Circulating Signals as Critical Regulators of Autonomic State – Central Roles for the Subfornical Organ

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Abstract

In order to maintain homeostasis autonomic control centers in the hypothalamus and medulla must respond appropriately to both external and internal stimuli. Although protected behind the blood brain barrier neurons in these autonomic control centers are known to be influenced by changing levels of important signaling molecules in the systemic circulation (e.g. osmolarity, glucose concentrations, and regulatory peptides). The subfornical organ, belongs to a group of specialized central nervous system structures, the circumventricular organs, that are characterized by the lack of the normal blood brain barrier, such that circulating lipophobic substances may act on neurons within this region, and via well documented efferent neural projections to hypothalamic autonomic control centers, influence autonomic function. This review focuses on the role of the subfornical organ in sensing peripheral signals and transmitting this information to autonomic control centers in the hypothalamus.
**Introduction**

In order to maintain homeostasis autonomic control centers in the hypothalamus and medulla must respond appropriately to both external and internal stimuli. It is well established that mechanisms exist for the relay of essential information derived in the periphery, detected by specialized sensory structures (ie baroreceptors, chemoreceptors), to hypothalamic and medullary autonomic nuclei through afferent neural inputs. It is also known that the activity patterns (i.e. outputs) of neurons in these autonomic control centers are influenced by changing levels of important signaling molecules in the systemic circulation (e.g. osmolarity, glucose concentrations, and regulatory peptides), despite the fact that all of the important hypothalamic and medullary autonomic control centers are protected from direct access to such circulating autonomic signals by the blood brain barrier (BBB).

The anatomical features of the BBB are well understood. The BBB consists not only of endothelial cells of cerebral microvessels joined together by tight junctions (in strict contrast to the fenestrations between endothelial cells in most capillaries), but also astrocytes, the end feet of which wrap around the brain side of the endothelial cell membrane (1). The tight junctions of the endothelial cells are more complex in the CNS than in the periphery, in that there are networks of strands formed by intramembranous particles joining adjacent cells (140). The continuity of the BBB ensures that all transport occurs across the cell membrane thus limiting movement across the barrier to substances that are
either lipophilic (and thus readily diffusible across the lipid bilayer), or substances that are transported across this barrier by alternative mechanisms. The existence of the BBB thus means that nearly all large hydrophilic molecules, such as peptides and proteins which do not have a specific transport system, are excluded from the CNS. The barrier develops during late prenatal and early postnatal life, and importantly has been shown to be compromised in a number of pathological states including inflammation and hypertension (1).

In this way, the BBB allows preferential maintenance of the constituents of the extracellular fluid of the brain in a number of important ways. Perhaps most importantly the BBB ensures that potential “toxins” in the circulation do not access CNS tissue and compromise CNS function. Secondly, by effectively creating a separate brain fluid compartment, the BBB permits the use of chemical messengers used in the circulation as hormones to also be utilized behind the BBB as chemical messengers (neurotransmitters/neuromodulators) in neuron to neuron communication within the CNS. This compartmentalization effectively precludes autonomic control centers within the CNS from directly monitoring the varying levels of many of these important peripheral indicators of physiological status as such peripheral access would effectively “short-circuit’ neurotransmitter function of the same signaling molecule.

As suggested above there is now a clear understanding that neurons in both medullary and hypothalamic autonomic control centers must receive information provided by these peripheral signals, many of which cannot diffuse across the BBB, in order to accurately assess the global state of the “milieu
interieur.” The recognition that this occurs leads to the critical question of “How do CNS structures, protected behind the BBB, sense circulating signals which do not cross the BBB?”

There are at least five primary mechanisms through which transfer of information might occur:

- The **vagus nerve** can transmit information from periphery to the CNS through access points in the medulla.
- Lipophilic substances can readily pass through the lipid bilayer of the cell membrane (e.g. steroids, barbiturates, and alcohol) by **simple diffusion** through the endothelial cell membranes of the cerebral vasculature to access to neural tissue behind the BBB.
- Specific **saturable transporters** may move lipophobic substances (e.g. glucose, leptin and cytokines) across the barrier (6).
- Signaling molecules may act on one side of the endothelial cell and, by **transendothelial cell signaling**, induce release of a second molecule on the other side of the barrier (89).
- Circulating lipophobic substances may act on neurons in specialized regions of the brain known as the **circumventricular organs** (CVOs) which lack the normal BBB (contain fenestrated capillaries).

