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(−)-*threo*-Chlorocitric Acid: A Novel Anorectic Agent

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SULLIVAN, A. C., W. DAIRMAN AND J. TRISCARI. *(−)-threo-Chlorocitric acid: A novel anorectic agent.* PHARMAC. BIOCHEM. BEHAV. 15(2) 303–310, 1981.—A new class of peripherally acting anorectics is described in these studies. The four stereoisomers of chlorocitric acid which are structurally similar to the known anorectic, (−)-*threo*-hydroxycitric acid, all suppressed food intake when administered at high doses to rats. Only one of these isomers, (−)-*threo*-chlorocitric acid, retained its anorectic activity at lower doses. The anorectic potency of (−)-*threo*-chlorocitric acid was approximately 40-fold greater in dogs than in lean and obese rats. The decreased food intake in rats resulted in a significant reduction of body lipid, without affecting protein levels. In contrast to the tolerance which was observed with the continued administration of mazindol or diethylpropion, tolerance to the anorectic effect of (−)-*threo*-chlorocitric acid did not develop. These studies suggest that (−)-*threo*-chlorocitric acid might be useful as an antiobesity agent.

(−)-*threo*-Chlorocitric acid Anorectic agent Antiobesity agent Obese Zucker rats Diethylpropion Mazindol (−)-*threo*-Hydroxycitric acid

CURRENT views on appetite regulation support the involvement of both central and peripheral processes [9,22]. The pharmacological treatment of obesity has been based on the use of centrally acting anorectic agents [1, 4, 6, 8, 10], and these drugs continue to be useful as short-term adjuncts to diet programs. However, the chronic use of many centrally acting appetite suppressants is limited because of side effects, potential addiction liabilities and, in particular, the development of tolerance. The initial anorectic effect of amphetamine and other appetite suppressants is diminished with repeated administration [3,5].

In an attempt to eliminate the development of tolerance which is characteristic of the centrally active anorectic drugs, we have been searching for peripherally acting anorectics. These efforts led to the identification of two antiobesity agents which have peripheral modes of action. (−)-*threo*-Hydroxycitric acid and (±)-*threo*-epoxyaconitic acid produced anorexia and decreased body weight gain by modulating hepatic and gastrointestinal rather than central mechanisms regulating appetite [13–15]. Neither CNS stimulation nor the development of tolerance was observed with these agents. (−)-*threo*-Hydroxycitric acid appeared to act primarily by inhibiting hepatic lipid synthesis [11, 17, 20] and altering carbohydrate metabolism [13]. The diversion of hepatic metabolites from fatty acid synthesis into glycogen synthesis was suggested to have affected hepatic glucoreceptors which are thought to monitor energy availability in the liver and play a role in appetite regulation [13]. In contrast to (−)-*threo*-hydroxycitric acid, (±)-*trans*-epoxyaconitic acid did not alter fatty acid or glycogen synthesis. Its anorectic activity appeared to be related to a selective reduction in the rate of gastric emptying [14].

This report describes the activity of a novel mono-substituted citric acid analog, (−)-*threo*-chlorocitric acid. The pharmacological effects of (−)-*threo*-chlorocitric acid on appetite, body weight and body composition were determined in laboratory animals. (−)-*threo*-Chlorocitric acid decreased food intake, body weight gain and body lipid. The potential for the development of tolerance to the anorectic activity of (−)-*threo*-chlorocitric acid was compared to that of the centrally acting anorectic agents, mazindol and diethylpropion.

EXPERIMENT 1: COMPARISON OF THE ANORECTIC ACTIVITY OF THE ISOMERS OF CHLOROCITRIC ACID IN RATS

Since (−)-*threo*-hydroxycitrate had been shown to have anorectic activity in rats [16], the possibility that other substituted analogs of citric acid, and citric acid itself, might demonstrate similar activity was explored. A comparison of the configurations of the hydroxycitric acids and chlorocitric acids is shown in Fig. 1. (−)-*threo*-Hydroxycitric acid, the active hydroxycitric acid which is equivalent in configuration to (+)-*erythro*-chlorocitric acid, will be referred to as (−)-hydroxycitrate in the ensuing discussion.

