Contribution of TRPV channels to osmosensory transduction, thirst, and vasopressin release

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Systemic osmoregulation is an integrated physiological process through which water intake and excretion are continuously balanced against salt intake and excretion to maintain the osmolality of the extracellular fluid near an optimal 'set-point' value. The behaviors (that is, thirst and sodium appetite) and renal responses (diuresis and natriuresis) that are modulated to mediate osmoregulatory homeostasis are mainly controlled by the nervous system. Appropriate regulation of these parameters depends in large part on specialized osmosensitive neurons, termed osmoreceptors, which convert changes in plasma osmolality into electrical signals that ultimately modulate effector functions to achieve homeostasis. Previous work has shown that mechanosensitive cation channels expressed in osmoreceptor neurons play a key role in the process of osmosensory transduction. Although the molecular identity of these channels remains unknown, a growing body of evidence, reviewed here, indicates that members of the transient receptor potential vanilloid family of ion channels may contribute to osmosensory transduction and to homeostatic responses implicated in the control of water balance.

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SYSTEMIC OSMOREGULATION AND OSMORECEPTORS

Changes in extracellular fluid osmolality resulting from the loss or gain of systemic water or electrolytes are experienced by all vascularized tissues, where they can interfere with the normal volume, metabolism, and function of individual cells. In the brain, acute changes in fluid osmolality can affect the excitable properties of neurons, and thus induce a progression of clinical symptoms that can range from headaches and mental confusion to convulsive seizures and death (reviewed by Verbalis[1] and Bourque *et al.*[2]). Fortunately, mammals have evolved efficient homeostatic responses that strive to maintain extracellular fluid osmolality near a relatively stable set point.[1–4] Small (±1%) changes in plasma osmolality trigger behavioral and neuroendocrine responses that balance the amount of water and salt gained through ingestion and metabolism with those lost through breathing, sweating, and urine (reviewed by Verbalis[1] and Bourque *et al.*[2]). Behavioral responses are mediated in part by the hypothalamus, which contributes to the osmotic control of thirst and appetite for salt (reviewed by Bourque *et al.*[2]). Neuroendocrine responses are also controlled by the hypothalamus, which participates in regulating the rate of renal excretion of sodium (natriuresis) and water (diuresis) through changes in the release of neurohypophysial natriuretic and antidiuretic hormones.[1–4] The present review focuses specifically on the mechanisms that mediate the osmotic control of water balance. As illustrated in Figure 1, systemic hypotonicity suppresses thirst to reduce water intake, and inhibits vasopressin (VP) release to promote water excretion by the kidney. Systemic hypertonicity, in contrast, is associated with an increase in thirst and VP release to increase the intake and retention of water.[1–4]

Previous work has shown that the orchestration of these osmoregulatory mechanisms occurs in the brain and depends on the existence of osmoreceptors. These types of neurons are defined not only by their intrinsic sensitivity to changes in fluid osmolality but also by their ability to regulate neuronal systems responsible for the production of specific osmoregulatory responses.[2] These osmoreceptors are located in several brain areas (for details, see review by Bourque *et al.*[2]), including the organum vasculosum laminae terminalis (OVLT),[5,6] as well as the supraoptic (SON)[7] and paraventricular[8] nuclei of the hypothalamus. Although many brain areas likely contain osmoreceptors involved in the osmotic

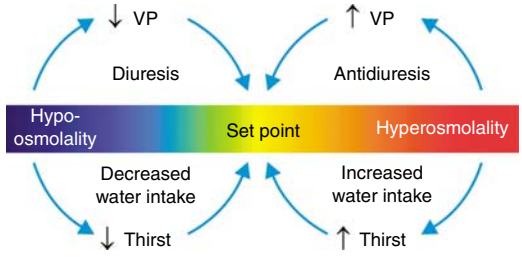

Figure 1 | Osmotic regulation of thirst and VP release. Changes in systemic water loss or intake provoke fluid hypo-osmolality, or hyperosmolality, relative to a predetermined set point (color-coded horizontal bar). Central osmoreceptor neurons calculate the difference between the prevailing osmolality and the set point, and then trigger proportional changes in VP release (upper arrows) or thirst (lower arrows) to achieve homeostasis.

control of osmoregulatory responses, this review will highlight findings obtained from OVLT and SON neurons, where recent studies have provided significant advances in our understanding of the cellular and molecular processes involved in osmoreception.