This latter possibility, and in particular the role of one of these CVOs – the subfornical organ (SFO) – in sensing such peripheral signals and transmitting
this information to autonomic control centers in the hypothalamus will be the focus of the remainder of this review.

**Specialized Anatomical Features of the CVOs**

The fenestrated capillaries of the CVOs are distinct from the rest of the CNS (43) in that they lack the typical tight junctions between adjacent endothelial cells (91). In addition, the CVOs possess an extensive and complex vascular supply as compared to other areas of the brain which includes capillary loops extending to the ependymal surface and large, perivascular spaces (Virchow-Robin spaces) surrounding the blood vessels (42), characteristics which maximize the time and area for exposure of blood borne substances to the cellular components of the CVOs (42). In addition to the lack of the normal BBB and the dense vascular supply, the sensory CVOs (SFO, organum vasculosum of the lamina terminalis and the area postrema), contain exceptionally dense aggregations of a variety of different receptors for peripheral signals including regulatory peptides (e.g. angiotensin, cholecystokinin, ghrelin, leptin) (78; 93; 115; 122), steroids (e.g. estradiol) (102), and specific ions (e.g. Ca^{2+}, Na^{+}) (85; 100), observations which clearly suggest the ability of neurons in these CVOs to sense circulating concentrations of these signaling molecules. These specialized anatomical features, uniquely position the sensory CVOs with the potential to directly monitor the constituents of peripheral circulation and communicate this information, via well-documented afferent projections, to autonomic control.
centers in the hypothalamus and medulla. The sensory CVOs thus represent potential windows in the brain for autonomic feedback to the CNS.

The Subfornical Organ

One such specialized CNS structure is the subfornical organ (SFO), located in the forebrain on the midline wall of the third ventricle. It consists primarily of neuronal cell bodies which receive afferent input from the circulation and communicate this information, via well documented efferent neural projections (see below), to hypothalamic autonomic control centers.

Anatomical Features of the SFO

The SFO is comprised of two distinct regions (a core region and peripheral outer zone) with differing neuronal projections and ligand binding abilities (74). The SFO sends both direct (monosynaptic) and indirect (polysynaptic) efferent projections to the hypothalamic autonomic control and neuroendocrine centers, such as the paraventricular (PVN) and supraoptic nuclei (SON) (71; 79), respectively. Specific excitatory projections have been found to vasopressin and oxytocin secreting magnocellular 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 (30)).

The SFO also sends efferents to neurons in the anteroventral third ventricle region, specifically to the median preoptic nucleus and the organum vasculosum of the lamina terminalis (71; 80), both of which in turn send additional axonal projections to hypothalamic centers including SON and PVN.
Less dense efferent connections 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 have been reported (68; 79), while one study has suggested efferent connections to the arcuate nucleus (44) (see figure 1).

The majority of anatomical data suggests that SFO neurons have relatively compact dendritic trees and do not receive extensive neural inputs (22), supporting the suggested principle role of this region in receiving afferent information from peripheral circulation. Limited afferent inputs to the SFO originate from the same areas that receive SFO efferents, including the lateral hypothalamus (70), and the median preoptic nucleus (70), as well as the lateral division of the parabrachial nucleus, nucleus tractus solitarius, midbrain raphé, nucleus reunions of the thalamus, and organum vasculosum of the lamina terminalis (for review see (18)), suggesting important reciprocal communication between these regions.

**Intrinsic Properties of SFO Neurons**

The description of SFO as a sensory CVO is effectively a consequence of the ability of SFO neurons to “sense” different physiological signals, responses which are manifested as changes in neuronal excitability. Many studies have described the basic firing patterns and sensitivity of SFO neurons to exogenous hormones and neurotransmitters, as well as important information regarding SFO connectivity and effects of synaptic input on SFO neuronal activity (for review see
Voltage clamp techniques have described voltage gated sodium, potassium and calcium currents, as well as hyperpolarization activated (I\textsubscript{h}), persistent sodium (I\textsubscript{NaP}), swelling activated chloride and non-selective cationic conductances in SFO neurons (18).

