Pharmacological Actions of the Peptide Hormone Amylin in the Long-Term
Regulation of Food Intake, Food Preference and Body Weight

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pramlintide
Abstract

The ability of amylin to reduce acute food intake in rodents is well established. Longer-term administration in rats (up to 24 d) shows a concomitant reduction in body weight, suggesting energy intake plays a significant role in mediating amylin-induced weight loss. The current set of experiments further explores the long-term effects of amylin (4-11 weeks) on food preference, energy expenditure, and body weight and composition. Furthermore, we describe the acute effect of amylin on locomotor activity and kaolin consumption to test for possible nonhomeostatic mechanisms that could affect food intake. Four wk subcutaneous amylin infusion of high fat-fed rats (3-300 μg/kg/d) dose-dependently reduced food intake and body weight gain (ED50 for body weight gain=16.5 μg/kg/d). The effect of amylin on body weight gain was durable for up to 11 wks, and associated with a specific loss of fat mass and increased metabolic rate. The body weight of rats withdrawn from amylin (100 μg/kg/d) after 4 wks of infusion returned to control levels 2 wks after treatment cessation, but did not rebound above control levels. When self-selecting calories from a low or high fat diet during 11 wks of infusion, amylin-treated rats (300 μg/kg/d) consistently chose a larger percentage of calories from the low fat diet versus controls. Amylin acutely had no effect on locomotor activity or kaolin consumption at doses that decreased food intake. These results demonstrate pharmacological actions of amylin in long-term body weight regulation, in part through appetitive-related mechanisms, and possibly via changes in food preference and energy expenditure.
Introduction

Amylin is a pancreatic β-cell hormone secreted simultaneous with insulin in response to carbohydrate (glucose) and protein-derived amino acids following a meal. Animal studies have shown that the glucoregulatory actions of amylin complement those of insulin by regulating the rate of glucose appearance in the circulation via suppressing nutrient-stimulated glucagon secretion and by regulating the rate of gastric emptying (1). Consistent with the effects of amylin in rodents, clinical studies in insulin-treated patients with diabetes have shown that pramlintide, an analog of human amylin, reduces postprandial glucose excursions by suppressing inappropriate glucagon secretion and by slowing gastric emptying (2, 3, 4).

Most research to date on the role of amylin in the regulation of body weight has focused on changes in food consumption. Central or peripheral administration of amylin has been shown to reliably decrease food intake in rodents (see 5 for review). This reduction in food intake may contribute to amylin’s ability to regulate post-prandial glucose via limiting the rate of nutrient appearance. Amylin’s effect on food intake is mediated through activation of amylin binding sites in the area postrema of the brain stem, and through activation of subsequent upstream pathways, notably the lateral parabrachial nucleus, central amygdala and lateral hypothalamus (6). Specifically, it has been established that amylin produces its anorexigenic effects through reducing meal size (7). Importantly, decreased food intake has been shown to occur in the absence of a conditioned taste aversion (8, 9). Amylin receptor antagonism also inhibits amylin-
suppression of food intake (10-13) as well as stimulates feeding in normal, untreated animals (12, 13), providing support for a role of amylin in mediating food intake.

Several single-dose studies have shown long-term amylin treatment in rodents to decrease body weight. Sustained administration of amylin for 10 days into the third ventricle via osmotic minipumps reduced food intake and subsequent body weight in rats (14). More informative of the role of amylin are data showing reduced body weight gain during 14-day peripheral (subcutaneous, or SC) amylin infusion (48 ug/kg/d) in rats maintained on a diet of moderate fat content (15) while 24-day SC amylin infusion (300 ug/kg/d) also durably reduced body weight gain, with a selective reduction in fat tissue, in diet-induced obesity-prone rats (16). Furthermore, Arnelo and colleagues (17) have reported a dose-dependent effect of amylin on body weight in lean rats following 8 days of SC infusion. These data, coupled with the finding that amylin knockout mice display a greater rate of weight gain compared to wildtype mice (18, 19), demonstrate amylin’s ability to reduce body weight.

