Comparative effects of amylin and cholecystokinin on food intake and gastric emptying in rats

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Veterans Affairs Medical Center, Omaha 68105; Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, Nebraska 68178; and Arvid Wretlind Laboratory for Metabolic Research, Department of Surgery, Karolinska Institutet at Huddinge University Hospital, S-14186 Stockholm, Sweden

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Reidelberger, Roger D., Urban Arnebo, Lars Granqvist, and Johan Permert. Comparative effects of amylin and cholecystokinin on food intake and gastric emptying in rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R605–R611, 2001.—CCK is a physiological inhibitor of gastric emptying and food intake. The pancreatic peptide amylin exerts similar actions, yet its physiological importance is uncertain. Objectives were to compare the dose-dependent effects of intravenous infusion of amylin and CCK-8 on gastric emptying and food intake in rats, and to assess whether physiological doses of amylin are effective. Amylin and CCK-8 inhibited gastric emptying with mean effective doses (ED50s) of 3 and 35 pmol/kg min1, respectively. Amylin and CCK-8 inhibited food intake with ED50s of 8 and 14 pmol/kg min1, respectively. The minimal effective amylin dose for each effect was 1 pmol/kg min1. Our previous work suggests that this dose increases plasma amylin by an amount comparable to that produced by a meal. 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. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
physiological inhibitors of gastric emptying (35). It remains to be determined whether postprandial plasma levels of amylin are sufficient to decrease gastric emptying in normal subjects.

Factors that produce gastric distension through inhibition of gastric emptying can reduce food intake. Thus it is reasonable to postulate that amylin may inhibit food intake in part by inhibiting gastric emptying. This idea is further supported by a recent study showing that amylin is a much less potent inhibitor of sham feeding (3), a condition in which ingested liquid food is not allowed to accumulate in the stomach. It remains to be determined whether amylin is at least as potent in inhibiting gastric emptying as in reducing food intake.

The aims of the present study were to 1) determine the dose-dependent effects of intravenous infusion of amylin on gastric emptying and food intake, 2) assess whether physiological doses of amylin are effective, and 3) compare amylin’s potency and efficacy in reducing gastric emptying and food intake with those of the gut-brain peptide CCK, an established physiological inhibitor of these events (25, 26).

METHODS

Experimental design. Five experiments were performed using five different groups of rats. The first and second experiments determined the dose-dependent effects of 3-h intravenous infusions of amylin and CCK-8 at dark onset on food intake and meal patterns in non-food-deprived rats. The third, fourth, and fifth experiments determined the dose-dependent effects of 15-min intravenous infusions of amylin and CCK-8 on gastric emptying of a 3-ml saline load during a 5-min period in unanesthetized rats. The Animal Studies Subcommittee of the Omaha Veterans Affairs Medical Center approved the experimental protocol.

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 off at 17:00).

Surgical procedures. The procedures for implantation of a jugular vein catheter for peptide infusions were described previously (33). Catheters were filled with heparinized saline (40 units/ml), plugged with stainless steel wire, and flushed with 0.5 ml of heparinized saline every other day to maintain patency. In animals used for feeding studies, jugular vein catheters were connected to 40-cm lengths of tubing passed through a protective spring coil connected between a light-weight saddle (IITC, Woodland Hills, CA) worn by the rat and an infusion swivel. The procedures for implantation of a gastric cannula for instillation of saline and retrieval of gastric contents were described previously (18, 27).

Effects of intravenous infusion of amylin on food intake and meal patterns. Excess amounts of fresh food were provided each day at 1400. Animals were adapted to experimental conditions for at least 1 wk before the start of experiments. Non-food-deprived rats received a 3-h jugular vein infusion (3 ml/h) of amylin (Peninsula Laboratories; 0, 1, 3, 10, 30, or 100 pmol·kg⁻¹·min⁻¹ in 0.15 M NaCl, 0.1% BSA) beginning 15 min before dark onset (1700). Food intake during the first 17 h after onset of infusion was determined, as described previously, from continuous computer recordings of changes in food bowl weight (33). Each rat (n = 14) received each dose of amylin in random order at 48-h intervals. Amylin was administered via a syringe infusion pump (model 22, Harvard Apparatus, South Natick, MA); pumps were turned on and off by a computer program.

Effects of intravenous infusion of CCK-8 on food intake and meal patterns. Experimental procedures were identical to those used in the preceding experiment. Food intake was measured in non-food-deprived rats (n = 10) receiving, in random order at dark onset on different days, 3-h jugular vein infusions of different doses of CCK-8 (Peninsula Laboratories; 0, 5, 17, 50, and 170 pmol·kg⁻¹·min⁻¹ in 0.15 M NaCl, 0.1% BSA).

