Endocrine controls of eating: CCK, leptin, and ghrelin

Nori Geary*

E.W. Bourne Laboratory, Weill Medical College of Cornell University, White Plains, NY 10506, USA

Abstract

The peripheral physiological and central nervous mechanisms contributing to the control of eating present formidable challenges to experimental analysis. One of the most productive approaches to these challenges has been endocrinological. This review introduces the endocrine control of eating by considering three hormonal signals that have been hypothesized to control hunger or satiation, cholecystokinin (CCK), leptin, and ghrelin. The roles of these molecules in humans and in rodents are considered against a set of criteria established in classical endocrinology for establishing physiological endocrine action. It is concluded that according to these criteria, CCK’s satiating action in humans is the best-established physiological endocrine action. In contrast, support for endocrine actions of leptin in satiation and of ghrelin in hunger is incomplete, and areas urgently requiring further research are identified. Finally, a review of work on these three hormones suggests the utility of a new conceptual scheme for understanding the endocrine control of eating. This scheme distinguishes between endocrine effects in which the stimuli for hormonal secretion and the effect of secretion on eating are tightly coupled and endocrine effects in which one or both of these links is uncoupled. The implications of this concept for research design and interpretation of data are discussed.

A vast literature links endocrine systems to the control of eating behavior. This is fortunate for ingestive science. Endocrinology is a well-developed discipline with an impressive armamentarium of intellectual and technical tools. In contrast, ingestive science, i.e., the study of eating, drinking, and drug use, is at a more rudimentary stage of development. My general thesis here is that the adaptation and application of some of the well-accepted intellectual tools of endocrinology is likely to accelerate progress in ingestive science.

The organization of the review is threefold. First, the treatment of endocrine controls of eating is selective. Just three hormones, CCK, leptin, and ghrelin, are considered. It is not clear that these are the three most important endocrine controls of eating, but they are each certainly interesting candidates, and comparisons among them are instructive. Second, I argue for the utility of the application of classical endocrine criteria for the identification of physiological effects of hormones, as adapted to eating behavior. This is done by introducing these criteria, considering each hormone’s status with respect to them, and identifying the areas where relevant evidence is currently available or is lacking. Third, I argue for the utility of making explicit the distinction between endocrine actions in which the stimuli for hormonal secretion and the effect of secretion on eating are tightly coupled and endocrine actions in which these links are uncoupled. This concept is assembled inductively in the course of the review.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Endocrine controls; CCK; Leptin; Ghrelin

1. Identification of physiological effects of hormones on eating

Endocrinology evolved into a distinct research discipline and medical specialty in the first half of the last century [9,35,47,58]. Endocrinology is based on a particular type of control mechanism, i.e., those involving the secretion of signaling molecules into the blood, their transport in the circulation to distant organs, and the initiation of physiological effects via the activation of specific receptors. As the understanding of these processes has grown, there have been developments in the definition of a hormone and the standards of evidence or criteria for identification of hormonal mechanisms [9,37]. In addition, criteria for identifying particular signaling molecules as hormones and criteria for identifying particular effects of these molecules as normal, physiological effects take slightly different forms. Table 1 lists a version of these criteria for the identification of particular effects of hormones as physiological effects.

The next sections of this review (1) evaluate the physiological statuses of the effects on eating of cholecystokinin...
The four phases are (1) hunger, the initiation of eating at meal onset (hunger); (2) the maintenance of eating during the intermeal interval, are all separate hypotheses. (CCK), leptin, and ghrelin against these criteria and (2) discuss several difficulties and subtleties that must be considered in the application of the criteria. Each discussion begins with a specific statement of the hypothesis(es) at risk. This is for two reasons. First, eating behavior has at least four functional phases, and a particular hormone may affect one of these, but not the others, or affect them differently. The four phases are (1) hunger, the initiation of eating at meal onset (hunger); (2) the maintenance of eating during the meal; (3) satiation, the termination of eating at meal end; and (4) postprandial satiety, the inhibition of eating during the postprandial intermeal interval (this terminology follows that of Blundell [13] and Smith [78]). Second, in at least one of the cases considered, important species differences have emerged, which are better treated separately.

2. CCK

The hypothesis is that hormonal CCK released from the small intestine during meals selectively signals satiation. This function seems selective to satiation because CCK injection reduces meal size but usually either fails to affect the intermeal interval or shortens it. Indeed, when CCK was injected during every spontaneous meal by remotely controlled intraperitoneal catheters, there was no tolerance in the satiating effect over several days, but intermeal intervals were shortened so that there was no decrease in total food intake after the third day of infusions [89]. The CCK satiation hypothesis is considered separately for humans and for experimental rodents because research has led to different conclusions.

2.1. CCK and satiation in humans

2.1.1. Secretion

Liddle et al. [52] developed the first sensitive and specific assay for CCK and reported that the ingestion of mixed meals, casein, amino acid or glucose solutions, or lipid emulsions led within minutes to similar marked increases in plasma CCK concentration (Fig. 1, top panel). Thus, CCK is secreted sufficiently rapidly to function as a satiation signal in humans. Prandial CCK secretion has been reported to be markedly greater in women than in men [66], which may be relevant to the apparent sexual differentiation of CCK satiation [28,30,43].

