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Studies on the Mechanism of Action of a Novel Anorectic Agent, (−)-*threo*-Chlorocitric Acid

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TRISCARI, J. AND A. C. SULLIVAN. *Studies on the mechanism of action of a novel anorectic agent, (−)-threo-chlorocitric acid.* PHARMAC. BIOCHEM. BEHAV. 15(2) 311–318, 1981.—(−)-*threo*-Chlorocitric acid, a novel and potent anorectic agent in rats and dogs, decreased food intake to a comparable extent in rats fed chow, low fat, or high fat diets. This inhibition of food intake was not the result of conditioned aversion. Unlike (−)-*threo*-hydroxycitric acid, a structurally related compound which inhibits fatty acid synthesis, (−)-*threo*-chlorocitric acid did not suppress fatty acid synthesis in an isolated hepatocyte system or *in vivo*. (−)-*threo*-Chlorocitric acid significantly delayed the rate of gastric emptying. Of the four stereoisomers of chlorocitric acid, (−)-*threo*-chlorocitric acid was the most active both in delaying gastric emptying and producing anorexia in rats. It is suggested that the mechanism by which (−)-*threo*-chlorocitric acid may suppress food intake is through a reduction of gastric emptying.

(−)-*threo*-Chlorocitric acid (−)-*threo*-Hydroxycitric acid Gastric emptying Conditioned aversion Anorexia Lipid synthesis

THE pharmacological treatment of obesity using centrally acting anorectic drugs on a chronic basis is restricted due to the development of tolerance [15,22], side effects, and addiction liabilities. Another approach to obesity therapy could involve antiobesity agents with primarily peripheral, rather than central, mechanisms of action. Several compounds have been described with this pharmacological profile. Some of these compounds produce anorexia which may be secondary to another metabolic alteration such as a decrease in fatty acid synthesis and/or an increase in glycogen synthesis. These metabolic effects have been observed following treatment with (−)-*threo*-hydroxycitric acid [29], oxytetracycline [1,9], and fenfluramine [3,4].

The gastrointestinal tract represents another site for pharmacological modulation of appetite and body weight. Some of the proposed signals from the gastrointestinal tract which may participate in appetite regulation include signals from the stomach such as osmoreceptors regulating gastric emptying [13], gastric satiety signals [7], gastrointestinal tension receptors [5, 6, 26], nutrient duodenal receptors [20] and others. Pharmacological agents which modulate these signals may demonstrate anorectic activity.

This report describes studies which were designed to investigate the mechanism of action of (−)-*threo*-chlorocitric acid, a novel and potent anorectic agent. Unlike (−)-*threo*-hydroxycitric acid a structural analog which decreases food intake by altering hepatic metabolism, (−)-*threo*-chlorocitric acid appears to function in the gastrointestinal tract where it delays gastric emptying.

GENERAL METHOD

Animals

Female Sprague-Dawley rats obtained from Charles River Breeding Laboratories were used in all experiments. Animals were housed in wire-bottomed group cages in temperature regulated (22°C) light-controlled rooms (light, 6 a.m. to 6 p.m.; dark, 6 p.m. to 6 a.m.) for at least one week prior to each study. Rats were transferred to individual wire-bottomed cages before the training and study periods began.

Statistics

All data were processed for outliers [8]. The Students' two-tailed *t*-test was used to determine significance [25].

EXPERIMENT 1: COMPARISON OF THE EFFECT OF (−)-*threo*- AND (+)-*threo*-CHLOROCITRIC ACID ON FOOD INTAKE

The object of this experiment was to determine whether (−)-*threo*-chlorocitric acid would affect food intake in rats fed low and high fat diets. Preliminary experiments suggested that the stomachs of (−)-*threo*-chlorocitric acid-treated rats contained large amounts of diet despite the fact that this drug caused a suppression of food intake [27]. Since the fat content of the diet is an important factor in the rate of gastric emptying [16] and since fat content in human diets is greater than the 4.5% lipid present in Purina Laboratory

Chow, it was important to determine if the anorectic effect of (−)-*threo*-chlorocitric acid was sustained in animals trained to consume a high fat diet. Food intake was measured in meal-fed rats at 1 hr intervals to determine whether the anorectic effect was transitory. The (+)-*threo*-chlorocitric acid was included for comparison because it had demonstrated some anorectic activity in previous studies [27].

