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Pharmacological actions of the peptide hormone amylin in the long-term regulation of food intake, food preference, and body weight

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Mack C, Wilson J, Athanacio J, Reynolds J, Laugero K, Guss S, Vu C, Roth J, Parkes D. Pharmacological actions of the peptide hormone amylin in the long-term regulation of food intake, food preference, and body weight. *Am J Physiol Regul Integr Comp Physiol* 293: R1855–R1863, 2007. First published September 12, 2007; doi:10.1152/ajpregu.00297.2007.—The ability of amylin to reduce acute food intake in rodents is well established. Longer-term administration in rats (up to 24 days) 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 wk) 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-week subcutaneous amylin infusion of high-fat fed rats (3–300 $\mu g \cdot kg^{-1} \cdot day^{-1}$) dose dependently reduced food intake and body weight gain (ED_{50} for body weight gain = 16.5 $\mu g \cdot kg^{-1} \cdot day^{-1}$). The effect of amylin on body weight gain was durable for up to 11 wks and was associated with a specific loss of fat mass and increased metabolic rate. The body weight of rats withdrawn from amylin (100 $\mu g \cdot kg^{-1} \cdot day^{-1}$) after 4 wks of infusion returned to control levels 2 wks after treatment cessation, but did not rebound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 $\mu g \cdot kg^{-1} \cdot day^{-1}$) consistently chose a larger percentage of calories from the low-fat diet vs. 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.

body composition; meal size; locomotor activity; kaolin consumption; pramlintide

AMYLIN IS A PANCREATIC β-CELL hormone secreted simultaneously 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 (38). 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 (6, 17, 36).

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 (for a review, see Ref. 11). This reduction in food intake may contribute to amylin's ability to regulate postprandial 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 (23). Specifically, it has been established that amylin produces its anorexigenic effects through reducing meal size (12). Importantly, decreased food intake has been shown to occur in the absence of a conditioned taste aversion (19, 32). Amylin receptor antagonism also inhibits amylin suppression of food intake (8, 18, 22, 33) as well as stimulates feeding in normal, untreated animals (18, 22), 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 (34). More informative of the role of amylin are data showing reduced body weight gain during 14-day peripheral (subcutaneous) amylin infusion (48 $\mu g \cdot kg^{-1} \cdot day^{-1}$) in rats maintained on a diet of moderate fat content (13), while 24-day subcutaneous amylin infusion (300 $\mu g \cdot kg^{-1} \cdot day^{-1}$) also durably reduced body weight gain with a selective reduction in fat tissue in diet-induced obesity-prone rats (27). Furthermore, Arnelo et al. (1) reported a dose-dependent effect of amylin on body weight in lean rats following 8 days of subcutaneous infusion. These data, coupled with the finding that amylin knockout mice display a greater rate of weight gain compared with wild-type mice (4, 7), 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 wk) on food intake, food preference, body weight and 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 subcutaneous infusion is established. Second, the effect of amylin on food intake and body weight following

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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. Last, the effect of amylin on food preference and energy expenditure is examined. A portion of this data has appeared in abstract form (14–16, 20, 24, 37).

METHODS

All experiments were approved by the Institutional Animal Care and Use Committee at Amylin Pharmaceuticals 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 12:12-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 diet-induced obesity-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, cat. no. D12331; Research Diets, New Brunswick, NJ) throughout the course of the experiment. Amylin was dissolved in 50% DMSO and delivered (flow rate = 2.5 μl/h) via 4-wk osmotic pumps (model 2ML4; Durect, Cupertino, CA). Pumps were implanted subcutaneously 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, cat. no. 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, cat. no. D1251M; Research Diets). Kaolin pellets (*experiment 6*) were supplied by Research Diets (cat. no. K5001). All drugs were administered as a single intraperitoneal 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 killed by cardiac puncture under isoflurane anesthesia and ~8 ml of blood was collected (EDTA-coated syringe). Whole blood was centrifuged at 1,000 *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 protease inhibitor B 1:100 volume (25 mg/ml chymostatin in DMSO) and stored at −70°C until analysis. Plasma amylin concentrations were quantified by using a two-site sandwich immunoenzymetric assay with fluorescent detection (21).

