Salmon calcitonin reduces food intake through changes in meal sizes in male rhesus monkeys

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Bello NT, Kemm MH, Moran TH. Salmon calcitonin reduces food intake through changes in meal sizes in male rhesus monkeys. Am J Physiol Regul Integr Comp Physiol 295: R76–R81, 2008. First published May 14, 2008; doi:10.1152/ajpregu.90327.2008.—Amylinergic mechanisms are believed to be involved in the control of appetite. This study examined the effects of the amylin agonist, salmon calcitonin, on food intake and meal patterns in adult male rhesus monkeys. Fifteen minutes before the onset of their 6-h daily feeding period, monkeys received intramuscular injections of various doses of salmon calcitonin (0.032, 0.056, 0.1, 0.32, and 1 μg/kg) or saline. Salmon calcitonin dose dependently reduced total daily and hourly food intake, with significant decreases at the 0.1, 0.32, and 1 μg/kg doses. Daily food intake was reduced by ~35%, 62%, and 96%, at these doses, respectively. An analysis of meal patterns revealed that size of the first meal was significantly reduced across the dose range of 0.056 to 1 μg/kg, while average meal size was reduced with the 0.32 and 1 μg/kg doses. Meal number was only affected at the 1 μg/kg dose. Repeated 5-day administration of the 0.1 μg/kg dose resulted in a reduction in daily food intake only on injection day 2, while significant reductions in food intake were observed on all five injection days with a 0.32 μg/kg dose. Daily food intake was also reduced for 1 day after the termination of the 5-day injections of the 0.32 μg/kg salmon calcitonin dose. These sustained reductions in intake were expressed through decreases in meal size. These data demonstrate that salmon calcitonin acutely and consistently decreases food intake mainly through reductions in meal sizes in nonhuman primates.

AMYLIN (ISLET AMYLOID POLYPEPTIDE, OR IAPP) is a 37-amino acid peptide that is cosecreted with insulin from the β-islet cells of the pancreas (13, 18). Similar to meal-related insulin, amylin levels are low prior to a meal and are elevated following a glucose load or a mixed meal (9, 19). Postprandial amylin levels have been shown to be higher following a high carbohydrate meal than following an isocaloric high-fat meal, and plasma amylin levels are positively related to subjective satiety ratings (15). Amylin’s potential role as a satiety peptide has been further supported by its ability to acutely reduce food intake in mice and rats without producing a conditioned taste aversion (10, 22, 24, 27, 34). More recently, the synthetic human amylin analog, pramlintide, has been shown to acutely and chronically (e.g., over 6 wk) reduce meal sizes and total food intakes in normal-weight and obese subjects (11, 38).

As a member of the calcitonin family of peptides, amylin shares some biological activities with calcitonin, adrenomedullin, and the calcitonin gene-related peptides (31). The purification and synthesis of human amylin are complicated by its tendency to self-aggregate and form amyloid fibrils and plaques (5). Moreover, synthetic or natural amylin analogs have been established as being more effective at reducing food intake in a variety of species (11, 12, 14, 25, 26, 34). Calcitonin of salmon origin (sCT) is one of these potent amylin analogs. Dose comparisons with amylin have demonstrated that sCT produces a more robust suppression of food intake through similar changes in meal patterns, and its effects on feeding can be blocked by an amylin-selective antagonist, AC187 (25, 34).

Although the effects of sCT on food intake have been extensively examined in rodent models, there is only a single report of the ability of sCT to affect food intake in nonhuman primates. In that study, the food intake of rhesus monkeys was suppressed for 3 days following a single high dose of sCT (6 μg/kg)(30). The aim of our study was to determine the dose range of sCT that would affect food intake and meal patterns in nonhuman primates. In addition, we examined whether repeated administration of sCT would produce long-lasting changes in food intake and meal patterns. This study is the first to demonstrate that sCT dose dependently reduces food intake predominantly through changes in meal sizes in nonhuman primates.

