Review

Energy regulatory signals and food reward

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ABSTRACT

The hormones insulin, leptin, and ghrelin have been demonstrated to act in the central nervous system (CNS) as regulators of energy homeostasis, acting at medial hypothalamic sites. Here, we summarize research demonstrating that, in addition to direct homeostatic actions at the hypothalamus, CNS circuitry that subserves reward and is also a direct and indirect target for the action of these endocrine regulators of energy homeostasis. Specifically, insulin and leptin can decrease food reward behaviors and modulate the function of neurotransmitter systems and neural circuitry that mediate food reward, the midbrain dopamine (DA) and opioidergic pathways. Ghrelin can increase food reward behaviors, and support midbrain DA neuronal function. We summarize discussion of behavioral, systems, and cellular evidence in support of the contributions of reward circuitry to the homeostatic roles of these hormones in the CNS. The understanding of neuroendocrine modulation of food reward, as well as food reward modulation by diet and obesity, may point to new directions for therapeutic approaches to overeating or eating disorders.

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1. Introduction

The causes underlying the modern epidemic of obesity have become an area of research focus across many disciplines evaluating nutrition, ingestive behavior, and metabolism. It has been proposed that the increased rates and severity of obesity may be in part related to the ready availability of highly palatable food. Consumption of palatable food is associated with CNS reward, motivational, and hedonic mechanisms, and simultaneously may be associated with dysregulation of CNS energy-regulatory systems. An early and continued research emphasis in ingestive behaviors has been on the actions of endocrine factors at the medial hypothalamus, which is a key site in the CNS regulation of metabolism, energy balance, and caloric intake in the context of physiological need. Extensive evidence that the hormones leptin and insulin act at the medial hypothalamus to provide negative feedback for food intake and body weight is summarized below (and see: Ahima and Osei, 2001; Barsh and Schwartz, 2002; Baskin et al., 1999; Beck, 2000; Figlewicz, 2003; Horn et al., 1999; Seeley and Schwartz, 1999). Discussion of more recent evidence, that insulin and leptin also act at reward circuitry in the brain, follows. Finally, the hormone ghrelin has been identified as an endocrine orexigen, that also interacts with both hypothalamic and reward circuitry, and those observations are summarized.

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2. Energy regulatory hormones signal in the CNS

In 1979, it was demonstrated in a primate model that insulin infused into the CNS caused a significant decline in food intake and body weight (Woods et al., 1979), and it was proposed that insulin served as an "adiposity signal" that completes a negative feedback loop linking the behavior of feeding with size of adipose stores (Porte and Woods, 1981). Many studies since have validated this basic concept (e.g., Air et al., 2002a,b,c; Brief and Davis, 1984; Chavez et al., 1995; McGowan et al., 1990). In the mid-1990's the candidate energy-regulatory signal and adipose hormone, leptin, was identified (Zhang et al., 1994), and has now been well-characterized as a regulator of energy homeostasis acting in the CNS. Two aspects of this hypothetical model had to be addressed in order to validate a role for energy regulatory signals in modulating any aspect of CNS function.

First, it had to be demonstrated that these circulating signals have access to CNS circuitry. The presence of insulin in the CNS was reported in 1979 (Havrankova et al., 1979), and many studies established that the predominant amount of insulin in the CNS can be accounted for by receptor-mediated transport across the blood–brain barrier into the CNS (Coker et al., 1990; Dernovsek et al., 1984; Dernovsek and Bar, 1985; Giddings et al., 1985; Israel et al., 1993; King and Johnson, 1985; Schwartz 1990a,b 1999). Although intermittent reports have suggested that insulin can be synthesized locally in the developed (adult) CNS, quantities appear to be negligible particularly when compared to the affinity of the receptor for peripherally circulating insulin. The relationship between CNS and plasma levels of insulin is saturable (non-linear), consistent with a receptor-mediated transport process. In the 1990's, when the adipose hormone leptin was identified, evidence rapidly accumulated that it likewise could be transported by multiple mechanisms into the CNS (Banks et al., 2000, Banks et al., 2002; Hileman et al., 2002; Kastin and Pan, 2000; Maness et al., 2000; Maresh et al., 2001; Munzberg, 2008). Relative levels of both leptin and insulin in the CSF are decreased in association with obesity (Banks et al., 1999; Banks et al., 2001; Caro et al., 1996; Kaiyala et al., 2000; Schwartz et al., 1990a, 1996; Stein et al., 1987). Recent studies from Banks et al. have shown increased transport of both insulin and ghrelin, but decreased transport of leptin, by serum triglycerides, suggesting a more complex relationship between nutritional status, obesity status, and transport of these endocrine signals into the CNS (Banks, 2008; Banks et al., 2008; Urayama and Banks, 2008).