METHOD

Two month old Sprague-Dawley female rats (Charles River Breeding Laboratories, Wilmington, MA) were housed in individual wire-bottomed cages in a temperature-regulated (22°C) light-controlled room (12 hr light, 6 a.m. to 6 p.m. and 12 hr dark, 6 p.m. to 6 a.m.). All rats were fasted for 48 hours, then meal-fed a nutritionally complete synthetic 70%

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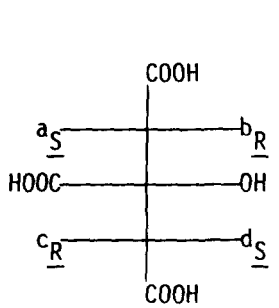
ABSOLUTE CONFIGURATIONS OF THE STEREOISOMERS OF CHLOROCITRIC ACIDS AND HYDROXYCITRIC ACIDS



Substituent in Above Formula				Compound	Nomenclature Convention	
a_S	b_R	c_R	d_S		R/S System	(pn_{cit})-System
H	H	H	H	Citric Acid		
				Chlorocitric Acids		
H	H	CL	H	(−)-threo-chlorocitric acid	1R, 2R	(4R)-CLcit-(pn_{cit})
CL	H	H	H	(+)-threo-chlorocitric acid	1S, 2S	(2S)-CLcit-(pn_{cit})
H	CL	H	H	(−)-erythro-chlorocitric acid	1R, 2S	(2R)-CLcit-(pn_{cit})
H	H	H	CL	(+)-erythro-chlorocitric acid	1S, 2R	(4S)-CLcit-(pn_{cit})
				Hydroxycitric Acids		
H	H	OH	H	(−)-erythro-hydroxycitric acid	1R, 2S	(4R)-OHcit-(pn_{cit})
OH	H	H	H	(+)-erythro-hydroxycitric acid	1S, 2R	(2S)-OHcit-(pn_{cit})
H	OH	H	H	(+)-threo-hydroxycitric acid	1R, 2R	(2R)-OHcit-(pn_{cit})
H	H	H	OH	(−)-threo-hydroxycitric acid	1S, 2S	(4S)-OHcit-(pn_{cit})

FIG. 1. Absolute configuration of the stereoisomers of chlorocitric acid, hydroxycitric acid and citric acid.

glucose diet [19] (Bioserv, Frenchtown, NJ) from 8 to 11 a.m., daily, for one week prior to the experiment. Rats which weighed 130 to 150 g and which had gained weight during the pretreatment period were divided into a control group (n=10) and 5 experimental groups (n=6). On the experimental day each rat received water or the indicated compound by oral intubation 30 minutes before the 3 hour meal.

All the data in the ensuing experiments were analyzed for statistical validity with the use of a two-tailed Students' *t*-test [7] after processing for outliers [2]. A p value of less than 0.05 was considered significant.

RESULTS

The effects of a single oral administration of the stereoisomers of chlorocitric acid on food intake were compared at 2 doses, 597 mg/kg and 150 mg/kg. The citric acid dose (505 mg/kg) was equimolar to the high dose of the chlorocitric acids. All four isomers demonstrated some anorectic activity at the high dose with the following order of potency: (−)-*threo-* > (+)-*threo-* > (−)-*erythro-* > (+)-*erythro-* (Table 1). Citric acid was inactive. Only (−)-*threo-*chlorocitric acid produced significant anorectic activity at the low dose (59% of control). (+)-*erythro-*Chlorocitric acid, the isomer equivalent in configuration to the anorectic (−)-hydroxycitrate (Fig. 1 and [16]), was the least active in reducing food intake.

EXPERIMENT 2: EFFECT OF THE ISOMERS OF CHLOROCITIRIC ACID ON BODY WEIGHT GAIN IN RATS

The purpose of this experiment was to determine whether

TABLE 1

EFFECT OF THE ISOMERS OF CHLOROCITRIC ACID ON FOOD CONSUMPTION IN RATS

Treatment*	Dose mg/kg	Food Intake % of control
Control	—	100 ± 9
Citric Acid	505	94 ± 13
(−)-*threo*-Chlorocitric Acid	597	16 ± 4‡
	150	59 ± 9†
(+)-*threo*-Chlorocitric Acid	597	38 ± 9‡
	150	83 ± 12
(−)-*erythro*-Chlorocitric Acid	597	53 ± 11‡
	150	85 ± 23
(+)-*erythro*-Chlorocitric Acid	597	78 ± 6†
	150	99 ± 20

*See Method section, Experiment 1, for details. Each value is the mean ± SE.
†$p \leq 0.05$, ‡$p \leq 0.001$.

the anorexia produced by (−)-*threo*-chlorocitric acid treatment would reduce body weight. The other chlorocitric acid isomers were also tested to determine if any of them had effects on body weight which might be independent of any potential anorectic activity. For this purpose an intermediate dose (300 mg/kg) was chosen to minimize the anorectic effects of the (+)-*threo*-, (−)-*erythro*- and (+)-*erythro*-chlorocitric acids.

METHOD

Three month old Sprague-Dawley female rats were housed and fed as outlined in Experiment 1. The pretreatment meal-feeding with the 70% glucose diet [19] lasted two weeks. Rats weighing 190 to 220 g were divided into a control group (n=16) and 4 experimental groups (n=8). On each of three experimental days the animals were given either water (control) or the chlorocitrates (300 mg/kg) by oral intubation 30 minutes prior to the start of the 3 hour meal.