MATERIALS AND METHODS

Five individually housed adult male rhesus monkeys (Macaca mulatta), weighing between 6 and 15 kg, were used in this study. Monkeys were maintained on a 12:12-h light-dark cycle (7:00 AM to 7:00 PM) in an environmentally controlled room with ad libitum access to water. Water intake was not measured in these studies. The monkeys were weighed every 2 wk. Food in the form of nutritionally complete 1-gm pellets (Bioserv, Frenchtown, NJ) was provided for 6 h/day beginning at 12:00 PM. Food pellets were available in response to lever pressing on a fixed ratio reinforcement (FR) schedule. The FR schedule was individually determined for each animal to prevent cheek pouching of pellets. FR ratios ranged from FR3 to FR15 lever presses per pellet. All procedures were reviewed and approved by the Johns Hopkins University Animal Care and Use Committee.

Five monkeys received an intramuscular injection of a dose range, 0 (saline; vehicle) 0.032, 0.056, 0.1, 0.32, and 1 μg/kg (0, 9.32, 16.31, 29.13, 93.24, 291.13 pmol/kg) salmon calcitonin (Bachem, King of Prussia, PA) 15 min before the onset of food access (11:45 AM). The order of salmon calcitonin administration was randomized. At the time of injection, monkeys had been fasted for ~18 h (end of the feeding program on the previous day). Approximately 5 days were allowed between injections to eliminate any effects from previous

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results

sCT administration resulted in dose-related suppression in 6-h food intake \[ F(5, 15) = 19.2, P < 0.001 \]. The 0.1, 0.32, and 1 \( \mu \)g/kg doses significantly reduced intakes to 65%, 38%, and 4% of vehicle intake, respectively (\( P < 0.01 \), Figure 1A). On the 1 \( \mu \)g/kg injection days, two of the five monkeys failed to eat during the 6-h daily access period. As a consequence, the 1 \( \mu \)g/kg dose was significantly different from all other doses tested (\( P < 0.05 \)). On the basis of the feeding suppression of the six sCT doses and a regression line fit, the \( ED_{50} \) for sCT in nonhuman primates was determined to be 0.176 \( \mu \)g/kg. Hourly food intake was also reduced by the sCT injections in a dose-related fashion, as indicated by a significant effect for sCT dose \( F(5, 15) = 19.8, P < 0.001 \), hour \( F(5, 15) = 35.3, P < 0.001 \), and the interaction of dose \( \times \) hour \( F(25, 75) = 6.6, P < 0.001 \). Post hoc testing revealed significant reductions across time with the 0.1, 0.32, and 1 \( \mu \)g/kg doses (\( P < 0.01 \), Figure 1B). Meal pattern analyses revealed sCT led to reductions in average meal size, \( F(5, 15) = 3.0, P < 0.05 \) that were significant at the 0.32, and 1 \( \mu \)g/kg doses (\( P < 0.05 \), Fig. 2). sCT also affected the size of the first meal \( F(5, 15) = 8.9, P < 0.005 \), with significant reductions at the 0.056, 0.1, 0.32, and 1 \( \mu \)g/kg doses (\( P < 0.05 \), Fig. 3). There was also an effect of sCT on meal frequency \( F(5, 15) = 8.7, P < 0.05 \).

However, this difference was due to a significant reduction in meal frequency with the 1 \( \mu \)g/kg sCT dose (\( P < 0.05 \), not shown), a dose that completely prevented intake in two of the monkeys. For the monkeys that ate, there were no effects of sCT on the latency to initiate the first meal \( F(5, 10) = 1.8, \text{n.s.} \), or on the intermeal intervals \( F(5, 10) = 0.9, \text{n.s.} \). Although there was a trend for monkeys with lower body weights to have larger reductions in food intake from vehicle following the 0.32 \( \mu \)g/kg sCT dose (\( R = -0.84, \text{n.s.} \)), the degree of suppression was fairly consistent across monkeys (59–69%), and there was no significant effect. The 0.32 \( \mu \)g/kg was chosen for this comparison because it effectively reduced food intake in all monkeys and significantly reduced average meal size.

