MINIREVIEW

Making Sense of It: Roles of the Sensory Circumventricular Organs in Feeding and Regulation of Energy Homeostasis

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Obesity is associated with significant health risks including stroke and heart disease. The prevalence of obesity has dramatically increased over the past 20 years. Although the development of obesity is clearly related to changing lifestyles, the central nervous system plays a key role in regulation of energy balance. To develop effective strategies for treating obesity, we must gain a clearer understanding of the neurocircuitry and signaling mechanisms involved. Toward this end, recent progress has been made in the understanding of the roles played by the sensory circumventricular organs (CVOs) of the brain. These areas lack the normal blood-brain barrier and thus act as transducers of signals between the blood, other centers in the brain, and the cerebrospinal fluid. This review focuses on the roles played by the sensory CVOs in detecting and responding to a number of signals that carry information regarding nutritional status, including cholecystokinin, amylin, ghrelin, peptide YY, pancreatic polypeptide, leptin, adiponectin, and glucose. Exp Biol Med 232:14–26, 2007

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Introduction

Obesity has become a problem of epidemic proportions within the past 20 years. The associated health risks include type 2 diabetes, hypertension, stroke, atherosclerosis, and cardiovascular disease. Hundreds of thousands of deaths each year are due to obesity-related complications, and it has been estimated that billions of dollars are spent annually in direct and indirect costs (1). It is well accepted that this increase in the prevalence of obesity in industrialized nations is in part related to an environment with increased visibility and availability of convenient, inexpensive, palatable, and calorie-dense foods as well as a lack of physical activity (2). However, to understand the development of obesity and to design effective therapies to treat the disease, it is essential to understand how the central nervous system (CNS) regulates energy homeostasis.

The neural systems regulating food intake and energy homeostasis are complex and are organized hierarchically (see Ref. 3 and references therein). Corticolimbic structures, including sensory cortex, orbitofrontal cortex, hippocampus, amygdala, and the nucleus accumbens, are thought to mediate aspects of energy homeostasis, such as procurement of food, sensory evaluation, and social and hedonistic aspects of feeding. Hypothalamic and brainstem structures, such as paraventricular nucleus, arcuate nucleus, nucleus of solitary tract, dorsal motor nucleus of the vagus, and others, are thought to be involved in the detection of satiety signals (metabolites, hormones, adipokines, and neuropeptides) and the translation of information provided by these signals to control autonomic and neuroendocrine outputs and, of course, ultimately, behavior.

Clearly, the regulation of energy homeostasis by the CNS involves detection of feedback signals, integration of these signals, and generation of appropriate outputs to effectors. Although numerous excellent reviews have focused on the sensing of signals in the hypothalamus and the modulation of hypothalamic circuits involved in regulation of energy homeostasis (4–10), the purpose of

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this review is to highlight the roles of the often-overlooked, specialized sensory areas of the CNS, the circumventricular organs (CVOs).

Blood-Brain Barrier and the CVOs

The vasculature of the CNS differs from that found in other tissues. The walls of the capillaries found in many tissues are "leaky": specifically, these blood vessels have highly fenestrated endothelial cells and numerous plasmalemmal vesicles, allowing rapid mass transport of solute from inside the capillary into the interstitium of surrounding tissues. In contrast, the capillaries of most areas of the CNS contain no fenestrations, numerous tight junctions between endothelia, and very few plasmalemmal vesicles, and the vessels are surrounded by a continuum of astrocyte end feet, resulting in a highly restricted permeability of solute molecules into the CNS. This restricted transcapillary movement of blood-borne molecules gave rise to the idea of the blood-brain permeability barrier (BBB). Molecules that cross the BBB under normal conditions are thought to do so by one of two mechanisms, diffusion or transport. Lipid-soluble molecules (for example, phenobarbital and ethanol) are able to diffuse across the cell membranes of cerebral capillaries and into the CNS with relative ease. The permeability of these lipophilic molecules is related to molecular size and partition coefficient. Alternatively, glucose, amino acids, and some other biological molecules cross the BBB by means of protein transporters. A few selected peptide hormones have also been demonstrated to be transported across the BBB *via* saturable (for example, insulin; Refs. 11, 12; and leptin; Ref. 13) and nonsaturable transport systems (for example, growth hormone; Ref. 14). However, for the most part, the BBB acts to isolate the CNS from circulating factors, such as hormones and cytokines, while at the same time, ensuring that many neuropeptides, transmitters, and growth factors that are produced in, and act in, the brain do not diffuse out into the circulation.