The excitability of subpopulations of neurons within the SFO have been shown to be influenced by osmolarity, calcium, or sodium concentrations in the systemic circulation (for review see (40)). Increases in extracellular [Ca\textsuperscript{2+}] depolarizes SFO neurons as a result of activation of the calcium sensing receptor leading to a modulation of non selective cationic conductance (NSCC), I\textsubscript{h}, and I\textsubscript{NaP} (138). Additionally, SFO neurons have been shown to be osmosensitive (3), although the channels underlying this effect have yet to be described. Interestingly, glial cells within the SFO have been shown to express the Na\textsubscript{X} channel and respond to changes in extracellular [Na\textsuperscript{+}] suggesting these cells to be true sodium sensors (85).

The presence of peptidergic receptors in SFO has also been used as a logical indicator of the sensory abilities of SFO neurons. In many instances this surrogate for sensory capability has been supported by later electrophysiological studies confirming neuronal sensitivity. Thus, the description of the presence of angiotensin II receptors within the SFO (41; 78), was given functional context by many electrophysiological studies showing that SFO neurons are depolarized by increases in angiotensin II concentrations (28; 31). These findings supported the classical view of the SFO as the primary central site at which circulating angiotensin II acted to induce drinking (110). More recently, a diverse and
growing literature describing actions (receptor localization and electrophysiological effects) of many other circulating factors on SFO neurons has begun to support the concept of a far broader sensory role for neurons in this CVO (see(40; 74) for review). SFO neurons have been shown to be sensitive to changes in acetylcholine (28), amylin (93; 97; 99; 115), atrial natriuretic peptides (12), calcitonin (7; 99; 107), endothelin (30; 134; 135), estrogen (136), ghrelin (93), interleukin 1-β (23), oxytocin (52), prokineticin 2 (17; 39), relaxin (123), vasopressin (5; 137), and, most recently, leptin (115) and adiponectin (2) (see figure 2). In addition, recent work using whole genome microarray technology has confirmed the presence of receptor and/or protein mRNA for many of the substances listed above (see figures 3 and 4), as well as identifying a number of novel transcripts (48), the physiological roles of which have yet to be elucidated. Thus, the perspective emerges of the ability of neurons within the SFO to sense a wide variety of circulating signals reflecting physiological status and, intriguingly, suggest that this structure may play a role in integrating such signals from multiple physiological systems, a number of which we will highlight in the following sections.

**Functional Roles for the SFO:**

**Body Fluid Homeostasis**

Classically, the SFO is known for its well established roles in the coordination of body fluid balance (30; 74). The initial finding that blood-borne
carbachol and angiotensin II acted at the SFO to elicit drinking behavior in rats (110; 111) and that destruction of the SFO abolished angiotensin II induced drinking (112) directed attention to the SFO in the regulation of body fluid balance. The functional importance of SFO in the control of fluid balance was confirmed by later studies showing that destruction of SFO eliminated both water and saline ingestion in rats acutely depleted of sodium (119; 131). In addition, studies showed SFO neurons are activated by water deprivation (20), intravenous injection of hypertonic saline (87; 101), sodium restriction (75), and furosemide induced sodium depletion (103), many of these effects being abolished by the angiotensin II antagonist, losartan, or the angiotensin converting enzyme inhibitor, captopril (75). A role for angiotensin II receptors within the SFO in controlling sodium appetite has been demonstrated by studies showing that administration of losartan prevents both the marked increase in salt intake induced by furosemide and captopril treatment, and the increase in salt and water intake in response to angiotensin II microinjection into the SFO, associated with serotonergic blockade of the lateral parabrachial nucleus (16; 77).

Very high levels of angiotensin converting enzyme have been localized in the SFO (14; 92; 104) and angiotensin-converting enzyme in SFO has been shown to mediate captopril-induced drinking (132), suggesting that local production of angiotensin II in the SFO may also contribute to the physiological effects of angiotensin II in this CVO.

Subsequently, the SFO was shown to be sensitive to the sodium and water content of plasma (9; 86; 96), while single cell recordings have shown that
SFO neurons respond to changes in sodium and osmolarity (4; 45). Interestingly, Na$_x$ channels on glial cells in SFO have been shown to sense increases in sodium levels, information which is then transmitted by lactate to influence the activity of GABAergic neurons in the SFO (108). Aquaporin-4, a selective channel specialized for water transport, has been localized in SFO (84), suggesting a mechanism which may contribute, at least in part, to the osmoregulatory role of SFO in volume homeostasis. Functional studies utilizing acute low intensity electrical stimulation of the SFO have been shown to elicit drinking in satiated rats (114; 117).