2.1.2. Receptors

CCK’s biological actions are mediated by cell-surface membrane receptors on its target cells [49,50]. CCK receptors are widespread in the gastrointestinal tract and also occur in the pancreatic acinar cells, the gall bladder, and the brain. Moran and Ladenheim [62] and Moran et al. [63] identified two subtypes of CCK receptors with different pharmacological characteristics and different tissue distributions in the periphery and CNS: CCK\textsubscript{A} receptors (or CCK\textsubscript{1} receptors, CCK1R), which have two affinity states, and CCK\textsubscript{B} receptors (CCK2R).

2.1.3. Physiological dose

The work of Liddle [49,50] and Liddle et al. [51,52] on CCK’s effect on gall bladder contraction serves as an excellent model for the consideration of the investigation of endocrine controls of eating. These investigators first established an intravenous infusion procedure that mimicked prandial changes in plasma CCK concentrations and then compared the effects of meals and of exogenous CCK administered at the physiological dose on gall bladder contraction, as indicated by abdominal ultrasonography [52]. The results clearly indicate that prandial gall bladder contraction meets the physiological dose criterion for a physiological effect of prandial hormonal CCK release (Fig. 1, middle panel).

Attempts to use this strategy to test the satiating action of CCK have had mixed results. Almost all early reports that intravenously infused CCK-8 produces satiation in humans used pharmacological doses (see Refs. [43,79–81] for reviews). The lowest effective dose in these studies was between 0.8 and 1.7 pMol/kg/min CCK-8 [31], which is much larger than the physiological dose of 0.2 pMol/kg/min established by Liddle et al. [52]. Importantly, however, even these pharmacological doses inhibit feeding without any subjective or physical side effects [79–81]. In two studies published in 1995, however, apparently, physiological doses of CCK were sufficient to reduce meal size in humans (Table 2). In the first of these landmark studies, Ballinger et
reported that intravenous infusions of 0.72 pMol/kg/min CCK-8 reduced the size (in kJ) of a buffet meal about 20% in a group of normal-weight men and women. A preliminary study had indicated that this infusion produced similar plasma CCK concentrations as did the ingestion of a similar meal. In the second study, Lieverse et al. [54] reported that intravenous infusion of only 0.24 pMol/kg/min CCK-33 significantly reduced both the size of a single-food test meal, again by about 20%, and postprandial “desire to eat” in a group of lean and obese women. This dose increased plasma CCK concentration from about 2 to about 10–14 pMol/l, which was the same increase reported to accompany the ingestion of a mixed meal using the same CCK radioimmunoassay ([26]; although it is somewhat higher than typically reported to accompany meals). These two studies indicate that, at least under some experimental conditions, CCK satiation meets the physiological dose criterion for a hormonal effect.

The cause of the apparent discrepancies among these experiments is not clear. Both of the two studies described above, as well as most previous experiments, attempted to maximize the satiating potency of CCK by using a preload design (Ballinger et al. [6] had participants drink 200 ml water at the beginning of the meal, and Lieverse et al. [54] served a small banana appetizer; neither of which should elicit CCK secretion). Ideal, rather than actual, body weight was used to compute dosages for obese women because this resulted in comparable plasma levels in the two groups.

Table 2
Intravenous infusion of physiological doses of CCK reduces meal size in humans

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Meal size (mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[kJ]</td>
</tr>
<tr>
<td>Saline</td>
<td>6418 ± 673</td>
</tr>
<tr>
<td>Ballinger et al. [6]</td>
<td>CCK-8 5092 ± 665</td>
</tr>
<tr>
<td>Leiverse et al. [54]</td>
<td>CCK-33 346 ± 31</td>
</tr>
</tbody>
</table>

In the Ballinger et al. [6] study, participants were four men and two women, all of normal weight (BMI 21–25 kg/m^2). Infusions were begun at 2000 h after an 8-h fast. After 25 min, the participants were offered a meal of meat, rice, bread, cake, chocolate, and crisps; 400 ml of water was drunk with the meal.

In the Lieverse et al. [54] study, participants were 10 normal-weight women (BMI 22 ± 3 kg/m^2) and 8 healthy obese women (BMI 39 ± 2 kg/m^2). Infusions were begun at 0800 h after an overnight fast. After 60 min, the participants consumed a 132-kcal preload of bananas, followed 15 min later by a banana-shake meal (bananas were used because they do not elicit CCK secretion). Ideal, rather than actual, body weight was used to compute dosages for obese women because this resulted in comparable plasma levels in the two groups.

Fig. 1. Endocrine action of CCK on gall bladder contraction in humans. Top panel: Ingestion of a meal elicits an increase in plasma CCK concentration and is associated with the contraction of the gall bladder. Middle panel: Intravenous infusion of 0.2 pMol/kg/min CCK-8 mimics the initial prandial increase in plasma CCK and is sufficient to produce normal prandial gall bladder contraction in men. Bottom panel: Oral administration of 10 mg of the CCK1R antagonist devazepide during the meal prevents prandial gall bladder contraction. Upper and middle panels are from Ref. [52]; lower panel is from Ref. [51]; used with permission.
when Lieverse et al. [53] omitted the preload, they obtained only an insignificant 13% decrease in meal size. Nor is the difference in the molecular form of CCK used likely to have been important. CCK-8 and CCK-33 have similar affinities for CCK receptors and similar potencies in most biological contexts [50]. CCK-33 is degraded more slowly by the liver than CCK-8 [22], but this difference is not likely to be significant here because Lieverse et al. [54] demonstrated that their infusions did not elevate plasma CCK concentrations more than did meals.

