Amylin receptor blockade stimulates food intake in rats

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D. David Smith,2 Courtney S. Schaffert,1 and Johan Perment3
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Reidelberger, Roger D., Alvin C. Haver, Urban Arnello, D. David Smith, Courtney S. Schaffert, and Johan Perment. Amylin receptor blockade stimulates food intake in rats. Am J Physiol Regul Integr Comp Physiol 287: R568–R574, 2004. First published May 6, 2004; 10.1152/ajpregu.00213.2004.—Amylin is postulated to act as a hormonal signal from the pancreas to the brain to inhibit food intake and regulate energy reserves. Amylin potently reduces food intake, body weight, and adiposity when administered systemically or into the brain. Whether selective blockade of endogenous amylin action increases food intake and adiposity remains to be clearly established. In the present study, the amylin receptor antagonist acetyl-[Asn30, Tyr32] sCT-(8–32) (AC187) was used to assess whether action of endogenous amylin is essential for normal satiation to occur. Non-food-deprived rats received a 3- to 4-h intravenous infusion of AC187 (60–2,000 pmol·kg−1·min−1), either alone or coadministered with a 3-h intravenous infusion of amylin (2.5 or 5 pmol·kg−1·min−1) or a 2-h intragastric infusion of an elemental liquid diet (4 kcal/h). Infusions began just before dark onset. Food intake and meal patterns during the first 4 h of the dark period were determined from continuous computer recordings of changes in food bowl weight. Amylin inhibited food intake by ~50%, and AC187 attenuated this response by ~50%. AC187 dose-dependently stimulated food intake (maximal increases from 76 to 171%), whether administered alone or with an intragastric infusion of liquid diet. Amylin reduced mean meal size and meal frequency, AC187 attenuated these responses, and AC187 administration alone increased mean meal size and meal frequency. These results support the hypothesis that endogenous amylin plays an essential role in reducing meal size and increasing the postmeal interval of satiety.

receptor antagonist; AC187; satiety; islet amyloid polypeptide

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 (9, 37). Amylin has also been detected in gut endocrine cells (23) and visceral sensory neurons (24) and throughout the brain (34). Exogenous amylin potently reduces food intake (2, 4, 29), body weight (2), adiposity (32), gastric emptying (8, 29), and gastric acid secretion (11) when administered systemically or into the brain.

CGRP, calcitonin (CT), and adrenomedullin (AM), together with amylin, form a family of structurally related peptides with overlapping biological actions. The teleost peptide salmon CT (sCT) appears to be significantly more potent than either amylin (20, 30) or mammalian CTs (7, 20, 30, 35) in decreasing food intake and gastric emptying. An sCT-like peptide has been isolated from rat diencephalon (12). Our laboratory recently compared the effects of continuous intravenous infusion of amylin, sCT, CGRP, AM, and CT during the first 3 h of the dark period on food intake in non-food-deprived rats (29, 30). sCT, amylin, CGRP, and AM dose-dependently inhibited food intake during the infusion period, with estimated half-maximal effective doses of 0.5, 8, 26, and 35 pmol·kg−1·min−1 and maximal inhibitions of 88, 78, 90, and 49%, respectively. In contrast, CT had no effect at doses up to 100 pmol·kg−1·min−1.

Amylin-related peptides share a predicted α-helical structure from amino acid residues 8–18, an amidated COOH-terminal aromatic residue, and an NH2-terminal 6–7 amino acid ring structure linked by a disulfide bridge. These structural characteristics are required for agonist activity (21). Amylin and CGRP share the highest sequence homology of ~50% (25, 36). Numerous studies have now demonstrated that compounds without the common NH2-terminal ring structure, CGRP-(8–37), amylin-(8–37), sCT-(8–32), and acetyl-[Asn30, Tyr32] sCT-(8–32) (AC187), are selective receptor antagonists (27, 28). The amino acid substitutions noted for AC187 confer on the sCT molecule a tertiary structure that is more similar to amylin. These compounds are proving useful not only in demonstrating the existence of multiple receptor subtypes for the homologous peptides, but also in defining their physiological actions.