RESULTS

Following three consecutive daily doses with the isomers of chlorocitric acid (300 mg/kg), (−)-*threo*-chlorocitric acid reduced food intake to 37% of control and decreased body weight gain by more than 22 g compared to control rats (Table 2). The (−)-*erythro*-chlorocitric acid also caused a significant decrease in food intake (86% of control), but this degree of anorexia was insufficient to significantly reduce weight gain during the three day period. (+)-*threo*-Chlorocitric acid and (+)-*erythro*-chlorocitric acid were inactive. These data suggest that the chlorocitrates had no metabolic effects which might result in decreased body weight gain.

EXPERIMENT 3: DEVELOPMENT OF TOLERANCE, COMPARISON OF (−)-*threo*-CHLOROCITRIC ACID TO MAZINDOL AND DIETHYLPROPION IN RATS

The purpose of this experiment was to determine whether tolerance developed to the anorectic effect of (−)-*threo*-chlorocitric acid. Two known anorectics, mazindol and diethylpropion, were used for comparison. The dose of (−)-*threo*-chlorocitric acid selected (100 mg/kg) was found, in a 1 day pilot study, to produce a reduction in food intake equivalent to that of 10 mg/kg of either mazindol or diethylpropion.

METHOD

Sprague-Dawley female rats were trained on a meal-feeding regimen as described in Experiment 1. Rats weighing approximately 200 g were divided into a control group (n=30) and 3 experimental groups (n=20). On each of 15 experimental days rats were given either water, 100 mg/kg of (−)-*threo*-chlorocitric acid, 10 mg/kg of mazindol or 10 mg/kg of diethylpropion by oral intubation 30 minutes before the three hour meal. Mazindol and diethylpropion were kindly provided by Sandoz Pharmaceuticals (Hanover, NJ) and Merrell-National Laboratories (Cincinnati, OH), respectively.

RESULTS

The potential for the development of tolerance to the anorectic activity of (−)-*threo*-chlorocitric acid (100 mg/kg), mazindol (10 mg/kg) and diethylpropion (10 mg/kg) was evaluated in a 15 day study. Higher concentrations of mazindol and diethylpropion could not be given because of toxicity. All three drugs decreased food intake to an equivalent extent on the first day of treatment (Fig. 2). The reduced food con-

TABLE 2

EFFECT OF THE ISOMERS OF CHLOROCITRIC ACID ON FOOD INTAKE AND BODY WEIGHT GAIN IN A THREE DAY STUDY

Treatment*	Three-Day Food Consumption		Three-Day Body Weight Gain	
	g	% of control	g	% of initial body weight
Control	28.6 ± 1.1	100	− 0.2 ± 1.1	− 0.1
(−)-*threo*-Chlorocitric Acid	10.5 ± 1.3‡	37	−22.4 ± 2.3‡	−11.0
(+)-*threo*-Chlorocitric Acid	26.5 ± 1.3	93	− 3.5 ± 1.2	− 1.7
(−)-*erythro*-Chlorocitric Acid	24.5 ± 1.8†	86	− 4.8 ± 3.2	− 2.4
(+)-*erythro*-Chlorocitric Acid	31.8 ± 2.3	111	− 1.5 ± 2.3	− 0.7

*See Method section, Experiment 2, for details. Each value is the mean ± SE.
†$p \leq 0.05$, ‡$p \leq 0.001$.

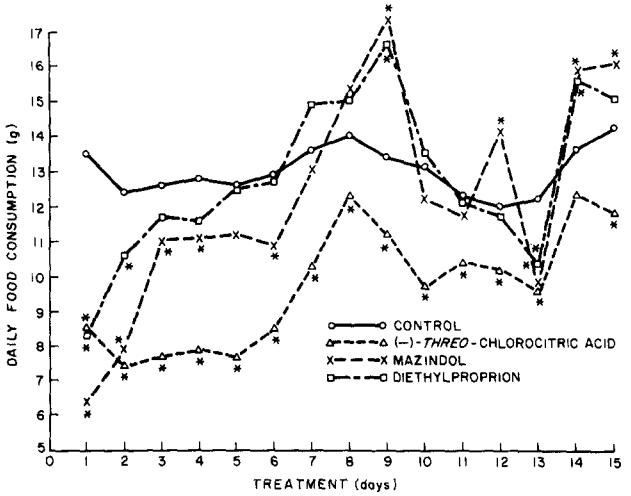


FIG. 2. Comparison of the effect of (−)-*threo*-chlorocitric acid, mazindol and diethylpropion on food intake in meal-fed rats. *$p<0.05$ compared to control.

