loads of the following solutions in doses of 2.63 mmoles/kg; (1) (−)-hydroxycitrate sodium (2) (−)-hydroxycitrate ethylenediamine (3) lithium chloride (4) sodium chloride. The testing procedure was repeated every 12 hr for four days. Since strong conditioned rejection was observed in all experimental groups, 24 hr two-bottle preference between water and the saccharin solution was measured on the second day following the end of training.

Results

Under these sensitive conditions, all experimental groups exhibited strong conditioned rejection of saccharin (Fig. 2), intakes of all groups remaining reliably lower than control intakes during all tests following the first training session (t's>1.84 to 4.80, $df = 18$, p's<0.05 to 0.001). The lack of any difference among experimental groups was apparently due to a floor effect since the 24-hr preference test after the end of training did indicate a systematic ordering of preferences – the LiCl group consuming less than 5% of its water from the saccharin solution, the (−)-hydroxycitrate ethylenediamine group consuming 20%, the (−)-hydroxycitrate sodium group 55%, and the controls 91%. Thus, strong conditioned rejection can be produced with both salts of (−)-hydroxycitrate, but the effects still are systematically smaller than observed with equimolar LiCl.

Neither salt of (−)-hydroxycitrate produced any reliable suppressions of feeding under the 12-hr measurement procedures employed in this experiment. Whether there

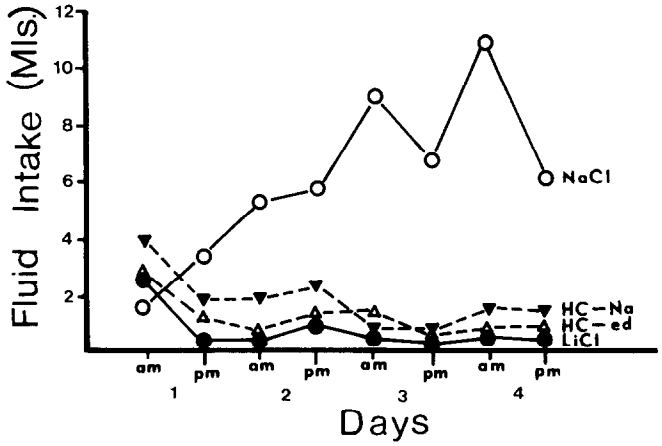

FIG. 2. One-hr saccharin intakes for Experiment 3.

might have been initial suppressions of feeding during the first few hours following drug treatment cannot, of course, be ascertained from the data.

EXPERIMENT 3

Although hydroxycitrate has been observed to reliably reduce feeding in previous experiments [8,9], the lack of such an effect in Experiment 2 may have been due to the use of the long 12-hr recording period. The major metabolic effects of (−)-hydroxycitrate in decreasing lipogenesis and increasing glycogenesis at the doses used are over in about eight hr [7,9]. Thus, compensatory adjustments of food intake could have occurred during a 12-hr recording period. In the following experiment, we measured both the degree of conditioned rejection of saccharin and suppression of chow intake under conditions where both could be expressed. Also, to further classify whether the conditioned rejection of saccharin following the tubing of (−)-hydroxycitrate was due to the drug or the specific form of salt used, citrate controls for each of three forms of (−)-hydroxycitrate were included.

Method

Fifty-five Long-Evans female rats weighing approximately 200 g were used. Animals were divided into nine groups of six rats apiece with one extra animal serving in the control group. Throughout this experiment, chow was available for 20 hr each day, all animals being deprived for the last four hours of the light cycle each day. Animals were also deprived of water for 23 hr each day, receiving their one-hr ration during the last hour of light (i.e., just before the start of the feeding period). During the first two days of the experimental procedure, animals were given tap water to drink and, thereafter, for five days of conditioning the 0.25% saccharin solution was provided. Immediately following each saccharin period, animals were injected with 2.63 mm/kg of the following solutions: (1) sodium chlroide (2) lithium chloride (3) ethylenediamine citrate (4) (−)-hydroxycitrate ethylenediamine (5) ethylenediamine dihydrochloride (6) citric acid (7) (−)-hydroxycitric acid (8) (−)-hydroxycitrate sodium, and (9) sodium citrate. This provided a comparison between three forms of (−)-hydroxycitrate and the corresponding citrate controls. The ethylenediamine dihydrochloride was included to determine whether this form of the salt might reduce conditioned aversion as compared to ethylenediamine in the simple citrate forms. All solutions were tubed in volumes of 10 ml/kg. Food intakes during the 1st, 4th, and 20th hr following treatment were measured each day. To assure that all groups were at the same level of hydration at the start of the daily feeding tests, animals in each group were tubed with enough water to bring their fluid intakes up to the average level of the group which had consumed the most saccharin during the preceeding one-hr test. Each day, the reference group turned out to be the one which had received the (−)-hydroxycitrate sodium.

