How Satiety Factors Reach CNS Appetite Centers

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Abstract: In this review we will consider how central structures involved in the regulation of energy balance gather information from the variety of different peripherally derived signaling molecules that we now believe provide an integrated perspective of energy status of the organism. The existence of the blood brain barrier means that the CNS is theoretically unable to directly monitor many of these circulating signals such as adiponectin, amylin, cholecystokinin (CCK), glucose, ghrelin, leptin, and peptide YY (PYY) which do not freely diffuse across this barrier. Studies have identified five primary mechanisms through which these circulating signals may transmit the afferent information they carry from the periphery to the CNS which we describe below. We will both discuss mechanisms and potential contributions of vagal afferent signaling, peptide transporters, vascular endothelial cell signaling, the arcuate nucleus of the hypothalamus, and brain structures which lack the blood brain barrier known as the sensory circumventricular organs (CVOs), in providing sensory information to the integrative centers of the hypothalamus and medulla which play such essential roles in the regulation of energy balance.

INTRODUCTION

Considerable work has been undertaken to elucidate the signals from the periphery that relay to the brain the start and end of meals, as well as states of hunger or satiety and overall energy balance [1-5]. These signals include peptides derived from the enteroendocrine system of the gut and pancreas, lipid mediators from the gut or adipose tissue, adipokines from adipose tissue and circulating nutrients. The complexity of peripheral signaling systems reflect the need to ensure that energy balance is tightly controlled and energy intake is maintained to meet the demands of the organism. The signaling systems provide an opportunity for the integration of digestive, endocrine, immune and metabolic functions to ensure optimal intake to support host-defense, reproductive, metabolic and other functions, effectively to establish what one might refer to as an integrated autonomic state.

Peptides play essential roles in the regulation of intake and satiety. To date we know that the only gut-derived peptide orexigen is ghrelin, which is synthesized and released from the stomach. There are numerous anorexigenic peptides from the gut that act in a paracrine manner on the vagus nerve which, as will be discussed later plays important roles in gut-brain communication, as well as in an endocrine manner within the central nervous system (CNS). Aside from these peptides, the lipid mediator anandamide which is an endogenous ligand for the cannabinoid CB_1 receptor is also increased during fasting and acts *via* the vagus to increase food intake [6]. Another lipid mediator, oleylethanolamide (OEA), is increased in the small intestine after feeding and induces satiety [7-9]. Circulating factors from adipose tissue that increase food intake are not known, but the receptors for anorexigenic factors are now being found to be widely distributed on the vagus nerve and, as we have shown, on the sensory structures of the brain that respond to circulating peptide hormones and other factors [10].

Many excellent reviews have focused on the CNS structures, pathways, and hierarchical organisation through which the brain controls energy balance [2,3,11-14]. However, the purpose of this review is to consider how these central structures gather information from the variety of different signaling molecules that we now believe provide an integrated perspective of energy status of the organism. Were it not for the existence of the blood brain barrier (for reviews on anatomical basis see [14]), this might not be considered a difficult problem as all circulating energy balance signals would be equally distributed in both the periphery and the CNS, such that the only requirement for an ability to sense any of these signals would be the presence of appropriate receptors on neurons in the relevant CNS control centres. However, the blood brain barrier restricts such free exchange of the majority of these signals as they are carried by lipophobic molecules which cannot readily diffuse across this barrier [15]. Thus, the CNS is theoretically unable to directly monitor circulating peptides such as adiponectin, amylin, cholecystokinin (CCK), ghrelin, leptin, and peptide YY (PYY) which do not freely diffuse across this barrier [16]. Studies have identified a number of potential mechanisms through which these circulating signals may transmit the afferent information they carry from the periphery to the CNS centres, where such information is gathered and integrated, thus allowing adjustments to the output systems of the autonomic and endocrine systems which under normal circumstances lead to appropriately regulated energy homeostasis. The five primary mechanisms through which such communication has been suggested to occur are: 1) vagal signaling; 2) specific transporters; 3) vascular endothelial cell signaling; 4) direct access to the arcuate nucleus of the hypothalamus; and 5) actions at brain structures which lack the blood brain barrier known as the sensory circumventricular organs (CVOs). We

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will consider each of these possibilities separately in the following sections of this review.

