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Neurophysiological characterization of mammalian osmosensitive neurones

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Abstract

In mammals, the osmolality of the extracellular fluid is maintained near a predetermined set-point through a negative feedback regulation of thirst, diuresis, salt appetite and natriuresis. This homeostatic control is believed to be mediated by osmosensory neurones which synaptically regulate the electrical activity of command neurones that mediate each of these osmoregulatory effector responses. Our present understanding of the molecular, cellular and network basis that underlies the central control of osmoregulation is largely derived from studies on primary osmosensory neurones in the organum vasculosum lamina terminalis (OVLT) and effector neurones in the supraoptic nucleus (SON), which release hormones that regulate diuresis and natriuresis. Primary osmosensory neurones in the OVLT exhibit changes in action potential firing rate that vary in proportion with ECF osmolality. This effect results from the intrinsic depolarizing receptor potential which these cells generate via a molecular transduction complex that may comprise various members of the transient receptor potential vanilloid (TRPV) family of cation channel proteins, notably TRPV1 and TRPV4. Osmotically evoked changes in the firing rate of OVLT neurones then regulate the electrical activity of downstream neurones in the SON through graded changes in glutamate release.

Introduction: mammals tightly regulate extracellular fluid (ECF) osmolality

Acute changes in ECF osmolality cause water to flow across the plasma membrane and therefore provoke cellular swelling or shrinking. Although many types of cells are endowed with an innate ability to restore their volume following osmotic perturbations (Wehner *et al.* 2003), this adaptation is often incomplete and can occur with a delay of seconds or minutes (McManus *et al.* 1995). Fragile tissues, such as brain, can thus be significantly damaged by the mechanical impact of acute pathological osmotic perturbations (Verbalis, 2006). Fortunately, animals have evolved behavioural and physiological mechanisms that together work to maintain systemic osmolality near a stable set-point despite the episodic nature of salt and fluid intake (Bourque *et al.* 1994). Mammals, in particular, aggressively maintain ECF osmolality near a value of 300 mosmol kg^{-1}. Although various species of mammals defend slightly different osmotic set-points (e.g. humans ~ 280 mosmol kg^{-1}, rats ~295

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mosmol kg^{-1}, mice ~ 310 mosmol kg^{-1}), individuals with free access to salt and water normally maintain ECF osmolality within 3% of their native set-point. In humans, for example, increases in plasma osmolality of about 9 mosmol kg^{-1} accompany a state of mild hypernatraemia (Andersen *et al.* 2002). Here we review our present understanding of the mechanisms by which the brain detects the body's hydration status and initiates responses that mediate osmotic homeostasis.

Systemic osmoregulatory responses are controlled by the central nervous system

Previous studies have shown that mammals maintain osmotic homeostasis by making proportional adjustments in the intake and excretion of sodium and water when blood osmolality deviates from the set-point value by more than 1%. These adjustments are mediated largely by concerted changes in behaviour, neurohypophysial hormone release and sympathetic outflow (see below). Although the basis for this concerted regulation is unclear, the central control of body fluid balance is presumably mediated by osmotically evoked changes in the electrical activity (i.e. action potential firing rate or pattern) of distinct subsets of 'command' neurones that regulate each of the osmoregulatory responses.

Osmotic control of water intake

Water intake is controlled through a modulation of thirst. Specifically, hypertonic conditions enhance the cognitive sensation of thirst to promote a homeostatic increase in water intake, whereas hypotonic conditions have the reverse effect (see Bourque *et al.* 1994; Denton *et al.* 1996). Studies involving electrical stimulation of different cortical areas in animals and functional brain imaging in humans have highlighted a number of regions that may be involved in the genesis and satiation of thirst (McKinley *et al.* 2006). Among these, the anterior cingulate cortex (ACx) stands out as a strong candidate area for the command of thirst. The ACx is coincidentally activated and inhibited under conditions which, respectively, promote thirst and satiation (Egan *et al.* 2003), and stimulation of this area reliably induces drinking in monkeys (Robinson & Mishkin, 1968). Direct evidence that subsets of ACx neurones serve as command neurones for the sensation of thirst remains to be obtained.