Results and Discussion

Data are presented only for the last three days of testing (Fig. 3). The first two days of conditioning are omitted because the initial novelty of the saccharin severely disrupted the immediately succeeding feeding. For instance, in the NaCl group, intake dropped more than 60% as compared to the previous two days when water had been available prior to the feeding period.

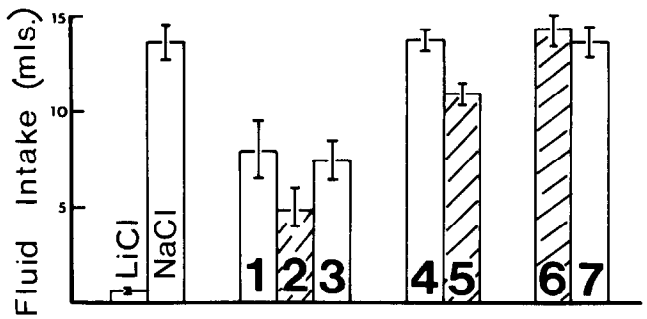

FIG. 3. Average one-hr saccharin intakes for last three days of Experiment 4. Conditions: (1) ethylenediamine citrate, (2) hydroxycitrate ethylenediamine, (3) ethylenediamine dihydrochloride, (4) citric acid, (5) hydroxycitric acid, (6) hydroxycitrate sodium, (7) sodium citrate.

In this experiment, LiCl treated animals exhibited the largest conditioned rejection of saccharin (Fig. 3). All three ethylenediamine groups exhibited moderate conditioned suppression compared to the NaCl group (t's>3.45, $df = 11$, p's<0.001) but they did not differ from each other. (−)-Hydroxycitric acid produced very mild, though statistically very reliable, suppression ($t = 4.13$, $df = 10$, $p<0.001$) as compared either to the citric acid or NaCl controls. The (−)-hydroxycitrate sodium produced no rejection of saccharin.

Analysis of food intakes during the first hour following treatment indicated that lithium chloride reliably decreased feeding by 24% compared to NaCl (4.1 g to 3.1 g), $t = 2.85$, $df = 11$, $p<0.01$; (−)-hydroxycitrate ethylenediamine reduced food intake by 30% when compared to either of the ethylenediamine or NaCl controls (4.0 to 2.8 g), t's>3.5, $df = 10$, $p<0.005$); hydroxycitric acid reduced intake by 25% compared to the citric and control group (5.1 to 3.8 g), $t = 3.03$, $df = 8$, $p<0.01$; and (−)-hydroxycitrate sodium depressed chow intake by 24% when compared to the sodium chloride control (4.1 g to 3.1 g), $t = 1.91$, $df = 11$, $p<0.05$). The effects of hydroxycitrate and LiCl were no longer apparent by the fourth and twentieth hr following treatment.

These results indicate that agents which produce conditioned rejection of saccharin also reduce food intake but, more importantly, that there tends to be a dissociation between the two effects as far as (−)-hydroxycitrate is concerned. Even though all three ethylenediamine compounds produced mild conditioned rejection of saccharin, the only agent which reliably decreased food intake was the (−)-hydroxycitrate salt. Also, the (−)-hydroxycitrate sodium produced reliable suppression in feeding, although it produced no conditioned aversion in the present experiment. Since all the (−)-hydroxycitrate compounds suppressed feeding as much as LiCl even though they produced relatively little conditioned aversion, it seems likely that the inhibition of feeding by (−)-hydroxycitrate is largely independent of possible aversive effects of the drugs.

