Effects of Intermittent Intraperitoneal Infusion of Salmon Calcitonin on Food Intake and Adiposity in Obese Rats

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Chelikani PK, Haver AC and Reidelberger RD. Effects of Intermittent Intraperitoneal Infusion of Salmon Calcitonin on Food Intake and Adiposity in Obese Rats. Chronic administration of anorexigenic substances to experimental animals by injections or continuous infusion typically produces either no effect or a transient reduction in daily food intake and body weight. Our aim here was to identify an intermittent dosing strategy for intraperitoneal (IP) 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 (646±10 g body weight, 27±1% body fat) with IP catheters tethered to infusion swivels had free access to a 45% fat diet. Vehicle-treated rats (n=16) had relatively stable food intake, body weight and adiposity during the 7-wk test period. 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 across the 7-wk period by 9% (587±12 vs. 651±14g) and 22% (20.6±1.2 vs. 26.5±1.1%), respectively. The declining inhibitory effect of sCT on daily food intake when inter-infusion interval was 6h appeared to be due in part to an increase in food intake between infusions. The declining inhibitory effect of sCT on daily food intake when inter-infusion interval was 2-3h suggested possible receptor down-regulation and tolerance to frequent sCT administration; however, food intake increased dramatically when sCT treatments were discontinued for 1 day following apparent loss in treatment efficacies. Together, these results demonstrate the activation of a potent homeostatic response to increase food intake when sCT treatments reduce food intake and energy reserves in diet-induced obese rats.

Key Words: salmon calcitonin, amylin, anorexia, body weight, body fat
An important early step in development of obesity drugs is determining whether chronic administration of anorexigenic substances, either alone or in combination, can produce a sustained decrease in daily food intake and adiposity in obese experimental animals. Methods of administration typically include either daily injections or insertion of an osmotic minipump beneath the skin or into the peritoneal cavity to deliver substances continuously for a week or more. Results from such studies are usually inconclusive. Reasons include development of a compensatory increase in food intake between injections, receptor down-regulation 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 (IV) or intraperitoneal (IP) administration of anorexigenic 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 every 20 seconds, permits daily assessment of the instantaneous effects of infused substances on food intake. Adjustments in dosing pattern can be performed daily in order to define a dosing strategy that minimizes both compensatory hyperphagia between doses and tolerance. We used this experimental model to show that IV 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 IV 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
to 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 IV infusions of several anorexigenic substances on short-term food intake in rats, including gastrointestinal peptides amylin, cholecystokinin octapeptide, PYY(1-36), PYY(3-36), and glucagon-like peptide-1, the adipose hormone leptin, melanocortin agonist melanotan II, cannabinoid antagonist AM251, opioid antagonist naloxone, and amylin homologs salmon calcitonin (sCT), calcitonin gene-related peptide, and adrenomedullin (9-11, 30, 31). The two most potent anorexigenic substances were sCT and amylin (Figure 1).

Amylin and sCT are structurally related peptides that appear to act at the same receptor to inhibit food intake (22). Previous studies have shown that continuous ICV administration of the amylin receptor antagonist acetyl-[Asn30, Tyr32] sCT-(8–32) (AC187) 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 wildtype mice (26). These results suggest that amylin may contribute to the long-term regulation of energy reserves, and that amylin agonists including salmon calcitonin may prove useful in treating obesity. However, chronic administration of amylin (6, 32, 34), sCT (38), and calcitonin (25) to rodents either by osmotic minipump or injections have been reported to only transiently reduce daily food intake and body weight. Our aim here was to identify an intermittent dosing strategy for IP infusion of sCT that produces a sustained reduction in daily food intake and adiposity in diet-induced obese rats.
MATERIALS AND METHODS

