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Enterostatin: A gut-brain peptide regulating fat intake in rat

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Summary – The effect of enterostatin on high-fat food intake has been investigated. After 18 h of food deprivation, rats were injected intravenously with enterostatin (VPGPR). A dose of 38 nmol of enterostatin gave a significant inhibition of high-fat food intake, while at a higher dose of 76 nmol the inhibiting effect was lost. During the first hour, after injection of enterostatin, there was even a slight increase in food intake. Binding studies of tritiated enterostatin to crude brain membranes indicated one binding site with high affinity (K_d = 0.5 nM) and one with low affinity (K_d = 170 nM). The two dissociation constants suggest different receptor subtypes and could explain why enterostatin both can inhibit and, at high doses, stimulate fat intake in rats.

binding / membranes / food intake / procolipase / pancreas

Introduction

Enterostatin is a peptide produced in the gut by tryptic cleavage of pancreatic procolipase, which has been shown to be a potent inhibitor of food intake (Erlanson-Albertsson and Larsson, 1988). Furthermore, the anorectic effect of enterostatin has been found to be specific for fat (Erlanson-Albertsson *et al*, 1991; Okada *et al*, 1991) as opposed to carbohydrate or protein, being more efficient with a high-fat diet than a low-fat diet.

In rat one molecular form of enterostatin has the structure VPGPR, determined by cloning of procolipase cDNA (Wicker and Puigserver, 1990). In this work we have investigated the feeding response of this peptide in rat using high-fat food. A biphasic dose-response curve was obtained, with an inhibition of fat intake at lower doses and a stimulation at higher doses of enterostatin.

Since enterostatin was shown to be effective when given either centrally (Shargill *et al*, 1991) or peripherally (Mei and Erlanson-Albertsson, 1992), and also found to be absorbed intact through rabbit intestine (Huneau *et al*, 1993), a possible site of action for enterostatin would be the brain. In this paper we have therefore investigated the binding of enterostatin to crude brain membranes. A Scatchard plot indicated a two-site model with dissociation constants 0.5 nM and 30 nM. We suggest that high-affinity binding is involved in inhibition of feeding, while the low affinity binding may involve a stimulation of feeding.

Materials and methods

Female Sprague-Dawley rats were obtained from ALAB, Stockholm, Sweden. All rats were housed in individual cages in a room maintained at 22 ± 1°C, with a 12-h light-dark cycle. The rats were adapted to feeding between 9.30 am and 3.30 pm. Free access to water was provided all the time.

Enterostatin was obtained from Senn Chemicals AG, Dielsdorf, Switzerland (> 95% purity by HPLC).

After 9 days of adaption to the above described feeding schedule, the rats were injected with peptide into the tail vein in 1-ml vehicle, control rats only with vehicle. Enterostatin was infused singly in different doses. Immediately after injection, rats were placed in individual cages and provided with a high-fat diet. Food intake was measured after 30, 60, 120, 180, 240 and 360 min.

Preparation of brain membranes

The rats were decapitated and the brains minus cerebelli were rapidly excised and cut into several pieces. The tissue was then homogenized with a teflon-glass homo-

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genizer in 10 volumes of icecold 0.3 M sucrose in Hepes-buffer (25 mM Hepes, 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, pH 7.2) containing a protease inhibitor cocktail (1 mM EDTA, 1 mM PMSF and 10 µg/ml leupeptin). The homogenate was centrifuged at 1 000 *g* for 15 min. The supernatant was saved on ice and the pellet suspended in sucrose-Hepes-buffer followed by centrifugation at 1 000 *g* for 15 min. The supernatants were pooled and centrifuged at 30 000 *g* for 30 min. The pellet was suspended in Hepes-buffer without sucrose and again centrifuged at 30 000 *g* for 30 min. This step was repeated twice. The resulting pellet was suspended in Hepes-buffer to a final protein concentration of 1 mg/ml and stored at –70°C until use. Protein concentrations were determined by the method of Lowry (Lowry *et al*, 1951).

Receptor binding studies

Saturation experiment

Portions of crude brain membranes were incubated in the above described Hepes-buffer, in a final volume of 500 µl, with tritiated enterostatin in different concentrations, at 10°C for 75 min. The non-specific binding was determined by adding 10 µM of unlabelled enterostatin. After incubation, the membranes were transferred to centrifuge tubes, layering 450 µl over 500 µl 50 mM Na-phosphate buffer containing 10% sucrose. The tubes were centrifuged at 10 000 *g* for 5 min, the supernatants aspirated and the pellets washed twice in phosphate buffer. Pellets were then suspended in buffer and transferred to scintillation vials for counting.

Results

Effect of enterostatin given intravenously on high-fat feeding

Enterostatin decreased high-fat food intake at lower doses, while at higher doses there was a slight stimulation of feeding. The biphasic dose-response curve is shown in figure 1. Thus, at a dose of 38 nmol a significant reduction in high-fat food intake was observed ($P < 0.05$, two-way ANOVA), while at a dose of 76 nmol, enterostatin significantly stimulated food intake ($P < 0.05$, two-way ANOVA). Lower doses than 38 nmol gave no significant change in high-fat food intake.