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 wk prior to treatment after which they were implanted with 4-wk osmotic pumps (n = 5–13/group). One group of rats received 3, 10, or 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ of amylin or vehicle (body wt at implant = 459 ± 4 g, mean ± SE), while another group received 30, 100, or 300 $\mu g \cdot kg^{-1} \cdot day^{-1}$ of amylin or vehicle (body wt at implant = 407 ± 4 g, mean ± SE). Plasma amylin concentrations were assessed at termination in animals treated with 3, 10, and 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ 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 wk and then implanted with 4-wk osmotic pumps delivering amylin (100 $\mu g \cdot kg^{-1} \cdot day^{-1}$) or vehicle (body wt at implantation = 452 ± 4 g, mean ± SE, n = 11–15/group). On *day 28*, all pumps were removed and half of the amylin-treated rats were reimplanted with 4-wk replacement pumps containing amylin (100 $\mu g \cdot kg^{-1} \cdot day^{-1}$). The remaining amylin-treated rats and all vehicle-treated rats were reimplanted with 4-wk pumps containing vehicle. The total treatment period was 8 wk.

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 intraperitoneal injection of vehicle prior to the onset of the dark cycle. The test chamber consists of a 13 × 10 × 9-inch Plexiglas cage equipped with a tunnel with a food hopper at the end. As the animal ate, food intake was measured at 1-min intervals via automated changes in scale weight (Dietpro Software, Accuscan Instruments, Columbus, OH). On test day, all rats received an intraperitoneal 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, intermeal 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.

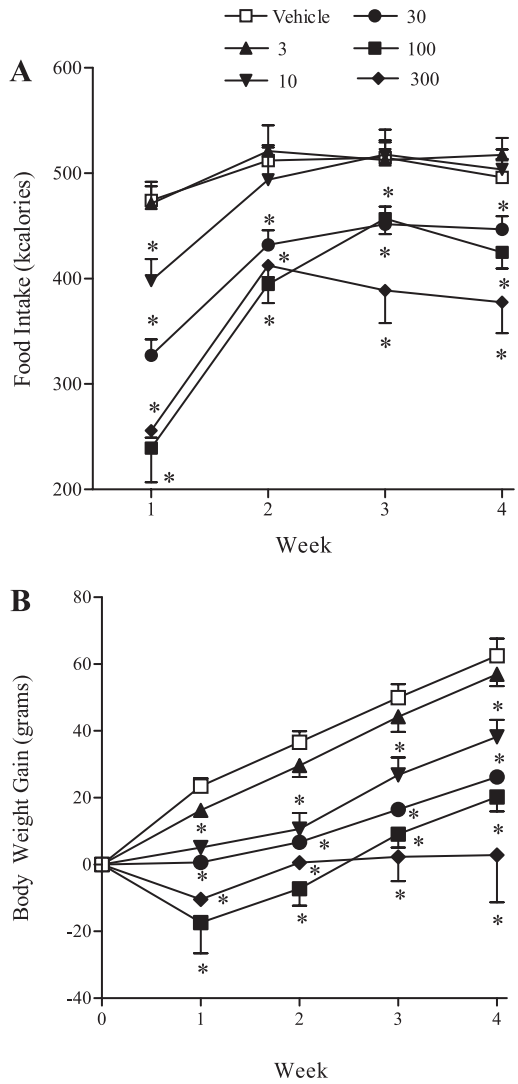

Fig. 1. Weekly food intake (*A*) and cumulative body weight gain (*B*) of high-fat fed rats during 4-wk sustained subcutaneous infusion of 3, 10, 30, 100, or 300 $\mu g \cdot kg^{-1} \cdot day^{-1}$ of amylin (n = 5–13/group). *$P < 0.05$ compared with vehicle at each time point.

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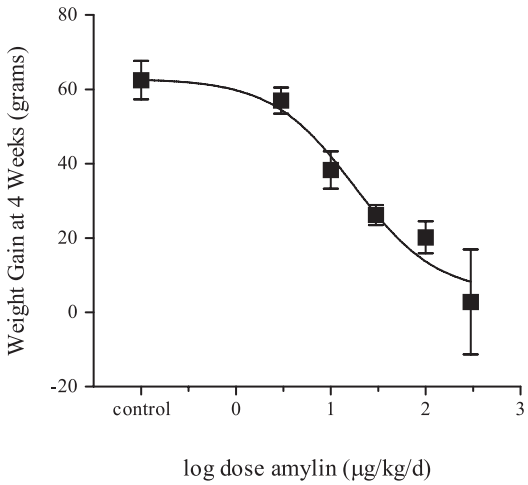

Fig. 2. Dose-response relationship for body weight gain of high-fat fed rats following 4-wk sustained subcutaneous infusion of amylin (ED_{50} = 16.5 $\mu g \cdot kg^{-1} \cdot day^{-1}$ ± 0.05 log units).