Additionally, the presence of functional insulin and leptin receptors in the CNS was established. Receptors for both insulin (Corp et al., 1986; Havrankova and Roth, 1978; Unger et al., 1991; Werther et al., 1987; Zahniser et al., 1984) and leptin (Elmquist et al., 1998; Leshan et al., 2006) are widely expressed throughout the CNS. The medial hypothalamus, a key center for the regulation of energy homeostasis and coordination of metabolic events, is a major target for both insulin and leptin action (Baskin et al., 1999; Mirshamsi et al., 2004; Niswender et al., 2001, 2003; Seeley and Schwartz, 1999). Other CNS sites and neural systems are targets for insulin and leptin action (Figlewicz et al., 1990; Figlewicz, 2003; Grill et al., 2002; Harvey, 2007; McNay, 2007). Studies utilizing antisense oligonucleotides against the insulin receptor and conditional, localized knockout of the insulin receptor, have elucidated the contribution of the brain insulin receptor to energy homeostasis and glucose homeostasis (Bruning et al., 2000; Koch et al., 2008; Obici et al., 2002; Obici and Rossetti, 2003). The leptin receptor, likewise extensively expressed, is present as different splice-variant isoforms in the CNS, with the "signaling" form OBRb having the major role in leptin action. The obese db/db mouse and Zucker fa/fa rat represent naturally occurring "knockouts" (Chua et al., 1996) of the leptin receptor, and recent use of receptor constructs with modifications in signaling capability validate the importance of CNS leptin action in energy homeostasis.

The role of the orexigenic peptide ghrelin in CNS regulation of metabolic processes and energy homeostasis, in contrast to that of insulin and leptin, is at present somewhat more equivocal. Originally isolated in the X/A-like endocrine cells of the oxyntic gastric mucosa (Kojima et al., 1999), which by most accounts are the preponderant source of circulating ghrelin (Ariyasu et al., 2001), expression of this peptide has also been demonstrated in a number of diverse tissues, including the small intestine (Date et al., 2000), pancreas (Volante et al., 2002), kidney (Mori et al., 2000), testis (Tanaka et al., 2001), ovary (Caminos et al., 2003), and CNS (Korbonits et al., 2001). Moreover, the diffuse sites of hypothalamic and extra-hypothalamic expression of ghrelin in the CNS suggest that this "brain-gut" peptide plays a much broader role in brain signaling than just the control of food intake. Since hypothalamic ghrelin is endogenous in origin (Cowley et al., 2003), being locally expressed in numerous axon terminals, its function as a neurotransmitter sets it apart from peripheral peptide hormones arriving at the hypothalamus via specific transport. Nevertheless, the demonstration of ghrelin-containing neurons innervating the arcuate (ARC), ventromedial (VMN), paraventricular (PVN) and dorsomedial nuclei, along with ghrelin expression in arcuate neurons terminating on pro-opiomelanocortin (POMC), neuropeptide Y (NPY) and other ghrelin-producing neurons is consistent with a major role in energy homeostasis.

Ghrelin is a natural ligand of the 1a isoform of the growth hormone secretagogue receptor (GHS-R) (Howard et al., 1996), which is distributed in diverse tissues both peripherally and in the CNS (Gnanapavan et al., 2002). Type 1a GHS-R has been identified in peripheral endocrine tissue such as the adrenal gland, testis, ovary, pancreas, and thyroid, as well as in the stomach, kidney, liver, arteries, heart and adipose tissue (Papotti et al., 2000). CNS distribution of 1a GHS-R is likewise widespread, with marked expression observed in the ARC and VMN of the hypothalamus (Mitchell et al., 2001). Although extra-hypothalamic sites of expression include the cerebral cortex, dentate gyrus, hippocampus, substantia nigra, ventral tegmental area, nodose ganglion, and dorsal vagal complex of the medulla, it is hypothalamic GHS-R and its co-expression with growth hormone-releasing hormone, NPY, POMC, somatostatin (SS), and tyrosine hydroxylase (TH) that lends further credence to ghrelin signaling comprising a viable component of nutritional homeostasis and metabolism.

The multiple effects of insulin and leptin on energy homeostasis depend upon interaction with key hypothalamic nuclei and peptides to regulate energy balance (Inui, 1999). Among the most extensively studied CNS mediators are NPY (Clark et al., 1984; Schwartz et al., 1992a, b; Sipols et al., 1995; White et al., 1990), POMC and its product α-melanocyte stimulating hormone (α-MSH), and the melanocortin antagonist, AgRP (for reviews, see: Benoit et al., 2000; Morton and Schwartz, 2001; Seeley et al., 1999; Woods et al., 2000). POMC and AgRP are selectively expressed in neurons of the ARC colocalized with receptors for insulin and leptin, which comprise endogenous circuitry capable of regulating food intake (Cheung et al., 1997; Mizuno et al., 1999; Mountjoy and Wong, 1997). Leptin and insulin increase expression of the anorexic peptide, α-MSH, and decrease expression of AgRP (see (Benoit et al., 2000) for reviews). Collectively, these data suggest that leptin and insulin act on ARC melanocortin (AgRP and POMC) neurons to regulate food intake and energy balance. It is likely that other endogenous neurotransmitters either directly or indirectly mediate the CNS effects of leptin and insulin. Among these, the orexigenic peptides orexin-A and melanin concentrating hormone (MCH) are expressed in the lateral hypothalamus (Broberger et al., 1998; Mondal et al., 2000; Peyron et al., 1998). Intriguingly, recent data suggests that orexin-A may be an important factor in the effects of drugs of abuse. Orexin antagonists blunt the behavioral response to cocaine and other psychostimulants and may be important for the rewarding effects of food as well (e.g., Clegg et al., 2002; for reviews see Boutrel and de Lecea, 2008; Hervieu, 2003).

