

Effects of intermittent intraperitoneal infusion of salmon calcitonin on food intake and adiposity in obese rats

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Chelikani PK, Haver AC, Reidelberger RD. Effects of intermittent intraperitoneal infusion of salmon calcitonin on food intake and adiposity in obese rats. *Am J Physiol Regul Integr Comp Physiol* 293: R1798–R1808, 2007. First published August 29, 2007; doi:10.1152/ajpregu.00386.2007.—Chronic administration of anorexigenic substances to experimental animals by injections or continuous infusion typically produces no effect or a transient reduction in daily food intake and body weight. Our aim was to identify an intermittent dosing strategy for intraperitoneal infusion of salmon calcitonin (sCT), a homolog of amylin that produces a sustained 25–35% reduction in daily food intake and adiposity in diet-induced obese rats. Rats (649 ± 10 g body wt, $27 \pm 1\%$ body fat), with intraperitoneal catheters tethered to infusion swivels, had free access to a 45% fat diet. Food intake, body weight, and adiposity during the 7-wk test period were relatively stable in the vehicle-treated rats ($n = 16$). None of 10 sCT dosing regimens administered in succession to a second group of rats ($n = 18$) produced a sustained 25–35% reduction in daily food intake for >5 days, although body weight and adiposity were reduced by 9% (587 ± 12 vs. 651 ± 14 g) and 22% (20.6 ± 1.2 vs. $26.5 \pm 1.1\%$), respectively, across the 7-wk period. The declining inhibitory effect of sCT on daily food intake with the 6-h interinfusion interval appeared to be due in part to an increase in food intake between infusions. The declining inhibitory effect of sCT on daily food intake with the 2- to 3-h interinfusion interval suggested possible receptor downregulation and tolerance to frequent sCT administration; however, food intake increased dramatically when sCT was discontinued for 1 day after apparent loss of treatment efficacy. Together, these results demonstrate the activation of a potent homeostatic response to increase food intake when sCT reduces food intake and energy reserves in diet-induced obese rats.

amylin; anorexia; body weight; body fat

AN IMPORTANT EARLY STEP in development of obesity drugs is determining whether chronic administration of anorexigenic substances, alone or in combination, can produce a sustained decrease in daily food intake and adiposity in obese experimental animals. Methods of administration typically include daily injections or insertion of an osmotic minipump beneath the skin or into the peritoneal cavity to deliver substances continuously for ≥ 1 wk. Results from such studies are usually inconclusive. Reasons include development of a compensatory increase in food intake between injections, receptor downregulation and tolerance (tachyphylaxis) to continuous or frequent administration of the anorexigenic substances, and redundancy and plasticity in the energy regulatory system (17, 25, 35).

We have developed a novel experimental model that permits precise intravenous or intraperitoneal administration of anorex-

igenic substances to rats tethered via infusion swivels to computer-controlled pumps. Rats are free to move, eat, and drink within their individual cages, and their indwelling catheters remain functional for many months. Measurement of food bowl weight, recorded by computer at 20-s intervals, permits daily assessment of the instantaneous effects of infused substances on food intake. Dosing pattern can be adjusted daily to define a dosing strategy that minimizes compensatory hyperphagia between doses and tolerance. We used this experimental model to show that intravenous infusion of the gut hormone peptide YY(3–36) [PYY(3–36)] dose dependently reduces short-term food intake in lean rats (10). We further demonstrated that intermittent intravenous infusion of PYY(3–36) produced a sustained decrease in daily food intake and adiposity in lean rats for 10 days, but only when intervals between PYY(3–36) infusions were shortened sufficiently to minimize compensatory hyperphagia between infusions (8). These studies helped resolve the intense debate regarding the inhibitory effects of exogenous PYY(3–36) on food intake and body weight (18, 27).

We previously determined the dose-response effects of 3-h intravenous infusions of several anorexigenic substances on short-term food intake in rats (9–11, 30, 31). These include the gastrointestinal peptides amylin, cholecystokinin octapeptide and adrenomedullin, PYY(1–36), PYY(3–36), and glucagon-like peptide-1, the adipose hormone leptin, the melanocortin agonist melanotan II, the cannabinoid antagonist AM-251, the opioid antagonist naloxone, and the amylin homologs salmon calcitonin (sCT), calcitonin gene-related peptide, and adrenomedullin. The two most potent anorexigenic substances were sCT and amylin (Fig. 1).

Amylin and sCT are structurally related peptides that appear to act at the same receptor to inhibit food intake (22). Previous studies showed that continuous intracerebroventricular administration of the amylin receptor antagonist acetyl-[Asn³⁰, Tyr³²]sCT-(8–32) (AC-187) by osmotic minipump increases daily food intake and body fat in lean rats (33) and that amylin-null mice gain weight at a higher rate than wild-type mice (26). These results suggest that amylin may contribute to the long-term regulation of energy reserves and that amylin agonists, including sCT, may prove useful in treating obesity. However, chronic administration of amylin (6, 32, 34), sCT (38), and calcitonin (25) to rodents by osmotic minipump or injections has been reported to only transiently reduce food intake. Our aim here was to identify an intermittent dosing strategy for intraperitoneal infusion of sCT that produces a sustained re-

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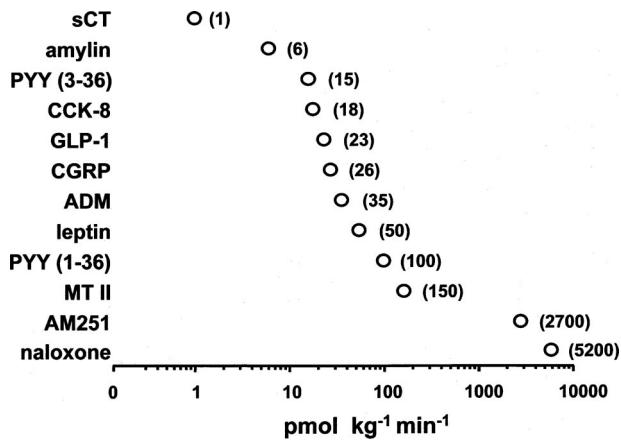


Fig. 1. Mean effective dose (ED_{50}) of 3-h intravenous infusion of anorexigenic substances [salmon calcitonin (sCT), peptide YY(3–36) [PYY(3–36)], cholecystokinin octapeptide (CCK-8), glucagon-like peptide-1 (GLP-1), calcitonin gene-related peptide (CGRP), adrenomedullin (ADM), melanotan II (MT II), AM-251, and naloxone] at the onset of the dark period on food intake in nondeprived lean rats (Refs. 9–11, 30, 31).