The current set of experiments extend these findings by further exploring the long-term effects of amylin (4-11 weeks) on food intake, food preference, body weight and body composition, and energy expenditure in high fat-fed rats. Furthermore, we describe the acute effects of amylin in two models testing for possible nonhomeostatic mechanisms that could result in decreased food intake. In the first set of experiments, a dose-response relationship for amylin’s effect on body weight after 4 wks of SC infusion is established.
Secondly, the effect of amylin on food intake and body weight following withdrawal is examined. A third set of experiments confirm the satiating actions of amylin and examines the effects of acute amylin on locomotor activity and kaolin consumption.

Lastly, the effect of amylin on food preference and energy expenditure is examined. A portion of this data has appeared in abstract form (20-25).

Methods

All experiments were approved by the Institutional Animal Care and Use Committee at Amylin Pharmaceuticals Inc. in accordance with Animal Welfare Act guidelines. All animals, with the exception of those in the energy expenditure study (Experiment 7), were individually-housed male Sprague-Dawley rats obtained from Harlan (San Diego, CA) that were maintained on a 12 h light/dark cycle (22 to 24°C with ambient humidity levels), and had ad libitum access to food and water unless otherwise noted. The animals in Experiment 7 were Levin DIO prone rats (Charles River, Kingston, NY) maintained under the conditions described above.

In long-term experiments, animals were maintained on a pelleted high fat diet (58% fat kcal; #D12331, Research Diets, New Brunswick, NJ) throughout the course of the experiment. Amylin was dissolved in 50% DMSO and delivered via 4-week osmotic pumps (model # 2ML4, flow rate = 2.5 µl/h, Durect Corp., Cupertino, CA). Pumps were implanted SC in the interscapular region under isoflurane anesthesia. Food intake and body weight were measured weekly. In all studies, animals were divided into treatment groups of equal body weight.
In acute experiments, animals were maintained on a pelleted standard laboratory diet (6% fat kcal; #LM-485, Harlan Teklad, Madison, WI) with the exception of Experiment 4 (assessment of amylin’s effect on meal patterns) in which animals were maintained on a powdered high fat diet (45% fat kcal, #D1251M, Research Diets). Kaolin pellets (Experiment 6) were supplied by Research Diets (#K5001). All drugs were administered as a single intraperitoneal (IP) injection (dissolved in 10% DMSO, injection volume of 1 ml/kg). Rat amylin was synthesized by Peptisyntha (Torrance, CA). Cisplatin was supplied by Sigma (Saint Louis, MO).

With the exception of Experiment 4, food was measured manually by pouring the food from the home cage lid into a bucket on a scale. This container was large enough to capture all pelleted food. Although the floor of the home cage was examined to account for spillage, food inside the home cage was rarely observed.

In Experiment 1, plasma was collected for analysis. Rats were sacrificed by cardiac puncture under isoflurane anaesthesia and approximately 8 ml of blood was collected (EDTA coated syringe). Whole blood was centrifuged at 1000 x g for 15 min in a 15 ml conical containing 1 ml Protease Inhibitor A (2.5 mg/ml Elastatinal, 50 μg/ml Leupeptin, 50 mg/ml DiSodium EDTA, and 200 μg/ml Antipain). Plasma was collected in eppendorf tubes containing PIB 1:100 volume (25 mg/ml Chymostatin in DMSO) and
stored at -70°C until analysis. Plasma amylin concentrations were quantified using a two-site sandwich immunoenzymetric assay with fluorescent detection (26).

