CHAPTER 2

Gut-to-Brain Mechanisms of Body Weight Regulation

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Although energy balance is ultimately regulated by the central nervous system (CNS), the organs of the alimentary canal and supporting organs of the peritoneal cavity supply numerous neuroendocrine signals that are necessary for the physiological and behavioral processes that affect energy balance. This chapter provides a brief overview of "gut hormones" and their contribution to energy balance regulation. Here, gastrointestinal (GI)- and pancreatic-derived peptides that have been extensively studied both in the context of normal physiology and in the pathophysiological state of obesity are discussed. Accordingly, the hormonal systems reviewed here are likely those systems that represent the greatest opportunity for future pharmacological targets to treat obesity. It is also worth noting that some of the gut peptides discussed in this chapter are also synthesized centrally within the brain and are more accurately referred to as *neuropeptides*. Thus, an important consideration for all of the peptides described is a discussion about the mechanisms (behavioral, endocrine, and autonomic) and neuroanatomical sites-of-action within the brain and periphery.

Gut peptides regulate feeding behavior by negatively or positively influencing food intake during a meal and/or between meals (influencing the intermeal interval and the frequency of meal taking; Grill & Hayes, 2012; Moran, 2006; Ritter, 2004). This process involves a constant stream of gut-to-brain communication through humoral mechanisms as well as neuronal signaling predominately via the vagus nerve and, to a smaller extent, splanchnic pathways. Once a meal has begun and food enters the oral cavity, cranial nerves VII, IX, and X relay various properties of the ingesta (e.g., taste and texture) to the brainstem, which promotes further feeding if the food is perceived as palatable (Norgren, 1983). As food is swallowed and enters the GI tract, information about the volume of the food is sensed through the mechanical distension of the stomach and subsequently relayed to the nucleus tractus solitarius (NTS) by the vagus nerve. These gastric inhibitory signals begin to counteract the positive meal-promoting signals from the oral cavity. The various chemical and nutritive properties of the food stimulate the release of gut peptides and neurotransmitters from the GI tract and supporting organs of the alimentary canal (e.g., pancreas). This process enhances communication to the brain via humoral (i.e., endocrine) and neuronal pathways about

the ongoing status of the meal. These within-meal food-intake-inhibitory signals are collectively referred to as *satiation signals* (Ritter, 2004). The accumulation of these satiation signals is perpetually sensed by the brain and eventually leads to *satiety,* or meal termination. Satiety then persists from the end of one meal to the start of the next meal.

Given the redundant number of gut peptides, neuropeptides, and neurotransmitter systems that exist in the body to inhibit food intake and promote satiety, it seems initially paradoxical that obesity rates continue to rise worldwide. One interpretation of this phenomenon is that humans lack true homeostatic equilibrium when it comes to energy balance. Many theories have been proposed, discussed, heavily reviewed, and cited on this topic (McAllister et al., 2009; Thomas et al., 2012). A growing evolutionary theory states that our energy balance neuroendocrine systems have developed not to maintain leanness or normal energy equilibrium, but rather to defend adiposity and the surplus of energy storage (Rosenbaum, Kissileff, Mayer, Hirsch, & Leibel, 2010). From this perspective, it is worth noting that the brain initiates autonomic, behavioral, and endocrine responses not just in response to the accumulation of a given neuropeptide signal and receptor activation, but also in response to the *reduction* of a given signal.

GI Satiation Signals

The abundant vagal afferent innervation and proximal location of the stomach within the GI tract provide an early monitoring system for the status of meal ingestion. Specifically, the food-intake-inhibitory signals produced by the stomach arise from the mechanical distension of the stomach (rather than the chemical/nutritive properties of the ingested food; Mathis, Moran, & Schwartz, 1998; Phillips & Powley, 1996; Powley & Phillips, 2004). Unlike the satiation signals that arise from the intestine, the intake-inhibitory signals arising from the stomach are not mediated by gut peptides. Rather, a portion of the dendritic vagal sensory endings innervating the stomach is specialized to be responsive to stretch and/or tension. These sensory endings are referred to as *intraganglionic laminar endings* and *intramuscular arrays* (see Ritter, 2004, for review). The vagal dendritic detection of tension and stretch within the gastric wall results in neuronal transmission from vagal axon projections to NTS neurons in the caudal brainstem (see Grill & Hayes, 2012, for review). In addition, as the gastric wall is distended, the neurotransmitter serotonin (5-HT) is secreted from gastric enterochromaffin (EC) cells and is thought to provide the principal stomach-derived intake-inhibitory signal. This 5-HT-mediated hypophagic response engaged by gastric distension occurs principally through the activation of ionotropic 5-HT type-3 receptors (5-HT3_R) expressed on the dendritic terminals of vagal afferents innervating the stomach (Glatzle et al., 2002; Hayes, Moore, Shah, & Covasa, 2004; Mazda, Yamamoto, Fujimura, & Fujimiya, 2004). Where appropriate, gastric distension and GI-derived satiation signaling interactions with gut peptides that control energy balance are discussed further in this chapter.