Peptides. sCT was obtained from Anaspec Inc. (San Jose, CA). Peptide purity was confirmed by HPLC, and molecular mass confirmed by electrospray mass spectrometry. sCT stock was prepared by dissolving the peptide in 0.15 M NaCl, 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; initially weighing 225-350 g) were housed in a room with controlled temperature (19-21°C) and a 12:12-h light-dark cycle (lights off at 1700 h). Rats were provided pelleted rat chow (Labdiet®, 5001 Rodent diet, PMI® Nutrition International, MO) and water ad libitum for about a week before being subjected to experimental procedures. The 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. Animals were provided both a high fat pelleted food (45, 35, and 20% calories from fat, carbohydrate, and protein, respectively; 4.73 kcal/g, D12451, Research Diets Inc, 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). The combined use of palatable high fat solid and liquid foods induces obesity in a high proportion of rats (3, 4, 20, 24). Total body fat was measured monthly in unanesthetized rats using an EchoMRI-700™ quantitative nuclear magnetic resonance (QMR) analyzer (Echo Medical Systems, LLC, Houston, TX). Obesity (≥25% body fat) was induced in 106 of the 166 rats (64%) during a 4 to 8 month period. Percent body fat of the remaining 60 rats
was 21.9 ± 0.4% (14 had percent body fat from 13.3 to 19.9%, and 46 had percent body fat from 20 to 24.9%). Our aim here was to test whether sCT treatment 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 consuming only the high fat solid food (D12451). The 106 obese rats were housed singly and provided this food for 6 weeks. We chose a 6-week 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 3-7 week period. Figures 2A and 2B show weekly body weight and body fat distributions during this period. Median body weight and body fat both decreased slightly by the end of the first week, yet distributions remained relatively stable during the next 6 weeks.

Surgical and post-surgical adaptation procedures. Eighty-six of these diet-induced obese rats (29.1 ± 0.4% body fat) were surgically implanted with an IP catheter under isoflurane anesthesia using procedures described previously (12). The IP catheter, which exited the skin in the dorsal cervical region, was plugged with stainless steel wire and kept patent by flushing weekly with 1 ml of normal saline. After surgery, rats were transferred to a room with a 12:12-h light-dark cycle with lights off at 1100 h. During the post-surgical recovery period, the animals had continued access to both the Ensure and pelleted high-fat food. Rats were allowed 3 weeks to regain lost body weight. The animals were then fitted with a light-weight harness (IITC, Woodlands, CA) used for tethering to an infusion swivel and were allowed a further three weeks to adapt to the harness. Of the 86 rats that had surgery, 37 were used in the sCT administration study because available resources did not permit larger treatment groups. The rats chosen for
testing at the end of the 8-week period of adaptation between surgery and 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 needed to ensure a sufficient number of stable, tethered obese subjects for experimentation.

*Experimental procedures:* Obese rats (n = 32) were then 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, which was fixed to a digital balance. The 32 balances in this 32-cage system were connected to a computer through a code-activated switch (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 cumulated hourly. Another 5 obese rats were housed in the same type metabolism cage with the same type food container, which was not fixed to a digital balance. For these animals, daily ingestion of food was determined by manually weighing 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 intakes were measured in all 37 rats and cumulative hourly intakes were measured each day in 32 of the 37 rats.

Each rat had its IP catheter 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. Rats were provided powdered high fat solid food (D12451) and water each day from 1100 to 0800h the next morning (dark period was from 1100-2300h). Experimental setup and routine maintenance were performed each day between 0800-1100h. Animals were allowed an additional two weeks to regain body weight and to adapt to tethering and experimental conditions. Thus, rats had access to high-fat foods for 7-11 months before testing. During an initial 7-day baseline period, all rats received an IP infusion of vehicle (0.15 M NaCl, 0.1% BSA; 0.9 ml/h) during intervals 0-3 and 9-12 h of the dark period (1100-1400h and 1700-2000h, 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. Two days later the animals were divided into two groups, one to receive vehicle (n = 18) and the other 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 first day of treatment, sCT was infused at 0.3 pmol/kg/min (12 pmol/h) during intervals 0-3 and 9-12 h of the dark period. We previously determined that IV infusion of this dose of sCT during the first 3 hours of the dark period produces a significant inhibition of food intake in lean rats (31). This initial twice daily dosing regimen permitted us to evaluate the extent to which (i) food intake is reduced during the first 3-hour treatment period, (ii) desensitization to sCT-induced anorexia occurs during the second 3-hour treatment period, (iii) an increase in food intake occurs between treatment periods, and (iv) 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 both the lowest dose and
frequency of sCT administration that would induce a sustained 25-35% reduction in average daily caloric intake for 2 weeks, when compared to average daily food intake in the rats administered vehicle at the same infusion rate during the same intervals. A dosing regimen was usually changed after observing two or more consecutive days of daily food intake reductions either below or above this 25-35% criterion for reduction in daily food intake. This criterion was chosen for two reasons: (i) 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 (ii) 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-week period (Figure 3). On 3 different occasions, sCT treatment was discontinued for 1 day to assess whether loss in efficacy of a treatment might be due to receptor down-regulation 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-week period. During this period, two vehicle-treated rats and one sCT-treated rat were removed from the experiment due to catheter malfunction.

