Effects of amylin-related peptides on food intake, meal patterns, and gastric emptying in rats

ROGER D. REIDELBERGER, LINDA KELSEY, AND DEAN HEIMANN
Veterans Administration Medical Center, Omaha 68105; and Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, Nebraska 68178

Received 1 October 2001; accepted in final form 9 January 2002

Reidelberger, Roger D., Linda Kelsey, and Dean Heimann. Effects of amylin-related peptides on food intake, meal patterns, and gastric emptying in rats. Am J Physiol Regulatory Integrative Comp Physiol 282: R1395–R1404, 2002.—We previously demonstrated that amylin inhibits food intake and gastric emptying in rats with half-maximal effective doses (ED_{50} s) of 8 and 3 pmol·kg^{-1}·min^{-1} and maximal inhibitions of 78 and 60%, respectively. In this study of identical design, rats received intravenous infusions of salmon calcitonin (sCT), rat calcitonin (rCT), rat calcitonin gene-related peptide (rCGRP), and rat adrenomedullin (rADM) for 3 h at dark onset, and food intake was measured for 17 h or for 15 min and gastric emptying of saline was measured during the final 5 min. sCT, rCGRP, and rADM inhibited food intake with estimated ED_{50} s of 0.5, 26, and 35 pmol·kg^{-1}·min^{-1} and maximal inhibitions of 88, 90, and 49%, respectively. rCT was not effective at doses up to 100 pmol·kg^{-1}·min^{-1}. sCT, rCGRP, rADM, and rCT inhibited gastric emptying with ED_{50} s of 1, 130, 160, and 730 pmol·kg^{-1}·min^{-1} and maximal inhibitions of 60, 66, 60, and 33%, respectively. These results suggest that amylin and sCT may act by a common mechanism to decrease food intake, which includes inhibition of gastric emptying.

calcitonin; calcitonin gene-related peptide; adrenomedullin; anorexia; potency

AMYLIN (ALSO CALLED ISLET amyloid polypeptide) is a 37-amino acid peptide that is cosecreted with insulin from the pancreas in response to a meal (11, 51). Amylin has also been detected in gut endocrine cells (35), visceral sensory neurons (36), and throughout the brain (44). Exogenous amylin potently reduces food intake (1, 3, 39), body weight (1), adiposity (41), gastric emptying (10, 39), and gastric acid secretion (15) when administered systemically or into the brain. We recently demonstrated that the minimal effective intravenous dose for amylin-induced inhibition of food intake and gastric emptying in rats (1 pmol·kg^{-1}·min^{-1}) increases plasma amylin by an amount comparable to that produced by a meal (3, 39). We also demonstrated that in suppressing feeding and gastric emptying, amylin is at least as potent and efficacious as CCK (39), a physiological inhibitor of food intake and gastric emptying. These results support the hypothesis that amylin acts as a hormonal signal to the brain to inhibit gastric emptying and food intake and that amylin produces satiety, in part, through inhibition of gastric emptying.

Calcitonin gene-related peptide (CGRP), calcitonin (CT), and adrenomedullin (ADM), together with amylin, form a family of structurally related peptides with overlapping biological actions (Fig. 1). Each of these peptides inhibits food intake (13, 22, 32, 45) and gastric emptying (8, 20, 30, 38) when administered systemically or into the brain. The teleost peptide salmon CT (sCT) appears to be significantly more potent than either amylin (29) or mammalian CTs (8, 29, 47) in decreasing food intake and gastric emptying. No study has directly compared the effects of systemic administration of these amylin-related peptides on food intake and gastric emptying in the same species. Factors that inhibit gastric emptying can indirectly reduce food intake by promoting gastric distention. If the anorexia produced by these amylin-related peptides is due, in part, to inhibition of gastric emptying, then it would be important to determine for each peptide whether its potency for reducing gastric emptying is greater than or equal to its potency for reducing food intake. Lutz et al. (26) concluded that amylin does not reduce food intake by inhibiting gastric emptying because they observed that a single anorexic dose of amylin had no effect on gastric emptying of spontaneous meals. In contrast, we recently demonstrated that amylin inhibits food intake and gastric emptying of a nonnutrient liquid with a similar potency and efficacy (39). The aims of the present study were to determine the dose-response effects of intravenous infusions of rat (r) CGRP, CT, ADM, and sCT on food intake and gastric emptying in rats and to compare these effects with those of rat amylin, which were determined previously using an identical experimental design (39).