Intracellular signaling secondary to insulin and leptin interaction with CNS receptors has been elucidated from studies in the medial

hypothalamus. Signaling for the CNS insulin receptor is comparable to post-receptor mechanisms in peripheral target tissues. The receptor is an autophosphorylating tyrosine kinase, and its activation leads not only to tyrosine phosphorylation of other proteins including the key signaling moiety, IRS, but also to a cascade of additional phosphorylation events, including activation of the PI3 kinase pathway (Hadari et al., 1992), and phosphorylation of Akt/PKB (Heidenreich, 1989; Heidenreich, 1993; Kenner and Heidenreich, 1991; Niswender et al., 2003; Shemer et al., 1989). The leptin receptor, upon leptin binding, can likewise initiate IRS phosphorylation, and activation of the PI3 kinase pathway (Niswender et al., 2003). However, the receptor does not have intrinsic tyrosine kinase activity, thus JAK-Stat signaling is a critical initial event, leading to transcriptional events, and, ultimately, to the generation of SOCS-3 which provides negative feedback on leptin signaling (Leshan et al., 2006; Robertson et al., 2008). Thus, there are both parallel and unique intracellular pathways by which insulin and leptin can mediate intracellular events related to ingestive behavior and caloric homeostasis (Carvalheira et al., 2001; Carvalheira et al., 2005, Pocai et al., 2005). In metabolic circumstances in which plasma insulin or leptin levels are low (starvation and reduced adiposity), signaling would be decreased and drive for food intake would be increased. Obesity (excessive adiposity) would represent a pathophysiologic state in which either energy regulatory signals are decreased in relative or absolute amount in the CNS, or there is direct CNS resistance to their action (Clegg et al., 2005; De Souza et al., 2005; Irani et al., 2007; Munzberg et al., 2004, 2008; Wang et al., 2001a,b). Collectively, this research has paved the way for studies of endocrine signaling within reward circuitry.

Electrophysiological studies by several investigators (Cowley et al., 2003; Seoane et al., 2003; Zigman et al., 2006) have similarly confirmed the ARC as a major site of ghrelin action in neurons controlling food intake. Although the neuronal expression of ghrelin within several hypothalamic regions has been reported, there has been some controversy regarding this expression, and it is likely that a significant contribution to medial hypothalamic effects comes from peripherally synthesized (i.e., gastric) ghrelin (please see Sakata et al., 2009; Castaneda et al., 2010 for discussion and references). Ghrelin-positive terminals have been reported to innervate a number of different ARC neurons expressing type 1a GHS-R that project mainly to the PVN. In a group of ARC neurons, ghrelin has been shown to directly increase firing of NPY/AgRP neurons, which then inhibit POMC neurons via GABA and NPY release. Given the putative roles of NPY and AgRP as potent orexigens and POMC as an anorexigen, the modulation of neuronal activity by ghrelin is entirely consistent with its hypothesized role in signaling nutritional insufficiency. In a group of medial PVN neurons, ghrelin was found to reduce inhibitory GABAergic input, an effect substantially dependent upon Y1 and Y5 NPY receptor signaling. This effect within the PVN, along with the ability of ghrelin to increase firing of ARC neurons inhibited by leptin (Traebert et al., 2002), further supports a role for ghrelin in maintaining energy balance.

Along with its ability to influence feeding pathways via direct synaptic processes, several studies suggest that ghrelin may also regulate neurotransmission via gene expression. Increased mRNA levels of NPY and AgRP in ARC neurons are associated with intracerebroventricular (ICV) doses of ghrelin that stimulate food intake (Nakazato et al., 2001; Seoane et al., 2003), suggesting an effect on *de novo* neurotransmitter synthesis. Moreover, direct ICV administration of ghrelin in many hypothalamic nuclei involved in feeding resulted in increased neuronal activation, as evidenced by elevated Fos expression (Lawrence et al., 2002), particularly in many NPY ARC neurons (Wang et al., 2002), a number of orexin neurons in the lateral hypothalamus (Toshinai et al., 2003), and some oxytocin neurons in the PVN (Olszewski et al., 2007). Together with recent observations of type 1 cannabinoid receptor inactivation blocking the ability of ghrelin to increase AMP-activated protein kinase in the hypothalamus (Kola et al., 2008), these findings illustrate the complex interactions of ghrelin with many neurotrans-

mitter systems crucial to the central regulation of feeding and energy homeostasis. An additional aspect regarding ghrelin and food intake, i.e. the motivational aspect, is discussed below.