Experiment 4. Assessment of locomotor activity. After an overnight fast, rats (400–440 g) were administered an intraperitoneal 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 traveled and vertical movements (rearing) were measured 30 min postinjection by using a locomotor activity monitor (Smart Frame Units; Hamilton-Kinder, San Diego, CA) consisting of a bi-level 4 × 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 postinjection.

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 intraperitoneal 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 METHODS) 2 wk prior to initiation of the study. Animals remained on this regimen for the duration of the study. On the test day, rats (494 ± 11 g) were scanned in an MRI (Echo MRI, Houston, Texas) to establish basal body composition and were then implanted with osmotic pumps containing amylin (300 $\mu g \cdot kg^{-1} \cdot day^{-1}$) or vehicle ($n$ = 7–8/group). Animals were reimplanted with 4-wk pumps at the beginning of *weeks 5* and *9*. Food intake and body weight were measured weekly for 11 wk. At 11.5 wk, 24-h energy expenditure was assessed by 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 h. Metabolic rate and

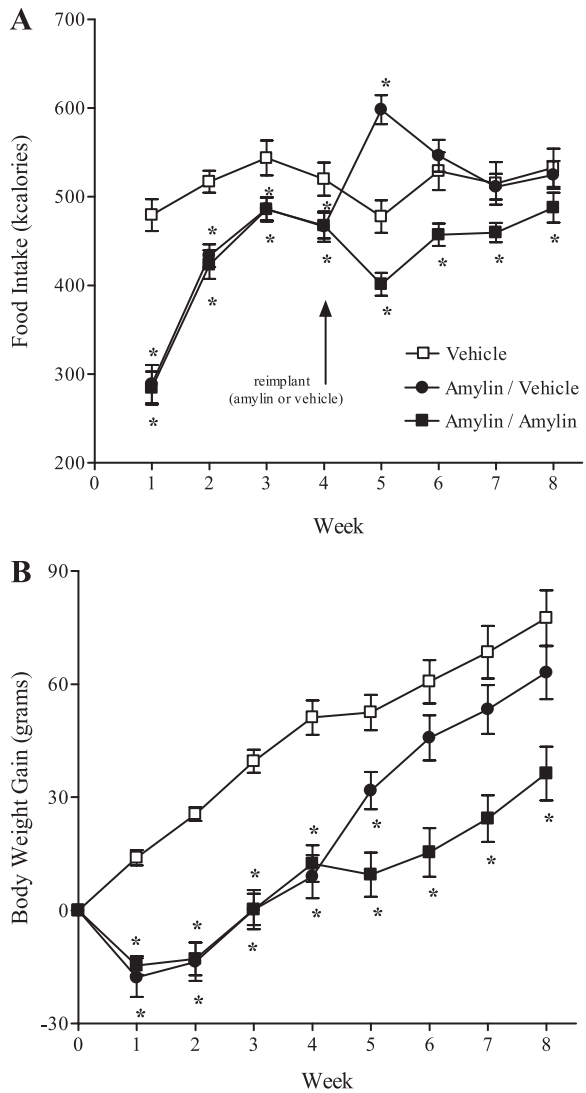

Fig. 3. Weekly food intake (*A*) and cumulative body weight gain of rats (*B*) during 8-wk sustained subcutaneous infusion of amylin (100 $\mu g \cdot kg^{-1} \cdot day^{-1}$) or 4-wk treatment with amylin (100 $\mu g \cdot kg^{-1} \cdot day^{-1}$) followed by 4-wk treatment with vehicle (n = 6–9/group). *P < 0.05 compared with vehicle group at each time point.

Table 1. *Plasma amylin concentrations in high-fat fed rats following 28-day sustained subcutaneous infusion of amylin*

Dose	Plasma Amylin Concentration
Vehicle	6.7 [0.8]
3	19.1 [2.7]
10	68.3 [18.6]
30	147.4 [33.9]

Values are means ± SE. Doses are in micrograms per kilograms per day, and concentrations are in picomolar.