METHODS

Subjects. Adult male Sprague-Dawley rats (Sasco, Charles River), weighing 350–400 g at the time of surgery, were housed individually in hanging wire mesh cages in a temperature-controlled room with a 12:12-h light-dark cycle (lights
Effects of intravenous infusions of sCT, rCT, rCGRP, and rADM on gastric emptying. The experimental design was similar to that described previously (39). Rats with gastric and jugular vein cannulas were adapted to a 17-h fast, followed by light restraint in a Bollman-type cage, flushing of the stomach with warm saline, and a 15-min intravenous infusion (3.2 ml/h) of 0.15 M NaCl, 0.1% BSA. On experimental days, the food-deprived rats received a 15-min jugular vein infusion of sCT (0, 1, 3, or 10 pmol·kg⁻¹·min⁻¹ in 0.15 M NaCl, 0.1% BSA). Ten minutes after infusion onset, 3 ml of saline containing 60 mg/ml phenol red were instilled into the stomach. Gastric contents were recovered 5 min later through the gastric cannula, the volume was measured, and the concentration of phenol red was determined spectrophotometrically to provide a measure of the amount of saline emptied during the 5-min period. Each rat (n = 10) received each dose of sCT in random order at intervals of at least 48 h.

In separate experiments of identical design for each peptide, rats randomly received a series of doses (0, 50, 170, and 500 pmol·kg⁻¹·min⁻¹) of rCT (n = 10), rCGRP (n = 12), and rADM (n = 10).

Statistical analyses. Values are presented as group means ± SE. For the feeding experiments, we separately evaluated the dose-dependent effects of jugular vein infusions of sCT, rCT, rCGRP, and rADM on amount of food ingested each hour, food intake cumulated hourly across the 17-h test period, first meal parameters [latency, meal size, postmeal interval, and satiety ratio (postmeal interval/meal size)], and mean meal parameters across the 3-h infusion period [number of meals, meal size, postmeal interval, satiety ratio, and eating rate (meal size/meal duration)] by repeated-measures ANOVA, with peptide dose and time being the within-group factors. For the gastric emptying experiments, the dose-dependent effects of jugular vein infusions of sCT, rCT, rCGRP, and rADM on volume of saline emptied from the stomach in 5 min were evaluated separately using a repeated-measures ANOVA, with peptide dose being the within-group factor. Planned comparisons of treatment means were evaluated by direct contrasts of means with the statistical program SYSTAT. In each analysis, differences were considered significant if P < 0.05. A one-tailed test was used for the postulated unidirectional effects of each peptide.

A general nonlinear, least-squares curve-fitting method was used as previously described (12) to fit the dose-response data for the effects of sCT, rCT, rCGRP, and rADM on food intake and gastric emptying to the following logistic equa-
Effects of intravenous infusions of sCT, rCT, rCGRP, and rADM on food intake. sCT infusion for 3 h at dark onset dose dependently inhibited cumulative food intake across the 17-h test period (Fig. 2). The minimal effective dose (0.1 pmol·kg⁻¹·min⁻¹), the lowest dose administered, inhibited cumulative intake at 2, 3, and 4 h by 27% (P < 0.05), 25% (P < 0.05), and 17% (P < 0.05), respectively. The maximal effective dose (10 pmol·kg⁻¹·min⁻¹), the largest dose administered, decreased cumulative intake throughout the 17-h test period, with a peak inhibition of 100% at 1 h (P < 0.001), decreasing to 85% inhibition by 17 h (P < 0.001). In this experiment, each rat received all doses of sCT in random order at 48-h intervals. We measured food intake on intervening days when sCT was not administered to determine if there was a carry-over effect of the peptide on food intake between the experimental days. The day before the sCT experiment began, food intake was 26.8 ± 1.7 g. Food intake on days immediately following delivery of the 0, 0.1, 0.3, 1, 3, and 10 pmol·kg⁻¹·min⁻¹ doses was 23.8 ± 1.0, 24.3 ± 1.4, 26.0 ± 2.5, 25.6 ± 1.2, 27.8 ± 1.6, and 24.9 ± 2.2 g, respectively. These data clearly show that food intake returned to normal within 48 h of sCT administration.

