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Afferent signals regulating food intake

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Food intake is a regulated system. Afferent signals provide information to the central nervous system, which is the centre for the control of satiety or food seeking. Such signals can begin even before food is ingested through visual, auditory and olfactory stimuli. One of the recent interesting findings is the demonstration that there are selective fatty acid taste receptors on the tongue of rodents. The suppression of food intake by essential fatty acids infused into the stomach and the suppression of electrical signals in taste buds reflect activation of a K rectifier channel (K 1·5). In animals that become fat eating a high-fat diet the suppression of this current by linoleic acid is less than that in animals that are resistant to obesity induced by dietary fat. Inhibition of fatty acid oxidation with either mercaptoacetate (which blocks acetyl-CoA dehydrogenase) or methyl-palmoxirate will increase food intake. When animals have a choice of food, mercaptoacetate stimulates the intake of protein and carbohydrate, but not fat. Afferent gut signals also signal satiety. The first of these gut signals to be identified was cholecystokinin (CCK). When CCK acts on CCK-A receptors in the gastrointestinal tract, food intake is suppressed. These signals are transmitted by the vagus nerve to the nucleus tractus solitarius and thence to higher centres including the lateral parabrachial nucleus, amygdala, and other sites. Rats that lack the CCK-A receptor become obese, but transgenic mice lacking CCK-A receptors do not become obese. CCK inhibits food intake in human subjects. Enterostatin, the pentapeptide produced when pancreatic colipase is cleaved in the gut, has been shown to reduce food intake. This peptide differs in its action from CCK by selectively reducing fat intake. Enterostatin reduces hunger ratings in human subjects. Bombesin and its human analogue, gastrin inhibitory peptide (also gastrin-insulin peptide), reduce food intake in obese and lean subjects. Animals lacking bombesin-3 receptor become obese, suggesting that this peptide may also be important. Circulating glucose concentrations show a dip before the onset of most meals in human subjects and rodents. When the glucose dip is prevented, the next meal is delayed. The dip in glucose is preceded by a rise in insulin, and stimulating insulin release will decrease circulating glucose and lead to food intake. Pyruvate and lactate inhibit food intake differently in animals that become obese compared with lean animals. Leptin released from fat cells is an important peripheral signal from fat stores which modulates food intake. Leptin deficiency or leptin receptor defects produce massive obesity. This peptide signals a variety of central mechanisms by acting on receptors in the arcuate nucleus and hypothalamus. Pancreatic hormones including glucagon, amylin and pancreatic polypeptide reduce food intake. Four pituitary peptides also modify food intake. Vasopressin decreases feeding. In contrast, injections of desacetyl melanocyte-stimulating hormone, growth hormone and prolactin are associated with increased food intake. Finally, there are a group of miscellaneous peptides that modulate feeding. β-Casomorphin, a heptapeptide produced during the hydrolysis of casein, stimulates food intake in experimental animals. In contrast, the other peptides in this group, including calcitonin, apolipoprotein A-IV, the cyclized form of histidyl-proline, several cytokines and thyrotropin-releasing hormone, all decrease food intake. Many of these peptides act on gastrointestinal or hepatic receptors that relay messages to the brain via the afferent vagus nerve. As a group they provide a number of leads for potential drug development.

Food intake: Afferent signals: Peptides: Satiety

Abbreviations: CCK, cholecystokinin; CNTF, ciliary neurotrophic factor; dMSH, deacetyl melanocyte-stimulating hormone; GRP, gastrin-releasing peptide; MSH, melanocyte-stimulating hormone.

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Modulation of food intake is a complex process. It has been well established that central monoaminergic and peptidergic systems play important roles in regulating food intake. Recently, a growing list of peptides has also been shown to affect food intake when given peripherally. The largest number of these peptides comes from the gut–brain group of neuroenteric peptides, and in most cases they inhibit feeding. A few peptides also increase food intake. The peptides that regulate food intake when given peripherally are the subject of the present paper. Monoamines and nutrients also affect feeding. The present review will examine all these peripheral signals that affect food intake. Table 1 lists the peptides, monoamines and metabolites that affect feeding.

Gastrointestinal signals

Cholecystokinin (CCK), bombesin, glucagon, insulin, enterostatin, cyclohistidyl-proline, somatomedin, amylin, leptin, and apolipoprotein A-IV all reduce food intake. β-Casomorphin is the only peptide known to us that increases food intake when administered peripherally. Of the peptides acting peripherally, leptin, which is produced in adipose tissue, is one of the most important because it reduces food intake and stimulates the sympathetic nervous system. CCK is the most clearly established gastrointestinal peptide that is physiologically involved in suppression of food intake.

A number of peptides modulate feeding when injected peripherally (Bray & York, 1971, 1979; Lee *et al*. 1994; Bray, 1995; Leibowitz & Hoebel, 1998). Table 2 pulls together information about some of the important peptides that may be the basis for therapy aimed at using peripheral satiety messages to treat obesity. Each peptide will be discussed individually. Table 2 indicates whether the effect has been documented for the peptide in question.