Experiment 1—Assessment of dose-related effects of peripherally administered amylin on food intake and body weight: Rats (300-350 g) were maintained on a high fat diet for 5 weeks prior to treatment after which they were implanted with 4-week pumps (n=5-13/group). One group of rats received 3, 10 or 30 μg/kg/d of amylin or vehicle (body weight at implant = 459 ± 4 g, mean±SEM) while another group received 30, 100 or 300 μg/kg/d of amylin or vehicle (body weight at implant = 407 ± 4 g, mean±SEM). Plasma amylin concentrations were assessed at termination in animals treated with 3, 10 and 30 μg/kg/d of amylin.

Experiment 2—Assessment of changes in food intake and body weight during and after amylin treatment: Rats (340 -370 g) were fattened for 5 weeks and then implanted with 4-week osmotic pumps delivering amylin (100 μg/kg/d) or vehicle (body weight at implantation = 452 ± 4 g, mean ±SEM, n=11-15/group). On Day 28, all pumps were removed and half of the amylin-treated rats were re-implanted with 4-week replacement pumps containing amylin (100 μg/kg/d). The remaining amylin-treated rats and all vehicle-treated rats were re-implanted with 4 week pumps containing vehicle. The total treatment period was 8 weeks.

Experiment 3—Assessment of amylin’s effect on meal patterns: Rats (450-540 g) were first housed in the test chamber for 10 days for habituation. On the last 3 days of habituation, rats received an IP injection of vehicle prior to the onset of the dark cycle.
The test chamber consists of a 13”X10”X9” plexiglass cage equipped with a tunnel with a food hopper at the end. As the animal eats, food intake is measured at one-minute intervals via automated changes in scale weight (Dietpro Software, Accuscan Instruments, Columbus, Ohio). On test day, all rats received an IP injection of amylin (100 µg/kg) or vehicle just prior to the onset of the dark cycle (n=7-8/group). Latency, meal size, meal duration, inter-meal interval, and satiety ratio were analyzed for the first meal. Minimum meal size was set at 0.2 g with an intermeal interval of ≥15 min.

**Experiment 4—Assessment of locomotor activity:** After an overnight fast, rats (400-440 g) were administered an IP injection of 1, 10 or 100 µg/kg of amylin or vehicle and immediately placed individually into the test chamber (n=5-7/group). Total distance travelled and vertical movements (rearing) were measured 30 min post injection using a locomotor activity monitor (Smart Frame Units, Hamilton-Kinder, San Diego, CA) consisting of a bi-level 4 x 8 photo beam frame surrounding the test chamber. To correlate activity levels with food intake, a second group of rats (450-510 g) was administered amylin at equivalent doses and food intake was measured for 30 min post injection.

**Experiment 5—Assessment of kaolin intake:** To assess kaolin intake, rats (315-380 g) were first introduced to kaolin pellets similar in size and shape to their chow for 3 days prior to experimentation. On Day 4, rats were assigned to treatment groups balanced for chow and kaolin intake. Rats were fasted overnight (both chow and kaolin removed) on Day 5. The following morning, rats received an IP injection of amylin (300 µg/kg), cisplatin (3 mg/kg) or vehicle (n=6/group). As amylin is rapidly metabolized, chow
intake was measured at 2 and 24 h. Kaolin intake was determined 24 h following the injection.

Experiment 6—Assessment of Food Choice and Energy Expenditure: Rats (460-520 g) were allowed to self-select from a low fat laboratory chow (6% kcal from fat) and high fat chow (58% kcal from fat, both described in General Methods section) 2 weeks prior to initiation of the study. Animals remained on this regimen for the duration of the study. On test day, rats (494 ± 11 g) were scanned in an MRI (Echo MRI, Houston, Texas) to establish basal body composition, and then implanted with osmotic pumps containing amylin (300 μg/kg/d) or vehicle (n=7-8/group). Animals were reimplanted with 4-week pumps at the beginning of week 5 and week 9. Food intake and body weight were measured weekly for 11 weeks. At 11.5 weeks, 24-hr energy expenditure was assessed using indirect calorimetry methods (Oxymax equal flow system, Columbus Instruments, Columbus, Ohio). Animals were placed individually in the metabolic chamber, with food and water and allowed to habituate to the chamber for 24 hr. Metabolic rate and RQ were then measured the following 24 hr in the absence of food. Pumps were then removed and body composition again assessed via an MRI scan.