Effects of intravenous infusion of amylin on gastric emptying. The experimental design was similar to that described previously (17). 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 amylin (0, 1.7, 17, or 170 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 was 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 = 12) received each dose of amylin in random order at intervals of at least 48 h.

A subsequent experiment was performed to determine the effects of infusion of lower amylin doses on gastric emptying. Each rat in one group (n = 11) received vehicle and a 0.5 pmol·kg⁻¹·min⁻¹ dose of amylin in random order; each rat in another group (n = 11) received vehicle and a 1 pmol·kg⁻¹·min⁻¹ dose of amylin in random order.

Effects of intravenous infusion of CCK-8 on gastric emptying. Experimental procedures were identical to those used in the preceding experiment. Gastric emptying of the 3-ml saline load was measured in rats (n = 8) receiving, in random order on different days, jugular vein infusions of different doses of CCK-8 (0, 1.7, 50, and 170 pmol·kg⁻¹·min⁻¹ in 0.15 M NaCl, 0.1% BSA).

Statistical analyses. Values are presented as group means ± SE. In the first two experiments we separately evaluated the dose-dependent effects of jugular vein infusions of amylin (or CCK-8) 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), and mean meal parameters across the 3-h infusion period (number of meals, meal size, postmeal interval, and satiety ratio) by repeated-measures ANOVA, with amylin (or CCK-8) dose and time being the within-group factors. In the third, fourth, and fifth experiments, the dose-dependent effects of jugular vein infusions of amylin (or CCK-8) on volume of saline emptied from the stomach in 5 min were evaluated separately using a repeated-measures ANOVA with amylin (or CCK-8) 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. A general non-linear, least-squares curve fitting method was used as previously described (12) to fit the dose-response data for the effects of amylin and CCK-8 on food intake and gastric emptying to the following logistic equation: \[ Y = \frac{(a - d)X}{(1 + (X/c)^b)} + d, \] where \( Y \) is the response; \( X \), the dose; a, the
response for 0 dose; d, the response for infinite dose; c, the 
ED_{50} (dose producing response halfway between a and d); 
and b, a slope factor that determines steepness of the curve. 
The method of Meddings et al. (21) was used to compare 
amylin and CCK-8 ED_{50} and maximal responses.

RESULTS

Effects of intravenous infusion of amylin on food 
intake. Amylin infusion for 3 h at dark onset dose depend-
ently inhibited cumulative food intake across the 17-h test period (Fig. 1). The minimal effective dose (1 pmol·kg^{-1}·min^{-1}) inhibited cumulative intake at 1 and 2 h by 37% (P < 0.05) and 26% (P < 0.05), respectively. The maximal effective dose (100 pmol·kg^{-1}·min^{-1}) decreased cumulative intake across the 17-h period, with a peak inhibition of 78% at 3 h (P < 0.001), decreasing to 10% by 17 h (P < 0.01). Figure 2 shows the dose-response effects of amylin on 3-h food intake. Nonlinear regression fitting of the data to the logistic equation gave the following relationship between food intake in grams and amylin dose in picomoles per kilogram per minute: food intake = 5.5 g/[1 + (amylin/8.0 pmol·kg^{-1}·min^{-1})^{1.4}] + 1.4 g (goodness of fit r^2 = 0.82). The estimated ED_{50} was 8 pmol·kg^{-1}·min^{-1}.

Effects of intravenous infusion of CCK-8 on food 
intake. CCK-8 infusion for 3 h at dark onset dose depend-
ently inhibited cumulative food intake across the 17-h test period (Fig. 3). The minimal effective dose (17 pmol·kg^{-1}·min^{-1}) inhibited cumulative intake at 2 and 3 h by 44% (P < 0.05) and 37% (P < 0.05), respectively. The two highest doses (50 and 170 pmol·kg^{-1}·min^{-1}) produced a similar maximal inhibition at 3 h of 69% (P < 0.001) and 63% (P < 0.001), respectively, which decreased to 12% (P < 0.05) and 17% (P < 0.01) by 17 h. Figure 2 shows the dose-
response effects of CCK-8 on 3-h food intake. Nonlinear regression fitting of the data to the logistic equation gave the following relationship between food intake in grams and CCK-8 dose in picomoles per kilogram per minute: food intake = 3.6 g/[1 + (CCK/13.7 pmol·kg^{-1}·min^{-1})^{1.7}] + 1.6 g (goodness of fit r^2 = 0.75). The estimated ED_{50} was 13.7 pmol·kg^{-1}·min^{-1}. The ED_{50} and maximal response for CCK-8 were not differ-
ent from those for amylin (F_{1,127} = 0.44, P = 0.51 and 
F_{1,127} = 0.02, P = 0.89, respectively).

