Published in final edited form as:

Brain Res. 2005 November 16; 1062(1-2): 26–31. doi:10.1016/j.brainres.2005.09.029.

5-HT_{1B} 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-HT_{2C} 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-HT_{2C} receptor mutant mice (saline 4.54 ± 0.47 kcal vs. Ent 2.53 ± 0.76 kcal) 1 h after injection. A selective 5-HT_{1B} 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 1B) 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 1 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-HT_{1B} receptors and that a neuronal pathway from the amygdala to the PVN regulates the enterostatin response through activation of 5-HT_{1B} receptors in PVN.

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

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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 subtypes have been identified and each subtype has more than one form [7]. Among these receptors, 5-HT_{1B} and 5-HT_{2C} 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-HT_{2C} 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-HT_{2C} 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-HT_{2C} receptor knockout mice (KO) and wild-type mice (WT) were used in these studies. Mice lacking functional 5-HT_{2C} 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-HT_{2C} 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 stereotactically 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 project 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 μ l) 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 μ l 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 measure with accuracy because the amounts are so small.) The 5-HT1B antagonist GR55562 or saline vehicle was administered 45 min before enterostatin injection.

The experiments with rats were also performed on overnight food-deprived animals (16 h [6 pm–10 am]). The rats were randomly assigned to four groups with injection of either the specific drug vehicle or 5-HT antagonists into the PVN, plus either saline vehicle or enterostatin into the amygdala. Injections into the PVN were given 10 min before the amygdala injections. Rats were then returned to their home cages and provided with high fat diet. The food intake of rats was recorded for the next 4 h allowing for all spillage. All of the 5-HT antagonist experiments were performed on the same animals with a minimum 7-day recovery period between the different tests. Rats were randomly assigned to experimental groups each time.

At the conclusion of testing, rats were anesthetized with pentobarbital sodium and perfused transcardially with 4% paraformaldehyde in 0.1 M PBS (pH 7.2). Brains were removed, and coronal sections (50 μ m) were cut on a cryostat and thaw mounted on glass slides. Cannula placements were determined after cresyl violet staining with reference to the atlas of Paxinos and Watson [31].

2.5. Data analysis

Cumulative intake of a high fat diet (gram) is presented as means $\pm$ SEM. The data were analyzed by ANOVA with repeated measures (time), and the Bonferroni test was used for post hoc analysis. $P < 0.05$ is considered as a significant difference.

3. Results

3.1. Food intake in 5-HT_{2C} receptor knockout (KO) mice

Enterostatin (120 nmol, i.p.) decreased the intake of the high fat diet in both female and male mice (see Fig. 1). The reduction was about 50% in wild-type (WT) [ANOVA showed overall enterostatin treatments: $F_{(1,4)} = 26.08$, $P = 0.007$] and 40% in KO male mice [$F_{(1,8)} = 7.252$, $P = 0.027$]; 60% (WT) and 46% (KO) in female mice 2 h after injection of enterostatin [main treatment effects: WT: $F_{(1,5)} = 7.717$, $P = 0.039$; KO: $F_{(1,6)} = 12.967$, $P = 0.011$]. There were no differences of the intake between treatment groups at 24 h.

Injection of the 5-HT_{1B} receptor antagonist GR55562 (40 mg/kg body weight, i.p.) prior to enterostatin (120 nmol, i.p.) blocked the hypophagia induced by enterostatin in male 5-HT_{2C} KO mice. GR55562 alone did not affect feeding (see Fig. 2). ANOVA showed a significant enterostatin treatment effect [$F_{(1,23)} = 5.574$, $P = 0.027$].

3.2. Food intake in rats with PVN and amygdala cannulas

3.2.1. Effects of metergoline on the response to enterostatin—The effect of the non-selective antagonist metergoline injected into the PVN, at a dose of 10 nmol, on the feeding response to enterostatin (0.01 nmol) injected into the amygdala, was investigated (Fig. 3). Enterostatin in the amygdala reduced food intake by 45% at 1 h (saline: 5.8 ± 0.59 g vs. enterostatin: 3.47 ± 0.56 g, $P < 0.05$), and the effect lasted through the next 4 h. The main treatment effect of enterostatin was significant [ANOVA: $F_{(1,21)} = 4.452$, $P = 0.047$]. Metergoline alone did not alter the food intake [metergoline treatment: $F_{(1,21)} = 0.027$, $P = 0.871$] over the time course of the experiment. However, at the 1-h time point, food intake of the metergoline-treated rats was reduced significantly below those of the control group although by 2 h, it had returned to control levels. Metergoline attenuated the anorectic response to enterostatin although the difference between vehicle/enterostatin and enterostatin/metergoline groups did not reach statistical significance until the 3- and 4-h time points.