Distinct receptors for amylin, CT, CGRP, sCT, and AM have been characterized pharmacologically in different tissues from different species. The receptors appear to be related functionally because the various peptides cross-react in binding studies and have overlapping biological actions. Recent evidence suggests that the two cloned receptors, the CT receptor and the CT receptor-like receptor (CL), form the basis of all of the receptors for this peptide family (22, 28, 40). Distinct peptide receptor phenotypes appear to be determined through modification of these receptors by one of three proteins called receptor activity modifying proteins (RAMPs). CL appears to be transformed by RAMP1 to a CGRP receptor, which is selectively antagonized by CGRP-(8–37) and by RAMP2 (or RAMP3) to an AM receptor. The most common variant of the CT receptor appears to be transformed by RAMP1 to a relatively high-affinity receptor for sCT, amylin, and CGRP, and by RAMP3 to a relatively high-affinity receptor for sCT and amylin. In the absence of RAMP transformation, the CT receptor is a high-affinity receptor for CT. sCT-(8–32) and AC187 appear to be potent, selective antagonists of the CT receptor, whether or not it is transformed by a RAMP.

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If action of endogenous amylin is necessary for normal satiation to occur, then pharmacological blockade of the CT/ RAMP1 and CT/RAMP3 receptor complexes with AC187 should increase food intake. Rushing et al. (31) demonstrated that acute and chronic intracerebroventricular (ICV) administration of AC187 increases food intake in nonfasted rats. In contrast, Lutz et al. (18) reported that ICV injection of CGRP-(8–37), but not AC187 or amylin-(8–37), stimulates feeding in food-deprived rats. However, only single doses of antagonists were employed in this study, and the ability of these doses to antagonize anorexic responses to ICV injection of amylin or CGRP was not assessed. Lutz et al. (17, 20) also observed no effect of bolus intraperitoneal injection of AC187 or CGRP-(8–37) on food intake in 24-h food-deprived rats. If AC187 has a relatively short half-life, bolus dosing of this compound may not be sufficient to attenuate the satiety effects of a prolonged meal-induced secretion of amylin. AC187 also may not be able to stimulate food intake in previously food-deprived animals that already express a significant drive to eat.

The aim of this study was to use the amylin receptor antagonist AC187 to assess whether action of endogenous amylin is essential for normal satiation to occur. Initial experiments determined the effects of short-term, continuous intravenous infusion of AC187 on food intake, and on the anorexia produced by intravenous infusion of amylin, during the early dark period in non-food-deprived rats. Subsequent experiments of similar design determined the dose-dependent effects of intravenous infusion of AC187 on food intake in rats receiving an intragastric infusion of an elemental liquid diet. We previously determined that CCK-receptor antagonists produce a greater stimulation of food intake in rats receiving an intragastric infusion of peptone. We reasoned that intragastric diet administration may reduce the "ceiling effect" that likely limits the expression of an orexigenic response to AC187 in hungry rats. Some of the work presented in this paper was previously reported (3).

METHODS

Subjects. Male rats (Sasco Sprague-Dawley, Charles Rivers Laboratories, Kingston, NY; 350 g at the start of the study) were housed individually in hanging wire-mesh cages in a temperature-controlled room with a 12:12-h light-dark cycle (lights off at 1600). The animals were provided rat chow (Purina no. 5001, 3.3 kcal/g) and water ad libitum. The Animal Studies Subcommittee of the Omaha Veterans Affairs Medical Center approved the experimental protocol. Animal experimentation was conducted in conformity with the guiding principles of the American Physiological Society for research involving animals and human beings (1).

Surgical procedures. The procedures for implantation of a gastric cannula for liquid diet infusion and a jugular vein catheter for administration of amylin and amylin receptor antagonist were described previously (38). Gastric and jugular vein cannulas were filled with water and heparinized saline (40 U/ml), respectively, plugged with stainless steel wire, and flushed every other day to maintain patency. Cannulas were connected to 40-cm lengths of tubing passed through a protective spring coil, connected between a lightweight saddle (IITC, Woodland Hills, CA) worn by the rat and either a single- or double-channel infusion swivel (Instech Laboratories, Plymouth Meeting, PA). The double-channel swivel permitted simultaneous administration of amylin antagonist intravenously and liquid diet intragastrically.

Synthesis and purification of AC187. Polyacrylic-polystyrene glycol-polystyrene resin for solid-phase peptide synthesis and N-(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridino-1-ylmethylene)-N-methylmethanaminium hexafluorophosphate N-oxide (HATU) were purchased from Applied Biosystems (Foster City, CA). Protected amino acid derivatives were purchased from Chem-Impex International (Wood Dale, IL). Dimethylformamide (DMF), piperdine, trifluoroacetic acid (TFA), and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA). Thiainosine, anisole, ethanethiol, and tert-butyl methyl ether were purchased from Aldrich (St. Louis, MO).