Taste

The taste and smell of food are important factors regulating its intake and ingestion. Sweet-tasting foods usually signify the presence of glucose or other sugars, and these foods are highly prized by many species. The umami, or savoury, taste may be related to proteins. Some tastes, such as bitter and

Table 1. Compounds which affect food intake when given peripherally

Effect on food intake			
Increase food intake		Decrease food intake	
Compound	Reference	Compound	Reference
Monoamines and metabolites			
2-Deoxy-D-glucose	Thompson & Campbell (1977)	Glucose	Russek (1963), Sakata & Kurokawa (1992)
2,5-Anhydromannitol	Tordoff *et al.* (1991), Sakata & Kurokawa (1992)	Lactate	Langhans (1996), Nagase *et al.* (1996*b*)
		Pyruvate	Langhans (1996), Nagase *et al.* (1996*b*)
Glucosamine	Sakata & Kurokawa (1992)	3-Hydroxybutyrate	Langhans (1996), Nagase *et al.* (1996*b*)
N-acetylglucosamine	Sakata & Kurokawa (1992)	3, 4-Dihydroxybutanoate	Oomura (1986)
1,5-Anhydroglucitol	Sakata & Kurokawa (1992)	2-Buten-4-olide	Oomura (1986)
2-Mercaptoacetate	Ritter & Taylor (1989)	5-Hydroxytryptophan	Cangiano *et al.* (1992)
Methylpalmoxirate	Horn & Friedman (1998)	β_2-Adrenergic agonists	Cangiano *et al.* (1992)
2, 4, 5-Trihydroxypentanoate	Oomura (1986)	β_3-Adrenergic agonists	Yamashita *et al.* (1994), Susulic *et al.* (1995), Tsujii & Bray (1998)
		Serotonin	Bray & York (1972), Blundell *et al.* (1995)
		Propylgallate	Glick *et al.* (1982)
		Simmondsin	Cokelaere *et al.* (1992)
Peptides			
Insulin	Diabetes Care and Complications Trial (1993), United Kingdom Prospective Diabetes Study Group (1998)	Apolipoprotein IV	Fujimoto *et al.* (1993)
		Bombesin	Lieverse *et al.* (1993), Muurahainen *et al.* (1993)
β-Casomorphin	Lin *et al.* (1998)	Cholecystokinin	Gibbs *et al.* (1973), Kissileff *et al.* (1981), Stacher *et al.* (1982), Gibbs & Smith (1988), Boosalis *et al.* (1992)
		Enterostatin	Erlanson-Albertsson *et al.* (1991), Okada *et al.* (1991), Shargill *et al.* (1991)
		Glucagon	Schulman *et al.* (1957), Penick & Hinkle (1963), Geary (1990), Geary *et al.* (1992), Flint *et al.* (1998)
		Gastrin-releasing peptide	Gutzwiller *et al.* (1994)
		Insulin	VanderWeele *et al.* (1982)
		Leptin	Campfield *et al.* (1995*a*), Halaas *et al.* (1995), Pelleymounter *et al.* (1995), Flier (1998), Zhang & Leibel (1998)
		Neuromedin B and C	Kirkham *et al.* (1995*b*)
		Somatostatin	Lieverse *et al.* (1995*b*)

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Table 2. Major peptides that affect feeding

	CCK*	Bombesin and neuromedin†	GRP‡	Enterostatin§	Glucagon and GLP-1‖	Leptin¶
Released by meals	Y	Y	Y	Y	Y	Y
Effective in rodents	Y	Y	Y	Y	Y	Y
Graded	Y	Y	Y	Y	Y	Y
Specific	Y	Y	Y	Y	Y	Y
Physiological	Y	Y	?	?	N(?)	Y
Effective in human subjects	Y	Y	Y	N	Y	Y
Graded	Y	?	Y	?	?	Y
Specific	Y	Y	Y	?	Y	Y
Physiological	Y(?)	?	?	–	Y(?)	Y
Blocked by antagonist						
Rat	Y	Y	?	?	Y	?
Human	Y(?)	?	?	?	N	?
Specific	Y(?)	?	?	?	?	?

Y, yes, that effect is produced by peptide; N, no, that effect is not produced by peptide; ?, unknown or conflicting data; CCK, cholecystokinin; GRP, gastrin-releasing peptide; GLP-1, glucagon-like peptide-1.
* Gibbs *et al.* (1973), Kissileff *et al.* (1981), Stacher *et al.* (1982), Gibbs & Smith (1988), Boosalis *et al.* (1992), Lieverse *et al.* (1995*a*,*b*).
† Lieverse *et al.* (1993, 1998), Muurahainen *et al.* (1993), Kirkham *et al.* (1995*b*), Rushing *et al.* (1996).
‡ Stein & Woods (1983), Gutzwiller *et al.* (1994).
§ Erlanson-Albertsson *et al.* (1991), Okada *et al.* (1991), Shargill *et al.* (1991), Rossner *et al.* (1995), Erlanson-Albertsson & York (1997).
‖ Schulman *et al.* (1957), Penick & Hinkle (1963), Geary (1990), Geary *et al.* (1992), Flint *et al.* (1998).
¶ Campfield *et al.* (1995*b*), Halaas *et al.* (1995), Pelleymounter *et al.* (1995), Flier (1998), Zhang & Leibel (1998), Heymsfield *et al.* (1999).

sour, may be signals that the food contains harmful substances. A salty taste generally signals the presence of Na, which is required by land-living animals. Recently, Gilbertson *et al*. (1998) provided evidence that there is an additional taste for selected fatty acids. Using isolated taste buds, they have shown that a specific K rectifier channel (K 1·5) is selectively inhibited by polyunsaturated fatty acids, but not by saturated or monounsaturated fatty acids. In animals that prefer fat and that become obese eating a high-fat diet, the suppression of this K rectifier channel is only about 50 % compared with nearly complete suppression by the same fatty acids in animals that prefer carbohydrates and that do not become obese eating a high-fat diet. Thus, a response system for polyunsaturated fatty acids may be a new taste system and may explain the greater reduction in food intake of S5B rats when oleic acid is infused into their intestine (Greenberg *et al*. 1999).