Statistical analyses. Values are presented as group means ± SE. Data were analyzed by analysis of variance (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 IP infusion of sCT on food intake, body weight, and adiposity in diet-induced obese rats. By the end of the 7-day baseline period, the two groups of rats that were to
receive either IP infusions of vehicle (n = 18) or sCT (n = 19) had distributions of body weight (648 ± 15 vs. 651 ± 13 g), percentage body fat (26.9 ± 1 vs. 26.7 ± 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) that were not statistically different (P > 0.05, Figure 3). Cumulative hourly food intakes on the last day of the baseline period (day -3) were also not different in these groups (Figure 4).

During the 7-week treatment period, daily food intake in vehicle-treated rats remained relatively stable (Figure 3), body weight increased slightly from 648 ± 15 to 672 ± 19 g (P < 0.01), and percent body fat remained the same (26.9 ± 1.1 vs. 27.9 ± 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 weeks. A dosing regimen was usually changed after observing two or more consecutive days of daily food intake reductions either below or above this criterion. Responses to the various dosing regimens were as follows:

a. On day 1, sCT was administered by infusion at 12 pmol/h during intervals 0-3 and 9-12 h of the dark period. Food intakes on days 1 and 2 were reduced by 31 and 41% compared to those of vehicle-treated rats (Figure 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 hours of the end of the first infusion on both days (Figure 4, Table 1).
b. On day 3, sCT dose was reduced to 3 pmol/h during intervals 0-3 and 9-12 h of the dark period to determine whether a lower dose would produce a sustained 25-35% reduction in daily food intake. Food intake that day was reduced by only 13% (Figures 3 and 4).

c. On day 4, sCT dose was increased to 6 pmol/h during intervals 0-3 and 9-12 h of the dark period. Food intakes on days 4 and 5 were reduced by 20 and 23%, respectively (Figures 3 and 4).

d. On day 6, sCT dose was increased to 12 pmol/h during intervals 0-3 and 9-12 h of the dark period, a dosing strategy which was identical to that used for paradigm ‘a’. Food intakes on days 6 through 12 were reduced by 34, 24, 30, 29, 27, 22, and 23%, respectively (Figures 3 and 5). During the 7-day period, food intake between the two intervals of sCT infusion gradually increased relative to that observed in vehicle-treated rats (Table 1).

e. On day 13, the inter-infusion interval for sCT at 12 pmol/h was reduced from 6 to 3 hours in an attempt to attenuate the increase in food intake that developed between infusion intervals. Food intakes on days 13 to 17 were reduced by 27, 28, 20, 26, and 19%, respectively (Figures 3 and 6). The second interval of sCT infusion did not inhibit food intake as well as the first interval. Rate of food intake after the second interval of infusion appeared to gradually increase during the 5-day period.

f. On day 18, frequency of sCT infusion at 12 pmol/h was increased to six, 2-hour intervals of infusion, each separated by 2 hours of no infusion, in an attempt to limit increases in food intake
between intervals of sCT infusion. Food intakes on days 18 to 22 were reduced by 42, 33, 33, 14, and 21%, respectively, suggesting that receptor down-regulation and tolerance to frequent infusion of sCT may have developed after 3 days (Figures 3 and 7).

g. On day 23, sCT treatment was replaced with vehicle. 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 (Figures 3 and 7). This strong rebound in food intake indicates that sCT had apparently not lost its efficacy during the previous dosing regimen, but that an orexigenic mechanism had been activated to counteract the inhibitory effect of sCT on food intake.

h. On day 24, sCT was administered at 12 pmol/h during intervals 0-3 and 6-9 h of the dark period, and 15-18 h of the light period. Food intakes on days 24 and 25 were reduced by 17 and 15%, respectively (Figures 3). Food intake during the first 3 hours of the dark period appeared to be reduced only slightly by sCT infusion.

