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CENTRAL AMYLIN ACTS AS AN ADIPOSITY SIGNAL TO CONTROL BODY WEIGHT AND ENERGY EXPENDITURE

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Abstract

The pancreatic B-cell hormone amylin has been proposed to be both a satiation signal and an adiposity signal. The effects of peripheral amylin on energy balance are well investigated, but the effects of central amylin are less clear. We determined the effects of low doses of amylin administered into the 3rd cerebral ventricle (i3vt) on food intake, body weight and other indices of energy balance. Amylin (2 pmol/h) significantly lowered body weight compared to saline after 2 weeks of infusion, independent of whether prior body weight was decreased by fasting, increased by voluntary overfeeding or unmanipulated. A bolus injection of amylin (10 pmol, i3vt) increased energy expenditure and body temperature, whereas chronic i3vt amylin infusion had no effect on energy expenditure above that of control rats even though body temperature was increased. Chronic amylin also reduced RQ, implying a preferential oxidation of fat. Overall, the data provide new evidence that amylin is an adiposity signal that acts within the brain, and informing the brain about the status of peripheral energy stores.

Keywords

amylin; fasting; overfeeding; energy expenditure; adiposity signal

INTRODUCTION

Amylin is a peptide hormone co-secreted with insulin by pancreatic B-cells in response to food intake (6). Analogous to cholecystokinin (CCK) (11), amylin exhibits the characteristics of a satiation signal; i.e., acute peripheral amylin administration reduces food intake dose-dependently, mainly by reducing meal size (18). This effect is not secondary to

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DISCLOSURE

The authors have nothing to disclose

a conditioned taste aversion, nor to unspecific effects such as a reduction of water intake (18;24). Further, antagonizing the effect of endogenous amylin stimulates eating, mainly by increasing meal size (31). The area postrema (AP) plays an important role in the anorectic effect of peripheral amylin. A lesion of the AP blocks the anorectic effect of peripherally injected amylin (20), and when an amylin antagonist is infused into the AP, food intake is increased due to an augmented meal size (23).

In addition to its action as an acute satiation signal (18;21), amylin also has body weight-lowering effects as seen during chronic administration in rats or during repeated administration of the amylin analogue pramlintide in humans (1;2;14;19;27;34;35). For these and other reasons, amylin has been proposed to act as an adiposity signal (21), similar to leptin and insulin (42). In agreement with this, plasma amylin levels are higher in diet-induced or genetically induced obesity (30), and amylin-deficient mice have increased body weight gain compared to wild-type control mice (12;25). Analogous to leptin, amylin has also been suggested to increase energy expenditure (34;41).

While the effects of systemic amylin have been investigated extensively on food intake and body weight, far less is known about the effects of centrally administered amylin. Previous studies have reported that the necessary effective doses are consistently lower when amylin is infused centrally than when injected peripherally (36). Therefore, because amylin shares properties of both an acutely-acting satiation signal and a long-term adiposity signal, the present experiments were intended to see if these two actions could be dissociated when amylin is administered directly into the brain. The paradigm was based on the work of Chavez et al. (8) who asked similar questions of centrally-administered insulin. We used this approach to determine for the first time the effects of amylin on food intake and body weight in normal rats and in rats that were force under- or overfed. One strategy was to *lower* the body weight of some subjects to a level below that normally achieved by a central amylin infusion, and then to administer amylin to those animals as well as to a normal-weight control group. If amylin functions mainly to reduce food intake, both groups should eat less when first administered amylin; conversely, if amylin acts in the brain to help maintain a lower-than-normal body weight (i.e., if it acts as an adiposity signal), the animals whose weight is already reduced should eat normally or even increase their food intake when administered central amylin. The second strategy was to *increase* body weight a priori and then determine the effect of amylin. We hypothesized that a given amount of amylin in the brain would determine a particular level of body weight maintained independent of the starting weight.

MATERIALS AND METHODS

Animals and housing

Male Wistar rats (Elevage Janvier, Le-Genest-St. Isle, France) weighing 300-330 g at the beginning of the studies were individually housed in wire-mesh cages under an artificial 12 h / 12 h light-dark cycle (lights on 0300 h) and at a room temperature of $21 \pm 2^\circ \text{C}$. Water and low-fat pelleted chow (GLP 3430, 13.8 kJ/g Provimi Kliba AG, Kaiseraugst, Switzerland) were available *ad libitum* unless otherwise stated. For indirect calorimetry, rats were housed in Plexiglas air-tight cages ($41 \times 41 \times 31 \text{cm}$) on a layer of wood shavings. Powdered chow (GLP 3433 [same composition as GLP 3430], Provimi Kliba AG, Kaiseraugst, Switzerland) and water were available *ad libitum* unless otherwise stated. All rats were adapted to the housing conditions for at least one week before experiments. Experiments were approved by the Veterinary Office of the Canton Zurich, Switzerland.