Osmotic control of sodium intake

The control of sodium intake is achieved through a modulation of appetite for salt. Specifically, hypotonic conditions have been shown to contribute to the homeostatic enhancement of salt appetite, whereas hypertonic conditions have the reverse effect (for review see Bourque *et al.* 1994; Daniels & Fluharty, 2004). A variety of brain areas have been shown to play important roles in the control of salt intake under various physiological conditions, and an integrative analysis of these studies has indicated that neural pathways between forebrain and brainstem systems are likely to be key components of the circuitry that gives rise to salt appetite (Daniels & Fluharty, 2004). Unfortunately, the identity of putative command neurones for the genesis of salt appetite has remained elusive.

Osmotic control of water excretion

The osmotic control of water excretion (diuresis) is primarily achieved through changes in the plasma concentration of vasopressin (VP, the antidiuretic hormone). Specifically, systemic hypotonic conditions suppress VP release from the neurohypophysis, thus reducing the kidney's ability to reabsorb water. Conversely, hypertonic conditions stimulate VP release, which promotes homeostatic water conservation. Vasopressin is synthesized in magnocellular neurosecretory cells (MNCs) located in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus. The release of VP into the circulation occurs at the neurohypophysial axon terminals of MNCs in response to action potential discharge. Vasopressin-secreting MNCs thus represent the 'command' neurones that control diuresis, which varies as an inverse function of the firing rate of these neurones (Bourque *et al.* 1994).

Osmotic control of sodium excretion

The osmotic control of sodium excretion (natriuresis) occurs at the kidney (Andersen *et al.* 2002), where it is regulated by the effects of various hormones (e.g. Antunes-Rodrigues *et al.* 2004; Bie *et al.* 2004) and by innervating sympathetic fibres (e.g. DiBona, 1977). Although peripheral organs can produce hormones that can regulate natriuresis (e.g. aldosterone, angiotensin II and atrial natriuretic peptide), oxytocin (OT) released by OT-synthesizing MNCs has been shown to act as a natriuretic hormone (Verbalis *et al.* 1991) and to stimulate natriuresis under hypertonic conditions (Huang *et al.* 1996). Thus the brain can directly contribute to the osmotic control of sodium excretion via the modulation of electrical activity in OT-MNCs, which operate as command neurones that can modulate this process.

Osmosensitive neurones form the core of the osmostat

The feedback regulation of osmoregulatory responses implies the existence of an 'osmostat', a specialized sensory apparatus through which ECF osmolality can be measured and compared with a predetermined set-point. Since various responses are modulated by distinct populations of command neurones, the osmostat must first transduce osmotic perturbations into a neural code (i.e. a change in action potential firing rate or pattern) that is subsequently used to regulate these distributed command neurones via axonal projections and synaptic contacts. Electrophysiological studies have shown that the core function of the osmostat is performed by osmosensitive neurones, i.e. neurones that can display significant changes in their rate or pattern of action potential discharge in response to osmotic perturbations.

Osmosensitive neurones are widely distributed

In principle, the central control of systemic osmoregulation could be mediated by a single osmostat wired to neurones located in integrative centres and/or directly to command neurones located in the 'effector' parts of the brain. However, it is now known that neurones with intrinsic osmosensory properties can be found in various parts of the central and peripheral nervous systems, including central nuclei thought to contain sensory, integrative and effector neurones (Bourque *et al.* 1994).

Intrinsically osmosensitive neurones have been found in the organum vasculosum of the lamina terminalis (OVLT; Vivas *et al.* 1990; Ciura & Bourque, 2006), a small brain area believed to serve as the brain's primary osmostat (Johnson *et al.* 1992; Denton *et al.* 1996; McKinley *et al.* 2006). Osmosensitive neurones have also been found in the subfornical organ (Anderson *et al.* 2000) and in the nucleus tractus solitarii (Izawa *et al.* 2000), central nuclei that appear to integrate osmotic information with humoral and ascending interoceptive sensory signals (Johnson *et al.* 1992; Daniels & Fluharty, 2004). Outputs from these nuclei may modulate osmoregulatory responses in a manner that co-ordinates osmotic homostasis with that of other cardiovascular parameters (e.g. ECF volume). Interestingly, osmotic signals can also be detected by primary chemosensory afferents (Gallego *et al.* 1979) and by afferent fibres in the hepatic branch of the vagus nerve (Adachi *et al.* 1976). Osmosensory information collected from the splanchnic mesentery is also known to be relayed to central areas via ascending projections carried in part via spinal pathways (Vallet & Baertschi, 1982; King & Baertschi, 1991). Finally, the VP and OT neurones of the hypothalamus are also intrinsically osmosensitive (Mason, 1980; Oliet & Bourque, 1993), indicating that at least some subtypes of osmoregulatory command neurones can also express the osmosensitivity phenotype.