i. On day 26, a 12 pmol loading dose of sCT was administered just prior to onset of the dark period, followed immediately by infusion of sCT at 12 pmol/h during intervals 0-3, 6-9, and 15-18 h. The loading dose was employed to quickly increase tissue levels of sCT at a time when rate of food intake is at its highest. Food intakes on days 26 through 30 were reduced by 28, 19, 26, 12 and 13% (Figure 3).
j. On day 32, a larger 16 pmol loading dose of sCT was administered just prior to onset of the dark period, infusion dose was increased to 24 pmol/h, and frequency of sCT administration was increased to six, 2 hour intervals of infusion, each separated by 2 hours of no infusion. Food intakes on days 32 through 40 were reduced by 38, 27, 14, 16, 8, 12, 5, 14 and 11%, respectively (Figures 3 and 8), again suggesting gradual development of receptor down-regulation and tolerance to frequent sCT administration.

k. On day 41, sCT treatment was again replaced with vehicle. Food intake in rats that had previously received sCT treatments was 50% greater than on day 40, and 41% greater than in the vehicle-treated control rats (Figures 3 and 8). This strong rebound in food intake indicates that sCT had not lost its efficacy during the previous dosing regimen, but that an orexigenic mechanism had been activated to counteract the inhibitory effect of sCT on food intake.

l. On day 42, a larger 32 pmol loading dose of sCT was administered just prior to onset of the dark period, infusion dose was increased to 48 pmol/h, and frequency of administration was changed to four, 3-hour infusions, each separated by 3 hours of no infusion. Food intakes on days 42 and 43 were reduced by 57 and 45%, respectively (Figures 3 and 9).

m. On day 44, a lower 24 pmol loading dose of sCT was administered just prior to onset of the dark period, infusion dose was reduced to 36 pmol/h, and frequency of administration remained the same (four, 3-hour infusions, each separated by 3 hours of no infusion). Food intakes on days 44 through 46 were reduced by 22, 11 and 6%, respectively (Figures 3 and 9), again suggesting receptor down-regulation and tolerance to sCT administration.
n. On day 47, sCT treatment was again replaced with vehicle. Food intake in rats that had previously received sCT was 41% greater than on day 46, and 33% greater than in vehicle-treated control rats (Figures 3 and 9). This strong rebound in food intake again indicates that sCT had not lost its efficacy, but that an orexigenic mechanism had been activated to counteract the inhibitory effect of sCT on food intake.

o. On day 48, sCT was administered using the strategy employed on day 44. Food intake that day was reduced by 30%, a level comparable to that observed on day 44 (Figures 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-week study are shown in Figure 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 ± 1.2%). In contrast, body weight in sCT-treated rats was reduced by 9% (587 ± 12 vs. 651 ± 14 g, P < 0.001), and percent body fat was reduced by 22% (20.6 ± 1.2 vs. 26.5 ± 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. First, vehicle-treated diet-induced obese rats had relatively stable food intake, body weight and adiposity during the 7-week test period. Second, none of the
10 sCT dosing regimens administered in succession during the 7-week period produced a sustained 25-35% reduction in daily food intake for more than 5 days, although body weight and adiposity across the test period were reduced by 9 and 22%, respectively. Third, the declining inhibitory effect of sCT on daily food intake when inter-infusion interval was 6h appeared to be due in part to an increase in food intake between infusions. Fourth, the declining inhibitory effect of sCT on daily food intake when inter-infusion interval was 2-3h suggested possible receptor down-regulation and tolerance to sCT administration; however, food intake increased dramatically when sCT treatments were discontinued for 1 day following apparent loss in treatment efficacies. 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 ICV injection once daily for 5 days to lean rats, food was available postinjection for only 90 min, and 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 SC or ICV 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, 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 cause desensitization and down-regulation of their receptors. Each of these anorexigenic substances including sCT act at G-protein coupled receptors. Numerous studies have demonstrated that prolonged exposure of G-protein coupled receptors to agonists can induce receptor down-regulation and tolerance (17, 35).