Recently, a number of studies have suggested the involvement of additional regulatory molecules (peptidergic and non-peptidergic) that act in the SFO to regulate volume homeostasis. Leptin, an adipose tissue derived hormone known primarily for its involvement in energy homeostasis, has been shown to decrease water intake when given systemically (10). Binding sites for stanniocalcin, a circulating hormone that regulates calcium/phosphate homeostasis, have recently been identified in the SFO (and hypothalamic projections sites involved in fluid homeostasis (SON)) further implicating action of this hormone at the SFO in the regulation of fluid homeostasis, electrolyte balance, and cardiovascular regulation (see below) (95). Peripheral administration of pilocarpine, a muscarinic receptor agonist, has been shown to facilitate drinking behaviour in rats (38; 106). Recent studies revealing c-fos immunoreactivity in SFO and patch clamp recording techniques demonstrating that pilocarpine directly depolarizes SFO neurons by suppressing the release of
an inhibitory transmitter (53), suggest that SFO may mediate the dipsogenic effects of exogenous pilocarpine administration. Serotonin receptor mRNA is one of the most highly expressed receptor mRNAs present in the SFO (48) and it has been shown that angiotensin II reduces the release of serotonin in the SFO, suggesting that serotonin receptors may be involved in the elicitation of drinking behavior by ANG II (127). In addition, it has been demonstrated that serotonergic pathways from the midbrain raphe system to the SFO are activated by hemorrhage in the rat (129) further supporting the role of serotonin in control of fluid balance at the level of the SFO.

Attention has been directed to the involvement of free radicals in SFO by studies showing rac1-dependent NADPH oxidase to be a primary source of angiotensin II – dependent superoxide production in the SFO and that genetic inhibition of this enzyme complex in the SFO attenuated the dipsogenic effects of ICV administration of angiotensin II (142). Specifically, Nox2 has been selectively linked to the dipsogenic effects of intracerebroventricular angiotensin II administration. (90)

Thus, it is evident that the SFO detects multiple peripheral signals reflecting fluid balance via afferent inputs from multiple sources and relay this information via efferent connections to autonomic control centers, thereby regulating and integrating the functions of many different controllers of overall body fluid homeostasis.
Cardiovascular Regulation

Microinjection studies showing that direct injection of angiotensin II into the SFO increased arterial pressure (73), an effect blocked by section of the ventral stalk of the SFO (which is the region in which efferent projections exit the SFO (69)), first focused attention on the potential roles for SFO neurons in cardiovascular regulation. Such roles were later confirmed by reports that stimulation of the SFO by chemical or electrical means causes a biphasic increase in blood pressure, which results in the secretion of vasopressin and oxytocin (33-35; 50; 109) and activation of sympathetic outflow (15; 32). The hypertensive effect SFO stimulation is prevented by ablation of the hypothalamic PVN (36), suggesting this to be one of the primary efferent outputs pathways through which SFO neurons elicit such effects. At the cellular level, systemic angiotensin II acts at the SFO to control the activity of PVN neurons (37; 65) and, interestingly, SFO neurons projecting to PVN have been shown to utilize angiotensin II as a neurotransmitter (66). Angiotensin II has been shown to influence the excitability of SFO neurons through the combined inhibition of potassium currents, potentiation of calcium currents, and activation of a non-selective cationic conductance (30; 88; 137). Collectively, these studies suggest that the SFO is a critical relay center through which the CNS is able to monitor circulating angiotensin II and influence cardiovascular function.

Recent work using site specific gene ablation techniques to knock down the renin angiotensin system exclusively in the SFO have intriguingly demonstrated that such manipulation abolished the pressor and bradycardic
effects of renin infused in the CNS, providing direct evidence that the local renin angiotensin system in the SFO plays a critical role in blood pressure regulation (113). In addition, the use of viral vectors to cause the overexpression of angiotensin-converting enzyme 2 (ACE2) specifically in SFO reduced the pressor (and dipsogenic) response elicited by intracerebroventricular angiotensin II and was associated with downregulation of the angiotensin II type 1 receptor expression (29). These observations suggest a new target for the treatment of hypertension and other cardiovascular diseases.

