REVIEW

# Enterostatin—A Peptide Regulating Fat Intake

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**Abstract**

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**A high fat intake, together with an inability to match lipid oxidation to fat intake, has been found to be correlated with obesity in humans. This review describes our current understanding of enterostatin, a peptide that selectively reduces fat intake. Enterostatin is formed in the intestine by the cleavage of secreted pancreatic procolipase, the remaining colipase serving as an obligatory cofactor for pancreatic lipase during fat digestion. Enterostatin is also produced in the gastric mucosa and the mucosal epithelia of the small intestine. Procolipase gene transcription and enterostatin release into the gastrointestinal lumen are increased by high-fat diets. After feeding, enterostatin appears in the lymph and circulation. Enterostatin will selectively inhibit fat intake during normal feeding and in experimental paradigms that involve dietary choice. Its anorectic effect has been demonstrated in a number of species. Both peripheral and central sites of action have been proposed. The peripheral mechanism involves an afferent vagal signaling pathway to hypothalamic centers. The central responses are mediated through a pathway that includes both serotonergic and opioidergic components. Chronically, enterostatin reduces fat intake, bodyweight, and body fat. This response may involve multiple metabolic effects of enterostatin, which include a reduction of insulin secretion, an increase in sympathetic drive to brown adipose tissue, and the stimulation of adrenal corticosteroid secretion. A possible pathophysiological role is suggested by studies that have linked low enterostatin production and/or responsiveness to strains of rat that become obese and prefer dietary fat. Humans with obesity also exhibit a lower secretion of pancreatic procolipase after a test meal, compared with persons of normal weight.**

**Key words: fat intake, insulin, bodyweight, obesity, pancreas, colipase, lipase**

## Introduction

Behavioral physiologists have come to recognize the complexity of feeding behavior. The frequency size and composition of individual meals may be regulated such that, over a medium time frame, energy balance is maintained. Although numerous brain centers control feeding behavior, they respond to a variety of preingestive and postingestive signals that include neural, endocrine, nutrient, and metabolic components that relay information on energy reserves, the nutrient status, and the size and composition of a meal (4,87,92). The ability to control the intake of specific nutrients is illustrated by the concept of sensory-specific satiety, first proposed by Rolls and colleagues (60). It is now recognized that overfeeding with one type of macronutrient may lead to a reduced intake of that particular macronutrient subsequently, but intake of other nutrients may be unaffected. This is true for rats (34) as well as humans (79). The ability of salt- or amino acid–depleted rats to select the missing dietary component (28,29) is an illustration of the existence of similar control on individual nutrients.

The molecular mechanisms underlying macronutrient selection are unclear. A number of peptides, neurotransmitters, and metabolic inhibitors (Table 1), may have selective effects to regulate the intake of a specific macronutrient (9,10,93). Carbohydrate intake is preferentially stimulated by neuropeptide Y (30,73) and by the $\alpha_2$ effects of noradrenaline (33), and κ-opioids (2,13,57) may selectively stimulate fat intake. The role of other peptides is less clear. Although galanin has been linked to fat intake in some studies (34), its effects are strongly influenced by background preferences such that it selectively stimulates carbohydrate in carbohydrate-preferring rats (67). Likewise, serotonin was initially linked to carbohydrate feeding (86), but more recent evidence suggests that serotonin agonists selectively inhibit fat intake (5,68). The inhibition of protein intake has been claimed to occur through the peptide glucagon (81). A peptide that has been found to selectively

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Table 1. The regulation of the intake of macronutrients

| Macronutrient | Stimulating | Inhibiting |
|---|---|---|
| Carbohydrate | NPY (30,73)* | |
| | $\alpha_2$-Adrenergic agonists (33) | |
| | Insulin (31) | |
| | Galanin† (67) | |
| | 2-Deoxy-D-glucose (32) | |
| | β-Mercaptoacetate (66) | |
| Fat | Galanin† (34) | Enterostatin (55,70) |
| | κ-Opioids (2,13) | Serotonin (5,68) |
| | | CRH (42) |
| Protein | Growth hormone–regulating hormone (78) | Glucagon (81) |

*Numbers in parentheses indicate specific references.
†Dependent on baseline preference of animals.

inhibit fat intake is enterostatin (55,70). Enterostatin is formed through the processing of procolipase, a protein necessary for intestinal fat digestion (21). This review will give an overview of the biological actions of enterostatin.

## Feeding Response of Enterostatin

Studies involving the injection of enterostatin, either centrally or peripherally, have demonstrated that enterostatin reduces food intake and has a dose-dependent and selective effect to inhibit fat intake in a number of dietary paradigms. In rats given a three-choice macronutrient diet of fat, carbohydrate, and protein (55,56) it reduces intake of the fat macronutrient. On a two-choice high-fat (HF) and low-fat (LF) diet paradigm, it only reduces intake of the HF diet (24,35). Similarly, enterostatin reduces intake of single diets when the diet is high, but not when it is low, in fat content (36). Enterostatin is effective intragastrically (91) and intraduodenally (48) and by intraperitoneal (38,55,56), intravenous (46), and intracerebroventricular (icv) (36,38,46) routes. With the exception of intravenous administration, when there is a delay in the response of 60 minutes to 120 minutes, the peptide has a rapid effect (<30 minutes) after administration by all other routes. In overnight-fasted rats, the inhibition of fat intake by enterostatin has been related to an early induction of satiety (37); the time spent eating and grooming and in physical activity were decreased, whereas the period of resting or sleeping was significantly increased. The behavior of the animals after the injection of enterostatin was thus similar to that after natural satiety, suggesting that enterostatin was not mediating its effects through nausea or other behavior. This is supported by the report that enterostatin did not initiate a conditioned taste aversion (46).