The significance of this distributed localization of osmosensitive neurones is presently unclear. One possibility is that some of the osmosensitive cells in these different areas may be wired to each other in a manner that amplifies osmotic signalling. Indeed, the osmotic control of the firing rate of OT- and VP-releasing MNCs has been shown to result from an integration of multiple factors, including information originating from central and peripheral osmoreceptors, and the intrinsic osmosensitivity of the neurones (Russell *et al.* 1988; Bourque *et al.* 1994; Hussy *et al.* 2000; Voisin & Bourque, 2002). The upregulation of 'redundant' osmostat mechanisms might also explain why animals eventually recover from the acute osmoregulatory deficits induced by lesions of the OVLT (e.g. Carithers & Johnson, 1986), and why transgenic animals which lack molecules that are important for specific osmosensory mechanisms can still osmoregulate (e.g. Liedtke & Friedman, 2003; Ciura & Bourque, 2006; Sharif-Naeini *et al.* 2006). Different osmosensitive neurones might also constitute functionally distinct osmostats. For example, osmosensory neurones might differ in terms of the type of stimulus which they are able to detect (i.e. responsiveness to hypotonic or hypertonic stimuli), or in the mechanism by which they regulate downstream neurones (e.g. by providing inhibitory or excitatory signals). Indeed, as illustrated in Fig. 1, osmotic perturbations lead to the simultaneous activation and inhibition of different subsets of osmoregulatory responses. The sensory and network processes that underlie this co-ordinated regulation remain to be defined. In the remainder of this article, we review our present understanding of the mechanisms by which neurones in the OVLT operate as primary osmostats.

Neurones in OVLT are excited by hypertonic stimuli

Electrophysiological recordings *in vivo* (Honda *et al.* 1990) and *in vitro* (Sayer *et al.* 1984; Vivas *et al.* 1990; Nissen *et al.* 1993) have shown that a majority of neurones in the OVLT are excited by hypertonic stimuli and inhibited by hypotonicity. The functional basis for this osmoresponsiveness has remained unknown until recently. Indeed, experiments on neurones

isolated from the OVLT of adult mice have provided definitive evidence that these cells are intrinsically sensitive to increases in the osmolality of the extracellular fluid (Ciura & Bourque, 2006). Hypertonic conditions were found to provoke increases in membrane non-selective cation conductance, thereby causing the generation of an inward current at normal resting potential. This inward current induces a depolarizing osmoreceptor potential that increases the probability of action potential discharge. Indeed, changes in firing rate induced by hypertonic stimuli are directly proportional to the magnitude of the depolarizing osmoreceptor potential (Ciura & Bourque, 2006). Thus, as commonly observed in cells that transduce other sensory modalities, OVLT neurones transduce osmotic signals by generating a depolarizing receptor potential. The intensity of the osmotic stimulus is encoded by the changes in firing rate that are graded in proportion with this potential.

