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Vesicular osmometers, vasopression secretion and aquaporin-4: A new mechanism for osmoreception?

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Abstract

This review cites new evidence suggesting a link between the recently discovered membrane bound water-selective channel, aquaporin-4 (AQP4), and the mechanism of central osmoreception. AQP4 is found in a number of brain regions associated with the osmoregulation of vasopressin secretion and thirst, including the supraoptic nucleus (SON) and subfornical organ (SFO). AQP4 expression is restricted to ependymal cell membranes in the SFO and astrocyte membranes in the SON, especially perivascular end foot processes, suggesting that glial cells may correspond to Verney's hypothalamic 'vesicular osmometers'. Information on osmotic status may thus be conveyed to the neuronal elements of the 'osmoreceptor complex' by a neurone–glial interaction. © 1998 Elsevier Science Ireland Ltd.

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1. Introduction

It is now 50 years since Verney first described the antidiuresis induced by intracarotid infusions of hypertonic saline [1]. The mechanisms mediating the control of vasopressin (AVP) secretion, and its action in the renal collecting duct, are once again in the limelight following the recent discovery of the aquaporin family of membrane bound water channels. One member of this family, aquaporin-2, is found exclusively in the apical membrane of the collecting duct principal cells, and is now recognized as the protein responsible for the AVP-induced increase in membrane permeability [2]. This article reviews new evidence linking another species of aquaporin, AQP4, with the mechanism of central osmoreception. If AQP4 is responsible for conferring osmosensitivity on specific cells, the accumulated data suggest a novel mechanism for Verney's hypothalamic 'vesicular osmometers'.

2. A brief overview of the osmotic control of AVP secretion

Verney [1] proposed that the antidiuresis he observed may be controlled by cells in the hypothalamus acting as 'vesicular osmometers', their size and function being altered under differing osmotic conditions. There has been considerable debate on the identity and sensitivity of such cells, focusing mainly on the neurones located in osmosensitive nuclei.

The magnocellular neurosecretory cells in the supraoptic nuclei (SON) appear to exhibit intrinsic osmosensitivity. The increase in firing rate observed in the AVP-secreting cells following direct application of hypertonic saline [3] is thought to be related to a reduction in cell volume [4]. This cellular shrinkage is

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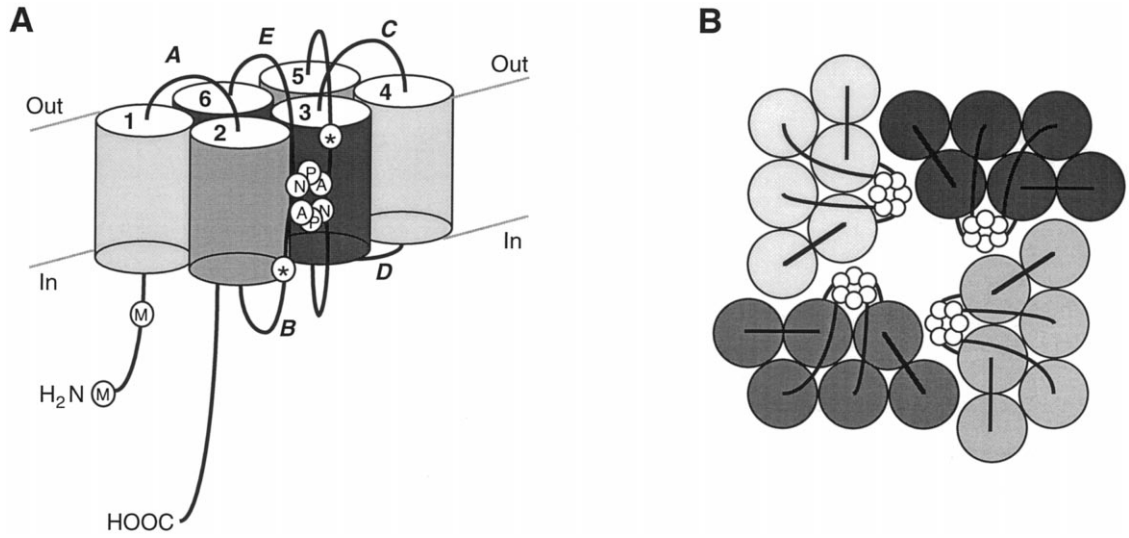