Effects of intravenous infusion of amylin and CCK-8 
on meal patterns. Amylin and CCK-8 had similar dose-
dependent effects on meal patterns (Tables 1 and 2). Lower doses of each peptide (≤10 pmol·kg^{-1}·min^{-1}) reduced food intake primarily by decreasing mean meal size during the 3-h infusion period. Higher doses also increased the latency to the first meal and reduced meal frequency. CCK-8, but not amylin, decreased the average rate at which meals were consumed during the infusion period.

Effects of intravenous infusion of amylin on gastric 
emptying. Amylin dose dependently reduced the vol-
ume of saline emptied from the stomach during the 5-min test period (Fig. 4; F_{3,33} = 30.7, P < 0.001). The minimal effective dose (1.7 pmol·kg^{-1}·min^{-1}), which was the lowest dose given in the initial experiment, decreased empying by 18% (P < 0.05). The maximal effective dose (170 pmol·kg^{-1}·min^{-1}) decreased empying 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 amylin in picomoles per kilogram per minute: gastric

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**Fig. 1.** Food intake response to intravenous infusion of amylin in 14 rats. Non-
food-deprived rats received a 3-h intrave-
nous infusion of amylin for 3 h beginning 
15 min before dark onset. *P < 0.05, †P < 
0.01, or ‡P < 0.001 compared with 0 
pmol·kg^{-1}·min^{-1} dose of amylin.
emptying = 1.3 ml/[1 + (amylin/2.9 pmol·kg⁻¹·min⁻¹)¹.0] + 0.9 ml (goodness of fit r² = 0.95). The estimated ED₅₀ was 2.9 pmol·kg⁻¹·min⁻¹.

A second experiment examined the effects of lower doses of amylin (0.5 and 1 pmol·kg⁻¹·min⁻¹) on gastric emptying. The 0.5 pmol·kg⁻¹·min⁻¹ dose had no significant effect on emptying [2.3 ± 0.1 ml vs. control 2.3 ± 0.1 ml; t(10) = 0.1, P > 0.05]; the 1 pmol·kg⁻¹·min⁻¹ dose significantly reduced emptying by 18% [2.0 ± 0.2 ml vs. control 2.3 ± 0.1 ml; t(10) = 2.1, P < 0.05].

Effects of intravenous infusion of CCK-8 on gastric emptying. CCK-8 dose dependently reduced the volume of saline emptied from the stomach during the 5-min test period (Fig. 4; F₃,₂₁ = 78, P < 0.001). The minimal effective dose (1.7 pmol·kg⁻¹·min⁻¹) decreased emptying by 13% (P < 0.01). The maximal effective dose (170
FEEDING AND GASTRIC EMPTYING RESPONSES TO AMYLIN AND CCK

Table 1. Effects of intravenous infusion of amylin on feeding patterns

<table>
<thead>
<tr>
<th>Dose (pmol·kg⁻¹·min⁻¹)</th>
<th>Latency</th>
<th>MS</th>
<th>PMI</th>
<th>SR</th>
<th>First Meal</th>
<th>All Meals During the 3-h Infusion Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47.7 ± 8.7</td>
<td>2.4 ± 0.3</td>
<td>50.8 ± 18.3</td>
<td>21.4 ± 5.2</td>
<td>2.9 ± 0.4</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>1</td>
<td>45.1 ± 10.7</td>
<td>1.8 ± 0.2</td>
<td>45.4 ± 11.4</td>
<td>27.8 ± 7.9</td>
<td>3.4 ± 0.3</td>
<td>1.9 ± 0.1*</td>
</tr>
<tr>
<td>3</td>
<td>36.6 ± 7.8</td>
<td>1.8 ± 0.3</td>
<td>55.4 ± 13.0</td>
<td>30.4 ± 5.8</td>
<td>3.1 ± 0.3</td>
<td>1.9 ± 0.2*</td>
</tr>
<tr>
<td>10</td>
<td>73.1 ± 15.5</td>
<td>1.5 ± 0.3†</td>
<td>62.1 ± 16.8</td>
<td>53.3 ± 15.3</td>
<td>2.3 ± 0.5</td>
<td>1.6 ± 0.2†</td>
</tr>
<tr>
<td>30</td>
<td>116.4 ± 21.0†</td>
<td>1.8 ± 0.3</td>
<td>76.3 ± 17.2</td>
<td>74.4 ± 30.9*</td>
<td>1.4 ± 0.3</td>
<td>1.6 ± 0.2†</td>
</tr>
<tr>
<td>100</td>
<td>131.4 ± 29.0‡</td>
<td>1.3 ± 0.3‡</td>
<td>71.8 ± 21.0</td>
<td>103.8 ± 35.7*</td>
<td>1.4 ± 0.3</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Nonfasted rats received a 3-h intravenous infusion of amylin beginning 15 min before dark onset, and food intake and meal patterns were determined from continuous computer recordings of changes in food bowl weight. *P < 0.05, †P < 0.01, ‡P < 0.001 compared with 0 pmol·kg⁻¹·min⁻¹ dose of amylin.