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

Parameter	Vehicle	Amylin
Latency, min	7.9 [2.5]	8.1 [4.8]
Meal size, g	1.50 [0.12]	0.79 [0.16]*
Duration	4.38 [1.59]	6.14 [1.91]
Rate	0.59 [0.15]	0.21 [0.06]*
Intermeal interval, IMI, in min	53.6 [6.0]	54.9 [11.8]
Satiety ratio, IMI/meal size	38.1 [6.5]	72.8 [11.2]*

Values are means ± SE. *P < 0.05 compared to vehicle; n = 7–8 rats/group.

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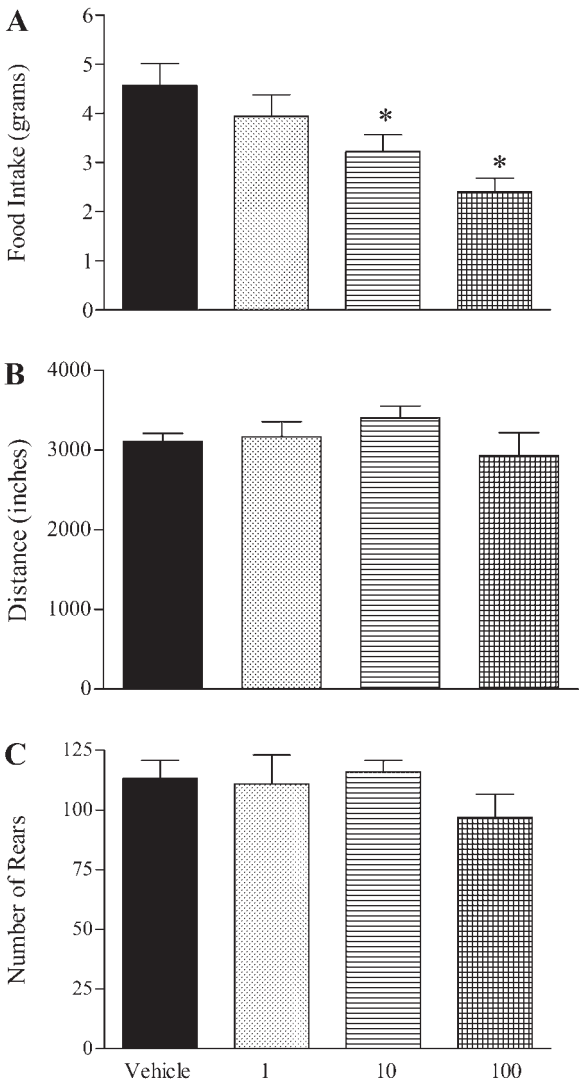

Fig. 4. Thirty-minute food intake (*A*) and 30-min locomotor activity (*B* and *C*) in chow-fed rats following intraperitoneal administration of 1, 10, or 100 μg/kg of amylin (n = 5–7/group). *$P < 0.05$ compared with vehicle group.

respiratory quotient (RQ) were then measured the following 24 h 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 means + SE. Group differences were analyzed using 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 $\mu g \cdot kg^{-1} \cdot day^{-1}$ of amylin and the other examining 30–300 $\mu g \cdot kg^{-1} \cdot day^{-1}$ 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 vs. 66.7 ± 6.4 g, at 4 wk, $P > .05$), 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 300 $\mu g \cdot kg^{-1} \cdot day^{-1}$ compared with controls throughout the 4 wk of treatment ($P < 0.05$, Fig. 1*A*). Subsequently, body weight gain was dose dependently reduced with amylin (Fig. 1*B*) with a maximal vehicle-corrected body weight loss of 14% at 4 wk. Rats treated with doses of ≥10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ had significantly less weight gain throughout the study ($P < 0.05$). The ED_{50} for reduction in body weight gain at 4 wk was 16.5 $\mu g \cdot kg^{-1} \cdot day^{-1}$ ± 0.05 log units (Fig. 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 with controls during the first 4 wk of 100 $\mu g \cdot kg^{-1} \cdot day^{-1}$ of amylin treatment ($P < 0.05$; Fig. 3, *A* and *B*). Following withdrawal of amylin at the end of 4 wk, caloric intake significantly increased compared with 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 ($P < 0.05$).