Statistical Analysis

All data are presented as mean + SEM. Group differences were analyzed using analysis of variance (ANOVA). In some experiments post hoc comparisons were carried out using Fisher’s Least-Significant Difference test (P level set at 0.05). Nonlinear regression (sigmoidal dose-response) was used to examine dose-response relationships.
Results

Experiment 1—Dose-Related Effect of Amylin on Food Intake and Body Weight:
Two dose-response experiments, one examining 3-30 ug/kg/d of amylin, and the other examining 30-300 ug/kg/d of amylin, were carried out. The weight gained by the control animals in both arms of the experiment did not differ from one another (+59.5 ± 7.8 g versus +66.7 ± 6.4 g, at 4 weeks, P>.05) and therefore, these data sets were combined. ANOVA at each time point showed amylin to dose-dependently reduced food intake, with significant reductions at 30, 100 and 300ug/kg/d compared to controls throughout the 4 weeks of treatment (P’s<0.05, Figure 1A). Subsequently, body weight gain was dose-dependently reduced with amylin (Figure 1B), with a maximal vehicle-corrected body weight loss of 14 % at 4 weeks. Rats treated with doses of ≥10 μg/kg/d had significantly less weight gain throughout the study (P’s<0.05). The ED_{50} for reduction in body weight gain at 4 weeks was 16.5 μg/kg/d ± 0.05 log units (Figure 2). Plasma amylin concentrations achieved at the three doses tested are shown in Table 1.

Experiment 2—Food Intake and Body Weight During and Following Cessation of Amylin Treatment: ANOVA at each time point showed significantly reduced caloric intake and body weight gain compared to controls during the first 4 weeks of 100 ug/kg/d of amylin treatment (P’s<0.05, Figures 3A and B). Following withdrawal of amylin at the end of 4 weeks, caloric intake significantly increased compared to controls for the first week following cessation of peptide treatment (P<0.05). Food intake values returned to control levels by week 6, and were indistinguishable from controls for the remainder of the study. The transient increase in food intake did not result in a rebound effect on body weight above controls; the body weight of these rats returned to control levels during the
second week of vehicle treatment (week 6) and remained indistinguishable from controls for the remainder of the experiment. For those rats continuing amylin treatment through week 8, the reductions in caloric intake and body weight gain were maintained (P’s<0.05).

**Experiment 3—Meal Pattern Analysis:** Analysis of the first meal at the onset of the dark-cycle showed a significant reduction in meal size and feeding rate in amylin versus control-treated rats (P’s<0.05, Table 2). The satiety ratio (the IMI divided by meal size) was also significantly increased with amylin treatment (P<0.05). There was no effect of amylin on latency, duration, or IMI.

**Experiments 4 and 5—Effect of Amylin on Locomotor Activity and Kaolin Consumption:** Following acute IP administration, amylin dose-dependently reduced food intake compared to controls, with significant reductions at 10 and 100 µg/kg doses (P’s<0.05, Figure 4A). At these same doses, amylin had no effect on distance traveled or number of rears (Figure 4B and C). In the kaolin consumption model, the known emetic agent cisplatin significantly increased kaolin consumption and decreased food intake 24 h post injection (P<0.05, Figure 5A and B). Amylin at 100 µg/kg did not elicit pica behavior at 24 hr post injection but significantly suppressed food intake at 2 and 24 hr post injection (P’s <0.05, Figure 5A and B).