Continuous delivery of sCT by osmotic minipump to rats has been shown to cause a dose-dependent down-regulation of sCT-binding receptors (7). Using autoradiography to measure $^{125}$I-sCT binding sites in rat kidney sections, 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 to 48 pmol/h) may produce some, but not extensive, receptor down-regulation. 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 following apparent loss in treatment efficacy. If loss of sCT-binding receptors was primarily responsible for loss in 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 hypothalamic concentration of the potent orexigenic peptide neuropeptide Y (5), suggesting that a similar counter-regulatory mechanism may have been
activated in our sCT-treated rats. Whether this orexigenic mechanism was induced by a decline in leptin signalling 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 anti-obesity 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 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 during a 7-week period produced a sustained 25-35% reduction in daily caloric intake for more than 5 days, although body weight and adiposity were reduced across the test period by 9 and 22%, respectively. sCT’s inability to sustain a reduction in food intake appears to have been due to activation of a potent homeostatic response to counteract sCT’s inhibitory effect on food intake and body weight, rather than to down-regulation 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 pre-obese individuals, remains to be determined.
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Table 1. Effects of intermittent IP infusions of sCT on food intake during specific time intervals each day in diet-induced obese rats.

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>0 - 5 h</th>
<th>5 - 9 h</th>
<th>9 - 14 h</th>
<th>14 - 24 h</th>
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<td>1</td>
<td>Veh</td>
<td>7.9 (0.8)</td>
<td>2.5 (0.3)</td>
<td>3.0 (0.4)</td>
<td>3.1 (0.4)</td>
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<tr>
<td></td>
<td>sCT</td>
<td>5.0 (0.6) †</td>
<td>2.5 (0.4)</td>
<td>1.2 (0.3) ‡</td>
<td>1.8 (0.4) *</td>
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<td>2.8 (0.3)</td>
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<td></td>
<td>sCT</td>
<td>4.0 (0.6) ‡</td>
<td>3.4 (0.3)</td>
<td>0.6 (0.2) ‡</td>
<td>2.3 (0.4)</td>
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<tr>
<td>6</td>
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<td>3.4 (0.5)</td>
<td>2.8 (0.4)</td>
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<tr>
<td>7</td>
<td>Veh</td>
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<td>3.4 (0.5)</td>
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<td>3.5 (0.4)</td>
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<tr>
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<tr>
<td></td>
<td>sCT</td>
<td>5.6 (0.7) ‡</td>
<td>4.0 (0.4)</td>
<td>1.4 (0.3) *</td>
<td>2.1 (0.4) *</td>
</tr>
<tr>
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<td>Veh</td>
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<td>3.7 (0.4)</td>
<td>2.5 (0.4)</td>
<td>3.3 (0.4)</td>
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<td>sCT</td>
<td>4.8 (0.5) ‡</td>
<td>4.0 (0.4)</td>
<td>1.9 (0.3) *</td>
<td>2.2 (0.3) *</td>
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<td>10</td>
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<td>9.5 (0.7)</td>
<td>3.8 (0.4)</td>
<td>2.3 (0.3)</td>
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<td>2.8 (0.5)</td>
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<td>4.4 (0.6) *</td>
<td>2.3 (0.5)</td>
<td>2.4 (0.4)</td>
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Data are from days 1 and 2, and 6 through 12 (paradigms a and d) of experiment described in Figure 3. sCT was infused at 12 pmol/h during intervals 0-3 and 9-12 h each day. Time 0 is the start of the 12-h dark period. Values are means ± (SE). Veh = vehicle, sCT = salmon calcitonin, * P < 0.05, † P < 0.01, ‡ P < 0.001 vs. vehicle.
Figure 1. Mean effective dose (ED$_{50}$) of 3-h IV infusion of various anorexigenic substances at dark onset on food intake in non-deprived lean rats (9-11, 30, 31). sCT (salmon calcitonin), PYY (3-36) [peptide YY (3-36)], CCK-8 (cholecystokinin octapeptide), GLP-1 (glucagon-like peptide-1), CGRP (calcitonin gene-related peptide), ADM (adrenomedullin), MT II (melanocortin agonist melanotan II), AM251 (cannabinoid 1 receptor antagonist), naloxone (opioid receptor antagonist).