Cholecystokinin

The neuropeptide cholecystokinin (CCK), released peripherally from intestinal "I" cells in response to ingestion of nutrients, is arguably one of the most well-studied satiation signals (see Moran, 2006, and Ritter, 2004, for review). Indeed, over four decades ago, Gibbs, Young, and Smith (1973) first reported that exogenous, systemic administration of CCK produced a dose-dependent decrease in meal size. This initial finding was the first to demonstrate that a GI-derived peptide could negatively influence food intake, providing the seminal discovery for future scientific fields investigating the gut-to-brain communication involved in the control of energy balance.

Systemic CCK acts via CCK-1 receptors (historically referred to as CCK-A receptors) that are densely distributed in the periphery on the afferent terminals of the vagus nerve and in select regions of the CNS. Importantly, though, the primary site-of-action for either endogenous or exogenous systemic CCK is not the CNS, but rather the vagal afferents (Smith, Jerome, & Norgren, 1985). Support for the physiological requirement of endogenous CCK-1 receptor signaling in

controlling meal size, as well as energy balance more broadly, comes from the findings that blockade of CCK-1 receptors, using selective antagonists, results in a short-term increase in food intake. Other supporting data show that rats with genetic deletion of the CCK-1 receptor are chronically hyperphagic and obese (see Moran, 2006, and Ritter, 2004, for review).

The suppression of food intake by CCK administration is enhanced when combined with other GI-derived satiation signals. For example, data from Schwartz and Moran show that CCK and gastric distension combine in a dose- and volume-dependent fashion to increase firing rate and total spike number of electrophysiological recordings made on single vagal afferent fibers (Schwartz, McHugh, & Moran, 1993; Schwartz & Moran, 1996). This vagal integration is further postulated to mediate the enhanced behavioral suppression of food intake when CCK and gastric distension are combined (Moran, Ladenheim, & Schwartz, 2001; Ritter, 2004; Schwartz & Moran, 1996). Interestingly, these two GI-derived satiation signals also mechanistically interact within a meal to suppress the ongoing meal. Specifically, CCK-1 receptor activation reduces gastric emptying and thereby enhances gastric distension as the animal/human continues to feed (Moran & McHugh, 1982). Further, this interaction between CCK and gastric distension involves participation of other GI-derived satiating signals, such as 5-HT, which is released in response to gastric distension (Mazda et al., 2004) and interacts with CCK to reduce food intake. Indeed, blockade of 5-$HT3_R$ attenuates the suppression of food intake by CCK (Daughters et al., 2001; Hayes et al., 2004).

In addition to the traditional role of CCK as a within-meal satiation signal, CCK interacts with hormonal systems such as insulin and leptin, which serve as a readout of long-term energy stores (see Begg & Woods, 2012, and Grill & Hayes, 2012, for review). Looking at leptin as the example of this interaction, leptin potentiates the anorectic effects of CCK and other GI-derived satiation signals (see Grill & Hayes, 2012, for review). This leptin–CCK interaction is not confined to one nucleus within the brain, but rather involves distributed sites of action throughout the body that include vagal afferents (Peters, Karpiel, Ritter, & Simasko, 2004; Peters, Ritter, & Simasko, 2006), the NTS (Hayes et al., 2010), the parabrachial nucleus (Flak et al., 2014), and hypothalamic nuclei (Barrachina, Martinez, Wang, Wei, & Tache, 1997; Emond, Schwartz, Ladenheim, & Moran, 1999).