An overarching question remains as to the origin of ghrelin that may be central to *in vivo* signaling of energy disequilibrium. While there is no dispute that ghrelin functions as a classical neurotransmitter across synapses to influence postsynaptic activity–as demonstrated in many of the above-cited studies–one cannot discount the possibility that circulating gastric ghrelin activates hypothalamic type 1a GHS-R populations via intermediary neurons in circumventricular organs such as the subfornical organ (SFO). As mentioned above, it is now nearly universally agreed that the circulating energy regulatory signals insulin and leptin gain access to their hypothalamic receptors via specific transport mechanisms across the blood–brain barrier (BBB). As a "brain-gut" peptide, the ghrelin pool that influences food intake could at least partly be extra-hypothalamic in origin, given that gastric secretion of ghrelin is many-fold that of hypothalamic release. Importantly, a substantial number (30%) of SFO neurons are responsive to ghrelin *in vitro* by increasing intracellular calcium secretion and the number of action potentials (Pulman et al., 2006). Therefore, ghrelin-sensitive SFO neurons that innervate the hypothalamus could well play a crucial role in relaying information about circulating ghrelin concentrations—which, among other humoral signals, reflect the state of energy homeostasis—to the ARC for integration into the neuronal feeding pathways.

3. Energy regulatory signals modulate food reward

A current focus of energy homeostasis research is the elucidation of how environmental factors such as diet composition interact with the energy regulatory signal-CNS feedback loop to modulate the effectiveness of these signals. For example, putting rats on a high fat diet results in an impairment in the action of insulin to decrease body weight (Arase et al., 1988; Chavez et al., 1996), and a similar observation has been made for leptin (Lin et al., 2001). The extrapolation of these observations is that endogenous energy regulatory signals in the CNS may also become ineffective at providing feedback signaling. Data collected by the Centers for Disease Control document a pervasive increase of obesity in adults across the United States in the 1990's and 2000's (Hill et al., 2003; Mokdad et al., 2001), and a high incidence of obesity in the pediatric age group as well (Kim et al., 2006), interpreted as a significant environmental influence over the neural circuitry associated with the physiological maintenance of energy homeostasis. The epidemiologic finding also emphasizes that attention should be focused on additional CNS circuitry which is either directly or indirectly connected with hypothalamic circuitry to modulate feeding behavior.

One obvious target for study is the CNS circuitry which mediates motivation and reward. Midbrain circuits intimately involved in reward signaling have been previously identified using reproductive behavior and drug addiction paradigms. Components of this circuitry are activated with, and contribute to, complex behaviors including food seeking and food intake ((Berridge, 1996; Berridge and Robinson, 1998; Berthoud, 2004) and see below). This circuitry includes specific subregions of the cerebral cortex; hippocampus and amygdala; and the striatonigral pathway, which is implicated in transposing motivational aspects of stimuli into motor responses, as well as hedonic evaluation of the stimulus and associative learning (Everitt et al., 1999; Ikemoto, 2007; Ishiwari et al., 2004; Petrovich and Gallagher, 2003; Robbins and Everitt, 1996; Rollins and King, 2000; Will et al., 2004; Wise, 2002). As discussed below, the major neurotransmitter pathways associated with motivation and hedonics are mesolimbic dopamine (DA) and certain CNS opioid pathways. In terms of neural connectivity, the hypothalamus is linked to the "motivational circuitry" of the CNS both anatomically and functionally (Berthoud, 2007; Kelley and Berridge, 2002; Kelley et al., 2002; Stratford and Kelley, 1999). Insulin and leptin receptors are expressed throughout the limbic forebrain, including the hippocampus;

the amygdala; and the lateral hypothalamic/zona incerta area (Figlewicz et al., 2003; Leinninger and Myers, 2008; Leshan et al., 2006). This provides one rationale for exploring the potential that the limbic forebrain may itself be a direct target for insulin or leptin. Food intake can be driven by energy demands, i.e., "homeostatic" feeding. However, food intake can also be driven by the palatability or pleasure associated with eating a preferred food, "non-homeostatic" feeding (Berridge and Robinson, 1998; Berthoud, 2004, 2007). The palatability of a particular food source is assumed to be related to the flavor and taste of that food and high-fat diets are generally considered more palatable than diets that are low in fat, as they are preferentially over-consumed. In humans, individual differences exist in the reinforcing value of food with obese individuals displaying a stronger preference for diets high in fat and carbohydrates relative to non-obese individuals (Drewnowski et al., 1992a,b; Drewnowski and Popkin, 1997).