Cholecystokinin

CCK-33 and the octapeptide of CCK (CCK-8) are produced in the gastrointestinal tract (Liddle, 1995). Two mechanisms exist that stimulate CCK release. The first is a so-called monitor peptide produced in pancreatic acinar cells and secreted into the intestine. The second is an intestinal factor (luminal CCK-releasing factor) that stimulates CCK release in response to ingestion of protein or fats or in response to protease inhibitors. This coordinate system (Kissileff *et al*. 1981; Stacher *et al*. 1982; Liddle, 1995; Herzig *et al*. 1996; Miyasaka & Funakoshi, 1997; Gibbs & Smith, 1998) can regulate cholecystokinin levels in the gastrointestinal tract. When injected parenterally CCK-8 produces a dose-related reduction in sham-feeding in experimental animals and in lean and obese human subjects (Table 2; Kissileff *et al*. 1981; Stacher *et al*. 1982; Baile & Della-Fera, 1984; Boosalis *et al*. 1992; Smith & Gibbs, 1994; Lieverse *et al*. 1995*a*; Gibbs & Smith, 1998). There are two cholecystokinin receptors, CCK_A and CCK_B. The former is located primarily in the gastrointestinal tract and the latter in the brain. CCK_A receptor antagonists have been shown to increase feeding, implying that they may mediate a satiety signal from CCK (Lieverse *et al*. 1995*c*). One hypothesis for this effect is that cholecystokinin acts on CCK_A receptors in the pyloric channel of the stomach to cause constriction of the pylorus and to slow gastric emptying (Corwin *et al*. 1991), suggesting an important role for this peptide-stimulated afferent pathway. The Otsuka Long-Evans Tokushima rat, which has no CCK_A receptors, is obese and does not respond to exogenous CCK, which supports the suggested physiological role of CCK (Moran *et al*. 1998). On the other hand, a transgenic mouse lacking the CCK_A receptor also does not respond to exogenous CCK, indicating that the CCK_A receptor has been eliminated.

Peptide analogues of CCK provide one avenue for drug development (Moran *et al*. 1992; Johnson *et al*. 1994). The recently described benzodiazepines, which are CCK agonists, are a second way to use this strategy (Henke *et al*. 1996). Antagonists to proteolytic degradation of CCK and CCK-releasing factors in the gastrointestinal tract are a third approach to enhancing the effect of CCK and to altering gastric emptying, gastric distention and food intake (Liddle, 1995). Although acute treatment with CCK reduces food intake, chronic reduction in weight or food intake has only been shown by injecting CCK into animals that receive food during a restricted period of time (schedule-fed) (Bray & York, 1979).

Vagatomy blocks the reduction in food intake produced by the peripheral injection of CCK, suggesting that afferent

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messages are generated in the gastroduodenal–hepatic circuit and relayed to the brain by the vagus nerve (Bray & York, 1979). These vagal messages initiated by the intraperitoneal or intravenous administration of CCK activate several neuronal complexes in the brain, including the nucleus tractus solitarius, the lateral parabrachial nucleus, and the central nucleus of the amygdala, as assessed by expression of the early gene product c-fos (Hamamura *et al*. 1991). The production of early satiety by CCK does not require an intact medial hypothalamus because it occurs in human subjects with hypothalamic injury and obesity (Boosalis *et al*. 1992).

In human studies CCK causes hypophagia, ranging from 6 to 63 (average 27) % in lean subjects and ranging from 13 to 33 (average 21) % in obese subjects. A small number of studies have reported gastrointestinal side effects (Bray & York, 1979).

In addition to its peripheral effects, CCK injected into the central nervous system will also decrease food intake (Crawley & Corwin, 1994) and increase sympathetic activity (Yoshimatsu *et al*. 1992) by acting through CCK receptors. A biological role for CCK in the brain in modulating feeding is suggested by the fact that food in the stomach is associated with the release of CCK in the hypothalamus, and that blockade of CCK in the brain with anti-CCK antibodies increases food intake (Baile & Della-Fera, 1984).