Table 2. Doses and response times to enterostatin in rats

| Route/Site | Threshold Dose (nmol) | Response Time (min) | References |
|---|---|---|---|
| Intragastric/duodenal | 11.0* | <30 | 48,90 |
| Intraperitoneal | 40 | 15–20 | 37,56 |
| Intravenous | 16.7 | 60–120 | 46 |
| Intracerebroventricular | 0.3 | <30 | 38 |
| Paraventricular nucleus | 0.1 | <10 | 39 |
| Amygdala | 0.01 | <5 | Figure 3 |

*Per kg bodyweight.

The effect of enterostatin injected into the amygdala and paraventricular nucleus on the microstructure of feeding has been investigated recently by the use of an automated feeding apparatus (L. Lin and D.A. York, unpublished observations). In normal feeding, at the beginning of the dark cycle, icv enterostatin delays the onset of the meal, shortens its duration, and reduces its size. Subsequent meals are unaffected, and there is no compensatory increase for the smaller initial meal. Similar effects were observed when enterostatin was given after an overnight starvation or at the beginning of the meal time in 6-hour meal fed rats, with one exception: the delayed onset of feeding was not observed in these situations.

The potency of the action of enterostatin is reflected in its long duration of action and its effect on feeding, lasting up to 6 hours after a single injection in rats adapted to a 6-hour feeding schedule (48) or lasting up to 24 hours after a single injection in ad libitum–fed rats (56). During chronic

icv administration of enterostatin for 11 days in Osborne-Mendel (OM) rats fed an HF diet (52) or Sprague-Dawley rats fed a two-choice high-protein/LF diet (35), there was a decrease in daily food intake, fat deposition, and bodyweight gain. However, in Sprague-Dawley rats chronically treated with enterostatin and fed an LF diet for 7 days, there was no significant change in bodyweight gain (47). An intriguing characteristic of the response to enterostatin in both acute chronic studies is that the reduction in intake of dietary feat is not compensated by any increase in the intake of other macronutrients.

## Sites of Enterostatin/Procolipase Production

Procolipase, the parent molecule of enterostatin, is present in the exocrine pancreatic cells, where it is secreted into pancreatic juice (Figure 1) (21). After tryptic activation, procolipase is split into colipase and enterostatin (21). The role of colipase is to activate pancreatic lipase, to which it binds, forming a 1:1 molar complex (22,80). The ratio between lipase and colipase is variable. In humans, the ratio is 1:1; in pig, there is an abundancy of colipase relative to lipase, whereas in the rat, there is a deficiency of colipase relative to lipase (22,26).

Colipase is an acid-stable molecule containing five disulfide bridges, whereas pancreatic lipase forms a flexible structure, sensitive to acid pH as well as to bile salts. The initial finding of procolipase mRNA in the rat antral stomach (54) and the subsequent confirmation by immunohistochemistry (71) may therefore indicate a significant alternative source of enterostatin and colipase. These conclusions are supported by the localization of the procolipase production to chief cells in the fundal region and the measurement of procolipase and enterostatin in rat gastric juice (71). At this time, it is not clear what proportion of secreted procolipase is hydrolyzed in the gastric juice or after gastric procolipase has passed into the duodenum or upper small intestine. To date, it has not been possible to demonstrate any enterostatin immunoreactivity in the chief cells of the stomach, only procolipase immunoreactivity. Hence, the activation of procolipase occurs only after the procolipase has been secreted into the gastric juice. Likewise, pancreatic procolipase is only activated, and enterostatin released, after entrance into the intestinal lumen (22,44). However, between gastric and duodenal enterostatin inhibits food intake with a short response time, although its absorption across the intestine is slow and limited (6), a gastrointestinal site of action seems likely. York has suggested that gastric procolipase may release enterostatin locally to act in a paracrine