Berridge and Robinson have provided a conceptual framework for the consideration of reward, proposing that there is "wanting" of food (or another stimulus) and "liking" of food. The behavior associated with non-homeostatic feeding is in part regulated by the mesolimbic dopamine system (Berridge and Robinson, 1998), and within this system, the neurotransmitter dopamine plays a substantial role in the regulation of food reward. They have identified nucleus accumbens (NAc) dopamine projections as central to "wanting". The NAc represents a functionally specialized subregion of the striatum which is a critical anatomical component of CNS reward circuitry, with the extensive projection of DA neurons from the midbrain ventral tegmental area (VTA) and associated DA cell groups (Ikemoto and Wise, 2004). Activation of these midbrain DA neurons has been implicated in the motivating, rewarding, reinforcing and incentive salience properties of natural stimuli such as food and water, as well as drugs of abuse (Ikemoto and Wise, 2004; Richardson and Gratton, 1996; Schultz, 2002; Smith, 1995). The neural mechanisms of food reward are believed to be similar, if not identical, to drug rewards (Carelli, 2002; Kelley and Berridge, 2002).

DA neuronal activation can be modulated by the experience (e.g., [Bassareo et al., 2002]) or nutritional status of an animal. Wilson et al. demonstrated that food-restricted rats trained to drink a palatable liquid food had greater dopamine release in the NAc than free-feeding rats (Wilson et al., 1995). One question, then, is whether these dopamine neurons are a target for neural or endocrine factors that change in association with fasting and food restriction. Indeed, a neuroendocrine milieu exists in fasted animals that would bias them towards enhanced dopaminergic function. Adrenal glucocorticoid levels are elevated with fasting, and Piazza and colleagues have provided evidence that glucocorticoids can facilitate dopamine release and dopamine-mediated behaviors (Marinelli and Piazza, 2002). Additionally, both insulin and leptin levels rapidly decrease in association with food restriction or fasting (Havel, 2000), and both inhibit performance in food reward behavioral tasks that are dopamine-dependent (Figlewicz et al., 2001, 2004, 2007). Conversely, new studies demonstrate that ghrelin–which is increased in association with food deprivation–can increase NAc dopamine levels (Jerlhag et al., 2006) and, acting at the VTA, can stimulate feeding (Abizaid et al., 2006; Naleid et al., 2005) and increase dopamine neuronal activity (Abizaid et al., 2006).

Ghrelin may also be involved in the expression and maintenance of behaviors exhibited prior to actual ingestion, including, but not limited to, the appetitive aspects of food seeking, selection, memory, and reward (Naleid et al., 2005). Application of nanomolar amounts of ghrelin to in vitro VTA slices resulted in increased action potentials in dopaminergic neurons from normal mice, but not from mice missing the GHS-R gene (Abizaid et al., 2006). Moreover, ghrelin appears to increase excitatory inputs on DA neurons in the VTA, consistent with increased activation of reward systems. Studies of GHS-R and DA receptor co-localization in the VTA of transgenic mice reveal a potentiation effect of ghrelin through the GHS-R on the DA receptor, such that an excess amount of DA is released in the NAc (Jiang et al.,

2006). Speculation as to the behavioral correlate of these neurophysiological findings–e.g., increased locomotion in search of food is suggested by a recent study (Blum et al., 2009) in GHS-R knockout mice–at present remains fairly hypothetical. However, it is clear that ghrelin is well positioned to signal not just feeding in a mechanical sense through its hypothalamic receptor populations, but also the antecedent or motivational attributes of feeding behavior through GHS-R populations in the VTA (Abizaid, 2009).

Food restriction or fasting indeed enhance the addictive or reinforcing properties of drugs of abuse, as found in both drug self-administration and relapse to drug-taking paradigms; intraventricular leptin can reverse food deprivation-induced relapse to heroin self-administration (Carroll and Meisch, 1984; Shalev et al., 2001, 2002). Data from the experimental approach of brain self-stimulation have shown that food restriction shifts the dose response curve for self-stimulation in some perifornical hypothalamus sites to the left, such that weaker electrical current that normally would not support sustained self-stimulation activity at these sites becomes efficacious when animals are maintained on a food-restriction paradigm (Carr, 1996, 2002). Intraventricular leptin shifts the dose–response curve in food restriction-sensitive perifornical hypothalamic sites to the right (i.e., reverses the effect of food restriction); and administration of insulin into the brain of either food-restricted or ad libitum feeding rats increases the threshold for self-stimulation, both reversing the decreased threshold observed with fasting, and elevating the threshold above its "free-feeding" level (Carr et al., 2000; Fulton et al., 2000). This evidence, although limited, suggests that insulin and leptin may play a major role in mediating the effects of altered metabolic status on reward paradigms in general.