EXPERIMENT 4

The following experiment addressed two issues concerning the nature of conditioned rejection observed with (−)-hydroxycitrate in the previous experiments. First, since

above work with saccharin solutions cannot be clearly interpreted with reference to feeding, flavored diets were employed in the following experiment. An attempt was made to unravel the mechanism of conditioned rejection more precisely. Animals may consume less of certain foods because they have been paired with aversive internal states or because they learn that certain foods have high satiety value. Such a distinction between conditioned aversions and conditioned satiety becomes especially important with agents which are thought to produce normal satiety. An attempt was made to distinguish between the two processes by giving animals repeated exposure to two novel palatable foods, only one of which was paired with rejection-mediating agents. After this training, preference for the two diets was measured in a choice situation. It was reasoned that, if an agent actually produces conditioned aversion, then the animal should reject the relevant food in a choice situation, whereas if only conditioned satiety had been produced, then the paired food would not be rejected even though less might be eaten of it. Since ethylenediamine alone is sufficient to mediate conditioned rejection of saccharin (Experiment 4), the sodium salt of (−)-hydroxycitrate was selected for study in this experiment. To maximize the possibility of observing conditioned aversion, animals were tested undeprived, as in Experiment 3.

Method

Twenty-one adult female Long-Evans rats having free access to food and water were used. During five daily pretests, the animals were given one-hr simultaneous choice between two diets concocted with saccharin and either almond or vanilla extracts. After some adjustment of the concentration of saccharin, recipes were obtained where both diets were readily consumed during the measurement period but where one diet was consistently preferred over the other. The final mixtures were as follows: Diet A (the more preferred one) consisted of 1:1 (weight:weight) powdered Wayne lab chow and water sweetened with 0.5% saccharin and odourized with 0.1% vanilla extract (Ann Page); Diet B (the less preferred) consisted of the same basic diet flavored with 0.05% saccharin and 0.1% almond extract (Ann Page). The results from the last two choice tests are presented in Table 1.

The 21 animals were divided into three equal groups and, for the next eight days, they were presented with only one of the flavored diets each day for one hr – the vanilla Diet A on the 1st, 3rd, 5th, and 7th days and the almond Diet B on the remaining days. Immediately following Diet A periods, the animals received one of three intragastric injections: (1) 2.63 mmoles/kg (−)-hydroxycitrate sodium, (2) 2.63 mmoles/kg lithium chloride, or (3) the water vehicle. Following the presentation of Diet B, all animals received equivolumetric 10 cc/kg water loads. Following this training period, all animals were again given two preference tests separated by two days when both diets were presented simultaneously for one hr.

Results

As summarized in Table 1, the various groups exhibited a weak 54%–69% preference for Diet A during the initial simultaneous choice tests. Daily intakes of the two diets during training are summarized in Fig. 4. Lithium chloride treatment indiscriminately reduced intake of both experimental diets, while (−)-hydroxycitrate sodium had no major effect on intake of either.

Posttraining preference data (Table 1) confirmed that the lithium chloride treatment had completely suppressed intake of both diets. The (−)-hydroxycitrate may also have produced some suppression since the average intake during the posttest was 62% of pretest levels, while intake was still 92% for the vehicle controls, but this difference was not

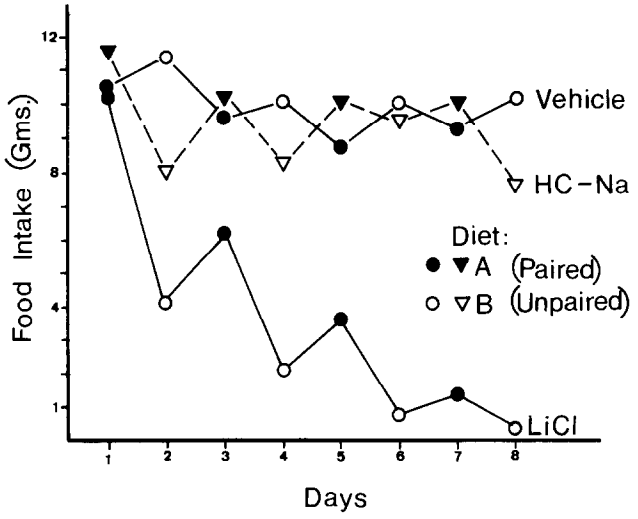

FIG. 4. One-hr intakes of diets during the conditioning phase of Experiment 5.

