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(−)*threo*-Chlorocitric Acid Decreases Sham Feeding of Sucrose in Rats

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WEATHERFORD, S. C., J. SALABARRIA, D. NELSON AND W. B. LAUGHTON (−)threo-*Chlorocitric acid decreases sham feeding of sucrose in rats*. PHARMACOL BIOCHEM BEHAV **40**(1) 75–78, 1991.—The contribution of changes in rate of gastric emptying to the anorectic effect of (−)-*threo*-chlorocitric acid (chlorocitrate) was assessed by examining the effect of this drug in sham feeding rats, a preparation where gastric distention does not occur. Gavage administration of chlorocitrate (100–400 $mg \cdot kg^{-1}$) decreased sham and real feeding of 20% sucrose in a dose-related manner. In sham-feeding rats, the minimal effective dose was 200 $mg \cdot kg^{-1}$. The anorectic effect was evident at 60 min after 200 $mg \cdot kg^{-1}$ and 30 min after 400 $mg \cdot kg^{-1}$. In real-feeding rats, the minimal effective dose was 100 $mg \cdot kg^{-1}$ and for all doses tested the effect was apparent at the 15-min time point. In a second experiment, the effect of chlorocitrate (100–400 $mg \cdot kg^{-1}$) on gastric emptying of 20% sucrose was examined. Chlorocitrate (200 and 400 $mg \cdot kg^{-1}$) had a modest but significant inhibitory effect on gastric emptying; however, the effect was not dose-related. Inasmuch as chlorocitrate decreased sham feeding, its anorectic effect cannot be solely attributed to inhibition of gastric emptying. However, because chlorocitrate was more potent in the real-feeding condition relative to sham feeding, and the time course of the response in the two feeding conditions was different, part of chlorocitrate's anorectic effect may depend on postingestive cues such as gastric distention.

Anorectic Food intake Gastric emptying Rat Chlorocitrate

(−)-*THREO*-CHLOROCITRIC acid (chlorocitrate) is an anorectic agent that has been shown to decrease food intake in a number of species including obese and lean rats (10), dogs (10) and humans (3). Unlike its close structural analog, (−)-*threo*-hydroxy citric acid (11), chlorocitrate's anorectic activity is not mediated through alteration of hepatic metabolism (12). In addition, chlorocitrate anorexia does not produce CNS side effects (10) associated with other centrally acting anorectics (e.g., fenfluramine and amphetamines) and is, therefore, thought not to be mediated through direct CNS stimulation. However, chlorocitrate-induced suppression of food intake is dependent on the integrity of the abdominal vagus nerve (5) and the drug has been shown to delay gastric emptying (12). Based on these findings, it has been proposed that the site of action of this compound is the upper gastrointestinal tract, with the anorexia possibly due to delayed gastric emptying (12).

The purpose of the present experiments was to assess the contribution of changes in gastric emptying to the anorectic effect of chlorocitrate. We approached this question in two ways. First, we examined the effect of chlorocitrate on the intake of 20% sucrose in sham-feeding rats, a preparation in which postingestive cues such as gastric distension and postabsorptive metabolic effects are minimized (9) or abolished (14). For comparison purposes we also examined the effect of chlorocitrate on sucrose intake in the real-feeding condition. Second, the potency of chlorocitrate for inhibiting gastric emptying of a 20% sucrose solution under similar conditions as the feeding tests were conducted was investigated. A preliminary report of these results has appeared in abstract form (13).

METHOD

Animals and Environment

Eight male Sprague-Dawley rats (Charles River, Raleigh, NC), weighing 200–225 g, were used in this experiment. Rats were housed and tested in individual hanging wire-mesh cages in a temperature-controlled environment (21 ± 1°C) with a 12:12 light/dark cycle (lights on 0600). Rats were maintained on ground Purina rodent chow and tap water.