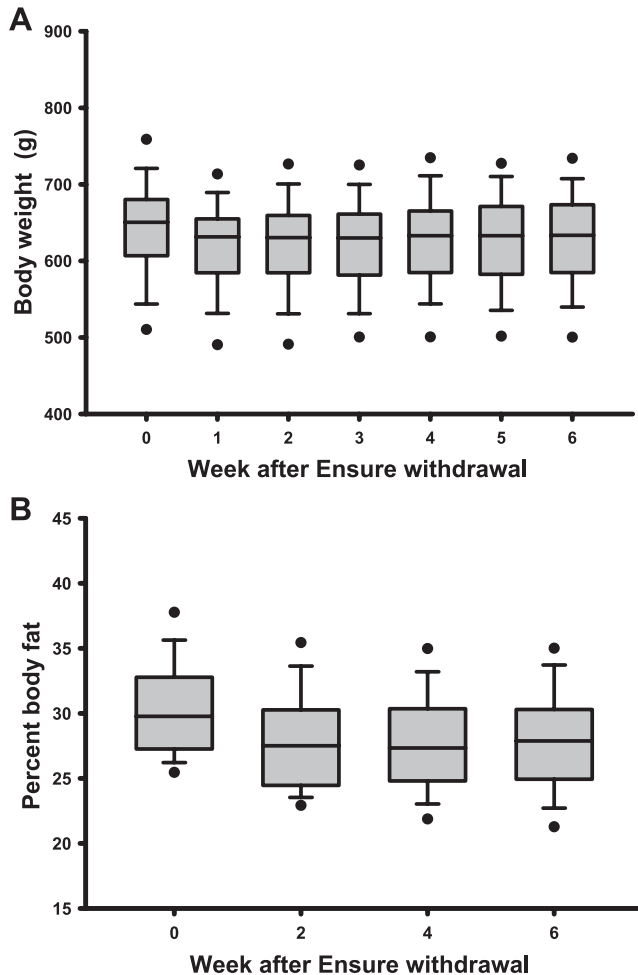


Fig. 2. Effects of Ensure liquid diet withdrawal on body weight distribution (A) and percent body fat distribution (B) in 106 diet-induced obese rats consuming 45% fat semipurified solid food. Rats had free access to Ensure and 45% fat solid diet for 4–8 mo; then Ensure was withdrawn for 6 wk. Upper border, middle line, and lower border of each box indicate 75th percentile, median, and 25th percentile, respectively; whiskers (error bars) above and below the box indicate 90th and 10th percentiles. ●, 95th and 5th percentiles.

duction in daily food intake and adiposity in diet-induced obese rats.

MATERIALS AND METHODS

Peptides. sCT was obtained from Anaspec (San Jose, CA). Peptide purity was confirmed by HPLC, and molecular mass was confirmed by electrospray mass spectrometry. sCT stock was prepared by dissolving the peptide in 0.15 M NaCl and 0.1% BSA to a concentration of 100 nmol/ml. Single-use aliquots were stored at -70°C .

Animals. Male Sprague-Dawley rats (Sasco, Charles River, Portage, MI; 225–350 g initial body wt) were housed in a room with controlled temperature ($19\text{--}21^{\circ}\text{C}$) and 12:12-h light-dark cycle (lights off at 1700). Pelleted rat chow (Labdiet, 5001 Rodent diet, PMI Nutrition International) and water were provided ad libitum for ~ 1 wk before the rats were subjected to experimental procedures. The

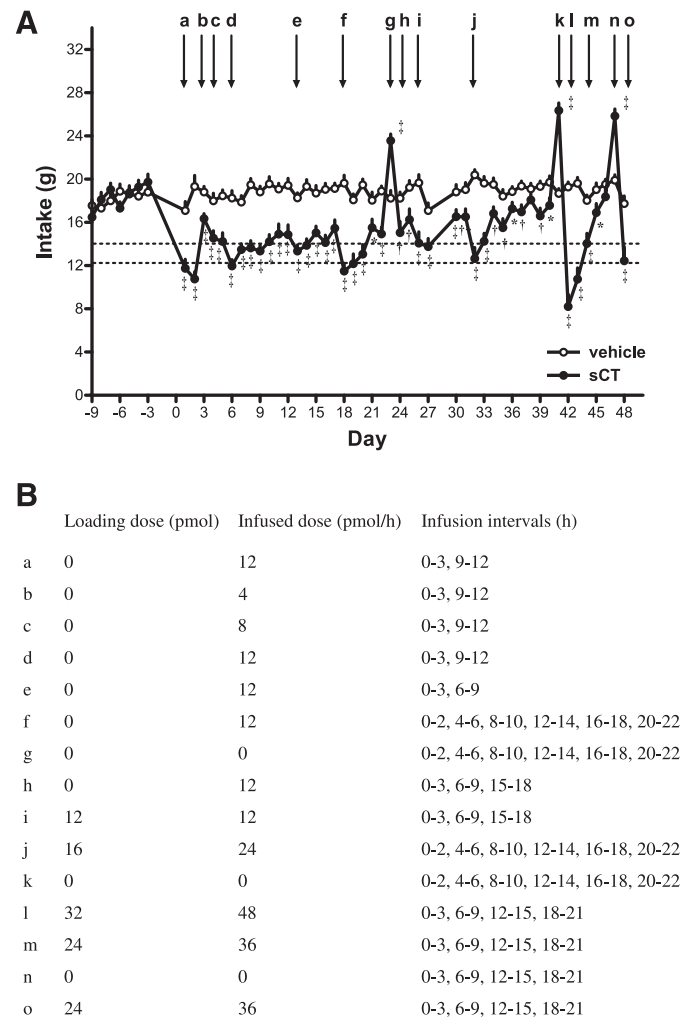


Fig. 3. Effects of intermittent intraperitoneal infusions of sCT on daily food intake in diet-induced obese rats. During a 7-day baseline period (days -9 to -3), rats ($n = 37$) received intraperitoneal infusions of vehicle during hours 0–3 and 9–12 of the dark period. During the 7-wk treatment period, separate groups of rats were infused intraperitoneally during the same periods with vehicle ($n = 16\text{--}18$) or sCT ($n = 18\text{--}19$) by 10 different dosing regimens. Dotted lines border a range in food intake that is $\sim 25\text{--}35\%$ less than that observed in vehicle-treated control rats. Values are means $\pm$ SE. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ vs. vehicle. B: dosing regimens. Some dosing regimens employed a loading dose of sCT administered just before food presentation at the onset of the dark period (time 0).

Animal Studies Subcommittee of the Omaha Veterans Affairs Medical Center approved the experimental protocol.

Dietary induction of obesity. Rats ($n = 166$) were housed in pairs in shoe-box cages with free access to water at all times. A high-fat pelleted food (45, 35, and 20% calories from fat, carbohydrate, and protein, respectively, 4.73 kcal/g; diet no. D12451, Research Diets, New Brunswick, NJ) and vanilla Ensure Plus liquid food (29, 56, and 15% calories from fat, carbohydrate, and protein, respectively, 1.5 kcal/ml; Ross Nutrition, Abbott Laboratories, Columbus, OH) were provided. The combined use of palatable high-fat solid and liquid foods induces obesity in a high proportion of rats (3, 4, 20, 24). An EchoMRI-700 quantitative nuclear magnetic resonance (QMR) analyzer (Echo Medical Systems, Houston, TX) was used for monthly measurements of total body fat in unanesthetized rats. Obesity ($\geq 25\%$ body fat) was induced in 106 of the 166 rats (64%) during a 4- to 8-mo period. Percent body fat of the remaining 60 rats was $21.9 \pm 0.4\%$ (13.3–19.9% body fat in 14 rats and 20–24.9% body fat in 46 rats). Our aim was to test whether sCT could reduce body weight and adiposity in obese rats, rather than just prevent weight gain. Thus we determined whether the obese rats could maintain a relatively stable body weight and adiposity while they consumed only the high-fat solid food (diet no. D12451). The 106 obese rats were housed singly, and diet no. D12451 was provided for 6 wk. We chose a 6-wk period to monitor body weight and body fat, because the study of Levin and Dunn-Meynell (20) suggests that when Ensure is withdrawn, a significant number of our rats might lose a significant amount of

body weight and fat over a 3- to 7-wk period. Figure 2 shows weekly body weight and body fat distributions during this period. Median body weight and body fat decreased slightly by the end of the 1st wk, yet distributions remained relatively stable over the next 6 wk.