**Experiment 6—Effect of Amylin on Food Choice and Energy Expenditure:** For total caloric intake (both diets combined), a main effect of Treatment and a Treatment X Week interaction were observed (P’s<0.05). Post-hoc analysis showed amylin-treated rats ate significantly less than controls during weeks 1-6 and week 8 (P’s<0.05, Figure
6A). By week 11, consumption levels were equal to those of control rats. Total intake at 11 weeks was significantly lower in amylin versus control treated rats (vehicle=6315 ± 111 kcal versus amylin= 5309 ± 202 kcal, P<0.05). As to food preferences, a main effect of Treatment and a Treatment X Week interaction were observed for high fat intake. At all time points, with the exception of week 11, amylin significantly reduced high fat intake (P’s<0.05, Figure 6B). Amylin also significantly increased low fat intake across the 11-weeks, indicated by a main effect of Treatment (P<0.05, Figure 6C). Total low fat intake at 11 weeks was significantly greater in amylin versus vehicle-treated rats (vehicle=270±42 versus amylin=633±146, P<0.05). Consequently, there was a main effect of Treatment for the percent of low fat food consumed, with a greater percentage of low fat kcal eaten with amylin treatment (P<0.05, Figure 6D). For body weight gain, a main effect of Treatment and a Treatment X Week interaction was observed (P’s<0.05); body weight gain was significantly decreased with amylin compared to controls at all time points (P’s<0.05, Figure 6E). The vehicle-corrected body weight loss at 11 weeks was 16%.

Figure 7 depicts baseline body composition (Figure 7A), terminal body composition (Figure 7b), the change in body composition (Figure 7C), and the percent change from baseline (Figure 7D) for both groups. For both the change in body composition and the change from baseline measures, amylin significantly increased fat mass compared to controls (P’s<0.05) There was no effect of amylin on lean tissue.
Changes in energy expenditure were also observed during both light and dark cycles of the 24-hr test. Compared to vehicle, a main effect of amylin (P<0.05) was observed, demonstrating a significant increase in 24-hr energy expenditure (Figure 8A). After the removal of food and start of recording, we observed in both groups a relatively sharp drop in energy expenditure which appeared to stabilize by 3 hours. For RQ, there was a main effect of light/dark cycle (P<0.05), with dark cycle RQ being significantly lower than light cycle RQ (Figure 8B). There was also a main effect of amylin on RQ (P<0.05); RQ was higher in amylin versus control-treated rats during both periods of the day.

Discussion

Several experiments were conducted to better understand the mechanism by which amylin influences body weight gain in rodents. The current results demonstrate for the first time a dose-dependent and durable effect (up to 11 wks) of sustained amylin infusion in rats to reduce body weight in a fat-specific manner. Furthermore, there was no rebound body weight gain (above control levels) following amylin withdrawal. Meal pattern analysis and side-effect modeling, in addition to human clinical data (27) point to satiation-related mechanisms as mediators of this effect. Lastly, it was demonstrated that increased metabolic rate and relative preference for standard versus palatable chow may also contribute to amylin-induced weight loss.

After 4 weeks of amylin infusion, a 14 % vehicle-corrected body weight loss was achieved at the highest dose tested, with an ED50 for body weight gain of 16.5 μg/kg/d.
Importantly, the durability of this effect was demonstrated for up to 11 wks of amylin treatment (16% body weight loss). It is noteworthy that on none of the test days did intake in amylin-treated rats exceed that observed in vehicle treated controls. Thus, when food intake is viewed as cumulative intake, amylin-treated rats overall eat less, implying that it is not a transient effect and the animals are still in net caloric deficit with treatment.

There also were no negative consequences on body weight and composition following amylin withdrawal. Following cessation of amylin after 4 wks of treatment, the body weight gain of treated rats returned to control levels within 2 wks and remained at controls levels at 4 wks after withdrawal.