Circumventricular organs, logically named because of their proximity to the ventricular system of the CNS, are specialized midline structures found in the brains of all vertebrates. They are unique in that they are extensively vascularized and they possess highly fenestrated capillaries. The CVOs are thus not "isolated" by the BBB but are uniquely situated to act as an interface between the brain and the periphery. Some of the initial observations that the CVOs were outside the BBB were made by Wislocki and colleagues when it was observed that intravitreally administered dyes stained specific circumventricular regions of the brain (15–18). In mammals, eight CVOs have been described: three sensory, four secretory, and one poorly defined. The sensory CVOs (Fig. 1) include the subfornical organ (SFO), the organum vasculosum of the lamina terminalis (OVLT), and the area postrema (AP). The term "sensory CVO" was coined in 1993 by Johnson and Gross (19) to highlight that these are the only CVOs containing

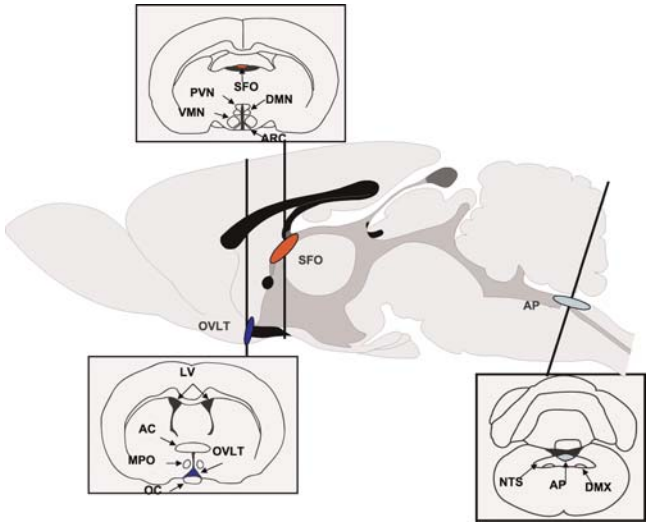

Figure 1. Sensory circumventricular organs and their anatomical relation to areas implicated in feeding and energy homeostasis. The center of this diagram shows a schematic representation of a midsagittal cut through a rat brain illustrating the anatomical locations of all three sensory circumventricular organs. The three insets show coronal sections highlighting the positioning of the SFO and OVLT within the hypothalamus, the AP in the medulla, and the relationship of these areas with other regions implicated in regulating energy homeostasis and feeding. Abbreviations: OVLT, organum vasculosum of the lamina terminalis; SFO, subfornical organ; AP, area postrema; PVN, paraventricular nucleus of the hypothalamus; VMN, ventromedial nucleus; DMN, dorsomedial nucleus; ARC, arcuate nucleus of the hypothalamus; NTS, nucleus of the solitary tract; DMX: dorsal motor nucleus of the vagus. Color figure is available in the on-line version.

neuronal cell bodies, that these neurons are exposed to factors circulating in the bloodstream, and that they respond to these factors and project axons to other nuclei transmitting this information. The secretory CVOs comprise terminals, axons, and glia and epithelial cells, and they include the median eminence, the neurohypophysis, the intermediate lobe of the pituitary gland, and the pineal gland. The subcommissural organ is poorly understood: it appears to lack the fenestrated capillaries of the other CVOs. However, it is thought to play both secretory and sensory roles (20). Some people also consider the choroid plexuses to be circumventricular organs. The reader should be aware that although numerous claims have recently been made as to the quality of the BBB at the arcuate nucleus (ARC) of the hypothalamus, all of the available anatomical data report tight junctions between endothelial cells characteristic of a normal BBB.

Only a few regulatory peptide signals controlling energy homeostasis have been demonstrated to cross the BBB, including leptin (13), insulin (11, 21), and amylin (22). Therefore, although many of these signals clearly affect electrophysiological properties of neurons in key homeostatic regulatory areas of the brain, such as the paraventricular nucleus (PVN) and the ARC of the hypothalamus (10), it remains difficult to understand how many of these peptides reach their targets. For example,

original experiments examining the mechanisms by which leptin enters the brain demonstrated movement of leptin into the ARC (13). It was unclear, however, how leptin actually got to the ARC: did it diffuse into the median eminence (a CVO adjacent to the ARC), and then through the extracellular space into the ARC; was leptin transported from the median eminence (or choroid plexus) into the cerebral spinal fluid, then into the ARC; or was leptin transported directly into the ARC from the vasculature. The first possibility, although attractive, was unlikely because the layer of tanycytes surrounding the median eminence acts as a barrier to the diffusion of molecules into the surrounding parenchyma (23), and the pattern of radiolabeled leptin observed deep in the ARC was inconsistent with a diffusion gradient from the median eminence. To our knowledge, there is no direct evidence that physiologically relevant levels of leptin can diffuse from the median eminence into the ARC. The second possibility was unlikely as well because leptin in the cerebral spinal fluid does not appear to effectively enter the ARC (24). The most likely possibility was that leptin was *transported across* the BBB into the ARC. Further controversy arises because, based on the rate of transport (across the BBB) of leptin into the ARC (13) and its reduced half-life in brain (13), one would expect the effective concentration of leptin in the ARC to be well below the EC_{50} observed in patch-clamp recordings from arcuate neurons in brain slices (25). Similarly, circulating ghrelin is thought to stimulate food intake as a consequence of actions in the ARC (26); however, transport of ghrelin across the BBB is not consistent across species (27). These discrepancies suggest two things: that more work is required to fully understand the transport of peptides across the BBB, and that alternative sites of action for peptide hormones, such as leptin and ghrelin, may exist.