METHOD

The effect of (−)-*threo*-chlorocitric acid and (+)-*threo*-chlorocitric acid on food intake was determined using several diets described previously [32]. Rats were fasted for 48 hours and then trained to consume a single meal daily from 8 to 11 a.m. for 14 days. The diets used were: Purina Rodent Chow, which contained 4.5% fat and 58% complex polysaccharides (Ralston Purina Co., St. Louis, MO); a low fat diet which contained 1% corn oil and 70% glucose (Bioserv, Frenchtown, NJ); and a high fat diet which contained 20% corn oil and 50% glucose (Bioserv, Frenchtown, NJ). On the day before the experiment, rats weighing 170 to 200 g were divided between control (8 to 10 rats) and experimental (5 to 6 rats) groups. Only animals which had demonstrated weight gain during the training period were used. On the day of the experiment each rat was treated by oral intubation with either water or the appropriate compound dissolved in water, 30 minutes before the 3 hour meal. The (−)-*threo* and (+)-*threo*-chlorocitric acids were administered at 75 or 150 mg/kg body weight. Food consumption was measured at 1, 2 and 3 hours following the initiation of the meal.

RESULTS

(−)-*threo*-Chlorocitric acid produced a dose-dependent decrease in food intake regardless of the diet employed (Table 1). Total food consumption over 3 hours was decreased from 49% to 56% of control at 150 mg/kg, and from 54% to 65% of control by 75 mg/kg of (−)-*threo*-chlorocitric acid. Food consumption was reduced in (−)-*threo*-chlorocitric acid treated rats during all 3 hours of each type of meal. The low fat-fed rats receiving 75 mg/kg of (−)-*threo*-chlorocitric acid demonstrated a significant increase in food intake during the third hour. (+)-*threo*-Chlorocitric acid, unlike its (−)-*threo* isomer, had no effect on food intake in the chow or high fat-fed rats. In the low fat-fed rats there was a significant suppression of food intake during the first hour after treatment with 150 mg/kg and during the second hour after treatment with 75 mg/kg. However, the total food intake for the three hour meal was not different from control values at either dose of (+)-*threo*-chlorocitric acid.

The suppression of food intake resulting from (−)-*threo*-chlorocitric acid administration was greater during the first two hours of the 3 hour meal in low fat-fed rats and returned to control or above control levels during the third hour of feeding. The reduction in food intake for the total 3 hour period was, however, equivalent in all three dietary groups. In the high fat-fed rats, food intake was suppressed consistently throughout the 3 hour meal. Since the control animals on the chow and high fat diets consumed a greater percentage of their meal during the third hour compared to the control rats on the low fat diet (25%, 23% vs 15%, respectively), it is possible that the higher fat content of these diets may have enhanced the anorectic potential of (−)-*threo*-chlorocitric acid during the latter stages of the meal, perhaps by itself decreasing gastric emptying during the early part of the meal.

EXPERIMENT 2: EFFECT OF CHLOROCITRIC ACIDS ON CONDITIONED AVERSION

This experiment was conducted in order to determine whether conditioned aversion was a factor in the anorectic effect produced by (−)-*threo*-chlorocitric acid. Lithium chloride ($LiCl_2$) was used as the positive control since it has been shown to produce aversive behavior in rats [21]. Citric acid and (+)-*threo*-chlorocitric acid were included for comparison.

METHOD

Rats weighing 170 to 200 g were allowed free access to Purina Rodent Chow throughout the duration of the study. Prior to each test day, the rats (8 per group) were water-deprived for 23 hours then given access to water (during pretreatment periods) or a saccharin solution (during experimental periods) for one hour, as described previously [21]. The 0.25% saccharin (Sigma, St. Louis, MO) solution contained 0.1% orange extract (Ehlers, Lake Success, NY) as an odorizer. On each of four successive days following the two day pretreatment period, each rat was given either water, lithium chloride at a concentration (112 mg/kg) shown to produce conditioned aversion [23], citric acid (75 mg/kg) or the (−) and (+) isomers of *threo*-chlorocitric acid (75 mg/kg) by oral intubation immediately after the 1 hr drinking period. The four day test period was followed by a three day extinction phase during which time the animals were not dosed. In order to prevent dehydration during the test period, rats which drank less than 10 ml of saccharin solution were intubated orally, with 10 ml of water, given three to four hr after the test period.