With chronic infusion, the magnitude of amylin’s effects on food intake and body weight decreased with time, possibly reflecting activation of other metabolic pathways to compensate for the negative state of energy balance. Due to its short half-life, amylin was administered via SCV infusion as a proof of concept for its potential use as an anti-obesity agent. Whether similar effects would be obtained with discrete dosing in rodents has not been investigated. Recent data have shown that intermittent administration of the peptide PYY3-36 is more effective at reducing food intake and body weight than is continuous infusion (28).

The current study also provides compelling evidence for a direct role of amylin in regulating food intake. Previous studies examining possible nonhomeostatic effects of amylin on food intake have employed the taste-aversion paradigm and have consistently reported a lack of a conditioned taste aversion following anorectic doses of peripheral
amylin (8, 9). The current findings show that at doses of IP-administered amylin that reduce food intake, amylin had no effect on either locomotor activity or kaolin consumption (a preclinical marker of emesis). Although it is difficult to generalize from assays of acute intake and locomotor activity to long-term effects on body weight, it is likely that the acute dosing used herein achieved much higher (albeit transiently) plasma levels of amylin than in our minipump studies (up to 300 μg/kg/d).

Administration of a single IP dose of amylin (100 μg/kg) also was found to reduce the size of the first meal after the onset of the dark-cycle. Previously, a selective reduction in meal size with amylin (5 μg/kg, IP) in rats was reported (7). However, the present report also found amylin to reduce the feeding rate and increase the satiety ratio. These differences between the studies may be due to the dose used, as we employed a 20-fold higher dose, and differences in potency for feeding microstructure variables have been observed following acute treatment with amylin or amylin receptor agonists (7, 29, 30). Consistent among these reports, however, is the finding that meal size is virtually always reduced, and this effect occurs at lower doses than the effects on other feeding variables.

Amylin also produced a durable effect on food preference across 11 weeks of treatment, shifting food preference, compared to controls, from a high fat chow to a more nutritionally balanced low fat chow. This is interesting in light of the fact that total intake (both diets combined) of amylin treated rats had reached control values by the end of the study. Thus, while total intake was statistically equal in amylin and control rats during the latter part of the study, the relative preference for low fat chow was maintained. Previously, studies have shown reduced preference for chocolate in mice
with the amylin agonist salmon calcitonin (31), and decreased preference for palatable sugar and increased preference for standard chow with amylin following restraint stress in rats (32). Thus, the effect of amylin on food preference can now be extended to normal, unstressed rats. Furthermore, the durability of amylin to reduce body weight gain for up to 3 weeks, with a specific loss in fat mass (16) can now be extended for up to 11 weeks of treatment. The magnitude of the reported reduction in body weight gain in the current studies is similar to that previously observed with the same dose of amylin in both lean (~365 g) and in bred-DIO prone (~585 g) rats (16), as well as weight-stable retired female breeder rats (33).

In this same study, amylin increased 24-hr metabolic rate in fasted rats after 11 weeks of treatment, suggesting that metabolic rate in rats treated with amylin was higher than would be expected given the same amount of weight loss in rats treated with vehicle. In parallel to the current results, a previous report in satiated rats showed increased metabolic rate in amylin treated, but not pair-fed rats, following 3 weeks of SC amylin infusion (100 µg/kg/d) (16). The initial apparent drop in energy expenditure seen in both groups is likely not due to the removal of food from the cage since rats are not commonly eating at this time of the day. Rather, the initial level of energy expenditure was probably high for this time of the day, likely due to a hyperthermic effect that resulted from disturbance created by the experimenter's entry into the room and removal of food from the cages. Paradoxically, RQ was increased with amylin treatment, indicating a shift from metabolism of fat tissue to lean tissue. However, lean tissue mass was unaffected by amylin treatment compared to controls, so the extent of metabolism of lean mass is
unclear. It is important to be mindful of the fact that the energy expenditure-related measures described here represent a snapshot of the complete 11-week time period for this study.