Several studies implicate insulin and leptin in food reward per se. In addition to normal feeding, DA activity has been implicated in behavioral paradigms that evaluate different aspects of reward or motivation: acute licking of palatable solutions (Davis and Smith, 1992; Schneider et al., 1990); self-administration (Ikemoto and Wise, 2004); and the conditioning of a place preference (Papp, 1988). Figlewicz and colleagues have demonstrated suppression of acute sucrose licking (intraventricular insulin) (Sipols et al., 2000); food-conditioned place preference (intraventricular insulin or leptin) (Figlewicz et al., 2004); and sucrose self-administration (intraventricular insulin or leptin) (Figlewicz et al., 2006) in rats fed ad libitum chow. DiLeone and colleagues (Hommel et al., 2006), and Morton and colleagues (Morton et al., 2009), have now demonstrated that direct administration of leptin into the VTA decreases chow intake in ad lib feeding rats. Taken together, the results of these different behavioral paradigms–self administration, lick rate task, conditioned place preference (CPP) and free-feeding of the baseline diet, chow–demonstrate that insulin and leptin, across a concentration range from fasting to free-feeding to elevated levels, are able to modify behaviors that reflect acute and learned reward evaluation. Some further resolution of these actions remain, for example, whether the effects of exogenous insulin and leptin reflect a simulation of physiological changes as would occur postprandially. Additionally, whether the rapid and chronic effects of both insulin and leptin are mediated via the same circuitry and the same neural mechanisms remains to be elucidated. There appear to be multiple anatomical loci implicated as targets for insulin- and leptin-induced suppression of food reward, including the lateral hypothalamus (Carr et al., 2000; Fulton et al., 2000; Leinninger and Myers, 2008).

Studies from a number of laboratories have focused on insulin and more recently, leptin, effects on the midbrain DA neurons at both the cellular and the behavioral level. Insulin receptors had historically been identified in the VTA and the striatum, using receptor autoradiography and receptor immunocytochemistry approaches (Unger et al., 1991; Werther et al., 1987), and insulin and leptin receptor mRNA is expressed in the substantia nigra (Elmquist et al., 1998). Figlewicz and colleagues have localized both insulin receptors and leptin receptors on midbrain DA neurons, including those of the VTA, as well as medial and lateral

substantia nigra (Figlewicz et al., 2003). The presence of functional receptors has been confirmed by work of Fulton (Fulton et al., 2006) and Hommel (Hommel et al., 2006). Recent studies have identified that insulin and leptin increase PI3kinase activity when given directly into the VTA (Figlewicz et al., 2007). Further, leptin (administered peripherally, intraventricularly, or directly into the VTA) increases JAK-stat phosphorylation, and this is critical for the effect of leptin in the VTA to decrease chow feeding (Morton et al., 2009). The synaptic or neural mechanisms that underlie insulin and leptin effects on food reward remain to be fully elucidated. Hommel et al. (2006) have reported that leptin decreases DA neuronal action potentials in VTA slices. One potential cellular target for insulin action is the dopamine re-uptake transporter (DAT), which inactivates DA signaling by transporting DA back into the DA nerve terminal from the synapse (Jaber et al., 1997). Both the synthesis, and the activity or synaptic concentrations, of the DAT can be regulated by intracellular signaling systems including PI3kinase (Garcia et al., 2005; Vaughan et al., 1997). Both *in vivo* and *in vitro* insulin (or its lack, in diabetic models) affects expression and activity of the DAT (Figlewicz et al., 1994; Patterson et al., 1998; Sevak et al., 2008): Insulin can increase mRNA levels of, and synaptic activity of, the DAT. The functional implication of this would be that increased DAT activity could result in increased clearance of DA from the synapse, and hence, decreased DA signaling. This would be consistent with an action of insulin to decrease the rewarding aspect of food. Thus, although there have been limited studies of direct insulin and leptin effects, findings to date suggest that, in non-obese animal models insulin and leptin should act overall to decrease DA signaling.