RESULTS

Water intake was similar in all groups of rats during the two pretreatment test periods (Fig. 1). Fluid intake was increased for all groups when the saccharin solution was substituted for water on day 0. Saccharin intake was suppressed in rats following treatment with lithium chloride ($p<0.05$ on day 1 and $p<0.001$ on days 2 to 4), and returned to normal on day 6 during the extinction phase. Saccharin intake was not altered by treatment with citric acid, (−)-*threo*-chlorocitric acid or (+)-*threo*-chlorocitric acid. No conditioned aversion was seen with the racemic isomers of chlorocitrate, (±)-*threo*-chlorocitric acid or (±)-*erythro*-chlorocitric acid, when they were tested at 150 mg/kg (data not shown). These data suggest that the anorectic activity of (−)-*threo*-chlorocitric acid is not the result of aversive behavior.

EXPERIMENT 3: *IN VITRO* AND *IN VIVO* EFFECT OF (−)-*threo*-CHLOROCITRIC ACID ON HEPATIC LIPID METABOLISM

(−)-*threo*-Hydroxycitrate, a close structural analog of (−)-*threo*-chlorocitric acid [27], is also an anorectic [29]. The anorectic activity of (−)-*threo*-hydroxycitrate may be related to its inhibition of fatty acid synthesis [29–31, 33, 34] and an alteration of hepatic metabolite flux [29]. Therefore it was essential to determine whether the chlorocitrates would inhibit lipogenesis. The four isomers of chlorocitrate were tested in an *in vitro* system to determine whether they inhibited fatty acid synthesis. The 2 mM concentration used was shown to be the IC_{50} for (−)-*threo*-hydroxycitrate inhibition of fatty acid synthesis in isolated hepatocytes [30]. In order to determine whether inhibition of fatty acid synthesis might

TABLE 1

THE EFFECT OF CHLOROCITRIC ACIDS AND DIET ON FEEDING BEHAVIOR IN MEAL-FED CHARLES RIVER RATS

Treatment*	Diet	Dose mg/kg	Food Intake (g)† 1st hr	2nd hr	3rd hr	total
Control	low fat		5.8 ± 0.7‡	4.8 ± 0.5	1.9 ± 0.5	12.6 ± 1.2
(+)-*threo*	low fat	150	3.2 ± 0.5§	5.1 ± 1.3	1.7 ± 0.3	10.1 ± 1.7
	low fat	75	6.2 ± 1.8	2.4 ± 0.8§	2.4 ± 0.5	11.0 ± 2.3
(−)-*threo*	low fat	150	2.7 ± 0.4§	1.7 ± 0.3¶	2.3 ± 0.5	6.7 ± 0.7¶
	low fat	75	2.2 ± 0.5¶	2.2 ± 0.5¶	3.8 ± 0.4§	8.2 ± 1.1§
Control	chow		7.7 ± 0.7	3.0 ± 0.5	3.5 ± 0.5	14.1 ± 0.9
(+)-*threo*	chow	150	7.7 ± 0.5	3.9 ± 0.5	1.8 ± 0.9	13.4 ± 0.9
	chow	75	7.4 ± 0.6	2.6 ± 0.7	2.2 ± 0.5	12.2 ± 0.4
(−)-*threo*	chow	150	3.6 ± 0.9¶	1.4 ± 0.2§	1.9 ± 0.4§	6.9 ± 1.2¶
	chow	75	4.2 ± 1.4§	1.6 ± 0.4	2.3 ± 0.7	8.1 ± 2.1§
Control	high fat		6.8 ± 1.0	3.1 ± 0.7	2.9 ± 0.8	12.7 ± 0.7
(+)-*threo*	high fat	150	6.7 ± 1.6	3.1 ± 0.6	2.2 ± 0.7	12.3 ± 1.6
	high fat	75	7.1 ± 1.2	3.9 ± 0.5	3.2 ± 0.6	14.2 ± 1.2
(−)-*threo*	high fat	150	3.6 ± 0.6§	2.2 ± 0.4	1.3 ± 0.4	7.1 ± 0.9§
	high fat	75	4.0 ± 0.6	1.6 ± 1.1	1.3 ± 0.5	6.8 ± 1.6¶

*See Method section, Experiment 1, for details.
†Food consumption was measured at the indicated times, following the initiation of the meal.
‡Each value is the mean ± SE.
§$p<0.05$, ¶$p<0.01$.

result *in vivo* from an effect other than the anorexia produced by (−)-*threo*-chlorocitric acid, a study in pair-fed rats was conducted. (−)-*threo*-Chlorocitric acid and (−)-*threo*-hydroxycitrate were compared at doses which produced equivalent reductions of food intake and maximized the inhibition of fatty acid synthesis by (−)-*threo*-hydroxycitrate. The control animals were pair-fed to the treated rats in order to separate the effect of caloric availability from any effect on lipogenesis.