Figure 3 shows the dose-response effects of sCT on intake during the 3-h infusion period. Nonlinear regression fitting of the data to the logistic equation gave the following relationship between cumulative intake in grams and sCT dose in picomoles per kilogram per minute: food intake = 7.6 g/[1 + (sCT/0.5 pmol·kg⁻¹·min⁻¹)⁰.⁹⁴] + 0.1 g (goodness of fit r² = 0.86). Thus sCT inhibited food intake during the 3-h infusion period with a threshold dose <0.1 pmol·kg⁻¹·min⁻¹ and an estimated ED₅₀ of 0.5 pmol·kg⁻¹·min⁻¹.

For comparison, Fig. 3 also shows the dose-response effects of intravenous infusion of amylin on 3-h cumulative intake using an identical experimental procedure (39). The ED₅₀ for sCT is significantly smaller than that for amylin (0.5 vs. 8 pmol·kg⁻¹·min⁻¹; F₁,₁₃₃ = 51.1, P < 0.001).
rCT infusion for 3 h at dark onset at doses from 3 to 100 pmol·kg\(^{-1}\)·min\(^{-1}\) had no significant effect on food intake (Figs. 3 and 4).

rCGRP infusion for 3 h at dark onset dose dependently inhibited cumulative food intake across the 17-h test period (Fig. 5). The minimal effective dose (10 pmol·kg\(^{-1}\)·min\(^{-1}\)) inhibited cumulative intake at 1, 2, and 3 h by 56% (P < 0.05), 47% (P < 0.01), and 30% (P < 0.01), respectively. The maximal effective dose (100 pmol·kg\(^{-1}\)·min\(^{-1}\)) was the largest dose administered, decreased cumulative intake throughout the first 10 h of the 17-h test period, with a peak inhibition of 89% at 1 h (P < 0.001), decreasing to 12% by 10 h (P < 0.05). Figure 3 shows the dose-response effects of rCGRP on intake during the 3-h infusion period. Nonlinear regression fitting of the data to the logistic equation gave the following relationship between cumulative intake in grams and rCGRP dose in picomoles per kilogram per minute: food intake = 4.1 g/[1 + (rCGRP/26 pmol·kg\(^{-1}\)·min\(^{-1}\)\(^{-1}\))]\(^{1.3}\) + 2.6 g (goodness of fit \(r^{2} = 0.94\)). Thus rCGRP inhibited food intake during the 3-h infusion period with a threshold dose between 10 and 30 pmol·kg\(^{-1}\)·min\(^{-1}\) and an estimated \(ED_{50}\) of 35 pmol·kg\(^{-1}\)·min\(^{-1}\). The \(ED_{50}\) for rADM is significantly larger than that for rCGRP (35 vs. 0.5 pmol·kg\(^{-1}\)·min\(^{-1}\); \(F_{1,139} = 24.8, P < 0.0001\), amylin (35 vs. 8 pmol·kg\(^{-1}\)·min\(^{-1}\); \(F_{1,108} = 104, P < 0.0001\), and rCGRP (35 vs. 26 pmol·kg\(^{-1}\)·min\(^{-1}\); \(F_{1,116} = 30, P < 0.0001\)). The maximal inhibitory response to rADM is not different from that to sCT, rCGRP, and amylin (rADM vs. sCT: \(F_{1,138} = 0.9, P = 0.34\); rADM vs. rCGRP: \(F_{1,115} = 0.26, P = 0.61\); and rADM vs. amylin: \(F_{1,107} = 1.21, P = 0.27\).

Effects of intravenous infusion of sCT, rCT, rCGRP, and rADM on meal patterns. Lower doses of sCT (≤0.3 pmol·kg\(^{-1}\)·h\(^{-1}\)) reduced food intake primarily by decreasing mean meal size during the 3-h infusion period (Table 1). Higher doses increased the latency to the first meal and reduced meal size and meal frequency. sCT had no significant effect on average eating rate during meals, as determined by dividing meal size by meal duration (data not shown).

The two highest doses of ADM (30 and 100 pmol·kg\(^{-1}\)·min\(^{-1}\)) reduced meal frequency and the size of the first meal following infusion onset (Table 1). CGRP reduced meal frequency at the 10- and 100-pmol·kg\(^{-1}\)·min\(^{-1}\) doses and reduced average meal size at the 30- and 100-pmol·kg\(^{-1}\)·min\(^{-1}\) doses. The highest dose of rCGRP significantly reduced eating rate.
during meals that were consumed during the 3-h infusion period (data not shown). However, only an average of 0.4 meals were consumed during this period, compared with 2.9 meals when vehicle was infused. rCT had no effect on meal size or frequency at any of the doses tested (Table 1).