Bombesin, neuromedin B and C, and gastrin-releasing peptide

Bombesin is a tetradecapeptide that was isolated from amphibian skin and is similar in structure to mammalian gastrin-releasing peptide (GRP) and neuromedin B (Table 2; Lee *et al*. 1994; Kirkham *et al*. 1995*a*,*b*). Bombesin acts through three different receptors, a GRP receptor, a neuromedin B receptor (Ladenheim *et al*. 1997*a*), and a bombesin-3 receptor. Studies on the contractile effect of bombesin in the gastric fundus show bombesin to be more potent at binding to the GRP-preferring receptors than either neuromedin B or neuromedin C receptors (Ladenheim *et al*. 1997*b*). The suppression of food intake showed the following order of potency: bombesin = acetyl neuromedin C > neuromedin C = GRP > neuromedin B = acetylneuromedin B (Ladenheim *et al*. 1996*b*). A mouse with a ‘knock-out’ of the bombesin-3 receptor has been reported to be moderately obese after at least 6–8 weeks of age (Okki-Hamazaki *et al*. 1997). Hyperphagia, however, is only a significant feature at greater than 12 weeks after the obesity has developed, suggesting that at least one of the three bombesin receptors may be involved in regulation of long-term fat stores.

Administration of bombesin parenterally to experimental animals or intravenously to human subjects (Lieverse *et al*. 1993, 1998; Muurahainen *et al*. 1993; Ladenheim *et al*. 1996*a*) will reduce food intake, but in contrast to CCK this effect is not completely blocked by vagotomy, although it can be blocked by vagotomy plus interruption of spinal afferents (Gibbs *et al*. 1979; Smith *et al*. 1981; Table 2). The effects of bombesin are independent of CCK, since drugs that block the effects of CCK do not block bombesin. Bombesin (Lieverse *et al*. 1993; Muurahainen *et al*. 1993) and GRP (Gutzwiller *et al*. 1994) decreased food intake in lean human subjects but not in obese women when compared with saline (9 g NaCl/l; Lieverse *et al*. 1998).

GRP has twenty-seven amino acids and inhibits food intake in rats (Stein & Woods, 1983; Table 2); it also reduces food intake in human subjects (Gutzwiller *et al*. 1994). In addition to the peripheral receptors for GRP, GRP receptors in the hind brain are also necessary for the peripheral response to GRP (Ladenheim *et al*. 1996*a*).

Peripheral or central injection of bombesin reduces food intake that is not blocked by vagotomy (Gibbs *et al*. 1979; Smith *et al*. 1981). Bombesin also activates the sympathetic nervous system (Barton *et al*. 1995). In animals that have been starved or have ventromedial hypothalamic lesions, bombesin produces a profound drop in temperature because the sympathetic nervous system cannot be activated (Barton *et al*. 1995). In intact animals bombesin will reduce body temperature if a ganglionic-blocking drug or the β-adrenergic antagonist, propranolol, is given that will eliminate the sympathetic activation of the thermogenic system in brown adipose tissue by bombesin.

Enterostatin and cyclohistidyl-proline

Enterostatin (val-pro-gly-pro-arg) is a pentapeptide produced by trypsin cleavage of pancreatic procolipase in the intestine (Lin *et al*. 1994; Erlanson-Albertsson & York, 1997; Table 2), and appears in chromaffin cells in the stomach as a result of local synthesis or accumulation of circulating enterostatin (Sorhede *et al*. 1996). Procolipase is secreted in response to dietary fat, and its signal peptide enterostatin is highly conserved across a number of species (Erlanson-Albertsson *et al*. 1991; Erlanson-Albertsson & York, 1997). Enterostatin decreases food intake whether given peripherally or centrally (Erlanson-Albertsson *et al*. 1991; Okada *et al*. 1991; Shargill *et al*. 1991). Peripheral injection of enterostatin selectively reduces fat intake by approximately 50 % in animals that prefer dietary fat (Erlanson-Albertsson *et al*. 1991; Okada *et al*. 1991). The peripheral effects of enterostatin are blocked by vagotomy or capsaicin treatment, indicating the importance of afferent vagal information for the action of this peptide (Tian *et al*. 1994). This afferent information activates c-fos expression in the nucleus of the nucleus tractus solitarius, in the lateral parabrachial nucleus, the central nucleus of the amygdala, and the supraoptic nucleus (Tian *et al*. 1994), which is similar to the effect of CCK. Injection of enterostatin also enhances serotonin turnover in the central nervous system (Erlanson-Albertsson & York, 1997). The dose response for enterostatin is ‘U’-shaped, with an optimal inhibitory effect on feeding in rats being achieved at 1 nmol peripherally. Higher and lower doses are less effective, and at high doses enterostatin actually stimulates food intake. Enterostatin stimulates the sympathetic nervous system at doses that decrease food intake (Nagase *et al*. 1996*c*), and chronic infusion will reduce body weight (Lin & York, 1998).

Enterostatin reduces food intake by intracerebroventricular injection, just as it does when given peripherally. It selectively reduces fat intake and is more potent when injected in the amygdala than in the paraventricular nucleus

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(Lin & York, 1995). There is almost no response when enterostatin is injected into the nucleus tractus solitarius (Lin & York, 1995). At high doses in non-deprived rats enterostatin has been reported to increase food intake (Rice & Corwin, 1998). In one clinical trial the administration of enterostatin intravenously did not reduce food intake in human subjects (Rossner *et al*. 1995).