VAGAL AFFERENT SIGNALING

The vagus nerve innervates the entire proximal gastrointestinal tract as far as the proximal colon and is composed of afferent (about 75%) and efferent fibers (about 25%) [17]. Vagal afferent fibers innervate the lamina propria of the intestinal villi, as well as the crypt region of the mucosa, but they do not penetrate the basal lamina of the epithelium [18]. Therefore, vagal afferents do not directly sense the luminal content of the gut, but respond indirectly to the presence of food or nutrients. The sensors that detect luminal contents of the gut appear to be enteroendocrine cells that are extensively found throughout the gut wall and possibly brush cells that have a more limited distribution [19, 20]. These specialised enterocytes contain a variety of peptides and amines that are released basolaterally in close proximity to the vagal nerve endings that they activate in a paracrine manner. Recently, it has been shown that these cells express the molecular components of taste receptors found in the tongue and elsewhere [19]. Activation of these receptors leads to the release of signalling molecules that activate vagal afferent terminals in the mucosa.

The peptides CCK, glucagon-like peptide-1 (GLP-1), PYY (3-36), ghrelin and others, as well as the amine serotonin (5-HT) signal to the brain by modulating vagal afferent activity. Vagal afferent neurons of the nodose ganglion express the cognate receptors for these signaling molecules including the CCK_1, GLP-1 receptor, Y_2 receptors for PYY (3-36), ghrelin receptor GHS-1 and 5-HT_3 receptors [21, 25]. The nodose ganglion also expresses the receptors for the lipid signaling molecules that are made on demand in the wall of the gut. These include the CB_1 receptors for endocannabinoids and the peroxisome proliferator activated receptor (PPAR) α receptor for OEA [8].

Burdyga *et al.* showed that the levels of expression and distribution of the CB_1 receptor are regulated by the state of satiety [21]. In fed rats, low levels of CB_1 expression are observed in the nodose ganglion. In fasted rats, message expression is increased substantially and is found in neurons that also express CCK_1 receptors as well as receptors for the peptide orexin. The expression of the CCK_1 and orexin receptors is not altered by fasting. Feeding downregulates CB_1 receptor expression, an effect that is mediated by CCK_1 receptors, since it is abolished by a CCK_1 receptor antagonist [21]. These results illustrate an interesting reciprocal action where inhibition of food intake by CCK occurs by blocking the orexigenic actions of endocannabinoids.

As noted above, vagal afferent neurons also express receptors for ghrelin [21], and these receptors are present on the same population of vagal afferents that express CB_1 and CCK_1 receptors and, as well, melanin-concentrating hormone [MCH]-1 and leptin receptors [26]. Activation of the ghrelin receptor prevents the down regulation of the CB_1 (and MCH-1) receptors by CCK, thereby limiting the extent of its action [21]. Not only are receptors for anorexigens and orexigens regulated, but the vagal afferent neurons contain anorexigenic and orexigenic peptides that are also regulated by gut signals. Vagal afferent neurons express the orexigenic peptide MCH [27] and the anorexigenic peptide cocaine- and amphetamine-regulated transcript (CART) [26, 28]. In fasted rats, MCH is expressed in vagal afferent neurons whereas CART is virtually undetectable. After feeding or the administration of CCK, CART expression is increased and this effect is blocked by a CCK_1 receptor antagonist. On the other hand, MCH levels fall after feeding and this effect is also regulated by CCK through CCK_1 receptors. Thus CCK reciprocally regulates CART and MCH in the same population of vagal afferent neurons [28]. Ghrelin inhibits the action of CCK of CART expression through a novel mechanism that involves the exclusion of phosphorylated CREB from the nucleus [28]. These findings illustrate a new role for ghrelin in modulating the expression of both receptors for other orexigens, and also anorexigenic and orexigenic peptide expression in vagal afferents. Thus the functional phenotype of vagal afferent neurons is governed by feeding and fasting, as well as endocrine and paracrine substances produced in the gut itself. This places the vagal afferent system in a pivotal position to filter and regulate how satiety signals reach the caudal brainstem.

