ISLET AMYLOID POLYPEPTIDE (IAPP) is a 37-amino acid peptide originally isolated from a human insulinoma and from pancreatic amyloid deposits of type 2 diabetic patients (27). The physiological function of IAPP is unknown, although roles in carbohydrate homeostasis (16) and calcium homeostasis (19) have been proposed. Numerous studies have measured plasma IAPP levels in fasted (2, 4, 8, 11, 13, 14, 18, 22, 23–25) and nonfasted subjects (3, 18, 24), in normal subjects after feeding (8, 18) and oral (8, 11, 13, 14, 25) or intravenous (24) glucose administration, in obese subjects (24, 25), and in diseased states (8, 11, 13, 14, 23, 25, 27). Only a single study has measured plasma IAPP levels produced by anorectic doses of IAPP (3). In general, maximal levels of plasma IAPP occurring physiologically or pathophysiologically in humans and rats (20–40 pM) are at the low end of the levels produced by anorectic doses of IAPP administered to rats by chronic subcutaneous infusion (35–240 pM). However, the differences in reported IAPP concentrations measured by the different assay procedures in these studies from different laboratories make it difficult to compare results from the separate investigations. Thus it remains to be determined within a single study whether postprandial plasma levels of IAPP are sufficient to produce satiety under normal physiological conditions.

The objectives of this study were to use a rat model to first measure meal-induced increases in plasma levels of IAPP and then to examine directly whether intravenous doses of IAPP that reproduce these levels are sufficient to independently inhibit food intake.

MATERIAL AND METHODS

Experimental design. Three experiments were performed in two groups of rats. The first study measured the arterial plasma IAPP response to food intake in rats with indwelling abdominal aortic catheters. The second determined a near-threshold dose for IAPP suppression of feeding when IAPP was infused intravenously into rats with jugular vein and aortic catheters. The third experiment was conducted in the...
same rats to determine the arterial plasma IAPP response to this near-threshold intravenous dose of IAPP.

Animals and housing conditions. Adult male Wistar rats (Harlan Sprague Dawley, Indianapolis, IN), 362–453 g at the start of experiments, were housed individually in metabolic cages (no. 5236; Lab Products, Maywood, NJ) modified to include a 10 cm × 7 cm × 10 cm side compartment with a 3 cm diameter hole in the base through which the animal ate finely powdered chow (Laboratory Rodent Diet no. 5001; PMI Feeds, St. Louis, MO). Water was available ad libitum throughout the experiments. Fresh food was provided daily; fresh water was provided twice weekly. The animal room was air and temperature controlled (22 ± 1°C) and had a 12:12-h light-dark cycle, with lights out at 2100 in the first experiment and at 1700 in the second and third experiments. The experimental protocols were approved by the Creighton University Animal Research Committee, and the studies were conducted in accordance with National Institutes of Health guidelines.

Surgical procedures and catheter maintenance. The procedures for fabrication and surgical implantation of jugular and abdominal aortic catheters have been described previously (4, 26). In the current experiment, the aortic catheter was modified by shortening the polyethylene tubing from 120 to 70 mm and placing the distal Dacron cuff 90 mm from the junction of the PE and silicone tubes to fit the lightweight saddle described below. A few days after arrival, the animals were anesthetized with pentobarbital sodium (Nembutal; Abbott, Chicago, IL; 50 mg/kg ip) and were given the prophylactic antibiotic Cefazolin (Apothecon, Princeton, NJ; 60 mg ip). Catheters were sterilized by ethylene oxide and implanted using aseptic surgical technique. Catheters were filled with 0.15 M NaCl and 40 U/ml heparin (heparin sodium; Lyphomed, Deerfield, IL) and were exteriorized 0.5 cm behind the occiput. The external part of the catheters was plugged with a metal stylet. Approximately 5 days after implantation of catheters, each rat was briefly anesthetized with ketamine (Ketaset, 100 mg/ml; 10–20 mg/kg iv; Aveco, Fort Dodge, IA) and each catheter was connected to a 40-cm length of PE-20 (venous catheter) or PE-50 (aortic catheter) tubing, which was passed through a protective spring coil connected to the light-detecting device (ITTC, Woodbridge, NJ) worn by the rat and to an infusion swivel above the cage (Instech Laboratories, Plymouth Meeting, PA). To prevent clotting, the catheters were flushed twice weekly with 0.5 ml of the heparin-saline solution. At the end of the second experiment, the patency of venous catheters was tested by injecting ketamine (13 mg/kg) intravenously. Animals not anesthetized within 30 s were excluded from the study.