Repeated 5-day administration of 0.1 \( \mu \)g/kg of sCT, resulted in an overall reduction in total daily food intake, \( F(8, 24) = 3.2621, P < 0.05 \). Post hoc tests revealed that intake for injection day 2 was significantly reduced from the baseline intakes (\( P < 0.05 \), see Fig. 4). For hourly food intake over the injection days, a two-way repeated-measures ANOVA demon-
strated overall effects for sCT administration \([F(8, 24) = 9.5, P < 0.01]\) and hour \([F(5, 15) = 19.8, P < 0.01]\), but no significant treatment \(\times\) hour interaction \([F(40, 120) = 0.9, n.s.]\). Planned comparisons revealed that intakes for hours 1 and 2 were significantly reduced on sCT injection days, compared with intakes at the same time on both preinjection and postinjections days \((P < 0.05)\). Even though average meal size was not significantly affected in response to repeated administration of this dose \([F(8, 160) = 1.5, n.s.]\), first meal size was significantly reduced in response to the sCT injections \([F(8, 24) = 3.4, P < 0.01]\). In addition, repeated 5-day injections of 0.1 \(\mu g/k g\) sCT did not affect meal frequency \([F(8, 24) = 0.2, n.s.]\), latency to first meal \([F(8, 24) = 1.4, n.s.]\) or duration of intermeal intervals \([F(8, 136) = 0.9, n.s.]\). On the basis of the 2-wk interval body weights, the 5-day repeated administration of 0.1 \(\mu g/k g\) sCT did not significantly affect body weight \((t = 1.26, n.s.)\).

For the daily repeated administration of 0.32 \(\mu g/k g\) of sCT, there was a reduction in total daily food intake over the course of the injection days, \([F(8, 24) = 21.1, P < 0.001]\). Post hoc tests revealed that food intakes were significantly reduced from the preinjection days on all five sCT injection days \((P < 0.05)\). Intakes on sCT injection days 2, 3, 4, and 5 were also significantly less than intakes on all postinjection days. Daily food intake was also significantly reduced on postinjection day 1 compared with baseline intakes \((P < 0.05, \text{see Fig. 5})\), but returned to baseline levels on postinjection days 2 and 3. On postinjection days 2 and 3, daily food intake never exceeded the monkey’s individual baseline intake levels. On injection days 3 and 4, one monkey failed to eat during the 6-h access period but resumed eating on injection day 5. A two-way repeated-measures ANOVA for hourly food intake over the 0.32 \(\mu g/k g\) injection series demonstrated overall effects of treatment \([F(8, 24) = 23.3, P < 0.001]\), and hour \([F(5, 15) = 24.1, P < 0.001]\). There was also a significant treatment \(\times\)
hour interaction [F(40, 120) = 7.8, P < 0.001]. Post hoc tests revealed that the food intakes on all five injection days were significantly reduced at each hour of the 6-h access compared with preinjection days and postinjection days 2 and 3 (P < 0.05). As illustrated in Table 1, meal pattern analysis revealed that average meal size significantly decreased over the repeated 5-day administration of 0.32 μg/kg sCT, [F(8, 24) = 3.0, P < 0.05]. Post hoc testing also demonstrated that the meal sizes on injection days 2, 3, and 4 were significantly reduced from the averaged baseline meal size (P < 0.05, see Table 1). Repeated 5-day administration of 0.32 μg/kg sCT led to significant reductions in first meal size [F (8, 16) = 4.0, P < 0.01]. Post hoc testing revealed this occurred on all five injection days and on postinjection days 1 and 2 (P < 0.05). There was also a significant overall effect of sCT on meal frequency [F (8, 16) = 3.6, P < 0.05]. For the monkeys that ate, the latency to first meal [F (8, 16) = 1.5, n.s.] and durations of the intermeal intervals were not significantly affected by the repeated administration of 0.32 μg/kg sCT . [F (8, 80) = 0.6, n.s.]. On the basis of the 2-kg body weights, the 5-day repeated administration of 0.32 μg/kg sCT did not significantly affect body weight (t = −1.78, n.s.).

**DISCUSSION**

The goal of these experiments was to determine the degree and behavioral mechanisms through which the anorexigenic amylin analog sCT (0.032–1 μg/kg), affects food intake in nonhuman primates. Daily food intake and meal patterns were determined during a daily access period of 6 h in rhesus monkeys trained to lever press for nutritionally complete pellets. A single sCT injection of 0.1, 0.32, and 1 μg/kg dose-dependently reduced intake by ~35%, 62%, and 96%, respectively. The feeding suppression observed in the present study was a result of reduction in first meal size with the 0.1, 0.32, and 1 μg/kg doses and reduction in average meal size with the 0.32 and 1 μg/kg doses. First meal size was also reduced with the 0.056 μg/kg sCT dose, but this dose did not affect total daily intake. This pattern of results is consistent with prior reports that amylin and sCT suppress feeding by reducing meal sizes, not meal frequencies in rodents (14, 24, 33, 42). We did observe a reduction in both meal size and frequency with the highest dose tested, 1 μg/kg. Notably, this dose completely prevented food intake in two out of the five monkeys tested. A reduction in both meal size and frequency has been previously reported for intravenous infusions of amylin and sCT in rats when they were administered doses that are ~10- to 30-fold higher than the minimum dose required to significantly reduce meal size (33, 34). In summary, the results of our single sCT dose experiments demonstrated that sCT decreased daily food intake by producing dose-dependent reductions in overall food intake that were expressed through reductions in first meal size and average meal size.