METHOD

Hepatocytes were prepared from meal-fed female Charles River rats (200 to 250 g) by the method of Berry and Friend [2] with modifications [30,33]. Rats were anesthetized with Nembutal (62.5 mg/kg IP), livers were perfused in situ and hepatocytes were isolated and washed in Krebs-Henseleit bicarbonate buffer pH 7.4. Cell integrity was determined using trypan blue; 90% to 95% of the cells excluded the dye. Sixty minute incubations with isolated hepatocytes were performed in triplicate at 37°C. Each incubation flask contained, in a total volume of 2.1 ml, 10 to 16 mg cells (dry weight), 1 ml of Krebs-Henseleit buffer, pH 7.4, 1 mCi ^3H_2O, 1 μCi [U-^{14}C] alanine, 1 μmole alanine, 0.3% glucose and 2 mM of the appropriate test compounds. Each flask was gassed for 15 seconds with a mixture of 95% O_2 and 5% CO_2 and stoppered during the incubation. Rates of fatty acid synthesis were determined after stopping the reaction with 2.1 ml of 5N NaOH. The flask contents were transferred to tubes, rinsed twice with 1 ml water, and saponified overnight at 90°C. The media were acidified with 2.5 ml of 5 N HCl, extracted 3 times with 5 ml petroleum ether, evaporated to dryness and radioactivity determined. Results are expressed

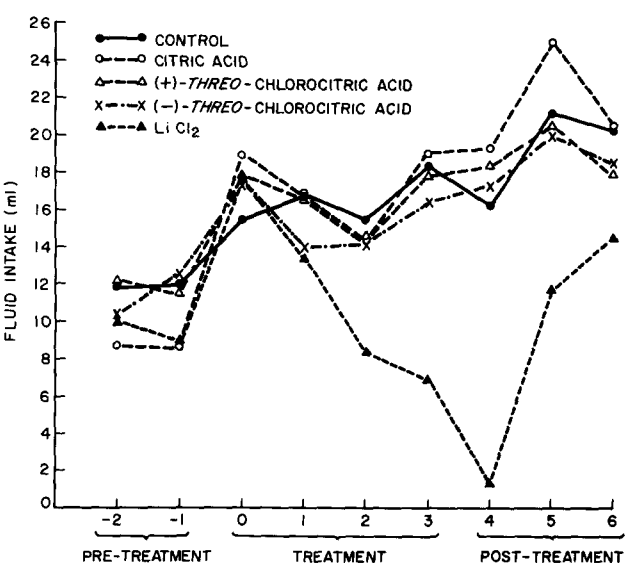

FIG. 1. Comparison of the effect of lithium chloride, citric acid, (−)-*threo*-chlorocitric acid and (+)-*threo*-chlorocitric acid on conditioned aversion in rats. Each value is mean daily fluid intake of 8 animals.

TABLE 2

THE EFFECT OF THE ISOMERS OF CHLOROCITRIC ACID ON FATTY ACID SYNTHESIS IN ISOLATED RAT HEPATOCYTES*

Compound	Concentration mM	Fatty Acid Synthesis ^3H_2O converted % of control	Fatty Acid Synthesis [^{14}C]alanine converted % of control
Control	—	100	100
(−)-*threo*-Hydroxycitric Acid	2	54‡	30‡
(−)-*threo*-Chlorocitric Acid	2	106	124†
(+)-*threo*-Chlorocitric Acid	2	93	102
(−)-*erythro*-Chlorocitric Acid	2	95	96
(+)-*erythro*-Chlorocitric Acid	2	94	97

*See Method section, Experiment 3, for details.
†$p \leq 0.05$, ‡$p \leq 0.01$.