Despite extensive examination of the CCK system, there are a number of hurdles blocking the development of safe and efficacious pharmacological tools targeting CCK as a means to facilitate weight loss in obese humans. One major obstacle is the pronounced tachyphylaxis that develops with repetitive CCK administrations (Crawley & Beinfeld, 1983). Perhaps most important for CCK-1 receptor agonists is the need for analogs that remain efficacious without producing pancreatitis, a well-known response to chronic CCK-like treatments in mammals (Lampel & Kern, 1977; Makovec et al., 1986). To this end, at least one compound, GI181771X (GlaxoSmithKline), appears to have little effect on pancreatic endpoints in overweight/obese humans (Myer, Romach, & Elangbam, 2014). Unfortunately, chronic treatment with this therapeutic did not reduce food intake and body weight in overweight and obese humans (Jordan et al., 2008), possibly for the aforementioned tachyphylaxis and reduced responsiveness that occurs with continuous CCK-1 receptor activation.

Glucagon–Like Peptide–1

Multiple biological processes are regulated by the glucagon-like peptide-1 (GLP-1) system, including insulin secretion; blood glucose regulation; suppression of gastric emptying; cardiovascular and thermogenic effects; modulation of reward- and goal-directed behaviors; and, importantly, a physiological role in food intake and energy balance (see Hayes, Mietlicki-Baase, Kanoski, & De Jonghe, 2014, and Holst, 2007, for review). Within the periphery, GLP-1 is principally secreted by enteroendocrine "L" cells of the distal small intestine and large intestine in response to the ingestion of food. GLP-1 is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-IV) to inactive metabolites, and therefore has a short circulating half-life of less than 10 minutes

(Holst, 2007), rendering native GLP-1 inappropriate for the treatment of obesity. GLP-1 acts on the GLP-1 receptor (GLP-1R), a G protein-coupled receptor, which has a varied tissue distribution in mammals (brain, pancreas, intestine, heart, etc.; see Holst, 2007, for review). Under normal physiological circumstances, intestinally derived GLP-1 activates GLP-1R, expressed on the dendritic terminals of the vagal afferents innervating the GI tract in a paracrine-like mode of action (see Hayes et al., 2014, for review). However, the relevant GLP-1R populations mediating the suppression of food intake through GLP-1R pharmacological agonists (e.g., liraglutide, exendin-4) or inhibitors of DPP-IV (e.g., sitagliptin) are more diverse. For example, when the long-lasting GLP-1R agonist liraglutide or exendin-4 is administered systemically, each can sufficiently penetrate the blood–brain barrier and gain access to the brain in amounts sufficient to drive physiological and behavioral responses. Indeed, activation of GLP-1R expressed in the CNS will recapitulate many of the same behavioral and physiological responses that are observed following peripheral GLP-1R ligand administration (see Hayes et al., 2014, for review). This makes it difficult to disentangle the effects originating in the periphery from those effects mediated by direct CNS activation. Therefore, one of the current challenges in the obesity field is to characterize the energy balance responses mediated by individual GLP-1R-expressing nuclei and the physiological mechanisms mediating these responses.

Whereas research pursuant to the exploration of GLP-1-mediated effects in a multitude of CNS nuclei is certainly warranted, of particular interest is research aimed at identifying GLP-1 modulation of food reward processes. Given that the excessive food intake that contributes to human obesity is not driven by metabolic need alone, a number of laboratories have made major advances in our understanding of the role that GLP-1 signaling in the nuclei of the mesolimbic reward system has on control of energy balance (Alhadeff, Rupprecht, & Hayes, 2012; Dickson et al., 2012; Dossat, Lilly, Kay, & Williams, 2011). Perhaps most attractive from the perspective of obesity treatment is the finding that some of these mesolimbic nuclei (e.g., ventral tegmental area [VTA]) are directly activated by systemic administration of GLP-1R agonists to suppress food intake and body weight (Mietlicki-Baase, Ortinski, et al., 2013). Recently, the GLP-1R agonist liraglutide (Saxenda) was approved for the treatment of obesity (Mordes, Liu, & Xu, 2015). We now need further basic science investigations to identify adjunct behavioral and pharmacological therapies that can be combined with GLP-1R ligands to enhance the suppression of food intake and body weight by these pharmacotherapies.