Mechanism of osmosensory transduction in OVLT neurones

Genetic and molecular biological studies have recently highlighted the possible role of different members of the transient receptor potential vanilloid (TRPV) proteins in osmosensory transduction (Liedtke, 2006). When expressed in heterologous cells, some of the genes coding for these proteins lead to the formation of non-selective cation channels whose probability of opening can be modulated by changes in osmolality. For example, homomultimeric channels assembled from TRPV2 (Muraki *et al.* 2003) and TRPV4 (Liedtke *et al.* 2000; Strotmann *et al.* 2000) can be activated by hypotonic stimuli. Although it is certainly possible that hypotonicity-activated cation channels could mediate osmosensory transduction, native osmosensory neurones showing intrinsic depolarizing (i.e. excitatory) responses to hypotonic stimuli have yet to be described. Since the majority of OVLT neurones are excited by hypertonic stimuli (Sayer *et al.* 1984; Vivas *et al.* 1990; Ciura & Bourque, 2006), it seems unlikely that the cation channels transducing these effects are homomultimers of TRPV2 or TRPV4. Nonetheless, products of the *Trpv2* or *Trpv4* genes could still contribute to a heteromultimeric osmosensory transduction complex in OVLT neurones. Indeed, *Trpv4*$^{-/-}$ mice show impaired thirst and VP responses to systemic osmotic stimuli, and the expression of Fos protein that normally occurs in OVLT neurones under hypertonic conditions is significantly reduced in these animals (Liedtke & Friedman, 2003). Interestingly, recent studies have shown that mice lacking normal expression of the *Trpv1* gene (i.e. *Trpv1*$^{-/-}$ mice) also display impaired thirst responses (Ciura & Bourque, 2006) and VP release (Sharif-Naeini *et al.* 2006) in response to hypertonic stimulation. Remarkably, electrophysiological analysis has shown that OVLT neurones obtained from *Trpv1*$^{-/-}$ mice lack the ability to respond to hypertonic stimuli *in vitro* (Ciura & Bourque, 2006). It is therefore likely that expression of the *Trpv1* gene is required for the osmosensitivity of these cells, as was also shown for MNCs in the SON (Sharif-Naeini *et al.* 2006). In agreement with this hypothesis, the responses of wild-type (WT) OVLT neurones to hypertonic stimuli can be blocked by Ruthenium Red, a generic inhibitor of TRPV channels (Ramsey *et al.* 2006). The molecular composition of the osmosensory transducer expressed in OVLT neurones is therefore likely to include a product of the *Trpv1* gene, and possibly other members of this family (e.g. products of *Trpv4*). Further studies are required to establish the molecular structure of the osmoreceptor and to establish the mechanisms by which osmotic stimuli regulate the opening probability of the transduction channel.

Neurones in OVLT modulate downstream effector neurones via glutamatergic synapses

Lesions of the OVLT have been shown to interfere with the osmotic modulation of most of the homeostatic responses illustrated in Fig. 1, and this area is now believed to be the brain's primary osmostat (Johnson *et al.* 1992; Denton *et al.* 1996). However, it is still not known how neurones in this area mediate the co-ordinated control of all effector responses. The OVLT is a relatively heterogeneous structure that contains neurones expressing a variety of chemical neurotransmitters, including biogenic amines, amino acids and neuropeptides (Landas & Phillips, 1987). Moreover, tracing studies have shown that the OVLT sends efferent projections to a wide variety of hypothalamic and extrahypothalamic brain regions (e.g. Camacho & Phillips, 1981; Armstrong *et al.* 1996). A recent study combining *in situ* hybridization and immunohistochemical detection has revealed that the OVLT contains both GABAergic (i.e. inhibitory) and glutamatergic (i.e. excitatory) neurones (Grob *et al.* 2003). Surprisingly, direct evidence concerning the chemical identity of osmosensitive OVLT neurones has yet to be obtained. However, some insight into this question has been provided by anatomical and electrophysiological studies examining the functional connectivity between OVLT neurones and the MNCs in the SON. In agreement with electron microscopic analysis indicating that both GABAergic and glutamatergic OVLT neurones send monosynaptic projections to the SON (Armstrong *et al.* 1996), electrical stimulation of the OVLT in *in vitro* hypothalamic explants elicits overlapping inhibitory (IPSPs) and excitatory postsynaptic potentials (EPSPs) in MNCs of this nucleus (Yang *et al.* 1994). When the spontaneous electrical activity of neurones in the OVLT is depressed by the application of a local inhibitory stimulus (e.g. via local delivery of GABA onto the OVLT), the rates of spontaneous EPSPs and IPSPs detected in SON neurones are both depressed, confirming that both glutamatergic and GABAergic OVLT neurones can synaptically modulate the electrical activity of these cells (Richard & Bourque, 1995). However, when a hypertonic stimulus is applied to the OVLT, the rate of spontaneous IPSPs detected in MNCs is unaffected (Richard & Bourque, 1995), whereas that of spontaneous excitatory synaptic events is increased (Richard & Bourque, 1995; Trudel & Bourque, 2003). These observations suggest that the subset of osmosensitive OVLT neurones that project to the SON comprises exclusively glutamatergic neurones. Indeed, in hypothalamic explants, osmotically evoked changes in the rate of spontaneous EPSPs are positively correlated with the rate at which action potentials are fired by SON neurones, and the excitatory responses of SON neurones to hyperosmotic stimulation of the OVLT can be inhibited by pharmacological blockade of ionotropic glutamate receptors (Richard & Bourque, 1995).