Other regulatory peptides known for effects on the cardiovascular system that have been shown to influence blood pressure as a result of actions in the SFO include vasopressin (116; 137) and atrial natriuretic peptide (105). In addition, the reproductive hormone, relaxin (82), the orexigenic and arousal neuropeptide, orexin (118), and the adipose derived hormone leptin (Smith and Ferguson, unpublished observation) have also been shown to influence blood pressure when microinjected into the SFO. Galanin has been shown to inhibit the electrical activation of angiotensin II-sensitive neurons in the SFO (54) and, while intracerebroventricular injection of galanin in conscious rats results in small pressor response, it has been shown to inhibit angiotensin II-induced pressor responses (49), suggesting a role in mediating cardiovascular control through actions at the SFO. It has also been suggested that cardiovascular responses seen in response to intravenous administration of sub-septic levels of lipopolysaccharide, a bacterial endotoxin (see immune regulation below), may be responsible for early interleukin 1-beta gene expression in the SFO (141).
Recent attention has focused on the role of oxidative stress on the development of hypertension and superoxide has been identified as a particularly important free radical in cardiovascular biology. Recent work has suggested a role for superoxide in SFO in mediating angiotensin II induced cardiovascular effects (143). Using adenovirus-mediated delivery of siRNA to the SFO it has been shown that NADPH oxidase homologues (Nox2 and Nox4) contribute to the cardiovascular responses elicited by intracerebroventricular administration of angiotensin II (90). Myocardial infarction (MI) has also been shown to increase superoxide production in the SFO, while prevention of such changes in the SFO following MI led to significantly improved myocardial function and diminished levels of cardiomyocyte apoptosis (72). Finally, scavenging superoxide in mouse forebrain is associated with improved cardiac function and survival following myocardial infarction (72). Together, these studies suggest that oxidative stress in the SFO plays a critical role in the deterioration of cardiac function following myocardial infarction and suggests a CNS-target for antioxidant therapy directed towards the treatment of myocardial infarction-induced heart failure.

The evidence presented above suggests that SFO involvement in cardiovascular regulation is multifaceted and involves a number of signaling molecules, many of which are not ‘classical’ cardiovascular mediators. This then further suggests an integrative role for the SFO in detecting and assimilating information from a variety of sources, relaying this information to the appropriate autonomic control centers to facilitate an appropriate coordinated autonomic response.
Immune Regulation

Systemic signaling of infection can occur directly through bacterial endotoxins such as lipopolysaccharides (LPS) or through pyrogenic cytokines, such as the interleukins (interleukin-1β and interleukin-6) which in turn act at the brain to cause an integrated immune response including changes in body temperature, hormone release, sleep patterns, and food intake. The CVOs have been suggested to provide both an access point to the brain for endotoxins and cytokines and, through their efferent connections to hypothalamic and medullary autonomic centers, potentially integrate the multiple components of this coordinated response.

The identification of interleukin-1β and CD14 (the classic marker for endotoxin binding) receptors in the SFO (19; 27; 60), as well as receptor mRNA for other known mediators of the immune response including ciliary neurotrophic factor (48) and tumour necrosis factor-α (48; 83), suggest that the SFO may play an important role in immune regulation.

Functional relevance to expression of these receptors in SFO is derived from studies demonstrating that peripheral administration of interleukin-1β induces c-fos expression in SFO neurons (26), which is further supported by electrophysiological studies showing that interleukin-1β depolarizes SFO neurons as a result of modulation of a non-selective cationic conductance (23). In addition, in vivo studies have revealed that SFO lesions inhibit LPS fever generation (124), a conclusion further supported by the demonstration that
microinjection of the interleukin-1 receptor antagonist into SFO also attenuates LPS induced fever (13).

While systemic administration of the high doses of LPS has been shown to cause a robust and widespread induction of c-fos mRNA within the CNS and induce global expression of pro-inflammatory cytokines in the brain, subseptic doses of LPS caused the selective triggering of c-fos expression within discrete brain regions, including the SFO (59), and caused the induction of interleukin-1β and tissue necrosis factor-α mRNA expression only in the choroid plexus, the circumventricular organs, including the SFO, and meninges. These results indicate that the actions of pro-inflammatory cytokines during subseptic infection may occur at the SFO, which in turn communicates this peripheral immune status to the autonomic control centers in the brain (94). It has also been suggested that differential cardiovascular responses observed in response to subseptic levels of endotoxins may underlie these differences (141).