Implantation of i3vt brain cannula and transmitters

Because the critical amylin receptors necessary for reducing food intake are located in the area postrema adjacent to the 4th ventricle, and because amylin reduces food intake when administered into the 3rd ventricle (36;38;39), we opted to access the area from the 3rd ventricle and take advantage of the normal flow of CSF to allow the amylin to reach the presumed receptive area. Rats were implanted with a 22-gauge stainless-steel guide cannula (Plastics One, Roanoke, VA) into the 3rd-cerebral ventricle (i3vt) 2.2 mm posterior to bregma and 7.5 mm ventral to dura (29). One week after surgery, cannula placement was confirmed by a positive dipsogenic response to 10 ng angiotensin II (Sigma-Aldrich, Buchs, Switzerland) in 2 μ l of saline. Animals that did not drink at least 5 ml of water within 60 min after angiotensin II were excluded. For assessment of indirect calorimetry, some rats were implanted at the same time with a transmitter (TA-F40, Data Science International, St. Paul, MN) into the peritoneal cavity (IP). This transmitter allowed telemetric assessment of body temperature and physical activity.

Indirect calorimetry

An open circuit calorimetry system (AccuScan Inc., Columbus, OH) was used in which room air was passed through each cage with a flow rate of approximately 2 L/min. Outcoming air was sampled for 20 sec every 5 min for each cage and analyzed for O₂ and CO₂ concentration. Food and water intake were measured continuously. Data were analyzed with AccuScan Integra ME software. Energy expenditure was calculated for each 5-min sample according to Weir (40) using the following equation, with O₂ consumption and CO₂ production normalized for body weight on the day of measurement: total energy expenditure (kcal/kg/h) = $3.9 \times V(\text{O}_2)\text{L/h} + 1.1 \times V(\text{CO}_2)\text{L/h}$. The means over 30 min and 60 min were calculated for each individual animal and expressed as kcal/kg/h. The respiratory quotient (RQ) was defined as the quotient of CO₂ production and O₂ consumption. Body temperature and physical activity were simultaneously monitored by the DataScience ART4.0 telemetry system (DataScience International, St. Paul, MN).

Experimental designs

Experiment 1 – Effects of chronic central amylin infusion on body weight and food intake in rats fasted for 48 h and then refed—While under brief isoflurane anaesthesia, rats with verified i3vt cannulas were implanted subcutaneously with osmotic minipumps (Alzet 2002, DURECT Corporation, Cupertino, CA, pumping 0.5 μ L/h) filled with either amylin (to deliver 2 pmol/h; Bachem AG, Bubendorf, Switzerland) or saline. The pumps were connected to an injector inserted into the guide cannula and that extended 1 mm beyond the guide cannula. Groups matched for body weight received i3vt amylin or saline beginning on Day 0. After implantation, one amylin group (FA: n = 7) and one saline group (FS: n = 7) were fasted for 48 h and then refed *ad libitum* with chow. The other amylin (AA: n = 9) and saline groups (AS: n = 6) were fed *ad libitum* throughout. Food intake and body weight were monitored daily (1 h prior to lights off) for 11 days. Energy efficiency was calculated by dividing body weight gain (in g) by the amount of ingested energy (in kcal).

In a separate cohort of comparable rats, 48-h fasting led to a body weight loss of approximately 40 g. While this decrease was in part due to the emptying of the entire gastrointestinal tract, total fat mass (subcutaneous and intra-abdominal) was reduced by about 10% from about 32 g to 29 g. Subcutaneous and intra-abdominal adipose tissue were analyzed in anesthetized rats between vertebrae L1 and L6 with a rodent computerized tomography (CT) scanner (La Theta, LCT-100, Aloka, Tokyo, Japan). This method provides accurate estimates of total subcutaneous and intra-abdominal fat pads as validated by dissection (13). Of note, lean body mass as determined here includes gut contents.