Experiment 3. Meal pattern analysis. Analysis of the first meal at the onset of the dark-cycle showed a significant

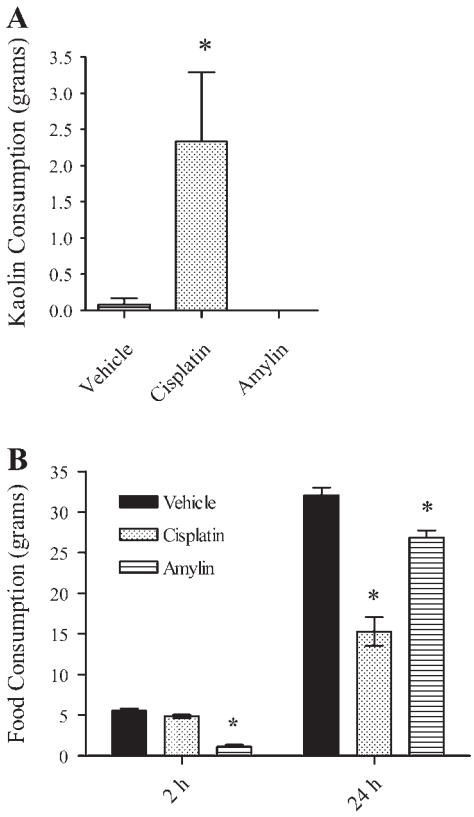

Fig. 5. Twenty-four-hour kaolin intake (*A*) and 2- and 24-h food intake (*B*) in rats following intraperitoneal administration of cisplatin (3 mg/kg) or amylin (300 μg/kg; n = 6/group). *$P < 0.05$ compared with vehicle.

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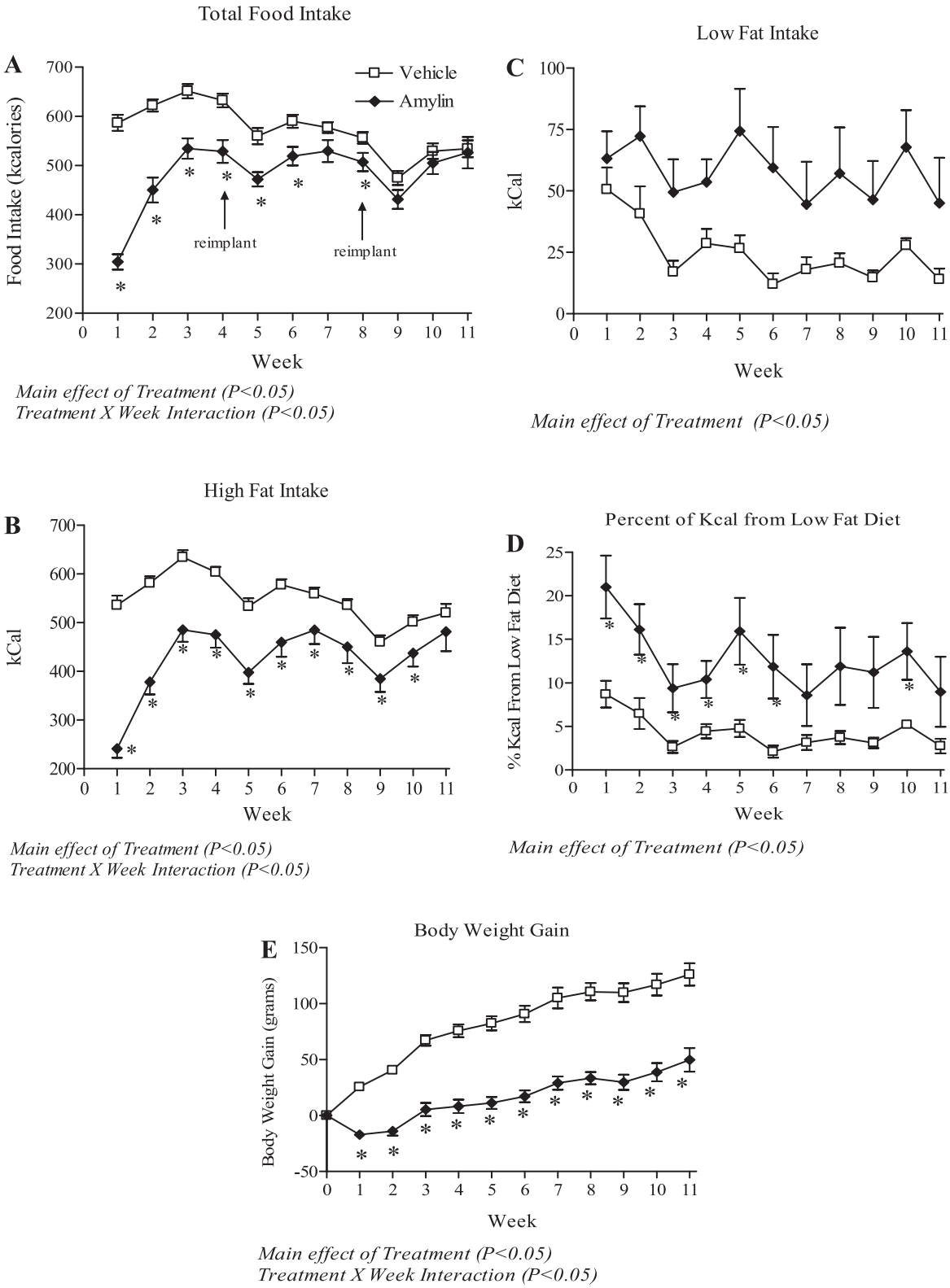

