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Research Report

5-HT1B receptors modulate the feeding inhibitory effects of enterostatin

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Abstract

Serotonin (5-HT) is considered to play an important role in control of appetite. Enterostatin has been shown to alter 5-HT release in the brain, and non-specific 5-HT antagonists blocked the anorectic response to icv enterostatin. The aim of this study was to further identify which 5-HT receptor subtype mediates the enterostatin feeding behavior and whether this effect occurs due to action in the PVN. Wild-type and 5-HT2C receptor−/− (KO) mice and normal Sprague–Dawley rats were used in these experiments. All animals were fed a high fat diet. Enterostatin (120 nmol, i.p.) reduced the intake of high fat diet in 5-HT2C receptor mutant mice (saline 4.54 ± 0.47 kcal vs. Ent 2.53 ± 0.76 kcal) 1 h after injection. A selective 5-HT1B antagonist (GR55526, 40 mg/kg body weight, i.p.) blocked the enterostatin hypophagic effects in these KO mice. Rats were implanted with cannulas into the amygdala and the ipsilateral PVN. The 5-HT receptor antagonists metergoline (non-specific receptor subtypes 1 and 2), or ritanserin (selective 2C), or GR55562 (selective l B) was injected into the PVN prior to enterostatin (0.01 nmol) injection into the amygdala. Enterostatin reduced food intake (saline: 5.80 ± 0.59 g vs. enterostatin 3.47 ± 0.56 g, $P < 0.05$ at l h). Pretreatment with either metergoline (10 nmol) or GR55526 (10 nmol) but not ritanserin (10 nmol) into the PVN attenuated the anorectic response to amygdala enterostatin. The data imply that the enterostatin anorectic response may be modulated by 5-HT1B receptors and that a neuronal pathway from the amygdala to the PVN regulates the enterostatin response through activation of 5-HTlB receptors in PVN.

Theme: Neural basis of behavior
Topic: Ingestive behaviors

Keywords: Enterostatin; Amygdala; PVN; 5-HT receptor 1B; Feeding

1. Introduction

Enterostatin, a pentapeptide cleaved from pancreatic procolipase during fat digestion, has been shown to selectively suppress the intake of dietary fat after both peripheral and central administration [8–10]. Eating a high fat diet elevates the levels of enterostatin in the circulation and increases procolipase gene expression [4,38,40]. Procolipase gene is also expressed in the stomach and brain [25,34]. Enterostatin has a conserved sequence containing X-pro-Y-pro-arg in various species, e.g., human, rat, chicken, pig, horse, and hagfish [10,23]. Previous studies have shown that enterostatin reduces the food intake in several animal species, including rat and sheep [8,21,28]. Peripherally, it acts on the stomach or proximal duodenum to reduce food intake through a pathway that depends on afferent vagal nerve activity [24]. Centrally, enterostatin acts in the amygdala and paraventricular nucleus of the hypothalamus (PVN) to suppress feeding, but it is more potent and feeding responses are faster after injection in the amygdala [20,22,23]. We have proposed that the central nucleus of amygdala may be its primary site of action in the central nervous system (CNS).

Serotonin (5-hydroxytryptamine, 5-HT) is considered to play an important role in the control of feeding behavior [2]. 5-HT or its receptor agonists suppress food intake and its antagonists stimulate feeding. At least seven receptor

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subtypes have been identified and each subtype has more than one form [7]. Among these receptors, 5-HT1B and 5-HT2C postsynaptic receptors are currently recognized as subtypes that process within-meal satiation and postmeal satiety [7]. Like enterostatin, 5-HT also preferentially suppresses the intake of fat when animals have dietary choice [3,33] but the receptor subtype responsible for this has not yet been identified. This action of 5-HT has been localized to the paraventricular nucleus [33]. A similar serotonergic effect on fat intake has been described in man [3]. We have previously shown that peripheral enterostatin increased 5-HT release and turnover in several brain regions, including the PVN (unpublished data). In addition, a non-specific 5-HT 1 and 2 receptors antagonist, metergoline, abolished the anorectic effects induced by intracerebroventricular (icv) injection of enterostatin in the rat [42]. The availability of mice lacking functional 5-HT2C receptors [35] and specific 1B receptor antagonist GR55562 [37] now make it possible to further identify the 5-HT receptor subtype that mediates the enterostatin effects. Both receptor subtypes appear to be important to the anorectic response to D-fenfluramine [12,30,36]. We were also interested to know if PVN serotonergic components would have functional connections that were activated by amygdala enterostatin. Therefore, we used 5-HT2C receptor knockout (KO) mice to examine the importance of 5-HT receptors in mediating the hypophagia induced by enterostatin, and used rats with PVN and amygdala double cannulas to study the interactions between 5-HT and enterostatin.