Gastric Inhibitory Polypeptide

Gastric inhibitory polypeptide (GIP) stimulates glucose-dependent insulin secretion, insulin transcription/translation, as well as betacell growth and preservation of betacell survival under normal physiological conditions (see Sadry & Drucker, 2013, for review). However, there is limited evidence supporting consistent effects of GIP on energy balance produced by GIP treatment alone. Further, much of the beneficial glycemic effect of GIP signaling is impaired in states of chronic hyperglycemia (Jones, Owens, Vora, Luzio, & Hayes, 1989). The latter fact has greatly precluded any significant pharmacological advancement for the GIP system as a primary treatment strategy for type 2 diabetes mellitus (T2DM). There is also a multitude of conflicting reports showing opposing metabolic effects arising from activation or inhibition of GIP receptors. For example, GIP administration in hyperglycemic patients with T2DM promotes glucagon secretion and worsens glucose tolerance (Chia et al., 2009), an effect contrary to GIP-mediated effects in euglycemic nondiabetic conditions. In mouse models, genetic deletion of the GIP receptor improves glucose tolerance and insulin sensitivity (see Sadry & Drucker, 2013, for review). Thus, although emerging combination therapies involving GIP signaling are being pursued as a potential treatment strategy for metabolic diseases (Sadry & Drucker, 2013), the cautious view at this moment indicates that further extensive preclinical and clinical trials for GIP-based pharmacotherapy are needed before considering any GIP-based compound as a viable treatment option for T2DM and/or obesity.

Peptide YY

Peptide YY (PYY) is co-released with GLP-1 from the L cells of the small and large intestines after the ingestion of food (Lundberg et al., 1982). PYY is initially secreted in a longer form (PYY[1–36]) that has no effect on food intake (Sloth, Davidsen, Holst, Flint, & Astrup, 2007), but like GLP-1, PYY is rapidly cleaved by DPP-IV) to form PYY(3–36) (Medeiros & Turner, 1994). Although first identified in the early 1980s (Tatemoto, 1982), the effects of PYY(3–36) on energy balance remained controversial for many years (Batterham & Bloom, 2003; Batterham et al., 2002; Boggiano et al., 2005; Tschop et al., 2004), due in part to the fact that the two endogenous circulating isoforms of PYY (PYY[1–36] and PYY[3–36]) bind to Y1, Y2, and Y5 receptors with different affinities (Blomqvist & Herzog, 1997; Silva, Cavadas, & Grouzmann, 2002). Importantly, PYY(1–36) binds to all of these receptors, whereas PYY(3–36) is thought to have the highest affinity for the Y2 receptor (Keire, Bowers, Solomon, & Reeve, 2002). Further contributing to the controversy is the bidirectional effect on feeding seen with peripheral versus central delivery. Specifically, peripheral administration of PYY(3–36) suppresses feeding and body weight in humans and in animal models (Batterham & Bloom, 2003), whereas central (lateral, third, and fourth intracerebroventricular [ICV]) administration of either PYY(1–36) or PYY(3–36) robustly *stimulates* food intake (Clark, Sahu, Kalra, Balasubramaniam, & Kalra, 1987). Thus, although there is growing research attention to the PYY system as a potential future target for obesity treatment, it is clear that an abundance of work is needed to discern the physiological effects mediated by Y-receptor signaling.

From the perspective of creating pharmacotherapies to treat obesity, it is worth noting that chronic systemic administration of PYY(3–36) reduces food intake (Reidelberger, Haver, Chelikani, & Buescher, 2008). This peptide can cross the blood–brain barrier (Nonaka, Shioda, Niehoff, & Banks, 2003) and is thought to act primarily within the CNS to exert its hypophagic effects. Like ghrelin (discussed later), particular attention has been paid to the actions of PYY on the neuropeptide Y (NPY) system (Ballantyne, 2006). Indeed, PYY(3–36) is thought to exert its anorectic effects, in part, via agonism of the Y2 receptor, and specifically the Y2 receptors expressed on arcuate nucleus of the hypothalamus (ARC) NPY/agouti-related peptide (AgRP) neurons (Teubner & Bartness, 2013). However, further research is needed to determine if all of the CNS action results in a decrease or increase in food intake, if there is a hope that PYY-based pharmacotherapies could be used to treat human obesity.