Electrophysiological recordings from osmoreceptors in the SON of anesthetized rats have revealed that the rate at which action potentials are discharged by these neurons varies as a positive function of plasma osmolality.[9] Intraperitoneal injection of water, which reduces plasma osmolality, reduces the firing rate of SON neurons,[10] whereas injection of hypertonic solutions provokes an increase in firing rate.[10] Similar responses have been observed *in vitro* in primary osmoreceptors in the OVLT,[5,6] where osmosensory information encoded by changes in neuronal firing is relayed to downstream effector neurons that are more directly responsible for controlling thirst and VP release.[2,11] Indeed, lesions encompassing the OVLT significantly impair the osmotic control of thirst and VP release from the neurohypophysis.[11]

CELLULAR MECHANISMS FOR OSMORECEPTION

In 1937, Gilman[12] demonstrated that drinking in dogs could be evoked by injection of hypertonic sodium chloride but not by an equivalent osmotic load of membrane-permeant urea. This finding suggested that the sensation of thirst arises as a consequence of 'cellular dehydration' (that is, reduced cell volume), which results from efflux of cytoplasmic water upon increased external osmotic pressure.[12] Similar to Gilman's finding on thirst, Verney[3] discovered that VP release in dogs could only be evoked by hypertonic solutions comprising an excess of sodium chloride, sodium sulfate, or sucrose, but not urea. Therefore, detection of hyperosmotic conditions requires that the excess solute be membrane impermeant, implying a role for cellular volume changes in the primary sensory event.

Osmotic stimuli modulate non-selective cation channels

Intracellular recordings from rat hypothalamic slices maintained *in vitro* have revealed that SON neurons become depolarized when exposed to hypertonic solutions.[7] Similarly, hypertonic stimulation of acutely isolated rodent OVLT and SON neurons voltage clamped at the resting potential have been shown to elicit a reversible inward (that is, depolarizing) current.[6,13,14] Current–voltage analysis indicated that these responses are associated with an increase in membrane conductance, and that the current induced by hyperosmolality reverses at a voltage consistent with the involvement of non-selective cation channels (that is, −40 to −10 mV, depending on ionic conditions).[6,13–15] Conversely, hypotonic stimuli were found to cause hyperpolarization because of the suppression of a non-selective cation current.[13,14] These observations provided the first evidence indicating that a single population of cation channels might mediate osmosensory transduction through a proportional modulation of their probability of opening during changes in fluid osmolality.

Role of cell volume in osmosensory transduction

Morphometric analysis of isolated rat SON neurons, using confocal laser scanning microscopy, combined with whole-cell patch-clamp measurements revealed that the increase in membrane conductance provoked by a transient hypertonic stimulus is reversibly linked to a decrease in cell volume,[13,14] supporting the notion that changes in cell volume play a key role in osmoreception. The close temporal association between changes in cell volume and changes in cation conductance further implied that generation of a long-lived second messenger was unlikely to participate in the production of these responses. Given these constraints, two mechanisms stand out as possible candidates for the regulation of cation conductance during changes in fluid osmolality. First, it is possible that changes in physical strain associated with osmotically induced volume changes could regulate an intrinsically mechanosensitive transduction channel. Alternatively, because variations in cell volume caused by osmotic stimuli result from a net loss or gain of water, it is possible that accompanying changes in intracellular solute concentration could play a direct role in osmosensory transduction.

To resolve this issue, the effects of swelling or shrinking of isolated SON neurons were examined in the absence of osmotic stimulation. It was observed that a decrease in cell volume provoked by pipette suction elicited depolarization, whereas pressure-evoked swelling caused hyperpolarization.[14] Examination of current–voltage relations during isotonic shrinking and swelling indicated that depolarizing and hyperpolarizing responses are associated with increases and decreases in membrane conductance, respectively, and that the currents underlying both responses reversed at voltages equivalent to osmotically modulated currents.[13,14] More importantly, responses to hypertonic stimuli could be reversed by inflating the cells via increased pressure in the recording pipette, whereas responses to hypotonic stimuli could be reversed by applying suction to the pipette.[14] The results of these experiments indicated that changes in cell volume, rather than variations in cytosolic solute

concentrations, are required for the osmotic modulation of the cation channels mediating osmosensory transduction.

