Published in final edited form as:

Nutrition. 2008 September ; 24(9): 843–847. doi:10.1016/j.nut.2008.06.028.

Novel functions of orexigenic hypothalamic peptides: From genes to behavior

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Abstract

The regulation of energy balance depends on the precise coordination of multiple peripheral and central systems. Much recent research has highlighted the importance of behavioral mechanisms in this control and suggested that the regulation of body weight shares CNS pathways in common with other complex behaviors, including learning and drug addiction. Here, we present a brief review of some of this work and highlight the novel functions for central orexigenic neuropeptides. We review evidence that organisms engage in critical regulatory behaviors both before and after ingestion has occurred. Additional evidence supports the idea that appetitive mechanisms are engaged that are critical for the regulation of intake during the act of ingestion. Finally, we briefly discuss the recent work on the potential role for CNS reward centers and how those might be critically linked to the central regulation of food intake, as well as how they may be dysregulated by the abundance of highly palatable, energy-dense foods.

Introduction

Energy balance requires that an organism match caloric intake relatively precisely with caloric expenditure. Over the past 40 years, the average body weight of American adults has increased at a rate of one pound per year, but the steady increase has yielded an increase of an average of 3 BMI points, bringing the average adult from a healthy weight into the overweight category (1). This increase brings with it a significantly increased risk of a number of health problems, including type 2 diabetes, high blood pressure, and cardiovascular disease (2). Here, we will review both peripheral and central signaling mechanisms relating to the food intake side of the equation, with special attention to novel functions of CNS systems that control food intake.

Hypothalamic Regulation

The critical forebrain regulation of food intake behavior is thought to occur in the hypothalamus. Early evidence suggested that lesions in this area had profound effects on ingestive behavior. Lesions of the ventromedial hypothalamus (VMH) result in drastically increased food intake and obesity, while lateral hypothalamic area (LHA) lesions yield hypophagia and reduced growth. These findings led to the hypothesis that the hypothalamus controlled food intake by acting as the “satiety” and “feeding” centers, respectively, in the brain (3). In fact, the hypothalamus is still considered the key region for central control of energy homeostasis and much more is now known regarding the molecular mechanisms at work in this area that act to control energy intake. The arcuate nucleus contains two populations

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of neurons that seem to be the first-order relay neurons in responding to adiposity signals from the periphery. One population co-expresses the peptides NPY and the melanocortin receptor antagonist AgRP, while another contains POMC, the pre-cursor to the melanocortin receptor agonist α -MSH, and CART. Central infusion of NPY or AgRP potently stimulates food intake (4), while icv administration of α -MSH or CART inhibits food intake (5), suggesting that these two populations represent primary orexigenic and anorexigenic pathways, respectively. In further support of this function, food deprivation increases expression of AgRP and NPY mRNA, while decreasing POMC and CART gene expression (6). Overexpression of agouti or AgRP yields hyperphagia and obesity, as does disruption of the genes encoding the melanocortin-4 receptor, POMC or CART (e.g., 7). Finally, ablation of these neurons in adult mice yields dramatic reductions in food intake and bodyweight, while the reverse occurs with ablation of POMC neurons (8).

Importantly, receptors for the adipocyte hormone leptin as well as insulin are expressed on both of these types of neurons, suggesting that they are responsive to circulating levels of these hormonal signals, acting as effectors for altering food intake in response to alterations in energy balance as indicated by body adiposity. Leptin and insulin both cross the blood-brain barrier indicating that peripheral production of these hormones can have central action. Indeed, as central insulin and leptin increase hypothalamic POMC expression, leptin increases activity in POMC neurons and melanocortin antagonists can block leptin-induced anorexia (9). These neurons project to other areas of the hypothalamus, such as the paraventricular nucleus (PVN) and the LHA where several additional peptides that influence food intake and body weight are synthesized. A number of second-order neurons in the PVN synthesize and release anorexigenic compounds, such as corticotrophin-releasing hormone (CRH), while those in the LHA and adjacent perifornical area (PFA) are orexigenic, such as melanin-concentrating hormone (MCH) and orexin A and B (aka hypocretin 1 and 2) (e.g., 10). In addition to leptin and insulin, receptors for ghrelin are also located on arcuate AgRP/NPY neurons, which are activated by central ghrelin administration (e.g., 11). Furthermore, peripheral ghrelin administration activates neurons in the ARC and AgRP and NPY have been demonstrated to be requisite mediators of the hyperphagia induced by systemic ghrelin.