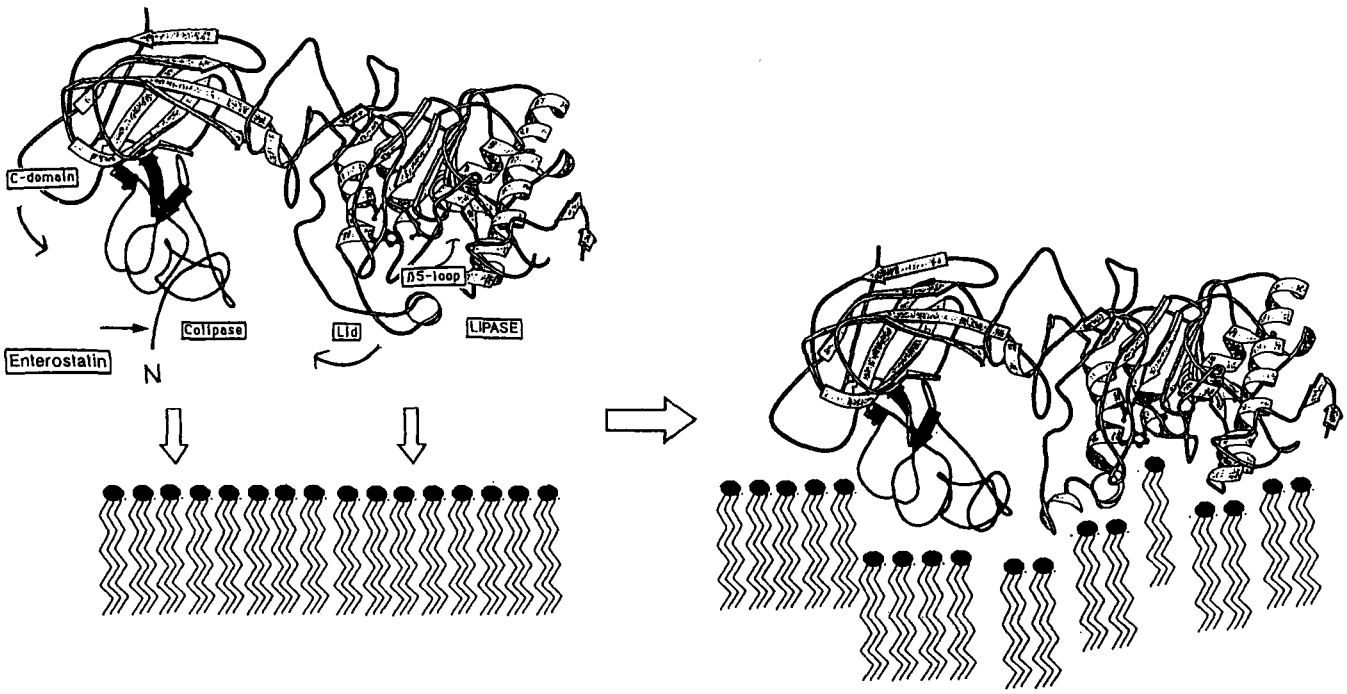


Figure 1: Intestinal fat digestion through the interaction of lipase and colipase. The active site of lipase is covered by a "lid," which is "opened" after the interaction of lipase with colipase. Colipase is secreted as procolipase. Before lipase activation, procolipase is cleaved by trypsin, yielding enterostatin released from the N-terminal end of procolipase, and colipase. The removal of enterostatin is important for the conformational change of lipase induced by colipase. The amount of colipase determines the amount of dietary fat digested.

manner to activate the hepatic vagal branch endings which innervate the stomach in the rat (91,92).

In addition to pancreatic procolipase, immunohistochemistry has revealed the existence of enterostatin in enterochromaffin cells of the gastrointestinal tract, being most abundant in the antral part of the stomach, less in the duodenum and jejunum, and only a few cells in the ileum (69). In some of these enterochromaffin cells, there is, moreover, colocalization of enterostatin and serotonin. The significance of this observation is not known. It may be that enterostatin and serotonin are concomitantly released during fat feeding.

## Regulation of Synthesis of Enterostatin/ Procolipase

To advance our understanding of the potential physiological role of enterostatin in mediating feeding behavior, information on the regulation of enterostatin synthesis and secretion under various physiological conditions is required. A significant finding is the increased production of enterostatin/procolipase in the pancreas (44) and the gastric mucosa (91) after feeding of fat. This increased procolipase synthesis occurs in proportion to the amount of fat ingested (44) and occurs within 24 hours of the presentation of an HF diet (84). It is consistent with enterostatin acting as a long-term feedback signal to attenuate the levels of fat ingested. Conversely, short-term starvation reduced pancreatic procolipase levels significantly (Figure 2) in Sprague-Dawley rats.

The mechanism through which enterostatin/procolipase production is increased by HF diets is not known. Gastric inhibitory polypeptide is released in the gastrointestinal tract during a fat-containing meal and has been shown to stimulate procolipase synthesis (19). Two other key hormones in energy metabolism, insulin and corticosterone, also regulate procolipase synthesis (16). Both hormones inhibit procolipase mRNA production (17,20), whereas cAMP has a stimulatory effect (18). This regulation of enterostatin synthesis by corticosterone was demonstrated further by adrenalectomy, which increased procolipase expression (53) as well as procolipase synthesis (17), concomitant with reduced intake of HF diet and bodyweight. However, because HF diets normally activate the hypothalamic-pituitary-adrenal axis (11), corticosteroids would appear to counteract the fat-induced increase of procolipase production.

To date, there is only one immunoassay for the measurement of the human enterostatin peptide ala-pro-gly-pro-arg (APGPR) (7). The antibodies obtained cross-react with val-pro-gly-pro-arg (VPGPR) (44), but exhibit no cross-reactivity with val-pro-asp-pro-arg (VPDPR) (44). By this method, immunoreactive enterostatin has been demonstrated in human serum and urine (8), as well as in rat intestinal contents (44). In humans, serum enterostatin increased in response to a satiating meal (8). Two peaks of immunoreactive enterostatin were identified, one being maximal after 40 minutes and a second being maximal 80 minutes after the onset of eating. The reason for the biphasic response is not known, but may correspond to an early,