AC187 was assembled by continuous flow-solid-phase methodology on the polycrylic-polystyrene glycol-polystyrene support at a 0.5-mmol scale by using a Pioneer Peptide Synthesizer (Foster City, CA). α-Amino groups were protected with the fluorenylmethoxycarbonyl (Fmoc) group. Side chains were protected with the trityl group for Gln, His, and Asn; tert-butyl group for Ser, G1u, Thr, and Tyr; tert-butylxocarbonyl group for Lys; and pentamethyldihydrobenzo-furanosulfonyl group for Arg. After removal of the Fmoc group from the resin with 20% piperdine/DMF (vol/vol), HATU-activated Fmoc-Tyr(tBu)-OH in DMF was coupled to the resin. This process was repeated with each amino acid derivative. After assembly, the peptide resin was washed with diethyl ether and dried under vacuum. Cleavage of peptide from the resin and removal of side chain protecting groups were accomplished in a 50-nl mixture of TFA, thioanisole, ethanethiol, and anisole (9:0.5:0.3:0.2, vol/vol/vol/vol). After stirring at room temperature, the peptide was precipitated by addition of cold tert-butyl methyl ether. The peptide/resin was filtered. The peptide was dissolved in TFA, reprecipitated in cold tert-butyl methyl ether, isolated by filtration, and lyophilized.

Purification of AC187 was accomplished by reverse-phase HPLC on a Waters (Milford, MA) model 600 HPLC system. The crude peptide was dissolved in 77% solvent A (0.1% TFA/water) and 23% solvent B (0.095% TFA/acetonitrile) and subjected to a gradient of 0.5-mmol scale by using a Pioneer Peptide Synthesizer (Foster City, CA). α-Amino groups were protected with the fluorenylmethoxycarbonyl (Fmoc) group. Side chains were protected with the trityl group for Gln, His, and Asn; tert-butyl group for Ser, G1u, Thr, and Tyr; tert-butylxocarbonyl group for Lys; and pentamethyldihydrobenzo-furanosulfonyl group for Arg. After removal of the Fmoc group from the resin with 20% piperdine/DMF (vol/vol), HATU-activated Fmoc-Tyr(tBu)-OH in DMF was coupled to the resin. This process was repeated with each amino acid derivative. After assembly, the peptide resin was washed with diethyl ether and dried under vacuum. Cleavage of peptide from the resin and removal of side chain protecting groups were accomplished in a 50-nl mixture of TFA, thioanisole, ethanethiol, and anisole (9:0.5:0.3:0.2, vol/vol/vol/vol). After stirring at room temperature, the peptide was precipitated by addition of cold tert-butyl methyl ether. The peptide/resin was filtered. The peptide was dissolved in TFA, reprecipitated in cold tert-butyl methyl ether, isolated by filtration, and lyophilized.

Purification of AC187 was accomplished by reverse-phase HPLC on a Waters (Milford, MA) model 600 HPLC system. The crude peptide was dissolved in 77% solvent A (0.1% TFA/water) and 23% solvent B (0.095% TFA/acetonitrile) and subjected to a gradient of 23% B to 35% B over 50 min on a semipreparative Vydac (Hesperia, CA) C18 column (10 × 250 mm). Flow rate was 4 ml/min, and the peptide was detected by UV absorbance at 230 nm. Fractions containing the pure peptide were collected and lyophilized.

Proof of AC187 structure was provided by matrix-assisted laser desorption ionization time-of-flight mass spectrometry on a CIPHERGEN PBS II (Fremont, CA) spectrometer and coelution with a known sample purchased from Peninsula Laboratories (San Carlos, CA).

Effects of AC187 on food intake and amylin-induced anorexia. Two experiments were performed. The first experiment determined the effects of continuous intravenous infusion of 500 pmol·kg⁻¹·min⁻¹ of AC187 on feeding and on the anorectic response to intravenous infusion of 5 pmol·kg⁻¹·min⁻¹ of amylin. The half-life of AC187 has not been previously determined. AC187 was, therefore, administered by continuous infusion to ensure a sustained blockade of amylin receptors during the test period. The 5 pmol·kg⁻¹·min⁻¹ dose of amylin was chosen because it reliably inhibits food intake by an amount that is comparable to that produced by the mean effective dose of 8 pmol·kg⁻¹·min⁻¹ (29). The 500 pmol·kg⁻¹·min⁻¹ dose of AC187 was initially chosen because it was assumed that a 100:1 ratio between AC187 and amylin doses might be sufficient to completely prevent binding of amylin to its receptors. The AC187 infusion was begun 1 h before the amylin infusion to give AC187 a competitive advantage in binding to the amylin receptors. The second experiment of similar design determined the effects of a 800-fold higher dose of AC187 (2,000 pmol·kg⁻¹·min⁻¹) compared with amylin (2.5 pmol·kg⁻¹·min⁻¹).