TRANSPORTERS

There is a clear recognition that some small lipophilic signaling molecules (including some peptides) may have physical properties that do in fact allow them to diffuse across the BBB by simple diffusion down a concentration gradient [16]. However, the majority of energy balance signals described to date do not have such properties, and therefore cannot simply diffuse from blood to brain, or for that matter in the opposite direction from CNS to blood. Despite these physical considerations it is well established that changes in the circulating concentrations of many of the theoretically non-diffusible signals influences both behaviours associated with energy metabolism, and the activity of neurons in regulatory centers of the hypothalamus and the medulla [16, 29]. One explanation for these observations is that there exist in the cerebral vasculature peptide-specific transporters which are able to move these substances across the blood brain barrier in a similar manner to the well recognised glucose transporters essential to the movement of this essential nutrient form blood to the neuropil of the CNS [30, 31]. Banks and Kastin were the first to provide empirical evidence in support of the existence of such transporters in their studies demonstrating the existence of a saturable blood to brain transport system for leptin [32]. In these studies, they were able to demonstrate the movement of radioiodinated leptin from blood to brain, and importantly were also able to show that this transport system was saturable, by showing that excess administration of unlabelled leptin inhibited the influx of iodinated leptin. Since the initial description of such transporters they have been shown to be differentially distributed in the CNS, and also been suggested to be regulated by other signals, observations which both argue in favour of functional roles for these transporters [33]. Transporters for a number of energy balance signals (glucose, ghrelin, leptin, PYY, orexin) have also been identified using these approaches, while other signals (adiponectin, nesfatin) have been shown not to be transported [34-37]. Despite the extensive literature describing these peptide-specific saturable transport systems, to date, none of

these have been molecularly characterized or cloned. Finally, the true physiological roles of these systems remains hard to define in view of our lack of a clear understanding of the concentrations of transported substances that are actually delivered to their site of action (the receptor) at the locus of their actions at CNS energy balance integrative centres.

VASCULAR ENDOTHELIAL CELL SIGNALING

While the transporters discussed above are believed to move signaling molecules from one side of the BBB to the other, a second mechanism for transduction of messages from such lipophobic substances has been described in central cardiovascular regulation. In this case it has been shown that the luminal surface of cerebral vascular endothelial cells expresses AT_1 receptors to which circulating angiotensin II binds and then stimulates the production of nitric oxide on the CNS side of the endothelial cell barrier. Such a system transmits peptidergic information from the circulation to the CNS through the production of substances such as nitric oxide which readily diffuse into the surrounding microenvironment [38,39]. It will be of interest to see if similar signaling mechanism may ultimately explain access of certain satiety signals to the CNS.

THE ARCUATE NUCLEUS OF THE HYPOTHALAMUS

Direct access of circulating signals to the neuropeptide Y (NPY) and proopiomelanocortin (POMC) neurons of the arcuate nucleus has been extensively described in the literature as a mechanism through which certain molecules influence the activity of neurons in these centers. This suggestion is based on the assertion that vasculature of the arcuate nucleus consists of a "leaky" blood brain barrier, an important suggestion the origins of which we will consider in more detail. The ARC has in a number of cases been referred to and adopted as an additional CVO, despite the fact that there is no anatomical evidence to date which suggests that the ARC lacks the normal BBB [40-42]. The idea really originates from studies carried out to assess blood brain barrier integrity by following the diffusion of systemically administered horseradish peroxidase (HRP) into the brain [43], with the conclusion being that HRP labelled regions lack a complete BBB. These studies demonstrated that, following systemic HRP, the primary areas labelled were the traditional CVOs [43], but also showed that 8 hours after infusion of HRP, other regions of the brain demonstrated HRP labelling including the arcuate nucleus. The authors of these studies concluded that this labelling of HRP in the ARC occurred as a consequence of retrograde axonal transport to arcuate neurons that project to the median eminence [43]. Detailed structural analysis of median eminence and arcuate nucleus capillaries has also confirmed that Type III capillaries with true fenestrations are located only in the median eminence and not in the arcuate nucleus [44]. Recent studies using more sensitive fluorescence tracers confirm this conclusion by showing that systemic administration of hydroxystilbamidine (fluorogold equivalent) results in labelling of arcuate astrocytes only (no neurons)[45, 46] an observation which suggests that these while these glial cells send processes to vascular endothelial cells, there is no leakage of this marker into the neuropil of the arcuate nucleus. Collectively, these observations do not provide any definitive evidence at this time that arcuate neurons are in a preferential position to directly access circulating substances. Despite these anatomical data it is clear that changes in concentrations of circulating signals such as leptin and ghrelin do influence the activity (assessed by c-Fos activation) of arcuate neurons [47-49]. In addition, both of these peptides have been shown to have direct effects on the activity of arcuate neurons assessed using *in vitro* electrophysiological techniques [50, 51], with the explanations for such actions being that leptin results from transporter mediated (see transporter section) delivery, while ghrelin actions could be similarly mediated by the ghrelin transporter, or alternatively by synaptic release from ghrelin-ergic neurons within the hypothalamus [50]. Thus, while it is clear that the arcuate nucleus does play important roles in integrating systemic appetite signals, the present anatomical evidence is similarly clear that this CNS region is protected from the systemic circulation by non-fenestrated capillaries in the same manner as the remainder of the CNS with the exclusion of the true circumventricular organs which will be considered in detail in the following section.