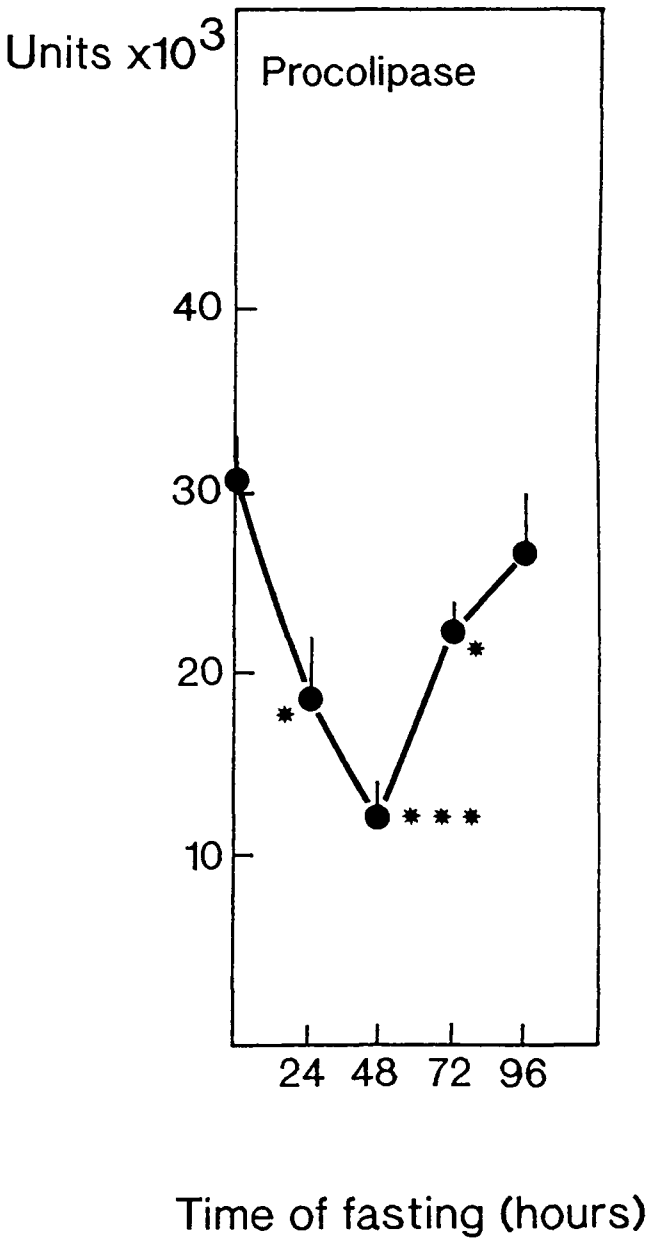


Figure 2: Effect of food deprivation on the activity of pancreatic procolipase in rat. Sprague-Dawley rats were fasted for various lengths of time, after which they were killed and the pancreas was excised and analyzed for procolipase content. After 48 hours of starvation, pancreatic procolipase was found to be reduced to 50% of the original value, but was thereafter specifically increased.

vagally stimulated peak of pancreatic secretion and a second later, intestinally mediated peak of pancreatic secretion. Immunoreactive enterostatin (APGPR) was also identified in urine, suggesting that enterostatin survives intact in the circulation for a sufficient time to allow systemic dispersal (8). The possibility that a postprandial rise in enterostatin is an important satiety signal needs further investigation of the temporal relationships between feeding behavior and circulating levels.

In the intestinal content, immunoreactive enterostatin is present in a 1:1 molar relationship with pancreatic colipase after a test meal, suggesting a complete cleavage of procolipase to colipase and enterostatin (22). The presence of immunoreactive enterostatin (APGPR) has also been demonstrated in the intestinal content of the rat, the concentration increasing after cholecystokoinin (CCK) stimulation as well as after 1 day of HF feeding (44). The rise in intestinal enterostatin levels after HF feeding occurred in parallel to an increase in pancreatic colipase synthesis (44), suggesting a close correlation between intestinal enterostatin and procolipase originating in the pancreas. Enterostatin has also been identified in lymph, where it was increased 4-fold after the ingestion of a cream meal (77), suggesting that enterostatin may associate with chylomicrons for delivery to the lymph rather than direct diffusion into the blood. Such a route of absorption would provide for slow uptake of enterostatin and suggests that enterostatin appearing in the circulation after a meal does not act as a satiety signal to reduce the intake of that immediate meal.

## Peripheral Response to Enterostatin

Dietary fat intake is reduced by the intragastric, intraduodenal, or intraperitoneal administration of enterostatin (38,48,55,56,91). Because enterostatin is released peripherally either from the pancreas or the gastrointestinal tract, it is relevant to question how the response to peripheral enterostatin is transmitted to the brain. Tian et al. (76) reported that afferent vagal-central nervous system connections were important for the feeding response to intraperitoneal enterostatin. Transection of the hepatic vagus completely blocked the inhibitory response to intraperitoneal enterostatin on HF diet consumption in rats after overnight starvation. The importance of neuronal transmission for the feeding response of enterostatin was also suggested by the attenuation of the feeding response to intraintestinal enterostatin after tetracain administration to block peripheral nerve endings (48). These studies thus suggest neuronal transmission of the enterostatin response from the intestine to the brain. Further support for this is provided by the demonstration that peripheral enterostatin induced c-*fos* immunoreactivity in specific brain sites, including the nucleus tractus solitarius, parabrachial, paraventricular, and supraoptic nuclei, and that this effect was absent in rats with selective hepatic vagotomies (76). Interestingly, β-mercaptoacetate, an inhibitor of fatty acid oxidation, activates a set of central nuclei different from those that respond to enterostatin (59). However, at this time, it is not clear if intraluminal enterostatin gains access to the vagal nerve terminals, and if it does, how this is achieved.

The enterostatin inhibition of food intake is only normally seen in rats adapted to an HF diet or allowed access to fat in a dietary choice paradigm. The fat signal that is perceived by the rat is unknown. The possibility that afferent vagal information was required for this "fat signal" was ruled out by the demonstration that capsaicin treatment, which destroys small nonmyelinated nerve fibers, prevented the response to peripheral enterostatin but not to icv enterostatin (94).

## Central Effects of Enterostatin

Intracerebroventricular enterostatin reduces food intake in rats, sheep, and baboons (38,49,82). Studies in rats have shown this effect to be selective toward dietary fat. The neurochemical pathways that mediate the feeding response to enterostatin have been studied by Barton and his colleagues (2,39,57,95). Both κ-opioidergic and serotonergic components have been implicated. The selective κ-opioid agonist U50488 increased HF feeding at high doses but reversed the enterostatin-induced inhibition of feeding at low doses that had no independent effects on food intake (2). Previous studies have shown that κ-opioid agonists stimulate feeding and that there is a concomitant preference for fat intake (13). More recently, Ookuma and colleagues have provided additional support for the involvement of a κ-opioid pathway by showing that the κ-antagonist nor-BNI would mimic the effects of enterostatin and that subthreshold doses of nor-BNI and enterostatin, when combined, reduce fat intake (57). Opiates are recognized to have an important role in preference acquisition (43).