Surgical Procedure

All rats were implanted with chronic stainless steel gastric cannulas according to previously described methods (14). Briefly, after overnight food deprivation, rats were anesthetized with Nembutal anesthesia (pentobarbital sodium; 60 $mg \cdot kg^{-1}$, in-

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traperitoneal) and a midline incision was made to expose the abdominal viscera. The stomach was isolated and the cannula (11 mm long, with a 15 mm diameter flanged end) was inserted through a small incision (1 mm) in the nonglandular portion of the stomach. A purse string suture was made through the gastric wall around the shaft of the cannula, thus preventing leakage of gastric contents into the peritoneum. To promote the adhesion of the gastric wall and peritoneum, a small piece of Marlex Mesh (Bard Implants Division, Billerica, MA) was stretched down over the shaft of the cannula so that it lay flush against the gastric wall. The cannula was then externalized through a stab wound in the left abdominal wall. A removable screw cap closed the cannula to prevent drainage of gastric contents while rats were not being tested.

Experiment 1. Effect of Chlorocitrate on Sham and Real Feeding of Sucrose

Testing procedure. Rats were allowed 2 weeks to recover from surgery at which time they were trained to sham feed 20% sucrose (w/v in distilled water). All rats were placed on a 17-h overnight food-deprivation schedule. At 0900 rats received 1 $ml \cdot kg^{-1}$ of 5% gum arabic (w/v in 0.9% saline; Fisher Scientific, Fairlawn, NJ) by gavage. Thirty min later the screw cap occluding the cannula was removed and the stomach was gently lavaged with tap water until the gastric drainage was free of food particles. To facilitate drainage and collection of gastric contents, a Silastic drainage tube surrounded by a flexible metal spring was threaded into the shaft of the cannula. Rats were returned to their cages and the drainage tube was passed through a longitudinal slit in the wire-mesh floor, thus allowing rats free movement throughout the experimental period. A plastic beaker was placed below the rats' cages to collect gastric drainage. Rats were then offered 20% sucrose (Fisher Scientific, Fairlawn, NJ) in a graduated cylinder adapted to fit a sipper tube. Sham intakes were recorded at the 15, 30, 60 and 120 min time points. Following the 120-min test, the drainage tube was removed and the screw cap was replaced. Chow and water were returned immediately after the test and were available until the next deprivation period began. Tap water was available at all times except during the test.

After baseline intakes (intake after 5% gum arabic) were established, drug testing with chlorocitrate was initiated. All rats received 3 doses of chlorocitrate (Hoffmann-La Roche, Inc., Nutley, NJ; Ro 21-7716; 100, 200 and 400 $mg \cdot kg^{-1}$) in ascending order by gavage administration. A vehicle (equimolar citric acid in 5% gum arabic) test always preceded and followed a drug test day.

After all rats received each dose of chlorocitrate one time in the sham-feeding condition they were trained to real feed 20% sucrose. For this experiment, seven rats from the sham-feeding experiment were tested using an identical protocol described for the sham-feeding condition, the only difference being that the screw cap was replaced after stomachs were lavaged. Hence, ingested sucrose accumulated in the stomach and emptied into the intestine as occurs with normal ingestive and digestive processes. Under the real-feeding condition, baseline intakes stabilized after 5 training sessions at which time drug testing was initiated. Rats received the same three doses of chlorocitrate as in the sham-feeding condition.

Statistical analysis. Before data could be included in analysis for the sham-feeding experiments, two criteria had to be met to insure complete collection of the sham-fed solutions (4): first, gastric contents had to begin draining from the tube by 15 seconds after the initiation of sham feeding; second, the gastric contents collected during the test session had to equal or exceed the volume consumed. Data from all rats met these criteria at all doses tested. The sham- and real-feeding conditions were analyzed with two-way (dose and time), repeated measures analysis of variance (ANOVA). For each feeding condition, differences in intakes following vehicle were not found, therefore, the pooled vehicle conditions were used as a zero level dose. Significant differences in main effects were analyzed with post hoc paired *t*-tests.