Experiment 2 – Effects of chronic central amylin infusion on body weight and food intake in rats after 3 weeks of voluntary overfeeding—Rats with verified i3vt cannulas were divided into two groups matched for body weight. One group had *ad libitum* access to chocolate flavored Ensure® Plus (5.8 kJ/g, kindly provided by Abbott AG, Baar, Switzerland) plus chow for 21 days, and the other was fed chow only. After 21 days, half of each group, again matched for body weight, received minipumps containing saline or amylin (2 pmol/h): chow-saline (CS: n = 8), chow-amylin (CA: n = 7), Ensure®-saline (ES: n = 8) and Ensure®-amylin (EA: n = 8). After implantation (Day 0), all rats received chow only for the following 15 days and food intake and body weight were monitored. At the end of the experiment, rats were fasted for 2 h in the middle of the light phase and anesthetized with pentobarbital sodium (80 mg/kg IP). Cerebrospinal fluid samples were taken from the cisterna magna and blood samples by heart puncture. A protease cocktail inhibitor (P2714, Sigma-Aldrich Fluka Chemie GmbH, Buchs, Switzerland) was used to avoid degradation of amylin. Samples were centrifuged and the supernatant stored at -80°C until further analysis. Amylin concentrations were analyzed using a rat endocrine lincoplex kit (RENDO-85K, Labodia SA, Yens, Switzerland). Plasma leptin was analyzed using a rat leptin radioimmunoassay (RL-83K, Labodia SA, Yens, Switzerland). Subcutaneous and intra-abdominal adipose tissue was analyzed in the frozen carcasses of rats as described above.

Experiment 3 – Effects of acute central amylin on energy expenditure, RQ, body temperature and physical activity—Rats maintained in the indirect calorimetry cages and with verified i3vt cannulas and implanted transmitters were handled and received i3vt saline (2 μL i3vt) daily for several days to adapt to the treatment procedure. On the test day, undeprived rats were administered i3vt saline (2 μL) or amylin (2 or 10 pmol in 2 μL saline) in the middle of the light phase. Rats had no access to food for 3 h after injection. Each rat received each treatment using a randomized cross-over design with at least 4 days between injections. Energy expenditure, RQ, body temperature and physical activity were assessed every 5 min for 3 h after the injections.

Experiment 4 – Effects of chronic central amylin on energy expenditure, RQ, body temperature, physical activity, body weight and food intake—Rats with implanted transmitters were adapted to the indirect calorimetry cages for 7 days. They were then divided into two groups matched for body weight receiving i3vt cannulas (3280P, 28 gauge, Plastics One, Roanoke, VA) connected to osmotic minipumps (model 2001, pumping rate 1 $\mu\text{L}/\text{h}$, Alzet, DURECT Corporation, Cupertino, CA) containing either amylin (10 pmol/h, n = 8) or saline (n = 8). Energy expenditure, RQ, body temperature, physical activity, food intake and water intake were measured every 5 min for 6 days. Body weight was measured daily. Cannula placement was verified by dye injection

Statistical analysis

Data are expressed as mean $\pm$ SEM. Two-factor ANOVA was used to test for the effect of diet (i.e., fasting vs. *ad libitum*; or Ensure® versus chow, respectively) and the effect of treatment (amylin vs. saline). Post-hoc Bonferroni tests were used to test for significant differences between groups (AS vs. AA, FS vs. FA, AS vs. FS and AA vs. FA in experiment 1 and CS vs. CA, ES vs. EA, CS vs. ES and CA vs. EA in experiment 2) for each time point when at least one factor was significantly different. To assess within-subjects effects for a treatment effect over time, a paired t-test was used to test between Day 0 and a specific day during infusion.

Acute energy expenditure, RQ and body temperature data were analyzed with one-way ANOVA with repeated measurements and post-hoc Bonferroni tests. Chronic effects were

analyzed with two-factor ANOVA with the factors time and treatment. Student's t-test was used for post hoc comparison for each time point. $P < 0.05$ was considered significant.

RESULTS

Experiment 1 – Effects of chronic central amylin infusion on body weight and food intake in rats fasted for 48 h and then refed