Enterostatin, although having the actions of a κ-opioid antagonist, does not directly interact with opioid receptors (M. Umahara and D.A. York, unpublished observations) but may modulate opioidergic activity through a serotonin pathway. Enterostatin increased the turnover of serotonin in several hypothalamic sites important for the regulation of feeding behavior (95). Further evidence for the importance of a serotonin pathway mediating enterostatin response is provided by the fact that the serotonin antagonist metergoline attenuates the inhibition of fat intake by enterostatin (39). This putative serotonergic component in the enterostatin-responsive pathway assumes some significance because Smith et al. (68) have recently shown that peripheral dexfenfluramine selectively inhibits fat intake in rats irrespective of their basal macronutrient preference. Initial mapping studies have shown that enterostatin is effective after local injection onto the central bed nucleus of the amygdala (Figure 3) and paraventricular nucleus, but not

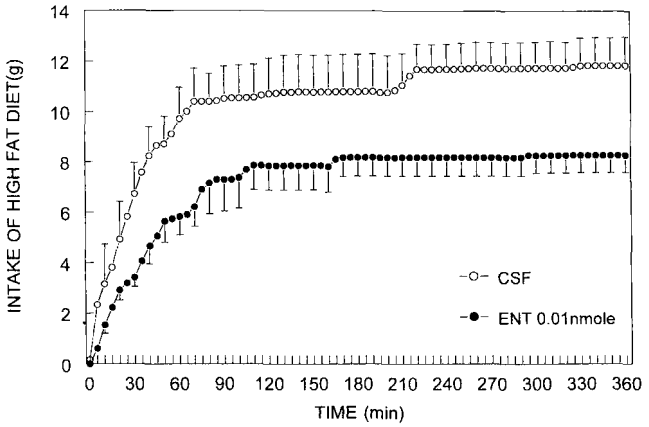


Figure 3: Effect of enterostatin (ENT; 0.01 nmol) injected into the amygdala on food intake of Sprague-Dawley rats after an overnight fast. CSF, cerebrospinal fluid.

onto the ventromedial nucleus, lateral hypothalamus, or nucleus tractus solitarius (40).

It has been suggested that galanin preferentially stimulates fat ingestion (1,34,75). However, this does not appear to be a robust effect, and we have presented data to suggest that galanin stimulates the intake of the preferred macronutrients (67). Although enterostatin does inhibit galanin-induced feeding (36), this may be the result of opposing actions rather than being indicative of the two peptides affecting the same pathway. This suggestion is supported by two observations; first, enterostatin does not displace galanin from its binding sites on hypothalamic membranes (36), and second, galanin-induced feeding appears to be modulated through a μ-opioid pathway rather than a κ-opioid pathway (2).

The uptake of enterostatin peptide from the circulation into the brain has not yet been demonstrated, nor has it been possible to demonstrate the presence of procolipase mRNA or immunoreactive enterostatin in the brain. However, the sensitivity of the central response suggests that enterostatin or a related peptide must have specific receptors within the brain to which it can gain access.

## Endogenous Levels of Enterostatin in Relation to Natural Dietary Preferences

Various rat strains have been shown to have a natural preference for dietary fat or dietary carbohydrate. The OM rat has a strong dietary preference for fat, in contrast to the S5B/P1 rat, which prefers dietary carbohydrate and highly restricts its fat intake (10,56). As an index of the endogenous levels of enterostatin, the activity of the precursor protein procolipase was measured in these two rat strains after adaptation to a three-choice macronutrient diet (56). S5B/P1 rats exhibited 2- to 3-fold higher levels of procolipase than the OM rats. Indeed, voluntary fat intake was reciprocally related to pancreatic procolipase levels, both within and across rat strains, when rats were allowed to choose their dietary macronutrients. Higher endogenous pancreatic procolipase activities were associated with lower intakes of dietary fat, and vice versa. The response to exogenous enterostatin was also strain dependent. Thus, the SSB/P1 rats with a high pancreatic procolipase activity did not respond to exogenously administered enterostatin, in contrast to OM rats, which had a robust response and had low endogenous levels of enterostatin release.

Observations regarding the obese *fa/fa* rat demonstrated a low expression of pancreatic procolipase mRNA compared with its lean *Fa/?* counterpart (53), supporting the suggestion that endogenous production of enterostatin may be important in regulating fat intake. It was, furthermore, shown that adrenalectomy of the obese *fat/fa* rat increased the levels of procolipase mRNA, concomitant with an inhibition of further obesity, and abolished the feeding response to exogenous enterostatin. These data again suggest a link between the endogenous production of enterostatin/procolipase and the response to exogenous peptide. Conversely, corticosterone treatment reduces endogenous production of colipase but enhances the response to exogenous enterostatin (50).

Several studies have demonstrated that Sprague-Dawley rats exhibit considerable individual differences in their preference for carbohydrate, protein, and fat (12,34,64). In studies of male rats, approximately 50% of the population showed a strong preference for carbohydrate, with a low consumption of fat. In contrast, 35% of the population preferred fat and consumed >40% of their diet in the form of fat. Furthermore, the HF eaters exhibited a higher bodyweight and a greater amount of adipose tissue than the carbohydrate eaters. They also consumed larger, less frequent meals than the carbohydrate eaters. Whether procolipase/enterostatin is involved in the determination of fat or carbohydrate preferences in these rats is presently under investigation. M. Sörhede et al. (unpublished observations) (Figure 4) demonstrated an inverse relationship between fat intake and the amount of pancreatic procolipase in Sprague-Dawley rats, confirming the previous observations in OM and S5B/P1 rats (56). Cook et al. (12) found that fat-preferring rats responded to exogenously administered enterostatin by a decrease in fat intake, whereas carbohydrate-preferring rats were unresponsive. One conclusion may be that the carbohydrate-preferring rats have an optimal level of endogenous enterostatin and hence do not respond to further administration of enterostatin, as has been demonstrated for the S5B/P1 rat (56). Further studies, using direct assay of circulating enterostatin, will be necessary to confirm this hypothesis. Nevertheless, it is possible that endogenous levels of enterostatin are one factor in the determination of macronutrient preferences. Other neuropeptides that might also have an important influence on mac-