Animals were permitted at least 1 wk to recover from surgery. They were then tethered to infusion swivels and adapted to experimental conditions for at least 1 wk before the start of experiments. Excess amounts of fresh ground rat chow were provided each day at 1300. In the first experiment, non-food-deprived rats (n = 16) received a 4-h intravenous infusion of AC187 (500 pmol·kg⁻¹·min⁻¹; infused at 1 ml/h) or vehicle (0.15 M NaCl, 0.1% BSA) beginning 1 h before receiving a 3-h intravenous infusion of amylin (5 pmol·kg⁻¹·min⁻¹, 1 ml/h; Peninsula Laboratories, San Carlos, CA) or vehicle (0.15 M NaCl, 0.1% BSA) beginning 1 h before receiving a 3-h intravenous infusion of amylin (5 pmol·kg⁻¹·min⁻¹, 1 ml/h; Peninsula Laboratories, San Carlos, CA).
0.1% BSA), which began 15 min before dark onset. Food intake and meal patterns during the first 4 h after dark onset were determined, as described previously, from continuous computer recordings of changes in food bowl weight (38). Infusions were administered by using a syringe infusion pump (Harvard Apparatus, South Natick, MA); pumps were turned on and off by a computer program. Each rat received each treatment in random order at intervals of at least 48 h.

At the end of the experiment, data from a rat were excluded if its jugular vein catheter was not patent. A catheter was deemed patent if the rat lost consciousness within 10 s of a bolus injection of the short-acting anesthetic brevital into the catheter. In the second experiment of similar design, rats (n = 16) received 3.25-h intravenous infusions of AC187 (2,000 pmol·kg⁻¹·min⁻¹) and amylin (2.5 pmol·kg⁻¹·min⁻¹), which began at the same time 15 min before dark onset.

Effects of AC187 on food intake in rats receiving intragastric infusion of a liquid diet. Two experiments were performed to examine the effects of intravenous infusion of AC187 on food intake in rats receiving an intragastric infusion of an elemental liquid diet during the first 2 h of the dark period. The first experiment assessed the effects of a 2,000 pmol·kg⁻¹·min⁻¹ dose of AC187, the same dose that was used in the preceding experiment when diet was not infused. The second experiment determined the effects of a range of AC187 doses from 60 to 2,000 pmol·kg⁻¹·min⁻¹. In the first experiment, non-food-deprived rats (n = 16) received a 3.5-h intravenous infusion of AC187 (2,000 pmol·kg⁻¹·min⁻¹, 2 ml/h) or vehicle (0.15 M NaCl, 0.1% BSA), beginning 15 min before receiving a 2.25-h intragastric infusion of Criticare HN (4 kcal/h, 1.06 kcal/ml, 3.8 ml/h; Mead Johnson Nutritional, Evansville, IN), which began 15 min before dark onset. We previously determined that this dose of Criticare HN inhibits food intake during the first 3 h of the dark period by ~50% (data not presented). The caloric composition of Criticare HN is 14.4% protein (hydrolyzed casein, amino acids), 4.5% fat (saflower oil, emulsifiers), and 81% carbohydrate (maltodextrin, modified corn starch). In the second experiment of similar design, rats (n = 16) received a 3.5-h intravenous infusion of AC187 (60, 200, 600, or 2,000 pmol·kg⁻¹·min⁻¹, 2 ml/h) or vehicle beginning 15 min before receiving the 2.25-h intragastric infusion of Criticare HN. In each experiment, food intake and meal patterns were measured during the first 4 h after dark onset, and each rat received each treatment in random order at intervals of at least 48 h.

Statistical analyses. Values are presented as group means ± SE. Our intent was not to compare data across experiments. Thus data from each experiment were analyzed separately. Effects of AC187 on feeding, on amylin-induced inhibition of feeding, and on feeding in rats receiving an intragastric infusion of an elemental liquid diet were evaluated by using a within-subjects repeated-measures ANOVA, with peptide dose and time being the within-group factors. Feeding data included cumulative hourly food intake during the 4-h test period and mean meal size and number of meals during the first hour and the first 3 h of the test period. Planned comparisons of treatment means were evaluated by direct contrasts of means by using the computer program SYSTAT. Differences between means were considered significant when P < 0.05. A one-tailed test was used for postulated unidirectional effects.