Behavioral mechanisms of molecular signals

Importantly, there are a number of ways by which these various peptides and neural systems may act to alter food intake. They may alter meal initiation (i.e., the likelihood of beginning an eating bout), which is generally observed as a change in meal frequency, or they may alter meal termination (i.e., how much is consumed prior to ending a meal), which is generally observed as a change in meal size. They may also affect the subjective feelings that an individual interprets as “hunger” or “fullness” and uses to determine when to begin or end a meal or the subjective palatability or reward value associated with eating particular foods.

When analyzing the component of food intake that is influenced by peptides found either peripherally or centrally, it is the size of a meal that is most often found to be affected, leading some to suggest that meal termination is more strongly controlled by biological processes, while there a vast number of environmental influences that are more likely to be involved in meal initiation (i.e., availability of food, time of day, cognitive factors, learned associations/signals) (12). The majority of the peripheral satiety hormones, including CCK, GLP-1 agonist exendin-4, amylin and leptin, appear to act by reducing meal size with little or no effect on meal frequency, while ghrelin plays a role in meal initiation (e.g., 13). Not surprisingly, as central leptin effectors, melanocortin agonists have been shown to reduce meal size, while MC antagonists have the opposite effect (e.g., 5).

In addition to influencing total energy intake via changes in these basic meal parameters when a constant test diet is used, some systems also differentially affect food selection or intake based on the macronutrient compositions of the diet. While NPY and AgRP both increase total caloric intake, NPY appears to induce greater appetitive and consummatory behaviors for foods high in carbohydrates, while the melanocortin system selectively affects fat intake and responding for fat-associated stimuli (e.g., 14,15). Not surprisingly, leptin, acting to inhibit both NPY and AgRP, reduces intake of carbohydrates and fats.

A number of these peripheral signals seem to be responsible for the subjective feelings of “hunger” or “satiety”. This was determined using an experimental design known as the “deprivation intensity discrimination paradigm”, in which rats are trained to discriminate between internal cues associated with 24 hours or 1 hour of food deprivation by receiving a reinforcer in a specific environment under only one of these conditions. The generalization between these deprivation states and those of a variety of potential hunger- or satiety-inducing peptides is tested by administering an exogenous dose of the peptide of interest and measuring the animal's behavior in the training environment. These types of experiments have demonstrated that ghrelin produces interoceptive cues similar to that of 24-hr food deprivation, while CCK and leptin produce cues similar to 1-hr food deprivation. Interestingly, other peptides that influence food intake, such as NPY, bombesin, and MC-R agonists and antagonists do not appear to produce cues that generalize to either deprivation state, suggesting that their mechanism of action is independent of inducing a subjective feeling of “hunger” or “satiety” (e.g., 16).

Pre-ingestive behaviors

Animals engage in several behaviors to find and acquire food. These appetitive acts are termed “pre-ingestive” since they occur prior to the animal engaging the food directly and consuming it. One hypothesis to be assessed is that hypothalamic peptides act on these appetitive approach and food-seeking behaviors, before the beginning of the more stereotyped consummatory behavior. The initiation of food seeking and ingestion is often considered to result from homeostatic signals that accumulate over the interval since food was last consumed. Specifically, food deprivation is thought to generate homeostatic signals related to the gradual depletion of energy stores and these signals may make animals more likely to engage in appetitive behaviors that ultimately lead to food consumption. The first requirement for changes in physiological systems related to food deprivation to be able to affect food-seeking and other pre-ingestive behaviors is that animals must be able to detect these changes. In fact, there is compelling evidence, using Pavlovian conditioning techniques, demonstrating that animals can learn to respond to the presence or absence of these internal signals.