β-Casomorphin

β-Casomorphin is the only peptide that stimulates food intake when given peripherally. It is a cleavage product of milk casein (Lin *et al*. 1998). It has seven amino acids with the sequence tyr-pro-phe-pro-gly-pro-ileu, in contrast to the val-pro-gly-pro-arg or ala-pro-gly-pro-arg sequences for enterostatin. Since there are 'pro-X-pro' similarities between enterostatin and β-casomorphin, the effects of β-casomorphin and its four and five amino acid N-terminal fragments on food intake have been tested (Lin *et al*. 1998). β-Casomorphin 1–7 stimulates food intake when injected peripherally. This effect is completely lost if the three carboxy-terminal amino acids gly-his-ileu are removed. However, β-casomorphine 1–4 still retains its opioid-like properties. Thus, the gly-his-ileu carboxy terminal tripeptide contains important information for modulating feeding.

Apolipoprotein A-IV

Apolipoprotein A-IV is produced by the intestine and is incorporated into lipoproteins and chylomicrons. When this peptide is injected peripherally there is a significant decrease in food intake. The release of apolipoprotein A-IV during the hydrolysis of lipoproteins by lipoprotein lipase in the periphery has been hypothesized to be a satiety signal related to fat digestion (Fujimoto *et al*. 1993; Okumura *et al*. 1995). The active component of apolipoprotein A-IV is a short amino acid sequence that may provide new clues for peripherally-acting agents that can reduce food intake.

Pancreatic hormonal signals

Insulin

The effects of insulin on food intake depend on the dose and route of administration. Although intraportal infusion of insulin did not affect food intake in rats, infusion of an anti-insulin antibody increased meal size, suggesting that the presence of insulin may be related to meal termination (Esler *et al*. 1995).

In doses that will lower blood glucose insulin is hyperphagic, probably because it produces hypoglycaemia (Diabetes Care and Complications Trial, 1993; United Kingdom Perspective Diabetes Study Group, 1998). Indeed, the transient declines in glucose that precede many meals may result from a brief transient rise in insulin (Campfield *et al*. 1996). In contrast, chronic infusion of low doses of insulin inhibits feeding (VanderWeele *et al*. 1982). Infusion of insulin into the ventricular system decreases food intake and body weight of baboons (Woods *et al*. 1979) and rodents (Brief & Davis, 1984; Arase *et al*. 1988; McGaowan *et al*. 1993; Schwartz *et al*. 1994; Porte *et al*. 1998). A similar finding was reported for animals eating a high-carbohydrate diet but not in those eating a high-fat diet (Arase *et al*. 1988). Schwartz *et al*. (1994) demonstrated that changes in cerebrospinal fluid insulin reflect blood levels and are related to food intake. The authors showed that the entry of insulin is a facilitated process, and that it may be a negative feedback for regulating fat stores. A low level of insulin secretion and enhanced insulin sensitivity both predict weight gain in Pima Indians (Ravussin & Swinburn, 1992; Schwartz *et al*. 1995).

The use of diazoxide is one approach to lowering insulin, and is successful in slowing weight gain in animals (Alemzadeh *et al*. 1996). Long-term octreotide treatment has caused weight loss, reduced insulin resistance and reduced acanthosis nigricans in a case report (Lunetta *et al*. 1996; Lustig *et al*. 1999). An agent that reduces insulin secretion and obesity in experimental animals has been reported by Campfield *et al*. (1995*b*) and opens the field to new potential pharmacological agents.

Glucagon

Glucagon is a twenty-nine amino acid peptide that reduces food intake after peripheral administration (Penick & Hinkle, 1961; Geary & Smith, 1983; Geary, 1990; Table 2). It produces a dose-dependent inhibition of food intake following portal vein administration in experimental animals. Antibodies that bind glucagon increase food intake, suggesting that the signals generated by pancreatic glucagon act in the liver and may be physiologically relevant in modulating feeding. Glucagon decreases food intake in human subjects when given alone but not when given simultaneously with CCK (Geary *et al*. 1992).

Glucagon-like peptide-1 (glucagon 6–29) is produced by the post-translational processing of pro-glucagon, and is thought to be one signal that enhances insulin release in response to glucose incretin (gastric inhibitory peptide and/or glucagon-like peptide; Nauck *et al*. 1993). Infusion of glucagon-like peptide-1 peripherally in human subjects will significantly reduce food intake (Flint *et al*. 1998).

Amylin

Amylin, or islet-associated polypeptide, is a thirty-seven amino acid peptide that is co-secreted with insulin from the pancreatic β-cell. Many of its biological activities mimic those of the calcitonin gene-related peptide that is not a β-cell peptide (Castillo *et al*. 1995). The level of amylin is related to the level of insulin, and is higher in older somewhat more obese animals than in lean animals (Pieber *et al*. 1994). Amylin:insulin is increased in genetically-obese animals. In individuals with type I insulin-dependent diabetes amylin is essentially absent from the plasma. The plasma level of amylin rises after a meal or a glucose load (Castillo *et al*. 1995). In a transgenic mouse model over-expressing amylin plasma levels were increased 15-fold, but there was no elevation in glucose or insulin, and obesity did not develop (Hoppener *et al*. 1993). Amylin will decrease food intake in mice (Morley *et al*. 1994; Lutz *et al*. 1995) and rats when given either peripherally (Chance *et al*. 1993) or intrahypothalamically (Chance *et al*. 1991). We are not

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aware of any studies of the effect of amylin on food intake in human subjects.