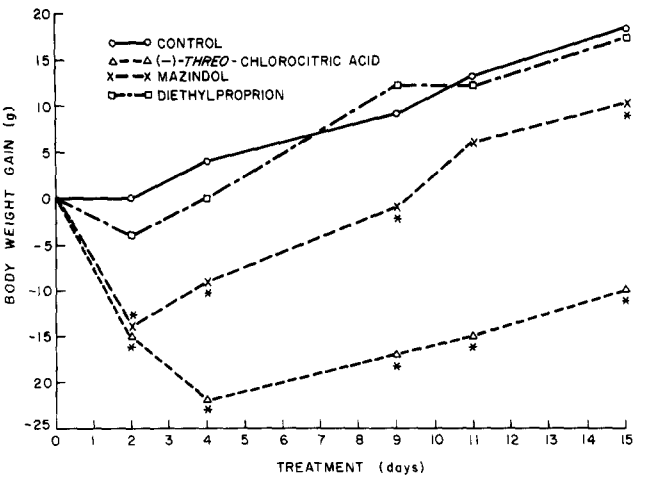


FIG. 3. Comparison of the effect of (−)-*threo*-chlorocitric acid, mazindol and diethylpropion on body weight gain in meal-fed rats. *$p<0.05$ compared to control.

sumption was maintained for 4 to 6 days in mazindol-treated rats and for 2 days in diethylpropion-treated rats, after which time tolerance developed to the anorectic activity of these drugs. A significant compensatory increase in food intake was observed on days 9, 12, 14 and 15 with mazindol and on days 9 and 14 with diethylpropion. In contrast to the development of tolerance and the compensatory hyperphagia noted with mazindol and diethylpropion, treatment with (−)-*threo*-chlorocitric acid resulted in significantly suppressed food intake throughout the 15 day study period. The most potent effects were observed during the first week of treatment.

Body weight gain was reduced significantly and equivalently by both mazindol and (−)-*threo*-chlorocitric acid 2 days after treatment began (Fig. 3). Diethylpropion had no effect on weight gain at any time during the study. Four days after the beginning of treatment, weight gain was further decreased by (−)-*threo*-chlorocitric acid, while mazindol treated rats began to increase their weight gain towards that of the control rats. At the end of the 15 day treatment period the diethylpropion-treated rats had gained as much weight as controls. The mazindol-treated rats had gained less weight than controls but were catching up, while the (−)-*threo*-chlorocitric acid-treated rats had maintained a markedly lower weight gain than either the controls, mazindol or diethylpropion-treated groups.

EXPERIMENT 4: EFFECT OF LONG TERM TREATMENT WITH (−)-*threo*-CHLOROCITRIC ACID ON FOOD INTAKE, BODY WEIGHT GAIN AND CARCASS COMPOSITION IN RATS

This experiment was conducted to determine whether the effects of (−)-*threo*-chlorocitric acid on food intake and body weight gain were dose-dependent and to evaluate the composition of the weight loss. A pilot study demonstrated that the minimally effective dose for anorexia produced by (−)-*threo*-chlorocitric acid was approximately 20 mg/kg. Other studies also indicated that the anorectic effect of (−)-*threo*-chlorocitric acid was greater in chow fed rats [21]. Therefore, these studies were conducted in chow fed rats at doses of 57, 114 and 227 mg/kg of (−)-*threo*-chlorocitric acid.

METHOD

Sprague-Dawley female rats were trained on a 3 hour meal-feeding schedule using Purina Chow diet (Ralston Purina Co., St. Louis, MO). Following the one week training period rats weighing 200 grams were divided into a control group (n=10) and three experimental groups (n=6). Rats were treated by oral intubation 30 minutes before the 3 hour meal. Controls received water and experimentals received 57, 114 or 227 mg/kg of (−)-*threo*-chlorocitric acid on each of 17 consecutive days. Body weights and food intakes were measured at the indicated intervals (Figs. 4 and 5). Data for body weight gain on day 14 for rats treated with 227 mg/kg of (−)-*threo*-chlorocitric acid are missing because of a weighing error.

Carcass analyses were done on a separate group of rats which were treated as described above with the following exceptions: the pretreatment period was five days, the duration of the experiment was 16 days, the control group contained 10 rats and the experimental group treated with 227 mg/kg of (−)-*threo*-chlorocitric acid contained 9 rats.

Total body lipid and protein levels were determined as described previously [18]. The rats were killed by decapitation and the livers and blood were removed. The carcasses were weighed, saponified in 10% potassium hydroxide in ethanol, acidified and extracted with petroleum ether. The petroleum ether supernatants were transferred to preweighed glass vials, immediately evaporated under nitrogen to dryness, and reweighed. Carcass lipid data are expressed in grams and percentages of carcass weight. An aliquot of the saponified carcass extract was neutralized and total carcass nitrogen was determined using the Kjeldahl procedure. Carcass protein data are expressed in grams and percentages of carcass weight.