Surgical and postsurgical adaptation procedures. In 86 of these diet-induced obese rats ($29.1 \pm 0.4\%$ body fat), an intraperitoneal catheter was surgically implanted under isoflurane anesthesia with use of procedures described previously (12). The intraperitoneal catheter, which exited the skin in the dorsal cervical region, was plugged with stainless steel wire and kept patent by weekly flushing with 1 ml of normal saline. After surgery, rats were transferred to a room with a 12:12-h light-dark cycle (lights off at 1100). During the postsurgical recovery period, the animals had access to Ensure and pelleted high-fat food. Rats were allowed 3 wk to regain lost body weight. The animals were then fitted with a light-weight harness (IITC, Woodlands, CA), which was used to tether them to an infusion swivel, and allowed a further 3 wk to adapt to the harness. Of the 86 rats with the surgically implanted intraperitoneal catheter, 37 were used in the sCT study, because available resources did not permit larger treatment groups. The rats chosen for testing at the end of the 8-wk period of adaptation between surgery and the start of experiments had functional catheters, the largest percent body fat, the most stable body weight, and the most stable daily food intake. For this first study using this experimental design, we surgically prepared approximately twice as many animals as

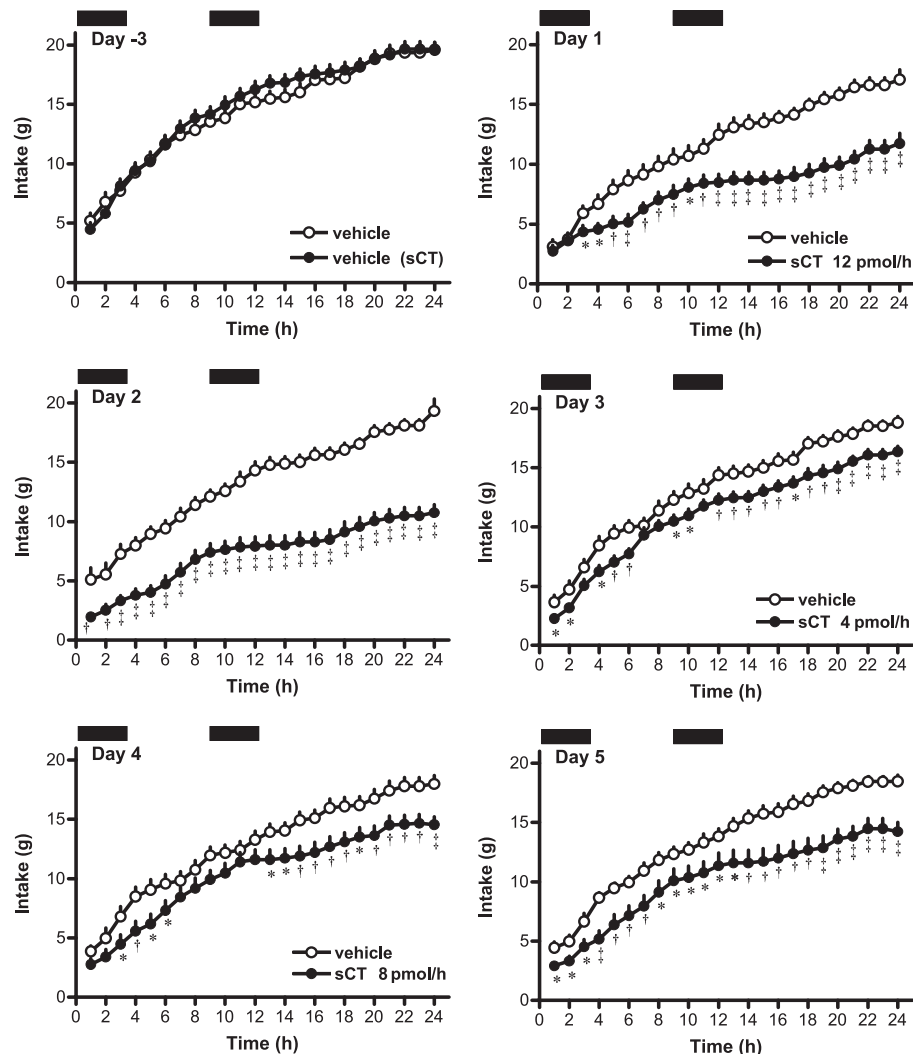


Fig. 4. Effects of intermittent intraperitoneal infusions of sCT on cumulative hourly food intake in diet-induced obese rats during the last day of the baseline period (day -3) and the first 5 days of sCT treatments. Data are from the experiment described in Fig. 3. Time 0, start of 12-h dark period. Horizontal bars indicate periods of infusion. Values are means $\pm$ SE. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ vs. vehicle.

needed to ensure a sufficient number of stable, tethered obese animals for experimentation.

Experimental procedures. Obese rats ($n = 32$) were housed individually in a metabolism cage modified to include a stainless steel side compartment with a 3-cm-diameter hole in the base. Below the hole was a food cup for powdered food that was fixed to a digital balance. The 32 balances in this 32-cage system were connected to a computer through a code-activated switch (model CAS-161, Western Telematic, Irvine, CA). Output from each balance was monitored at ~20-s intervals, and changes in food container weight were recorded. Data were processed to determine the amount of food ingested each hour, and total food intake was cumulated hourly. Another five obese rats were housed in the same-type metabolism cage with the same-type food container that was not fixed to a digital balance. For these animals, daily ingestion of food was determined by manual weighing of the food container at the start and end of each day. Manual and automated weighing of the food containers gave the same results. Thus daily food intake was measured in all 37 rats, and cumulative hourly intake was measured each day in 32 of the 37 rats.

The intraperitoneal catheter of each rat was connected to a 40-cm length of tubing passed through a protective spring coil connected between the light-weight harness worn by the rat and a single-channel infusion swivel (Instech Laboratories, Plymouth Meeting, PA), which allowed free movement of each rat in its individual cage. Powdered high-fat solid food (diet no. D12451) and water were provided each day from 1100 to 0800 on the next morning (dark period 1100–2300). Experimental setup and routine maintenance were performed each day between 0800 and 1100. Animals were allowed an additional 2 wk to regain body weight and adapt to tethering and experimental conditions. Thus rats had access to high-fat foods for 7–11 mo before testing. During an initial 7-day baseline period, all rats were intraperitoneally infused with vehicle (0.15 M NaCl and 0.1% BSA, 0.9 ml/h) during hours 0–3 and 9–12 of the dark period (1100–1400 and 2000–2300, respectively). Rats were weighed at the beginning and end of the baseline period, and their total body fat was determined by QMR at the end of the baseline period. After 2 days, the animals were divided into two groups: one to receive vehicle ($n = 18$) and the other to receive sCT ($n = 19$). The groups were matched for average daily caloric intake during the last 3 days of the baseline period, weight gain during the baseline period, and body weight and percent body fat at the end of the baseline period.

On the 1st day of treatment, sCT was infused at $0.3 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (12 pmol/h) during hours 0–3 and 9–12 of the dark period. We previously determined that intravenous infusion of this dose of sCT during the first 3 h of the dark period significantly inhibits food intake in lean rats (31). This initial twice-daily dosing regimen permitted us to evaluate the extent to which 1) food intake is reduced during the first 3-h treatment period, 2) desensitization to sCT-induced anorexia occurs during the second 3-h treatment period, 3) an increase in food intake occurs between treatment periods, and 4) daily food intake is reduced in response to the two treatment periods. On subsequent days, dosing level and/or pattern of administration in the sCT-treated group was adjusted, as necessary, in an attempt to define the lowest dose and frequency of sCT administration that would induce a sustained 25–35% reduction in average daily caloric intake for 2 wk compared with average daily food intake in the rats treated with vehicle at the same infusion rate during the same intervals. A dosing regimen was usually changed after 2 consecutive days of daily food intake reductions below or above this 25–35% criterion for reduction in daily food intake. This criterion was chosen for two reasons: 1) a sustained daily caloric restriction of this magnitude has been reported to prevent the spontaneous occurrence of obesity and metabolic disease in Sprague-Dawley male rats (19), and 2) a similar daily caloric restriction is generally recommended for weight loss in obese humans. We tested the effects of 10 different sCT treatments during the 7-wk period (Fig. 3). On three different occasions, sCT treatment was discontinued for 1 day to allow us to assess whether loss in efficacy of a treatment

might be due to receptor downregulation and tolerance to sCT administration or activation of orexigenic mechanisms to counter the inhibitory effect of sCT on food intake and adiposity. Rats were weighed weekly, and their body fat was measured by QMR at the end of the 7-wk period. During this period, two vehicle-treated rats and one sCT-treated rat were removed from the experiment because of catheter malfunction.