Somatostatin

Somatostatin is a fourteen amino acid peptide that is present in the pancreas, gastrointestinal tract and brain. Somatostatin serves to inhibit gastrointestinal motility as well as exocrine and endocrine secretions (Table 1). In experimental animals somatostatin decreases food intake (Lotter *et al*. 1981). Somatostatin also decreases food intake in healthy human subjects (Lieverse *et al*. 1995*b*). During the first 1 h of somatostatin infusion there was a significant decrease in feelings of hunger. When an intraduodenal fat load was given at this time it tended to reverse the feelings of satiety. The intake of sandwiches 90 min after the fat load tended to be higher during the somatostatin infusion than during the saline infusion. Feelings of hunger were less in the 5 h after terminating the somatostatin infusion than with the control infusion.

Signals arising from adipose tissue

Leptin

Leptin was discovered by cloning the *ob* gene in the obese hyperglycaemic mutant mouse, which is a widely-studied model of diabetes and insulin resistance. It is a 167 amino acid peptide whose receptor is a member of the gp 130 cytokine superfamily (Campfield *et al*. 1995*a*; Halaas *et al*. 1995; Pelleymounter *et al*. 1995; Flier, 1998; Zhang & Leibel, 1998). Since its discovery in 1994 (Zhang *et al*. 1994), there has been a logarithmic increase in publications concerning this peptide (Zhang & Leibel, 1998). Leptin is synthesized and secreted primarily from adipocytes, but can also be made by the placenta. Circulating levels of leptin are highly correlated with the level of body fat. Leptin production by adipocytes is stimulated by insulin and glucocorticoids, and it is inhibited by β-adrenergic stimulation (Bray, 1996*a*; Flier, 1998; Zhang & Leibel, 1998). Circulating leptin may be bound to a 'carrier' protein. Deficiency of leptin in mice (Zhang *et al*. 1994) and in human subjects (Montague *et al*. 1997) is associated with massive obesity. Conversely, chronic administration of leptin to animals or overexpression of leptin in transgenic mice (Ogawa *et al*. 1999) reduces body fat in a dose-related manner. Leptin reduces food intake and increases the activity of the sympathetic nervous system.

These effects of leptin occur through leptin receptors. Several variants of the leptin receptor have been identified and cloned. In the brain the long form of the leptin receptor is located in the medial hypothalamus. Activation of leptin receptors produces stimulation of Janus kinase, which activates signal transduction and translation molecules. Absence of the leptin receptor produces obesity in mice and in human subjects (Clement *et al*. 1998). The interaction of leptin with receptors in the brain activates neurons in the arcuate nucleus that produce pro-opiomelanocortin, and coordinately reduces activity of arcuate nuclei producing neuropeptide-Y (Flier, 1998; Zhang & Leibel, 1998). These two peptide systems are believed to mediate the effects of leptin on food intake in the central nervous system. Lesions in the ventromedial hypothalamus abolish the effects of leptin (Stricker & Rowland, 1978).

Ciliary neurotrophic factor

The receptor subunits of leptin share sequence similarities with the hypothalamic receptor for ciliary neurotrophic factor (CNTF), a neurocytokine (Gloaguen *et al*. 1997). Leptin and CNTF produce similar patterns of activation of signal transduction and translation molecules. Treatment with CNTF of either *ob/ob* mice (which lack leptin) or *db/db* mice (which lack the leptin receptor) reduced the adiposity, hyperphagia and hyperinsulinaemia. CNTF was similarly effective in mice with diet-induced obesity. These findings, coupled with the fact that the overexpression of leptin in transgenic mice will almost completely eliminate body fat, suggest that this system may be a valuable one to target. In a published trial using CNTF in patients with amyotrophic lateral sclerosis (ALS CMTF Treatment Study Group, 1996) weight loss and anorexia were among the most notable side effects.

Data from one clinical trial with leptin was presented in 1999 (Heymsfield *et al*. 1999). A total of fifty-four lean (72 kg) and seventy-three obese subjects (90 kg) were assigned to 4 weeks of treatment with three daily peripheral injections of recombinant methionyl human leptin at doses of 0·01, 0·03, 0·1 or 0·3 mg/kg, and were randomized within each dose level to placebo or leptin and stratified according to BMI (Herzig *et al*. 1996). At the end of 4 weeks, sixty of the seventy obese patients who remained in the study elected to continue for an additional 20 weeks. Subjects were on a diet that was 2090 kJ (500 kcal)/d below maintenance energy throughout the study. Using subjects who completed the study (fifty-three lean subjects at 4 weeks and forty-seven obese subjects at the end of 20 weeks), the authors found a significant dose response for weight loss from baseline at 4 weeks, and from baseline in the obese subjects treated for 24 weeks (weight changes for obese subjects at 24 weeks were: placebo −1·7; leptin (mg/kg) 0·01 −0·7 kg, 0·03 −1·4, 0·10−2·4 kg, 0·3−7·1 kg). Injection-site reactions were the most common adverse event, but only two subjects withdrew for this reason. Glycaemic control was unchanged during the study. Leptin treatment of a child with leptin deficiency also lowered food intake and body weight (Farooqi *et al*. 1999). These studies show that human leptin can produce weight loss in human subjects. The route of delivery needs to be improved if it is to become acceptable.