Effects of intravenous infusion of sCT, rCT, rCGRP, and rADM on gastric emptying. sCT dose dependently reduced the volume of saline emptied from the stomach during the 5-min test period (Fig. 7; \( F_{3,27} = 19.9, P < 0.001 \)). The minimal effective dose (1 pmol·kg\(^{-1}\)·min\(^{-1}\)), which was the lowest dose given, decreased emptying by 32% (\( P < 0.001 \)). The maximal effective dose (10 pmol·kg\(^{-1}\)·min\(^{-1}\)), the largest dose given, decreased emptying by 60% (\( P < 0.001 \)). Nonlinear regression fitting of the data to the logistic equation gave the following relationship between gastric emptying in milliliters and sCT dose in picomoles per kilogram per minute:

\[
\text{gastric emptying} = 2.1 \text{ ml} / [1 + (sCT/1.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})^{1.1}] + 0.9 \text{ ml} \quad (\text{goodness of fit} \ r^2 = 0.91) \]

Thus sCT inhibited gastric emptying with a threshold dose of 1 pmol·kg\(^{-1}\)·min\(^{-1}\) and an estimated \( \text{ED}_{50} \) of 1 pmol·kg\(^{-1}\)·min\(^{-1}\). For comparison, Fig. 7 also shows the dose-response effects of intravenous infusion of amylin on gastric emptying using an identical experimental procedure (39). The estimated \( \text{ED}_{50} \) for sCT is significantly smaller than that for amylin (1 vs. 2.9 pmol·kg\(^{-1}\)·min\(^{-1}\); \( F_{1,83} = 10.6, P = 0.0016 \)); maximal inhibitory responses to sCT and amylin are not different (\( F_{1,82} = 2.0, P = 0.16 \)).

rCGRP dose dependently reduced the volume of saline emptied from the stomach during the 5-min test period (Fig. 7; \( F_{3,33} = 33.8, P < 0.001 \)). The minimal effective dose (170 pmol·kg\(^{-1}\)·min\(^{-1}\)), the largest dose given, decreased emptying by 60% (\( P < 0.001 \)). Nonlinear regression fitting of the data to the logistic equation gave the following relationship between gastric emptying in milliliters and rCGRP dose in picomoles per kilogram per minute:

\[
\text{gastric emptying} = 2.1 \text{ ml} / [1 + (rCGRP/170 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})^{1.1}] + 0.9 \text{ ml} \quad (\text{goodness of fit} \ r^2 = 0.91) \]

Thus rCGRP inhibited gastric emptying with a threshold dose of 170 pmol·kg\(^{-1}\)·min\(^{-1}\) and an estimated \( \text{ED}_{50} \) of 170 pmol·kg\(^{-1}\)·min\(^{-1}\). For comparison, Fig. 7 also shows the dose-response effects of intravenous infusion of amylin on gastric emptying using an identical experimental procedure (39). The estimated \( \text{ED}_{50} \) for rCGRP is significantly smaller than that for amylin (170 vs. 2.9 pmol·kg\(^{-1}\)·min\(^{-1}\); \( F_{1,82} = 10.6, P = 0.0016 \)); maximal inhibitory responses to rCGRP and amylin are not different (\( F_{1,82} = 2.0, P = 0.16 \)).

rADM dose dependently reduced the volume of saline emptied from the stomach during the 5-min test period (Fig. 7; \( F_{3,33} = 33.8, P < 0.001 \)). The minimal effective dose (500 pmol·kg\(^{-1}\)·min\(^{-1}\)), the largest dose given, de-
Table 1. Effects of intravenous infusions of sCT, rCGRP, rADM, and rCT on meal patterns