On the basis of data from the dose-response experiment, we chose two doses of sCT to examine the effects of repeated administration, one dose that was subthreshold for reducing meal size and a second dose that produced reliable suppressions in overall food intake and meal size (0.10 and 0.32 μg/kg, respectively). Both doses produced consistent reductions in first meal size over the 5-day administration periods. A more robust and sustained reduction in daily food intake was observed with the 0.32 μg/kg sCT dose. Specifically, the size of the first meal was reduced by an average of ~75%, and average meal size was reduced by ~33% to 54%. Daily food intake remained suppressed with this dose, even 1 day after the 5-day injection series was terminated. This reduction in intake can be attributed to a ~50% reduction in first meal size. Two studies in rats have reported a sustained suppression in food intake following repeated administration. One study employed intraperitoneal sCT infusions with the goal of reducing percent body fat and body weight in obese rats fed a high-fat diet (12). To achieve this effect, infusions were done intermittently and at various doses. Over the 7-wk experiment, sCT potently reduced food intake to sustain a body weight reduction of 9% and percent body fat reduction of 22% from preinfusion levels. Interestingly, on the infusion days when sCT was replaced with saline, food intake dramatically increased >41% compared with previous sCT infusion days. In the other study, sCT was repeatedly administered into the lateral cerebroventricle in rats for 5 days (40). Unlike the primates in our study, rats demonstrated a reduction in food intake for only the first three days of the injections. By injection day 4 and 5, rats were consuming the same amount of food as vehicle-injected rats. Our data indicate that monkeys did not develop tolerance to the suppressive effects of sCT on daily food intake nor increase food intake above baseline levels following secession of treatment.

The observation that repeated 5-day administration of sCT did not reduce its efficacy to suppress food intake indicates alterations in the function or expression of the amylin receptor dimer (CTR/RAMP1; calcitonin receptors/receptor activity modifying protein 1) (41). Sustained peripheral 7-day infusions of sCT have been shown to cause a dose-dependent decrease in renal calcitonin-binding sites (8). The inability to demonstrate behavioral tolerance and the prolonged suppression of sCT on the food intake following termination, however, suggests a continued activation of the CTR/RAMP1 downstream effects (17). Peripheral injections of amylin or sCT that acutely suppressed food intake also reduced orexin gene expression in the lateral hypothalamus (LH). Only the injections of sCT

<table>
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<tr>
<th>Preinjection 3-Day</th>
<th>Average</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Post-Injection</th>
<th>Average</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
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<tbody>
<tr>
<td>Average meal size, g</td>
<td>24.6±3.8</td>
<td>16.6±2.4</td>
<td>14.9±3.1</td>
<td>12.9±4.0</td>
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<td>18.1±2.4</td>
<td>21.8±4.4</td>
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<td>First meal size, g</td>
<td>52.4±11.7</td>
<td>14.3±3.9</td>
<td>11.5±6.8</td>
<td>20.0±12.6</td>
<td>13.0±5.7</td>
<td>6.5±1.1</td>
<td>24.5±7.7</td>
<td>21.8±7.2</td>
<td>50.5±12.8</td>
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<td>Latency to first meal, min</td>
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<td>0.2±0.1</td>
<td>27.6±26.4</td>
<td>62.1±37.2</td>
<td>3.1±1.8</td>
<td>20.7±11.6</td>
<td>1.37±0.7</td>
<td>0.3±0.1</td>
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<tr>
<td>Meal frequency</td>
<td>7.9±1.2</td>
<td>7.5±1.3</td>
<td>5.0±1.4</td>
<td>4.7±1.7</td>
<td>6.0±1.2</td>
<td>5.8±0.7</td>
<td>7.8±1.4</td>
<td>8.5±1.4</td>
<td>8.0±1.5</td>
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</tr>
<tr>
<td>Average IMI, min</td>
<td>40.0±5.1</td>
<td>45.3±6.6</td>
<td>68.1±18.0</td>
<td>63.2±22.5</td>
<td>57.9±16.2</td>
<td>62.7±10.0</td>
<td>43.0±6.8</td>
<td>39.7±5.7</td>
<td>39.0±6.8</td>
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sCT, salmon calcitonin; IMI, Intermeal interval. *P < 0.05 significantly different from preinjection baseline.
though suppressed melanin-concentrating hormone in the LH, but neither sCT nor amylin altered arcuate nucleus (ARC) gene expression of neuropeptide Y or agouti gene-related protein (3). Long-term peripheral infusions (22 days) of amylin in obesity-prone rats, however, has been shown to increase pro-opiomelanocortin (POMC) gene expression in the ARC (36). There was no change in POMC expression in rats that underwent a similar level of calorie restriction, suggesting that amylneric mechanisms alter central anorexigenic pathways to reduce feeding.