Properties of ion channels underlying osmoreception

Cell-attached single-channel recordings from SON neurons revealed the existence of mechanosensitive cation channels that could be reversibly modulated by slight changes in pipette pressure, or by modifying the osmolality of the extracellular fluid, identifying these channels as possible mechanotransducers for osmoreception.[13] In these cells, the average opening probability (P_o) of the mechanosensitive channels recorded in cell-attached patches was found to be maximal near zero pressure and to decrease in response to membrane stretch caused by increasing or decreasing pipette pressure.[13] Because their activity appears to be inhibited by membrane stretch, the channels expressed in SON neurons are functionally designated as being stretch-inhibited (SI). As illustrated in Figure 2, the presence of SI cation channels in SON neurons is consistent with the osmotic regulation of cell volume, macroscopic conductance, and membrane potential in these cells. Thus, by increasing membrane stretch, hypotonic cell swelling suppresses SI channel activity, reduces whole-cell cation conductance, and provokes membrane hyperpolarization. Conversely, relaxation of membrane stretch during hypertonic shrinkage increases channel activity and membrane cation conductance, thereby depolarizing the cell.

Efforts aiming to enhance our molecular understanding of osmosensory transduction have been hampered by the lack of information regarding the identity of the SI channel and associated proteins. It is reasonable to state that any molecularly defined channel identified as a putative transduction channel of osmoreceptor neurons in the SON should display the following properties: (1) be activated by cell shrinkage and inhibited by cell swelling during whole-cell recordings; (2) be directly mechanosensitive, that is, be inhibited by membrane stretch during cell-attached single-channel recordings; (3) display ion permeability characteristic of a non-selective cation channel; (4) be inhibited by pharmacological inhibitors of osmosensory transduction such as ruthenium red and gadolinium. Ultimately, the identification of gene(s) encoding the osmosensory transduction channel complex will lead to a better understanding of the mechanisms through which changes in extracellular fluid osmolality control this channel, and reveal how mutations in these genes might contribute to pathologies related to hydromineral homeostasis.[1]

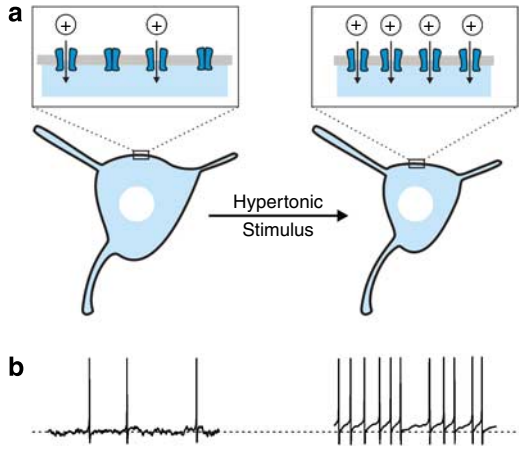

Figure 2 | Proposed mechanism of osmosensory transduction in osmoreceptors. Under resting conditions (**a**, left side), a proportion of the SI cation channels is active and allows the influx of positive charge, contributing to membrane depolarization and triggering of action potentials (**b**, left side). Hypertonic stimulation provokes cell shrinking and increases the proportion of active SI channels (**a**, right side). The resulting increase in positive charge influx depolarizes the membrane (**b**, right side, see dotted line) and increases neuronal action potential firing frequency. Under hypotonic conditions, the SI channels are inhibited and the loss of cation influx causes hyperpolarization and inhibits firing (data not shown).