<table>
<thead>
<tr>
<th>pmol·kg⁻¹·min⁻¹</th>
<th>First Meal</th>
<th>All Meals During 3-h Infusion Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latency</td>
<td>MS</td>
</tr>
<tr>
<td>sCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>44.4 ± 7.5</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>0.1</td>
<td>55.4 ± 11.3</td>
<td>1.4 ± 0.2*</td>
</tr>
<tr>
<td>0.3</td>
<td>52.7 ± 15.7</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>1</td>
<td>103.2 ± 23.0</td>
<td>1.3 ± 0.3*</td>
</tr>
<tr>
<td>3</td>
<td>243.0 ± 77.0</td>
<td>0.9 ± 0.2*</td>
</tr>
<tr>
<td>10</td>
<td>230.0 ± 76.0</td>
<td>0.9 ± 0.2*</td>
</tr>
<tr>
<td>rCGRP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>50.4 ± 13.1</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>51.5 ± 10.2</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>74.6 ± 12.1</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>30</td>
<td>66.0 ± 13.6</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>100</td>
<td>190.5 ± 21.8*</td>
<td>3.7 ± 0.6*</td>
</tr>
<tr>
<td>rADM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>48.5 ± 14.1</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>44.9 ± 11.6</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>10</td>
<td>27.6 ± 6.5</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>30</td>
<td>39.8 ± 9.8</td>
<td>1.5 ± 0.2*</td>
</tr>
<tr>
<td>100</td>
<td>48.9 ± 11.7</td>
<td>1.4 ± 0.3*</td>
</tr>
<tr>
<td>rCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>40.2 ± 12.4</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>47.8 ± 13.8</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>54.2 ± 13.1</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>30</td>
<td>48.1 ± 8.6</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>100</td>
<td>59.3 ± 15.2</td>
<td>2.0 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Nonfasted rats received a 3-h intravenous infusion of peptide beginning 15 min before dark onset; food intake and meal patterns were determined from continuous computer recordings of changes in food bowl weight. Latency, time to first meal (min); MS, meal size (g); PMI, postmeal interval (min); SR, satiety ratio (PMI/MS (min/g)); sCT, salmon calcitonin; rCGRP, rat calcitonin gene-related peptide; rADM, rat adrenomedullin; and rCT, rat calcitonin. *P < 0.05, †P < 0.01, and ‡P < 0.001.

creased emptying by 66% (P < 0.001). Nonlinear regression fitting of the data to the logistic equation gave the following relationship between gastric emptying in milliliters and rCGRP dose in picomoles per kilogram per minute: gastric emptying = 2 ml/[1 + (rCGRP/130 pmol·kg⁻¹·min⁻¹)¹.⁹] + 0.9 ml (goodness of fit r² = 0.94). Thus rCGRP inhibited gastric emptying with a threshold dose between 50 and 170 pmol·kg⁻¹·min⁻¹ and an estimated ED₅₀ of 160 pmol·kg⁻¹·min⁻¹. The ED₅₀ for rADM is significantly larger than that for sCT (160 vs. 1 pmol·kg⁻¹·min⁻¹; F₁,₇₅ = 47, P < 0.0001) and amylin (160 vs. 2.9 pmol·kg⁻¹·min⁻¹; F₁,₈₅ = 50, P < 0.0001), and it is similar to that for rCGRP (160 vs. 130 pmol·kg⁻¹·min⁻¹; F₁,₈₃ = 2.4, P = 0.12). The maximal inhibitory response to rADM is not different from that to sCT, rCGRP, and amylin (rADM vs. sCT: F₁,₇₄ = 0.11, P = 0.74; rADM vs. amylin: F₁,₈₂ = 2.6, P = 0.11; and rADM vs. rCGRP: F₁,₈₂ = 0.06, P = 0.81).