RESULTS

Effects of AC187 on food intake and amylin-induced anorexia. Figure 1A shows the individual and combined effects of intravenous infusions of AC187 (500 pmol·kg⁻¹·min⁻¹) and amylin (5 pmol·kg⁻¹·min⁻¹) on food intake during the early dark period. ANOVA demonstrated significant main effects of amylin and AC187 on cumulative food intake at 1, 2, 3, and 4 h, and no significant interaction of amylin and AC187 on cumulative intake at any time point (Table 1). Comparisons of individual treatment means showed that AC187 infusion alone produced a significant increase in cumulative food intake during the 4-h test period compared with the response to vehicle infusion, with a peak stimulation of 76% at 1 h (P < 0.001), decreasing to 11% stimulation at 4 h (P < 0.05). Amylin infusion alone produced a significant, sustained reduction in cumulative food intake across the 4-h test period compared with the response to vehicle infusion, with a peak inhibition of 43% at 3 h (P < 0.001), decreasing to 32% inhibition at 4 h (P < 0.001). AC187 appeared to significantly attenuate amylin-induced inhibition of food intake compared with the response to AC187 infusion alone and at 1, 2, 3, and 4 h by 50% (P < 0.05), 52% (P < 0.05), 57% (P < 0.01), and 48% (P < 0.01), respectively. The magnitudes of these AC187-induced effects were not statistically different from those produced by AC187 alone, as indicated by the nonsig-
significant interactions between AC187 and amylin. A similar analysis of the effects of these treatments on meal patterns during the first 3 h of the feeding period when peptides were infused indicated that amylin treatment alone decreased mean meal size by 25% during both the first hour (P < 0.05) and the 3-h period (P < 0.01), and decreased the number of meals during the 3-h period by 23% (P < 0.01) (Table 2). AC187 treatment alone increased the number of meals during the first hour by 38% (P < 0.05), but had no effect on the number of meals during the 3-h period or mean meal size during the first hour or the 3-h period. AC187 appeared to attenuate the amylin-induced decrease in mean meal size during the 3-h period by 50% (P < 0.01).

Figure 1B shows the individual and combined effects of intravenous infusions of a fourfold higher dose of AC187 (2,000 pmol·kg⁻¹·min⁻¹) and a 50% lower dose of amylin (2.5 pmol·kg⁻¹·min⁻¹) on food intake during the early dark period. ANOVA demonstrated significant main effects of amylin and AC187 on cumulative food intake at 1, 2, 3, and 4 h, and no significant interaction of amylin and AC187 on cumulative intake except at 1 h (Table 1). Comparisons of individual treatment means showed that AC187 alone produced a significant, sustained increase in cumulative food intake during the 4-h test period compared with the response to vehicle infusion, with a peak inhibition of 50% at 2 h (P < 0.001), decreasing to 24% inhibition at 4 h (P < 0.001). AC187 appeared to significantly attenuate amylin-induced inhibition of food intake compared with the response to AC187 infusion alone at 2, 3, and 4 h by 46% (P < 0.01), 51% (P < 0.001), and 49% (P < 0.01), respectively. The magnitudes of these AC187-induced effects were not statistically different from those produced by AC187 alone, as indicated by the nonsignificant interactions between AC187 and amylin. A similar analysis of the individual and combined effects of these treatments on meal patterns indicated that amylin treatment alone decreased mean meal size during the first hour and the 3-h period by 50% (P < 0.05) and 38% (P < 0.01), respectively, and decreased number of meals during the 3-h period by 27% (P < 0.01) (Table 2). AC187 treatment alone increased mean meal size during the first hour by 150% (P < 0.01) and increased the number of meals during the first hour and the 3-h period by 89% (P < 0.01) and 19% (P < 0.05), respectively. AC187 appeared to attenuate the amylin-induced decrease in mean meal size during the 3-h period by 62% (P < 0.01) and the amylin-induced decrease in the number of meals during the first hour and the 3-h period by 27% (P < 0.05) and 65% (P < 0.01), respectively.

Table 1. Repeated-measures ANOVA results for experiments determining individual and combined effects of AC187 and amylin on cumulative food intake.