Table 2. Effects of intravenous infusion of CCK-8 on feeding patterns

<table>
<thead>
<tr>
<th>Dose (pmol·kg⁻¹·min⁻¹)</th>
<th>Latency</th>
<th>MS</th>
<th>PMI</th>
<th>SR</th>
<th>First Meal</th>
<th>All Meals During the 3-h Infusion Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>53.4 ± 12.1</td>
<td>1.3 ± 0.2</td>
<td>47.8 ± 10.0</td>
<td>55.5 ± 20.4</td>
<td>2.6 ± 0.3</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>74.0 ± 17.1</td>
<td>1.9 ± 0.3</td>
<td>25.0 ± 9.8</td>
<td>13.7 ± 3.8*</td>
<td>2.8 ± 0.3</td>
<td>1.6 ± 0.2*</td>
</tr>
<tr>
<td>17</td>
<td>85.9 ± 21.3</td>
<td>1.5 ± 0.3</td>
<td>52.4 ± 12.2</td>
<td>40.7 ± 9.5</td>
<td>2.1 ± 0.5</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>50</td>
<td>105.5 ± 23.7*</td>
<td>1.4 ± 0.3</td>
<td>61.1 ± 15.7</td>
<td>73.9 ± 25.1</td>
<td>1.6 ± 0.4*</td>
<td>1.0 ± 0.2*</td>
</tr>
<tr>
<td>170</td>
<td>189.2 ± 32.2‡</td>
<td>2.1 ± 0.4*</td>
<td>33.9 ± 8.9</td>
<td>22.1 ± 8.5</td>
<td>1.1 ± 0.6*</td>
<td>1.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Nonfasted rats received a 3-h intravenous infusion of CCK-8 beginning 15 min before dark onset, and food intake and meal patterns were determined from continuous computer recordings of changes in food bowl weight. *P < 0.05, †P < 0.01, ‡P < 0.001 compared with 0 pmol·kg⁻¹·min⁻¹ dose of CCK-8.

DISCUSSION

If pancreatic amylin acts as a hormonal signal to the brain to reduce gastric emptying and food intake, then it would be important to determine whether intravenously infused amylin decreases gastric emptying and food intake at physiological doses that reproduce meal-induced increases in plasma amylin. Intraperitoneal administration is not an appropriate method to use, because amylin administered in this manner could act locally to produce a response before being absorbed into the circulation, which would preclude establishing a meaningful correlation between the amylin-induced response and a specific increase in plasma amylin. Aims of the present study were to 1) determine the dose-dependent effects of intravenous infusion of amylin on gastric emptying and food intake; 2) assess whether physiological doses of amylin are effective; and 3) compare amylin’s potency and efficacy in reducing gastric emptying and food intake with those of the gut-brain peptide CCK, an established physiological inhibitor of these events.

Amylin and CCK-8 each dose dependently decreased gastric emptying of saline during a 5-min period when the peptides were administered by continuous intravenous infusion beginning 10 min before the test period. Minimal effective doses were 1 and 1.7 pmol·kg⁻¹·min⁻¹, ED₅₀ were 3 and 35 pmol·kg⁻¹·min⁻¹, and maximal inhibitions were 60 and 65%, respectively. In feeding experiments, amylin and CCK-8 each dose dependently decreased food intake when administered to non-food-deprived rats by intravenous infusion for 3 h beginning 15 min before dark onset. Minimal effective doses were 1 and 17 pmol·kg⁻¹·min⁻¹, ED₅₀ were 8 and 14 pmol·kg⁻¹·min⁻¹, and maximal suppressions were 78 and 69%, respectively. Amylin and CCK-8 had similar dose-dependent effects on meal patterns during the infusion period: lower doses (<10 pmol·kg⁻¹·h⁻¹) reduced food intake primarily by decreasing mean meal size; higher doses also increased the latency to the first meal and reduced meal frequency. These results suggest that amylin inhibits gastric emptying and food intake with a similar potency and efficacy and that amylin is at least as potent and efficacious as CCK in producing these effects.