Fig. 6. Weekly total food intake (both diets combined) (*A*), high-fat intake (*B*), low-fat intake (*C*), percent of food intake from the low-fat diet (*D*), and cumulative body weight gain (*E*) of rats that could self select between a high-fat or low-fat diet during 11 wk of sustained SC infusion of amylin (300 $\mu g \cdot kg^{-1} \cdot day^{-1}$, n = 7–8/group). $*P < 0.05$ compared with vehicle group at each time point.

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reduction in meal size and feeding rate in amylin vs. control-treated rats ($P < 0.05$; Table 2). The satiety ratio (the intermeal interval divided by meal size) was also significantly increased with amylin treatment ($P < 0.05$). There was no effect of amylin on latency, duration, or intermeal interval.

Experiments 4 and 5. Effect of amylin on locomotor activity and kaolin consumption. Following acute intraperitoneal administration, amylin dose dependently reduced food intake compared with controls, with significant reductions at 10 and 100 μg/kg doses ($P < 0.05$; Fig. 4*A*). At these same doses, amylin had no effect on distance traveled or number of rears (Fig. 4, *B* and *C*). In the kaolin consumption model, the known emetic agent cisplatin significantly increased kaolin consumption and decreased food intake 24 h postinjection ($P < 0.05$; Fig. 5, *A* and *B*). Amylin at 100 μg/kg did not elicit pica behavior at 24 h postinjection but significantly suppressed food intake at 2 and 24 h postinjection ($P < 0.05$; Fig. 5, *A* 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 by week interaction were observed ($P < 0.05$). Post hoc analysis showed amylin-treated rats ate significantly less than controls during *weeks 1–6* and *week 8* ($P < 0.05$; Fig. 6*A*). By *week 11*, consumption levels were equal to those of control rats. Total intake at 11 wk was significantly lower in amylin- vs. control-treated rats (vehicle = 6,315 ± 111 kcal vs. amylin = 5,309 ± 202 kcal, $P < 0.05$). As to food preferences, a main effect of treatment and a treatment by week interaction were observed for high-fat intake. At all time points, with the exception of *week 11*, amylin significantly reduced high-fat intake ($P < 0.05$, Fig. 6*B*). Amylin also significantly increased low-fat intake across the 11 wk, indicated by a main effect of treatment ($P < 0.05$; Fig. 6*C*). Total low-fat intake at 11 wk was significantly greater in amylin- vs. vehicle-treated rats (vehicle = 270 ± 42 vs. 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 ($P < 0.05$, Fig. 6*D*). For body weight gain, a main effect of treatment and a treatment by week interaction was observed ($P < 0.05$); body weight gain was significantly decreased with amylin compared with controls at all time points ($P < 0.05$; Fig. 6*E*). The vehicle-corrected body weight loss at 11 wk was 16%.