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<th>df</th>
<th>F</th>
<th>P</th>
<th>F</th>
<th>P</th>
<th>F</th>
<th>P</th>
<th>F</th>
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<tbody>
<tr>
<td>Amylin (5 pmol·kg⁻¹·min⁻¹)</td>
<td>1,14</td>
<td>9.8</td>
<td>0.007</td>
<td>11.1</td>
<td>0.005</td>
<td>33.4</td>
<td>&lt;0.001</td>
<td>56.8</td>
<td>&lt;0.001</td>
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<td>AC187 (500 pmol·kg⁻¹·min⁻¹)</td>
<td>1,14</td>
<td>16.6</td>
<td>0.001</td>
<td>17.7</td>
<td>&lt;0.001</td>
<td>8.2</td>
<td>0.013</td>
<td>13.7</td>
<td>0.002</td>
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<tr>
<td>Interaction</td>
<td>1,14</td>
<td>0.72</td>
<td>0.404</td>
<td>0.0</td>
<td>0.914</td>
<td>4.5</td>
<td>0.051</td>
<td>1.1</td>
<td>0.307</td>
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<tr>
<td>Amylin (2.5 pmol·kg⁻¹·min⁻¹)</td>
<td>1,13</td>
<td>33.2</td>
<td>&lt;0.001</td>
<td>25.2</td>
<td>&lt;0.001</td>
<td>54.4</td>
<td>&lt;0.001</td>
<td>26.5</td>
<td>0.001</td>
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<tr>
<td>AC187 (2,000 pmol·kg⁻¹·min⁻¹)</td>
<td>1,13</td>
<td>22.0</td>
<td>&lt;0.001</td>
<td>18.0</td>
<td>&lt;0.001</td>
<td>66.0</td>
<td>&lt;0.001</td>
<td>17.2</td>
<td>&lt;0.001</td>
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<tr>
<td>Interaction</td>
<td>1,13</td>
<td>8.3</td>
<td>0.013</td>
<td>0.4</td>
<td>0.543</td>
<td>0.7</td>
<td>&lt;0.417</td>
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df, Degrees of freedom; AC187, acetyl-[Asn⁶⁰, Tyr⁶⁹] sCT-(8–32).

Table 2. Individual and combined effects of AC187 and amylin on meal parameters during first 3 h of test period.

<table>
<thead>
<tr>
<th></th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
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<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of meals</td>
<td>1.3±0.2a</td>
<td>1.6±0.2a</td>
<td>3.9±0.4a</td>
<td>2.0±0.2a</td>
</tr>
<tr>
<td>MS</td>
<td>1.6±0.2a</td>
<td>1.2±0.2b-c</td>
<td>3.6±0.3a</td>
<td>2.3±0.2a</td>
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<tr>
<td>Amylin (5 pmol·kg⁻¹·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of meals</td>
<td>0.9±0.2a</td>
<td>1.0±0.3a</td>
<td>3.7±0.4a</td>
<td>2.1±0.2e</td>
</tr>
<tr>
<td>MS</td>
<td>0.6±0.2a</td>
<td>0.5±0.2a</td>
<td>2.7±0.4a</td>
<td>1.3±0.1b</td>
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<tr>
<td>AC187 (500 pmol·kg⁻¹·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of meals</td>
<td>1.7±0.3a-c</td>
<td>1.6±0.2-c</td>
<td>3.5±0.5-b</td>
<td>1.9±0.1a</td>
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<tr>
<td>MS</td>
<td>1.8±0.3a</td>
<td>1.9±0.2a</td>
<td>3.6±0.3a</td>
<td>2.3±0.2a</td>
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<tr>
<td>Amylin + AC187</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>No. of meals</td>
<td>0.9±0.3-s-c</td>
<td>0.9±0.2-s-b</td>
<td>3.8±0.4-c</td>
<td>1.9±0.2a</td>
</tr>
<tr>
<td>MS</td>
<td>1.1±0.2-s</td>
<td>1.4±0.3-s</td>
<td>3.5±0.3-s</td>
<td>1.9±0.1b</td>
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<tr>
<td>Vehicle (2.5 pmol·kg⁻¹·min⁻¹)</td>
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<td></td>
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</tr>
<tr>
<td>No. of meals</td>
<td>1.2±0.4a</td>
<td>1.1±0.3-s</td>
<td>3.2±0.6</td>
<td>2.5±0.3-s</td>
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<tr>
<td>MS</td>
<td>1.2±0.3-s</td>
<td>0.9±0.3-s</td>
<td>3.3±0.5-s</td>
<td>2.1±0.2-s</td>
</tr>
<tr>
<td>AC187 (2,000 pmol·kg⁻¹·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of meals</td>
<td>1.2±0.3</td>
<td>1.3±0.3</td>
<td>4.0±0.5</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>MS</td>
<td>1.2±0.2</td>
<td>1.7±0.2</td>
<td>3.4±0.4</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>Amylin (2.5 pmol·kg⁻¹·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of meals</td>
<td>1.6±0.3-s</td>
<td>1.7±0.3-s</td>
<td>4.2±0.6-s</td>
<td>2.2±0.2-s</td>
</tr>
<tr>
<td>MS</td>
<td>1.6±0.3-s</td>
<td>1.7±0.3-s</td>
<td>4.2±0.6-s</td>
<td>2.2±0.2-s</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 13–15 rats for each experiment. MS, mean meal size (g). Treatment means labeled with the same letter are not statistically different (P > 0.05).
Effects of AC187 on food intake in rats receiving an intragastric infusion of a liquid diet. Figure 2A shows the effects of an intravenous infusion of AC187 (2,000 pmol·kg⁻¹·min⁻¹) during the early dark period in rats receiving an intragastric infusion of Criticare HN (4 kcal/h) to reduce voluntary intake. AC187 significantly increased cumulative food intake at 2, 3, and 4 h by 41, 41, and 36%, respectively. AC187 significantly increased mean meal size during the 3-h period by 21% (P < 0.05) and increased the number of meals during the first hour and the 3-h period by 31% (P < 0.05) and 25% (P < 0.01), respectively (Table 2).