Appetitive behaviors

Another well-validated procedure for evaluating the effects of pharmacological manipulations on appetitive behaviors is also based in Pavlovian conditioning. In this paradigm, animals are trained to associate a stimulus with food presentation. In one version of this procedure, rats are trained to expect a small drop of peanut oil following the presentation of one stimulus (e.g., a light) and to expect a small drop of a sucrose solution following the presentation of a different stimulus (e.g., a tone). The animals must then learn to approach a food cup to obtain the oil or the sucrose when the appropriate stimulus is presented (e.g., 17). After extensive training, the animals are treated with a pharmacological manipulation that is known to alter food intake, and they are given a test trial in which the “oil” and the “sucrose” stimuli are presented, but no oil or sucrose is delivered. Whether or not the animal approaches the food cup during each specific stimulus (oil or sucrose) is the dependent variable of interest. The extinction condition (i.e., no oil or sucrose available) during the test trial is an important feature of this paradigm. This allows us to conclude that any changes in responding are indicative of pre-ingestive

mechanisms affected by the peptide of interest and are not confounded by changes in palatability or energy intake.

In addition to influencing overall approach behavior toward food-paired stimuli, different peptides and hormones might be critically involved in the selection of specific macronutrients. The use of cue-nutrient learning has allowed our lab to use this paradigm to assess hypotheses about how specific hypothalamic neuropeptides alter responses to sucrose and oil-specific stimuli while simultaneously assessing the effect on general appetitive behaviors. Data from our lab using this paradigm indicate that NPY appears to have a general effect to increase appetitive behavior in response to the context in which both foods were provided, while appetitive behavior following administration of the AgRP is nutrient-specific, increasing in response to the oil-paired cue and decreasing in response to the sucrose-paired cue (18,15).

Motivational mechanisms

The paradigm described above is an ideal tool for assessing the effects of hypothalamic peptides involved in food intake when animals are faced with cues previously associated with the delivery of food. However, those studies do not address possible effects of these peptides on motivation to obtain food. A progressive ratio response schedule allows determination of the degree of effort a rat will expend to earn a reinforcer and, as commonly employed in the drug self-administration literature, is often considered a measure of the animal's "motivation" to obtain that particular reinforcer. In this procedure, the number of responses required to obtain each morsel of food is progressively increased during a meal. As expected, food deprivation increases the number of responses rats will make to obtain food whereas having just eaten decreases that number (19). This use of the paradigm has demonstrated that NPY is capable of producing increases in operant responding and that response levels are similar to 36-48 hr food deprivation. Orexin-A and peripheral insulin also increase progressive ratio breakpoints for sucrose, although not to the same extent as NPY or significant (i.e., 24-hr or greater) food deprivation (e.g., 20). On the other hand, central administration of insulin and leptin reduce operant responding for a sucrose reinforcer (21).

Consummatory mechanisms

Taste and palatability

Another significant division of the controls of food intake comes into play during the ingestive behavior itself. Palatability (defined here as an indication of food's hedonic value) influences both the amount and type of food that is ingested. 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. It is possible that one set of physiological signals might reduce ingestion by making foods less palatable. Consistent with this general idea, some hypothalamic peptides (e.g., melanocortins) project to CNS areas important for taste processing and food hedonics, including the ventral tegmental area, nucleus accumbens, and substantia nigra (e.g., 34). Further, leptin receptors are expressed in the VTA, leptin deficiency leads to mesolimbic dopamine dysfunction and leptin, like being sated, has been found to regulate the hedonic qualities of brain self-stimulation (e.g., 22). The implication is leptin or its effector neuropeptides modulate taste-related properties, or "palatability" during ingestion. In fact, a recent study found that centrally administered orexin-A, MCH and NPY increased intake of saccharin, while AgRP, ghrelin and dynorphin had no effect (23).

Since food must contact receptors on the tongue in order for palatability to be assessed, and this generally results in swallowing of the food, post-ingestive (i.e., gastrointestinal) factors can easily confound interpretations of experiments designed to determine the specific effects of palatability. A procedure known as "sham feeding" is one method that can be used to assess

the effects of taste and orosensory stimulation independent of post-ingestive effects. The sham feeding method involves surgical implantation of a fistula in the stomach, which can be opened during testing to allow food to drain out of the stomach, eliminating post-oral stimulation. Manipulations that alter intake during sham feeding, therefore, can be attributed to effects on the orosensory characteristics of the ingested solution. Central administration of NPY enhanced sham feeding of a sucrose solution to an even greater extent than real feeding, leading to the conclusion that the NPY acts primarily via consummatory mechanisms to increase food intake (24).