2.1.4. Removal and replacement

There is no selective surgical technique for the removal of the intestinal cells secreting CCK. Some spontaneous mutations of the CCK1R gene, however, indicate that missense mutations of this gene produce a phenotype of obesity and gallstones (the latter due to a flaccid gall bladder; [55,59]). These data are consistent with this criterion but cannot be considered compelling evidence because of the numerous complications that limit the interpretation of phenotypes arising from gene deletions (these complications are considered further below).

2.1.5. Antagonism

Liddle et al. [51] continued their study of CCK’s role in meal-stimulated exocrine pancreatic secretion by testing intravenous infusions of an antagonist of the CCK1 receptor (CCK1R), devazepide (formerly designated L-364,718 or MK-329). Oral administration of 10 mg devazepide completely prevented gall bladder contraction stimulated either by meals or by intravenous infusions of CCK-8 that produced high physiological plasma CCK concentrations (Fig. 1, bottom panel). These data beautifully fulfill the antagonism criterion for a physiological effect of CCK in prandial gall bladder contraction in humans [51]. Shortly thereafter, Beglinger et al. [10] and Matzinger et al. [57] used an analogous strategy to demonstrate that the prandial antagonism of CCK1R by intravenous infusion of the antagonist loxilumide (1) blocks the satiating effect of intraduodenal infusions of fat emulsions, which stimulate endogenous CCK secretion, (2) increases the perception of hunger and decrease the perception of fullness during meals, and (3) increases meal size (Fig. 2). These data indicate that CCK satiation meets the antagonism criterion in humans.

2.2. CCK and satiation in rats and mice

2.2.1. Secretion

There little evidence that CCK is secreted into the blood during meals in rats or mice. Using the assay developed by Liddle et al. [52], Yox et al. [94] have shown that intraduodenal infusions of oleate and other nutrients first increased plasma CCK only after about 30 min, which is longer than the typical meal duration in rats. In contrast, Mamoun et al. [56], using reverse-phase high-performance liquid chromatographic assay, were able to detect three- to fourfold increases in plasma CCK during meals in rats. Because Mamoun et al. [56] delivered food by intrarot infusion, which artificially determines the ingestion rate, it
would be useful to repeat these tests in naturally eating animals. Nevertheless, their data indicate that hormonal CCK is a plausible satiation signal under at least some conditions in rats.

2.2.2. Receptors
The satiating actions of intestinal CCK in the rats arise from the activation of peripheral, low-affinity CCK1R, most likely localized in the circular muscle layer of the pyloric sphincter or nearby vagal afferent nerve endings [62,81,79]. Direct evidence for this localization comes from the demonstration of Cox [18] that CCK inhibits eating and that the CCK1R antagonist devazepide stimulates eating more effectively when infused via the superior pancreaticoduodenal artery, which perfuses these sites, than via the jugular vein (Fig. 3).

2.2.3. Physiological dose
In contrast to the human data reviewed above, intravenously infused CCK inhibited eating much less effectively in rats and dogs than it stimulated pancreatic exocrine secretion [69,71]. Furthermore, intravenous infusion of a CCK antibody reduced pancreatic exocrine secretion but failed to affect eating [72]. In addition, the satiating potency of some intraduodenal nutrient infusions that did not elicit CCK secretion was reversible by CCK1R antagonism with devazepide [14]. One possible explanation of these data is that CCK inhibits eating in rats via a paracrine, rather than via an endocrine, mode of action. This conclusion is directly supported by the finding that the satiating potency of intraperitoneally injected CCK was markedly larger than that of CCK intravenously infused into the hepatic portal vein [34,83]. The close proximity of the CCK-secreting cells in the small intestine to the pyloric site of the crucial receptors for satiation is also consistent with a paracrine mode of action. The prandial changes in local CCK concentration at these receptors are unknown, however; thus, whether a physiological dose of exogenous CCK is sufficient to elicit satiation via this paracrine mechanism has not been investigated.

2.2.4. Removal and replacement
The effects of the absence of CCK have not been described in rats. As for humans, there are data about genetic defects in CCK1R. The Otsuka Long–Evans fatty rat bears a null mutation of the CCK1R gene and displays a phenotype including chronically increased meal size, increased food intake, obesity, and diabetes [61]. In contrast, however, CCK1R knockout mice do not display hyperphagia, obesity, or diabetes [45]. It is possible that the difference in these models has to do with brain, rather than hormonal, CCK. That is, in the OLETF rat, the lack of brain CCK1R is associated with the overexpression of the orexigenic peptide NPY in the dorsomedial hypothalamus, whereas in the mouse, it is not yet clear that this is the case [12]. The extent of the involvement of deranged NPY signaling in the phenotype of the OLETF rat is not yet clear. In summary, studies of CCK1R knockout animals have not yet provided evidence that abdominal CCK1R signaling alone is necessary for normal control of meal size. It would support this hypothesis if CCK1R knockout mice displayed increased meal size, but this has not yet been tested. The general point is that most signaling molecules, CCK included, have a variety of roles in a variety of tissues. Hence, it is unwise to interpret changes in complex phenotypes, such as eating in animals with knockouts of signaling systems, as the specific result of the defect in a specific site. Some other complications in the interpretation of genetic models are considered in the Leptin section.