SENSORY CIRCUMVENTRICULAR ORGANS

The circumventricular organs of the brain are a group of specialised structurally unique CNS structures in that they all lack the normal blood brain barrier. The subfornical organ (SFO), area postrema (AP), and the organum vasculosum of the lamina terminalis (OVLT), are further specialised as containing neuronal cell bodies (as opposed to nerve terminals), and classified as the "sensory CVOs" primarily as a consequence of their roles in the sensation of circulating peptides which elicit central actions in the regulation of cardiovascular and neuroendocrine function [10, 52, 55] A growing body of evidence now suggests that these sensory CVOs, the SFO and AP in particular may play similar roles in sensing a variety of circulating energy balance signals and, through efferent projections to the medullary (AP), and hypothalamic (SFO) centres, transmit this information to critical nuclei involved in the regulation of energy balance.

Studies showing that glucosemimetic antimetabolites had their largest orexigenic effects following injection into the fourth ventricle of the brain [56], were perhaps the first to focus attention on the brainstem and ultimately the AP as an appetite control center. Numerous lesion studies since this time have demonstrated that AP ablation results in hypophagia and reductions in body weight [57,58], although it is not clear whether these effects are a result of a loss of recognition of hunger, a false feeling of satiety, or an aversion to food. The AP sends efferents to and receives afferents from a variety of autonomic centers in the medulla, pons and forebrain [59,60]. The main efferent projections from the AP are directed to the nucleus of the solitary tract (NTS) and parabrachial nucleus [59,60], with minor projections going to the A1 region of the nucleus ambiguus, the dorsal motor nucleus of the vagus, and dorsal regions of the tegmental nuclei [59,60]. Similar to the other sensory CVOs, inputs to the AP originate in many of the same regions to which area postrema neurons project. The NTS, paraventricular nucleus of the hypothalamus (PVN), vagus nerve and parabrachial nucleus all send afferents to AP [59-61].

The AP and NTS are major sites of abdominal sensory input to the CNS [58]. The AP is capable of sensing toxins in the blood stream and triggering nausea and emesis [64], while inhibition of AP neurons can induced by anti-emetic agents such as propofol, maybe as a result of the inhibition of GABA currents [65]. The AP was therefore established as a CNS structure that could sense a toxic insult *via* cytokines, pathogens and vagal inputs, trigger vomiting to remove the insult, and teach future avoidance of the toxic substance.

More recent work has focused on the potential sensory roles of AP neurons in the detection of physiologically relevant blood-borne satiety signals. Amylin is secreted with insulin in response to food intake, and is structurally and functionally similar to calcitonin and calcitonin gene-related peptide. Both peripheral and central amylin causes hypophagia [66] effects which are independent of vagal afferents [67]. The high density of amylin receptors in AP [68] when combined with observations that AP lesions reduce this effect [69,70] identify the AP as one important site for the actions of amylin, a suggestion which gains support from single cell work showing that amylin increases the excitability of AP neurons through cGMP mediated mechanisms [71]. Interestingly, the majority of cells excited by amylin are also glucose sensitive, implying an integrative role for these cells in transducing nutritional signals to feeding centers of the brain [69]. The AP is also under the influence of other circulating hormones believed to play important roles as signals of metabolic status including GLP-1 [72], CCK [73,74], adrenomedullin [75,76] adiponectin [77], and ghrelin [10,78]. The orexins/hypocretins also influence the excitability of AP neurons through activation of a non-selective cationic conductance [79]. In combination, these data support a complex and integrative role for AP in the control of feeding.

Until recently the AP was thought to be the predominant CVO involved in the regulation of food intake despite the literature showing that OVLT or SFO lesions result in anorexia and emaciation. The SFO sends both monosynaptic, and polysynaptic projections to the PVN and supraoptic nucleus (SON) of the hypothalamus [80, 81]. Specific excitatory projections have been found to vasopressin and oxytocin neurons in the SON and PVN, as well as to parvocellular areas of the PVN that in turn project either to the median eminence, the medulla, or the spinal cord (for review see [54]). The SFO also sends efferent projections the anteroventral third ventricle region, specifically to the median preoptic nucleus and the OVLT [81,82] both of which in turn send additional axonal projections to hypothalamic autonomic centers including SON and PVN (hence polysynaptic connections from SFO) [82]. There are less dense efferents from the SFO to the zona incerta, raphé nuclei, infralimbic cortex, rostral and ventral portions of the bed nucleus of the stria terminalis, lateral preoptic area, lateral hypothalamus/dorsal perifornical region, and the arcuate nucleus[80,83,84].