Figure 2B shows the dose-response effects of intravenous infusion of AC187 (60, 200, 600, and 2,000 pmol·kg⁻¹·min⁻¹) during the early dark period in rats receiving the intragastric infusion of Criticare HN (4 kcal/h). The minimal effective dose of 600 pmol·kg⁻¹·min⁻¹ significantly increased food intake and mean meal size during the first hour by 128% (P < 0.05) and 61% (P < 0.01), respectively (Fig. 2B and Table 2). The largest dose administered (2,000 pmol·kg⁻¹·min⁻¹) significantly increased intake at 1, 2, 3, and 4 h by 163, 44, 47, and 24%, respectively, and increased number of meals during the 3-h period by 31% (P < 0.05).

**DISCUSSION**

The amylin receptor antagonist AC187 was used to assess whether action of endogenous amylin is essential for normal satiation to occur. Our results demonstrate several important properties of the effects of intravenous infusion of AC187 during the early dark period on food intake in non-food-deprived rats: 1) AC187 appears to attenuate, but does not completely block, amylin-induced anorexia; 2) AC187 administration alone stimulates food intake; and 3) AC187 dose-dependently stimulates food intake in rats receiving an intragastric infusion of a liquid diet. These results support the hypothesis that amylin plays an essential role in the production of satiety.

Because AC187 is an antagonist of amylin action, then it would be reasonable to assume that AC187 would specifically attenuate amylin’s effects on meal patterns. Our laboratory (39) addressed the validity of such an assumption in our prior study of the effects of CCK receptor blockade on meal pattern responses to exogenous CCK. In the present study, amylin (2.5 and 5 pmol·kg⁻¹·min⁻¹) significantly reduced both mean meal size and meal frequency, and AC187 appeared to attenuate the meal size response in both experiments and the meal frequency response in one experiment. In the three experiments in which AC187 (2,000 pmol·kg⁻¹·min⁻¹) stimulated food intake, mean meal size was increased in two experiments, and meal frequency was increased in all three experiments. Together, these results suggest that amylin acts by an essential mechanism to both reduce meal size and increase the postmeal interval of satiety.

It remains to be established that the orexigenic response to AC187 is specifically due to blockade of endogenous amylin action at either CT/RAMP1 or CT/RAMP3 receptor complexes. AC187 also antagonizes binding of CGRP to the CT/RAMP1 complex, sCT to the CT/RAMP1 and CT/RAMP3 complexes, and CT to the CT receptor (28). Our laboratory (30) previously demonstrated that 3-h intravenous infusions of amylin, CGRP, CT, and sCT during the early dark period suppress feeding in rats with a rank order of potency of sCT > amylin > CGRP >> CT. These results suggest that CT is not likely to be a physiological regulator of food intake. CGRP-producing cells are widely distributed within the central and peripheral nervous systems in rats (9). An sCT-like peptide has also recently been isolated from rat diencephalon (12). Thus it remains to be determined whether endogenous amylin, CGRP, and/or an sCT-like peptide acts at CT/RAMP receptor complexes to reduce food intake.

