

# How Does the Brain Sense Osmolality?

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## ABSTRACT

For nearly 60 years, we have known that the brain plays a pivotal role in regulating the osmolality of body fluids. Over this time period, scientists have determined the structure and function of arginine vasopressin and its receptors, the role of the posterior pituitary as a storage site, and the determinants of vasopressin release. The cellular mechanisms by which the kidney responds to vasopressin are also well understood. One area that remains unclear is the neural mechanisms underlying osmoreception. New findings have implicated the TRPV family of cation channels as osmo-mechanoreceptors that may mediate the neuronal responses to changes in systemic tonicity. This topic is reviewed here.

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Body fluid homeostasis is directed at maintaining the stability of the osmolality of body fluids (osmotic homeostasis) and the intravascular blood volume (volume homeostasis). Osmotic regulation serves to minimize osmotically induced perturbations in cell volume, which has adverse effects on multiple cellular functions. Body fluid osmolality in humans is maintained between 280 and 295 mOsm/kg H<sub>2</sub>O, representing one of the most highly regulated parameters of body physiology. This is accomplished through an integration of thirst, arginine vasopressin (AVP) secretion, and renal responsiveness to AVP. To preserve plasma osmolality within such narrow tolerances, pituitary AVP secretion must vary in response to small changes in plasma osmolality, which is achieved through the activation or inhibition of central osmoreceptor cells. Understanding where and how the brain senses the osmolality of body fluids and transduces this information into mechanisms that regulate AVP secretion and thirst is the subject of this commentary.

## WHERE ARE OSMORECEPTORS LOCATED?

The pioneering investigations of Verney in the 1940s<sup>1</sup> found infusion of hyperosmotic solutions into blood vessels that perfused the anterior hypothalamus produced an antidiuresis in dogs, thereby identifying this area as the site of osmo-responsive elements in the brain. The most parsimonious explanation for these findings would be that the AVP-secreting magnocellular neurons themselves are the osmoreceptors. Although these neurons do display osmoreceptive characteristics,<sup>2</sup> their location inside the blood–brain barrier does not position them to respond quickly to small changes in osmolality in the circulation. Subsequent studies strongly implicated the circumventricular organ named the organum vasculosum of the lamina terminalis (OVLT), which lacks a blood–brain barrier, as well as areas of the adjacent hypothalamus near the anterior wall of the third cerebral ventricle as the site of the principle brain osmoreceptors. Destruction of this area of the brain abolishes both AVP secretion and thirst re-

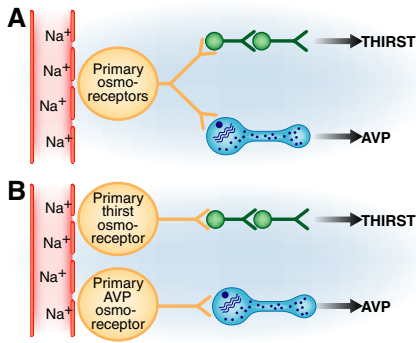
sponses to hyperosmolality in experimental animals<sup>3</sup> and in human subjects with brain damage that infarcts the region around the OVLT, who typically are unable to maintain normal plasma osmolalities even under basal conditions.<sup>4</sup> In contrast to the effects of such lesions to eliminate both osmotically stimulated thirst and AVP secretion, diabetes insipidus caused by destruction of the magnocellular AVP neurons in the supraoptic (SON) and paraventricular (PVN) nuclei eliminates dehydration-induced AVP secretion but not thirst, clearly indicating that osmotically stimulated thirst must be generated proximally to the AVP-secreting cells themselves (Figure 1A). Other regions have also been reported to contain putative osmoreceptors, including the hepatic portal circulation, leading to the suggestion that osmoreceptors are widely distributed.<sup>5</sup> However, cells in these areas likely act to modulate the activity of the primary OVLT osmoreceptors because they are not able to maintain osmotically stimulated AVP secretion or thirst after lesions of the OVLT.