All groups had comparable baseline body weights on Day 0 (mean 384 g). After two days of fasting, two-factor ANOVA revealed a significant effect of fasting ($F_{1,25} = 63.46$, $P < 0.001$), a significant treatment effect ($F_{1,25} = 5.66$, $P < 0.05$) and a significant interaction ($F_{1,25} = 6.19$, $P < 0.05$). Fasted saline rats (FS) had a significantly lower body weight gain (-40.3 ± 2.4 g) after 48 h of fasting than *ad libitum*-fed saline rats (AS; 1.0 ± 4.3 g) ($t_{25} = 7.053$, $P < 0.001$); and amylin-infused fasted rats (FA; -39.9 ± 4.0 g) had a significantly lower body weight gain than *ad libitum*-fed amylin-infused rats (AA; -18.2 ± 3.5 g) ($t_{25} = 4.08$, $P < 0.001$). Further, AA lost weight relative to AS ($t_{25} = 3.47$, $P < 0.01$), whereas FS and FA did not differ in terms of weight change over the 2 days (see Figure 1A). Over the ensuing infusion period, body weight of FS approached that of AS, and body weight of FA approached that of AA. On Day 11 there was a significant effect of treatment ($F_{1,25} = 20.59$, $P < 0.001$), but not of prior fasting ($F_{1,25} = 9.64$, $P > 0.05$) and no significant interaction ($F_{1,25} = 4.30$, $P > 0.05$). In other words, at the termination of the study, i3vt amylin resulted in a significant reduction of body weight gain of about 25 g, independent of the initial 48-h fasting period.

Food intake data are summarized in Figure 1B. AA ate significantly less than AS on Day 1 ($t_{13} = 2.26$, $P < 0.05$), and on Day 2 the difference just failed to reach significance ($t_{13} = 1.62$, $P > 0.05$). On Day 3, there was a significant effect of diet condition ($F_{1,25} = 4.37$, $P < 0.05$) and of treatment ($F_{1,25} = 7.76$, $P < 0.01$). However, post-hoc tests did not indicate significant differences between individual groups. Analysis of total food intake over Days 3-11 revealed a significant effect of diet condition ($F_{1,25} = 5.96$, $P < 0.05$); i.e., previously fasted rats ate more than *ad libitum*-fed rats; and there was a significant effect of treatment ($F_{1,25} = 8.54$, $P < 0.01$); i.e., amylin-infused rats ate less than saline controls. There was no significant interaction ($F_{1,25} = 4.58$, $P > 0.05$); i.e., the amylin effect was not influenced by the initial fasting period. Post-hoc tests revealed a significant difference between FS and FA ($t_{1,25} = 2.53$, $P < 0.05$) and between FS and AS ($t_{1,25} = 2.10$, $P < 0.05$) (Figure 1B). Energy efficiency was significantly lower ($F_{1,25} = 18.49$; $P < 0.0001$) in amylin-treated rats (0.009 ± 0.007 and 0.006 ± 0.006 g/kcal for *ad libitum*-fed and fasted rats, respectively) compared to saline treated rats (0.035 ± 0.003 and 0.030 ± 0.005 g/kcal for *ad libitum*-fed and fasted rats, respectively).

Experiment 2 – Effects of chronic central amylin infusion on body weight and food intake in rats after 3 weeks of voluntary overfeeding

Baseline body weight was the same for both groups (mean 374 g). Rats with access to Ensure® plus chow gained on average 148.6 ± 8.3 g whereas rats fed chow only gained 87.1 ± 4.5 g ($t_{29} = 6.38$, $P < 0.0001$) over 3 weeks (Days -21 to 0) (Figure 2A). On Days 1 to 4 of the infusion period, there was a significant effect of diet (Day 1: $F_{1,27} = 28.8$, $P < 0.001$; Day 2: $F_{1,27} = 24.2$, $P < 0.001$; Day 3: $F_{1,27} = 18.1$, $P < 0.001$; Day 4: $F_{1,27} = 17.9$, $P < 0.001$), but not of treatment, and no interaction. From Days 5 through 15, both diet and treatment effects were significant, with no interaction between the two factors. On Day 16, there was only a significant treatment effect ($F_{1,27} = 9.6$, $P < 0.01$). In other words, after 16 days of amylin infusion, body weight was significantly lower than in saline controls, independent of the initial Ensure® feeding period; i.e., body weight was similar for CA and EA rats. The body weight difference in rats fed chow (CS-CA) was comparable to that

observed in Experiment 1 (~25 g). In rats with previous access to Ensure® (ES-EA), it was ~35 g; however, considering the higher body weight at initiation of infusion, the relative reduction in body weight by amylin was comparable.