Experiment 2. Effect of Chlorocitrate on Gastric Emptying Rate of 20% Sucrose

A second experiment was conducted to determine the effect of chlorocitrate on gastric emptying of 20% sucrose in rats using a protocol similar to those described in Experiment 1. In this experiment a second group of male Sprague-Dawley rats (~200 g) (N = 7) was implanted with gastric cannulas and allowed to recover as described in Experiment 1. To insure that the second group of rats had a similar response to chlorocitrate as the subjects in Experiment 1, they were tested with chlorocitrate using the protocol described for the real feeding condition. Testing with chlorocitrate (100, 200 and 400 $mg \cdot kg^{-1}$) was initiated after 10 training sessions of real feeding 20% sucrose. The second group was found to respond to the anorectic effect of chlorocitrate in an ideal manner as rats in Experiment 1, and were, therefore, used in the subsequent experiment examining the effect of chlorocitrate on gastric emptying rate.

To measure the rate of gastric emptying, rats were adapted to the following procedure. As in Experiment 1, rats were placed on a 17-h overnight food deprivation schedule. At 0900 they received 5% gum arabic by gavage. Thirty min later stomachs were lavaged and a Silastic tube surrounded by a protective metal spring was attached to the cannula. Ten ml of 20% sucrose containing phenol red (0.6 $mg \cdot ml^{-1}$) was instilled into the stomach through the drainage tube and the tube was occluded with an alligator clip. Fifteen min later the sucrose was aspirated from the stomach and the volume recorded. Two ml of Na_2PO_4 (27.5 $g \cdot l^{-1}$) was added to a 1 ml aliquot of the aspirate. This was then diluted to a total volume of 10 ml with distilled water and read at 550 nm in a Beckman DU-64 spectrophotometer. Rate of emptying was measured from the following formula (1).

$$\text{Rate of emptying (ml/15 min)} = \frac{(V_i C_i) - (V_r C_r)}{(C_i + C_r)/2}$$

where V_i and V_r are the initial and recovered volumes, respectively and C_i and C_r are the initial and recovered concentrations of phenol red, respectively.

Rats were adapted to the procedure for measuring gastric emptying for four training trials at which time drug testing with chlorocitrate was initiated. Rats received three doses of chlorocitrate (100, 200 and 400 $mg \cdot kg^{-1}$) in ascending order. A vehicle test day always preceded and followed a drug test day.

Data analysis. Data were analyzed with a one-way repeated measures ANOVA with drug doses as the repeated measure. Emptying rate after vehicle did not differ; therefore, pooled data were used as a zero-level dose. Significant differences in main effects were analyzed with post hoc paired *t*-tests.

RESULTS

Experiment 1

Chlorocitrate decreased sham feeding in a dose-related manner; there was a significant main effect of dose of chlorocitrate

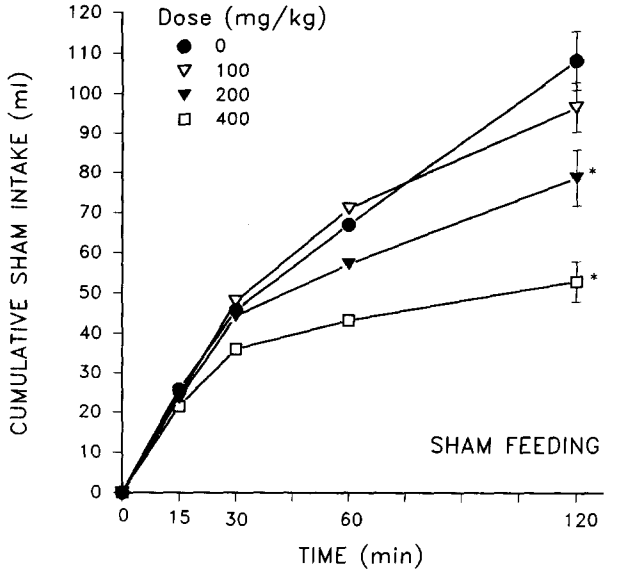

FIG. 1. Mean cumulative sham intake (ml) of rats sham feeding 20% sucrose 30 min after gastric intubation of chlorocitrate (0, 100, 200 and 400 mg/kg). *$p<0.01$ by paired t-tests after significant ANOVA. N=8. For visual clarity, SEM and asterisks indicating significance are only shown at the 120-min time point.

