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Amylin and GLP-1 target different populations of area postrema neurons that are both modulated by nutrient stimuli

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HIGHLIGHTS

- Protein attenuates amylin-induced hypophagia and activation of the area postrema.
- Fasting enhances GLP-1 dependent activation of the area postrema.
- Amylin activates other the area postrema neurons compared to GLP-1, LiCl or AngII.

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ABSTRACT

The area postrema mediates the hypophagic effect of the pancreatic hormone amylin and is also sensitive to glucagon-like peptide 1 (GLP-1). Protein seems to modulate amylin responsiveness because amylin seems to produce a stronger hypophagic effect and a stronger c-Fos expression when protein is absent from the diet. Accordingly, amylin induces a stronger c-Fos expression in the AP when injected in fasted compared to ad libitum fed rats. Here we tested the hypothesis that diet-derived protein attenuates the amylin dependent suppression of feeding and AP activation using isocaloric diets that differed in their protein content. Moreover, we investigated whether peripheral amino acid injection attenuates amylin-induced c-Fos expression in fasted rats. Since recent evidence suggests that GLP-1 may also reduce eating via the AP we tested whether 24 h fasting also increases neuronal AP responsiveness to GLP-1 similar to the fasting-induced increase in amylin responsiveness. Finally, we used the calcitonin receptor (CTR) as an immunohistochemical marker for amylin-receptive AP neurons to investigate whether amylin's target neurons differ from GLP-1 responsive AP neurons. We also dissociated amylin responsive cells from neurons implicated in other AP-mediated functions such as aversion or blood pressure regulation. For this purpose, we conducted c-Fos/CTR double staining after LiCl or angiotensin II treatment, respectively. Amylin (5 μg/kg s.c.) was more effective to reduce the intake of a 1% vs. an 8% or 18% protein diet and to induce c-Fos expression in the AP in rats receiving 1% vs. 18% protein diet. Increased protein intake was associated with increased blood amino acid levels. Peripheral injection of amino acids (1 g/kg i.p.) attenuated the amylin-induced AP activation in 24 h fasted rats. Similar to amylin, GLP-1 (100 μg/kg i.p.) elicited a significant c-Fos response only in fasted but not in ad libitum fed rats. However, in contrast to a high co-localization of amylin-induced c-Fos and CTR (68%), no c-Fos/CTR co-localization occurred after treatment with GLP-1 or the GLP-1R agonist exendin 4 (2 μg/kg ip). Similarly, LiCl (76 mg/kg ip) or AngII (50 μg/kg sc) led to c-Fos expression only in CTR negative AP neurons.
In conclusion, our findings support a protein-dependent modulation of behavioral and neuronal amylin responsiveness under equicaloric feeding conditions. Amino acids might contribute to the inhibitory effect of diet-derived protein to reduce amylin-induced neuronal AP activation. Neuronal AP responsiveness to GLP-1 is also increased in the fasted state suggesting that diet-derived nutrients may also interfere with AP-mediated GLP-1 effects. Nevertheless, the primary target neurons for amylin appear to be distinct from cells targeted by GLP-1 and by stimuli producing aversion (LiCl) or contributing to blood pressure regulation (AngII) via the AP. Since amylin and GLP-1 analogs are targets for the treatment of obesity, the nutrient-dependent modulation of AP responsiveness might entail implications for such therapeutic approaches.

1. Introduction

The pancreatic hormone amylin acts as a satiation signal via an excitation of area postrema (AP) neurons [1,2]. Peripheral injection

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of amylin induces a c-Fos expression in noradrenergic AP neurons. Similar to a complete AP ablation [3], a specific chemical lesion of noradrenergic AP neurons blocks amylin's hypophagic affect [4]. The functional amylin receptor consists of the calcitonin receptor (CTR) as core component co-expressed with receptor activity modifying protein 1 or 3 (RAMP1, RAMP3) [5,6]. The CTR and RAMP3 are expressed in the AP [7,8]. We recently demonstrated that amylin induces a stronger c-Fos expression in 24 h fasted vs. ad libitum fed rats [9]. This effect appeared to depend on diet-derived protein because the amylin-induced c-Fos expression was attenuated when rats were fed a non-caloric diet supplemented with protein while glucose or fat supplementation did not affect the amylin response [9].

In our current work, we extended these studies by using isocaloric diets with different protein content (1%, 8%, and 18%) in order to minimize the possible influence of the diet's caloric density. Differences in protein intake are likely to result in altered levels of circulating amino acids, which might exert a direct or indirect signaling function modulating the central amylin sensitivity. Due to the lack of a functional blood–brain-barrier in the AP, blood-borne amino acids might directly act on AP neurons. In order to assess the possible role of circulating amino acids in the modulation of the responsiveness of AP neurons to amylin we investigated whether the amylin-induced c-Fos response in the AP of fasted rats is attenuated by intraperitoneal administration of amino acids.

The AP may also represent a target structure for glucagon-like peptide 1 (GLP-1). GLP-1 is released by L-cells in response to nutrient stimuli [10,11]. In addition, GLP-1 can also be released locally in the AP from GLP-1ergic nerve terminals of enteroceptive neurons located in the nucleus of the solitary tract (NTS) [12,13]. GLP-1 receptor (GLP-1R) mRNA is highly abundant in the AP and peripherally injected radiolabeled GLP-1 binds to these receptors [12,14]. Both exogenous GLP-1 and the GLP-1R agonist exendin-4 induce a c-Fos response in the AP [15] and both peptides reduce meal size in rats [16]. The AP mediates the hypophagic effect of GLP-1 infused into the hepatic portal vein [17]. Due to the similarities between amylin and GLP-1 we hypothesized that neuronal GLP-1 responsiveness of the AP might also be affected by the feeding status. To test this we compared the GLP-1-induced c-Fos expression in fasted vs. ad libitum fed rats.