Between Days -21 and 0, mean energy intake (Figure 2B) was significantly higher (approx. 33 kcal or 30% per day, $t_{29} = 7.56$, $P < 0.0001$) in rats with access to Ensure® plus chow compared to rats with chow only. Ensure®-fed rats consumed approximately 85% of their total energy intake from Ensure®. Energy intake of CA rats was lower than that of CS rats from Day 2 until Day 16, although this difference did not always reach significance. Both Ensure® groups had a dramatic decrease in energy intake from Day 1 until the end of the experiment compared to the average energy intake during Ensure® feeding. EA rats consumed only 4 kcal on Day 1 (i.e., about 3% compared to the mean of day -21 to 0). On Days 2 to 15, two-factor ANOVA revealed a treatment effect and a diet effect, but no interaction. This indicates that amylin reduced food intake compared to saline, and that the previous Ensure® overfeeding period resulted in lower food intake compared to chow-fed controls for at least 16 days upon refeeding with only chow. In other words, even at the end of the experiment, 16 days after switching rats back from Ensure® plus chow to chow only, energy intake was still lower than in respective chow-fed controls. The effect of amylin on food intake was similar in rats with or without prior exposure to Ensure®.

At the end of the experiment (Day 16), body adiposity was assessed using a CT-scanner. Total body fat was significantly higher in rats previously fed Ensure® compared to rats fed chow only ($F_{1,27} = 5.58$; $P < 0.05$) (Figure 2C). The difference in total fat was due to qualitatively similar differences in the amount of intra-abdominal fat ($F_{1,27} = 4.60$; $P < 0.05$ for diet and $F_{1,27} = 0.45$; $P > 0.05$ for treatment) and subcutaneous fat ($F_{1,27} = 12.53$; $P < 0.01$ for diet and $F_{1,27} = 0.45$; $P > 0.05$ for treatment), respectively. Post-hoc Bonferroni tests indicated that ES had significantly more total fat ($t_{1,27} = 2.39$, $P < 0.05$) and subcutaneous fat ($t_{1,27} = 3.19$, $P < 0.01$) than CS. Body adiposity did not differ between amylin-treated rats and saline controls ($F_{1,27} = 0.24$; $P = 0.63$). Other differences between groups failed to reach significance. Even though EA had a slightly lower body weight compared to CS on Day 16, EA rats had more subcutaneous fat than CS rats when corrected for body weight ($t_7 = 4.92$, $P < 0.01$). These data indicate that, despite the lack of significant differences in body weight on Day 16, body fat was increased in rats previously fed Ensure® and that amylin had no influence on this differential body adiposity. Plasma leptin levels were not significantly different among groups (Figure 2D), but trends were similar as for total fat.

Cerebrospinal fluid (csf) samples were analyzed for amylin concentration. For each group, 4 csf samples were measured. Amylin-infused rats had an approximately 30-fold increase in csf amylin concentration (301.8 ± 66.6 pM) compared to saline-infused rats (11.2 ± 3.2 pM). Plasma amylin levels did not differ between amylin-infused rats (24.3 ± 5.9 pM) and saline-infused rats (22.3 ± 4.3 pM).

Experiment 3 – Effects of acute central amylin on energy expenditure, RQ, body temperature and physical activity

At a dose of 2 pmol, i3vt amylin had no significant effects on energy expenditure, RQ or body temperature compared to saline. At 10 pmol, i3vt amylin significantly and robustly increased energy expenditure in the first 30 min compared to saline controls ($t_{13} = 5.63$; $P < 0.001$; Figure 3A). The significant difference was maintained between 30 and 60 min ($t_{13} = 2.73$; $P < 0.05$); thereafter, energy expenditure did not differ between groups. RQ was not affected by amylin (data not shown). Mean body temperature ($n = 11$ per group; Figure 3B) had a similar profile as total energy expenditure; i.e., amylin (10 pmol) significantly increased mean body temperature compared to saline for 60 min (0-30 min: $t_{10} = 10.12$; $P <$

0.001; 30-60 min: $t_{10} = 7.77$; $P < 0.001$). Locomotor activity was not influenced by amylin (data not shown).

Experiment 4 - Chronic effects of central amylin on energy expenditure, RQ and body temperature

Food and water intake data of one rat in the amylin group had to be excluded due to technical problems. Amylin significantly lowered body weight gain compared to saline beginning on the first day of infusion ($F_{1,13} = 31.27$; $P < 0.001$) (Figure 4A). Total 6-day food intake was significantly reduced in amylin-treated rats (58.3 ± 13.4 g) compared to saline (105.9 ± 4.7 g; $F_{1,12} = 14.71$; $P < 0.01$). Post hoc analysis on individual days revealed significance on Days 1 and 3 (Figure 4B). Total 6-day water intake was significantly lower in amylin-treated rats (76.3 ± 16.9 g) compared to saline (131.0 ± 6.9 g) ($F_{1,12} = 9.01$; $P < 0.05$), and post hoc analysis on individual days revealed significance on Day 1 (Figure 4C).