As mentioned above with respect to energy homeostasis, some effects of insulin and leptin are mediated through secondary hypothalamic peptide effector systems, including melanocortins and orexin-A. For example, melanocortin receptors (MC3R and MC4R) are also expressed in brain regions implicated in addictive behavior (e.g., Alvaro et al., 1996) and pharmacological studies have outlined functional roles for these receptors in the modulation of drug-taking behavior. Antagonism of these receptors in NAc inhibits operant responding for cocaine, while central agonism of this system augments amphetamine-induced behaviors (e.g., Hsu et al., 2005). Orexin neurons exhibit diverse projections in the CNS to sites including the VTA (e.g., Fadel and Deutch, 2002). Orexin-expressing neurons of the LH have mu-opioid receptors; and the molecular physiology of these neurons is altered with morphine administration or withdrawal, emphasizing their role in CNS reward circuitry (Georgescu et al., 2003). Orexinergic projections signal specifically on a majority of dopamine neurons to activate the mesolimbic pathway (Zheng et al., 2007), and VTA neuron populations express both orexin receptor subtypes (Marcus et al., 2001). Exogenous orexin can increase VTA dopaminergic neuron firing rates. A specific role for endogenous orexin action in the VTA on reward-seeking behavior is implied in the findings that an orexin antagonist could block the reinstatement of an extinguished place preference for morphine, and that intra-VTA orexin-A was sufficient to reinstate the place preference, in rats (Harris et al., 2005). Additional evidence comes from studies of genetic models demonstrating the inability of orexin-deficient mice to form morphine-conditioned place preferences (Narita et al., 2006). Orexin action in the LH also appears necessary for the acquisition and expression of morphine-induced CPP (Harris et al., 2007). Finally, Borgland (Borgland et al., 2006) reported that intra-VTA administration of an orexin antagonist effectively prevents behavioral sensitization and neurophysiological changes that typify chronic cocaine use. The important point is that to the degree that peripheral energy regulatory signals may affect reward function, they are likely to do so in part through these critical effector peptides. One important consequence of this could be that neither insulin nor leptin would have to act directly on VTA or NAc cells to exert regulatory control. Rather, as an additional mechanism, they could activate (or inactivate) effector systems in hypothalamic neurons that in turn project to the reward circuitry. This is supported by a recent study evaluating the specific CNS targets of intraventricular insulin to suppress food reward: The effect of insulin to decrease sucrose self-administration was found to be due to action at the ARC (Figlewicz et al., 2008).

4. Energy regulatory signals modulate palatability

Endogenous opioid neural networks appear to play a role in the regulation of food intake, food hedonics, and food choice in animals (Glass et al., 1999; Levine et al., 2003), and in human subjects (Drewnowski et al., 1992a,b, 1995; Yeomans et al., 1990). Although experimental evidence demonstrates that DA and the opioids play somewhat different roles in the mediation of food reward, the neuroanatomical circuitry that is implicated in opioid effects overlaps significantly with motivational circuitry. Opioids injected into numerous brain regions, including the VTA or the NAc, stimulate food intake (Badiani et al., 1995; Figlewicz et al., 2008; Lamonte et al., 2009; Noel and Wise, 1995); and food-induced DA release in the NAc is dependent upon opioid action in the VTA (Tanda and DiChiara, 1998). Further, activation of opioid receptors in the VTA is reinforcing (McBride et al., 1999); mu opioid receptor-induced inhibition of GABA neurons therein, which impinge on DA neurons, results in disinhibition of these DA neurons and facilitated DA release (Johnson and North, 1992; Cameron et al., 1997; Ford et al., 2006). The relevance of this for feeding resides in the observation of MacDonald et al. (2004) that stimulation of feeding by VTA administration of a mu opioid agonist is dependent upon DA1 receptor activity in the nucleus accumbens. Thus, there is functional crosstalk between opioids and dopamine.

Endogenous opioids and synthetic opiate peptides can enhance food intake (Arjune et al., 1990, 1991; Carr et al., 1991; Frisina and Sclafani, 2002; Islam and Bodnar, 1990; Levine et al., 1990; Levine et al., 1991; Jarosz and Metzger, 2002; Kirkham and Blundell, 1986; Kirkham and Cooper, 1988a,b; Lang et al., 1981; Marks-Kaufman et al., 1984; Yu et al., 1997, 1999) One ongoing question is whether opioids are responsible for intake of food in general, or only responsible for pleasurable food intake. The opioid antagonist naloxone has been shown to preferentially decrease motivation for, and consumption of, a palatable food (vs. rat chow) in non-deprived rats (Barbano and Cador, 2006; Cleary et al., 1996; Giraudo et al., 1993). Thus, opioids appear to signal hedonic value of a food independent from nutritional needs. However, in a study where animals were deprived of food for 24 hours, then given a choice between a preferred diet and a non-preferred diet, the general opioid antagonist, naltrexone, injected into the PVN decreased intake of both diets, but naltrexone injected into the amygdala decreased intake only of each animal's preferred diet (Glass et al., 2000; Naleid et al., 2007). Since the PVN plays a larger role in energy homeostasis, and the amygdala mediates portions of the emotional response to feeding, the study concluded that opioids affect different aspects of food intake in different CNS sites. Some have argued that opioids specifically enhance intake of fat. Indeed, many studies support a role for opioids in fat appetite (Glass et al., 1996; Islam and Bodnar, 1990; Kelley et al., 2002; Weldon et al., 1996; Yanovski and Yanovski, 2002; Zhang et al., 1998). Other studies, however, suggest that opioids modulate intake of an animal's preferred food, regardless of nutrient content (Glass et al., 1996, 1997; Gosnell and Krahn, 1992; Levine et al., 2003; Marks-Kaufman et al., 1985; Pomonis et al., 1997). Palatability influences both the amount and type of food that is ingested (Berridge, 1996). For example, it is well known that even non-caloric solutions will elicit drinking behavior in sated rats if they are made to taste sweet (Capaldi et al., 1997). Several hypothalamic peptides project to CNS areas important for taste processing (nucleus of the solitary tract), and hedonics and reward. With regard to such CNS-intrinsic signals, as reviewed by Olszewski and Levine (Olszewski and Levine, 2004), the opioid nociceptin may enhance or sustain feeding by interacting with feeding-termination neuropeptide pathways such as a-MSH, oxytocin, or CRH.