These observations provide strong evidence indicating that osmostat signalling between the OVLT and effector (VP/OT) neurones in the SON is mediated in part by excitatory synapses. Specifically, glutamatergic neurones in the OVLT encode ECF osmolality via proportional changes in their rate of spike discharge, and this information is transmitted to MNCs in the form of a glutamate-dependent excitatory synaptic drive whose intensity varies in proportion with the firing rate of the OVLT neurone. Although the results imply that GABAergic OVLT neurones projecting to the SON are not osmosensitive, it must be cautioned that the studies cited were performed in hypothalamic explants (e.g. Richard & Bourque, 1995) or slices

(e.g. Trudel & Bourque, 2003) in which the contribution of an osmosensitive GABAergic input might be impaired or absent. Thus the possibility that osmosensitive (or osmoresponsive) GABAergic neurones also participate in the osmotic control of SON neurones cannot be excluded. Indeed, previous studies have suggested that an active inhibitory process may be involved in the control of VP release under hypotonic conditions (e.g. Verbalis & Dohanics, 1991), and the osmotic control of firing rate in SON neurones *in vivo* appears to require a coactivation of excitatory and inhibitory inputs onto these neurones (Leng *et al.* 2001). The nature and origin of this putative osmotically modulated inhibitory input has yet to be identified. Whether the control of other osmoregulatory effector neurones relies on a direct excitatory modulation mediated by osmosensitive glutamatergic OVLT neurones or osmosensitive GABAergic OVLT neurones remains to be determined.

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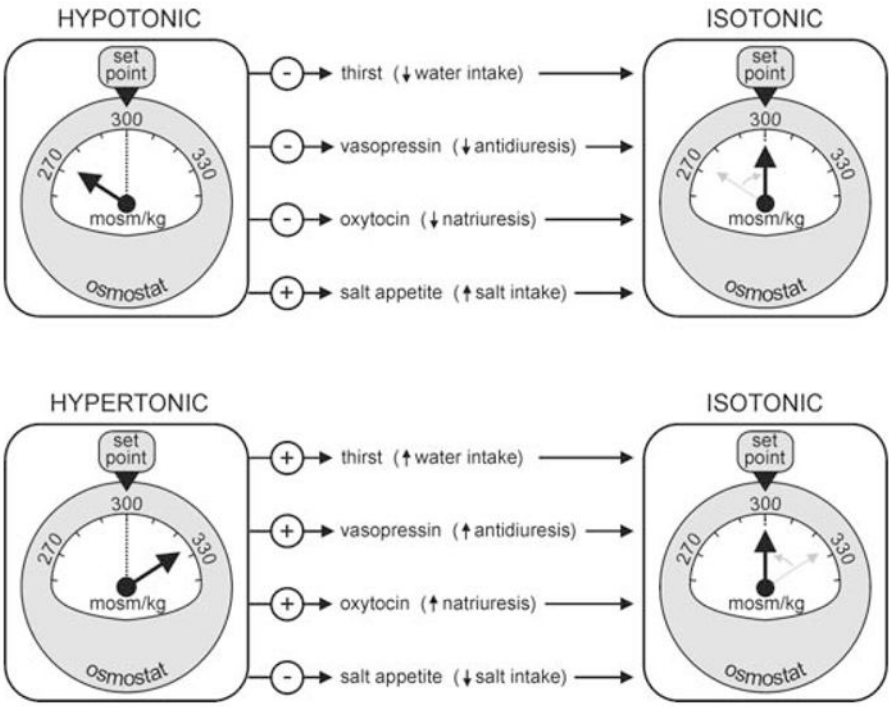

Figure 1. Osmostatic control of systemic osmoregulatory responses

Schematic diagram illustrating some of the features of osmostat function. The dashed line shows the desired set-point (300 mosmol kg^{-1}), and the arrow points to the prevailing ECF osmolality which is measured by the device. Arrows leading from the osmostat on the left indicate which of the effector responses are inhibited (−) or excited (+) under hypotonic (upper panel) or hypertonic conditions (lower panel) to restore homeostasis (i.e. osmostats on the right side, where ECF osmolality = set-point).