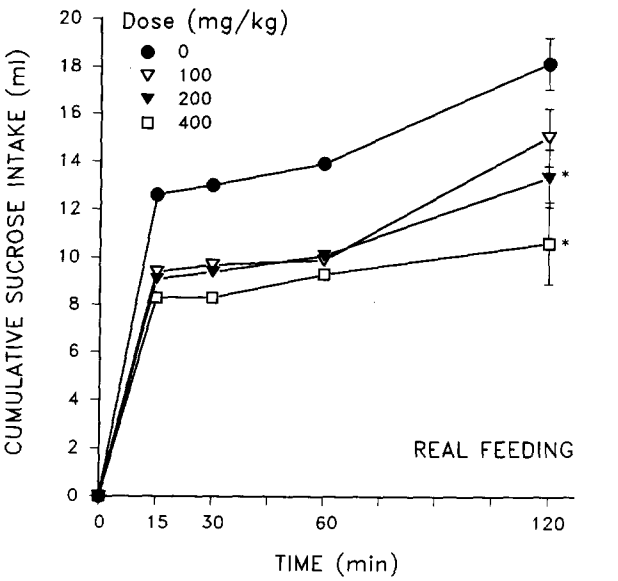

FIG. 2. Mean cumulative intake (ml) of rats real feeding 20% sucrose 30 min after gastric intubation of chlorocitrate (0, 100, 200 and 400 mg·kg^{-1}). *$p<0.01$ by paired t-tests after significant ANOVA. N=7. For visual clarity, SEM and asterisks indicating significance are only shown at the 120-min time point.

on sham intake, $F(3,21)=17.41$, $p<0.01$. In addition, the time course of the anorectic effect of each dose differed; a significant interaction between dose of chlorocitrate and time was found, $F(9,63)=10.22$, $p<0.01$. After 400 mg·kg^{-1} the first time point at which there was a significant effect on sham feeding was 30 min ($p<0.01$) and for the 200 mg·kg^{-1} dose it was 60 min ($p<0.01$). There was no effect of the 100 mg·kg^{-1} dose at any time point examined (Fig. 1).

Chlorocitrate also decreased real feeding of sucrose in a dose-related manner, however, the dose-response was shallower and shifted to the left relative to the sham-feeding condition. There was a significant main effect of dose on sucrose intake, $F(3,18)=12.78$, $p<0.01$, as well as a significant interaction between dose of chlorocitrate and time, $F(9,54)=3.54$, $p<0.01$. Post hoc t-tests indicated that all doses were significantly different from intake after vehicle at the 15-, 30- and 60-min time points. At the 120-min time point, only the two highest doses (200 and 400 mg·kg^{-1}) were significantly different from control values (Fig. 2).

Experiment 2

Chlorocitrate significantly delayed gastric emptying, $F(3,23)=3.94$, $p<0.05$, of 20% sucrose, but the effect was not dose-related: at all three dose levels, gastric emptying was reduced by 18–20%, although the effect was statistically significant only at the doses of 200 and 400 mg·kg^{-1} ($p<0.01$; see Table 1).

DISCUSSION

In real-feeding rats, chlorocitrate caused a rapid (15 min) and dose-related inhibition of 20% sucrose intake, with a minimal effective dose of 100 mg·kg^{-1}. Chlorocitrate was also found to inhibit gastric emptying, although the effect was not dose-related. These results confirm previous reports (10,12) demonstrating anorexic properties of orally administered chlorocitrate and are consistent with the hypothesis that retardation of gastric emptying may be involved in this effect (12).

However, chlorocitrate also deceased intake of 20% sucrose in sham-feeding rats. In contrast to the real-feeding condition the minimal effective dose was two times higher (200 mg·kg^{-1}) for sham-feeding rats as for real-feeding rats, and the onset of the anorexia was delayed. In the sham-feeding rat preparation, post-ingestive cues such as postabsorptive metabolic effects are minimized (9,14) and gastric distension is essentially abolished. Our results showing that chlorocitrate retains anorectic activity in sham-feeding rats, then, would argue against decreased rates of gastric emptying as being the sole mechanism responsible for the anorexic action of chlorocitrate.