Fig. 1. The hourglass model for aquaporins. (A) 3-D structure relating to AQP4 showing the bilayer-spanning domains (1–6), interconnecting loops (A–E), the highly conserved 'NPA' motifs located on loops B and E, the location of cysteine residues (94 and 210) that confer mercurial sensitivity on other aquaporins (*) replaced by glycine or arginine in AQP4, and the two translation initiation sites (M1 and M23). (B) Extracellular view showing the arrangement of AQP4 monomers into stable tetramers in the membrane.

associated with modulation of the activity of mechanosensitive cation channels, probably stretch-inactivated potassium channels [5], thereby regulating membrane voltage and action potential discharge. Some have confidently asserted that these magnocellular neurones correspond to Verney's 'vesicular osmometers'.

However, the magnocellular neurones do not act alone. Osmotic stimulation of these cells is abolished by deafferentation of the SON, or by lesions of the anteroventral third ventricular (AV3V) region. This region contains the organum vasculosum of the lamina terminalis (OVLT), a circumventricular organ (CVO) which lie outside the blood–brain-barrier. Neurones in the OVLT and median preoptic nucleus (MnPo) have also been shown to be intrinsically osmosensitive, and the integrity of a circuit of neuronal connections linking the SON, AV3V and MnPo is necessary for SON neurones to respond to osmotic stimulation. It appears that these neurones function as an 'osmoreceptor complex', amplifying the magnocellular response to hypertonicity [6] (see Fig. 2).

Nevertheless, the question remains concerning the mechanism conferring osmosensitivity on specific cells. Is it that all cells are capable of detecting changes in osmolality, but only certain cells respond (e.g. by the specific expression of stretch-inactivated potassium channels)? Alternatively, is detection specificity produced by a mechanism rendering the membrane of osmosensitive cells permeable to water? The discovery of a novel aquaporin, AQP4, expressed in the SON and paraventricular nucleus (PVN), may provide the answer, but at the same time, looks set to challenge the concept of primary neuronal osmosensitivity.

3. Aquaporin-4

The aquaporin family of membrane bound water transporting channels is expressed in a variety of organisms [7], with six species described to date in mammalian tissues. Channel-forming integral protein of 28 kDa (CHIP 28; AQP1) was the first aquaporin to be identified [8] and characterized, and the expression of this specialized water pore in erythrocyte membranes accounts for their high water permeability.

Aquaporin-4 is a newly discovered member of this family, the cDNA being derived simultaneously from rat brain [9] and rat lung [10]. This 323-aa polypeptide has 41% amino acid identity with AQP1, and in common with other aquaporins, is thought to contain six membrane spanning domains with five interconnecting loops (Fig. 1(a)). Loops B and E fold into the lipid bilayer and each contain an 'NPA' motif (highly conserved in other aquaporins). The folding of the protein is thought to bring these motifs together, to form the water selective transmembrane pore in an hourglass structure [11–13]. Like other aquaporins, single AQP4 units aggregate in the membrane to form a more stable tetrameric complex [14] (Fig. 1(b)).

The water transporting properties of AQP4 have been demonstrated in the *Xenopus* oocyte, where AQP4 expression increases the low diffusional permeability of the membrane by 10–20-fold [9,10]. In contrast with previously described aquaporins, the water transporting properties of AQP4 are not inhibited by the application of $HgCl_2$, since the cysteine residues that confer mercurial sensitivity are replaced by glycine and arginine (Fig. 1(a)). AQP4 has thus been called the mercurial-insensitive water channel [10].

4. Distribution and regulation of AQP4

Aquaporin-4 is widely distributed, but expression of AQP4 mRNA transcripts in the CNS is at least 10-fold higher than in other tissues [9]. In situ hybridization studies in adult rat brain have revealed AQP4 mRNA expression in cells lining the ventricular system, the pial surface, the SON and PVN, the granule cell layer of the dentate gyrus and the Purkinje cell layer of the cerebellum. AQP4 mRNA is not present in the choroid plexus where AQP1 is abundant [9,10]. Little is known at present about the regulation of AQP4, except that renal expression is unaffected by dehydration [15], and AQP4 transcripts are absent from the 17 day fetal rat brain [9].