POTENTIAL ROLE OF TRANSIENT RECEPTOR POTENTIAL VANILLOID CHANNELS IN OSMORECEPTION

The first evidence indicating a role for transient receptor potential vanilloid (TRPV) channels in osmoreception came from experiments performed on the worm *Caenorhabditis elegans* by Colbert *et al.*[16] They demonstrated that worms lacking a functional OSM-9 protein (which shares homology with the *Drosophila* TRP channel) fail to display a stereotypical avoidance behavior when encountering a high osmolality solution. The possible involvement of TRPV channels in mammalian central osmoreceptors was also suggested by observation that hypertonicity-induced responses of osmoreceptors can be blocked by ruthenium red,[6,17] a broad-spectrum antagonist of TRPV channels.[18]

TRPV2 and TRPV4 are osmosensitive channels

In an attempt to identify vertebrate homologs of the *osm-9* gene, two independent groups identified TRPV4, the first mammalian TRPV channel shown to be osmosensitive.[19,20] When expressed as a homomeric channel in heterologous systems, TRPV4 can be activated by cell swelling,[19,20] but is unresponsive to cell shrinkage.[19] Strotmann *et al.*[20] showed that this channel is not directly mechanosensitive, as no response to changes in pipette pressure was observed in cell-attached single-channel recordings. Because of its activation by cell swelling, its unresponsiveness to cell shrinkage, and its lack of direct mechanosensitivity, TRPV4 channels are unlikely to function on their own (that is, as homomultimeric complexes) as the transduction channels of central osmoreceptor neurons in rodents.

TRPV2 is another member of the TRPV family that can be modulated by osmolality and might therefore play a role in osmoreception. Indeed, Muraki *et al.*[21] demonstrated that TRPV2 is expressed in murine vascular smooth muscle cells, where it can be directly activated by membrane stretch. When freshly isolated cells from mouse aorta were exposed to hypotonic solutions, the cell swelling that followed was associated with the activation of a non-selective cation current and an increase in $[Ca^{2+}]_i$.[21] Treatment of mouse aorta with TRPV2 antisense oligonucleotides resulted in

suppression of these hypotonicity-induced responses. Finally, stimulation of TRPV2-transfected Chinese hamster ovary cells by application of membrane stretch through the recording pipette and hypotonic stimulation consistently activated single non-selective cation channels.[21] Although these experiments suggest that homomultimeric TRPV2 channels may be directly mechanosensitive, the channels functionally operate as stretch-activated channels, rather than SI channels. It would appear therefore that homomultimeric TRPV2 channels are unlikely to function as the mechanosensitive transducers in rodent osmosensory neurons.

The possibility remains, however, that a heteromultimeric complex comprising a variety of TRPV channel subunits (including TRPV2 and/or TRPV4, or splice variants of these channels) might display SI gating, and thus play an integral role in the central osmosensory transducer channel. Indeed, TRPV channel subunits have been shown to heteromerize both in heterologous[22] and in native[23] systems. Furthermore, evidence that channels encoded by both *trpv2*[24] and *trpv4*[19,25] genes are expressed in osmoreceptor neurons supports the hypothesis that these channels might play a role in osmoreception. Indeed, studies using *trpv4*-knockout ($trpv4^{-/-}$) mice have demonstrated that the *trpv4* gene contributes to fluid balance and to the osmotic control of VP release in mice.[25,26] For instance, Liedtke and Friedman[25] observed that under basal conditions, $trpv4^{-/-}$ mice drank less water and became more hyperosmolar than did wild-type (*WT*) littermates. In addition, $trpv4^{-/-}$ mice injected intraperitoneally with hypertonic saline had a significantly lower increase in circulating plasma VP concentration and a markedly lower expression of c-FOS in the OVLT than *WT* mice. These data suggest that TRPV4 channels play a role in osmosensation and in the generation of thirst in response to systemic hypertonicity.