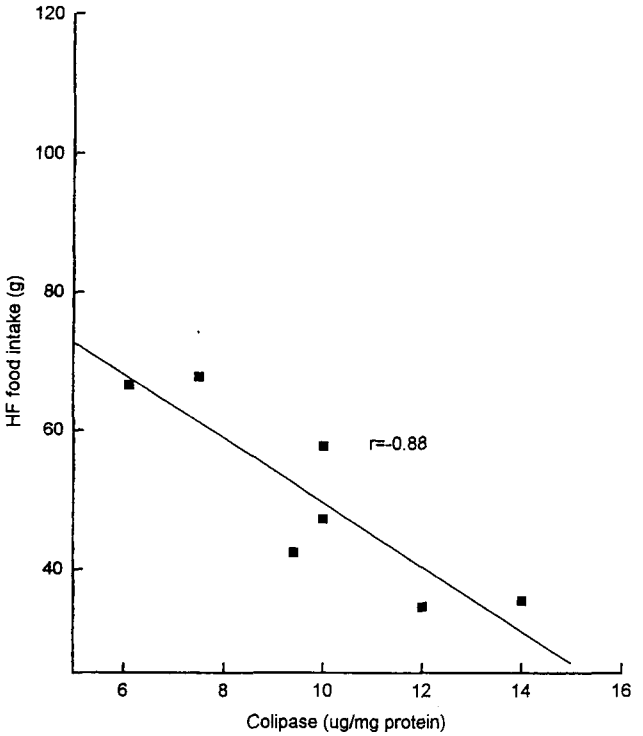


Figure 4: Inverse relationship between fat intake and pancreatic procolipase in Sprague-Dawley rats.

ronutrient selection include NPY (carbohydrate), κ-opioid agonists (fat), and galanin (fat or carbohydrate), in addition to the serotoninergic (fat) and $\alpha_2$-adrenergic (carbohydrate) pathways (Table 1).

### *Structure-Function Relationships*

Enterostatin has been identified in three molecular forms through peptide sequencing and cDNA analysis—VPDPR, APGPR, and VPGPR. The proportion of these forms varies between species (72). The major form in human, mouse, and rat is APGPR, whereas the dominant form is VPDPR in pig, dog, and horse. The explanation for the multiple molecular forms is unclear. It is unlikely to reflect multiple genes but may result from polymorphic changes in the triplet code for these sequences. Studies of the bioactivity of enterostatin analogs in relation to feeding behavior have demonstrated a similar efficacy of VPDPR and APGPR in the rat after central injection, whereas after peripheral injection, VPDPR was effective but APGPR had no effect on the intake of an HF diet (38). In contrast, APGPR was found to reduce HF food intake after intraduodenal administration (48), whereas VPGPR showed an inhibition of food intake after intravenous administration (70). The dose-response curve to enterostatin is U shaped, exhibiting an inhibition of food intake at lower doses, but stimulation of food intake at higher doses (25,38,63). This biphasic response may be explained in a number of ways. There could be two receptor subtypes with differing affinity-binding sites for enterostatin, one high-affinity site, suggested to be inhibitory to fat intake, and one low affinity-binding site, suggested to be stimulatory on fat intake (38,70). Alternatively, at higher doses, enterostatin may become a partial antagonist. Future identification of enterostatin receptors will differentiate between these possibilities.

A structure-function analysis of the feeding response of enterostatin analogs showed that the critical amino acid sequence was the aspartyl-proline (DP) structure at amino acids 3 to 4. This structure, when cyclized into the diketopiperazine peptide cyclo-Asp-Pro, has all of the biological activities of enterostatin. The two peptides have similar potency, both are selective toward fat, both have U-shaped dose-response curves, and both are effective after peripheral and central administration (38,89). The actual production of the DP peptide was observed in studies of the metabolism of enterostatin (6), opening the possibility for a physiological role of the dipeptide in appetite regulation. However, it is not clear at this time if enterostatin must be converted into cyclo-aspartyl-proline for its biological activity or whether both molecules have a similar three-dimensional structure that allows them to interact individually with the receptor.

The structure-function relationships for enterostatin inhibition of insulin secretion differ somewhat from those characteristics described above for the feeding response. For instance, the tripeptide pro-asp-pro (PDP) inhibited food intake but failed to inhibit insulin secretion in vivo (47).

## Gastrointestinal Effects of Enterostatin

Feelings of hunger and satiety have long been associated with changes in gastrointestinal motility. Gastric emptying is a very important mechanism that prevents overfilling of the gut with nutrients in excess of the digestive capacity. A slowing of gastric emptying has been found to occur in proportion to the fat content of a meal in humans (85). Gastric distention has also been found to be linked with increased feelings of satiety (14). This could be through either a direct effect on gastric stretch receptors or the effect of a prolonged interaction of meal components with intestinal chemoreceptors regulating intestinal motility and satiety. Intracerebroventricular enterostatin reduces gastric emptying of a methylcellulose test meal in rats (41). However, this response appears to be independent of the inhibitory effect of enterostatin on the consumption of an HF diet in comparisons made across three strains of rat. Likewise, intragastric and intraperitoneal enterostatin will reduce food intake but had no effect on stomach emptying.

After the food has entered the small intestine, absorption of the digested products is determined by the intestinal transit time, in addition to the intestinal surface available for absorption. Mechanisms determining the transit time are

complex; a main determinant appears to be fat, which increases the transit time (83). The motility of the intestine during fasting has been characterized as consisting of three phases: phase I, consisting of quiescence; phase II, exhibiting irregular contractions; and phase III, recognized by regular contractions of high frequency. After intraintestinal administration of enterostatin in the pig, a prolongation of phase I, a phase with no electrical activity, was observed (58). Such a prolongation would be expected to slow down the absorption of nutrients and prolong intestinal transit time, in agreement with the situation after intestinal fat infusion. Enterostatin also inhibits pancreatic enzyme secretion in the pig (27), consistent with a role to slow digestion and absorption.

