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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.

1. 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 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;

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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.

2. Materials and methods

2.1. 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 ± 2 °C. Water and low-fat pelleted chow (GLP 3430, 13.8 kJ/g [3.3 kcal/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 × 41 × 31 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.

2.2. Implantation of 3rd ventricle 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 μL 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.

2.3. 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 s every 5 min for each cage and analyzed for O_2 and CO_2 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 O_2 consumption and CO_2 production normalized for body weight on the day of measurement: total energy expenditure $(\text{kcal/kg/h}) = 3.9 \times V(O_2)\text{L/h} + 1.1 \times V(CO_2)\text{L/h}$. 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 CO_2 production and O_2 consumption. Body temperature and physical activity were simultaneously monitored by the DataScience ART4.0 telemetry system (DataScience International, St. Paul, MN).

2.4. Experimental designs

2.4.1. 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 μL/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 was 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.

2.4.2. 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 [1.38 kcal/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.

2.4.3. 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