Ghrelin: The Sole GI "Hunger Hormone"

The conscious decision made by humans and animals to procure food and initiate ingestion of a meal is made after neural assimilation of a multitude of internal and external stimuli. Meal initiation occurs in response to internal hunger signals that communicate energy need, as well as to external environmental cues and appetite signals that include entrainment and the social, memory, cognitive, and sensory aspects of feeding behavior. With regard to the subjective feeling of hunger, it is important to note that hunger is generated by an accumulation of central and peripheral orexigenic signals that promote feeding, as well as the reduction of GI-derived satiation signals once the previous meal has been digested and absorbed. To date, ghrelin represents the sole gut peptide that is classified as a hunger (orexigenic) hormone.

Upon its original discovery, ghrelin was recognized for its ability to promote growth hormone secretion (Kojima et al., 1999). The role of this peptide as a hunger hormone was identified shortly after its discovery (Tschop, Smiley, & Heiman, 2000). Ghrelin is produced primarily in the X/A-like cells of the stomach (Date et al., 2000), although some reports suggest that ghrelin is also synthesized centrally (Cowley et al., 2003). Within the circulation, ghrelin exists in two major forms: des-acyl ghrelin and acylated ghrelin (Hosoda, Kojima, Matsuo, & Kangawa, 2000). Acylation is accomplished by the actions of the enzyme ghrelin-*O*-acyltransferase (GOAT; Yang, Brown, Liang, Grishin, & Goldstein, 2008). Circulating levels of acylated ghrelin are lower than des-acyl

ghrelin (Hosoda et al., 2000); however, the orexigenic effects of ghrelin are attributed predominantly to its acylated form. Toshinai et al. (2006) have reported a hyperphagic effect of des-acyl ghrelin, but more recent studies show a reduction in food intake and body weight with des-acyl ghrelin administration (Heppner et al., 2014). In fact, there is a growing consensus that des-acyl ghrelin may act as an endogenous competitive antagonist for the hyperphagic effects of acylated ghrelin (Delhanty, Neggers, & van der Lely, 2012). Given the controversy and limited number of reports on des-acyl ghrelin's actions on food intake, for the duration of this section, *ghrelin* refers to the acylated form of the peptide.

One of the most remarkable and unique features of ghrelin with regard to GI-derived hormones is that circulating levels of ghrelin increase with fasting (Tschop et al., 2001). Individuals who take meals on a regular schedule from day to day will eventually exhibit an entrainment of ghrelin levels to their mealtimes (Cummings et al., 2001). This temporal link between peak ghrelin levels and the onset of feeding has led to the notion that ghrelin may serve as a meal initiation signal (Cummings et al., 2001). Once food is ingested, circulating ghrelin declines (Tschop et al., 2001). Interestingly, the postprandial suppression of ghrelin is related to the macronutrient content of the meal, with ghrelin being more effective at reducing carbohydrates than intake of fats or proteins (Overduin, Frayo, Grill, Kaplan, & Cummings, 2005).

The ghrelin receptor, or growth hormone secretagogue receptor (GHS-R), is widely distributed throughout the body, including the brain (Asakawa et al., 2003; Shuto et al., 2002). Given that ghrelin crosses the blood–brain barrier (Banks, Burney, & Robinson, 2008), circulating ghrelin can potentially activate both central and peripheral receptor populations. Indeed, most of the research on ghrelin's hyperphagic effects has focused on its actions within the brain. Some of the initial research on the neuronal mechanisms mediating ghrelin's orexigenic effects examined the ability of ghrelin to regulate feeding via effects in the ARC nucleus of the hypothalamus (Cowley et al., 2003). Specifically, ghrelin activating the GHS-R expressed on NPY/AgRP neurons in the ARC (Willesen, Kristensen, & Romer, 1999) increases expression of NPY and AgRP, neuropeptides with orexigenic effects (Kamegai et al., 2001). At the same time, ghrelin is thought to activate GHS-R expressed on adjacent ARC NPY/AgRP neurons to stimulate the release of gamma-aminobutyric acid (GABA) onto proopiomelanocortin (POMC) neurons within the ARC (Cowley et al., 2003), reducing the activity of this hypophagia-producing neuronal population. These complementary effects of increasing NPY/AgRP activity, while concomitantly suppressing POMC activity, contribute to the overall stimulation of feeding by ghrelin.