In contrast to neurons of the PVN and ARC, the neurons of the sensory CVOs are outside the BBB and are in direct contact with the blood. There are no issues with regard to transport across the BBB, and therefore, the sensory CVOs are in a unique position to detect changes in circulating signals, integrate that with other data regarding the interior milieu (blood osmolarity, for example), and transmit that information to other brain centers. In fact, the sensory CVOs are known to be particularly well-endowed with a variety of receptors for circulating signaling molecules (28). The anatomy and connectivity of the SFO, OVLT, and AP are briefly reviewed below.

Anatomical Connections of the CVOs

Subfornical Organ. The SFO is located dorsal to the anterior commissure, at the dorsal area of the lamina terminalis, and it projects into the third ventricle from the rostral wall (Fig. 1). Three morphological areas of the SFO have been identified comprising a central core, containing compact neuronal cell bodies and glia, and rostral and caudal regions, both of which contain mostly axonal fibers (29). The microcirculation within the SFO is extraordinarily complex, with at least three subtypes of capillaries and numerous pools of interstitial fluid surrounding capillaries (Virchow-Robin spaces). This anatomical relationship may serve to facilitate the sensory function of the organ (30). The SFO sends direct and indirect projections to vasopressin- and oxytocin-secreting neurons of the PVN and the supraoptic nucleus of the hypothalamus (31), along with projections to the parvocellular neurons of the PVN (32). The SFO also projects to the median preoptic nucleus of the hypothalamus, OVLT, zona incerta, raphe nuclei, infralimbic cortex, rostral and ventral portions of the bed nucleus of the stria terminalis, lateral preoptic area, lateral hypothalamus, and the arcuate and the dorsal perifornical region (32–39). Afferent projections to the SFO include the median preoptic nucleus of the hypothalamus, the nucleus of the solitary tract (NTS), the lateral hypothalamus, the midbrain raphe, and the nucleus reunions of the thalamus (35, 37, 40). Thus, the SFO is in direct contact with the systemic circulation, sends extensive efferent projections to important hypothalamic autonomic control centers, and shows the highest density of a large number of peptide receptors within the CNS (28, 41, 42). Together, these observations suggest essential roles for the SFO in sensing circulating signals and integrating information derived from them.

Organum Vasculosum of the Lamina Terminalis. The OVLT is an anteroventral CVO that sits ventral to the median preoptic nucleus and dorsal to the optic chiasm within the third ventricle (Fig. 1). The OVLT can be divided into a rostromedial vascular region, dorsal cap, and a lateral/posterior region based on projections to other nuclei (42, 43). Major projections from OVLT include direct and indirect efferents (*via* the median preoptic nucleus) to magnocellular neurons of the PVN and supraoptic nucleus (SON; Refs. 35, 44–46). Additionally, projections descend to corticotropin-releasing hormone (CRH) neurons of the PVN (46), the stria medullaris, and basal ganglia (47). Major inputs to the OVLT include those originating in the SFO, NTS, and median preoptic nucleus (48), whereas inputs derived from the ventromedial nucleus, ARC, and anterior, posterior, and dorsal hypothalamus compose a group of nuclei contributing minor inputs to the OVLT (45, 47, 49).

Area Postrema. The AP is located in the fourth ventricle, situated on the dorsal surface of the medulla immediately adjacent to the NTS (Fig. 1). The AP is divided into three or four regions (depending on species and investigator), based on the morphology of the neurons within and their projections. These regions include the mantle zone, the central zone, and the ventral zone (which has been subdivided into the ventral-junctional zone and the lateral zone; (30, 50). Virchow-Robin spaces are also evident in the AP (30). The AP, together with the NTS and the dorsal motor nucleus of the vagus, make up the dorsal vagal complex, a major site for integration of afferent information (predominantly from the gut and viscera). Thus,

the AP is in a position to interact with both the circulation and sensory information from the periphery. Electrophysiological and tracer studies show that the AP sends projections to a variety of targets, most notably, the NTS, the parabrachial nucleus, the nucleus ambiguus, and the dorsal regions of the tegmental nuclei (51–53). Reciprocal innervation exists with these structures, but the AP also receives projections from the PVN (53), glossopharyngeal (54), carotid sinus (55), and aortic depressor nerves (56), underscoring the importance of AP in regulation of autonomic function.

CVOs as Detectors of Signals Regulating Energy Homeostasis

Although the AP is a well-known site of action for circulating satiety signals, the SFO and OVLT are, to date, better known for their involvement in salt appetite and fluid regulation. Recently, however, a number of energy homeostasis–related peptide hormones have been shown to affect activity and electrical properties of OVLT and SFO neurons. Receptors for a number of energy homeostasis–related peptide hormones have been localized to the sensory CVOs, strongly implicating these areas in the regulation of energy homeostasis. Below, we review evidence that the sensory CVOs detect selected circulating energy homeostasis–related signals (including satiety signals, adiposity signals, and metabolites), and thus, play a crucial role in the regulation of energy homeostasis.