The sham-feeding preparation, in which ingested food drains from gastric cannulas, provides an acute removal strategy. Rats that are offered palatable food to sham feed after an overnight food-deprivation sham feed essentially indefinitely, and CCK injection both terminates sham feeding and reproduces the species typical behavioral display of postprandial satiety [3,33]. These data are strong support that CCK satiation meets this removal aspect of the criterion. It is not clear if the data meet the replacement aspect because it is not certain if the doses of CCK required to produce satiation were physiological. The production of satiation in sham-feeding rats by intraduodenal infusions of CCK secretagogues and the

---

**Fig. 3.** Superior pancreaticoduodenal (SPD) artery injection of the CCK1R antagonist devazepide stimulates eating in rats at doses that are ineffective when infused via the jugular vein (intraventricular). Injections were done immediately before the 15-min access to 30% sucrose solutions after a 6-h food deprivation during the light phase. Open bars, control; filled bars, devazepide. The superior pancreaticoduodenal artery perfuses the pyloric region of the stomach and the proximal small intestine. *Significantly different from control. From Ref. [18], used with permission.
reversal of this satiation by pretreatment with devazepide [14,94], however, may be considered support for a variant of the replacement criterion.

2.2.5. Antagonism

This criterion was amply fulfilled in the rat by several experiments, around 1990, that demonstrated that the antagonists of CCK1R, but not of CCK2R, (1) blocked the satiating effects of exogenous CCK and (2) increased meal size [18,21,40,60,70,82]. There are now dozens of such reports [79]. One result is shown in Fig. 3.

2.3. Formulation: CCK is a fully coupled endocrine satiation signal

The criteria for a physiological endocrine effect have been investigated in more detail for CCK than for any other hypothesized hormonal control of eating. Available evidence indicates that CCK released from the small intestine during meals is a physiological control of satiation in both rats and humans, but that CCK’s mode of action is paracrine in the rat and endocrine in humans. The support for these hypotheses is so strong that hypotheses have been considered to be proven [79–81]. Nevertheless, not all the classical criteria enumerated in Table 1 are met for either species.

CCK’s satiation signaling mechanism in humans may be characterized as a fully coupled endocrine control of eating. This is because discrete, apparently time-locked, causal physiological cascades appear to link (1) a specific food stimulus with increased CCK secretion into the blood and (2) increased plasma CCK with a change in eating, i.e., the termination of ongoing eating, or satiation. The situation in rats seems identical, except for the mode of signaling (a distinction that some authors consider of little interest [35]). The linkage is very tight, or coupled: The stimulus producing CCK secretion (Link 1) is the presence in the small intestine of nutrients that were ingested just minutes prior, and satiation ensues just a few minutes after CCK secretion (Link 2). As described below, some other endocrine controls of eating have very different functional characteristics, with the first, the second, or both links of the chain loosely linked, or uncoupled. These variations are schematized in Fig. 4. In a fully coupled control, such as CCK’s, the close temporal associations among eating, secretion, and satiation should inform the designs and interpretations of experimental dissection of the phenomena. For example, (1) knowledge of the adequate stimuli for CCK secretion and their temporal dynamics facilitates the manipulation of secretagogues; (2) experiments in which the natural temporal patterns are not closely mimicked, e.g., by administering CCK long before meal onset or by producing unnatural rates of increases in CCK concentrations, fail to recapitulate normal physiology and, in the worst case, may produce artifactual results; and (3) a neuronal correlate of increased peripheral CCK that occurs more slowly than satiation cannot be considered a part of the cause of satiation.

3. Leptin

Because available evidence indicates that leptin selectively affects meal size and not meal number [23,25,42], the hypothesis considered here is that leptin signals meal-ending satiation. In contrast to CCK, however, chronically decreased meal size induced by chronic leptin treatment is
not compensated for by changes in meal frequency [23,42]. This suggests that leptin (1) may inhibit hunger and meal initiation as well as stimulate satiation and (2) may be more relevant to body weight than CCK is. So far, there appears to be no reason not to consider the leptin’s satiating effects in humans and in rats and mice together.

3.1. Secretion

Adipocytes are the main source of circulating leptin, with leptin secretion increasing with increasing adipocyte size [1,27,48]. The characteristics of leptin secretion are complex and fundamentally different from those of CCK. Leptin secretion in adults displays a prominent circadian rhythm and is not affected by individual meals [76]. Fig. 5 shows this circadian rhythm of plasma leptin levels and the lack of influence of meals. In addition, plasma leptin concentration is positively correlated with body adiposity. As shown in Fig. 6, the relationships between body adiposity and morning fasting plasma leptin concentrations levels are linear in adults and women, but leptin concentrations are two–three times higher in women than in men and are affected by the hypothalamic–pituitary–gonadal axis function in women [74]. Finally, leptin secretion drops markedly during fasting, possibly disinhibiting eating.

3.2. Receptors

Although leptin receptors are widespread in the body, local-infusion experiments indicate that the populations of leptin receptors that mediate its effects on eating are in the brain, in the arcuate nucleus of the hypothalamus and in the

---

Fig. 5. Circadian rhythms of (top panel) serum leptin concentration (expressed as percent change from the 0800-h fasting level of 12.0 ± 4.4 ng/ml), (middle panel) insulin (μU/ml), and (bottom panel) glucose (mg/dl). Note that meals, indicated by arrows in the x axis of the bottom panel, clearly affect insulin and glucose concentrations but do not affect leptin concentration. Data are from normal-weight men and women. The leptin pattern shown was similar in obese participants (BMI 38.8 ± 2.5 kg/m²), but the 0800-h leptin level was significantly elevated (41.7 ± 9.0 ng/ml). From Ref. [76], used with permission.