Energy expenditure was not significantly influenced by amylin ($F_{1,13} = 1.23$; $P > 0.05$) (Figure 4D). The RQ was lower after the surgery in both treatment groups (baseline vs. dark phase 1: $t_{30} = 8.60$; $P < 0.001$) and slowly increased towards baseline levels. RQ was still significantly lower at the end of the experiment on Day 6 than at baseline ($t_{30} = 3.72$; $P < 0.01$) (fig. 4E). Repeated measures ANOVA indicated that RQ was significantly reduced by amylin compared to saline ($F_{1,13} = 14.88$; $P < 0.01$), with post hoc significant differences on the dark phases of Days 1, 2 and 4. Body temperature was significantly higher in amylin-treated rats compared to controls ($F_{1,13} = 27.15$; $P < 0.001$) (Figure 4F), with post hoc significant differences in the light phase of Day 1 and the dark phase of Day 2.

DISCUSSION

We investigated the effects of centrally administered amylin on various aspects of energy balance in rats. We were especially interested in dissociating the satiating and adiposity-signalling aspects of amylin, and consequently used a modified version of a prior paradigm asking similar questions of insulin (8); i.e., we changed the weight of some groups of rats prior to administering amylin to them. The main findings are that rats centrally infused with amylin had lower body weight gain than saline-infused controls independent of the test situation. We observed this effect for the first time in rats after forced under- and overfeeding; i.e., the effect was observed whether the rats' body weight was initially reduced by fasting during the initial phase of the infusion, whether body weight was increased by Ensure® (over-)feeding prior to the infusion, or if the rats were fed *ad libitum* throughout. These data provide additional novel evidence for amylin having a key role in the brain to control body weight. The body weight-lowering effect was at least partly due to an effect of amylin to reduce energy intake. Furthermore, a single bolus of amylin into the brain also stimulated energy expenditure. However, energy expenditure in rats receiving a chronic central amylin infusion was not different from that of control rats even though body temperature was increased. This has to be considered in view of the lower food intake and body weight gain that was also occurring. RQ was also reduced by chronic i3vt amylin infusion implying an increased oxidation of fat. Ideally, a group of rats pair-fed to the amylin group would have been included to compare levels of energy expenditure achieved following central amylin administration. However, food restriction generally leads to decreased as opposed to increased energy expenditure, as we have previously reported when amylin was administered systemically (41). Hence, we conclude that chronic central amylin prevented the decrease in energy expenditure that would be expected under conditions of lower body weight gain. Consistent with this view, amylin decreased energy efficiency (i.e., the body weight gain that is achieved by a given amount of ingested energy) in Experiment 1.

Rats with chronically elevated amylin in the brain achieved a significantly lower body weight than saline controls in every condition, and the magnitude of the effect was comparable. This supports the hypothesis that amylin functions as an adiposity signal. In this respect, amylin seems to differ fundamentally from “pure” satiation signals like CCK because rats infused continuously with CCK do not have sustained reductions in food intake and body weight (10). Hence, high levels of amylin in the brain may be perceived as being indicative of increased body weight or body adiposity, such that animals administered central amylin consequently initiate metabolic and behavioral responses resulting in reduced food intake and increased energy expenditure and consequently reduced body weight.

The observed effects of i3vt amylin on body weight in chow-fed rats are consistent with earlier data of chronic central amylin infusion that did not involve the paradigm of forced under- and overfeeding (27;38). The data are also consistent with previous findings on female rats with lowered body weight subsequent to food restriction (33). Generally, food intake was significantly lower in all amylin-treated rats. This was particularly clear in Experiment 2, where rats had been initially overfed by providing them access to Ensure®. Thus, we believe that amylin’s persistent action to reduce food intake explains at least partially its body weight-reducing action.

Central infusion of amylin also influenced energy expenditure. When unanesthetized rats without access to food received a bolus injection of amylin into the 3rd cerebral ventricle, energy expenditure was acutely increased. These data are consistent with our unpublished findings that daily peripheral injections of an anorectic dose of the long-acting amylin agonist salmon calcitonin (sCT) to 48-h fasted rats resulted in a larger decrease in body weight than in saline-treated, fasted control rats. Presumably, sCT increased energy expenditure. The data are also consistent with recent findings by Osaka et al. in anesthetized rats (28). Of note, the effective dose used here was two- to ten-fold lower than in the study by Osaka et al., and our rats were left undisturbed in their home cage representing a more physiological situation.