3.2.2. Effect of 5-HT receptor antagonists on the response to enterostatin—Injection of GR55562, a selective 5-HT_{1B} receptor antagonist alone into the PVN had no effects on food intake [$F_{(1,14)} = 2.504$, $P = 0.136$], but it completely abolished the enterostatin inhibition of food intake (Fig. 4). The interactions between GR55562 and enterostatin treatment were significant [$F_{(1,14)} = 4.694$, $P = 0.048$]. In contrast, the selective 5-HT_{2C} antagonist ritanserin injected into PVN did not block the amygdala enterostatin effects [$F_{(1,19)} = 0.001$, $P = 0.976$] (see Fig. 5). Food intake of enterostatin treated rats was significantly different from the vehicle (V + S) control rats [$F_{(1,19)} = 8.429$, $P = 0.009$] at all time points as was the ritanserin and enterostatin treated rats. Ritanserin alone induced a temporary reduction in food intake in the first 30 min, after which food intake returned to control levels at all time points. There were no significant differences between the food intakes of the vehicle/enterostatin and the ritanserin/enterostatin groups at any time point.

4. Discussion

The present study reported that peripheral administration of enterostatin decreased the intake of a high fat diet in both WT and 5-HT_{2C} receptor KO mice. In addition, a 5-HT_{1B} receptor antagonist reversed the hypophagia induced by both i.p. and amygdala injection of enterostatin, whereas a 5-HT_{2C} receptor antagonist had no effect in the intact rat, suggesting that the 5-HT_{2C} receptor is not required for the effects of enterostatin on feeding. The significance of this study is to suggest that 5-HT_{1B} receptors contribute to the satiety effects of enterostatin. The data suggest that enterostatin activates a neuronal pathway from the amygdala to PVN that enhances 5-HT activity through which 5-HT_{1B} receptor signaling modulates food (fat) intake.

Our previous data had shown the attenuation of enterostatin-induced hypophagia by the non-selective 5-HT antagonist metergoline in rat indicating that the 5-HT 1 or 2 receptors are involved in the feeding response to enterostatin [42]. Lack of specific 5-HT antagonists made it difficult to identify the specific receptor subtypes responsible for enterostatin-induced hypophagia. As an alternative to classic pharmacological approaches, mice lacking receptors are useful tools for explaining behavioral and physiological responses. 5-HT_{2C} receptor knockout mice are mildly hyperphagic and overweight and have a reduced response to the serotonergic drug *D*-fenfluramine [35,36]. In this paper, we report that the hypophagia in response to enterostatin is still observed in 5-HT_{2C} receptor KO mice, indicating that the 2C receptor is not required for its response. In contrast, the 5-HT_{1B} receptor activity appears to be essential for enterostatin effects, as evident by the ability of a 1B receptor antagonist to block the enterostatin hypophagic effects in 5-HT_{2CR} KO mice. This conclusion is further supported by the experiments performed on rats which showed that the 5-HT_{1B} antagonist GR55562 administered into the PVN completely reversed the anorexia caused in response to amygdala enterostatin, while the general 5-HT receptor antagonist metergoline partially blocked the effect. In contrast, the 5-HT_{2C} receptor antagonist ritanserin had no effect on the enterostatin response. Both metergoline and ritanserin caused a small acute independent reduction in food intake in the first 30 min. This may have been due to a mild sedative effect, since this response rapidly disappeared at subsequent time intervals. Previous studies have shown that 5-HT can act through the PVN to inhibit food intake [11,16]. Micro-injection of 5-HT agent decreased the intake of the fat diet [33]. Our studies here clearly demonstrate the importance of the PVN 5-HT_{1B} receptor to the amygdala enterostatin. High densities of 5-HT_{1B} receptor binding sites are located in the PVN and central nucleus of the amygdala, both of which receive serotonergic innervation from raphe nuclei [1,5,26,32]. Further, enterostatin has been shown to enhance the release of 5-HT in the PVN and other brain regions after peripheral or central administration [15]. We have shown *c-Fos* induction in the PVN in response to amygdala enterostatin. The current data suggest that this is a direct consequence of activation of 5-HT_{1B} receptors [20]. However, we cannot rule out that stimulation of 5-HT_{1B} receptors in other brain regions might also be involved through a polysynaptic mechanism. Our previous neuro-tracing studies have shown that the arcuate nucleus has direct anatomic connections from the amygdala [20] and contains afferents to the PVN [29]. The arcuate nucleus has a very high density of 5-HT_{1B} receptors [5].