Anatomical data suggests that SFO neurons have compact dendritic trees and do not receive extensive neural inputs [85], as might be predicted in view of the suggested primary role for this region in receiving afferent information from the systemic circulation. In most cases, the limited afferents to the SFO appear to originate from the same areas that receive SFO efferents. The SFO receives inputs from the lateral hypothalamus [86], and the median preoptic nucleus [86], as well as the lateral division of the parabrachial nucleus, NTS, midbrain raphé, and nucleus reunions of the thalamus (for review see [53]). Single cell recordings have shown effects of calcitonin [87] and amylin [71,88] on SFO neurons, while our own recent work showing that separate subpopulations of SFO neurons are responsive to amylin and ghrelin [89], does suggest potentially important roles for SFO neurons in monitoring an increasing number of satiety signals. Our own recent genomic and electrophysiological analysis of SFO has suggested potential roles for neurons in this CVO in sensing adiponectin, endocannabinoids, leptin, orexins and PYY, and future work will be necessary to identify the real physiological relevance of these actions on SFO neurons in the physiological regulation of energy balance [90].

SUMMARY AND CONCLUSIONS

We have outlined five mechanisms that explain how peripherally produced satiety factors or molecules involved in energy balance signal to the CNS. Currently, we recognize the importance of the vagal afferent system, transporters and the arcuate nucleus of the hypothalamus. The role of the sensory CVOs and vascular endothelial signalling in promoting satiety and regulating energy balance remains to be more fully explored.

The nature of the various signals produced in the periphery is incompletely understood. Whilst we have recognized many of the signals as satiety factors, relatively few studies have explored other central regulatory roles for these agents. Such studies are potentially very important in understanding integrated homeostatic control, and may ultimately help explain why there are so many signalling molecules and why they act at many sites in the CNS. Using the orexigenic peptide ghrelin as an example, it was shown that peripheral administration failed to increase food intake but not growth hormone secretion if animals were treated neonatally with monosodium glutamate [91]. Neonatal monosodium glutamate treatment extensively damages the arcuate nucleus and to some extent the sensory CVOs, suggesting that another signalling pathway was being utilized by this single molecule and that the functional consequences of this activation lead to an integrated physiological response, in this case, matching up of growth with the energy demands that are required to support it. Ghrelin also acts at the level of the vagus (as noted above) and not only is it an orexigen, but has recently been shown to reduce intestinal injury and inhibit inflammation [92]. This illustrates the diversity of actions on one peripheral signalling molecule and illustrates the vast potential that they have for regulatory control beyond that of satiety.

An obvious question is, why are there so many systems for signalling to the brain? Whilst we cannot answer this with any degree of certainty, we consider the answer also likely to be physiological integration. Endocrine and paracrine signaling molecules not only act on homeostatic centers in the brain to promote satiety, but also centers that integrate metabolic, cardiovascular, reproductive and immune functions. We know relatively little about how integra-

tion occurs, but we speculate the CNS structures such as the sensory CVOs are critically involved in this process because of their positions in the brain and their widespread anatomical projections. Importantly, we also recognize that cognitive, reward and executive brain functions, may be modulated by peripheral signals [18]. A good example of this is the neuronal activity stimulated in the orbitofrontal cortex by peripheral administration of PYY (3-36) that accurately predicts subsequent feeding behaviour in test subjects [93]. These higher brain functions are ultimately in control of appetite, which, as we all have experienced, can override satiety. We do not yet know the relative contributions of the peripheral signalling mechanisms to homeostatic and non-homeostatic brain functions, but given the complexity of these actions it is hardly surprising that there are many and varied routes for the brain to "listen" to what the body is telling it. A more thorough understanding of the mechanisms of appetite control will include a detailed knowledge of how the peripheral signalling mechanisms we have outlined are integrated within the brain to provide a comprehensive view of the short and long term energy status of the body. In addition we will also ultimately need to understand how these "energy balance" centers integrate such information with neural systems regulating cardiovascular, immune, reproductive and other autonomic functions which together are essential to the survival of the organism.

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