RESULTS

(−)-*threo*-Chlorocitric acid produced a significant, dose dependent suppression of cumulative food intake when given

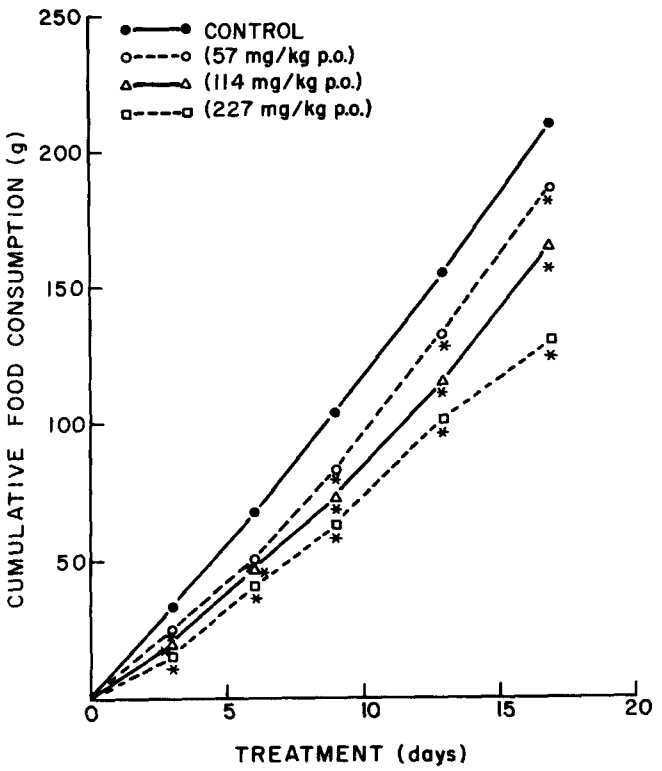


FIG. 4. Dose-dependent effect of (−)-*threo*-chlorocitric acid on food intake in rats meal-fed a chow diet. *$p<0.05$ compared to control.

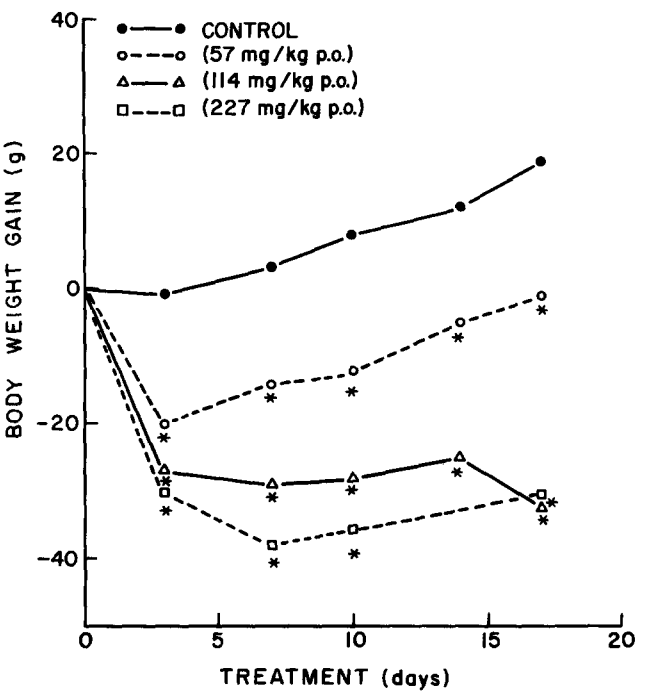


FIG. 5. Dose-dependent effect of (−)-*threo*-chlorocitric acid on body weight gain in rats meal-fed a chow diet. *$p<0.05$ compared to control.

over a 17 day period to rats meal-fed a chow diet (Fig. 4). This effect was sustained over the entire 17 day treatment period and was associated with a concomitant, dose related reduction in body weight (Fig. 5). The 16-day food intakes and body weight gains for the rats used in the carcass composition studies were 211±6 g and 23±2 g, respectively, for the control and 139±6 g and −27±4 g, respectively, for the (−)-*threo*-chlorocitric acid-treated rats. (−)-*threo*-Chlorocitric acid produced a significant decrease in carcass lipid (Table 3), whether expressed as total grams of fat or percent of carcass weight (4.4% for control rats compared to 1.9% for treated rats). Total grams of protein were also significantly decreased, but protein as percent of carcass weight was similar in treated (26.7%) and control (26.9%) groups.