TABLE 3

EFFECTS OF (−)-*THREO*-CHLOROCITRIC ACID AND (−)-*THREO*-HYDROXYCITRATE ON HEPATIC FATTY ACID SYNTHESIS *IN VIVO* IN A PAIR-FEEDING EXPERIMENT*

Treatment	Dose mg/kg	Fatty Acid Synthesis μmoles ^3H_2O converted/g liver/30 min	Fatty Acid Synthesis nmoles [^{14}C]alanine converted/g liver/30 min
Pair-fed control	—	21.3 ± 2.3	714 ± 86
(−)-*threo*-Hydroxycitric Acid	805	14.4 ± 1.6†	279 ± 36‡
Pair-fed control	—	25.6 ± 3.9	639 ± 69
(−)-*threo*-Chlorocitric Acid	150	21.4 ± 1.6	809 ± 67

*See Method section, Experiment 3, for details. Each value is the mean ± SE.
†$p \leq 0.05$, ‡$p \leq 0.01$.

as nmoles of radiolabelled precursor recovered as fatty acids per mg dry cells per 60 minutes.

In the *in vivo* pair-feeding experiment four groups of rats (140 to 160 g) containing 8 to 10 rats each were used. Rats were meal-fed a low-fat diet as described in Experiment 1. Control rats were pair-fed to the drug-treated rats and food intake was monitored daily over a three day period. Rats were given oral doses of water, (−)-*threo*-hydroxycitrate (805 mg/kg) or (−)-*threo*-chlorocitric acid (150 mg/kg) immediately before the three hour meal on three consecutive days; doses of the compounds were selected to produce comparable reductions in food intake. On days two and three, the pair-fed animals were given the same quantity of food that the matched drug-treated rats had eaten on the previous day. Rates of hepatic fatty acid synthesis *in vivo* were determined 30 minutes after the intravenous injection of a radioactive pulse consisting of 1 mCi of ^3H_2O, 5 μCi of [U-^{14}C]alanine, 12.3 mg of alanine, and 30.6 mg of α-ketoglutarate (as an amine acceptor for transaminase) in 0.25 ml of saline solution [34]. The radioactive precursor was administered immediately after the 3 hour meal. The animals were killed by decapitation 3.5 hours after drug treatment and livers were immediately excised, saponified in 10% potassium hydroxide in ethanol, acidified, and extracted with petroleum ether to determine *in vivo* rates of fatty acid synthesis [34]. Data are expressed as μmoles of ^3H_2O or nmoles of [^{14}C]alanine converted to fatty acids per gram of liver per 30 minutes.

RESULTS

In contrast to (−)-*threo*-hydroxycitric acid, none of the isomers of chlorocitric acid inhibited fatty acid synthesis from either ^3H_2O or [^{14}C]alanine in isolated rat hepatocytes (Table 2). (−)-*threo*-Hydroxycitric acid significantly reduced fatty acid synthesis from both precursors.

In vivo rates of hepatic fatty acid synthesis from both radio-labelled precursors, ^3H_2O and [^{14}C]alanine, were reduced significantly following three consecutive daily doses of (−)-*threo*-hydroxycitric acid, compared to pair-fed controls (Table 3). Fatty acid synthesis was not inhibited in rats treated with (−)-*threo*-chlorocitric acid.

EXPERIMENT 4: GASTRIC EMPTYING AS A MECHANISM FOR THE ANORECTIC ACTIVITY OF CHLOROCITRATE

During the course of these studies it was often noted that although (−)-*threo*-chlorocitric acid reduced food intake, the treated rats appeared to have full stomachs. This experiment was conducted to determine whether (−)-*threo*-chlorocitric acid influenced gastric emptying.

METHOD

In preliminary experiments fasted rats which were given a single radiolabelled glucose load (1 g/rat) by oral intubation 30 minutes after being treated with (−)-*threo*-chlorocitric acid retained a larger portion of the radioactive glucose in their stomachs then did the controls. In this model (−)-*threo*-chlorocitric acid inhibited gastric emptying to a greater extent than any of the other chlorocitric acid isomers (data not shown). To determine whether the delay in gastric emptying could also be demonstrated under the physiological conditions of a meal, rats weighing 180–200 g were fasted overnight and then trained for two weeks to consume a high fat (10% corn oil and 60% glucose [32]) meal during a two hour period daily (8 to 10 a.m.). Within this time, the rats were able to consume sufficient quantities of this diet (approximately 12 g/day) to gain weight at near normal rates. Experimental high fat diets were prepared by mixing the radioactive lipid tracer, glycerol tri[1-^{14}C]oleate (4.3×10^5 dpm per g of diet) with corn oil prior to mixing with the dry ingredients and [2-^{3}H]glucose (9.2×10^5 dpm per g of diet). (−)-*threo*-Chlorocitric acid (150 mg/kg) or saline were administered orally 30 minutes prior to meal feeding; the animals were killed by decapitation 30, 60 or 120 minutes after the end of the two hour feeding period. The pH of (−)-*threo*-chlorocitric acid was adjusted to 7 after dissolving in saline in order to eliminate the possibility of pH effects on gastric emptying. Blood was collected, the tissue and contents from stomachs, intestines and livers were removed and homogenized, and radioactivity in aliquots of the respective tissue homogenates were determined in a liquid scintillation counter.