Fig. 6. Linear relationships between plasma leptin concentration (ng/ml) and body fat mass (kg, measured by hydrodensitometry) in men (aged 19–41 years) and premenopausal (aged 19–45 years) and postmenopausal women (aged 49–87 years). Regression coefficients were .99, .95, and .92 in the three groups, respectively (all Ps < .0001). From Ref. [74], used with permission.
dorsal vagal complex [27,36]. This localization complicates normal endocrine function: Leptin must pass through the blood–brain barrier to reach these receptors, which it does via a selective active transport mechanism [7].

3.3. Physiological dose

In his authoritative review of the physiology of leptin, Friedman [27] cites two data sets to support the claim that physiological doses of leptin are sufficient to affect eating. Neither is convincing. The first data set is a comparison of one experiment demonstrating that plasma leptin levels increase in mice in which body weight increase are induced by feeding chow enriched with 10% or 45% fat [88] with another experiment demonstrating that continuous subcutaneous leptin infusions, in doses that produce leptin levels in the same range as high-fat feeding does, decreased body weight [38]. There are several limitations to the interpretation of this comparison. (1) Only body weight, not food intake, was measured, and the degree to which the weight loss mirrored hypophagia is unclear. Indeed, the authors reported increases in energy expenditure that may have accounted for much of the weight change. (2) Due to the changes in leptin transport and postreceptor function, leptin is much more potent in lean or underweight mice than in overweight, fat-fed mice. This is known as leptin resistance. The implication here is that the comparison is between essentially different animals. That is, there is no evidence that the elevated endogenous leptin levels in the overweight mice affected eating in them, and these levels can be considered pharmacological in the lean mice because they never occur in them. (3) The association between plasma leptin level and weight loss was not close, especially in the lower ranges of leptin concentration, and not statistically substantiated. In sum, these data are no more than encouraging. In the second data set, rats’ leptin levels were monitored over 14 days, during which they received chronic subcutaneous control or leptin infusions by osmotic mini-pump [2]. Although control rats progressively increased daily food intake and increased body weight about 80 g, their plasma leptin levels remained constant throughout the experiment, at about 4 ng/ml diurnally and 2.5 ng/kg nocturnally. Infusion of 2 or 4 μg/h leptin produced dose-related decreases in food intake and weight gain. Plasma leptin levels during the infusions, however, were 5–7 ng/ml diurnally and 4–7 ng/ml nocturnally for the two doses, which is more than the endogenous levels measured at any time in control rats. Thus, these data provide no direct evidence for the physiological dose criterion for leptin’s satiating effect. The fact that leptin infusions that produced only mild pharmacological levels of plasma leptin in rats was considered encouraging because obese rats have been reported to display such leptin levels. This comparison is subject to the complications discussed above.

There are several indications that leptin’s physiological role may be to disinhibit eating (and inhibit energy expenditure) when body adiposity is low rather than to inhibit eating (and stimulate energy expenditure) when adiposity is high [1,48,75]. Rosenbaum et al. [73] recently devised an elegant experiment to test the physiological status of leptin in this context. Several indices of energy expenditure were measured in participants at their usual body weight, while stable at 90% usual weight, and during a 5-week period at 90% usual weight during which they received twice-daily leptin injections that restored leptin concentration measured at 0800 h to the level at usual body weight. Normalizing leptin levels while the participants were at 90% of their usual weight reversed the decreases in plasma T3 and T4 levels and in nonresting energy expenditure that, otherwise, were caused by weight loss. These data suggest that the inhibitory effect on energy expenditure of decreased leptin levels meets the physiological dose criterion in humans. It would be fascinating to learn the effects of this or a similar chronic regimen on eating behavior in humans or animals. Such results would certainly have much more import than will the commonly used strategy of infusing leptin in rats following 2–4 days of total starvation.

3.4. Removal and replacement

Although the extirpation of the leptin-secreting cells, i.e., the adipocytes, is possible, this has not been done in the context of a test of leptin on eating. Nor, of course, would the removal of the adipose tissue result in a selective loss of leptin. Genetic models of leptin removal are more interesting. Obese (ob/ob) and diabetic (db/db) mice display identical syndromes of increased meal size, hyperphagia, obesity, and diabetes. It is now known that these syndromes arise from disruptions of leptin signaling: the wild-type ob gene, lep, encodes leptin, and the wild-type db gene, lep-r, encodes the leptin receptor [1,27,48]. Classic studies of the parabiosis of normal, ob/ob, and db/db mice [16] had indicated that these animals had a defective hormone and hormone receptor, respectively, long before either was identified. Leptin infusion has also been shown to reverse the hyperphagia and obesity of ob/ob mice [27,38]. In addition, transgenic reconstitution of lep-r in db/db mice ameliorates their hyperphagia and obesity [46]. Humans with two defective lep genes and a phenotype analogous to that of the ob/ob mouse have been identified, and chronic leptin treatment has been shown to ameliorate their syndrome [24,68].