Involvement of the OVLT and surrounding areas of the anterior hypothalamus in osmoreception is also supported by studies using immunohistochemical

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**Figure 1.** Brain osmoreceptor pathways. The primary brain osmoreceptors lie outside the blood–brain barrier in the OVLT. Different neural projections connect the primary osmoreceptors to brain areas responsible for AVP secretion and thirst. Whether the same (A) or different subsets (B) of osmoreceptors project to both areas is presently unknown. Although osmoreceptors can both stimulate as well as inhibit AVP secretion and thirst in response to systemic hyper- and hypotonicity, respectively, it is also not known whether there are separate subsets of excitatory and inhibitory osmoreceptor cells, or whether this is a property of single osmoreceptive cells.

techniques to detect early gene products in rats, which serve as markers of cell activation after dehydration. Intense expression of the cFos protein in and around the OVLT confirms this area is strongly activated by induced dehydration, and retrograde tracing studies verify that a subset of the activated neurons send projections to the magnocellular AVP neurons in the hypothalamus.<sup>6</sup> Although many of the neural pathways connecting the OVLT and other circumventricular organs with the magnocellular AVP-secreting cells in the SON and PVN have been identified, the neural circuits in the forebrain that stimulate thirst after osmoreceptor activation are still largely unknown. Recent studies using functional magnetic resonance imaging in humans have shown that the anterior cingulate area of the cortex is reliably activated in conjunction with the sensation of thirst.<sup>7</sup> Although data from lesion studies in both animals and man support the concept of a single group of osmoreceptive neurons that control both AVP

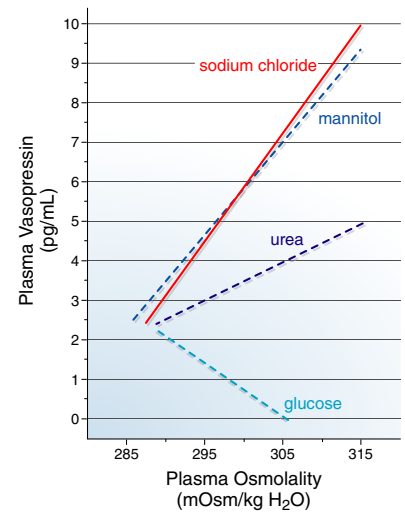
secretion and thirst, this has not been definitively confirmed. Separate but parallel pathways for these complementary functions remain possible (Figure 1B) and could account for the lower osmotic threshold for activation of AVP secretion compared with thirst.<sup>8</sup>

## WHAT DO BRAIN OSMORECEPTORS RESPOND TO?

Neither AVP secretion nor thirst is equally sensitive to all plasma solutes. Sodium and its anions, which normally contribute >95% of the osmotic pressure of plasma, are the most potent solutes in terms of their capacity to stimulate AVP secretion and thirst, although some sugars such as mannitol and sucrose are also equally effective when infused intravenously.<sup>8</sup> In contrast, increases in plasma osmolality caused by solutes such as urea or glucose cause little or no increase in plasma AVP levels in humans or animals (Figure 2).<sup>8,9</sup> These differences in response to various plasma solutes are independent of any recognized nonosmotic influence, which indicates they are an intrinsic property of the osmoregulatory mechanism itself. Thus, it is clear that osmoreceptor cells in the brain primarily respond to plasma tonicity rather than to total plasma osmolality. The physiological relevance of this finding is that osmoreceptors function primarily to preserve cell volume; elevations of solutes such as urea, unlike elevations of sodium, do not cause cellular dehydration and consequently do not activate the mechanisms that defend body fluid homeostasis by preserving or increasing body water stores.