Immunoperoxidase staining of rat brain has confirmed the presence of AQP4 protein in the meningeal cells of the pia mater (where it is co-expressed with AQP3), the ventricular lining, cerebellum, spinal cord, hippocampus, SON, subfornical organ (SFO), area postrema (AP) and median eminence [16–18]. However, in all of these locations the distribution of AQP4 is non-neuronal, being restricted to astrocytes in the SON and a subpopulation of ependymal cells in the SFO [17]. Postembedding immunogold labelling for AQP4 in glial cells is highly differentiated, being particularly abundant in the membranes of perivascular processes. This polarization is less pronounced in the astrocytes of the SON and PVN. In the SFO, immunogold labelling of AQP4 in the ependymal cells is restricted to the basolateral membrane [17]. The localization and expression of AQP4 in other regions of the brain thought to be intrinsically osmosensitive, such as the OVLT and MnPo, remains unknown.

This distribution of AQP4 suggests a particular functional significance. Jung et al. [9] proposed that AQP4 "may facilitate water balance between brain parenchyma and the fluid compartment". The movement of water into and out of brain cells through AQP4 may lie at the heart of the central mechanism of osmoreception, and the osmoregulation of AVP secretion and dipsogenesis. The expression of AQP4 in the magnocellular nuclei, SFO and AP makes this a distinct possibility. However, the glial-specific distribution of AQP4 in these areas implies that it may be glial cells, rather than neurones, that exhibit intrinsic osmosensitivity.

5. Glial cells as hypothalamic 'vesicular osmometers'

Glial cells in the SON and PVN have a striking morphological appearance, with long astrocytic processes radiating between the magnocellular neurones, effectively insulating neurosecretory cells from each other. These astrocytes are capable of considerable structural plasticity, especially in relation to the oxytocin (OXY)-secreting neurones. Following both acute and chronic dehydration the astrocytic processes are withdrawn, permitting an increase in neuronal juxtaposition [19]. This increase in both soma-somatic and dendro-dendritic apposition is accompanied by the appearance of double synapses, where one presynaptic terminal is in contact with two neurosecretory cell soma [19,20]. It is thought that these morphological changes may enhance the synchronization of neuronal activity [21]. Although this structural plasticity appears to be dependent upon a paracrine action of OXY [22], the presence of AQP4 in these cells raises the possibility of a direct osmotic effect.

Recent evidence demonstrates that direct osmotic stimulation induces both gene transcription and translation in astrocytes of the SON. Microdialysis of the SON with hypertonic saline (i.e. producing non-synaptic neuronal activation) elevates the expression of the immediate-early gene c-*fos* and the Fos protein in the glial cells, but not in the magnocellular neurones. In contrast, the opposite effect is seen following peripheral (i.e. synaptic) osmotic stimulation [23]. The authors of this study interpret their data by suggesting that the direct osmotic stimulation of the SON activates the glial cells via the neighbouring magnocellular neurones. The fact that glial cells, rather than neurones, express AQP4 indicates that the reverse may be true. Peripheral osmotic stimulation, on the other hand, may be insufficient to provoke an observable rise in c-*fos* expression in the glial cells, although by virtue of the amplifying effect of the neuronal connections in the 'osmoreceptor complex' an increase in c-*fos* expression in the magnocellular neurones is detectable.

Intrinsic osmosensitivity of glial cells does not necessarily preclude the possibility that neuronal elements in the 'osmoreceptor complex' are also osmosensitive (Fig. 2). In the SON, the magnocellular neurosecretory cells appear to have all the characteristics demanded by Verney's hypothalamic 'vesicular osmometer' hypothesis, but they do not express a known water-selective channel. Should astrocytes in the SON be intrinsically osmosensitive, elevation of plasma osmolality would elicit a withdrawal of water through AQP4 expressed in the perivascular glial end-foot processes. The more ubiquitous expression of AQP4 throughout the glial membranes in the SON would enable water movement into the dehydrated glial cells through the membrane facing the neuropil. Withdrawal of water from neuronal elements of the 'osmoreceptor complex', to effect cellular shrinkage and depolarization, could involve the action of an as yet unidentified neuronal aquaporin. Thus glial cells may function as the primary 'vesicular osmometer', conveying information on the state of water homeostasis to the neuronal elements of the 'osmoreceptor complex' by transcellular water movement, or some other neurone-glial interaction.