Statistical analyses. Values are group means $\pm$ SE. Data were analyzed by ANOVA. Planned comparisons of treatment means were evaluated by t -tests and paired t -tests. Differences were considered significant if $P < 0.05$.

RESULTS

Effects of intermittent intraperitoneal infusion of sCT on food intake, body weight, and adiposity in diet-induced obese rats. By the end of the 7-day baseline period, distributions of body weight (648 ± 15 vs. 651 ± 13 g), percent body fat (26.9 ± 1 vs. $26.7 \pm 1\%$), weight gain during the baseline period (6 ± 2 vs. 6 ± 2 g), and average daily food intake (20.0 ± 0.6 vs. 19.8 ± 0.4 g) in the two groups of rats that were to receive intraperitoneal infusions of vehicle ($n = 18$) or sCT ($n = 19$) were not statistically different ($P > 0.05$). Cumulative hourly food intake on the last day of the baseline period (day -3) was also not different in these groups (Fig. 4).

During the 7-wk treatment period, daily food intake in vehicle-treated rats remained relatively stable (Fig. 3), body weight increased slightly from 648 ± 15 to 672 ± 19 g ($P <$

Table 1. Effects of intermittent intraperitoneal infusions of sCT on food intake in diet-induced obese rats

	Intake, g			
	0–5 h	5–9 h	9–14 h	14–24 h
Day 1				
Vehicle	7.9 ± 0.8	2.5 ± 0.3	3.0 ± 0.4	3.1 ± 0.4
sCT	$5.0 \pm 0.6^\dagger$	2.5 ± 0.4	$1.2 \pm 0.3^\ddagger$	$1.8 \pm 0.4^*$
Day 2				
Vehicle	9.0 ± 0.6	3.2 ± 0.4	2.8 ± 0.3	2.9 ± 0.4
sCT	$4.0 \pm 0.6^\ddagger$	3.4 ± 0.3	$0.6 \pm 0.2^\ddagger$	2.3 ± 0.3
Day 6				
Vehicle	8.0 ± 0.6	3.4 ± 0.4	3.4 ± 0.5	2.8 ± 0.4
sCT	$3.7 \pm 0.7^\ddagger$	3.8 ± 0.4	$1.5 \pm 0.4^\dagger$	2.7 ± 0.4
Day 7				
Vehicle	7.7 ± 0.6	3.4 ± 0.5	2.6 ± 0.4	3.5 ± 0.4
sCT	$4.8 \pm 0.5^\ddagger$	3.8 ± 0.6	$1.4 \pm 0.3^\dagger$	2.6 ± 0.4
Day 8				
Vehicle	9.4 ± 0.9	3.2 ± 0.4	2.4 ± 0.4	3.4 ± 0.3
sCT	$5.6 \pm 0.7^\ddagger$	4.0 ± 0.4	$1.4 \pm 0.3^*$	$2.1 \pm 0.4^*$
Day 9				
Vehicle	9.0 ± 0.7	3.7 ± 0.4	2.5 ± 0.4	3.3 ± 0.4
sCT	$4.8 \pm 0.5^\ddagger$	4.0 ± 0.4	1.9 ± 0.3	$2.2 \pm 0.3^*$
Day 10				
Vehicle	9.5 ± 0.7	3.8 ± 0.4	2.3 ± 0.3	3.5 ± 0.4
sCT	$5.0 \pm 0.6^\ddagger$	4.3 ± 0.4	1.8 ± 0.3	3.2 ± 0.5
Day 11				
Vehicle	9.1 ± 0.6	3.3 ± 0.5	3.3 ± 0.3	3.1 ± 0.5
sCT	$5.5 \pm 0.7^\ddagger$	$4.3 \pm 0.3^*$	$1.6 \pm 0.4^\dagger$	2.4 ± 0.3
Day 12				
Vehicle	10.1 ± 0.6	3.0 ± 0.5	2.8 ± 0.5	2.9 ± 0.4
sCT	$5.7 \pm 0.8^\ddagger$	$4.4 \pm 0.6^*$	2.3 ± 0.5	2.4 ± 0.4

Values are means $\pm$ SE from experiment described in Fig. 3. Salmon calcitonin (sCT) was infused at 12 pmol/h during hours 0–3 and 9–12 each day. Time 0 is onset of the 12-h dark period. * $P < 0.05$; $^\dagger P < 0.01$; $^\ddagger P < 0.001$ vs. vehicle.

0.01), and percent body fat remained the same (26.9 ± 1.1 vs. $27.9 \pm 1.8\%$, $P > 0.05$).

Our goal was to define the lowest dose and frequency of sCT administration that would induce a sustained 25–35% reduction in average daily caloric intake for 2 wk. A dosing regimen was usually changed after 2 consecutive days of daily food intake reductions below or above this criterion. Responses to the various dosing regimens were as follows. On *day 1*, sCT was administered by infusion at 12 pmol/h during *hours 0–3 and 9–12* of the dark period (Fig. 3*B, a*). Food intake on *days 1 and 2* was reduced by 31 and 41%

compared with that in vehicle-treated rats (Fig. 3). The second interval of sCT infusion inhibited food intake at least as well as the first interval of infusion, and a normal rate of food intake returned within 2 h of the end of the first infusion on both days (Fig. 4, Table 1). On *day 3*, the sCT dose was reduced to 4 pmol/h during *hours 0–3 and 9–12* of the dark period to determine whether a lower dose would produce a sustained 25–35% reduction in daily food intake (Fig. 3*B, b*). Food intake on *day 3* was reduced by only 13% (Figs. 3 and 4). On *day 4*, sCT dose was increased to 8 pmol/h during *hours 0–3 and 9–12* of the dark period (Fig. 3*B, c*). Food intake on *days*