Gut Signals. *Cholecystokinin (CCK).* CCK is perhaps the most thoroughly studied peptide satiety signal. It is rapidly secreted by the duodenum and jejunum (57) in response to the presence of fat in the gut (58). Administration of CCK rapidly decreases meal size and duration in rodents and humans (59, 60).

The neuronal circuitry through which CCK exerts such effects include activation of vagal afferents and direct activation of AP neurons. Administration of CCK to rodents causes AP neuron activation (as determined by c-fos staining), with the level of activation being significantly reduced, but not abolished, by bilateral vagotomy (61–63). A well-recognized caveat of using c-fos staining is that only neurons that undergo a significant increase in action-potential frequency are labeled, and alterations in firing patterns and inhibition of neurons are likely to be missed using this technique (10, 64). More direct evidence that AP neurons are sensitive to CCK comes from electrophysiological studies demonstrating that application of CCK causes an increase in action-potential firing rate in a subpopulation of AP neurons (65, 66).

Amylin. Amylin is a 37 amino acid peptide hormone co-secreted with insulin from pancreatic beta cells (67, 68). Like insulin, it is secreted into circulation upon stimulation by food intake and acts as a satiety signal. Amylin has been shown to have profound effects on several aspects of glucose metabolism, including inhibition of glucagon secretion (69), inhibition of insulin secretion (Refs. 70–72, but see also Refs. 73, 74), inhibition of glucose uptake, and stimulation of glycolysis in skeletal muscle (75).

The effects of amylin are similar to those elicited by CCK, in that amylin also potently inhibits food intake by acting at the AP. However, in contrast to CCK, amylin does not appear to act *via* vagal inputs (76–78). SFO, OVLT, and AP all express high densities of amylin-binding sites as determined by autoradiography (79–81). Furthermore, Barth *et al*. (82) demonstrated that administration of amylin results in a profound increase in c-fos immunostaining in AP and SFO and that these areas expressed mRNA encoding the proteins that constitute functional amylin receptors. Amylin reversibly increases action potential frequency in a subset of both AP (83, 84) and SFO neurons (Figs. 2 and 3; Refs. 85–87). Of significant interest is the observation that administration of amylin in rats (86) and goats (88) induced drinking behavior, indicating that feeding and drinking behaviors may be linked *via* CVO signaling.

Polypeptide-Fold (PP-Fold) Hormones. The PP-fold hormones are 36 amino acid peptides, characterized by a common structure containing several tyrosine residues and a tertiary structure showing an α-helix and polyproline-helix connected by a β-turn, which forms a U-shaped loop (known as a PP-fold). C-terminal amidation is necessary for the biological activity of these peptides. The family includes polypeptide Y (PYY), pancreatic polypeptide (PP), and neuropeptide Y (NPY). PYY is predominantly released from L cells of the distal gut upon stimulation of the lumen by ingested food (89, 90), and production in the brain has not been reported. Two forms of PYY have been described: the full length 1–36 amino acid peptide, and the 3–36 amino acid form, which is the product of cleavage by dipeptidyl peptidase IV (91, 92). PP is predominantly produced by the endocrine pancreas, and, like PYY, its release is also stimulated by the presence of food in the gut (93, 94). NPY is predominantly a neuropeptide transmitter, potentially the most widely used peptide neurotransmitter. The single largest source of NPY production in the CNS is the ARC, with other sources including the dorsal vagal complex, the PVN and SON of the hypothalamus, the dorsal medial nucleus of the hypothalamus, the cerebral cortex, and the hilar region of the hippocampus (95–100). NPY is also extensively used by postganglionic fibers of the sympathetic nervous system (101–103). The PP-fold hormones bind to the Y family of receptors (Y1, Y2, Y4, Y5, and Y6) with differing levels of affinity. $PYY_{(1–36)}$ exhibits affinity for all the Y receptor subtypes, whereas $PYY_{(3–36)}$ exhibits greatest affinity for the Y2 receptor and some affinity for the Y1 and Y5 subtypes. In contrast, PP shows the greatest affinity for the Y4 receptor. Expression of these receptors has been observed in CVOs by various means, including *in situ* hybridization, immunocytochemistry, and binding of labeled ligands: Y1, Y2, Y4, and Y5 have been observed in AP (104–109); Y5 receptors have been observed in OVLT (110); and Y1 receptors have been observed in SFO (109).