Constitutive CD14 labeling has been demonstrated in the SFO and LPS treatment has been shown to cause a robust increase in CD14 expression and a rapid induction of interleukin-1β, interleukin--6, and tumour necrosis factor-α within SFO (60). Tumor necrosis factor acts via two cell-surface receptors, the p55 and p75 tumor necrosis factor receptors. Constitutive expression of p55 mRNA has been demonstrated in the SFO and functional activation of this receptor by circulating tumor necrosis factor-α has been shown to induce c-fos expression in the SFO (83).
Toll-like receptors have been suggested to be to play a key role in the innate immune response and, not only has Toll-like receptor 4 mRNA has been localized in the SFO, but a decreased receptor expression is also seen in response to systemic LPS or interleukin-1β challenge (60). In contrast to the strong up-regulation of the gene encoding mCD14 during endotoxemia, neither LPS nor interleukin -1β caused a convincing increase in the toll-like receptor 4 mRNA. The constitutive expression of both mCD14 and toll-like receptor 4 may explain the innate immune response in the brain, which originates from the structures devoid of blood-brain barrier, such as the SFO, in presence of circulating LPS (60).

Interleukin-6, an endogenous mediator of LPS-induced fever, has been shown to directly activate SFO neurons as revealed by the demonstration of a nuclear translocation (and thus activation) of the transcription factor, signal transducer and activator of transcription 3 (STAT3) within SFO (46; 58). In addition, suppressors of cytokine signaling (SOCS), cytokine-inducible proteins, are rapidly induced by interleukin-6 and peripheral LPS administration has been shown to cause a profound transcriptional activation SOCS-3 mRNA in SFO in a time dependent manner (63).

Thus, although the data suggesting sensory roles for the SFO in the regulation of immune function is not as extensive as that for fluid balance there is strong evidence that support the proposed role of the SFO as a sensor of humoral signals (endotoxins or cytokines) produced by the activated immune system which act centrally to initiate the integrated immune response.
Reproduction

A role for the SFO in the control of reproductive function was first suggested by lesions studies showing that removal of SFO disrupted the estrous cycle and forced rats into a prolonged state of diestrous (67). In addition, the proestrous follicle stimulating hormone surge was absent while the luteinizing hormone secretion remained unchanged in SFO lesioned rats suggesting that the SFO may play a role in the control of the cyclic release of these two gonadotropins (67). The SFO has also been found to contain significant levels of gonadotropin releasing hormone (57), although the specific impact of this expression is not known. However, studies have demonstrated excitatory inputs from SFO to putative GnRH neurons (24), and that activation of SFO neurons results in increased circulating concentrations of luteinizing hormone (25).

Although it is known that circulating estrogens influence the electrical activity of the hypothalamic magnocellular neurons which synthesize vasopressin or oxytocin and regulate body fluid homeostasis and reproduction, none of these magnocellular neurons express the estrogen receptor. Estrogen receptors have been localized within the SFO (102) and it has been demonstrated that SFO neurons expressing the estrogen receptor project to the SON thus providing a route through which circulating estrogen could exert its effect on the excitability of SON neurons (133). It has also been suggested that circulating estrogens act at receptors in SFO to modulate the role of angiotensin II in the regulation of fluid and electrolyte balance (102; 126; 128). The SFO has also been shown to have
high densities of binding sites for relaxin, a peptide produced primarily by the ovary of pregnant animals as well as in the brain. Relaxin has been shown to influence the activity of SFO neurons (123), suggesting that this CVO may be involved in controlling the duration of gestation (121) and the milk ejection reflex (120). Relaxin has also been shown to cause hypertension as a result of actions at the SFO (121). Exogenous administration of relaxin has been shown have a profound dipsogenic response (51; 123; 130). Blockade of the angiotensin II receptor negates several central actions of relaxin while expression of AT₁ receptors in the SFO increases in parallel with the increase in circulating relaxin seen in the second half of pregnancy (51), suggesting that relaxin may be important for the normal physiology of pregnancy.