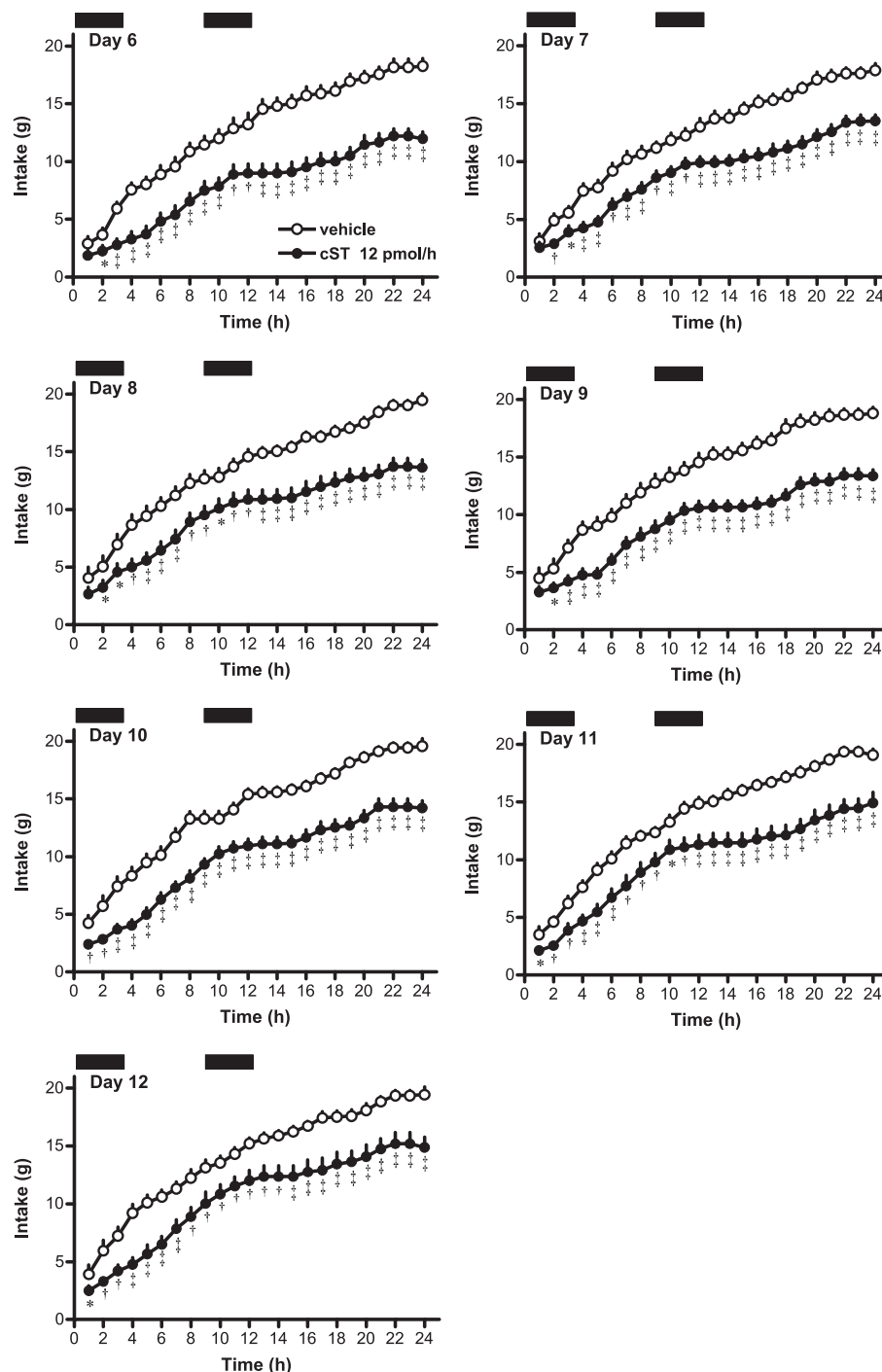


Fig. 5. Effects of intermittent intraperitoneal infusions of sCT on cumulative hourly food intake in diet-induced obese rats during *days 6–12* of sCT treatments. Data are from the experiment described in Fig. 3. *Time 0*, start of 12-h dark period. Horizontal bars indicate periods of infusion. Values are means $\pm$ SE. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ vs. vehicle.

4 and 5 was reduced by 20 and 23%, respectively (Figs. 3 and 4). On *day 6*, sCT dose was increased to 12 pmol/h during *hours 0–3* and *9–12* of the dark period, a dosing strategy identical to that used on *day 1* (Fig. 3*B, d*). Food intake on *days 6, 7, 8, 9, 10, 11*, and *12* was reduced by 34, 24, 30, 29, 27, 22, and 23%, respectively (Figs. 3 and 5). During the 7-day period, food intake between the two intervals of sCT infusion gradually increased relative to that in vehicle-treated rats (Table 1). On *day 13*, the interinfusion interval for sCT at 12 pmol/h was reduced from 6 to 3 h in an attempt to attenuate the increase in food intake that developed between infusion intervals (Fig. 3*B, e*). Food intake on *days 13, 14, 15, 16*, and *17* was reduced by 27, 28, 20, 26, and 19%, respectively (Figs. 3 and 6). The second interval of sCT infusion inhibited food intake as well as the first interval. The rate of food intake after the second interval of infusion appeared to gradually increase during the 5-day period. On *day 18*, the frequency of sCT infusion at 12 pmol/h was increased to six 2-h intervals of infusion, each separated by 2 h of no infusion, in an attempt to limit increases in food intake between intervals of sCT infusion (Fig. 3*B, f*). Food intake on *days 18, 19, 20, 21*, and *22* was reduced by 42, 33, 33, 14, and 21%, respectively, suggesting that receptor downregulation and tolerance to fre-

quent infusion of sCT may have developed after 3 days (Figs. 3 and 7). On *day 23*, sCT was replaced with vehicle (Fig. 3*B, g*). Food intake in rats that had been previously treated with sCT was 59% greater than on *day 22* and 29% greater than in the vehicle-treated control rats (Figs. 3 and 7). This strong rebound in food intake indicates that sCT had apparently not lost its efficacy during the previous dosing regimen but, rather, that an orexigenic mechanism had been activated to counteract the inhibitory effect of sCT on food intake. On *day 24*, sCT was administered at 12 pmol/h during *hours 0–3* and *6–9* of the dark period and *hours 15–18* of the light period (Fig. 3*B, h*). Food intake on *days 24* and *25* was reduced by 17 and 15%, respectively (Fig. 3). Food intake during the first 3 h of the dark period appeared to be reduced only slightly by sCT infusion. On *day 26*, a 12-pmol loading dose of sCT was administered just before onset of the dark period, followed immediately by infusion of sCT at 12 pmol/h during *hours 0–3, 6–9, and 15–18* (Fig. 3*B, i*). The loading dose was employed to quickly increase tissue levels of sCT when the rate of food intake is at its highest. Food intake on *days 26, 27, 29*, and *30* was reduced by 28, 19, 12, and 13%, respectively (Fig. 3). On *day 32*, a 16-pmol loading dose of sCT was administered just before the onset of the dark period, the infusion dose was increased to 24