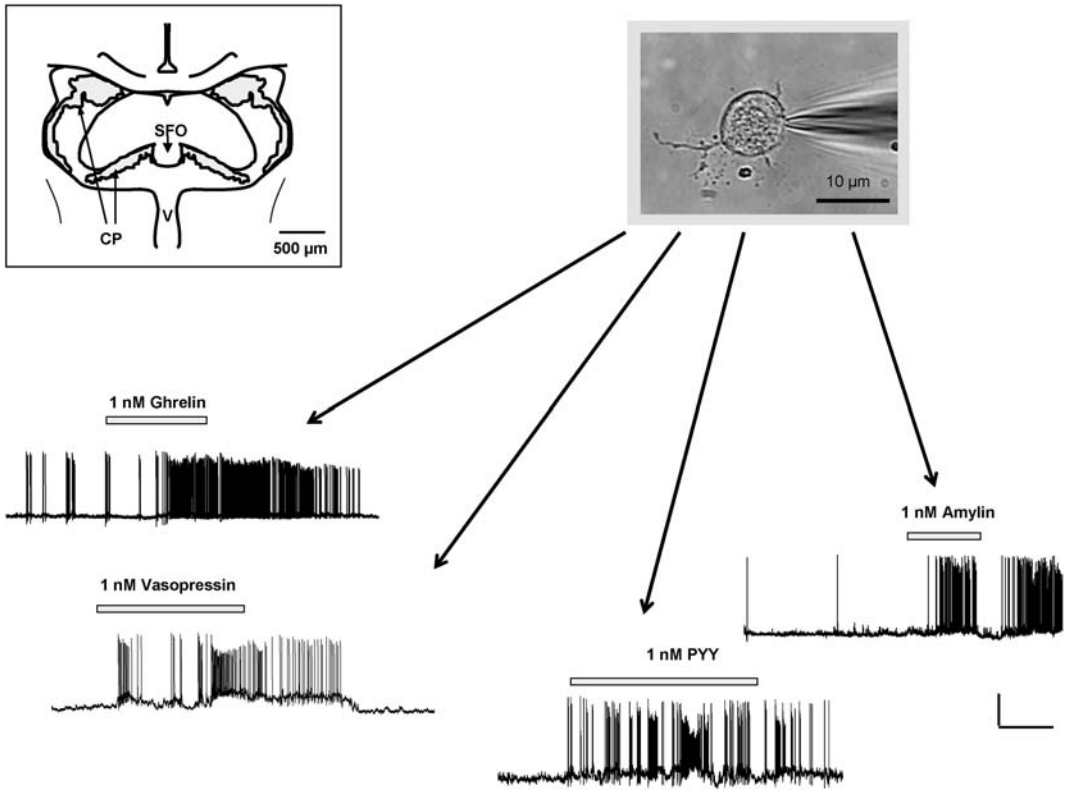

Figure 2. Circulating signals regulating feeding influence activity of SFO neurons. (Upper left) Schematic representation of coronal section through the region of the subfornical organ demonstrating its location within the third ventricle (–0.8 mm from bregma). In our laboratory, we routinely microdissect this CVO and dissociate the neurons to perform patch-clamp recordings. SFO, subfornical organ; CP, choroids plexus; V, third ventricle. (Upper right) photomicrograph showing an example of an SFO neuron that has been maintained in culture and subjected to patch-clamp analysis. (Lower panel) Representative voltage traces (from different cells) from current clamp experiments examining the effects of application of four selected "feeding signal" peptides. Although only one response is shown for each peptide, the same peptide may hyperpolarize or depolarize different neurons. The bar above each trace indicates the duration of application for each peptide. (Bottom right) Scale bar represents 50 mV and 50 sec for ghrelin experiment; 30 mV and 20 sec for vasopressin experiment; 60 mV and 50 sec for PYY experiment; 60 mV and 50 sec for amylin experiment.

Administration of PYY exerts a variety of effects on the gastrointestinal tract, including inhibition of gastric acid secretion, gallbladder and gastric emptying, and increasing the rate of fluid absorption (111, 112). These effects are, at least in part, mediated by direct activation of AP neurons (113). Studies have demonstrated that administration of $PYY_{(3–36)}$ to mice and humans has a significant anorectic effect (114–116) *via* activation of Y2 receptors; however, this result remains controversial (117, 118). Interestingly though, in contrast to the anorexic effect of peripheral PYY, central administration of PYY results in a powerful orexigenic effect (119). Although electrophysiological experiments have reported direct effects of PYY on ARC (120), emphasizing the presence of Y receptors, the endogenous physiological ligand (PYY or NPY) or source (circulating PYY or central NPY) are yet to be identified. In our laboratory, we have focused on again identifying potential actions of circulating PYY on SFO and AP neurons, both of which show concentration-dependent effects on membrane potential with thresholds at near physiological (1 n*M*) concentrations (Figs. 2 and 3). These observations support the possibility that SFO and AP may play important roles in sensing this apparently important circulating signal.

Administration of PP has also had profound effects on gastrointestinal motility and caused anorexia (114, 121). These effects of increased levels of circulating PP are thought to be mediated by activation of Y4 receptors at the AP and subsequent modulation of gastrointestinal networks in the dorsal vagal complex.