EXPERIMENT 5: COMPARISON OF THE ANORECTIC EFFECT OF (−)-*threo*-CHLOROCITRIC ACID IN GENETICALLY OBESE AND LEAN ZUCKER RATS

The obese Zucker rat has been shown to overeat compared to its lean littermate [23]. Its response to dietary caloric dilution, however, was different from that of its lean littermate [14], i.e., the obese rat did not increase its food intake to compensate for the dietary caloric dilution as the lean rat did. It was thus important to determine whether comparable anorexia would be produced in lean and obese rats by (−)-*threo*-chlorocitric acid.

TABLE 3

EFFECT OF (−)-*THREO*-CHLOROCITRIC ACID ON CARCASS LIPID AND PROTEIN CONTENT IN A 16 DAY STUDY

Treatment*	Carcass Weight†	Carcass lipid		Carcass Protein	
	g	g	% of carcass weight	g	% of carcass weight
Control	207 ± 4	9.2 ± 0.8	4.4 ± 0.4	55.8 ± 2.0	26.9 ± 0.8
(−)-*threo*-Chlorocitric Acid	155 ± 4‡	2.7 ± 0.2‡	1.9 ± 0.1‡	41.1 ± 1.9‡	26.7 ± 1.4

*See Method section, Experiment 4, for details. Each value is the mean ± SE.
†Carcass weight does not include blood and liver.
‡$p \leq 0.001$.

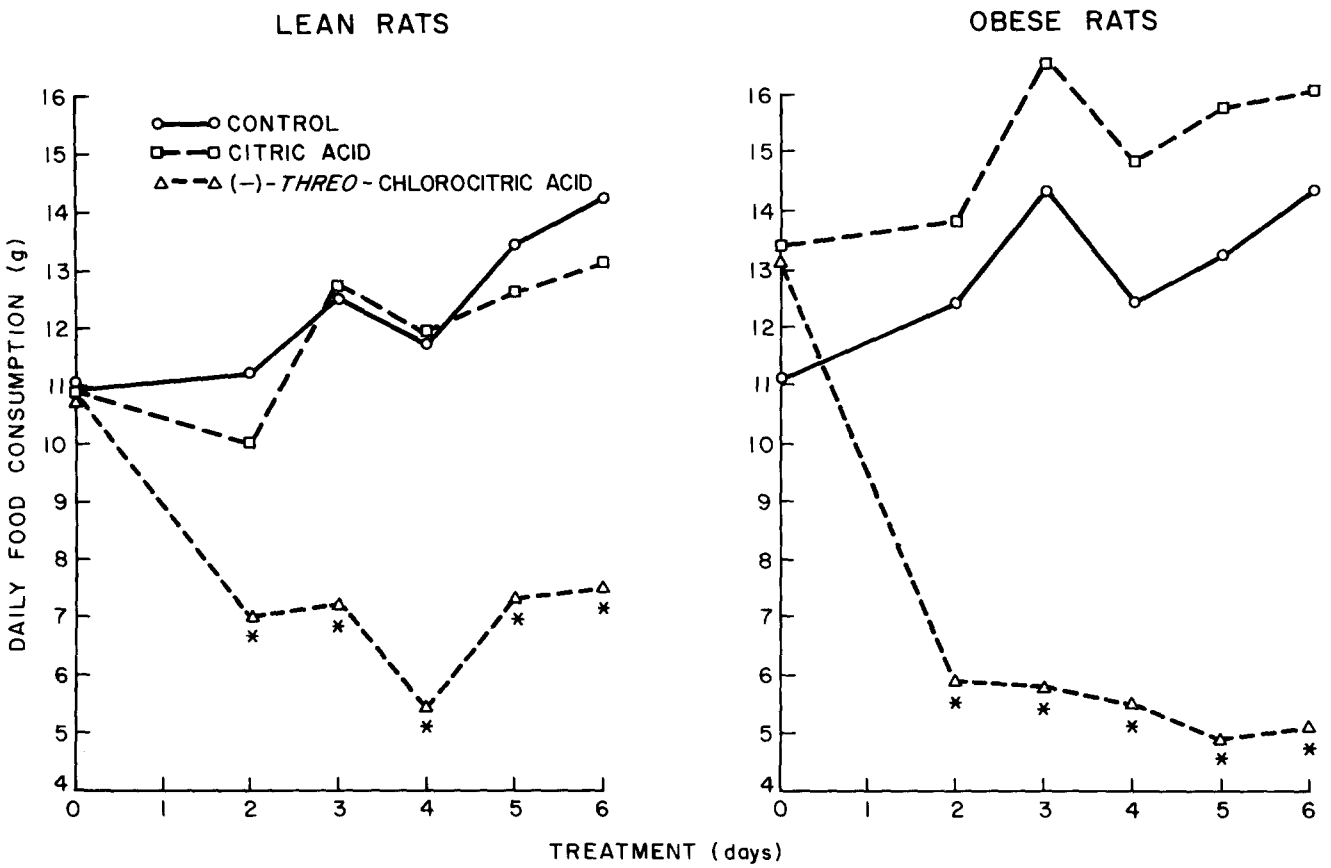


FIG. 6. Effect of (−)-*threo*-chlorocitric acid on food consumption in lean and obese Zucker rats. *$p<0.05$ compared to control.