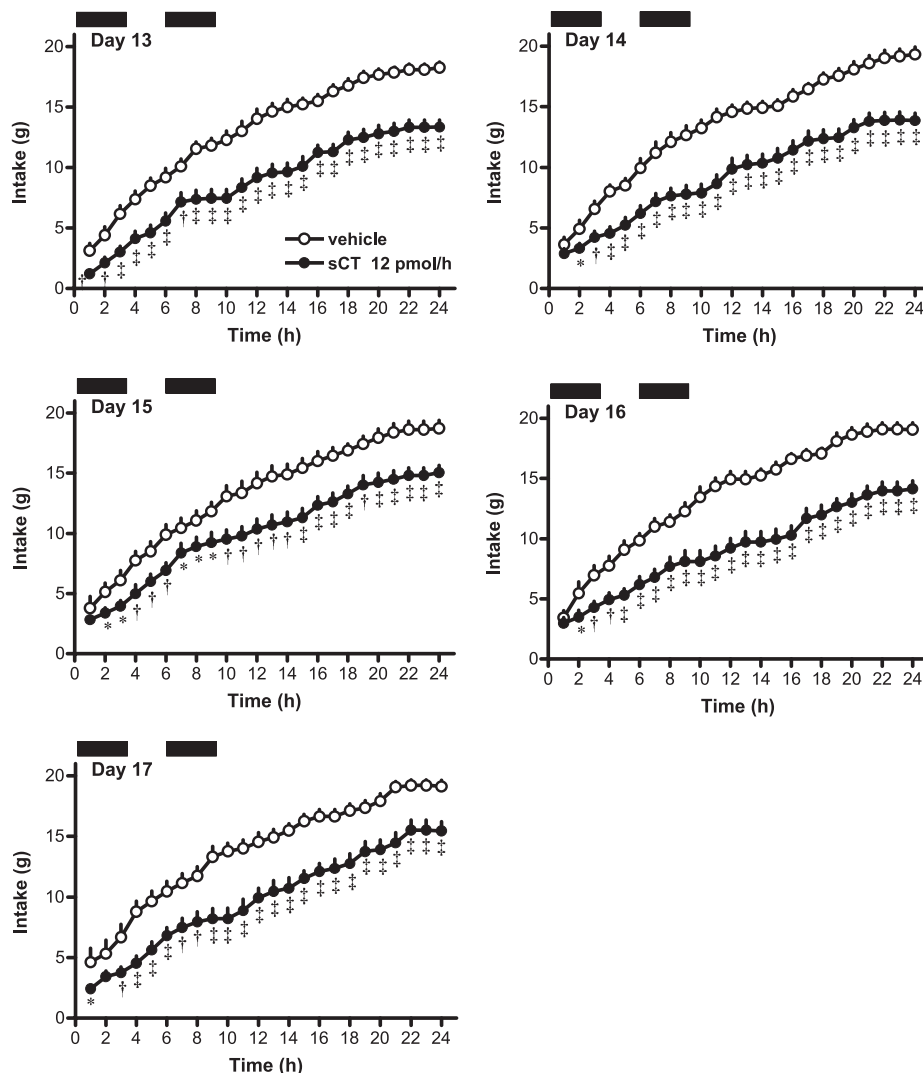


Fig. 6. Effects of intermittent intraperitoneal infusions of sCT on cumulative hourly food intake in diet-induced obese rats during *days 13–17* of sCT treatments. Data are from the experiment described in Fig. 3. *Time 0*, start of 12-h dark period. Horizontal bars indicate periods of infusion. Values are means $\pm$ SE. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ vs. vehicle.

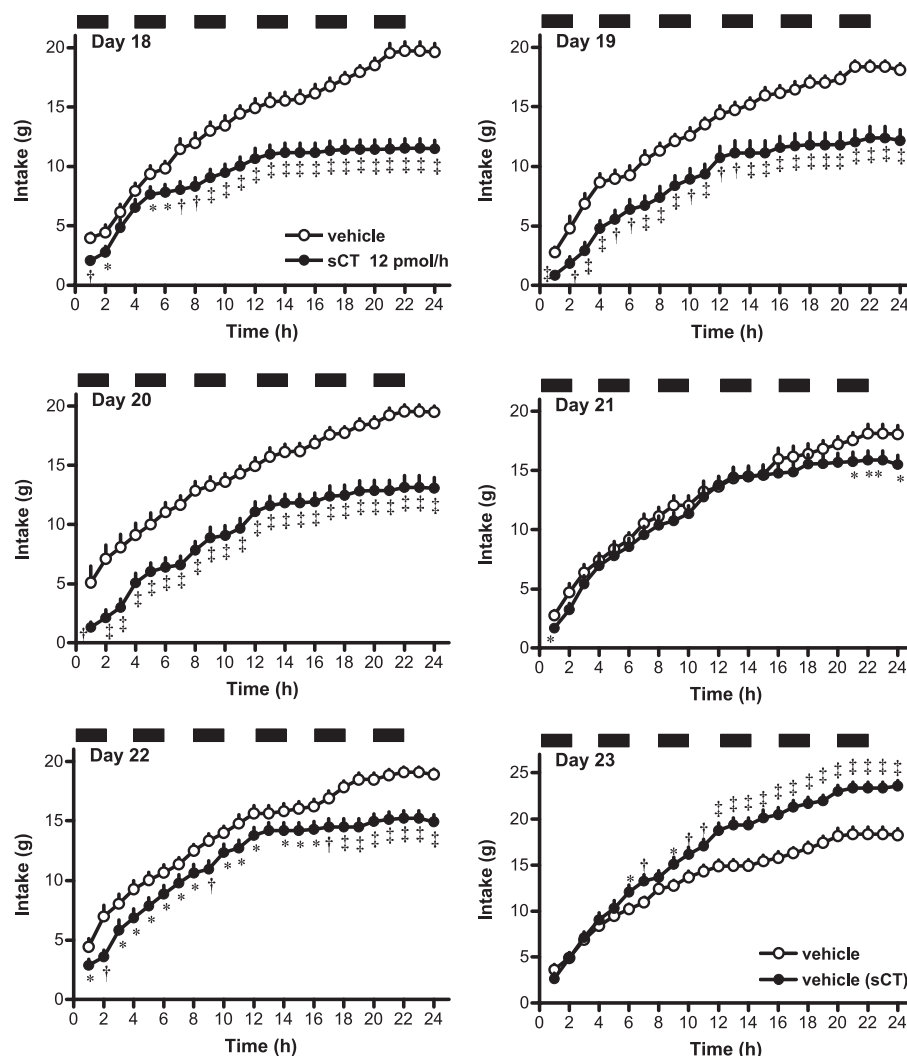


Fig. 7. Effects of intermittent intraperitoneal infusions of sCT on cumulative hourly food intake in diet-induced obese rats during *days 18–23* of sCT treatments. Data are from the experiment described in Fig. 3. *Time 0*, start of 12-h dark period. Horizontal bars indicate periods of infusion. Values are means $\pm$ SE. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ vs. vehicle.

pmol/h, and the frequency of sCT administration was increased to six 2-h intervals of infusion, each separated by 2 h of no infusion (Fig. 3*B, j*). Food intake on *days 32, 33, 34, 35, 36, 37, 38, 39, and 40* was reduced by 38, 27, 14, 16, 8, 12, 5, 14, and 11%, respectively (Figs. 3 and 8), again suggesting gradual development of receptor downregulation and tolerance to frequent sCT administration. On *day 41*, sCT was again replaced with vehicle (Fig. 3*B, k*). Food intake in rats that had previously been treated with sCT was 50% greater than on *day 40* and 41% greater than in the vehicle-treated control rats (Figs. 3 and 8). This strong rebound in food intake indicates that sCT had not lost its efficacy during the previous dosing regimen but, rather, that an orexigenic mechanism had been activated to counteract the inhibitory effect of sCT on food intake. On *day 42*, a 32-pmol loading dose of sCT was administered just before the onset of the dark period, the infusion dose was increased to 48 pmol/h, and the frequency of administration was changed to four 3-h infusions, each separated by 3 h of no infusion (Fig. 3*B, l*). Food intake on *days 42 and 43* was reduced by 57 and 45%, respectively (Figs. 3 and 9). On *day 44*, a 24-pmol loading dose of sCT was administered just before the onset of the dark period, the infusion dose was reduced to 36 pmol/h, and the frequency of administration

remained the same, i.e., four 3-h infusions, each separated by 3 h of no infusion (Fig. 3*B, m*). Food intake on *days 44, 45, and 46* was reduced by 22, 11, and 6%, respectively (Figs. 3 and 9), again suggesting receptor downregulation and tolerance to sCT administration. On *day 47*, sCT was again replaced with vehicle (Fig. 3*B, n*). Food intake in rats that had previously been treated with sCT was 41% greater than on *day 46* and 33% greater than in vehicle-treated control rats (Figs. 3 and 9). This strong rebound in food intake again indicates that sCT had not lost its efficacy but, rather, that an orexigenic mechanism had been activated to counteract the inhibitory effect of sCT on food intake. On *day 48*, sCT was administered using the strategy employed on *day 44* (Fig. 3*B, o*). Food intake on *day 48* was reduced by 30%, a level comparable to that observed on *day 44* (Figs. 3 and 9). Thus an intervening day of no treatment restored the inhibitory effect of the dosing strategy on food intake.