Ghrelin. Ghrelin is the only peripheral, appetite-stimulating hormone described to date. It is secreted predominantly from the stomach in response to fasting, and secretion appears to be inhibited by elevated circulating glucose and insulin (122). In humans, preprandial increases in circulating ghrelin strongly suggest a role in meal initiation (123), and administration of ghrelin potently stimulates release of growth hormones and feeding in both rodents and humans (124–127). The only known receptor for ghrelin, the growth hormone secretagogue receptor (GHS-R), is highly expressed in the hypothalamus but is also found in the brainstem, pituitary, gastrointestinal tract, and other peripheral tissues (128). Although the majority of studies focusing on its role in regulation of energy

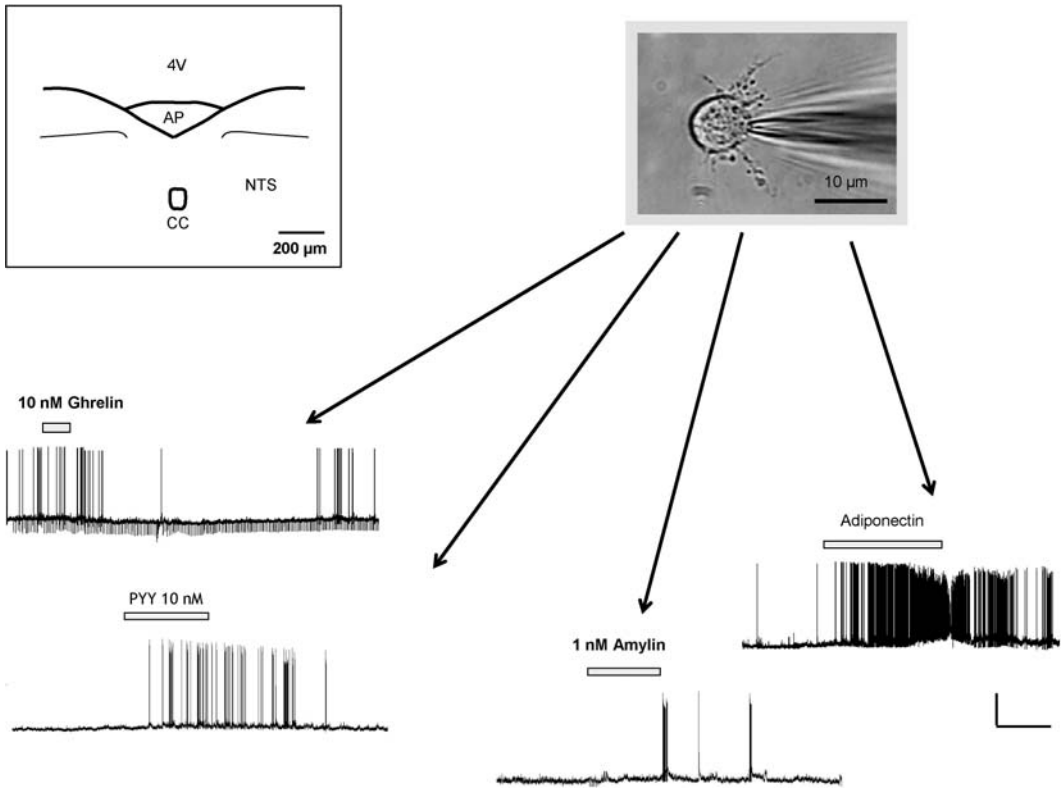

Figure 3. Circulating signals regulating feeding influence activity of AP neurons. (Upper left) Schematic representation of coronal section through the region of the brainstem demonstrating its location within the fourth ventricle (–13.8 mm from bregma). In our laboratory, we routinely microdissect this CVO and dissociate the neurons to perform patch-clamp recordings. AP, area postrema; 4V, fourth ventricle; NTS, nucleus of the solitary tract; CC, central canal. (Upper right) photomicrograph showing an example of an AP neuron that has been maintained in culture and subjected to patch-clamp analysis. (Lower panel) Representative voltage traces (from different cells) from current clamp experiments examining the effects of application of four selected ''feeding signal'' peptides. Although only one response is shown for each peptide, the same peptide may hyperpolarize or depolarize different neurons. The bar above each trace indicates the duration of application for each peptide. (Bottom right) The scale bar represents 50 mV and 300 sec for ghrelin experiment; 50 mV and 60 sec for PYY experiment; 50 mV and 60 sec for amylin experiment; 50 mV and 75 sec for adiponectin experiment.

homeostasis have examined the activity of ghrelin in the hypothalamus (129–132), the transport of this peptide across the BBB may not be ubiquitous across all mammalian species (27). Thus, the CVOs are also logical potential targets for its action. Recent work from our laboratory has demonstrated that the GHS-R is expressed in SFO, and using patch-clamp recording, we found that ghrelin depolarizes SFO neurons *via* a nonspecific cation conductance (Fig. 2; Ref. 87). Preliminary work also suggests that ghrelin affects membrane properties of AP neurons (Fig. 3), and thus, there exists a developing body of evidence in support of the notion that actions of ghrelin at these CVOs may compliment the orexigenic effects of this peptide at the ARC.