Figure 7 depicts baseline body composition (Figure 7*A*), terminal body composition (Fig. 7*B*), the change in body composition (Fig. 7*C*), and the percent change from baseline (Fig. 7*D*) for both groups. For both the change in body composition and the change from baseline measures, amylin significantly increased fat mass compared with controls ($P < 0.05$). 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-h test. Compared with

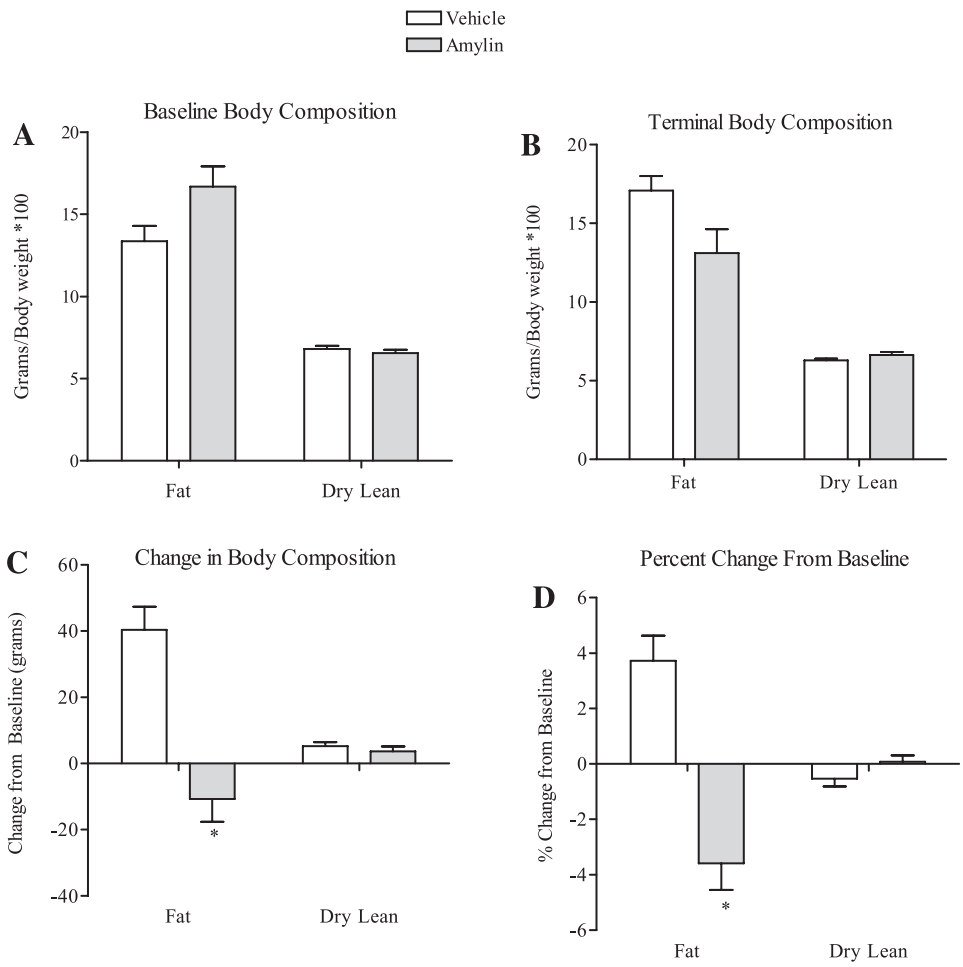

Fig. 7. Baseline (*A*), terminal (*B*), change from baseline (*C*), and %change from baseline (*D*) fat mass and lean mass in rats receiving 11.5 wk of subcutaneous infusion of amylin (300 $\mu g \cdot kg^{-1} \cdot day^{-1}$)($n$ = 7–8/group). *$P < 0.05$ compared with vehicle group.