Changes in body weight of vehicle- and sCT-treated rats completing the 7-wk study are shown in Fig. 10. Body weight in vehicle-treated rats increased by 3.7% (672 ± 19 vs. 648 ± 17 g, $P < 0.01$), while percent body fat remained the same (27.9 ± 1.8 vs. $26.8 \pm 1.2\%$). In contrast, body weight in sCT-treated rats was reduced by 9% (587 ± 12 vs. 651 ± 14 g,

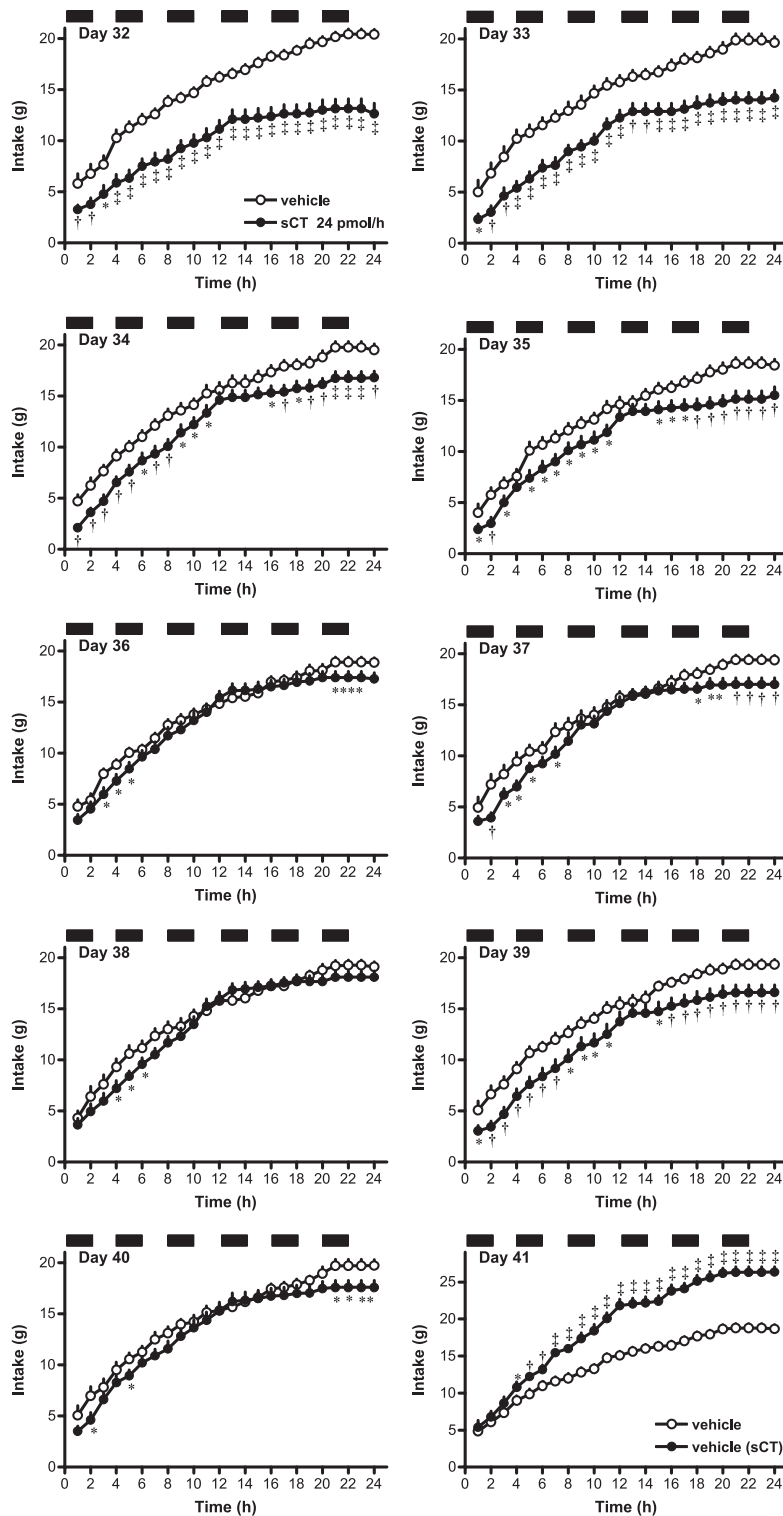


Fig. 8. Effects of intermittent intraperitoneal infusions of sCT on cumulative hourly food intake in diet-induced obese rats during days 32–41 of sCT treatments. Data are from the experiment described in Fig. 3. Time 0, start of 12-h dark period. Horizontal bars indicate periods of infusion. Values are means $\pm$ SE. * P < 0.05; † P < 0.01; ‡ P < 0.001 vs. vehicle.

P < 0.001), and percent body fat was reduced by 22% (20.6 ± 1.2 vs. $26.5 \pm 1.1\%$, P < 0.001).

DISCUSSION

Our results demonstrate several important properties of the effects of chronic administration of sCT, a homolog of the gastrointestinal peptide amylin, on food intake, body weight,

and adiposity in diet-induced obese rats. 1) Food intake, body weight, and adiposity were relatively stable during the 7-wk test period in vehicle-treated diet-induced obese rats. 2) None of the 10 sCT dosing regimens administered in succession during the 7-wk period produced a sustained 25–35% reduction in daily food intake for >5 days, although body weight and adiposity across the test period were reduced by 9 and 22%,