rCPT dose dependently reduced the volume of saline emptied from the stomach during the 5-min test period (Fig. 7; F₃,₃₇ = 7.5, P < 0.001). The minimal effective dose (170 pmol·kg⁻¹·min⁻¹) decreased emptying by 21% (P < 0.05). The maximal effective dose (500 pmol·kg⁻¹·min⁻¹), the largest dose given, decreased emptying by 33% (P < 0.001). Nonlinear regression fitting of the data to the logistic equation gave the following relationship between gastric emptying in milliliters and rCT dose in picomoles per kilogram per minute: gastric emptying = 2 ml/[1 + (rCT/730 pmol·kg⁻¹·min⁻¹)¹.⁹] + 0.9 ml (goodness of fit r² = 0.99). Thus rCT inhibited gastric emptying with a threshold dose between 50 and 170 pmol·kg⁻¹·min⁻¹ and an estimated ED₅₀ of 730 pmol·kg⁻¹·min⁻¹. The ED₅₀ for rCT is significantly larger than that for sCT (730 vs. 1 pmol·kg⁻¹·min⁻¹; F₁,₇₅ = 40, P < 0.0001), amylin (730 vs. 2.9 pmol·kg⁻¹·min⁻¹; F₁,₈₅ = 57, P < 0.0001), rCGRP (730 vs. 130 pmol·kg⁻¹·min⁻¹; F₁,₈₃ = 16, P = 0.01), and rADM (730 vs. 160 pmol·kg⁻¹·min⁻¹).
Fig. 7. Gastric emptying responses to intravenous infusions of rAMY, sCT, rCGRP, rADM, and rCT in 12, 10, 12, 10, and 10 rats, respectively. Eighteen-hour food-deprived rats received a 15-min intravenous infusion of peptide beginning 10 min before gastric instillation of 3 ml of saline containing phenol red. Gastric contents were recovered 5 min later. Individual curves represent nonlinear regression fitting of data to a logistic equation. Resulting best-fit equations were as follows: rAMY: volume emptied (ml) = 1.3 ml/[1 + (rAMY dose/2.9 pmol·kg⁻¹·min⁻¹)¹⁰] + 0.9 ml; sCT: volume emptied (ml) = 2.1 ml/[1 + (sCT dose/1.0 pmol·kg⁻¹·min⁻¹)¹⁰] + 0.9 ml; rCGRP: 2.0 ml/[1 + (rCGRP dose/130 pmol·kg⁻¹·min⁻¹)¹⁰] + 0.9 ml; rADM: 2.0 ml/[1 + (rADM dose/160 pmol·kg⁻¹·min⁻¹)¹⁰] + 0.7 ml; and rCT: 2.0 ml/[1 + (rCT dose/730 pmol·kg⁻¹·min⁻¹)¹⁰] + 0.9 ml. *P < 0.05 and ‡P < 0.001 compared with 0- pmol·kg⁻¹·min⁻¹ dose of the same peptide.

R.1401

AMYRIN-RELATED PEPTIDES

DISCUSSION

The present study demonstrates that when sCT, rCGRP, rADM, and rCT are administered by continuous intravenous infusion to nonfood-deprived rats during the first 3 h of the dark period, sCT, rCGRP, and rADM dose dependently inhibit food intake during the infusion period with estimated ED₅₀ₐₜ of 0.5, 26, and 35 pmol·kg⁻¹·min⁻¹ and maximal inhibitions of 88, 90, and 49%, respectively. In contrast, rCT has no effect at doses up to 100 pmol·kg⁻¹·min⁻¹. We previously demonstrated that under identical experimental conditions, rat amylin inhibits food intake with an estimated ED₅₀ of 8 pmol·kg⁻¹·min⁻¹ and a maximal inhibition of 78%. Thus the rank order of potency for the anorectic effects of these structurally related peptides is sCT > amylin > rCGRP > rADM > rCT. Lutz et al. (29) reported a similar rank order of potency for sCT, rat amylin, and rCT when administered to rats by bolus intraperitoneal injection. In contrast, Morley et al. (32, 33) reported similar potencies for amylin and rCGRP when administered by bolus intraperitoneal injection to rodents. Our work clearly shows that amylin inhibits feeding at lower doses and for a longer duration than rCGRP [present study (Ref. 39)]. These discrepancies may be due to the use of different mouse strains and bolus doses in the studies by Morley et al (32, 33).

Results from our gastric emptying experiments show that sCT, rCGRP, rADM, and rCT dose dependently inhibit gastric emptying of saline during a 5-min period when peptides are administered by continuous intravenous infusion beginning 10 min before the test period. Estimated ED₅₀ values are 1, 130, 160, and 730 pmol·kg⁻¹·min⁻¹ and maximal inhibitions are 60, 66, 60, and 33%, respectively. We previously demonstrated that under identical experimental conditions, amylin inhibits gastric emptying with an ED₅₀ of 3 pmol·kg⁻¹·min⁻¹ and a maximal inhibition of 60%. Thus the rank order of potency for the inhibitory effects of these structurally related peptides on gastric emptying is sCT > amylin > rCGRP = rADM > rCT. Vine et al. (50) previously demonstrated that sCT is more potent than amylin, and ADM is ineffective, in decreasing gastric emptying when peptides are administered by bolus intraperitoneal injection to rats. Others have reported that sCT is significantly more potent than mammalian CTs in decreasing gastric emptying (8).