The function of the central amygdaloid complex in feeding behavior and energy balance has not been fully explored. The amygdala complex consists of the central nucleus of amygdala and more than 20 subnuclei. We have shown that the central nucleus of the amygdala is involved in the regulation of the feeding behavior in response to enterostatin [17,18,20] but does not appear to be involved in the enterostatin regulation of energy expenditure [19]. The amygdala is involved in learned taste aversion [41], but growing evidence suggests that the amygdala is also involved in feeding regulation, especially fat/carbohydrate selection [13,39]. Lesions in this area in rats modify fat and carbohydrate selection [14]; injection of a GABA agonist into the amygdala blocks the fat craving that is triggered by opioids [39]. Recently, our group (Primeaux et al., unpublished observation) has shown that NPY administration into the amygdala influences macronutrient choice without affecting total energy intake. Taken together, these data suggest that amygdala is an important extrahypothalamic region that regulates the selection of dietary fat.

In conclusion, our data strongly suggest that the enterostatin inhibition of dietary fat intake is dependent upon the activity of 5-HT_{1B} receptors, and that these receptors may play a role in the satiety response to dietary fat.

Acknowledgment

We thank Dr. Brenda Smith-Richards for providing 5-HT2C knockout mouse and for her professional opinion.

References

1. Alheid, GF.; de Olmos, JS.; Beltramino, CA. Amygdala and extended amygdala. In: Paxinos, G., editor. *The Rat Nervous System*. London: Academic; 1995. p. 495-500.
2. Blundell JE. Is there a role for serotonin (5-hydroxytryptamine) in feeding? *Int. J. Obes* 1977;1:15–42. [PubMed: 361584]
3. Blundell JE, Halford JC. Serotonin and appetite regulation. *CNS Drugs* 1998;6:473–495.
4. Bowyer RC, Rowston WM, Jehanli AM, Lacey JH, Hermon-Taylor J. Effect of a satiating meal on the concentrations of procolipase propeptide in the serum and urine of normal and morbidly obese subjects. *Gut* 1993;34:1520–1525. [PubMed: 8244136]
5. Bruinvels AT, Landwehrmeyer B, Gustafson EL, Durkin MM, Mengod G, Branchek TA, Hoyer D, Palacios JM. Localization of 5-HT1B, 5-HT1D alpha, 5-HT1E and 5-HT1F receptor messenger RNA in rodent and primate brain. *Neuropharmacology* 1994;33:367–386. [PubMed: 7984275]
6. Currie PJ, Coscina DV. Metergoline potentiates natural feeding and antagonizes the anorectic action of medical hypothalamic 5-hydroxytryptamine. *Pharmacol. Biochem. Behav* 1996;53:1023–1028. [PubMed: 8801574]
7. Dourish CT. Multiple serotonin receptors: opportunities for new treatments for obesity? *Obes. Res* 1995;3:449S–462S. [PubMed: 8697043]
8. Erlanson-Albertsson C, Mei J, Okada S, York D, Bray GA. Pancreatic procolipase propeptide, enterostatin, specifically inhibits fat intake. *Physiol. Behav* 1991;49:1191–1194. [PubMed: 1896501]
9. Erlanson-Albertsson C, Westrom B, Pierzynowski S, Karlsson S, Ahren B. Pancreatic procolipase activation peptide-enterostatin-inhibits pancreatic enzyme secretion in the pig. *Pancreas* 1991;6:619–624. [PubMed: 1780322]
10. Erlanson-Albertsson C, York D. Enterostatin—A peptide regulating fat intake. *Obes. Res* 1997;5:360–372. [PubMed: 9285845]
11. Grignaschi G, Mantelli B, Samanin R. The hypophagic effect of restraint stress in rats can be mediated by 5-HT2 receptors in the paraventricular nucleus of the hypothalamus. *Neurosci. Lett* 1993;152:103–106. [PubMed: 8515859]
12. Grignaschi G, Samanin R. Role of 5-HT receptors in the effect of d-fenfluramine on feeding patterns in the rat. *Eur. J. Pharmacol* 1992;212:287–289. [PubMed: 1601071]
13. Kelley AE. Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neurosci. Biobehav. Rev* 2004;27:765–776. [PubMed: 15019426]
14. King BM, Rossiter KN, Stines SG, Zaharan GM, Cook JT, Humphries MD, York DA. Amygdaloid-lesion hyperphagia: impaired response to caloric challenges and altered macronutrient selection. *Am. J. Physiol* 1998;275:R485–R493. [PubMed: 9688684]
15. Koizumi M, Kimura S. Enterostatin increases extracellular serotonin and dopamine in the lateral hypothalamic area in rats measured by in vivo microdialysis. *Neurosci. Lett* 2002;320:96–98. [PubMed: 11849772]
16. Leibowitz SF. Neurochemical-neuroendocrine systems in the brain controlling macronutrient intake and metabolism. *Trends Neurosci* 1992;15:491–497. [PubMed: 1282749]
17. Lin L, York DA. Enterostatin actions in the amygdala and PVN to suppress feeding in the rat. *Peptides* 1997;18:1341–1347. [PubMed: 9392835]
18. Lin L, York DA. Changes in the microstructure of feeding after administration of enterostatin into the paraventricular nucleus and the amygdala. *Peptides* 1998;19:557–562. [PubMed: 9533645]
19. Lin L, York DA. Different metabolic responses to central and peripheral enterostatin. *Obes. Res* 2001;9:116S.
20. Lin L, York DA. Amygdala enterostatin induces c-Fos expression in regions of hypothalamus that innervate the PVN. *Brain Res* 2004;1020:147–153. [PubMed: 15312796]
21. Lin L, Okada S, York DA, Bray GA. Structural requirements for the biological activity of enterostatin. *Peptides* 1994;15:849–854. [PubMed: 7984504]