Beyond the hypothalamus, ghrelin has been well documented to activate a number of other nuclei within the brain to promote positive energy balance. These include hindbrain sites such as the dorsal vagal complex (Faulconbridge, Cummings, Kaplan, & Grill, 2003), as well as a number of forebrain nuclei, including the paraventricular nucleus of the hypothalamus (Currie, Mirza, Fuld, Park, & Vasselli, 2005), the lateral hypothalamus (Olszewski et al., 2003), the hippocampus (Kanoski, Fortin, Ricks, & Grill, 2013), and the amygdala (Alvarez-Crespo et al., 2012). Several recent studies have focused on the ability of ghrelin to modulate activity of the mesolimbic dopamine pathway, including the VTA, and to increase food intake and the motivation to obtain palatable food (Egecioglu et al., 2010). Collectively, these studies suggest that ghrelin may have interesting effects on reward and motivational processes involved in feeding.

Though it may seem paradoxical, obese individuals typically have lower plasma levels of ghrelin than do lean individuals. This is observed in fasting levels of ghrelin. Additionally, the postprandial suppression of ghrelin is not as large in obese individuals as in lean individuals (Tschop et al., 2001). Weight loss results in an increase in plasma ghrelin (Cummings et al., 2002), which may contribute to the increased hunger experienced during and after dieting-induced weight loss. Because ghrelin stimulates hunger and increases feeding, a number of laboratories have attempted to reduce the bioactivity of ghrelin in an attempt to promote weight loss. In animal models, reduction of

ghrelin levels has been accomplished through the use of anti-ghrelin immunoglobulins (Teubner & Bartness, 2013). Unfortunately, the bioavailability of these compounds, as well as multiple undesired side effects, have limited the translation of this type of pharmacological approach for humans.

Pancreatic Beta-Cell-Derived Hormones

Meal taking presents a challenge to many aspects of metabolic homeostasis, including glycemia. Maintenance of blood glucose levels is largely regulated by pancreatic-derived hormones glucagon and insulin, released from pancreatic alpha and beta cells, respectively. As nutrients enter the GI tract during meal taking, it is critical that adequate and rapid communication occur between the GI tract, brain, and pancreas to facilitate glycemic control. Interestingly, some of the hormonal signals produced by the pancreas also have potent effects on feeding and body weight.

Insulin

In addition to insulin's ability to regulate glucose levels, insulin receptor signaling also affects food intake, although the reliability of insulin-mediated energy balance effects are sometimes questioned (see Begg & Woods, 2012, and Woods & Langhans, 2012, for review). Although insulin obviously promotes the lowering of plasma blood glucose—an effect that, in itself, may affect subsequent food intake—the energy balance effects of insulin receptor signaling are thought to be independent from its effects on glycemia (Woods, Stein, McKay, & Porte, 1984). Thus, whereas some of the energy balance effects of insulin may be mediated by peripheral organs such as the liver (see Begg & Woods, 2012, for review), the majority of the intake-suppressive effects of peripheral insulin are thought to be centrally mediated (Woods, Seeley, Baskin, & Schwartz, 2003), following CNS penetrance via facilitated transport (Baura et al., 1993).

Within the CNS, insulin receptor signaling reduces food intake and body weight in baboons, rats, sheep, mice, and possibly humans (Brown, Clegg, Benoit, & Woods, 2006). Many studies have examined the ability of insulin to regulate feeding via actions in the hypothalamus, particularly in the ARC. Insulin receptors are tyrosine kinase receptors that are expressed on NPY-containing, as well as on POMC-expressing, ARC neurons. To this end, ICV administration of insulin reduces NPY expression in the ARC (Schwartz et al., 1991), as well as in the paraventricular nucleus (Schwartz et al., 1992), possibly via recruitment of GABAergic circuits. Insulin also increases POMC expression (Kim, Grace, Welsh, Billington, & Levin, 1999), consistent with an overall reduction in food intake.

Activation of the insulin receptor results in rapid phosphorylation of insulin receptor substrates (IRS). In particular, IRS-2 appears to be important for the control of energy balance by insulin, in that whole-body (Burks et al., 2000) or hypothalamic knockdown (Kubota et al., 2004) of IRS-2 promotes obesity in murine models. A number of intracellular pathways downstream of IRS have been documented as required signaling events to mediate the suppression of food intake by activation of insulin receptors. Principal among these is the . . . phosphatidylinositol-3-kinases (PI3K) signaling pathway (Niswender et al., 2003). Pharmacological inhibition of PI3K attenuates the ability of centrally delivered insulin to suppress food intake (Niswender et al., 2003), indicating the requirement of PI3K activation to facilitate the anorectic effects of insulin. Engagement of the PI3K pathway is also important for insulin-mediated control of energy balance in extrahypothalamic sites such as the amygdala (Castro et al., 2013).