Factors that inhibit gastric emptying can indirectly reduce food intake by promoting gastric distention. If the anorexia produced by amylin, sCT, CGRP, and ADM is mediated, in part, through their effect on gastric emptying, then it would be important to determine for each peptide whether its potency for inhibiting gastric emptying is greater than or equal to its potency for inhibiting food intake. We found this to be true for amylin and sCT (3 vs. 8 pmol·kg⁻¹·min⁻¹ and 1 vs. 0.5 pmol·kg⁻¹·min⁻¹, respectively) but not for CGRP or ADM (130 vs. 26 pmol·kg⁻¹·min⁻¹ and 160 vs. 35 pmol·kg⁻¹·min⁻¹, respectively). rCT had no effect on food intake at doses up to 100 pmol·kg⁻¹·min⁻¹ and very little effect on gastric emptying at doses up to 500 pmol·kg⁻¹·min⁻¹. Peptides were infused for a significantly longer period of time in feeding experiments, 180 vs. 15 min in gastric emptying experiments, which may have produced higher peptide levels in tissues and thus more potent effects. Nevertheless, our results are consistent with the hypothesis that sCT and amylin produce anorexia, in part, by inhibiting gastric emptying.

The mechanisms of action of peripherally administered amylin, sCT, CGRP, ADM, and CT on food intake and gastric emptying have not been clearly defined. Several lines of evidence suggest that these peptides may act directly within the brain. 1) Central nervous system (CNS) administration of sCT (13, 20), amylin (1, 5, 10, 41), CGRP (20, 22, 38), and ADM (30) appears to be more potent than systemic administration in reducing food intake and gastric emptying. 2) Subdiaphragmatic vagotomy does not attenuate anorectic responses to sCT (34) and amylin (25, 26). 3) Capsaicin denervation of peripheral sensory nerves does not at-
tenuate the anorexic response to amylin (24). 4) Amylin and ADM can penetrate the blood-brain barrier (6, 21). 5) Area postrema lesions block anorexic responses to amylin and CGRP (28). The effect of vagotomy, capsaicin, and lesioning of the area postrema on gastric emptying responses to amylin-related peptides has not been determined. Most of the studies cited above examined the effects of specific neural lesions on anorexic responses to single intraperitoneal doses that are not likely to be physiological. Other studies of similar design suggest that outcomes can vary depending on the dose of agonist administered. For example, subdiaphragmatic vagotomy has been shown to attenuate the pancreatic exocrine response to physiological, but not pharmacological, doses of the gut peptide CCK (37). Thus it remains to be determined whether lesions of putative sites of peptide action attenuate a stimulatory effect of antagonists of endogenous peptide action on food intake and gastric emptying.

Recent evidence suggests that the two cloned receptors, the CT receptor (CTR) and the CT receptor-like receptor (CRLR), form the basis of all receptors for sCT, amylin, CGRP, CT, and ADM (42). Unique receptor phenotypes appear to be determined through modification of these receptors by proteins called receptor activity-modifying proteins (RAMPs). The CRLR appears to be transformed by RAMP1 to a relatively high-affinity receptor for CGRP and by RAMP2 (or RAMP3) to a relatively high-affinity receptor for ADM. Similarly, the CTR is transformed by RAMP1 to a relatively high-affinity receptor for amylin and CGRP and by RAMP2 (or RAMP3) to a relatively high-affinity receptor for amylin. In contrast, sCT binds with high affinity to the CTR whether or not it is associated with an RAMP, and CT has at least a two- to threefold lower affinity than sCT to this receptor (9, 19, 46). CTR-RAMP complexes are therefore likely to be the primary mediators of the inhibitory effects of sCT, amylin, CGRP, and ADM on food intake and gastric emptying. CT has a relatively low affinity to these complexes, which may explain its significantly lower potency in reducing food intake and gastric emptying. Receptor autoradiography indicates widespread distributions of high-affinity binding sites for sCT, amylin, CGRP, and ADM in periphery and brain (7, 18, 43, 48). The anatomic distribution of CTR-RAMP receptor complexes has yet to be determined.