RESULTS

Rats treated with (−)-*threo*-chlorocitric acid demonstrated an increase in the stomach content of radioactivity at 30, 60 and 120 minutes after ingestion of the glycerol tri[1-^{14}C]oleate; this increase was significant at 120 minutes (Fig. 2). The total radioactivity in the intestine, plasma and liver was reduced significantly at 30, 60 and 120 minutes in comparison to the controls, as would be expected with an agent which delays stomach emptying. (−)-*threo*-Chlorocitric acid produced an increased gastric retention of radioactivity from the [2-^{3}H]glucose containing meal in a similar manner to that observed after glycerol tri[1-^{14}C]oleate ingestion; the changes were significant at 30 and 120 minutes (Fig. 3). Significant decreases in liver [^{3}H] radioactivity were observed 30 and 60 minutes after the meal.

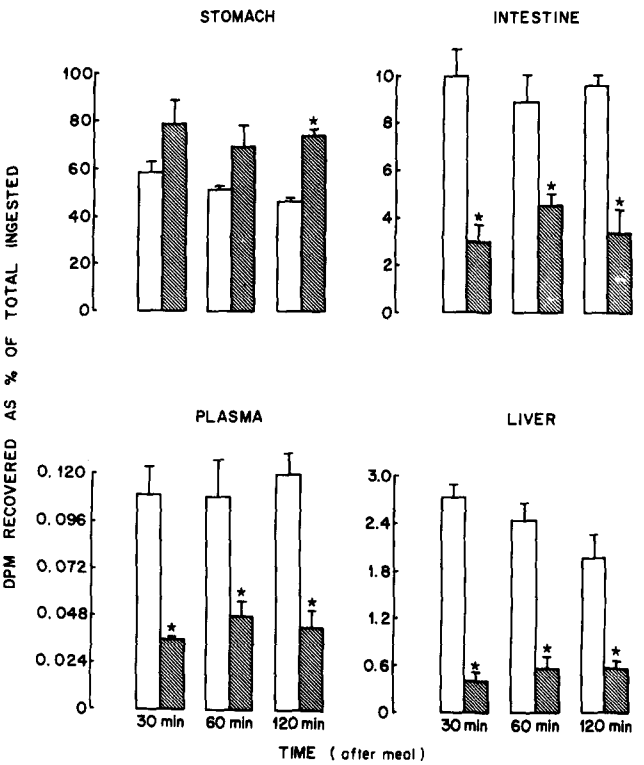

FIG. 2. Effect of (−)-*threo*-chlorocitric acid on gastric emptying and absorption of a glycerol tri[1-^{14}C]oleate containing meal in rats. Control values are represented by the open bars, while the values for (−)-*threo*-chlorocitric acid treated rats (150 mg/kg) are represented by the hatched bars. Each value is the mean±SE of five determinations. *$p<0.05$ compared to the control at each time period.

GENERAL DISCUSSION

The anorectic activity of the chlorocitric acids appears to be specific for (−)-*threo*-chlorocitric acid, since none of the other three isomers show a comparable reduction in food intake [27]. The mechanism of action of (−)-*threo*-chlorocitric acid does not appear to be similar to that of (−)-*threo*-hydroxycitric acid which is a potent competitive inhibitor of ATP citrate lyase [28,35]. Although the active chlorocitric and hydroxycitric acids are both (−)-*threo* isomers, the absolute configurations of these compounds are different (see Fig. 1 in [27]). According to the parent numbering system which is based on the parent molecule citric acid [12], the (−)-*threo*-hydroxycitric acid is the (4*S*)-OH cit-(pn_{cit}) whereas the (−)-*threo*-clorocitric acid is the (4*R*)-Cl cit-(pn_{cit}). However, the stereospecificity of the substitution does not appear to play as great a role in the inhibition of fatty acid synthesis as does the exchange of the -Cl for the -OH moiety. The chlorocitric acids, including the (+)-*erythro*-chlorocitric acid which has the same absolute configuration as the (−)-*threo*-hydroxycitric acid had no effect on fatty acid synthesis *in vitro* (Table 2).