In the context of obesity, insulin is often referred to as a lipostatic signal (Benoit, Clegg, Seeley, & Woods, 2004), in that circulating concentrations of insulin reflect levels of adiposity (Ahren, 1999). This concept fails, to some extent. Despite an accumulating magnitude of insulin signaling as adiposity increases, insulin resistance develops, and the obese individual does not subsequently reduce energy intake and increase energy expenditure to reduce adiposity levels. Thus, despite their higher plasma insulin, obese individuals are resistant to the

intake- and body-weight-suppressive effects of the peptide (see Begg & Woods, 2012, for review). These effects are not entirely due to an insufficient penetration of insulin into the CNS (Kaiyala, Prigeon, Kahn, Woods, & Schwartz, 2000), as direct, central administration of insulin is less effective at reducing food intake in obese animals maintained on a high-fat diet.

Amylin (Islet Amyloid Polypeptide)

The peptide hormone amylin is co-secreted with insulin at a 1-to-100 ratio from pancreatic beta cells after food is consumed (Ogawa, Harris, McCorkle, Unger, & Luskey, 1990). As one might expect, given its association with insulin release, amylin has complementary effects to insulin on glycemic control (Schmitz, Brock, & Rungby, 2004), mainly mediated through delayed gastric emptying (Clementi et al., 1996), inhibition of glucagon release (Fehmann et al., 1990), and potent anorectic effects (Lutz, 2010). Specifically, amylin is well documented as a satiation signal, given its robust ability to reduce food intake via suppression of meal size (Lutz, 2010).

Because surgical vagotomy does not block amylin-induced hypophagia (Lutz, Del Prete, & Scharrer, 1995), the effects of the peptide on feeding are thought to be mediated by direct activation of amylin receptors in the brain (Lutz, 2005). Amylin receptors are fairly unique, in that they contain one of two splice variants of the calcitonin receptor (CTa/CTb; a G-protein-coupled receptor) that heterodimerizes with one of the receptor activity modifying proteins (RAMP1, RAMP2 or RAMP3; Hay, Christopoulos, Christopoulos, & Sexton, 2004). Despite the widespread expression of amylin receptors throughout the central neuraxis, investigations of CNS nuclei and neuronal mechanisms mediating the anorectic effects of amylin have, until recently, focused on classic homeostatic circuitry (Hilton, Chai, & Sexton, 1995; Sexton, Paxinos, Kenney, Wookey, & Beaumont, 1994). The majority of reports describing the hypophagic effect of amylin have focused on its ability to regulate food intake via actions at the area postrema (AP) of the hindbrain, because lesions of this nucleus attenuate the hypophagic effects of systemic amylin administration (Lutz, Mollet, Rushing, Riediger, & Scharrer, 2001; Lutz et al., 1998). Importantly, though, amylin binding is distributed throughout the brain (Paxinos et al., 2004; Sexton et al., 1994), and amylin can cross the blood–brain barrier (Banks & Kastin, 1998; Banks, Kastin, Maness, Huang, & Jaspan, 1995). These data suggest that amylin's access to the CNS is not limited to circumventricular structures such as the AP. Indeed, recent research has shown that amylin can act directly in the VTA to control food intake (Mietlicki-Baase et al., 2015; Mietlicki-Baase, Rupprecht, et al., 2013). VTA amylin receptor activation appears to have especially potent suppressive effects on palatable food intake, as well as on the motivation to obtain a palatable food (Mietlicki-Baase et al., 2015; Mietlicki-Baase, Rupprecht, et al., 2013). This is an interesting finding given the role of the VTA and the mesolimbic system in regulating the intake of palatable and rewarding ingesta. Additionally, a few studies have investigated the actions of amylin in the ventromedial nucleus of the hypothalamus (VMH). Results indicate that amylin may enhance the intake-suppressive effects of the adipose-derived hormone leptin through actions in the VMH (Le Foll et al., 2014; Turek et al., 2010).