Recent data from a 6-week clinical study in obese patients treated SC three times daily (before meals) with pramlintide (180 μg), a synthetic analog of amylin, have also shown significant and durable decreases in food intake and body weight (34). Consistent with the current findings in preclinical models, the effect of pramlintide on food intake and body weight were dissociable from the occurrence of nausea. The amylin plasma concentrations producing body weight effects in rats (EC₅₀=264 pM) are within range of effective pramlintide concentrations in humans: in normal weight (70-75 kg) patients with type I diabetes, antidiabetic doses of pramlintide (100-300 ug) result in peak plasma concentrations of 75-200 pM 30 minutes post SC injection (35).

**Perspectives and Significance**

Peptide hormone therapeutics like amylin, with their ability to harness the body’s natural energy homeostatic signaling pathways, represent a promising potential therapeutic avenue. However, there has been limited efficacy achieved with current monotherapy approaches to obesity, likely attributable to redundant and counter-regulatory adaptations within neurohormonal pathways mediating energy balance. Although clinical studies with the amylin analog pramlintide suggest that some peptide hormones may have therapeutic potential as a monotherapy, there is increasing evidence of the potential of combinatorial
regimens. Recently, we reported that various combinations of several peptides (amylin, leptin and PYY$_{3-36}$) resulted in additive and/or synergistic interactions and caused marked weight loss in the diet-induced obese rat model (36, 37, 38). If confirmed in ongoing translational clinical studies, these findings may provide a rationale for a novel, integrated neurohormonal approach to pharmacotherapy for obesity.

385 **Acknowledgements**

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synergy and weight loss additivity. Endocrinology, e pub ahead of print,
doi:10.1210/en.2007-0898

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Multihormonal treatment with amylin, PYY (3-36), and leptin elicited marked,
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A57-58, 2006.
Figure 1. Weekly food intake (A) and cumulative body weight gain (B) of high fat-fed rats during 4 week sustained SC infusion of 3, 10, 30, 100 or 300 µg/kg/d of amylin (n=5-13/group). *P<0.05 compared to Vehicle at each time point.

Figure 2. Dose-response relationship for body weight gain of high fat-fed rats following 4 week sustained SC infusion of amylin (ED$_{50}$ = 16.5 µg/kg/d ± 0.05 log units).

Figure 3. Weekly food intake (A) and cumulative body weight gain of rats (B) during 8 week sustained SC infusion of amylin (100 µg/kg/d), or 4 week treatment with amylin (100 µg/kg/d) followed by 4 week treatment with Vehicle (n=6-9/group). *P<0.05 compared to Vehicle group at each time point.

Figure 4. 30 min food intake (A) and 30 min locomotor activity (B and C) in chow-fed rats following IP administration of 1, 10 or 100 µg/kg of amylin (n=5-7/group). *P<0.05 compared to Vehicle group.

Figure 5. 24 hr kaolin intake (A) and 2 and 24 hr food intake (B) in rats following IP administration of cisplatin (3 mg/kg) or amylin (300 µg/kg(n=6/group). *P<0.05 compared to Vehicle.

Figure 6. Weekly total food intake (both diets combined) (A), high fat intake (B), low fat intake (C), percent of food intake from the low fat diet (D), and cumulative body weight
gain (E) of rats that could self select between a high fat or low fat diet during 11 weeks of sustained SC infusion of amylin (300 µg/kg/d, n=7-8/group. *P<0.05 compared to Vehicle group at each time point.

Figure 7. Baseline (A), terminal (B), change from baseline (C), and percent change from baseline (D) fat mass and lean mass in rats receiving 11.5 weeks of SC infusion of amylin (300 µg/kg/d)(n=7-8/group). *P<0.05 compared to Vehicle group.