The lack of inhibition of fatty acid synthesis with the four isomers of chlorocitric acid in isolated hepatocytes suggests that these compounds are devoid of metabolic effects on the lipogenic pathway. In the same experiment (−)-*threo*-hydroxycitric acid produced a 46% reduction in the incorporation of $^{3}H_2O$ into fatty acids which is consistent with previous reports [30,33]. Pair-feeding studies were utilized to determine the effects on hepatic fatty acid synthesis *in vivo* (Table 3). (−)-*threo*-Chlorocitric acid did not inhibit fatty acid synthesis *in vivo* compared to pair-fed controls. How-

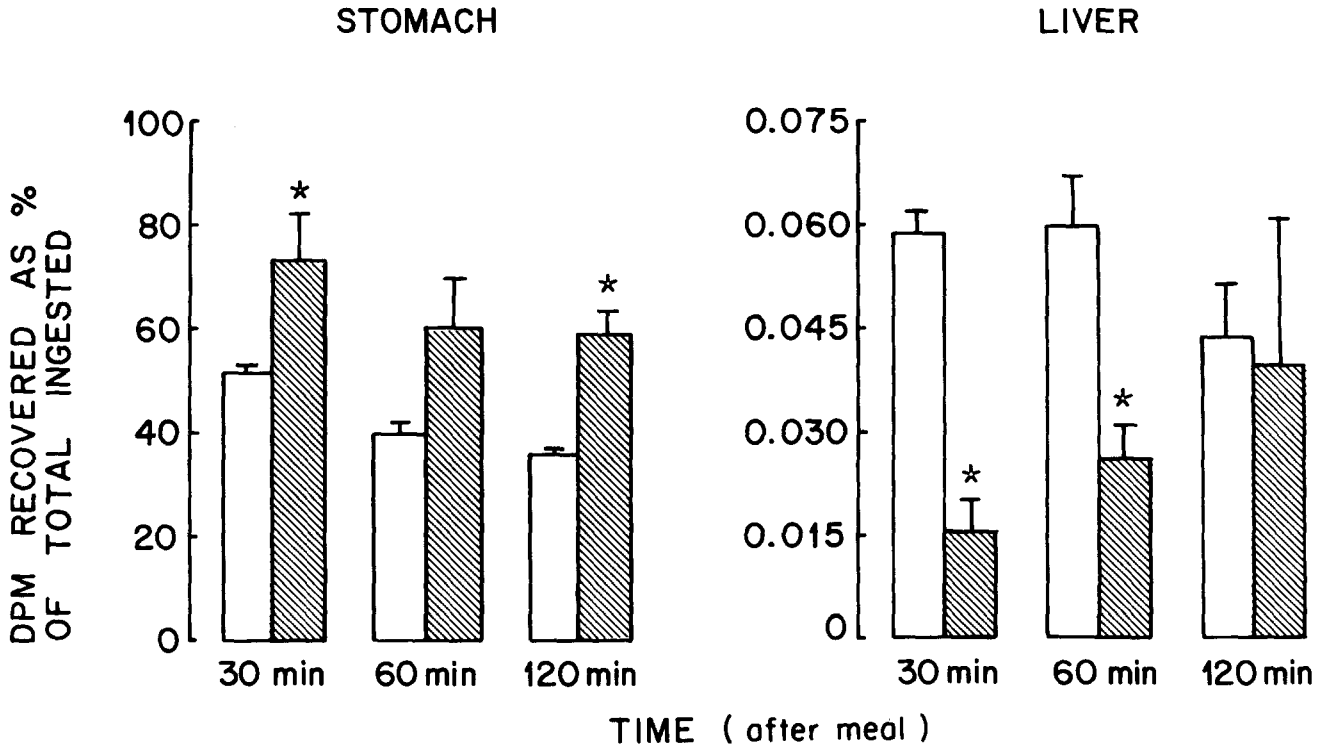

FIG. 3. Effect of (−)-*threo*-chlorocitric acid on gastric emptying and absorption of a [2-^{3}H]glucose containing meal in rats. See legend to Fig. 2 for details.

ever, (−)-*threo*-hydroxycitric acid significantly suppressed fatty acid synthesis, as previously demonstrated [30,31]. Therefore, despite a reduced entry of labelled glucose (precursor of fatty acids) into the liver of (−)-*threo*-chlorocitric acid treated rats after one hour of feeding (Fig. 3), hepatic fatty acid synthesis was not impaired after three hours of feeding (Table 3). It is concluded that (−)-*threo*-chlorocitric acid does not affect fatty acid synthesis either by acting on some step in the lipogenic pathway or by reducing the availability of hepatic fatty acid precursors.