Binding studies

Saturation studies, to crude brain membranes, with increasing concentration of [^{3}H]-enterostatin were performed. The saturation curve indicated a two-site model. Figure 2b shows a Scatchard plot

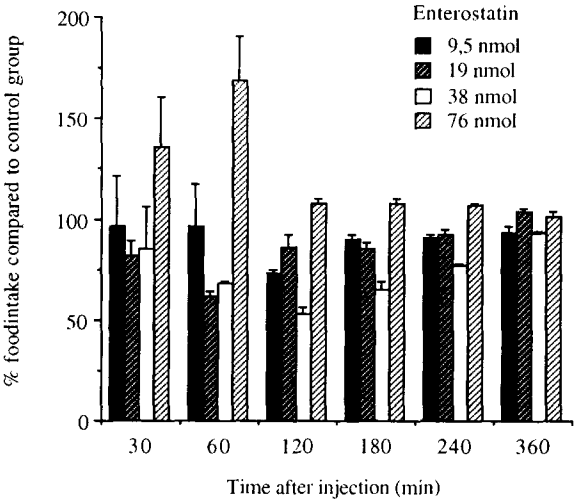

Fig 1. Dose-reponse curve for the effect of enterostatin, when administrated intravenously, on high-fat food intake. The food intake of the control group was set to 100%. Values are expressed as means ± SEM.

reflecting K_d-values of 0.5 nM and 30 nM respectively.

Discussion

In this work we show that enterostatin (VPGPR) after intravenous injection into rat decreases high-fat food intake. The dose-response curve was biphasic, as shown in figure 1, with an inhibition of feeding at low concentrations of enterostatin and no effect or even a slight stimulation of feeding at higher concentrations of enterostatin. The biphasic response of enterostatin is in agreement with previous findings (Erlanson-Albertsson and Larsson, 1988), being observed both after central and peripheral administration of the peptide (Shargill *et al*, 1991; Mei and Erlanson-Albertsson, 1992). In this work we also show for the first time that tritiated enterostatin binds specificially to crude brain membranes. A Scatchard plot indicates two binding sites, one high-affinity binding site (K_d = 0.5 nM) and one low-affinity binding site (K_d = 30 nM).

One major conclusion from this work is that enterostatin has a central site of action, explaining the feeding response of enterostatin when given centrally (Shargill *et al*, 1991; Mei and Erlanson-Albertsson, 1992). Since enterostatin is produced in the intestine (Mei *et al*, 1993) and yet has a

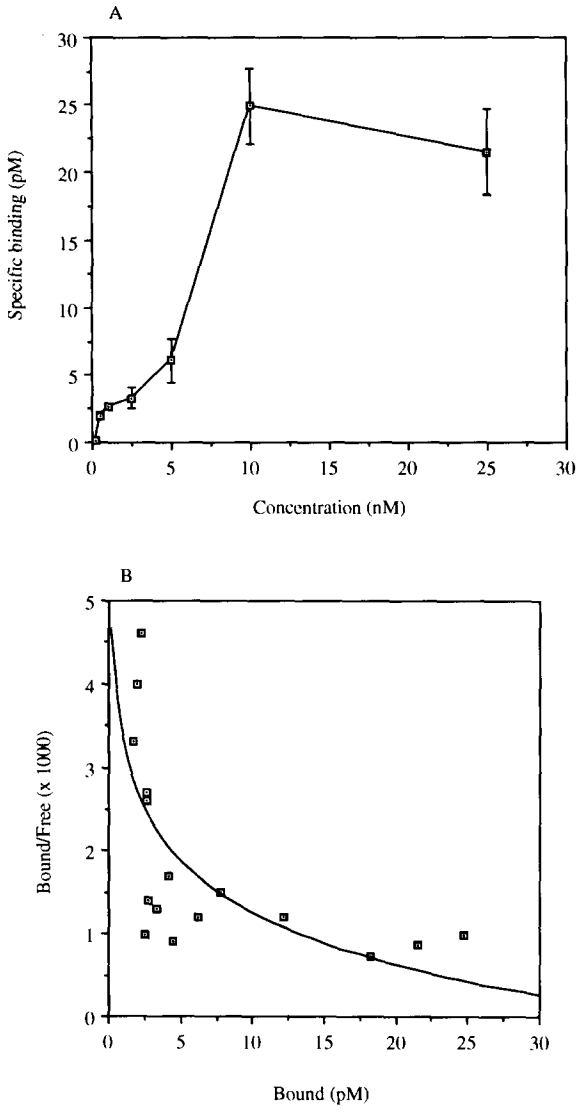

Fig 2. A. Saturation curve for specific binding of [^{3}H]-enterostatin to crude brain membranes. Data represent the average of results from three experiments performed in triplicate. **B.** Scatchard analysis of specific [^{3}H]-enterostatin binding to crude brain membranes indicates a two binding site model with one high affinity site (K_d = 0.5 nM) and one low affinity site (K_d = 30 nM).

central site of action, the peptide is indeed a gut-brain peptide.

Another conclusion from the above work, is that the biphasic feeding response of enterostatin could be explained by the presence of two different receptor subtypes, one with high affinity, being involved in the inhibition of feeding and the other with low affinity, being involved in the slight stimulation of feeding. The identity of the receptor subtypes are presently under investigation. Previous observations suggest the involvement of opiate receptors for enterostatin (Barton *et al*, 1993), possibly in the stimulating feeding response. We cannot at present time exclude that there are also peripheral sites of action for enterostatin, *ie* in the intestine.

Acknowledgment

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