These data from mice and humans support the removal and replacement criterion for physiological hormonal action. They are not, however, definitive evidence. As discussed above (see CCK), most signaling molecules, including leptin, are synthesized in sites other than the site of interest for the hormonal action in question. Hence, the phenotypes of interest in individuals with null mutations may arise from the effects of the molecule other that the hypothesized effect. Other limitations of such data are that (1) null mutations prevent any hormone secretion, not just the particular aspect of secretion relevant to the hypothesized effect; (2) null
mutations can produce pathophysiological effects beginning at any time in development when the gene is normally expressed, including effects unrelated to the hormonal action in question; and (3) such chronic developmental effects can produce compensatory physiological responses. Thus, in several respects, the adult organism bearing a null mutation falls far short of an ideal test, in which a hormone is suddenly removed, and cannot be considered crucial data. Many of these problems will be averted by the development of acutely inducible, tissue-specific gene knockouts and knock-ins, which will lead to more compelling evidence.

3.5. Antagonism

Effective leptin receptor antagonists have not yet been developed. A test of leptin antagonism on eating, using a passive immunization strategy, has been reported [15]. Food intake increased about 25% in normal rats in the 20 h following an intracerebroventricular injection of rabbit antismouse leptin antibodies; there was no effect on eating in Zucker fa/fa rats, which have defective leptin receptors (Fig. 7). The antibodies’ effect on exogenous leptin’s satiating action was not tested. These data provide support for one aspect of the antagonism criterion, but they are not proof because (1) there remains a question as to how an intracerebroventricularly injected antibody reaches the brain parenchyma and (2) the interpretation of acute tests of what may be a tonic physiological signal raises caveats, which are discussed below.

3.6. Formulation: leptin may be uncoupled endocrine satiation signal

Leptin research has catalyzed a revolution in behavioral neuroscience, especially regarding the genetic and hypothalamic mechanisms of eating. Despite this progress, however, the hypothesis that satiation is a normal hormonal effect of leptin remains unproven and suspect. Leptin satiation has not yet fulfilled several classical endocrine criteria for a physiological effect, notably the crucial physiological dose and antagonism criteria. Further work is required to better substantiate this hypothesis.

Many of the controls of leptin secretion, including circadian changes and changes during fasting, are not well understood. Furthermore, neither the temporal linkage nor the mediating mechanisms between any parameter of the pattern of leptin secretion and satiation during individual meals are understood. Leptin’s hypothetical satiation effect, therefore, appears fully uncoupled (Fig. 4).

The uncoupled nature of leptin signaling greatly complicates the evaluation of the endocrine criteria for physiological function. What, in the complex pattern of leptin secretion, is the effective stimulus for satiation—maximum nocturnal or diurnal plasma level, the level at some specific time of day, the average level over the day, or some other parameter? How long after a change in endogenous leptin does eating change? Do the effects of a single bolus injection of leptin have any physiological relevance? These are difficult questions. Their answers will determine how and when leptin should be administered and when eating should be measured to best test the physiological dose criterion for this hormone (as well as to optimize leptin’s pharmacotherapeutic use). It may be that slow infusion is the ideal way to test leptin’s effects, but this creates its own problems. For example, chronic leptin administration can lead to dynamic responses that cloud interpretation. When recombinant human leptin was administered by daily subcutaneous injections to obese human participants for nearly 6 months, it required 10- to 40-fold elevations of plasma leptin to produce significant weight loss, and this insensitivity was, in part, due to the neutralization of leptin’s biological activity by the development of antibodies in the majority of participants [41]. The administration of leptin to leptin-deficient humans also led to the production of antibodies [68]. At this point, effective strategies to avoid such difficulties have not been developed either in experimental physiology or pharmacological therapeutics.

4. Ghrelin

Ghrelin robustly stimulates eating, suggesting the hypothesis that ghrelin is a fully coupled signal for hunger and meal initiation [85,92,93]. In addition, basal ghrelin
secrection is affected by body adiposity, suggesting that ghrelin also may have an uncoupled hunger effect. Whether ghrelin affects spontaneous eating solely by increasing meal frequency, as these hypotheses predict, however, is not yet known.

4.1. Secretion

Ghrelin is synthesized and released primarily by endocrine cells in the stomach. Although the specific stimuli for ghrelin secretion are unknown, the increases in plasma concentration of ghrelin before meals and rapid decreases after meals are consistent with a hunger-inducing action in both humans and rats [18,86]. However, plasma ghrelin concentration in humans also increases during the evening and decreases in the early morning hours, in the absence of eating, [19] suggesting that ghrelin is not sufficient for a secretion-coupled hunger effect. Basal ghrelin levels are also decreased in obese humans and increased by weight loss [19,87], suggesting that this hormone may also be linked to the control of eating by adiposity. The circadian pattern of plasma ghrelin concentrations in humans is shown in Fig. 8. Ghrelin is also synthesized in neurons in the arcuate nucleus of the hypothalamus; thus, the molecule may also have central, nonendocrine actions on eating [17].