The AP is not only involved in the physiological control of eating, but also has a crucial function as a so-called chemoreceptor trigger zone for the sensing and processing of aversive and nausea-inducing stimuli [18]. Unlike amylin [1,19], GLP-1 plays a role in the induction of nausea and conditioned taste aversion [20]. Interestingly, c-Fos expression induced in the AP by the aversive stimulus lithium chloride (LiCl) is mediated by GLP-1 [21]. The divergence in the aversive responses induced by amylin and GLP-1 suggests the existence of discrete subpopulations of AP neurons that are targeted by amylin or GLP-1, respectively. Assuming that amylin-responsive AP neurons express the CTR we conducted immunohistological CTR/c-Fos double staining studies in order to analyze whether GLP-1 or its agonist exendin-4 activate AP neurons that differ from cells that are activated by amylin. As a commonly used model for aversion, we also included LiCl-treated animals in these studies. Furthermore, we used angiotensin II (AngII) as an additional functional stimulus because AngII is thought to primarily modulate the autonomic control of blood pressure but not feeding behavior via the AP [22].

2. Materials and methods

2.1. Animals and housing conditions

Male Wistar rats (Elevage Janvier, Le Genest-St-Isle, France) were used in all experiments. Their mean body weight was 250 g at the start of the experiments. The rats were adapted to the housing conditions and to handling for 10 days before the experiments started. The animals had ad libitum access to tap water and to standard laboratory rodent chow (#3430, 18.5% protein; Provimi Kliba, Gossau, Switzerland) except during periods when the animals received specific test diets or during food deprivation as described below. All rats were individually housed in hanging, stainless steel wire cages (50 × 25 × 18 cm) under controlled conditions of illumination (12:12 h light–dark cycle), humidity and temperature (21 ± 1 °C). In some experiments isocaloric custom-made diets (Provimi Kliba AG; Kaiseraugst, Switzerland) were used which differed in their protein (18%, 8%, and 1%) but not fat content. Caloric density (15.1–15.4 MJ/kg) was adjusted by variable contents of carbohydrates (mainly starch). With marginal differences the 18% protein diet had a nutrient composition that corresponded to the standard chow. The Veterinary Office of the Kanton Zurich, Switzerland, approved all animal procedures and experiments.

2.2. Effect of diet-derived protein on amylin-induced hypophagia and c-Fos expression in the AP

In order to adapt the animals to the respective test diets, the 1%, 8%, or 18% protein diets were offered on 2 single days during a 7–10 day habituation period. Before the feeding trials that were conducted in intervals of 5 days, the animals received regular chow for 3 days, chow in combination with the respective test diet for one day and only the test diet for another day. At dark onset, amylin (5 μg/kg s.c.; Bachem, Bubendorf, Switzerland) or saline (control) were injected; for the feeding tests, treatments were conducted in a crossover design for each diet in weight-matched groups. Cumulative food intake was measured 30 min, 1 h and 2 h after injection with a precision of 0.1 g and correction for spillage. The animals were kept on the diet until 24 h after the injection and then switched back to chow. The amylin-induced c-Fos expression was investigated in separate groups of rats fed 1% vs. 18% protein diet. The adaptation of the animals and the treatments were the same as for the feeding trials.

2.3. Effect of amino acids on amylin-induced c-Fos expression in the AP

To test the effect of circulating amino acids on the amylin-induced c-Fos expression in the AP the amino acid solution Aminoven (Fresenius Kabi Schweiz AG, Oberdorf, Switzerland) was used. Aminoven contains 16 different amino acids at a concentration of 15% and is used for medical purposes in parenteral nutrition. Four weight-matched groups of rats including a saline control group (n = 5), an amylin-treated group (n = 7), an Aminoven control group (n = 8) and a group that was treated with Aminoven and amylin (n = 7) were used.

The animals were food-deprived for 24 h and injected with Aminoven (1 g/kg i.p.) or saline 20 min before dark onset. At dark onset, amylin (5 μg/kg s.c.) or saline were injected. Two hours after these treatments, the animals were deeply anesthetized and transcardially perfused with ice-cold phosphate buffer solution (PB 0.1 M, pH 7.2) followed by 4% paraformaldehyde solution (4% PB 0.1 M) for fixation. Blood was sampled and the glucose concentration was measured as described below. The immunohistological detection of c-Fos is described in Section 2.5.

2.4. Measurements of blood glucose, amino acids and amylin levels

To measure the effects of the different protein diets on blood glucose and amino acid levels, blood samples were taken at dark onset from the lingual vein under isoflurane anesthesia. The experimental conditions were the same as in the respective feeding trials and c-Fos studies. Immediately after blood sampling, the concentration of glucose was measured using a glucose-oxidase based glucometer (Glucometer Elite; Bayer, Zürich).

The blood was then transferred into serum tubes (Microvette 500 Z, Sarstedt, Germany) and centrifuged at 2800 rpm for 10 min. A protease inhibitor mixture (P 2714, Sigma-Aldrich) was immediately