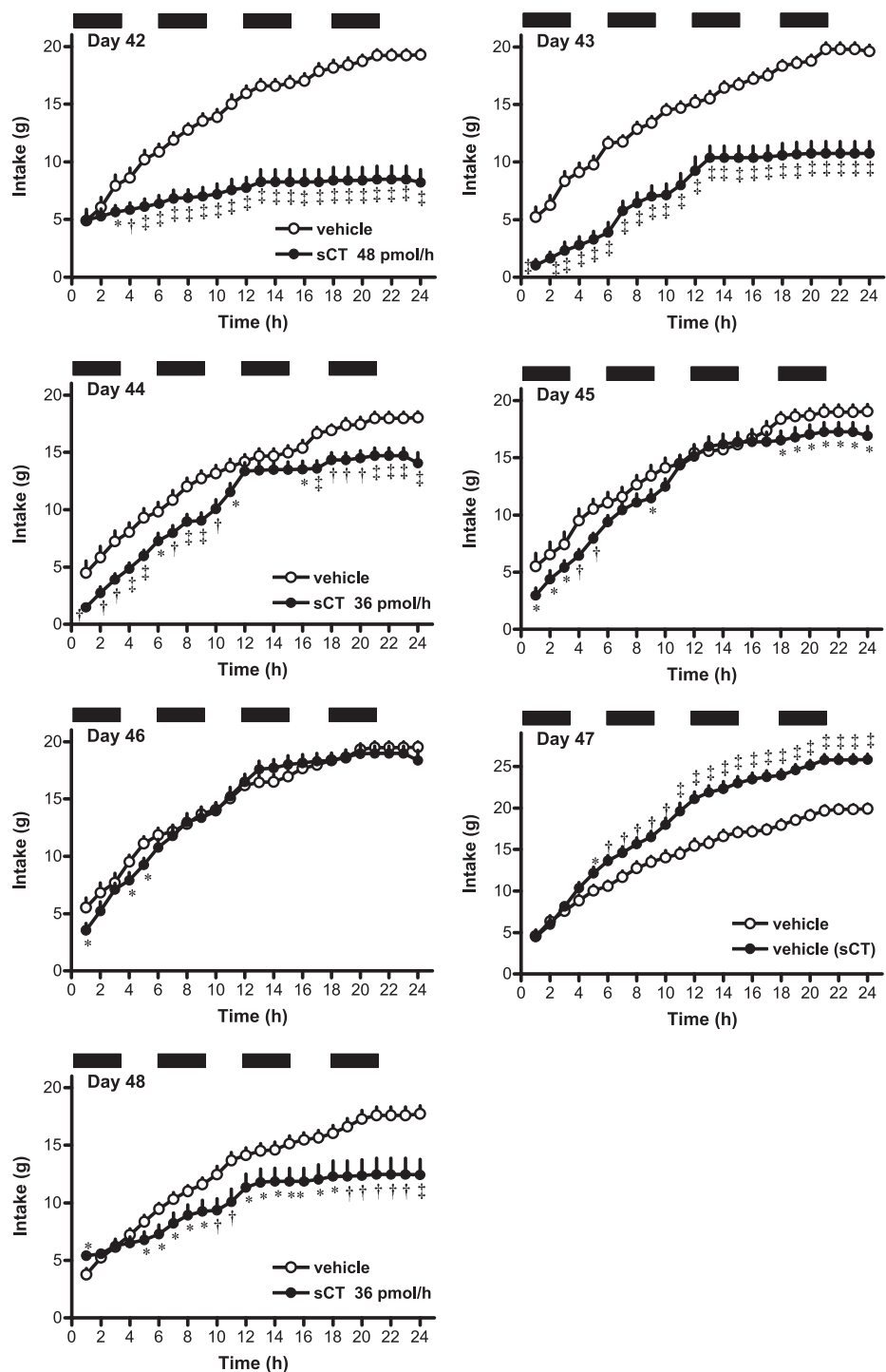


Fig. 9. Effects of intermittent intraperitoneal infusions of sCT on cumulative hourly food intake in diet-induced obese rats during days 42–48 of sCT treatments. Data are from the experiment described in Fig. 3. Time 0, start of 12-h dark period. Horizontal bars indicate periods of infusion. Values are means $\pm$ SE. * P < 0.05; † P < 0.01; ‡ P < 0.001 vs. vehicle.

respectively. 3) The declining inhibitory effect of sCT on daily food intake when the interinfusion interval was 6 h appeared to be due in part to an increase in food intake between infusions. 4) The declining inhibitory effect of sCT on daily food intake when the interinfusion interval was 2–3 h suggested possible receptor downregulation and tolerance to sCT administration; however, food intake increased dramatically when sCT treatments were discontinued for 1 day following apparent loss of treatment efficacy. Together, these results demonstrate the development of a potent homeostatic response to increase food

intake when sCT treatments reduce food intake and energy reserves in diet-induced obese rats.

We and others have demonstrated that acute systemic administration of the amylin agonist sCT potently inhibits short-term food intake in lean and diet-induced obese rodents (15, 22, 31, 38). We are aware of only one study that has examined the effects of chronic administration of sCT on daily food intake and body weight (38). In that study, sCT was given by intracerebroventricular injection once daily for 5 days to lean rats, food was available after injection for only 90 min, and

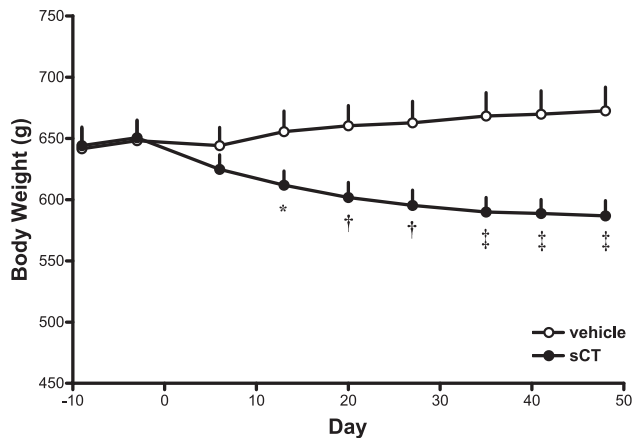


Fig. 10. Effects of intermittent intraperitoneal infusions of sCT on body weight in diet-induced obese rats. Data are from the experiment described in Fig. 3. Values are means $\pm$ SE. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ vs. vehicle.

sCT reduced daily food intake only during the first 3 days of administration. Other studies have shown that twice-daily calcitonin injections for 5 days produced a transient 3-day reduction in food intake in lean mice (25) and that continuous infusion of amylin by osmotic minipump, whether by subcutaneous or intracerebroventricular administration, produced a transient reduction in daily food intake in lean and obese rodents (6, 32, 34). Continuous minipump administration of other known anorexigenic substances [PYY(3–36), glucagon-like peptide-1 receptor agonists, cholecystokinin, and melanocortin receptor agonists] has also been reported to produce transient reductions in daily food intake in rodents (1, 13, 14, 21, 23, 25, 28, 29, 36, 37, 39, 40). One possible explanation for these transient responses is that early substance-induced reductions in daily food intake and adiposity induce a delayed homeostatic response to increase food intake and adiposity mediated by a reduction in leptin signaling to the brain (2, 16). Another possibility is that continuous or frequent administration of the substances causes desensitization and downregulation of their receptors. Each of these anorexigenic substances, including sCT, acts at G protein-coupled receptors. Numerous studies have demonstrated that prolonged exposure of G protein-coupled receptors to agonists can induce receptor downregulation and tolerance (17, 35).

Continuous delivery of sCT by osmotic minipump to rats has been shown to cause a dose-dependent downregulation of sCT-binding receptors (7). Autoradiographic measurement of 125 I-sCT-binding sites in rat kidney sections showed that continuous sCT infusion at 2 pmol/h for 7 days reduced binding sites by 15%, while sCT infusion at 146 pmol/h reduced binding by 80%. These results suggest that intermittent infusion of sCT at doses used in our study (3–48 pmol/h) may produce some, but not extensive, receptor downregulation. Thus transient feeding responses to the various sCT dosing paradigms used in our study were probably not due to receptor loss. This conclusion is further supported by our finding that food intake increased dramatically on each of three occasions when sCT treatment was discontinued for 1 day after apparent loss in treatment efficacy. If loss of sCT-binding receptors was primarily responsible for loss of sCT efficacy, then discontinuing sCT treatment should have had little, if any, effect on food

intake. Rather, our results suggest that an orexigenic mechanism was activated to offset the anorexigenic response to intermittent administration of sCT. Chronic osmotic minipump administration of amylin has been shown to increase the hypothalamic concentration of the potent orexigenic peptide neuropeptide Y (5), suggesting that a similar counterregulatory mechanism may have been activated in our sCT-treated rats. Whether this orexigenic mechanism was induced by a decline in leptin signaling to the brain remains to be determined. It also remains to be determined whether intermittent administration of sCT with leptin or other anorexigenic substances can produce a sustained reduction in daily food intake and adiposity in diet-induced obese rats.

Perspectives and Significance

There is an extensive body of evidence indicating that a sustained reduction in caloric intake in obese individuals will produce steady weight loss. Thus an important early step in discovery of antiobesity drugs is defining methods of administration of anorexigenic agents that can produce a sustained reduction in daily food intake and body weight in obese experimental animals. We previously showed that infusion of the amylin homolog sCT rapidly and potently suppresses food intake in rats. In contrast, several days of sCT administration would likely be required to produce a measurable reduction in body weight. Thus, monitoring the effects of specific sCT dosing strategies on daily food intake in diet-induced obese rats enabled us to quickly rule out potentially ineffective strategies for producing significant weight loss. Because we were able to measure the instantaneous effects of dosing strategy on the pattern of food intake each day, we were able to adjust sCT dosing daily in an attempt to optimize reduction in daily food intake. Here we showed that none of the 10 dosing strategies tested in the obese rats over a 7-wk period produced a sustained 25–35% reduction in daily caloric intake for >5 days, although body weight and adiposity were reduced across the test period by 9 and 22%, respectively. The inability of sCT to sustain a reduction in food intake appears to have been due to activation of a potent homeostatic response to counteract the inhibitory effect of sCT on food intake and body weight, rather than downregulation of receptors. Together, these results suggest that sCT administration alone would likely not produce significant weight loss in obese subjects. Whether a specific dosing strategy could be designed to produce modest weight loss in obese individuals or prevent weight gain in preobese individuals remains to be determined.

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