4.2. Receptors

Ghrelin was originally identified as the endogenous ligand for the pituitary growth hormone secretogogue receptor [44]. Local injection studies suggest that ghrelin receptors in the paraventricular and arcuate nuclei of the hypothalamus mediate its eating effect [5,67,93]. Ghrelin is transported across the blood–brain barrier [8], but whether the primary ligand for hypothalamic ghrelin receptors is hormonal ghrelin or ghrelin released by neurons projecting from the arcuate nucleus remains unclear. The arcuate nucleus, however, is not the only site where ghrelin may act to stimulate eating. Similar to leptin [36], there are ghrelin receptors in the brainstem, and ghrelin injection into the dorsal vagal complex stimulates eating. Furthermore, ghrelin receptors also exist in abdominal vagal afferent neurons, and abdominal vagotomy or capsaicin deafferentation eliminated the effect of intravenous infusions of ghrelin on eating, suggesting that these peripheral ghrelin receptors mediate the endocrine hunger effect of ghrelin [20]. Some of these data are shown in Fig. 9.

4.3. Physiological dose

This criterion has been addressed so far only in a study by Wren et al. [92]. In normal-weight men and women,

![Fig. 8. Circadian rhythms of plasma ghrelin (pg/ml) in men and women before (BMI 35.6 ± 1.6 kg/m²) and after (BMI 29.4 ± 1.5 kg/m²) diet-induced weight loss. Note that (1) ghrelin levels increase progressively prior to meals and decrease after meals, (2) ghrelin also increases during the evening before decreasing during the early morning hours, and (3) ghrelin levels are greater in normal-weight than in obese participants. From Ref. [19], used with permission.](image-url)
intravenous infusion of 5 pMol/kg/min human ghrelin increased hunger ratings and meal size (Table 3). These infusions increased plasma ghrelin concentrations about 2.5-fold over the fasting level, indicating that the infusions were out of the physiological range. Indeed, the aphysiological nature of the infusions is indicated by the fact that the participants perceived that hunger did not decrease even after their large lunches. Thus, more work is required to determine if ghrelin meets this criterion for a fully coupled physiological hormonal effect.

Chronic subcutaneous or intracerebroventricular administration of ghrelin has been shown to increase eating and body weight in mice and rats [65,86], but no efforts have yet been made to determine if physiological doses are sufficient for these potential secretion-uncoupled effects. A major issue that requires clarification in this context is that ghrelin often seems to stimulate energy metabolism and increase body weight more rapidly and more potently than it affects eating [86].

4.4. Removal and replacement

There is some evidence that ghrelin meets this criterion. Cummings et al. [19] showed that plasma ghrelin concentrations were maintained at a very low, constant level in obese patients who had had proximal Roux-en-Y gastric bypass surgery (Fig. 8), and patients who undergo gastric bypass have been reported to be hungry less often and eat fewer meals than they had prior to surgery [39]. Of course, it is also possible that such changes in eating are due to alterations in the actions of ingested food at postgastric sites following gastric bypass rather than to reduced ghrelin.

A transgenic animal lacking ghrelin has also been constructed [84]. Extensive efforts to demonstrate a phenotype of decreased eating in this animal met with no success. Although these data do not encourage the view that ghrelin is a necessary signal for eating, the same caveats that limit the interpretation of the positive effects of mutations of the CCK and leptin signaling systems prevent these negative data from being a crucial test of the ghrelin hunger hypothesis.

4.5. Antagonism

There are at least four reports that ghrelin antagonism reduces eating. Intracerebroventricular injections of ghrelin antibodies decreased eating both acutely (i.e., 1- or 2-h food intake measures in rats refed after fasting) and chronically (i.e., during a day or two following injection; [5,64,65]). Some of these data are shown in Fig. 10. The effect of intravenous infusion of ghrelin antibodies, however, has not been tested as yet. As antibodies do not penetrate the blood–brain barrier, such an experiment has the potential to differentiate hormonal and neural ghrelin as the source of the ghrelin blocked by intracerebroventricular ghrelin antibody injection. Intracerebroventricular injections of a peptide ghrelin receptor antagonist both decreased eating and blocked the acute stimulatory effect of intraperitoneally injected ghrelin on eating [4]. This same antagonist also decreased eating when injected intraperitoneally, but whether the site of action was peripheral or central was not established. Taken together,

Table 3

<table>
<thead>
<tr>
<th>Plasma ghrelin (nMol/l)</th>
<th>Hunger (cm)</th>
<th>Meal size (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>2.5 ± 0.5</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>5.9 ± 0.3*</td>
<td>6.9 ± 0.7*</td>
</tr>
</tbody>
</table>

Participants were five men and four women (BMI 23 ± 1 kg/m²). Infusions of saline or 5 pMol/kg/min human ghrelin were begun at 0830 h. Participants ate a fixed breakfast (1550 kJ) at 1030 h and were offered a buffet lunch at 1230 h. Data (mean ± S.E.M.) are plasma ghrelin level measured just before lunch, hunger rated on a 10-cm visual analog scale just before lunch, and lunch size.

* Significantly different from saline. Data are from Ref. [92].
these data suggest that both forms of the ghrelin hunger hypothesis may meet the antagonism criterion.

4.6. Formulation: ghrelin may have both coupled and uncoupled endocrine satiation effects

The investigation of the role of ghrelin in hunger is in early days. Initial data encourage the hypotheses that preprandial ghrelin secretion may be a fully coupled endocrine hunger signal and that basal ghrelin may be a partially or fully uncoupled hunger signal. As yet, however, none of the criteria for a physiological endocrine effect has been fully met for either hypothesis. The research programs described above for the endocrine effects of CCK and leptin should help inform work on ghrelin. As in the other cases, however, this peptide presents has its own special difficulties, such as the relative effect of hormonal ghrelin and of neuronal ghrelin.