The *trpv1* gene contributes to osmosensory transduction and osmoregulation

Relatively few reports have investigated the potential osmosensitivity of TRPV1. Liedtke *et al.*[19] reported that TRPV1-transfected HEK293 cells did not display any change in $[Ca^{2+}]_i$ in response to hypotonic stimulation. Furthermore, the transgenic expression of TRPV1 in OSM-9-mutant worms failed to rescue their hyperosmotic avoidance behavior defect.[27] In two recent studies,[6,17] we examined the role of *trpv1* gene products to the intrinsic osmosensitivity of osmoreceptors. Through a combination of reverse transcriptase-PCR and immunohistochemistry, we found that SON neurons express a capsaicin-insensitive splice variant of TRPV1, in which a portion of the N-terminal domain is truncated.[17] Moreover, electrophysiological analysis of SON and OVLT neurons of TRPV1-knockout ($trpv1^{-/-}$) mice revealed that products of the *trpv1* gene may contribute to the intrinsic osmosensitivity of these neurons. Indeed, although hyperosmolality still caused a reduction in cell volume in SON[17] and OVLT[6] neurons isolated from $trpv1^{-/-}$ mice, this effect was no longer associated with the increase in membrane conductance, membrane depolarization, or increase in firing frequency that is normally observed in cells obtained from *WT* mice (Figure 3). In agreement with these observations, hypertonicity-evoked thirst[6] and VP release[17] were both found to be significantly attenuated in $trpv1^{-/-}$ mice. Moreover, serum osmolality was significantly higher in $trpv1^{-/-}$ mice than in *WT* mice (321.2 ± 0.7 compared to $312.0 \pm 0.4\ mOsmol\ kg^{-1}$; $P = 1 \times 10^{-9}$),[17] a difference equivalent to that caused by 24-h dehydration in rats.[9]

CONCLUSION

Osmosensory transduction in central osmoreceptors involves a mechanical modulation of SI cation channels during changes in cell volume. Recent studies have shown that the *trpv1* gene may play an important role in the assembly of these SI channels and animals lacking either *trpv1* or *trpv4* gene show defects in systemic osmoregulation. As TRPV channel subunits are known to heteromerize,[22,23] it is possible that the osmosensory transducer of native osmoreceptors is a complex comprising more than one subtype of TRPV subunit. Identifying the molecular channel complex

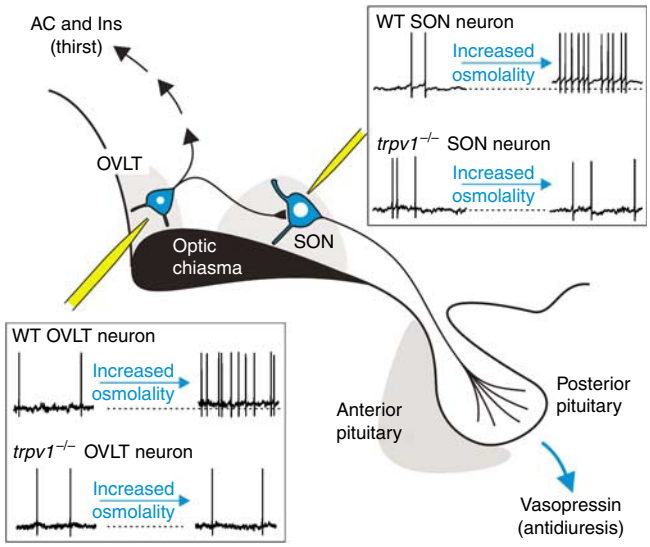

Figure 3 | *Trpv1* gene is required for osmosensory transduction. Sagittal diagram illustrating the positions of OVLT and SON in the rodent brain, relative to other hypothalamic structures. Osmoreceptor neurons in the OVLT of wild-type (WT) animals are normally depolarized by hypertonic conditions, thus increasing the rate of action potential discharge (upper traces in the inset shown at lower left). Such neurons send axonal projections to the SON, where they release the excitatory transmitter glutamate to synaptically excite the neurons that release VP. OVLT neurons also project via unspecified pathways to cortical areas such as the insula (Ins) and the anterior cingulate gyrus (AC), which are believed to be involved in the perception of thirst (reviewed by McKinley *et al.*[28]). The depolarization and excitation of VP-releasing neurons in the SON induced by hyperosmolality (upper traces in the inset shown at upper right) are caused by the combined action of SI channels and ionotropic glutamate receptors expressed in these cells. Experiments have shown that OVLT and SON neurons from $Trpv1^{-/-}$ mice lack excitatory responses to hypertonic stimuli (lower sets of traces in the insets).

that underlies osmosensory transduction will help us understand how changes in cell volume are coupled to changes in electrical activity in osmoreceptors, and how mutations in relevant genes might contribute to central pathologies related to hydromineral homeostasis.[15]

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