The increase in energy expenditure after acute central amylin was associated with a concomitant increase of body temperature. We presume that the amylin-induced increase in body temperature was due to an action on amylin receptors rather than cross-reactivity at receptors for the related peptide calcitonin-gene related peptide (CGRP). At a dose comparable to that used here, sCT which has high affinity for amylin but not CGRP receptors (e.g., 24), increased body temperature by about 1.5° C when injected directly into the AP. This increase lasted for about 6 h after administration, irrespective of whether the animals had access to food or not (unpublished). Previous reports also observed an increased body temperature following amylin administration into the 3rd ventricle (5) or directly into the paraventricular nucleus of the hypothalamus (PVN) (7). However, the previous studies used much higher doses (10 to 100 times higher) compared to the present study. Increased energy expenditure might be caused by an activated sympatho-adrenergic system (28), but this was not assessed here.

The effect of central amylin on energy expenditure and in particular on body temperature was observed despite the reduction in eating and body weight gain. Interestingly, when rats are chronically food restricted, they more typically reduce their energy expenditure and thermogenesis by down-regulation of uncoupling protein 1 (UCP-1) (26). In the present study, and despite the reduction in food intake and body weight by chronic amylin, total energy expenditure was not altered compared to controls. Hence, it is plausible that chronic central amylin, similar to chronic peripheral amylin (12), not only prevented the decrease in energy expenditure that would otherwise occur with weight loss, but even increased thermogenesis. In line with this possibility are data indicating that chronic peripheral amylin

administration is not associated with a decrease in UCP-1 expression (32;34). Those authors concluded that amylin prevents the compensatory decrease which is normally observed after a reduction in food intake and which was apparent in a pair-fed control group. Hence, even though we did not include a pair-fed control group in our study, we believe that our present data are consistent with the results of Roth et al. (34) and that our conclusions are justified. Interestingly, RQ was decreased in our study, suggesting a shift toward fat oxidation despite unaltered total energy expenditure. While reduced energy intake likely contributed to the reduced RQ, we have previously observed that administering salmon calcitonin, an agonist at the amylin receptor, is able to reduce RQ independent of a change of eating (41).

In our rats, amylin treatment had no influence on physical activity, rendering increased activity unlikely to have contributed to the observed increase in energy expenditure. Previously, Clementi et al. (9) reported that amylin (though at a 100-times higher dose than we used) reduced locomotor activity after central infusion. However, this would result in a decrease rather than an increase in energy expenditure. Further, other studies utilizing peripheral administration of amylin and a more sensitive method of assessing physical activity are consistent with the present study and did not observe effects on locomotor activity (22).

In the first experiment, rats had been initially fasted for 48 h to decrease body weight. This decrease can only partly be explained by a decrease in body fat; a pilot experiment performed under comparable conditions revealed that rats lose about 10% of their total body fat during a 48-h- fast. Presumably, a larger part of the difference in body weight between fasted and ad libitum-fed controls initially was accounted for by reduced gut contents. Gut contents cannot be exactly differentiated from lean body mass by the CT scanning technique used here. Body water was most likely not affected because animals had free access to drinking water and they did not have any sign of dehydration. Hence, under our feeding conditions, we saw some, albeit relatively small decrease in body adiposity during the first two days of the study. It will be instructive to perform similar studies with chronic central amylin infusions in animals that have been food restricted for an extended period of time to lower their adipose mass more than what occurred here.

In our second experiment, intra-abdominal, subcutaneous and total body fat were significantly higher in rats previously fed Ensure®, consistent with reports from Levin et al. (15;16). In those studies, when Ensure® was removed and rats were fed chow only, body weight returned to the level of control rats. However, the retroperitoneal fat depot remained significantly larger than in controls (16). Our findings are comparable because there was no significant difference of body weight between CS and ES rats at the end of infusion even though body fat was significantly higher in ES rats. On the other hand, at the end of infusion body weight was significantly lower in amylin-treated rats, independent of prior exposure to Ensure®, but body adiposity was not affected; i.e., there were no differences in body fat between CS and CA or between ES and EA rats, respectively. Roth et al. reported that rats receiving a chronic peripheral infusion of amylin had a lower percentage of body fat compared to pair-fed controls (33;34), suggesting an effect of peripheral amylin on body adiposity. Those data and our own previous data (37) are not consistent with the observation made here that central amylin did not influence body adiposity. The reasons for this discrepancy are not clear, although we did observe a trend towards reduced adiposity induced by amylin in the Ensure® fed rats. In our previous study (37), fat mass was determined by fat pad dissection while we now used CT scanning tomography. While the CT technique may allow a more complete assessment of body mass and body fat mass than fat dissection, it includes fat located in areas other than specific fat pads; e.g., ectopic fat in any of several organs. At present, we cannot exclude that peripheral but not central amylin can reduce body adiposity. Alternatively, the infusion period may have been too short in our

study to detect significant differences among groups. Chronic i3vt amylin did reduce RQ in our study indicating enhanced fat oxidation which, over longer time periods, may have resulted in lower body adiposity.