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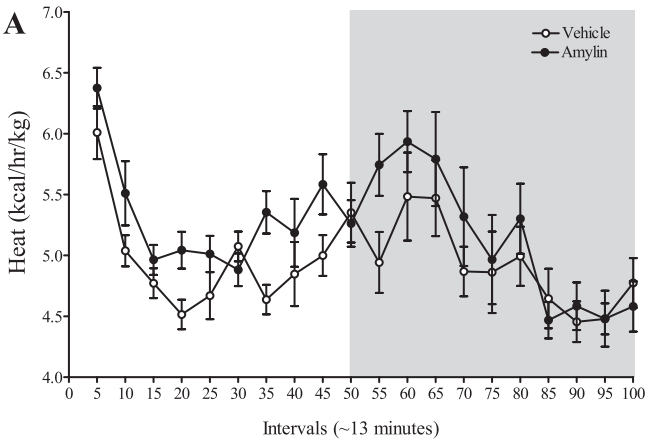
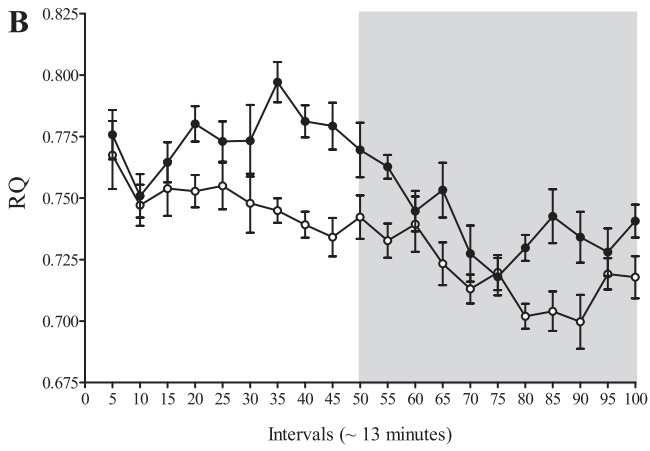
vehicle, a main effect of amylin ($P < 0.05$) was observed, demonstrating a significant increase in 24-h energy expenditure (Fig. 8*A*). After the removal of food and start of recording, we observed in both groups a relatively sharp drop in energy expenditure, which appeared to stabilize by 3 h. For RQ, there was a main effect of light-dark cycle ($P < 0.05$), with dark cycle RQ being significantly lower than light cycle RQ (Fig. 8*B*). There was also a main effect of amylin on RQ ($P < 0.05$); RQ was higher in amylin vs. control-treated rats during both periods of the day.



*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



*Group	**Light	Dark
Vehicle	0.749 ± 0.004	0.719 ± 0.003
Amylin	0.775 ± 0.004	0.741 ± 0.004

*P<0.05 for main effect of Amylin
**P<0.05 for main effect of Light/Dark Cycle

Fig. 8. Twenty-four-hour metabolic rate (*A*) and respiratory quotient (RQ; *B*) of rats treated for 11.5 wk with amylin (300 $\mu g \cdot kg^{-1} \cdot day^{-1}$, 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 with vehicle group.

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 (2), points to satiation-related mechanisms as mediators of this effect. Lastly, it was demonstrated that increased metabolic rate and relative preference for standard vs. palatable chow may also contribute to amylin-induced weight loss.

After 4 wk of amylin infusion, a 14% vehicle-corrected body weight loss was achieved at the highest dose tested, with an ED_{50} for body weight gain of 16.5 $\mu g \cdot kg^{-1} \cdot day^{-1}$. 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 subcutaneous infusion as a proof of concept for its potential use as an antiobesity 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 (3).

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 (19, 32). The current findings show that at doses of intraperitoneally 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 $\mu g \cdot kg^{-1} \cdot day^{-1}$).

Administration of a single intraperitoneal 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 (12). However, the present report also found amylin to reduce the feeding rate and increase the satiety ratio. These

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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 (12, 25, 26). 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 wk of treatment, shifting food preference, compared with 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 (5), and decreased preference for palatable sugar, and increased preference for standard chow with amylin following restraint stress in rats (10). 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 wk, with a specific loss in fat mass (27) can now be extended for up to 11 wk of treatment. The magnitude of the reported reduction in body weight gain in the present studies is similar to that previously observed with the same dose of amylin in both lean (~365 g) and inbred diet-induced obesity-prone (~585 g) rats (27), as well as weight-stable retired female breeder rats (28).

In this same study, amylin increased 24-h metabolic rate in fasted rats after 11 wk 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 wk of subcutaneous amylin infusion (100 $\mu g \cdot kg^{-1} \cdot day^{-1}$) (27). 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 with 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-wk clinical study in obese patients treated subcutaneously 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 (35). 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 (EC_{50} = 264 pM) are within range of effective pramlintide concentrations in humans: in normal weight (70–75 kg) patients with type 1 diabetes, antidiabetic doses of pramlintide (100–300 μg) result in peak plasma concentrations of 75–200 pM 30 min postsubcutaneous injection (9).

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 counterregulatory 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 PYY3-36) resulted in additive and/or synergistic interactions and caused marked weight loss in the diet-induced obese rat model (29–31). If confirmed in ongoing translational clinical studies, these findings may provide a rationale for a novel, integrated neurohormonal approach to pharmacotherapy for obesity.

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