Adiposity signals. *Adiponectin.* Adiponectin is a recently discovered peptide produced exclusively by adipocytes. In contrast to leptin (below), levels of adiponectin are decreased with increasing levels of adiposity (133–135) and obesity-related diseases, such as insulin resistance, metabolic syndrome, and hypertension (135, 136). Adiponectin is thought to primarily act as an insulin-sensitizing hormone, with skeletal muscle and liver being the main targets. Administration of adiponectin lowers hepatic gluconeogenesis, lowers serum glucose, and ameliorates insulin resistance in normal mice and mice with disturbances in glucose metabolism (136–139). Recently, Qi *et al*. (140) demonstrated that the brain is also a target of adiponectin, as central administration caused changes in glucose and lipid levels similar to those observed with peripheral administration of adiponectin. Immunostaining for c-fos indicated that central administration caused activation of neurons in the PVN without activation of the neurons in the arcuate nucleus. Although Spranger *et al.* (141) suggest that adiponectin may exert its central activity *via* modulation of cytokine release from vasculature, our experiments indicate that adiponectin acts directly on neurons of the AP. We have observed expression of both subtypes of receptor, AdipoR1 and AdipoR2, in AP neurons. Our experiments indicated that approximately 60% of AP neurons tested were influenced by adiponectin (globular form), with subsets being either depolarized or hyperpolarized by the peptide. Single-cell reverse-transcription polymerase chain reaction (RT-PCR) indicated that both subtypes of receptor, AdipoR1 and AdipoR2, were

expressed in most cells exhibiting sensitivity to adiponectin (submitted). Our experiments confirm a central mechanism of action of adiponectin and indicate a role for the CVOs in adiponectin-mediated metabolic regulation (Fig. 3).

Leptin. Leptin is a peptide hormone secreted primarily by adipocytes, with circulating concentrations positively correlated with levels of obesity, such that leptin is a peripheral signal indicating metabolic status and adiposity level to feedback systems. Leptin levels are decreased by fasting (142), and acute administration of leptin reduces food intake, body weight, and increases energy expenditure in fasted animals (143). Long-term administration in rodents also reduces food intake, body mass, and total fat (144). The primary site at which leptin acts to modulate food intake is thought to be within the ARC. Increasing levels of leptin alter mRNA expression levels of hypothalamic satiety signals NPY, Agouti-related protein (AGRP), and CRH. Leptin also causes activation of the anorexigenic proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) neurons, whereas concomitantly causing a reduction in the activity of the orexigenic NPY/AGRP neurons (145, 146).

However, leptin appears to play a key, yet indirect, role in the signaling of CCK at the AP. Whether or not leptin receptors are expressed in the CVOs is currently unclear, with contradictory data suggesting them to be absent (147, 148) or present (149, 150), and clearly, this is an area that deserves further clarification. Leptin potentiates the ability of CCK and bombesin (another putative satiety peptide) to inhibit food intake, apparently by increasing the ability these signals to activate the dorsal vagal complex neurons (151–153). It is presently unclear as to where leptin is acting to exert this influence; however, these results are important because they demonstrate that satiety signaling at the AP can be modulated. Moreover, these results highlight the suggestion that the sensory CVOs are multimodal integration sites and raise the possibility that energy homeostasis–related circuits in the CVOs may be subject to plasticity.

Metabolites. *Glucose.* In light of the recent explosion of discoveries in the field of peptidergic, energy homeostasis signals (satiety and adiposity signals), glucose is often overlooked, perhaps, because glucose metabolism does not appear to hold the same potential as a target for antiobesity pharmaceuticals. However, the fact remains that the concentration of circulating glucose is one of the most important circulating signals providing information concerning immediate metabolic status. Early studies demonstrated that hyperglycemia (induced twice per day) could reduce daily food intake in rats (Ref. 154; although later experiments indicated that acute glucose injection before a meal did not; Refs. 155–157). Moreover, though, hypoglycemia can potently stimulate food intake. Hypoglycemia induced by insulin, and glucopenia induced by administration of nonmetabolizable glucose analogues, such as 2-deoxyglucose (2-DG) and 5-thioglucose, potently stimulates food intake in many (but not all) mammals tested (158–164). Evidence suggests that the AP (but not vagal inputs) is a key glucose-detection site in mediating hypoglycemic feeding (165–167). In addition, lesions of AP (including parts of the NTS) attenuate 2-DG–induced feeding (168, 169). Although there are concerns that experiments using insulin or glucose analogues do not represent physiological hypoglycemia related to normal feeding behavior, but instead represent a "glucose emergency," the evidence is convincing that AP plays a role. In addition to this, glucokinase, an enzyme postulated to play a key role in glucose detection in glucose-sensing neurons is expressed in AP (170). Thus, under normal conditions, the lack of a BBB enables CVOs to effectively detect changes in circulating glucose as soon as they occur. Indeed, AP neurons have been shown to change firing rates in response to changing glucose concentration (84, 171, 172). More recent work suggests that lactate is also monitored by the AP (173). Furthermore, the integrative nature of the CVOs is again underscored by the observation that glucose-responsive neurons in the AP were also sensitive to the satiety signal amylin (84).