## Metabolic Effects of Enterostatin

Chronic icv and peripheral administration of enterostatin reduces bodyweight and body fat, even though the effects on food intake may be short lived (35,52). This suggests that enterostatin may have other metabolic effects that contribute to the weight loss. Initial studies support this suggestion. Both acute administration and chronic administration of enterostatin modulate insulin and glucocorticoid levels, hormones that influence energy metabolism. Thus, intraduodenal, intraperitoneal, and icv enterostatin all decrease the secretion of insulin in situ (45,47,52). This inhibition of glucose-stimulated insulin secretion has also been demonstrated on isolated rat islets (45), in perfused islets (23), and in a perfused pancreas (65). With the perfused rat pancreas, it was furthermore demonstrated that enterostatin also inhibited insulin secretion in response to stimulation by arginine or by tolbutamide (65). This inhibition of insulin secretion by enterostatin was not related to β-cell damage; furthermore, the inhibition was specific to insulin secretion and did not influence glucagon or somatostatin secretion.

The inhibition of insulin release by enterostatin has been reported after both acute and chronic administration (47,52). There is normally no associated change in blood glucose (35,47), but a small rise in blood glucose levels was observed in one study after central injection of enterostatin (52). The data suggest that insulin sensitivity may be improved either directly or indirectly by enterostatin. The mechanism through which central enterostatin inhibits insulin secretion is unclear. The speed of the acute response and the low dose used suggest it is unlikely that central enterostatin leaks to the periphery for its effects. It is possible that enterostatin, like several other peptides, might modulate the autonomic nervous system output to the β-cell in the islet of Langerhans and, hence, reduce insulin secretion. By relief of the insulin inhibition of adipose tissue hormone-sensitive lipase (3), or enhanced β-oxidation of fatty acids, enterostatin would increase the utilization of lipids and decrease energy stores in agreement with the reduced adipose tissue mass that was observed after the chronic administration of enterostatin (35,52).

Another hormone affected by enterostatin is corticosterone. Serum corticosterone was increased by enterostatin, after both central and peripheral administration (52). Furthermore, this stimulation of corticosterone secretion was observed after acute as well as chronic administration of enterostatin (52). The observation that serum corticosterone levels were greatly elevated in rats chronically infused with enterostatin suggests that enterostatin might stimulate corticotropin-releasing hormone (CRH) secretion. By using a CRH antagonist, it was, however, demonstrated that enterostatin did not mediate its anorectic effect through CRH (52). CRH may, however, be important in preventing an adaptive increase in the consumption of other nutrients when enterostatin reduces fat intake in experimental paradigms in which rats have choices of diets or macronutrients (88).

The physiological significance of the activation of the hypothalamic-pituitary-adrenal axis by enterostatin is not known. It is, however, well known that HF feeding activates the hypothalamic-pituitary-adrenal axis (11,88). Because the production of enterostatin increases after HF feeding (44), it could be argued that this activation is mediated through enterostatin. The release of corticosterone by enterostatin and during HF feeding could enhance the sensitivity of hormone-sensitive lipase to catecholamines and so stimulate adipose tissue lipolysis. Thus, by coordinately lowering insulin levels and raising corticosterone levels, enterostatin may promote the catabolic effects of glucocorticoids and promote the utilization of fat as an energy source (74) (Figure 5).

In addition to its endocrine effects, enterostatin influences energy metabolism through its activation of the sympathetic drive to brown adipose tissue, which would be expected to increase thermogenesis (51). This stimulation was observed only in rats that had been adapted to an HF diet, suggesting the involvement of additional factor(s) for the enterostatin response to occur. This effect would be consistent with an activation of the CRH pathway. Whatever the mechanism, this enterostatin stimulation of brown adipose tissue (BAT) function is likely to contribute to the weight loss observed during chronic treatment with enterostatin.

Figure 5 presents a schematic representation of the multiple feeding and metabolic effects of peripheral and central enterostatin. We suggest that enterostatin promotes weight loss through coordinate actions to reduce fat intake and enhance peripheral oxidation of lipids through both autonomic and endocrine pathways.

## Obesity and Enterostatin

In addition to the changes in enterostatin levels and responses that have been described in animal models of obesity (see above), there are also data to suggest that en-

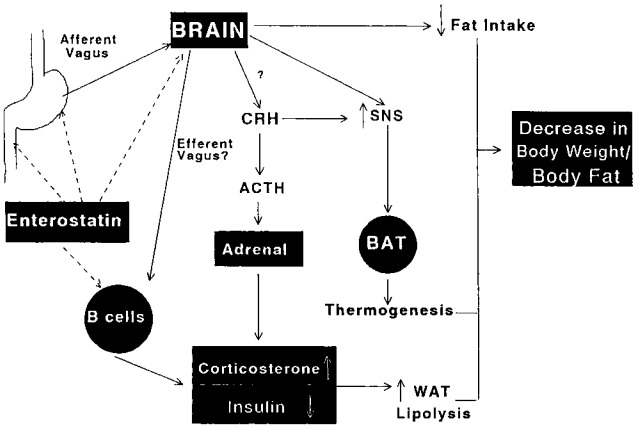


Figure 5: Schematic representative of the feeding and metabolic responses to peripheral and central enterostatin. Dotted arrows show sites of action of enterostatin. Peripherally, enterostatin activates the afferent vagus and has a direct action on pancreatic β-cells to suppress insulin secretion. Centrally, it not only modulates fat intake but also enhances sympathetic drive to BAT and increases corticosterone secretion. The combined effects of increased glucocorticoids and reduced insulin are likely to enhance the mobilization of lipid. This, together with the increased BAT thermogenesis and decreased fat ingestion, results in the reduction in bodyweight and body fat during chronic treatment with enterostatin.