If amylin, sCT, CGRP, and ADM act through a common receptor complex to inhibit food intake, then they would be expected to produce similar effects on meal patterns. Our results demonstrate that amylin and sCT do affect meal patterns similarly. Lower doses reduce only meal size, whereas higher doses reduce both meal size and meal frequency. Neither peptide reduces average eating rate during meals as determined by dividing meal size by meal duration. In contrast, ADM appears to reduce meal frequency and the size of the first meal following infusion onset. CGRP also appears to affect both meal frequency and meal size, although the data are somewhat inconsistent. These results suggest a role for at least two different receptor complexes in mediating the effects of amylin, sCT, CGRP, and ADM on food intake.

The present study demonstrates that sCT produces a prolonged suppression of food intake. Three-hour sCT infusions of 0.3, 1, 3, and 10 pmol·kg\(^{-1}\)·min\(^{-1}\) decreased 17-h food intake by 8, 20, 55, and 85%, respectively. Other studies also demonstrated prolonged actions of exogenous sCT on food intake (29) and gastric emptying (8). This unique characteristic of sCT appears to be dependent on its ability to irreversibly bind to the active state of the most common variant of the CTR (16).

CNS administration of ADM has been reported to inhibit food intake and gastric emptying in rats (30, 45). The present study is the first to demonstrate that peripherally administered ADM also reduces food intake and gastric emptying. ADM is a 52-amino acid peptide (rADM has 50 amino acids) that is expressed in virtually every tissue of the body with the possible exception of the thyroid and thymus (18). ADM has a range of biological actions that include vasodilation, cell growth, regulation of hormone secretion, natriuresis, and antimicrobial effects. A growing body of evidence suggests that endogenous ADM does not act like a conventional hormone but rather like an autocrine, paracrine, or neurocrine factor. In the present study, the threshold intravenous doses of rADM for inhibition of feeding (between 10 and 30 pmol·kg\(^{-1}\)·min\(^{-1}\)) and gastric emptying (between 50 and 170 pmol·kg\(^{-1}\)·min\(^{-1}\)) are significantly larger than ADM doses shown previously to produce hemodynamic and natriuretic effects in rats and humans [1 to 6 pmol·kg\(^{-1}\)·min\(^{-1}\) (23, 49)]. It remains to be determined whether blockade of endogenous ADM action affects food intake or gastric emptying.

An important role for endogenous amylin in the physiological control of food intake and gastric emptying remains to be established. A growing body of evidence suggests that amylin action may be important. In rodents, meal-induced increases in plasma amylin appear to be sufficient to inhibit both food intake and gastric emptying (3, 39). Blockade of endogenous amylin action has also been reported to increase food intake, body weight, and adiposity (2, 14, 40). The mechanism by which food intake stimulates amylin release and the source (pancreas, gut, and brain), mode (endocrine, paracrine, and neurocrine), and site of action (brain, visceral sensory nerves, stomach, and liver) of endogenous amylin to decrease food intake and gastric emptying remain to be determined.

There is little information regarding possible physiological roles for endogenous CGRP and CT in control of food intake and gastric emptying. CGRP-producing cells are widely distributed within the central and peripheral nervous systems (11). CGRP receptor blockade has been reported both to stimulate (27) and to
have no effect (2) on food intake. CT appears to be produced primarily by endocrine cells in the thyroid (4). CT is thought to act primarily as a hormone to increase bone resorption and renal Ca\textsuperscript{2+} excretion in response to a rise in plasma Ca\textsuperscript{2+} levels. Our results suggest that physiological CT doses are not sufficient to inhibit food intake or gastric emptying. Recently, a sCT-like peptide was isolated from rat diencephalon (17). Thus the possibility exists that an endogenous sCT-like peptide may act as a neurotransmitter or neuromodulator in the brain to inhibit food intake or gastric emptying.

In conclusion, we previously demonstrated that amylin inhibits gastric emptying and food intake in rats with a similar potency (ED\textsubscript{50} of 8 and 3 pmol·kg\textsuperscript{-1}·min\textsuperscript{-1}, respectively). In this study of identical design, only sCT inhibited gastric emptying and food intake with a similar potency (ED\textsubscript{50} of 0.5 and 1 pmol·kg\textsuperscript{-1}·min\textsuperscript{-1}, respectively). Our results also show that amylin and sCT inhibit gastric emptying and food intake with similar efficacies, and they produce similar changes in meal patterns. Previous studies suggest that amylin and sCT act by the same receptor system to inhibit food intake. Together, these results suggest that amylin and sCT may act by a common mechanism to decrease food intake, which includes inhibition of gastric emptying.

REFERENCES