## WHAT ARE THE CELLULAR MECHANISMS UNDERLYING OSMORECEPTION?

“Effective” solutes are those that penetrate cells slowly, or not at all, thereby creating an osmotic gradient that causes an efflux of water from osmoreceptor cells. The resultant shrinkage of osmo-



**Figure 2.** Solute specificity of brain osmoreceptors. The lines represent the relationship of plasma AVP to plasma osmolality in healthy adults during intravenous infusion of hypertonic solutions of different solutes. Note that effective solutes, *i.e.*, those compartmentalized to the extracellular fluid (NaCl and mannitol), are much more effective at eliciting AVP secretion than the non-effective solutes, urea and glucose, that distribute across cell membranes into the intracellular fluid as well (adapted from Zerbe and Robertson GL.<sup>8</sup>)

sensitive neurons has been found to activate membrane nonselective cationic conductances that generate inward current; if of sufficient magnitude, the resulting depolarization of the osmoreceptor neuron then produces an action potential.<sup>10</sup> Conversely, “ineffective” solutes that penetrate cells readily create no osmotic gradient and thus have little to no effect on the cell volume of the osmoreceptors. Electrophysiological studies of neurons in the OVLT show they display changes in action potential firing rate that vary in proportion to the tonicity of extracellular fluid, supporting the likelihood that these cells represent osmosensory neurons.<sup>5</sup> Osmotically evoked changes in the firing rate of the OVLT neurons in turn synaptically regulate the electrical activity of downstream effector neurons, importantly including the magnocellular AVP neurons in the SON and PVN, through graded changes in release of the excitatory neurotransmitter glutamate. This mechanism agrees well with

the observed relationship between the effect of specific solutes such as sodium, mannitol, and glucose on AVP secretion (Figure 2).

The presumption that the cell volume of the osmoreceptor cells represents the primary signaling event by which osmoreceptors detect changes in the tonicity of the extracellular fluid raises some interesting dilemmas. First, most cells in the body regulate their volume to prevent or minimize the detrimental effects of cell swelling or shrinkage on cellular functions. However, if osmoreceptors displayed volume-regulatory increases or decreases in response to changes in extracellular tonicity, this would not allow for an absolute plasma osmolality around which body fluid homeostasis is maintained; that is, chronic hyperosmolality would not elicit sustained stimuli to AVP secretion and thirst. Results using OVLT neurons in short-term dispersed cultures indeed suggest that these cells do not volume-regulate, consistent with their putative function as the primary brain osmoreceptors.<sup>11</sup> Whether this is also true after longer periods of sustained changes in tonicity has not been studied. Second, in response to chronic changes in tonicity, the magnocellular AVP neurons undergo effects opposite of those expected. These neurons enlarge in response to chronic hypertonicity<sup>12</sup> and shrink in response to chronic hypotonicity.<sup>13</sup> This is postulated to be a result of changes in cell synthetic machinery; upregulation of the many proteins required for increased AVP synthesis during chronic hypertonicity causes cell hypertrophy, and downregulation of these proteins during chronic hypotonicity produces the opposite effects. Thus, the true determinant of osmoreceptor activity must be the degree of stretch of the osmoreceptor cell membrane, with subsequent effects on stretch-activated or stretch-inactivated channels, rather than the absolute size of the neurons.<sup>10</sup> In this sense, osmoreceptors function as mechanoreceptors that detect the degree of membrane stretch at the cellular level, similar to

the function of baroreceptors at the vascular level.

The cellular osmosensing mechanism utilized by the OVLT cells is an intrinsic depolarizing receptor potential, which these cells generate through a molecular transduction complex. Recent results suggest this likely includes members of the transient receptor potential vanilloid (TRPV) family of cation channel proteins. These channels are generally activated by cell membrane stretch to cause a nonselective conductance of cations, with a preference for  $\text{Ca}^{2+}$ . Multiple studies have characterized various members of the TRPV family as cellular mechanoreceptors in different tissues.<sup>14</sup>