Figure 8. 24-hour metabolic rate (A) and respiratory quotient (B) of rats treated for 11.5 weeks with amylin (300µg/kg/d, n=7-8/group). There was a main effect of amylin for metabolic rate, and main effect of amylin and time (light/dark) for RQ (*P<0.05 compared to Vehicle group).
Figure 1

A

Food Intake (kcalories)

Week

B

Body Weight Gain (grams)

Week
Figure 2

- Weight Gain at 4 Weeks (grams)
- log dose amylin (µg/kg/d)
Figure 3

A

Food Intake (kcalories)

Week

Vehicle
Amylin / Vehicle
Amylin / Amylin reimplant (amylin or vehicle)

B

Body Weight Gain (grams)

Week
Figure 4

A

Vehicle

0

Food Intake (grams)

B

Distance (inches)

C

Number of Rears

0

100

2000

3000

4000

5000

100

250

50

125

25
Figure 5

A

B
Figure 6

A

**Total Food Intake**

- Main effect of Treatment \( (P<0.05) \)
- Treatment X Week Interaction \( (P<0.05) \)

B

**High Fat Intake**

- Main effect of Treatment \( (P<0.05) \)
- Treatment X Week Interaction \( (P<0.05) \)
C

Low Fat Intake

Main effect of Treatment (P<0.05)

D

Percent of Kcal from Low Fat Diet

Main effect of Treatment (P<0.05)

E

Body Weight Gain

Main effect of Treatment (P<0.05)
Treatment X Week Interaction (P<0.05)
Figure 7

A. Baseline Body Composition

B. Terminal Body Composition

C. Change in Body Composition

D. Percent Change From Baseline
Figure 8

A

*Group | Light       | Dark       |
-------|-------------|------------|
Vehicle| 4.95 ± 0.06 | 4.94 ± 0.11|
Amylin | 5.44 ± 0.15 | 5.13 ± 0.10|

*P<0.05 for main effect of Amylin
### B

![Graph showing RQ over intervals (~13 minutes).]

<table>
<thead>
<tr>
<th>*Group</th>
<th><strong>Light</strong></th>
<th><strong>Dark</strong></th>
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</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.749 ± 0.004</td>
<td>0.719 ± 0.003</td>
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<tr>
<td>Amylin</td>
<td>0.775 ± 0.004</td>
<td>0.741 ± 0.004</td>
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*P<0.05 for main effect of Amylin

**P<0.05 for main effect of Light/Dark Cycle
Table 1. Plasma amylin concentrations in high fat-fed rats following 28-day sustained SC infusion of amylin.

<table>
<thead>
<tr>
<th>Dose (ug/kg/d)</th>
<th>Plasma Amylin Concentration (pM)</th>
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<tr>
<td>Vehicle</td>
<td>6.7 [0.8]</td>
</tr>
<tr>
<td>3</td>
<td>19.1 [2.7]</td>
</tr>
<tr>
<td>10</td>
<td>68.3 [18.6]</td>
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<tr>
<td>30</td>
<td>147.4 [33.9]</td>
</tr>
</tbody>
</table>

Table 2. First-meal analysis of rats receiving a single IP injection of amylin (100 µg/kg) at the onset of the dark cycle.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle</th>
<th>Amylin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency (min)</td>
<td>7.9 [2.5]</td>
<td>8.1 [4.8]</td>
</tr>
<tr>
<td>Meal size (g)</td>
<td>1.50 [0.12]</td>
<td>0.79* [0.16]</td>
</tr>
<tr>
<td>Duration</td>
<td>4.38 [1.59]</td>
<td>6.14 [1.91]</td>
</tr>
<tr>
<td>Rate</td>
<td>0.59 [0.15]</td>
<td>0.21* [0.06]</td>
</tr>
<tr>
<td>Intermeal Interval (IMI, in min)</td>
<td>53.6 [6.0]</td>
<td>54.9 [11.8]</td>
</tr>
<tr>
<td>Satiety Ratio (IMI/meal size)</td>
<td>38.1 [6.5]</td>
<td>72.8* [11.2]</td>
</tr>
</tbody>
</table>

*P<0.05 compared to Vehicle; n=7-8/group