While eliminating confounding post-ingestive effects, the sham feeding technique still requires animals to locate and actively ingest the selected food. A clever technique to separate the consummatory from the pre-ingestive phase is the intraoral intake paradigm. This procedure allows for direct assessment of liquid (e.g., 10% sucrose) intake without the potential confounds of general activity or differences in appetitive approach behaviors. Briefly, rats receive an implanted intra-oral catheter through which small amounts of flavored solutions can be infused directly into the oral cavity. In this way, the experiment can circumvent the usual pre-ingestive behaviors such as the approach to a bottle, and instead directly assess the consummatory effects of a particular manipulation. Further, the technique can be used in combination with intake from a bottle on the homecage to dissociate the specific pre- and ingestive behavioral effects of a treatment. For example, treatments that increase bottle-intake but not intra-oral intake are thought to be involved in appetitive or pre-ingestive behaviors. In an experiment using the intraoral intake paradigm, Seeley and colleagues (25) demonstrated that centrally administered NPY increased rats' intake of sucrose from a bottle on the homecage, but had no effect on sucrose delivered directly into the oral cavity (intraoral administration). Based on these observations, Seeley et al. concluded that NPY exerted its orexigenic effects predominantly via appetitive or pre-ingestive, rather than consummatory, mechanisms.

Reward

Increasing evidence suggests that key hypothalamic peptide systems also play important roles in the processing of hedonics and reward, including the melanocortins and orexin-A. For example, melanocortin receptors (MC3R & MC4R) are also expressed in brain regions implicated in addictive behavior (e.g., 25,26) and pharmacological studies have outlined functional roles for these receptors in the modulation of drug taking behavior. Antagonism of these receptors in nucleus accumbens inhibits operant responding for cocaine, while central agonism of this system augments amphetamine related behaviors (e.g., 28).

While the field of reward neurobiology is largely focused on drugs abuse, there is now a growing understanding of the novel and shared neural mechanisms involved in natural reward processing like ingestive behaviors (e.g., 29). Recently, it was demonstrated that leptin acts on mesolimbic dopamine neurons in the VTA and regulates reward-related behaviors (e.g., 30). It is thought that the dysregulation of such mechanisms may be significant contributors to the obesity crisis (29,31). Importantly, the neural mechanisms of food reward are believed to be similar, if not identical, to drug rewards, namely the mesolimbic dopamine pathway (see 32 for review). High fat food is considered highly palatable and rewarding and in many cases, despite state of satiation, animals and humans will exhibit hyperphagia of a high fat food (see 31 for review).

As discussed, orexin neurons exhibit diverse projections in the CNS to sites including the VTA (e.g., 33). Importantly, VTA neuron populations express both orexin receptor subtypes (34) and orexinergic projections signal specifically on a majority of dopamine neurons to activate the mesolimbic pathway. In fact, exogenous orexin can increase VTA dopaminergic neuron firing rates. A specific role for orexin action in the VTA on reward-seeking behavior was first

implicated in studies demonstrating that an orexin antagonist could block the reinstatement of an extinguished place preference for morphine and that intra-VTA orexin-A itself was sufficient to reinstate an extinguished place preference for morphine in rats (35). Furthermore, LH orexin also appears necessary for the acquisition and expression of morphine-CPP (37). Evidence to support these findings came from genetic model studies showing the inability of orexin-deficient mice to form morphine-CPP's (38). Most recently, evidence indicates that intra-VTA administration of an orexin antagonist effectively prevents behavioral sensitization and neurophysiological changes that are typically caused by chronic cocaine use (39). Collectively, these studies are consistent with the idea that orexin signaling in the VTA could also promote intake of high fat food by reward –related mechanisms.

Conclusions

In summary, ample genetic, pharmacological and behavioral evidence implicates several key hypothalamic neuropeptides in the regulation of food intake. However, other previous data as well as a growing body of new research implicates these same systems and peptides in novel functions. Rather than simply increasing (or decreasing as the case may be) food intake, it is now apparent that hypothalamic peptides including NPY, AgRP and the melanocortins, orexin and MCH may play significant roles more complex behavioral and psychological processes. Specifically, these signals may play important roles in the generation of state cues (e.g., hunger), macro-nutrient selection, cognitive and learning processes, as well as reward and intake of drugs of abuse. We suggested that a more comprehensive and inclusive understanding of these ingestive processes may lead to novel therapeutic interventions for the treatment of obesity and potentially related behavioral disorders.

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