**Feeding and Metabolism**

Until recently the area postrema, located in the medulla, was thought to be the predominant CVO involved in the regulation of food intake. Interestingly, recent work reporting receptor mRNA for a number of feeding related peptides in the SFO (48), and single cell recordings showing effects of functional activation of these receptors on the excitability of SFO neurons (see Figure 2) has begun to focus attention on potential roles for this forebrain CVO in the sensation and integration of satiety signals. Calcitonin (107) and the satiation signal, amylin (97; 98), have been shown to influence the activity of SFO neurons. Our own recent work has shown neurons within the SFO to be excited by amylin or the meal initiation peptide, ghrelin, although none of these cells were excited by both
peptides (93), suggesting that different sub populations of neurons in the SFO differentially mediate the appetite suppressing and appetite stimulatory effects these peptides.

Systemic ghrelin administration has been shown to induce the neural expression of c-Fos protein in the SFO suggesting that ghrelin, released into the circulation, may stimulate SFO neurons (125). Peripherally administered amylin has been shown to induce an anorexigenic effect as a consequence of a reduction in meal size, as has peripheral administration of the amylin-related peptide, salmon calcitonin (21).

We have also demonstrated that the SFO possesses receptors for the adiposity signals leptin (115) and adiponectin (2) and that functional activation of these receptors influences the excitability of SFO neurons (2; 115). In addition, leptin and amylin, which both decrease food intake, have been shown to depolarize the same SFO neurons (115).

The melanocortin and neuropeptide Y systems have been shown to play important roles in feeding and metabolism. Both melanocortin 4 receptor (48; 56) and Y1 receptor mRNA (48; 55) have been observed in the SFO suggesting a role for the SFO in mediating the effects on feeding elicited by alpha melanocyte stimulating hormone and neuropeptide Y, the endogenous ligands for melanocotin 4 receptor and Y1 receptor, respectively.

SIRT1, a NAD-dependent deacetylase, has been suggested to be an important link between energy metabolism and aging, and mRNA for this enzyme
has been localized in the SFO, with intriguing age and gender related differential expression reported (61). Resveratrol, an activator of SIRT1 (11), inhibits the electrical activity of SFO neurons perhaps due to blockade of L-type voltage-gated calcium channel (64). Interestingly, resveratrol has been shown to improve the survival of mice fed a high-calorie diet (8) and it has been suggested to protect against metabolic disease through the activation of SIRT1 (62). SIRT1 activation by resveratrol has also been shown to downregulate AT1 receptor expression (81). Thus, it appears that the inhibition of the renin-angiotensin system may contribute, at least in part, to the resveratrol-induced longevity and antiatherogenic effect of resveratrol, and, although not yet investigated, an effect which may be mediated at the SFO.

Finally, our own recent behavioral studies have shown that short duration, low intensity electrical stimulation in SFO induces feeding in satiated rats (117) providing functional evidence for the involvement of SFO neurons in the control of energy homeostasis.

The Subfornical Organ as a Controller of Integrated Autonomic Function

As illustrated in the previous sections, it is apparent that a single peptide (ie angiotensin) can have a variety of physiological actions including behavioral (drinking), neuroendocrine (control of ACTH, oxytocin, vasopressin secretion)
and autonomic (sympathetic activation) effects as a direct consequence of actions in the CNS and that many of these action are mediated, at least in part, by actions at the SFO.

As highlighted above, the SFO is sensitive to a wide variety of circulating signals and it is now becoming clear that the sensory abilities of SFO neurons are far more sophisticated than was initially imagined. In addition to its unique position at the blood brain interface, the SFO combines broad ranging sensory abilities with the capability to exert control over interrelated, yet diverse physiological systems. Nearly all studies examining the sensitivity of SFO neurons to these circulating signals report that between 25% and 60% of neurons are influenced suggesting that single neurons must have multiple sensory abilities. This concept, at the minimal level of two signals, has been confirmed in SFO neurons which are angiotensin II and osmosensitive (3), angiotensin II and atrial natriuretic peptide sensitive (47), angiotensin II and calcitonin responsive (107), angiotensin II and estrogen responsive (128), and leptin and amylin sensitive (115). Whether single SFO neurons are therefore angiotensin II, atrial natriuretic peptide, calcitonin, estradiol, leptin and osmosensitive represents an intriguing and important question, as well as a somewhat daunting experimental challenge. However, in the advent of single cell RT-PCR, single cell gene array analysis there is potential in the immediate future to provide answers to this important question.