22. Lin L, Chen J, York DA. Chronic ICV enterostatin preferentially reduced fat intake and lowered body weight. *Peptides* 1997;18:657–661. [PubMed: 9213358]
23. Lin L, Umahara M, York DA, Bray GA. Beta-casomorphins stimulate and enterostatin inhibits the intake of dietary fat in rats. *Peptides* 1998;19:325–331. [PubMed: 9493865]
24. Lin L, Bray G, York DA. Enterostatin suppresses food intake in rats after near-celiac and intracarotid arterial injection. *Am. J. Physiol.: Regul., Integr. Comp. Physiol* 2000;278:R1346–R1351. [PubMed: 10801306]
25. Lin L, Braymer HD, York DA. Procolipase gene and enterostatin expression in the rat brain. *FASEB J* 2002;A783.
26. Ma QP, Yin GF, Ai MK, Han JS. Serotonergic projections from the nucleus raphe dorsalis to the amygdala in the rat. *Neurosci. Lett* 1991;134:21–24. [PubMed: 1815148]
27. Milatovich A, Hsieh CL, Bonaminio G, Tecott L, Julius D, Francke U. Serotonin receptor 1c gene assigned to X chromosome in human (band q24) and mouse (bands D-F4). *Hum. Mol. Genet* 1992;1:681–684. [PubMed: 1302605]
28. Miner JL, Erlanson-Albertsson C, Paterson JA, Baile CA. Reduction of feed intake in sheep by enterostatin, the procolipase activation peptide. *J. Anim. Sci* 1994;72:1578–1582. [PubMed: 8071183]
29. Morris BJ. Neuronal localisation of neuropeptide Y gene expression in rat brain. *J. Comp. Neurol* 1989;290:358–368. [PubMed: 2592617]
30. Neill JC, Cooper SJ. Evidence that d-fenfluramine anorexia is mediated by 5-HT₁ receptors. *Psychopharmacology (Berlin)* 1989;97:213–218. [PubMed: 2498930]
31. Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. 2nd edition. Sydney: Academic Press; 1982.
32. Sawchenko PE, Swanson LW, Steinbusch HW, Verhofstad AA. The distribution and cells of origin of serotonergic inputs to the paraventricular and supraoptic nuclei of the rat. *Brain Res* 1983;277:355–360. [PubMed: 6357352]
33. Smith BK, York DA, Bray GA. Activation of hypothalamic serotonin receptors reduced intake of dietary fat and protein but not carbohydrate. *Am. J. Physiol* 1999;277:R802–R811. [PubMed: 10484498]
34. Sorhede M, Mulder H, Mei J, Sundler F, Erlanson-Albertsson C. Procolipase is produced in the rat stomach—A novel source of enterostatin. *Biochim. Biophys. Acta* 1996;1301:207–212. [PubMed: 8664330]
35. Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, Julius D. Eating disorder and epilepsy in mice lacking 5-HT_{2c} serotonin receptors. *Nature* 1995;374:542–546. [PubMed: 7700379]