METHOD

Five month old male and female Zucker heterozygous lean (250 to 270 g) and homozygous obese (390 to 460 g) rats bred in our laboratories from an original stock provided by Dr. L. M. Zucker (Red Acre Farm, Stowe, MA) were trained for 5 days to consume a 70% glucose diet [19]. Rats (10 per group) were fed from 8 a.m. to 4 p.m. daily. This 8 hour meal-feeding period was selected because obese rats failed to maintain or increase their body weights on shorter meal-feeding schedules. On each of 6 experimental days rats received either water, (−)-*threo*-chlorocitric acid (150 mg/kg) or citric acid (254 mg/kg, which was twice the molar dose of the chlorocitric acid) twice a day at 7:30 a.m. and 12 p.m.

RESULTS

Daily b.i.d. administration of (−)-*threo*-chlorocitric acid (150 mg/kg/day) produced a consistent suppression of food intake in both lean and obese rats over a six day period (Fig. 6). This effect was greater in obese (56% decrease) than lean (43% decrease) rats. Citric acid had no effect in lean or obese rats at a dose of 254 mg/kg b.i.d. Thus, hyperphagic genetically obese rats appeared to respond to the anorectic effect of (−)-*threo*-chlorocitric acid like lean Sprague-Dawley rats.

EXPERIMENT 6: ANORECTIC EFFECT OF (−)-*threo*-CHLOROCITRIC ACID IN DOGS

This experiment was conducted to determine whether the anorexia produced by (−)-*threo*-chlorocitric acid in rats could be duplicated in another species. The doses of (−)-*threo*-chlorocitric acid used were based on results of pilot studies.

METHOD

Two male and two female beagle dogs, weighing 6.9 to 10.1 kg (Marshall Research Animals) were given (−)-*threo*-chlorocitric acid in gelatin capsules over a 5 week period. Eight hundred grams of diet consisting of Wayne Chunk dog food, Triumph canned beef and water were made available to each animal. Food was presented 2 hours after the oral administration of the gelatin capsules. Food intake in this study was measured at 6.5 hours and 24 hours after the beginning of the meal. Any uneaten food which remained after the 6.5 hour measurement was returned to the cages and left overnight. The food was removed the following morning and total food consumption was recorded.

During the first week of the study, each animal received 1 empty gelatin capsule each day for seven days and food consumption was measured daily. During the second week of

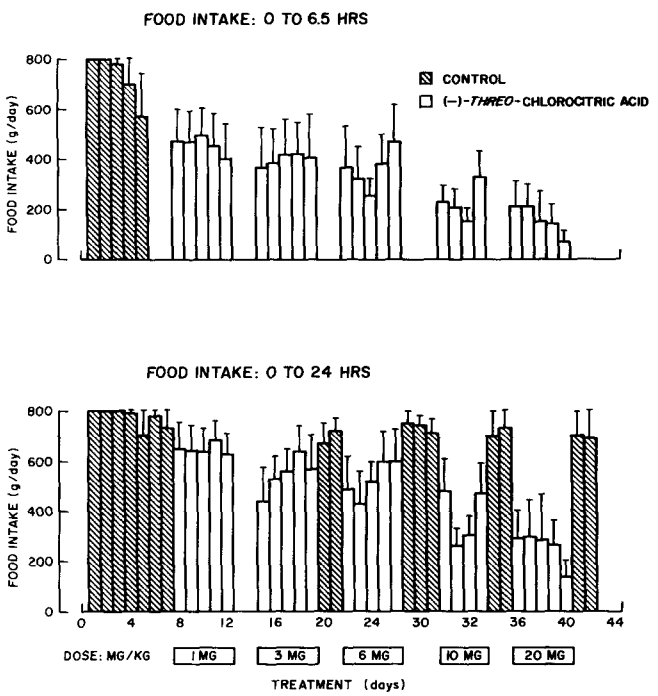


FIG. 7. Effect of (−)-*threo*-chlorocitric acid on food consumption in beagle dogs. (−)-*threo*-Chlorocitric acid significantly reduced food intake at each dose level ($p<0.05$).

the study, each animal received (−)-*threo*-chlorocitric acid at 1 mg/kg/day and food consumption was measured daily for five of seven days. During the third through sixth weeks, each animal received 3, 6, 10 or 20 mg/kg/day of (−)-*threo*-chlorocitric acid, respectively, for five of seven days and food consumption was measured daily.