terostatin secretion may be reduced in human obesity. Because obesity has been associated with high levels of fat intake (15,61), it is of obvious interest to investigate the production of enterostatin in patients with obesity compared with persons of normal weight. The outcome of such investigations has been hampered by the lack of an assay sensitive enough to detect serum levels of enterostatin. Bowyer et al. (8) investigated the urine of three morbidly obese and six normal-weight individuals after a satiating meal and reported that enterostatin was present in the urine of two patients with obesity at a significantly lower level than that in the subjects of normal weight. Further, the early peak in urine was absent and was replaced by a slow rise in urinary enterostatin after 4 hours to 6 hours in two of the three subjects with obesity. Enterostatin immunoreactivity could not be detected in serum of the individuals with obesity but was detectable, but highly variable, in the individuals of normal weight. This variability may be a reflection of the assay sensitivity and detection threshold. O. Wisén et al. (unpublished observations) measured procolipase activity in intestinal content after stimulation with CCK in patients of normal weight and patients with obesity. As shown in Figure 4, the patients with obesity had a 3-fold to 4-fold reduction in the production of pancreatic procolipase compared with the patients of normal weight, although this level

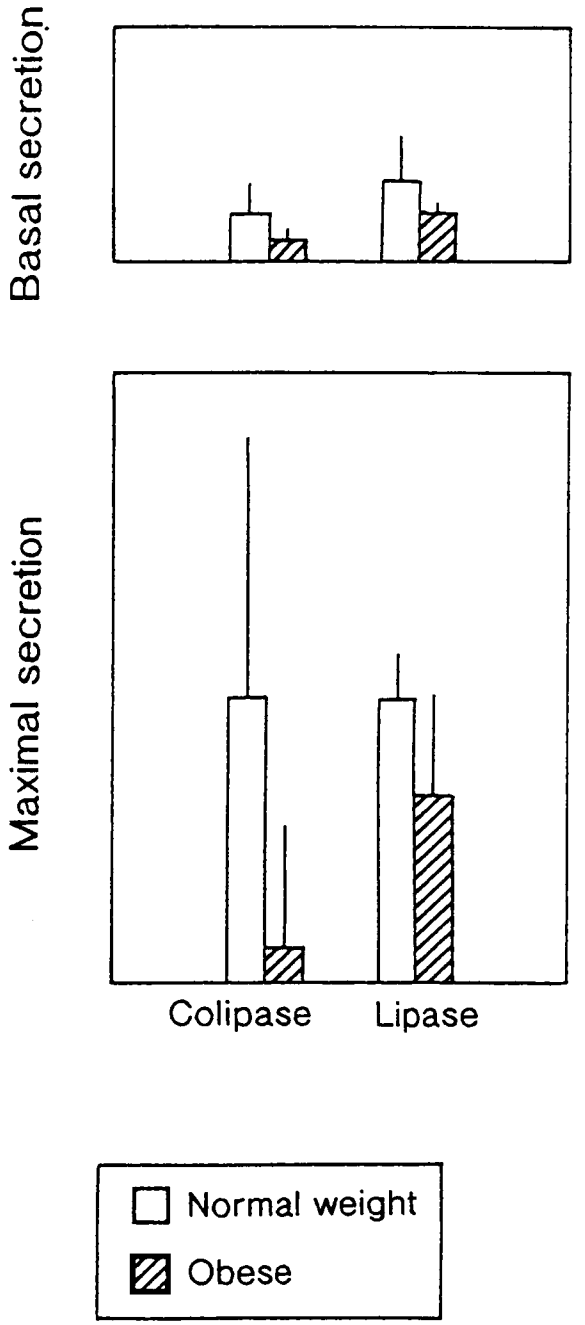


Figure 6: Secretion of pancreatic colipase and lipase in patients with obesity compared with subjects of normal weight. Patients were provided with intestinal catheters for the collection of duodenal juice. After stimulation with CCK, the duodenal content was aspirated and immediately frozen. Assay for pancreatic lipase and colipase was performed with pH-stat titration with tributyrine in bile salt as substrate. The patients with obesity (n = 6) had a severely reduced production of colipase compared with patients of normal weight (n = 6).

was still sufficient to maintain normal fat digestion and fat absorption. One explanation for the reduced pancreatic secretion in patients with obesity is vagal dysfunction, which would lead to decreased secretion of both gastric and pancreatic juice. These preliminary results identify the need for further study to determine the reliability of these observations and the relationship of enterostatin secretion to feeding behavior in subjects of normal weight and subjects with obesity.

A preliminary study of the efficacy of enterostatin after the acute ingestion of a meal in human subjects was negative (62). This may reflect the experimental design used in which enterostatin was given intravenously, a route known from animal studies to have a long delay in the response.

## Summary

High levels of dietary fat are thought to convey health risks. The ability to regulate fat intake is important for the regulation of bodyweight and energy balance, because fat oxidation must be coupled to fat intake for energy balance. Understanding the mechanisms that determine fat preference and fat intake may thus provide new approaches toward reducing the consumption of dietary fat. Experimental work with animal models has shown that enterostatin may have both peripheral and central sites of action to inhibit fat intake selectively. The peripheral and central responses may differ in their effects on feeding behavior, controlling the intake of an immediate meal or fat appetite, respectively. Metabolic effects consistent with enterostatin-promoting energy expenditure and fat mobilization have also been described. Only future work will show if the interesting biology of enterostatin reflects a physiological role of this peptide to modulate fat intake and body composition. Such progress will require the development of good antibodies for the assay of enterostatin and the identification of a specific enterostatin receptor.

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