An animal's energy state can impact activity of the opioid system and the behaviors mediated by opioids (Levine et al., 1995; Rudski et al., 1994). Food restricted rats show significant reductions in mu- and increases in kappa-opioid receptor binding in several forebrain areas related to food reward and in the hindbrain parabrachial nucleus (Wolinsky et al., 1994, 1996a,b). Conversely, mu-opioid receptor binding is increased in reward-related sites in animals made obese on a high-fat diet (Smith et al., 2002). As described above, leptin and insulin modulate brain self-stimulation (Carr et al., 2000; Fulton et al., 2000), for which CNS opioidergic signaling has been implicated (Carr, 1996). The question of whether energy regulatory signals can blunt palatability-induced feeding has been evaluated to a limited extent. Insulin and leptin decrease intake of sucrose in non-deprived rats, and modulate opioid effects on sucrose intake. Intraventricular insulin decreases sucrose pellet intake stimulated by a kappa opioid agonist, and acts cooperatively with a subthreshold dose of a kappa opioid antagonist to decrease baseline intake of sucrose pellets (Sipols et al., 2002). This would be consistent with an action at the medial hypothalamus, where dynorphin receptors have been localized. Further, sucrose pellet intake stimulated by direct intra-VTA injection of the mu-opioid agonist DAMGO, can be inhibited by concurrent injection of insulin or leptin into the same site (Figlewicz et al., 2007). Since the feeding effect of mu opioids in the VTA is dependent upon dopamine release (MacDonald et al., 2004), one may speculate that insulin and leptin block the DAMGO effect by blocking DA neuronal activity. The observation of Hommel et al. (2006), that leptin can directly modulate DA neuronal activity within the VTA, provides early support for this, however, clearly, further study is warranted. Given the identification in human eating disorders of a role for opioids, particularly mu opioids (Drewnowski et al., 1992a,b), such studies have potential clinical relevance. This is underscored by the recent report that leptin treatment in two obese leptin-deficient patients was sufficient to reduce food intake, reduce self-report ratings of preference for images of food, and reduce neural activity in the striatum (Farooqi et al., 2007).

5. Conclusion

The table below summarizes the effects of insulin, leptin, and ghrelin on reward behaviors. Clearly, there is much yet to be learned about ghrelin-related peptide effects on motivation and palatability-driven feeding. However, the generalized effect of food restriction on drug-seeking (Carroll and Meisch, 1984), and the observation of leptin reversal of food restriction-induced heroin relapse (Shalev et al., 2001), suggest that insulin, leptin, and ghrelin may act by modulation of dopaminergic or opioidergic function.

Effects of insulin, leptin, and ghrelin on reward behaviors

Behavior	Insulin	Leptin	Ghrelin
Brain self-stimulation	decrease (ICV)	decrease (ICV)	not determined
Relapse to heroin seeking	not determined	decrease (ICV)	not determined
Acute sucrose licking	decrease (ICV)	not determined	no effect (ICV)*
Food CPP	decrease (ICV)	decrease (ICV, SC)	not determined
Sucrose self-administration	decrease (ICV,ARC)	decrease (ICV)	increase (ICV)*
Chow intake (4–24 hr)	decrease (ICV)	decrease (ICV,VTA)	increase (ICV,VTA)
Acute sucrose intake	decrease (ICV,VTA)	decrease (VTA)	not determined

*"Ghrelin affects motivational but not hedonic aspects of feeding", J. Overduin and D.E. Cummings, North American Association for the Study of Obesity annual meeting 2007.

In conclusion, studies over the past decade have demonstrated that food deprivation- or restriction-induced sensitization of brain reward circuitry and function is due in part to the contributions of insulin, leptin,

and ghrelin interactions with major dopaminergic and opioidergic networks in the CNS. These studies serve as a model for the testing of future candidate energy regulatory signals' (endocrine, or endogenous to the CNS) role in the modulation of food reward and palatability. There is also the possibility that dysregulation of those circuits may adversely affect body weight regulation. For example, studies in humans (Wang et al., 2001a,b) and animals (Bina and Cincotta, 2000) suggest that changes in central dopamine may contribute to the development of obesity. Further, some human studies report that obese individuals have a decreased propensity to engage in the use of recreational drugs and a decreased frequency of substance abuse disorders (Simon et al., 2006). One implication of these findings is that obesity is capable of altering processes within the endogenous reward system of the brain. As recent research into animal models of obesity (Davis et al., 2008; Fulton et al., 2006; Hommel et al., 2006) suggests downregulation of dopaminergic pathways, future studies will need to focus on other CNS pathways or networks that may subserve food reward, and may provide appropriate targets for obesity therapeutics.

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