We investigated the effects of low doses of *central* amylin on energy balance in rats. Even though amylin is produced only in the periphery, the effects on food intake are known to occur in the brain (17), and several studies have found that amylin is transported across the blood brain barrier (3;4). Acute or chronic i3vt administration of the amylin antagonist AC187 produces profound increases of food intake and body weight, in particular body fat (37). AC187 treatment resulted in a small 5 g increase in total body weight, but body fat mass was increased by about 30% over a two-week period. Brown fat was not tested in that study. Overall, the clear-cut effects of antagonism to endogenous amylin support the hypothesis that amylin's control of body weight occurs in the brain. The exact site of action of i3vt-administered amylin, and the possible effects on the expression of hypothalamic or hindbrain neuropeptides involved in the control of energy balance, need to be addressed in future studies.

Because central amylin seems to control body adiposity, it may contribute to the relative constancy of body weight throughout adult life. The underlying mechanisms remain to be explored. Due to the effect of central amylin on body temperature, this should also include studies that test whether amylin affects UCP expression in brown fat or heat dissipation. Preferably, these studies should include pair-fed controls. Further, the role of endogenous amylin in the effects described in this paper needs to be more thoroughly tested, e.g., by using amylin antagonists or amylin-deficient mice. This should also include tests on the potential development of obesity-related resistance to the effects of amylin, as is the case for the other two adiposity signals leptin and insulin (40). Of note, leptin and insulin resistance are phenomena that at least in part involve disturbed peptide transport across the blood brain barrier; the latter effect is unlikely to be important for amylin which acts at the AP being devoid of this barrier.

In summary, our data indicate that central amylin has an important role in the control of body weight and body adiposity. Chronic amylin infusion leads to lower body weight, independent of increased or decreased initial body weight. Further, central amylin stimulates energy expenditure acutely. Together with higher plasma amylin levels in obese rat models (30), these data support the suggestion that amylin may act as an adiposity signal that informs the brain about body adiposity. Further characteristics of adiposity signals (e.g., whether baseline amylin levels change with body adiposity in static and dynamic situations; i.e., within the same animal that gains or loses body adiposity, and with the same temporal pattern) remain to be investigated in the future.

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Figure 1.

Effects of chronic central infusion of amylin (2 pmol/h) on body weight change relative to the start of the experiment on day 0 (A) and daily energy intake (B). Two groups were fasted for 48 h and then refed ad lib (FS and FA), the two other groups were fed ad lib throughout the entire experiment (AS and AA). Letters indicate significant differences ($P < 0.05$) between the groups (post-hoc comparisons: a = AS vs. AA, b = FS vs. FA, c = AS vs. FS and d = AA vs. FA).

Figure 2.

Effects of chronic central infusion of amylin (2 pmol/h) on body weight change (A), energy intake (B), body fat (C) and plasma leptin (D) after a period of voluntary overfeeding with Ensure®. Rats had ad libitum access to Ensure® in addition to chow, or to chow only. On day 0, rats were implanted with minipumps infusing either amylin or saline into the third ventricle. Ensure® was removed and all rats had access to chow only for the remainder of the study. Letters indicate significant differences ($P<0.05$) between groups in the post-hoc comparisons: a = CS vs. CA, b = ES vs. EA, c = CS vs. ES and d = CA vs. EA. * $P<0.05$ intra-abdominal fat CS vs. ES, ** $P<0.01$ subcutaneous fat CS vs. ES.

Figure 3.

Energy expenditure (A) and mean body temperature (B) after an acute central amylin injection. Baseline measurements show energy expenditure and body temperature of the hour prior to injection. * P<0.05, *** P<0.001 vs saline.

Figure 4.

Effects of 6 days of amylin infusion (2 pmol/h i3vt) on A) body weight change, B) food intake, C) water intake, D) energy expenditure, E) respiratory quotient and F) body temperature. In figures D-F, the gray shading represents the dark phase, the white shading the light phase. No data are shown for the light phase of the day of surgery, because the rats had to be removed from the cages and data were therefore incomplete. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.