In the period shortly after drug administration (15 and 30 min), decreases in the rate of gastric emptying of 20% sucrose may be responsible for the inhibition of intake in the real-feeding condition; the absence of the resulting increases in gastric distention in the sham-feeding condition may explain why the

TABLE 1

EFFECT OF (−)-*THREO*-CHLOROCITRIC ACID ON GASTRIC EMPTYING OF 20% SUCROSE IN RATS

Dose mg·kg^{-1}	Volume Emptied ml·15 min^{-1}
0	6.33 ± 0.17
100	5.19 ± 0.37
200	5.04 ± 0.34*
400	5.14 ± 0.25*

Values are means ± SEM. Chlorocitrate or vehicle was administered 30 min before instillation of 20% sucrose. N=7, *$p<0.01$ by paired t-tests after significant effect of one-way ANOVA.

drug was less effective at this time point. The relatively flat dose-response curve for chlorocitrate anorexia at 15, 30, and 60 min as well as for chlorocitrate inhibition of gastric emptying is consistent with alterations in gastric emptying mediating at least part of the effect at those earlier time points.

Conversely, the effectiveness of the drug in decreasing sham feeding at 30, 60 and 120 min must reflect mechanisms unrelated to postingestive cues such as gastric distention. Conceivably, the anorectic effect of the drug may be dependent on interaction with changes in plasma glucose or other consequential metabolic changes reported to occur in the sham-feeding preparation (9). These metabolic changes may be more apparent over time as the sham-feeding test progresses, which would explain the delay in the onset of the anorectic effect of chlorocitrate in sham-feeding rats. Additional experiments examining the effect of chlorocitrate in rats sham feeding nonnutritive solutions such as saccharin or mineral oil (7) may help resolve this issue.

An alternative explanation for the anorectic effect of chlorocitrate in sham-feeding rats is that the drug may have caused malaise or discomfort. However, inasmuch as it has been demonstrated that chlorocitrate is not an effective unconditioned stimulus for taste aversion learning in rats (12), this explanation seems improbable.

It is unlikely that the difference in the potency and time course of the effect of chlorocitrate in the two feeding conditions is due to "wash out" of the drug during the sham-feeding procedure. In both the sham- and real-feeding experiments, the rats' stomachs were lavaged to a similar extent 30 min after gastric intubation of the drug. It could be argued that the actual process of sham feeding may have washed out residual drug that may have remained in the stomach even after lavage. However, this would not explain why the drug had similar potency in the two feeding conditions at the 120-min time point; in both sham- and real-feeding rats, 400 $mg \cdot kg^{-1}$ inhibited sucrose intake by ~50%. It should be pointed out, however, that the process of lavaging the stomach does decrease the potency of the drug. When rats with gastric cannulas are allowed to real feed sucrose without lavage, the potency of the drug is enhanced (unpublished observations).

The effectiveness of chlorocitrate in reducing food intake in sham-feeding rats, combined with the lack of effect of the compound in vagotomized rats (5), seemingly presents a paradox explainable only by mediation of the anorexic effects by a vagally innervated structure other than gastric mechanoreceptors. Possible candidates include hepatic (2,8) or intestinal chemoreceptors (6).

Alternatively, the experimental design used in the vagotomy experiments described above (5) may simply have been insufficiently sensitive to detect residual responsiveness to the anorexic effect of chlorocitrate: a single dose (100 $mg \cdot kg^{-1}$) of chlorocitrate was administered, and food intake measured at 30 min. The reduction in food intake observed in drug-treated sham animals was totally abolished in the vagotomized rats. Similarly, in the present experiments, there was no effect of 100 $mg \cdot kg^{-1}$ of chlorocitrate in sham-feeding rats at the 30-min time point. Quite possibly, if higher doses of the drug had been tested in vagotomized rats, a similar blunting and rightward shifting of the dose-response curve would have been seen.

In summary, the results of these experiments demonstrate that chlorocitrate caused a dose-related decrease in both sham and real feeding of 20% sucrose in rats. The anorectic effect was more potent in the real-feeding condition and the onset of the anorectic effect was earlier. These results are consistent with the possibility that the anorectic effect of chlorocitrate is dependent on both postingestive cues such as gastric distension as well as nongastric peripheral cues.

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