RESULTS

The anorectic potency of (−)-*threo*-chlorocitric acid was approximately 40-fold greater in dogs than rats. (+)-*threo*-Chlorocitric acid was significantly less active in rats and completely inactive in dogs at oral doses of 1 mg/kg to 1000 mg/kg per day (data not shown). (−)-*threo*-Chlorocitric acid produced a dose-dependent decrease in food consumption when intake was measured at 6.5 hr and 24 hr after food was presented or 8.5 and 26 hour after drug administration (Fig. 7). At the 1 mg/kg dose level, the 6.5 hour food intake was suppressed by 37% and the 24 hour intake was reduced by 16%. At the 6 mg/kg dose, a 50% and 31% decrease was observed at 6.5 and 24 hours, respectively. At the highest dose tested, 20 mg/kg, the 6.5 hour intake was reduced by 70% and the 24 hour intake by 66%. Food consumption returned to normal on each day of empty capsule administration. There was a rapid return to control levels of food intake during drug-free periods and after discontinuing treatment at the end of the study. There was no compensatory hyperphagia following periods of decreased intake.

GENERAL DISCUSSION

This report describes the efficacy of a novel anorectic agent, (−)-*threo*-chlorocitric acid. Although the other three isomers of chlorocitric acid decreased food intake at doses of 597 mg/kg, only the (−)-*threo*-chlorocitric acid isomer retained potent anorectic effects at 150 mg/kg (Table 1). Thus, it appears that the stereospecific substitution of chlorine on the citric acid molecule (Fig. 1) may be responsible for the selective anorectic effects of (−)-*threo*-chlorocitric acid. (−)-*threo*-Chlorocitric acid was 40 times more potent in suppressing food intake in beagle dogs than in rats. Significant anorexia was demonstrated at a 1 mg/kg dose in dogs (Fig. 7). In contrast to (−)-*threo*-chlorocitric acid, the (+)-*threo*-chlorocitric acid isomer was completely inactive in dogs even at a dose of 1000 mg/kg, confirming the stereoselectivity of the anorectic action of the chlorocitric acids.

(−)-*threo*-Chlorocitric acid is the third mono-substituted citric acid analog reported to suppress food intake. (−)-*threo*-Hydroxycitric acid reduced food intake and body weight due to a selective reduction in body lipid in several obese rodent models [15,16]. The primary mechanism of weight loss and anorexia appeared to be mediated through its effects on lipid synthesis inhibition [13,17]. The other three stereoisomers of (−)-*threo*-hydroxycitric acid were inactive or slightly active in reducing lipid synthesis [12, 17, 20]. However, the most potent anorectic of the chlorocitric acid stereoisomers, (−)-*threo*-chlorocitric acid, has a different absolute configuration from (−)-*threo*-hydroxycitric acid, the active isomer of the hydroxycitric acids (Fig. 1). In contrast to (−)-*threo*-hydroxycitric acid, (−)-*threo*-chlorocitric acid had no effect on lipid synthesis [21]. A second anorectic drug, (±)-*trans*-epoxyaconitic acid, is another substituted analog of citric acid which, in contrast to (−)-*threo*-hydroxycitric acid, had no effect on lipid synthesis [14]. Its anorectic activity appeared to be related to a selective reduction in gastric emptying. This mechanism may also be important in the anorectic activity of (−)-*threo*-chlorocitric acid [21].

Chronic treatment with (−)-*threo*-chlorocitric acid produced a readily reversible, dose-dependent reduction in food intake in Sprague-Dawley rats and in beagle dogs. (−)-*threo*-Chlorocitric acid also produced a significant anorectic effect in both lean and obese Zucker rats over a six day period (Fig. 6). This suggested that the mechanism through which this drug acts was intact in obese rats. The dose-dependent reductions in body weight in rats produced by (−)-*threo*-chlorocitric acid treatment were reflected in a selective suppression of body lipid without a change in percent protein composition (Table 3). There was no evidence of tolerance development or rebound eating in rats and dogs treated with (−)-*threo*-chlorocitric acid, as occurred with mazindol and diethylpropion in rats (Figs. 2, 3). Both of these agents, and the amphetamines [3,5] are well known for their central stimulatory properties and the development of tolerance to their anorectic effects. Although we cannot exclude a central site of action for (−)-*threo*-chlorocitric acid, treated rats showed none of the usual signs of central stimulation associated with centrally active anorectic agents such as amphetamines, or sedation associated with fenfluramine.

In summary, (−)-*threo*-chlorocitric acid appears to fulfill many of the criteria for an "ideal" anorectic agent. It provides a long lasting, but readily reversible suppression of food intake which is associated with significant weight loss in experimental animals. There is no evidence of central nervous system stimulation or the development of tolerance to this drug. These studies suggest that (−)-*threo*-chlorocitric acid might be useful as an antiobesity agent.

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