To explore the possibility that food intake behavior may have been influenced by an aversion to the administration of (−)-*threo*-chlorocitric acid caused by a general feeling of malaise or gastrointestinal distress, the isomers of chlorocitric acid were compared to lithium chloride ($LiCl_2$) which has been used to produce conditioned aversion to novel food stuffs [21]. While $LiCl_2$ clearly produced long lasting aversion to saccharin, neither the chlorocitric acids nor citric acid itself had any effect on saccharin intake, suggesting that these compounds did not produce conditioned aversion (Fig. 1). A similar observation regarding lack of conditioned aversion was reported with the sodium salt of (−)-*threo*-hydroxycitric acid, although its ethylene diamine salt did produce aversion [23]. This effect was caused by the ethylene diamine and not the (−)-*threo*-hydroxycitric acid itself. More importantly, the lack of effect of (−)-*threo*-chlorocitric acid on drinking suggested that the body weight loss observed during long term administration of this drug [27] is not the result of decreased fluid intake.

Another mechanism that could play a role in the regulation of food intake might be the peripheral or hepatic utilization of nutrients. In humans, gastric emptying was delayed following the parenteral administration of a high caloric dextrose and amino acid solution [18], suggesting that the systemic availability of calories may play a role in the regulation of gastric emptying. The present studies show that (−)-*threo*-chlorocitric acid treatment actually decreased the amount of circulating and hepatic glucose or triolein (Figs. 2 and 3), indicating that systemic caloric availability may not be the component responsible for the delayed gastric emptying produced by this drug.

Since (−)-*threo*-chlorocitric acid had no apparent effect on fatty acid synthesis and preliminary studies suggest that it does not enter the brain, the possibility that its anorectic activity is linked to the observed inhibition of gastric emptying should be considered. The relationship between the regulation of gastric emptying and appetite, and its possible role in obesity was recently reviewed [13]. The regulation of gastric emptying was so precise in several experimental models that it was suggested to be an important modulator of preabsorptive satiety and the control of food intake [19]. Rapid transfer of energy from the stomach to the duodenum appeared to be associated with increased food intake [13]. It is of interest that a positive correlation between body weight and the amount of energy delivered into the duodenum 80 min after consuming a liquid meal was reported in humans [14]. In overweight subjects, a subjective feeling of satiety was positively correlated with gastric emptying time [36].

The control of food intake and the regulation of gastric emptying appear to have several components in common. Infusions of cholecystokinin [10,11] and surgical or pharmacological vagotomy [17,24] reduced gastric emptying and

were associated with decreased food intake. Cholecystokinin is released primarily in response to nutrients in the duodenum. Since (−)-*threo*-chlorocitric acid reduces the presence of nutrients in the intestines, it is unlikely that release of cholecystokinin is the primary way in which (−)-*threo*-chlorocitric acid inhibits gastric emptying. (−)-*threo*-Chlorocitric acid does not bind to cholinergic receptors in the stomach (M. Zanko and R. A. O'Brien, personal communication) so that vagal blockade in the stomach does not appear to be the primary site of its action. Preliminary evidence indicates that atropine methyl nitrate may block the chlorocitric acid-induced reduction of gastric emptying (J. Triscari and A. C. Sullivan, unpublished observation). Therefore, the possibility of a cholinergic effect at some peripheral site other than the stomach is plausible. Another possibility is the direct suppressant effect of (−)-*threo*-chlorocitric acid on gastric smooth muscle fibers or other unidentified hormonal effects on gastric emptying as explanations of its anorectic effects. While it remains uncertain whether the inhibition of gastric emptying alone is sufficient to explain the long term decreases in food intake observed during chronic (−)-*threo*-chlorocitric acid administration [27], this effect represents a possible explanation for the acute reductions in food intake observed in the present study.

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