There is to date no information regarding the ability of single CVO neurons to sense multiple signals which would traditionally be viewed as related to
different physiological systems. For example: Are leptin and amylin (energy homeostasis) sensitive SFO neurons also sensitive to angiotensin II (body fluid homeostasis)? Again, the fact that over 60% of SFO neurons are angiotensin II sensitive and leptin influences the excitability of 60% of SFO neurons (115) implies that there must be a population of SFO neurons that would be responsive to both angiotensin II and leptin. Behavioural studies demonstrating that, in addition to the decrease in food intake observed in response to peripheral administration of leptin, water intake was also reduced (10) and that angiotensin converting enzyme (ACE) inhibitors decrease body weight in mice fed a high fat diet (139) also supports this hypothesis. The pregnancy hormone relaxin may also play a role in body fluid homeostasis as exogenous administration of relaxin into the brain causes a profound drinking response (51; 123; 130) and expression of AT1 receptors in the SFO increases in parallel with the increase in circulating relaxin seen in the second half of pregnancy (51).

**Perspectives and Significance**

In light of the above evidence, the capacity of SFO neurons to sense multiple signals and thus control, in an integrated manner, broader based physiological outputs (ie energy homeostasis, cardiovascular regulation, reproduction and body fluid homeostasis) is emerging. However, research directed toward understanding central control of fluid balance, cardiovascular regulation, immune regulation, reproductive function, and energy homeostasis remains clearly separated into distinct fields. Although experimentally, divisions along the lines of
distinct autonomic function make sense, this separation, physiologically, does not. Humans very seldom eat without drinking. Impaired energy homeostasis (ie obesity) is often associated with cardiovascular pertubuations (hypertension) and reproductive dysfunction. Lack of available nutrients or the converse, profound obesity, has clear inhibitory effects on reproductive function, and systemic infections not only activate the neuroimmune axis but also profoundly influence fluid and food intake.

Thus, it seems that SFO neurons, which are uniquely positioned at the blood brain interface, have the capacity to coordinate physiological responses elicited by the various autonomic control signals, thereby establishing and maintaining an autonomic state for the entire organism, rather than for isolated individual parameters. It would seem, then, that in order to understand such potentially integrative roles of the SFO, we will need to take a far more integrative approach to the design of experiments which can assess the coordinated contributions of the SFO.

**Figure Legends:**

**Figure 1:**

This figure shows schematic representations of SFO efferent projections to autonomic nuclei subserving body fluid homeostasis, cardiovascular control, immune regulation, reproductive function and energy homeostasis.
**Figure 2:**

This figure shows that signals related to body fluid homeostasis (osmolarity), cardiovascular control, reproductive function (vasopressin), immune regulation (interleukin 1β), feeding, and metabolism (adiponectin and ghrelin) influence the activity of dissociated SFO neurons. Time and duration of peptide application or changes in osmolarity are indicated by the bar at the top of each current clamp recording.

**Figure 3:**

This summary figure highlights relative expression levels of mRNA for a number of receptors present SFO (characterized using microarray technology). This data not only confirm the presence of documented receptors shown to be present in SFO but also demonstrate the presence of many novel receptor transcripts not classically associated with the SFO or its primary functions. The presence of novel transcripts, such as the apelin, endocannabinoid (CB1), and thyroid hormone receptor α (Thyroid Horm Rec α) receptors, highlight these as potential targets for future study.
**Figure 4:**

This figure shows the relative expression levels of mRNA for peptides present SFO (characterized using microarray technology). This data not only confirm the presence of documented peptides shown to be present in SFO but also demonstrate the presence of many novel peptide transcripts not classically associated with the SFO or its primary functions. The presence of novel transcripts, such as nesfatin and CART (cocaine and amphetamine regulated transcript), highlight these as potential targets for future study.

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Smith and Ferguson Figure 1
Smith and Ferguson Figure 4

Adiponectin
Ang Conv Enzy
Angiotensinogen
Apelin
CART
CCK
CNTF
End Conv Enzy 2
Enkephalin
Fat
Glucokinase
MIF
Nesfatin
NPY
Orexin
Oxytocin
PMCH
Sim2
STAT 3
TNFα
UCP 2
Vasopressin

Relative Expression Level