5. Discussion

Eating is a complex function of the brain that has been profitably analyzed from a number of scientific perspectives, including psychological, nutritional, neuroanatomical, neurochemical, electrophysiological, endocrinological, and, most recently, molecular genetic perspectives (see Refs. [11,32] for synthetic overviews). Endocrine signals have emerged as an especially important aspect of the physiology of eating. In the analysis of these signals, it is of vital importance to capitalize on the critical tools of classical endocrinology. Failure to do so precludes establishing the true physiological nature of endocrine signals, leaving the rest of the research edifice built around them without a reliable foundation. This review has attempted to crystallize some methodological and interpretive guidelines for the application of classic endocrine criteria for establishing a physiological effect of a hormone using the examples of three hypothesized endocrine controls of eating, CCK, leptin, and ghrelin.

The endocrine criteria for a physiological hormonal effect (Table 1) are stringent, and fulfilling them to the level of scientific certainty is a formidable task. At present, of the many hypothesized endocrine controls of eating ([90] and Table 4), CCK’s satiating effect in humans comes closest to fulfilling these criteria. The physiological statuses of other hormonal eating signals are best considered, more or less, open questions that require further research. It is appropriate to emphasize that these criteria address only the endocrine function of these hormones up to and including binding to their cognate receptors. The criteria, and this review, have not considered the physiological mechanisms activated consequent to hormone receptor activation, in particular, the altered peripheral and central neural information processing. It is important to note that progress in analyzing postreceptor mechanisms mediating endocrine controls of eating does not necessarily parallel progress in analyzing

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Hypothesized endocrine controls of eating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitory</td>
<td>Excitatory</td>
</tr>
<tr>
<td>CCK</td>
<td>Ghrelin</td>
</tr>
<tr>
<td>Leptin</td>
<td>Testosterone</td>
</tr>
<tr>
<td>Ghrelin</td>
<td></td>
</tr>
<tr>
<td>Pancreatic glucagon</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
</tr>
<tr>
<td>Amylin</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein A-IV</td>
<td></td>
</tr>
<tr>
<td>Gastrin-releasing peptide</td>
<td></td>
</tr>
<tr>
<td>Neuropeptide B</td>
<td></td>
</tr>
<tr>
<td>Somatostatin</td>
<td></td>
</tr>
<tr>
<td>Neurotensin</td>
<td></td>
</tr>
<tr>
<td>Glucagon-like peptide 1</td>
<td></td>
</tr>
<tr>
<td>Glucagon-like peptide 2</td>
<td></td>
</tr>
<tr>
<td>Peptide YY(3-36)</td>
<td></td>
</tr>
<tr>
<td>Enterostatin</td>
<td></td>
</tr>
<tr>
<td>Estradiol</td>
<td></td>
</tr>
</tbody>
</table>
their peripheral endocrine aspects. The substantial progress on the molecular genetic and neural mechanisms of leptin and ghrelin signaling, e.g., encourages further research into their roles in eating independent of their present status as endocrine controls. Ultimately, however, all these aspects of the molecule’s function must be explicitly tied to a proven endocrine effect on eating or must be considered either nonendocrine or nonphysiological effects.

This review developed the concepts of coupled and uncoupled hormonal effects. As discussed above, a fully coupled endocrine control is characterized by two apparently time-locked causal physiological cascades, the first linking a specific food stimulus with altered hormone secretion and the second linking the hormonal change with a specific change in eating, e.g., meal initiation or termination (Fig. 4, upper panel). CCK apparently exemplifies fully coupled endocrine control. In contrast, an endocrine effect is fully uncoupled when neither the stimuli controlling the hormone secretion nor the temporal linkage or mediating mechanisms between hormone secretion and changed eating is clearly established (Fig. 4, lower panel). If only one of these two links appears coupled, the endocrine effect may be called partially coupled.

The distinction between coupled and uncoupled hormonal effects emphasizes differences in the putative causal relationships between endocrine function and eating. Each of the endocrine controls of eating considered here is also a feedback control, in that hormone secretion is tied, directly or indirectly, to past eating behavior. This is not the crux of the coupling concept, however, and not all endocrine controls are feedback controls [29].

The chief advantage of the coupling concept is that it provides concrete empirical approaches and interpretative criteria applicable to a large number of endocrine controls of eating, as exemplified in the previous discussions. The coupling concept is distinct from, and complements and extends, previous concepts that have been applied to endocrine controls of eating, such as phasic and tonic effects [28], direct and indirect effects [77], and meal and adiposity signals [91]. For example, the direct and indirect controls of meal size of Smith [77] relate only to satiation and are not restricted to endocrine mechanisms. Similarly, although the hypothesized uncoupled effects of leptin and ghrelin meet the criteria for adiposity signals [91], other endocrine effects that are not adiposity signals, such as endocrine signals affected by metabolism or reproductive function [28–30], also fit the uncoupled concept. Thus, the scheme of coupled and uncoupled endocrine effects on eating may provide a new and useful conceptual framework for the study of eating.

Acknowledgements

The author is supported by NIH research grants DK54523, AA526403, and MH529977.

References

[23] Eckel LA, Langhans W, Kühler A, Campfield LA, Smith FJ, Geary N. Chronic administration of OB protein decreases food intake by selec-


[64] O'Rahilly S, Farooqi IS, Yeo GS, Challis BG. Minireview: human