Plasma IAPP response to food intake. Rats (n = 9) with abdominal aortic catheters were adapted to a daily 12:12-h light-dark cycle (lights off at 2100 h) and a 6-h feeding period (1500–2100). Blood samples (1.5 ml each) were taken from each rat immediately before presentation of food (time 0) and at 15, 30, 60, 120, and 240 min thereafter. Three blood samples were collected from each rat on each of two experimental days separated by 5 days: at 0, 30, and 120 min on 1 day and at 15, 60, and 240 min on the other. The sampling schedule was randomized among the rats, and four or five rats were assigned to each schedule on each experimental day. To replace the lost blood volume, 1.5 ml of 0.15 M NaCl and heparin (4 U/ml) was injected into the aortic catheter after each sample was taken. Six-hour food intake was determined for each blood collection day by noting the weight change in the food bowl from 0 to 360 min. Food intake was determined similarly on the days preceding and following the sampling days.

Food intake response to IAPP infusion. Rats with jugular vein and abdominal aortic catheters were adapted to a daily 12:12-h light-dark cycle (lights off at 1700) and had free access to food and water. Animals were also adapted to the experimental conditions, which required 1–2 wk. This included several days of intravenous saline infusion, which was administered using a syringe pump (Harvard Apparatus, South Natick, MA). Pumps were turned on and off by a computer program. Experimental conditions also included a strict daily regimen from 1030 to 1230, during which feeding data from the previous day were collected and the animals were maintained. Experiments were begun when food intake had stabilized.

Our previous work indicates that in rats, the threshold dose for IAPP-induced inhibition of feeding is <8 pmol·kg⁻¹·min⁻¹ when IAPP is administered by continuous intravenous infusion (2). In the present study, a three-part experiment was performed to more clearly define a near-threshold intravenous dose of IAPP for suppression of feeding. Rats (n = 16) first received 5 pmol·kg⁻¹·min⁻¹ of IAPP or vehicle (0.15 M NaCl, 0.1% BSA; 2 ml/h) for 4 h beginning 1 h before lights out. Each rat received both treatments in random order at a 48-h interval. Meal patterns were monitored for 16 h beginning 30 min after the start of infusion. At the end of the infusion period, samples of infusate (1.5 ml) were collected from the ends of infusion lines and were stored at −80°C until assayed for IAPP concentration. This part of the experiment was then followed by two other parts of identical design using first 1 pmol·kg⁻¹·min⁻¹ of IAPP and vehicle (n = 13) and then 3 pmol·kg⁻¹·min⁻¹ of IAPP and vehicle (n = 14). Synthetic rat IAPP (Multiple Peptide Systems, San Diego, CA) was dissolved in DMSO, diluted to 10 nmol/ml with an equal volume of 0.15 M NaCl and 0.1% BSA, and stored in aliquots at −80°C. The final infusate concentration of DMSO was <0.32% vol:vol. On the day of each infusion, an aliquot of IAPP was thawed and diluted to the appropriate infusate concentration with 0.15 M NaCl and 0.1% BSA.

Procedures for recording meal patterns have been described previously (28). Briefly, each rat ate from a food cup that was fixed to a digital balance (Denver Instruments, South Natick, MA). The food cup was adapted to a 16-balance system connected to a IBM XT-compatible computer through a code-activated switch (CAS-16; Western Telematic, Irvine, CA). Output from each balance was monitored at 16-s intervals. Changes in food bowl weight were recorded and data processed to determine latency to the first meal, individual meal sizes, postmeal intervals (PMIs), satiety ratios (PMI/meal size), the amount of food ingested each hour, and food intake and number of meals cumulated hourly. Individual meals were defined using a minimum meal size criterion of 50 mg and an intermeal interval criterion of 5 min. These criteria minimize the possible influence of intrameal periods of no eating in the analysis of meal patterns (28).