In our earlier study using the same experimental model (2), several small intravenous doses of amylin were administered to define the threshold amylin dose for suppression of feeding. The threshold was determined to be between 1 and 3 pmol·kg⁻¹·min⁻¹ when amylin was infused intravenously for 4 h beginning 1 h before dark onset. In the present study amylin was
infused intravenously for 3 h beginning only 15 min before dark onset, and the minimal effective dose was 1 pmol·kg⁻¹·min⁻¹. This was also the minimal effective dose observed in the present study for amylin-induced inhibition of gastric emptying. Our previous work suggests that this dose increases plasma amylin in rats by ~10 pM, which is comparable to that produced by a chow meal (2). Together, these results suggest that postprandial plasma levels of amylin are sufficient to inhibit gastric emptying and food intake. Other studies suggest that amylin may interact synergistically with CCK (7) and insulin (30) to produce satiety.

A convergence of evidence indicates that the gut-brain peptide CCK is an important physiological inhibitor of gastric emptying (25) and food intake (26). In contrast to amylin, which appears to act as a bloodborne signal from pancreas to brain, CCK appears to act locally within the small intestine to stimulate a vagally mediated mechanism. No previous study has compared the potencies and efficacies of amylin and CCK-8 in reducing gastric emptying and food intake. Ideally, such a study would compare the effects of similar increases in concentration of amylin in plasma and CCK in intestinal extracellular fluid. Because plasma CCK is more easily measurable than CCK in intestinal extracellular fluid and because a change in plasma CCK concentration likely produces a similar change in intestinal extracellular CCK concentration, a more practical approach would be to compare the effects of identical increases in plasma concentrations of amylin and CCK-8 above baseline values on gastric emptying and food intake. Previous work suggests that when amylin and CCK-8 are administered to rats by continuous intravenous infusion, similar doses produce similar increases in plasma peptide concentration (2, 8, 13). For each peptide, steady-state plasma levels appear to increase by ~10 pM for each 1 pmol·kg⁻¹·min⁻¹ increase in dose administered. Thus, in the present study, graded doses of amylin and CCK-8 were administered by continuous intravenous infusion, and potencies (ED₅₀) were determined using an established mathematical method (12) to fit dose-response data to the following logistic equation: Y = (a - d)/[1 + (X/c)^b] + d, where Y is the response; X, the peptide dose in pmol·kg⁻¹·min⁻¹; a, the response at 0 dose; d, the response for infinite dose; c, the ED₅₀ (dose producing response halfway between a and d); and b, a slope factor that determines steepness of the curve.

Amylin and CCK-8 reduced food intake with a similar potency and efficacy. In contrast, amylin was 10 times more potent than CCK-8, yet equally efficacious in reducing gastric emptying. In an earlier study, amylin was reported to be 10 times more potent than CCK-8 in reducing gastric emptying in mice when administered by intraperitoneal injection (35). These results lend further support to the argument that amylin is an important physiological regulator of gastric emptying and food intake.

In the present study, amylin and CCK-8 produced similar dose-dependent effects on meal patterns when administered during the early dark period to non-food-deprived rats consuming ground rat chow. Lower doses (~10 pmol·kg⁻¹·min⁻¹) reduced food intake primarily by decreasing meal size; higher doses also increased first meal latency and reduced meal frequency. Because the same low “physiological” dose of amylin (1 pmol·kg⁻¹·min⁻¹) reduced gastric emptying of saline as well as meal size, we propose that endogenous amylin contributes to the control of meal size in part through modulation of gastric emptying. This idea is further supported by a recent study showing that amy-
lin is a much less potent inhibitor of sham feeding (3), a condition in which ingested liquid food is not allowed to accumulate in the stomach. It remains to be determined whether physiological doses of amylin inhibit gastric emptying of typical meals of solid food, whether amylin inhibits gastric emptying during, as well as after, gastric filling, and whether blockade of endogenous amylin action increases gastric emptying, meal size, and food intake.

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