Nutrient and monoamine signals

Hexose analogues and metabolites

The glycostatic hypothesis (Mayer, 1953), which might be better called the glucodynamic hypothesis (Bray, 1996*b*), proposes that rates of glucose utilization or changes in glucose concentration may be signals to eat or stop eating. The most convincing data that glucose plays this role comes from Louis-Sylvestre & LeMagnen (1980) and Campfield *et al*. (1996) who have shown that a dip in glucose can precede and trigger the onset of meals in animals and human

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subjects. Peripheral infusions of glucose decrease food intake in experimental animals (Niijima, 1983); the vagus nerve may be the connection between the peripheral glucoreceptors and the brain. When glucose is infused into the portal circulation, vagal afferent firing is reduced as the glucose concentration increases. Infusion of either glucose or arginine will lower the vagal firing rate and increase sympathetic efferent firing of nerves to brown adipose tissue (Inoue *et al*. 1991).

5,7-Anhydro-mannitol (or deoxy-fructose) is an analogue of fructose that stimulates food intake when given peripherally (Tordoff *et al*. 1991). One mode of action proposed for this compound is a decrease in hepatic ATP concentration. Another fructose analogue (2,5-anhydro-mannitol) will stimulate food intake when given intracerebroventricularly, but not when given intra-peritoneally (Sakata & Kurokawa, 1992). The likely explanation for the actions of both compounds is their ability to interfere with glucose metabolism. Pyruvate and lactate, two metabolites of glucose, also decrease food intake when injected peripherally (Langhans, 1996; Nagase *et al*. 1996*a*). Analogues of these various metabolites might be interesting molecules to test for anti-obesity effects. Glucosamine and N-acetylglucosamine both increase food intake when given orally to rats (Sakata & Kurokawa, 1992). The stimulation of feeding by N-acetylglucosamine was blocked by vagotomy, but the effect of glucosamine was only modestly attenuated. When glucosamine was given intracerebroventricularly it stimulated food intake. N-acetylglucosamine, on the other hand, was without effect centrally. Fujimoto *et al*. (1986) found that glucosamine accelerated lateral hypothalamic neuronal activity and decreased ventromedial hypothalamic neuronal activity.

Two other compounds deserve brief mention. The first are two polyphenols, gallic acid and its ester propylgallate (propyl-(3, 4, 5-trihydroxybenzoate); Glick, 1981). In feeding studies of lean and obese animals with ventro-medial hypothalamic lesions propylgallate was more potent than gallic acid (Glick *et al*. 1982). The second compound is simmondsin (2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl-β-D-glucoside), which is isolated from jojoba meal, an extract of *Simmondsia chinensis* that grows in the southwest USA (Cokelaere *et al*. 1992). Simmondsin decreases food intake within 1 h and remains effective when added to the diet. It may act through CCK_A receptors, since devazepide blocked the effect of simmondsin (Cokelaere *et al*. 1995).

Ketones, fatty acids, and lipoproteins

Intraperitoneal administration of 3-hydroxybutyric acid, a key metabolic product of fatty acid oxidation, decreases food intake (Blundell *et al*. 1995; Langhans, 1996). Increased circulating levels of this metabolite have been proposed as a satiety signal (Arase *et al*. 1988; Fisler *et al*. 1989). The inhibition of food intake by 3-hydroxybutyrate is dependent on an intact vagus nerve; both vagotomy and capsaicin treatment destroy afferent vagal nerve fibres, so blocking the inhibitory effects of 3-hydroxybutyrate on feeding (Langhans *et al*. 1985).

Oomura and his colleagues (Oomura, 1986) have identified three endogenous fatty acid derivatives in the circulation of rats and human subjects that affect feeding. Two of these derivatives, 3, 4-dihydroxybutanoate (Terada *et al*. 1986) and its lactam (2-buten-4-olide; Fukuda *et al*. 1988; Matsumoto *et al*. 1994), are inhibitory of food intake. The third derivative, 2, 4, 5-trihydroxypentanoate (Sakata *et al*. 1989), stimulates feeding. Produced peripherally, the most active stereoisomers are the 3-S isomer of 3, 4-dihydroxybutanoate and the 2-S, 4-S isomer of 2, 4, 5-trihydroxypentanoate. The biological significance of the molecules that modulate neuronal activity in the lateral hypothalamus is unclear (Silverstone *et al*. 1992).

Inhibition of fatty acid oxidation by 2-mercaptoacetate (Ritter *et al*. 1992), an inhibitor of acetyl-CoA dehydro-genase, or with methyl palmoxirate (Friedman, 1995; Horn & Friedman, 1998), an inhibitor of carnitine acyltransferase I, will increase food intake. Studies in animals indicate that this increased food intake is predominately carbohydrate and/or protein but not fat, even when fat is the only available nutrient (Singer *et al*. 1997). The peripheral effects of 2-mercaptoacetate are blocked by hepatic vagotomy, but the effects of methyl palmoxirate are not (Ritter & Taylor, 1989, 1990).