Rushing et al. (31–33) previously demonstrated that ICV administration of AC187 attenuates amylin-induced anorexia and increases food intake and adiposity. These studies were the first to suggest that action of an endogenous ligand at CT/RAMP receptor complexes within the brain reduces food intake and regulates energy reserves. In contrast, Lutz et al. (18) reported that ICV injection of 10 µg of CGRP-(8–37), but not 10 µg of AC187 or amylin-(8–37), stimulates feeding in...
rats when administered alone, suggesting that endogenous ligands act at CT/RAMP receptor complexes rather than CT/RAMP receptor complexes within the brain to inhibit food intake. However, only single doses of antagonists were employed in this study, and the ability of these doses to antagonize anorectic responses to ICV injections of amylin and CGRP were not assessed. Thus the negative results with AC187 and amylin-(8–37) may have occurred because insufficient doses were administered.

In contrast to our results, Lutz et al. (20) showed that systemic administration of AC187 attenuates sCT-induced anorexia but has no effect on food intake when administered alone. If AC187 has a relatively short half-life, the single bolus dose of AC187 used in their study may not have been sufficient to attenuate the satiety effects of a prolonged meal-induced secretion of amylin, CGRP, or an sCT-like peptide. There are no available pharmacokinetic data for AC187 that can be used to resolve this question. Lutz et al. deprived their rats of food for 24 h before AC187 administration. AC187 may not be able to stimulate food intake in previously food-deprived animals that already express a significant drive to eat. To obviate these concerns in the present study, different doses of AC187 were administered to non-food-deprived rats by continuous intravenous infusion, and AC187 dose-dependently stimulated food intake.

In the present study, AC187 did not completely block the anorexia produced by exogenous amylin, despite the use of AC187 doses that were 100- to 800-fold greater than those of amylin. Under similar conditions, we observed that a 400 nmol·kg^{-1}·h^{-1} dose of AC187 completely blocked the inhibitory effect of a 1 nmol·kg^{-1}·h^{-1} dose of amylin on gastric emptying (unpublished observations). These results suggest that AC187 may have limited access to CT/RAMP receptors mediating the anorectic response to systemic administration of amylin, or amylin may inhibit food intake, in part, by acting at a receptor subtype not blocked by AC187. Systemically administered amylin is postulated to act within the brain to inhibit food intake, and amylin has been shown to penetrate the blood-brain barrier (6, 13). Whether AC187 can similarly penetrate the blood-brain barrier remains to be determined. Recent evidence suggests that endogenous amylin acts through a receptor other than the CT receptor to regulate bone resorption (10). It remains to be determined whether amylin also acts, in part, through this unknown receptor subtype to inhibit food intake.

The mechanisms that mediate the inhibitory effect of amylin on food intake have not been clearly defined. Several lines of evidence suggest that amylin may act directly within the brain: 1) central nervous system administration of amylin (2, 5, 8, 32) appears to be more potent than systemic administration in reducing food intake and gastric emptying; 2) subdiaphragmatic vagotomy does not attenuate anorectic responses to amylin (15, 16); 3) capsaicin denervation of peripheral sensory nerves does not attenuate the anorectic response to amylin (14); 4) amylin can penetrate the blood-brain barrier (6, 13); and 5) area postrema lesions block anorectic responses to amylin (19).

Most of these studies examined the effects of specific neural lesions on anorectic responses to single intraperitoneal doses of amylin 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 cholecystokinin (26). Thus it remains to be determined whether feeding responses to physiological doses of amylin are mediated by visceral sensory nerves or a direct action of amylin in the area postrema or some other brain site. It also remains to be determined whether lesions of putative sites of amylin action attenuate the stimulatory effect of amylin receptor blockade on food intake.

The prevailing hypothesis is that amylin acts as a hormonal signal from the pancreas to the brain to reduce food intake and regulate energy reserves. However, amylin has also been detected in gastric and intestinal endocrine cells (23), visceral sensory neurons (24), and throughout the brain (34). The source of the amylin that is likely to contribute to the control of food intake has not been established.

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GRANTS

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REFERENCES

AMYLIN AND FOOD INTAKE