Plasma IAPP response to IAPP infusion. Fifteen of the rats used in the preceding experiment received an intravenous infusion of either 3 pmol·kg⁻¹·min⁻¹ of IAPP or vehicle for 4 h, as described above. Each rat received both treatments in random order at a 24-h interval. Blood samples (1.5 ml) were collected from the abdominal aortic catheter during the dark period (red lights on) at 1800 and 1900, which were 2 and 3 h, respectively, after the start of infusions. Arterial blood was allowed to drip into chilled tubes containing 2.5 mg EDTA and 50 μl aprotinin (Trasylol 400 KIU/ml; Bayer, Leverkusen, Germany). An equal volume of 0.15 M NaCl and heparin (4 U/ml) was then injected into the aortic catheter. Plasma was
separated by centrifugation at 4°C (1,700 g for 12 min). At the end of the infusion period, samples of infusate (1.5 ml) were collected from the ends of infusion lines. Plasma and infusate samples were stored at −80°C until assayed for IAPP concentration.

The procedures used to extract the plasma and infusate and to measure IAPP by radioimmunoassay were as described previously (23), with the exception that samples were acidified with acetic acid (final concentration of 0.25 M) and centrifuged before extraction of supernatant using Sep-Pak C18 reverse-phase cartridges (Waters, Milford, MA). The antisera employed (Peninsula Laboratories, Belmont, CA) cross-reacts equally well with rat and human IAPP. Gel-permeation chromatography revealed that >90% of immunoreactive IAPP in human plasma elutes in a single peak at an identical position to that of pure synthetic IAPP. Using rat IAPP as standard, the assay detected changes between adjacent samples of 2.0 pmol with 95% confidence. The antiserum showed no significant cross-reaction with calcitonin gene-related peptide. Within-assay coefficient of variation for IAPP was <8%, and IAPP recovery during Sep-Pak extraction was >80%.

Statistical analyses. Mean daily 6-h food intakes across the 7-day period in the first series of experiments were compared using repeated-measures ANOVA. Planned comparisons were evaluated by direct contrasts of means (least-significant difference method) using the statistical program SYSTAT. Mean plasma IAPP levels at different times after presentation of food were compared using repeated-measures ANOVA. Planned comparisons of mean IAPP levels at the different times with the mean basal level were evaluated by direct contrasts of means. The effects of each IAPP dose on feeding parameters were evaluated using separate single-factor repeated-measures ANOVA. Experiments were not designed to compare feeding responses across IAPP doses, but rather to systematically define a near-threshold IAPP dose for suppression of feeding. Planned comparisons were evaluated by direct contrasts of means. The effects of IAPP infusion on plasma IAPP levels were evaluated by a single-factor repeated-measures ANOVA. Planned comparisons were evaluated by direct contrasts of means. In each analysis, differences were considered significant if P < 0.05. Values are group means (±SE).

RESULTS

Plasma IAPP response to food intake. The 18-h-fasted rats immediately began eating food when presented at the beginning of the 6-h daily feeding period. In rats weighing 384 ± 4 g, food intake increased plasma IAPP levels significantly [F(5,40) = 7.8, P < 0.0001] from a fasting level of 10.8 ± 0.5 pmol·kg⁻¹·min⁻¹ to a plateau value of 16.2 ± 0.6 pmol·kg⁻¹·min⁻¹ at 1 h, which was sustained for the remaining 3 h of the experimental period (Fig. 1). Peak levels averaged 19.0 ± 1 pmol·kg⁻¹·min⁻¹ [F(8) = −6.8, P < 0.001 vs. fasting level] at a mean time of 2.2 ± 0.5 h. The 6-h daily food intakes on the two blood sampling days were not significantly different from each other, 18.8 ± 1.4 vs. 19.2 ± 0.7 g (P > 0.05) or from the average of the intakes on the days before and after blood collection, 18.8 vs. 19.0 g (P > 0.05) for the first blood collection day and 19.2 vs. 20.1 g (P > 0.05) for the second blood collection day.