Salmon calcitonin and the amylinergic control of food intake appear to be mediated by central mechanisms. For instance, although exogenously administered amylin and sCT both reduce gastric emptying (6, 16, 34, 43, 44), this action does not appear to fully account for their satiety effects. A comparison of intravenous doses of amylin or sCT has shown a similar dose range that reduces meal sizes, also inhibits gastric emptying (33, 34). Following subdiaphragmatic vagotomy, however, amylin no longer inhibits gastric emptying, but it is still effective at reducing food intake (28, 43). Likewise, a low dose of amylin that was effective at reducing feeding in rats had no effect on gastric emptying (23), and a 10-fold higher dose of amylin was needed to reduce sham feeding compared with real-feeding of 0.8 M sucrose (1). Although amylin also synergistically reduces food intake when injected with CCK (6), this effect appears to be mediated by separate mechanisms since CCK-1 antagonist doses that increase gastric emptying and food intake, do not influence amylin’s feeding reducing potency (20, 28). Taken together, these data suggest that amylin’s (and sCT) ability to reduce food intake is neither vagal afferent driven nor secondary to its inhibitory response on gastric emptying. Multiple laboratories have demonstrated that central amylin or sCT injections potently reduce food intake (2, 7, 21, 29). A dose comparison between peripheral and central (third ventricle intracerebroventricular) chronic administration of amylin in rats revealed that the minimum effective dose at suppressing food intake was 10-fold lower when delivered intracerebroventricularly (29). A relatively high density of amylin receptors are located in brain regions involved in the control of ingestive behaviors, such as the dorsal medial hypothalamus, ventromedial hypothalamus, nucleus accumbens, nucleus of the solitary tract and area postrema (4, 37).

Synthetic salmon calcitonin (e.g., Clacinmar and Miacalcin) is a Food and Drug Association-approved nonestrogetic treatment for osteoporosis in postmenopausal women and for the treatment of Paget’s disease (osteitis deformans). Prolonged sCT treatments have the therapeutic benefit of preventing bone fractures (32). Typically, before the availability of intranasal sCT, intramuscular injections of 100 IU (20 mg) of sCT every other day or 100 IU every day were prescribed for those suffering from osteoporosis or Paget’s disease, respectively. The two most commonly reported side effects with injectable sCT were a transient nausea with or without vomiting and a local site injection inflammatory response. Both of these side effects occur with a frequency of 10% in treated patients, while “loss of appetite” or “reduced body weight” has not been extensively reported with sCT treatments for osteoporosis (Miacalcin, Novartis product literature, 2002) (35, 39). More preclinical research is needed to clarify the discrepancy between the potent anorexigenic effect produced with low doses of sCT and the decrease in hypercalcemia and suppression of bone resorption shown with hundred- or thousand-fold higher doses of sCT used to treat bone maladies.

Perspectives and Significance

In these experiments, we have shown that the amylin analog, sCT, had a robust feeding suppressive effect mainly through its ability to reduce meal sizes in nonhuman primates. Neither tolerance nor rebound hyperphagia developed during the repeated 5-day administration of sCT at a dose that suppressed average meal size in the single-dose experiments. The observation that sCT potently suppresses food intake, first meal size, and average meal size, and these suppressions persisted following termination of treatment, further suggests that sCT is a potent anorexigenic peptide with lasting effects on feeding behavior.

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