Noradrenaline and related compounds

Peripheral injection of noradrenaline in experimental animals reduces food intake (Russek, 1963). Either β_2- and/or β_3-adrenergic receptors may mediate this effect. Treatment with β_2-adrenergic agonists will reduce food intake with little effect on thermogenesis (Yamashita *et al*. 1994). Clenbuterol was ten to thirty times more potent than a β_1-agonist (dobutamine) or a β_3-agonist (ICI D-7114) in reducing food intake (Yamashita *et al*. 1994). However, β_3-agonists do acutely reduce food intake in lean and obese rats (Tsujii & Bray, 1992; Grujic *et al*. 1997) and in lean mice (Susulic *et al*. 1995), but this effect is lost with continued treatment. In mice, knocking out the β_3 adrenergic receptors in white fat blocks the reduction in food intake by β_3-agonists, indicating that there are peripheral β_3-adrenergic receptors involved in the modulation of food intake that act on fat cells, and possibly other tissues, to produce inhibitory signals for feeding (Susulic *et al*. 1995).

α-Adrenergic receptors are widely distributed and have many functions. During weight loss induced by diet, phentermine or fenfluramine, the α-receptor binding by platelets of ligand NB4101 (2-([2′, 6′ dimethoxy] phenoxy-ethylamino) methylbenzodioxan) was increased in all groups. The significance of lower binding of α-adrenergic receptors on platelets of obese subjects is unclear (Sundaresan *et al*. 1983).

Serotonin

Peripheral injection of serotonin reduces food intake and specifically decreases fat intake (Bray & York, 1972; Orthen-Gambil & Kanarek, 1982). Since the majority of serotonin is located in the gastrointestinal tract, it may be that serotonin receptors in this tissue play an important role in the modulation of food intake, in response to enteral

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signals, or to the rate of gastric emptying. Tryptophan, the precursor of serotonin, also reduces food intake in human subjects (Cangiano *et al*. 1992).

Pituitary hormones

Vasopressin

At least four pituitary peptides have been shown to modulate food intake (Table 3). The first of these is vasopressin, the anti-diuretic hormone that enhances water re-absorption from the renal tubal. Vasopressin significantly reduced food intake over a 4 h period in experimental animals. The reduction in food intake, particularly in the first 30 min of feeding, was not significantly impaired by vagotomy, suggesting that its peripheral mechanism of action is different from that of CCK or enterostatin (Langhans *et al*. 1991).

Melanocyte-stimulating hormone

The yellow obese mouse inherits obesity as a dominant trait (Klebig *et al*. 1994). The demonstration that these animals have an increased amount of desacetyl(d) melanocyte-stimulating hormone (MSH) in their pituitary glands led to studies on the effect of dMSH on food intake and weight gain (Shimizu *et al*. 1989). In yellow mice treated with dMSH there was a substantial increase in food intake and weight gain which was thirty to 100 times greater than that of the acylated form (α) of MSH. In contrast, injection of the αMSH produced a much more potent darkening of the melanocyte than did dMSH.

Prolactin

Following treatment with growth hormone, hypophysectomized animals increase their food intake and grow. Whether this finding is a direct effect of growth hormone on feeding centres or a consequence of the enhanced flux of amino acids into new protein and a second stimulation of feeding is unclear, but the latter appears to be a more reasonable hypothesis. Lactation increases food intake, suggesting that prolactin may increase feeding. Gerardo-Gettens *et al*. (1989) found a dose-dependent increase in food intake in response to treatment with prolactin. Injection of prolactin into the cerebroventricular system of pigs also increases food intake. Bromocriptine, a dopamine (3, 4-dihydroxyphenylethylamine)-agonist that reduces prolactin secretion, has been reported to modulate the seasonal fattening of hibernating and migratory animals. The clinical relevance of prolactin to human obesity has not been established. The fact that prolactinomas do not produce obesity would argue against an important role.

Calcitonin

Calcitonin decreases food intake in genetically-obese (*db*/*db*) and in non-obese animals (Morley *et al*. 1982). A strong dose-dependent suppressive effect of calcitonin on food intake can be demonstrated in animals whose feeding has been stimulated by tail pinching, a technique for increasing food intake (Levine & Morley, 1981). As with β-casomorphin, the effect of calcitonin is blocked by vagotomy, suggesting vagally-transmitted afferent messages to the central nervous system (Table 4).

Table 3. Pituitary peptides shown to modulate food intake

Peptide	Amino acids	Food intake		Blocked by vagotomy
		Animals	Man	
Vasopressin	9	↓	?	Yes
dMSH	13	↑	?	?
Growth hormone	191	↑	↑	No
Prolactin		↑	?	?

dMSH, desacetyl melanocyte-stimulating hormone.

Table 4. Miscellaneous peptides affecting food intake

Peptide	Amino acids	Food intake		Blocked by vagotomy
		Animals	Man	
β-Casomorphin	7	↑	?	Yes
Calcitonin	32 or 37	↓	?	Yes
Apo A-IV		↓	?	?
Cyclo-his-pro	2	↓	?	Yes
Cytokines		↓	↓	Partial

Apo A-IV, apolipoprotein A-IV; cyclo-his-pro, cyclohistidyl-proline.

Summary

The present paper has briefly reviewed the peptides that act after peripheral administration to reduce food intake. Most of the peptides in this group decrease food intake, and many are blocked by vagotomy. Agonists to these peptides may provide ways of reducing food intake clinically. A few peptides in this group increase food intake, either directly (β-casomorphin) or indirectly (insulin growth hormone or prolactin). Modulation of this latter group with antagonists may provide insights into new therapies for obesity.

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