Food intake response to IAPP infusion. Figure 2 shows the cumulative food intake responses to 4-h intravenous infusions of IAPP at 1, 3, and 5 pmol·kg⁻¹·min⁻¹ in the three parts of the experiment. Radioimmunoassay of IAPP infused concentrations indicated that the actual intravenous doses were 1.0 ± 0.1, 2.7 ± 0.2, and 4.5 ± 0.2 pmol·kg⁻¹·min⁻¹, respectively. Of these, only the 4.5 pmol·kg⁻¹·min⁻¹ dose was significantly different (P < 0.05) from its calculated value of 5 pmol·kg⁻¹·min⁻¹. In rats weighing 409 ± 5 g, the 5 pmol·kg⁻¹·min⁻¹ dose significantly inhibited cumulative food intake at each time point during the 16-h recording period except during the first hour. At 2, 3, and 4 h, intakes were suppressed by 34 (P < 0.05), 25 (P < 0.05), and 29% (P < 0.01), respectively. In the subsequent experiment in the same rats, 1 pmol·kg⁻¹·min⁻¹ of IAPP had no significant effect on cumulative intake. In the next part of the experiment in the same animals, an intermediate dose of IAPP (3 pmol·kg⁻¹·min⁻¹) significantly inhibited cumulative food intake at each time point from 2 to 6 h. At 2, 3, and 4 h, intakes were reduced by 34 (P < 0.01), 22 (P < 0.05), and 25% (P < 0.05), respectively. Thus the threshold IAPP dose for suppression of feeding appeared to be between 1 and 3 pmol·kg⁻¹·min⁻¹.

Analysis of the effects of the IAPP infusions on meal parameters (Table 1) demonstrated that the 1 pmol·kg⁻¹·min⁻¹ dose was without an effect and that the 3 and 5 pmol·kg⁻¹·min⁻¹ doses decreased 4-h food intake by decreasing meal frequency rather than meal size. This effect of IAPP was further supported by the observation that IAPP significantly increased (3 pmol·kg⁻¹·min⁻¹ dose) or tended to increase (5 pmol·kg⁻¹·min⁻¹ dose) the PM1 of the first meal and the PM1s of all meals during the first 4-h period.

Plasma IAPP response to IAPP infusion. Radioimmunoassay of IAPP infused concentrations indicated that the actual intravenous dose administered in this experiment was 3.8 ± 0.2 pmol·kg⁻¹·min⁻¹ (P < 0.05 vs. calculated dose of 3 pmol·kg⁻¹·min⁻¹). Figure 3 shows that in the same rats as used in the preceding experiment, this IAPP dose significantly increased plasma
IAPP levels above those observed during vehicle infusion. Repeated-measures ANOVA demonstrated a significant main effect of IAPP infusion [F(1,14) = 324, P < 0.001], no significant main effect of time of blood sampling [F(1,14) = 0.4, P > 0.05], and no significant interaction between IAPP infusion and time of blood sampling [F(1,14) = 0.3, P > 0.05]. Compared with vehicle infusion, continuous intravenous infusion of 3.8 pmol·kg⁻¹·min⁻¹ of IAPP significantly increased plasma IAPP levels by ~30 pM, from 12.7 ± 0.7 to 44.0 ± 2.4 pM at 2 h and 12.8 ± 1.8 to 41.9 ± 1.7 pM at 3 h. The rats used in the studies examining the effects of IAPP infusion on food intake and plasma IAPP concentration increased in weight from 409 ± 4 to 425 ± 6 g during the experimental period.

**DISCUSSION**

Our objective was to study whether IAPP produces satiety by an endocrine mechanism. We used a rat model to determine whether postprandial plasma levels of IAPP are comparable to those required to inhibit feeding when IAPP is administered by continuous intravenous infusion. Intraperitoneal administration is not an appropriate method to use, because IAPP administered in this manner could act locally to affect feeding before being absorbed into the circulation, which would preclude the establishment of a meaningful correlation between the feeding response and an increase in plasma IAPP.

No previous study has both characterized the temporal profile of the plasma IAPP response to food intake and determined whether feeding is suppressed by an intravenous IAPP dose that mimics this plasma profile. In the present study, food intake in 16-h-fasted rats increased plasma IAPP gradually from a fasting level of 11 pM to a plateau of 17 pM at 60 min, which was sustained for the remaining 3 h of the sampling period. Peak levels averaged ~19 pM (an 8-pM increase). We then determined that a threshold anorectic dose for continuous intravenous infusion of IAPP was between 1 and 3 pmol·kg⁻¹·min⁻¹ and that a 3.8 pmol·kg⁻¹·min⁻¹ dose increased plasma IAPP by ~30 pM to a plateau level of 44 pM after 2 h of infusion. Assuming that plasma IAPP is cleared by first-order kinetics and has a half-life in rats of ~10 min (15, 29), we estimate that a continuous intravenous infusion of a threshold IAPP dose for suppression of feeding in our model would produce a steady-state increase in plasma IAPP concentration between 9 and 24 pM and that a 0.9 pmol·kg⁻¹·min⁻¹ dose of IAPP would be required to produce a steady-state increase in plasma IAPP similar to the peak response to food intake measured in our experiment. Because the threshold dose for suppression of feeding was between 1 and 3 pmol·kg⁻¹·min⁻¹, our results suggest that postprandial plasma levels of IAPP are not quite sufficient to independently produce satiety under normal physiological conditions.