A unique feature of amylin receptor activation as a potential treatment for obesity is that it remains effective in its ability to suppress food intake and body weight in obese rodents and humans (Boyle, Rossier, & Lutz, 2011). Studies using the amylin agonist pramlintide, which is FDA approved for the treatment of diabetes (Singh-Franco, Robles, & Gazze, 2007), show that pramlintide treatment in obese humans reduces body weight and enhances control over feeding behavior (Chapman et al., 2005; Ravussin et al., 2009; Roth et al., 2008). This ability of amylin to exert its effects in obese individuals contrasts with other hormonal signals, such as those of leptin and insulin, where sensitivity to suppressing food intake is reduced in the obese state (Munzberg, Flier, & Bjorbaek, 2004). Thus, the absence of amylin resistance in obesity has intensified interest in amylin-based pharmaceuticals as potential future treatments for obesity (Mietlicki-Baase & Hayes, 2014).

Adipose-Derived Hormones

Leptin

Research examining the adipose-tissue-derived hormone leptin has transformed our understanding of the function of white adipose tissue from one of a simple energy storage depot to the view that adipose tissue is an active endocrine organ. We now appreciate that the greater the fat mass of an individual, the larger the available circulating levels of leptin. Given that leptin, acting on its receptors (LepRb, a.k.a. ObRb) in the brain, is known to suppress food intake and energy expenditure, it seems somewhat paradoxical that obesity, a chronic state of elevated adipose mass, is able to occur. Under normal physiological conditions in a lean human or animal, both the total amount of adiposity and the fluctuation in adiposity levels are minimal. Under these conditions, slight variations in circulating leptin levels, which communicate energy storage within the adipose tissue, are sensed by the brain. Appropriate CNS signaling pathways are engaged to either increase or decrease food intake and energy expenditure to normalize energy balance. Unfortunately, however, in the case of obesity, leptin levels are chronically elevated and the brain fails to correctly perceive and respond to the overaccumulation of the leptin signal. Such a response is known as *leptin resistance*.

Under conditions in which the body is challenged by a constant oversupply of nutrients, the normal functioning of the physiological mechanisms maintaining energy balance is disrupted. A state of chronic nutrient excess (caused by overconsumption of calorically dense foods) leads eventually to a blunting of signaling in the insulin and leptin pathways, a concept referred to as *resistance*. As described previously, under normal conditions, elevated leptin levels act centrally to decrease feeding and prevent obesity. Under conditions of excess (i.e., obesity), even though large amounts of leptin circulate in the blood, there are disruptions in the receptor and intracellular signaling responses for these hormones. In short, the oversaturation of the hormone at the transporter into the CNS, as well as at the LepRb themselves, decreases the receptor response to the hormone such that leptin fails to appropriately suppress food intake and increase energy expenditure. Thus, weight gain continues, further exacerbating the obesity phenotype. A vicious cycle develops, in which the person who is already consuming too many calories now has less sensitivity to the normal neurochemical signals that should be leading to meal termination. Over time, this resistance to leptin signaling further predisposes the individual toward T2DM and obesity.

Conclusions and Future Directions for Obesity Treatment

Although this chapter considers the individual contributions of several gut-derived and pancreatic hormonal signals to energy balance control, it is crucial to reiterate that these signals do not act in isolation in mammals. Ingestion of food affects many neural and hormonal processes, including those described here, as well as numerous other peripheral and central systems, each of which contributes to the overall control of food intake and body weight. The redundancy of some of these signals is important for preserving and maintaining energy storage, but has also presented a major challenge to the development of pharmacological strategies for the treatment of obesity.

Historically, attempts to treat obesity by targeting a single neuroendocrine system have failed to produce meaningful and long-lasting suppression of body weight, and some have been plagued by serious side effects (Gadde, 2014; James et al., 2010). New monotherapeutic strategies continue to be developed and tested as potential anti-obesity drugs. However, the notion that combination approaches will be more effective for producing sustained reductions in body weight has become increasingly accepted by the scientific community (Phelan & Wadden, 2002). Such approaches include using pharmacotherapy in conjunction with behavioral intervention (Vetter, Faulconbridge, Webb, & Wadden, 2010) and/or pharmacologically targeting more than one neurotransmitter/neuropeptide system (Bray, 2014; Rodgers, Tschop, & Wilding, 2012). It is clear that further development of effective, noninvasive pharmacological strategies for obesity treatment is urgently required.

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