36. Vickers SP, Clifton PG, Dourish CT, Tecott LH. Reduced satiating effect of d-fenfluramine in serotonin 5-HT_{2C} receptor mutant mice. *Psychopharmacology (Berlin)* 1999;143:309–314. [PubMed: 10353435]
37. Walsh DM, Beattie DT, Connor HE. The activity of 5-HT_{1D} receptor ligands at cloned human 5-HT_{1D} alpha and 5-HT_{1D} beta receptors. *Eur. J. Pharmacol* 1995;287:79–84. [PubMed: 8666030]
38. Wicker C, Puigserver A. Changes in mRNA levels of rat pancreatic lipase in the early days of consumption of a high-lipid diet. *Eur. J. Biochem* 1989;180:563–567. [PubMed: 2469576]
39. Will MJ, Franzblau EB, Kelley AE. The amygdala is critical for opioid-mediated binge eating of fat. *NeuroReport* 2004;15:1857–1860. [PubMed: 15305124]
40. Winzell MS, Lowe ME, Erlanson-Albertsson C. Rat gastric procolipase: sequence, expression, and secretion during high-fat feeding. *Gastroenterology* 1998;115:1179–1185. [PubMed: 9797373]
41. Yamamoto T, Shimura T, Sako N, Yasoshima Y, Sakai N. Neural substrates for conditioned taste aversion in the rat. *Behav. Brain Res* 1994;65:123–137. [PubMed: 7718144]
42. York, DA.; Lin, L.; Smith, BK.; Bray, GA. Enterostatin and 5HT: modulation of fat intake. In: Bray, GA.; Ryan, DH., editors. *Nutr. Genet. Obes.* vol. 9. Baton Rouge: Louisiana State University Press; 1999. p. 246-266.

Fig. 1.

Effects of i.p. enterostatin (120 nmol) on the food intake of wild-type (WT; $n = 5$ male, $n = 6$ female) and 5-HT_{2C} receptor knockout (KO; $n = 9$ male, $n = 7$ female) mice. Mice were food-deprived overnight. Data are expressed as means $\pm$ SEM of the cumulative intake (g). * $P < 0.05$ compared with respective saline vehicle group.

Fig. 2. Effects of 5-HT_{1B} receptor antagonist GR55562 on hypophagia induced by i.p. enterostatin in 5-HT_{2C} receptor knockout mice. * $P < 0.05$ compared with respective saline/saline (sal + sal) vehicle group.

Fig. 3.

The effects of PVN injection of 5-HT receptors 1 and 2 antagonist metergoline on the hypophagia induced by amygdala injection of enterostatin. * $P < 0.05$ compared with respective vehicle + saline (V + S) control group.

Fig. 4.
The effects of PVN injection of 5-HT_{1B} antagonist GR55562 on the hypophagia induced by amygdala injection of enterostatin. * $P < 0.05$ compared with respective saline + saline (S + S) vehicle group.

Fig. 5.

The effects of PVN injection of 5-HT_{2C} antagonist ritanserin on the hypophagia induced by amygdala injection of enterostatin. * $P < 0.05$ compared with respective vehicle + saline (V + S) control group.