Our conclusion depends crucially on the characteristics of the radioimmunoassay used in this study to measure IAPP. The usefulness of the assay to measure endogenous IAPP has been described in detail (23). In the present study, plasma IAPP concentrations were ~10 pM in fasted animals, 12 pM (120% of basal) in ad libitum-fed animals at 1 and 2 h after the start of the dark period, and 17 pM (170% of basal) in previously fasted animals at 1, 2, and 4 h after ingestion of a large meal. In animals refed after a 24-h fast, plasma IAPP was recently reported to increase from a fasting level of ~3 pM to 14 pM after the first meal (466% of basal) (18). In the same study, plasma IAPP in non-food-deprived animals after the first nocturnal meal was ~3 times higher compared with fasting levels. In humans, postprandial levels increased from a fasting level of ~5 pM to a maximal of 9 pM (180% of basal) 90 min after...
Ingestion of a mixed meal (8). Thus the maximal meal-induced changes in plasma IAPP previously reported for rats and humans, 11 pM and 4 pM, respectively, are similar to those found in the present study (8 pM).

Two issues need to be considered before concluding that postprandial plasma IAPP levels may not be sufficient to produce satiety under physiological conditions. First, diet macronutrient compositions different from that used in the present study may produce a plasma IAPP response sufficient to inhibit food intake. No study has systematically examined the effects of diet composition on plasma IAPP. Second, the route of IAPP administration may be important with respect to its effects on food intake. Endogenous IAPP is released from the endocrine pancreas into the portal circulation, and it is possible that IAPP may be acting at hepatic sites to inhibit feeding. In rats, portal IAPP levels are larger than those in aortic blood (18, 24). If IAPP inhibits feeding by a hepatic-portal mechanism, intraportal administration of IAPP should be more effective than intravenous, and intravenous doses required to suppress feeding may appear to be unphysiologically high. No study has compared the effects of intraportal and intravenous administration of IAPP on food intake, although intraportal injection does appear to be less effective than intraperitoneal injection (17). Thus it remains to be determined whether the anorexia produced by IAPP is mediated by a hepatic-portal mechanism.

With respect to the effects of exogenous IAPP on meal patterns, the present study using acute intravenous infusion of IAPP (3 and 5 pmol·kg⁻¹·min⁻¹) confirms and extends our previous results with chronic subcutaneous administration of IAPP (2, 7, and 25 pmol·kg⁻¹·min⁻¹) (3) that administration of low doses of IAPP to rats fed ad libitum reduces food intake by decreasing meal frequency rather than meal size. In contrast, intraperitoneal injection of IAPP (260 pmol/kg) has been reported to decrease the size of the first meal in 24-h-fasted rats and to increase latency to the first meal as well as to decrease first meal size in ad libitum-fed rats (17). In the same study, meal size was also decreased by acute portal vein infusion of IAPP (680 pmol/kg). We conclude from these data that more physiological doses of IAPP decrease meal frequency in freely feeding rats while larger pharmacological doses are also likely to decrease meal size.

**Perspectives**

A role for endogenous IAPP in the physiological control of food intake remains to be established. The present study suggests that IAPP may not be acting as a satiety hormone or that other factors may need to interact with circulating IAPP in an additive or potentiating manner to produce satiety. Recently, it was reported that the combined administration of IAPP and CCK by intraperitoneal injection suppressed feeding by an order of magnitude greater than when either peptide was given alone (7). If endogenous IAPP is interacting with other factors to produce satiety by an endocrine mechanism, it would be important to determine whether interactions occur when IAPP is infused intravenously in amounts that reproduce meal-induced in-
creases in plasma IAPP. Failure to find an effect under these circumstances would not rule out the possibility that endogenous IAPP is acting by paracrine or other mechanisms. Furthermore, if endogenous IAPP action is necessary for normal satiety to occur, it would be important to determine whether selective IAPP receptor blockade stimulates food intake.

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Address reprint requests to U. Arnelo.

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