Figure 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 semi-purified solid food. Rats had free access to Ensure and 45% fat solid diet for 4-8 months, then Ensure was withdrawn for 6 weeks. The upper border, middle line, and lower border of each box indicate the 75$^{th}$ percentile, median, and 25$^{th}$ percentile, respectively. Whiskers (error bars) above and below the box indicate the 90$^{th}$ and 10$^{th}$ percentiles. Filled circles above and below whiskers indicate the 95$^{th}$ and 5$^{th}$ percentiles.

Figure 3. Effects of intermittent IP 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 IP infusions of vehicle during intervals 0-3 and 9-12 h of the dark period. During the 7-week treatment period, separate groups of rats received IP infusions during the same periods of either vehicle (n = 16 to 18) or sCT (n = 18 or 19) at 10 different dosing regimens. Letters designate a change in dosing strategy. Some dosing regimens employed a loading dose of sCT administered just prior to food presentation at dark onset. Dotted lines border a range in food intake that is about 25-35% less.
than that observed in vehicle-treated control rats. Values are means ± SE. * P < 0.05, † P < 0.01, ‡ P < 0.001 vs. vehicle.

<table>
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<th>Loading dose (pmol)</th>
<th>Infused dose (pmol/h)</th>
<th>Infusion intervals (h)</th>
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Figure 4. Effects of intermittent IP 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 Figure 3. Time 0 is the start of the 12-hour dark period. Bars indicate periods of infusion. Legends indicate dose of sCT given. Values are means ± SE. * P < 0.05, † P < 0.01, ‡ P < 0.001 when compared to vehicle-treated group.

Figure 5. Effects of intermittent IP infusions of sCT on cumulative hourly food intake in diet-induced obese rats during days 6 to 12 of sCT treatments. Data are from the experiment described in Figure 3. Time 0 is the start of the 12-hour dark period. Bars indicate periods of
infusion. Legends indicate dose of sCT given. Values are means ± SE. * P < 0.05, † P < 0.01, ‡ P < 0.001 when compared to vehicle-treated group.

Figure 6. Effects of intermittent IP infusions of sCT on cumulative hourly food intake in diet-induced obese rats during days 13 to 17 of sCT treatments. Data are from the experiment as described in Figure 3. Time 0 is the start of the 12-hour dark period. Bars indicate periods of infusion. Legends indicate dose of sCT given. Values are means ± SE. * P < 0.05, † P < 0.01, ‡ P < 0.001 when compared to vehicle-treated group.

Figure 7. Effects of intermittent IP infusions of sCT on cumulative hourly food intake in diet-induced obese rats during days 18 to 23 of sCT treatments. Data are from the experiment as described in Figure 3. Time 0 is the start of the 12-hour dark period. Bars indicate periods of infusion. Legends indicate dose of sCT given. Values are means ± SE. * P < 0.05, † P < 0.01, ‡ P < 0.001 when compared to vehicle-treated group.

Figure 8. Effects of intermittent IP infusions of sCT on cumulative hourly food intake in diet-induced obese rats during days 32 to 41 of sCT treatments. Data are from the experiment as described in Figure 3. Time 0 is the start of the 12-hour dark period. Bars indicate periods of infusion. Legends indicate dose of sCT given. Values are means ± SE. * P < 0.05, † P < 0.01, ‡ P < 0.001 when compared to vehicle-treated group.

Figure 9. Effects of intermittent IP infusions of sCT on cumulative hourly food intake in diet-induced obese rats during days 42 to 48 of sCT treatments. Data are from the experiment as described in Figure 3. Time 0 is the start of the 12-hour dark period. Bars indicate periods of infusion. Legends indicate dose of sCT given. Values are means ± SE. * P < 0.05, † P < 0.01, ‡ P < 0.001 when compared to vehicle-treated group.
Figure 10. Effects of intermittent IP infusions of sCT on body weight in diet-induced obese rats. Data are from experiment described in Figure 3. Values are means ± SE. * P < 0.05, † P < 0.01, ‡ P < 0.001 when compared to vehicle-treated group.
Week after Ensure withdrawal

Percent body fat

0 2 4 6

Week after Ensure withdrawal
DIO sCT FI BW BC 2 rrc.pzf:days 6-12 - Tue Aug 21 20:05:09 2007

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