2. Materials and methods

2.1. Animals

Both male and female 5-HT2C receptor knockout mice (KO) and wild-type mice (WT) were used in these studies. Mice lacking functional 5-HT2C receptors (C57BL/6J-*Htr2c*^{tm1Jul}) were obtained from The Jackson Laboratory (Bar Harbor, ME) and subsequently bred in the Pennington Biomedical Research Center vivarium. The 5-HT2CR gene is X-linked [27]; mice were shown to be homozygous for the Htr2c mutation if female and hemizygous for the Htr2c mutation if male. The functional knockout of the Htr2c gene in KO mice was confirmed by a PCR genotyping assay of DNA obtained from tail biopsies (The Jackson Laboratory, Bar Harbor, ME).

Male Sprague–Dawley rats (average body weight was 320 g at beginning of the study) were purchased from Harlan Laboratory Inc (Indianapolis, IN). All of the mice and rats were individually housed in stainless steel, wire-mesh bottom hanging cages under a 12-h light/dark cycle (lights off at 1900 h) with ad libitum access to a high fat diet (4.78 kcal/g, 56% of energy as fat) and tap water. The mice were given plastic tubes in the cages. The composition of the diet has been described elsewhere [22]. The experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee.

2.2. Brain cannulation in the rat

Rats were anesthetized with pentobarbital sodium (Nembutal; 0.1 ml/100 g body weight, i.p.) and stereotaxically implanted with 2 unilateral 25-gauge stainless steel cannulas into the PVN and central nucleus of the amygdala ipsilaterally. The coordinates (AP/L/DV to bregma) were PVN: −1.9/−0.4/6.0 mm; amygdala: −2.4/−3.8/−6.0 mm [17,31]. The cannulas were secured in place with 3 anchor screws and dental acrylic and occluded with a 30-gauge stylet. The injectors for the PVN and amygdala were designed to projected 2 mm beyond the guide cannula tip. The animals were returned to their home cages after recovery from the anesthesia and were not used for experiments until they had regained their preoperative weight (approximately 7 days).

2.3. Chemicals

Enterostatin (APGPR) was synthesized by the Core Laboratory of Louisiana State University Health Science Center (New Orleans, LA). The 5-HT receptor non-specific antagonist metergoline [6] was purchased from Sigma-Aldrich Co. (St. Louis, MO), the 1B antagonist GR55562 from Tocris Cookson Inc. (Ellisville, MO) [37] and the 2C receptor antagonist ritanserin [11] from Sigma-Aldrich Co. (St. Louis, MO).

Enterostatin and GR55562 were soluble in saline (0.9% w/v). Metergoline was dissolved in a small amount of 5% tartaric acid initially and diluted to the required concentration by using 0.05M phosphate-buffered saline (pH7.2). Ritanserin was dissolved in 1% (v/v) Tween 80 in 0.05M phosphate-buffered saline (pH7.2) vehicle.

In the mouse study, enterostatin was given as a single injection of 120 nmol intraperitoneally (i.p.) per mouse; GR55562 was injected i.p. at a dose of 40 mg/kg body weight in a volume of 0.1 ml saline. In the rat study, enterostatin (0.01 nmol/0.3 μl) was injected into the rat central nucleus of amygdala. The enterostatin doses chosen have previously been shown to induce a maximal feeding inhibitory effect [8,17,18]. The doses of 5-HT receptor antagonists injected into rat PVN (10 nmol in 0.3 μl volume of vehicle) were based on previous reports of the responses to metergoline [6,33].

2.4. Experimental protocols

Mice were food-deprived overnight (16 h [6 pm–10 am]) and either saline vehicle or enterostatin was injected prior to the provision of a preweighed food cup. Diet consumption was measured at 2, 4 and 24 h with correction for the spillage. (One-hour food intakes in mice are difficult to