Both *in vitro* and *in vivo* studies of the TRPV family of cation channel proteins provides evidence supporting roles for TRPV1, TRPV2, and TRPV4 proteins in the transduction of osmotic stimuli in mammals.<sup>15</sup> An N-terminal *trpv1* variant is expressed in OVLT cells, and *trpv1*-null mice have defects in osmotically stimulated AVP secretion and thirst.<sup>5</sup> Heterologous expression of the *trpv2* gene in Chinese hamster ovary (CHO) cells causes an activation of  $\text{Ca}^{2+}$  influx in response to hypotonicity, a response that can be mimicked by cell membrane stretch.<sup>15</sup> *trpv4*-Transfected cells respond similarly to hypotonicity and mechanical stretch, and they display deficient volume-regulatory decreases in response to hypoosmolality.<sup>16</sup> But *in vivo* studies have yielded inconsistent findings. *trpv4*-Null mice have a potentiated AVP response to a combined hypertonic and hypovolemic stimulus in one study<sup>17</sup> but blunted responses of both AVP secretion and thirst to a selective hypertonic stimulus in another.<sup>18</sup> These findings are not necessarily contradictory because both AVP secretion and thirst are likely under bimodal control; that is, they are stimulated by hypertonicity and inhibited by hypotonicity.<sup>19</sup> In support of this possibility, treatment with desmopressin leads to hyponatremia in *trpv4*-null mice but not wild-type controls, indicating a failure of osmotic inhibition of drinking.<sup>18</sup> Thus, different channels and/or different sets of osmoreceptor cells may mediate opposite responses to

cell membrane stretch, although osmosensitive inhibitory neurons have not yet been identified in the OVLT.<sup>5</sup>

The combined studies to date therefore strongly support the characterization of TRPV1, TRPV2, and TRPV4 as osmomechano-TRPs.<sup>15</sup> However, despite the very promising nature of these findings, several dilemmas are evident with regard to their involvement in brain osmoreception. First, it is striking that animals with gene deletions of individual members of the TRPV family manifest blunted AVP secretion and thirst but have a normal basal plasma osmolality. These results stand in marked contrast to animals with lesions that destroy the OVLT and surrounding hypothalamus, in which osmotically stimulated AVP secretion and thirst are virtually abolished, leading to chronically elevated plasma osmolality. This raises the likelihood that different ion channels, or possibly combinations of subunits from different channels, mediate osmosensitivity in the brain and compensate for the absence of individual ion channels. Second, it is surprising that all of the TRPV channels appear to be activated by membrane stretch, including the cell swelling induced by extracellular hypotonicity, whereas *in vitro* studies of putative OVLT osmoreceptors have indicated that the mechanism responsible for hyperosmolar activation of these cells is activation of a stretch-inactivated cationic conductance that responds to cell shrinkage.<sup>10</sup> These and other questions remain to be answered before we fully understand brain osmoreceptors and how they function.

## UNRESOLVED QUESTIONS

Although the details of exactly how and where various members of the TRPV family of cation channel proteins participate in osmoregulation in different species remains to be ascertained by additional studies, a strong case can be made for their involvement in the transduction of osmotic stimuli in the neural cells in the OVLT and surrounding hypothalamus that regulate osmotic homeostasis, which appears to be highly conserved

throughout evolution.<sup>15</sup> Future studies will be necessary to address still unanswered questions, including the exact structure of the molecular transduction complex that regulates the opening of cationic channel(s) in response to changes in tonicity; whether different heteromultimeric combinations of TRPV1, TRPV2, and TRPV4 and possibly other cationic channels, mediate differential responses to changes in tonicity; whether separate excitatory and inhibitory osmoreceptors control AVP secretion and thirst; and the potential involvement of the TRPV family of ion channels in the responses of different tissues to changes in tonicity, particularly the kidney and the vasculature. But with promising candidate cells and gene products now clearly identified, answers to these questions should be forthcoming.

## DISCLOSURES

None.

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