Flip Side of the Coin: Energy Expenditure

The "thrifty gene hypothesis" (174) suggests that evolution would favor those who are best able to store excess energy for times when food is scarce. Because survival is more likely to be threatened by acute energy deficits than excesses, the circuitry driving feeding behavior aspects of energy homeostasis may well be weighted in favor of energy consumption and storage. Although feeding is the most overt aspect of regulating energy homeostasis and, perhaps, one of the most important for short-term survival, there are other components of the system. Regulation of reproduction, growth, energy partitioning, and energy expenditure are also critical for long-term survival of the individual and species. Although it is beyond the scope of this review to consider the role of the sensory CVOs in regulation of each of these aspects, possible roles in energy expenditure deserve mentioning.

Regulation of energy expenditure may be considered to be the flip side of the coin of food intake: what does an animal do with excess energy (beyond what is stored as fat)? Energy expenditure occurs by three main routes: (i) energy required for basic physiological requirements of all cells; (ii) energy required for physical work, including movement; (iii) energy required for adaptive thermogenesis, which is the process of generating excess heat in response to a cold environment or the consumption of excess (or specific types of) food, also known as diet-induced thermogenesis (DIT). DIT occurs when energy is used without work being done, resulting in production of heat. The mechanism can be achieved by the uncoupling of ATP synthesis and proton transport across the mitochondrial membrane. DIT in rodents is stimulated by the sympathetic innervation of brown fat stores releasing norepinephrine and acting on beta

adrenergic receptors, causing an upregulation of a mitochondrial uncoupling protein, UCP-1 (175). Even though mature humans lack brown fat, DIT is still thought to occur, although the mechanism is yet unclear. Although there is no direct evidence yet for a role of the sensory CVOs in DIT, the position of CVOs outside the BBB, allowing detection of circulating signals, and their demonstrated connectivity to autonomic control centers that modulate sympathetic tone makes them ideally suited to regulate this aspect of energy homeostasis. Indeed, peripherally administered regulatory signals leptin and ghrelin (see above) significantly alter UCP-1 expression in brown fat stores and regulate white fat mass in mice (176–178). It is intriguing to hypothesize that signaling at the sensory CVOs contributes to the modulation of sympathetic activity regulating thermogenesis. Thus, it should be emphasized that, at this time, there are few studies that have specifically investigated the roles of the sensory CVOs in controlling energy expenditure. Therefore, we would suggest that the lack of evidence in this area should not yet be interpreted as evidence for lack of function, until future studies have investigated this important area more thoroughly.

Concluding Comments

Intuitively, the lack of BBB and the connectivity to the hypothalamic circuitry suggests that the CVOs play critical roles in detecting and integrating humoral and neural signals that regulate energy homeostasis. A significant body of evidence appears to confirm this hypothesis and indicate that the functional importance of these sensory roles played by the CVOs in detection of circulating satiety signals deserves careful, systematic investigation. In addition, as the list of peptide satiety factors and their receptors grows, our understanding of the regulation of energy homeostasis will likely benefit by the detailed investigation of dynamic gene expression within the CVOs**.**

In reality, intake of energy and water are inextricably linked. This is supported by numerous studies (179–182) and by the casual observation that we seldom eat without drinking. Although experimental evidence suggests that feeding and drinking behaviors can be separated, their underlying physiologies are clearly related by more than the fact that food and water enter the body through the mouth. Increasing evidence suggests one signal, acting at the sensory CVOs, may play roles in both feeding and drinking. For example signals controlling feeding, such as amylin and ghrelin, can significantly change neuronal activity at the SFO (87), a CVO primarily recognized for its ability to sense angiotensin II, osmolarity, and vasopressin, the latter two of which have also been reported to have effects on food intake (183). The role of AP in food intake and fluid balance is made clear by studies demonstrating that lesions of the AP cause alterations in feeding behavior and salt appetite (184–186) The complex interaction between feeding and drinking behaviors is likely to be, at least in part, a result of signal convergence for both homeostatic processes on the sensory CVOs. Moreover, given the clearly established links between cardiovascular dysregulation and obesity, one would expect there to be potential sites within the brain at which these systems are regulated in an integrated way. We would propose that the sensory CVOs represent one such potential site for combined regulatory control, a possibility that we believe is worthy of future investigation.

Although we have provided a sampling of the roles of satiety signals at the sensory CVOs, a scan of the current literature will reveal volumes of work indicating the roles of numerous other blood-borne signals (peptide and otherwise) in the regulation of energy homeostasis. With the panoply of such regulatory signals being described, it is unlikely that a single one will emerge to be the lynchpin of the homeostatic program. The cloning of leptin, for example, was initially hailed by many as the beginning of the end of obesity. Unfortunately, this has not happened; however, we are now truly beginning to move away from the myopic and piecemeal approach of attempting to understand the functions of individual signals in isolation, to investigating their roles in a more integrated manner. In the present review, we do not propose that the sensory CVOs are the sole sites of action for circulating signals to regulate pathways involved in energy homeostasis; however, we do suggest that CVOs play key roles in the process. The presence of multiple signaling pathways (ghrelin acting *via* the ARC and the CVOs, for example) underscores the integrative nature of the CVOs and perhaps the importance of the signals, whereas the simple, multisensory abilities of the CVOs in monitoring the multitude of signals described in the present review argues persuasively that these structures play important integrative roles in the control of fluid and energy balance.

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