TABLE 1

ONE-HOUR FOOD INTAKES DURING PRE- AND POSTTRAINING TWO DIET PREFERENCE TESTS

	Pretraining				Posttraining			
	Test 1		Test 2		Test 1		Test 2	
	Diet A	Diet B	Diet A	Diet B	Diet A	Diet B	Diet A	Diet B
Vehicle	5.5 (±2.2)	3.3 (±0.4)	5.3 (±2.0)	4.2 (±3.0)	5.0 (±.18)	2.7 (±1.7)	4.6 (±2.5)	4.4 (±2.2)
(—)-Hydroxycitrate Sodium Salt	7.1 (±2.8)	4.8 (±3.1)	6.6 (±1.4)	5.4 (±2.3)	4.1 (±3.2)	3.2 (±2.4)	4.3 (±3.1)	4.4 (±2.5)
Lithium Chloride	6.0 (±2.5)	4.4 (±3.2)	5.7 (±1.4)	4.8 (±2.2)	0.3 (±0.2)	0.4 (±0.3)	0.3 (±0.3)	0.3 (±0.1)

Values are $\overline{X}$ ± S.D.
Diet A: 0.1% vanilla extract + 0.15% saccharin diet (1:1,Wayne Lab Chow:Water).
Diet B: 0.1% almond extract + 0.05% saccharin diet (1:1,Wayne Lab Chow:Water).

statistically significant ($t = 1.4$, $df = 12$, $p > 0.10$). Furthermore, there was no indication that the relative reference for the two diets had been changed in the (−)-hydroxycitrate group.

These data indicate that under sensitive nondeprivation conditions rats do not exhibit any reliable conditioned rejection of a diet which has been paired with (−)-hydroxycitrate sodium. This is in contrast to findings under similar conditions with saccharin solutions (Experiment 2). This difference may be due to the use of food vs. fluid or to the fact that animals in the present experiment were exposed to the experimental diets on several occasions prior to the start of conditioning. In any case, the results again indicate that the effects of relatively high doses of sodium salt of (−)-hydroxycitrate are very small in comparison to lithium chloride.

This experiment also presents an interesting failure of discrimination in the lithium chloride group. Though only a single diet had been paired with the unconditional stimulus (LiCl), behavior generalized to the other diet presented 24 hr later. Whether this indicates that some characteristic other than odor was the most salient feature of each diet or whether it indicates the weakness of discrimination learning in visceral conditioning cannot be ascertained from the present data. At the very least, quite a deal of parametric work is needed to provide diets which will yield an acceptable level of discrimination for the proper execution of this kind of an experiment.

GENERAL DISCUSSION

The present results indicate that (−)-hydroxycitrate at doses which have been found to be very effective in inhibiting lipid synthesis [7,9] does not produce marked conditioned aversion in situations where the effects of lithium chloride are unmistakable and profound. However, the present results also highlight that some forms of (−)-hydroxycitrate, such as the ethylenediamine salt, could mediate conditioned rejection, but this effect was not due to the (−)-hydroxycitrate since the ethylenediamine in any form is quite sufficient to produce the effect. The fact that the sodium salt of (−)-hydroxycitrate could provoke conditioned rejection of saccharin in the most sensitive of situations (i.e., under low thirst conditions) does leave the matter of (−)-hydroxycitrate has any aversive property open, but it is noteworthy that the suppression under the most effective of conditions was very weak by traditional norms. Thus, in Experiment 3, saccharin intake was suppressed during a one-hr test but not when the animal was allowed a choice of the saccharin or water for a whole day. Under other conditions (Experiments 1, 4, and 5), the sodium salt yielded no conditioned rejection at all.

The present experiments also highlight the fact that the reduction of appetite seen after hydroxycitrate administration is modest – a 25% reduction in feeding under the most favorable testing conditions (Experiment 3), where feeding was measured during the first hour after drug administration but not apparent during longer testing periods such as in Experiment 2. The results also suggest that the inhibition of feeding is not due to aversive side effects of the drug. The agent could reduce feeding while producing no conditioned rejection of recently consumed solutions.

In summary, within the limits of conditions employed, (−)-hydroxycitrate sodium appears to be a safe and benign drug while certain other salts, specifically the ethylenediamine forms, can have undesirable behavioral effects. Careful evaluation of new pharmaceutical agents by the procedures used herein appears to be useful in selecting forms which may prove to be more acceptable than others in medical practice.

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