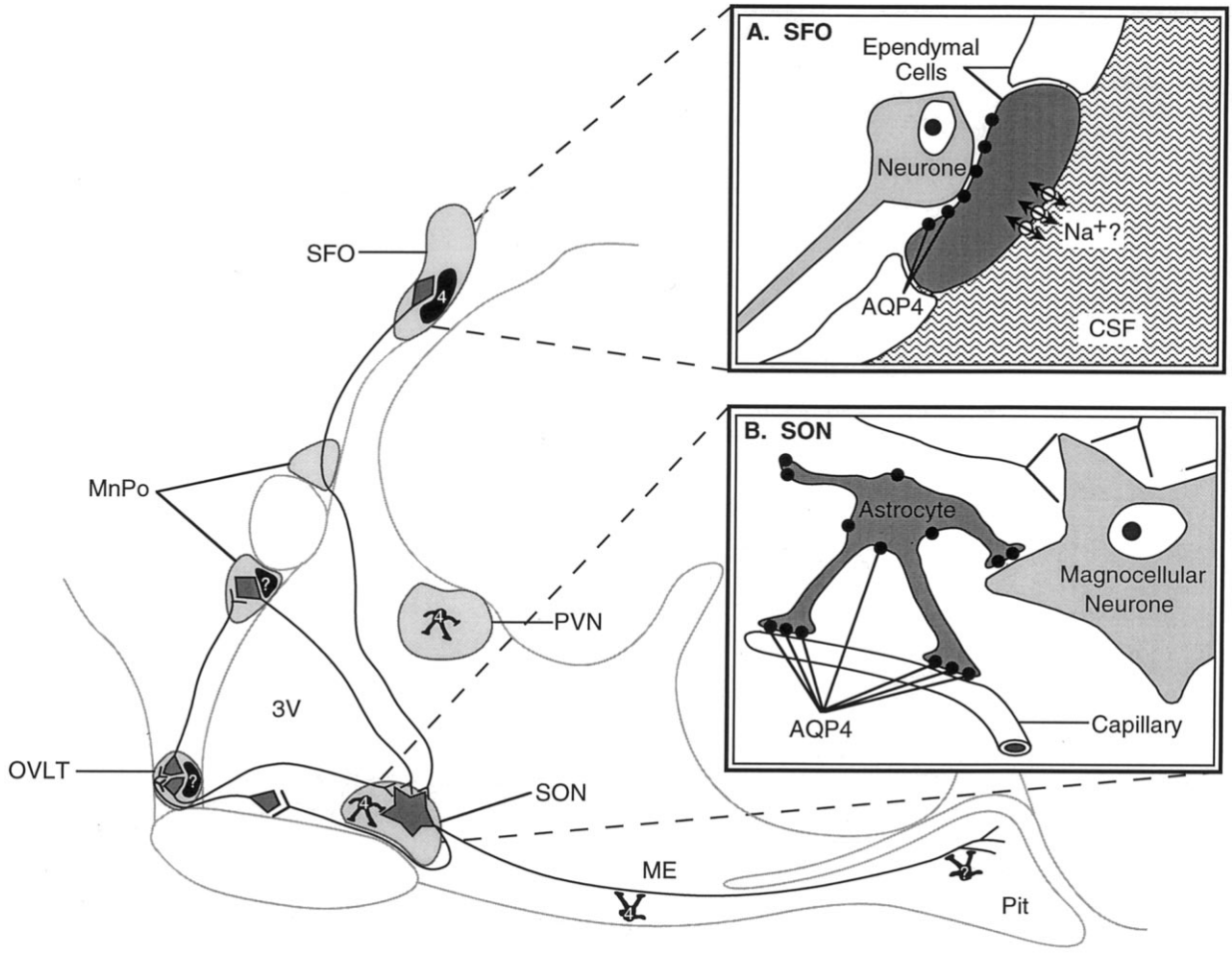

Fig. 2. Schematic representation of the interaction between AQP4-positive cells and the neuronal elements of the 'osmoreceptor complex'. Glial cells known to express AQP4 (4), or possibly expressing AQP4 (?), are shown in black. Inset A shows the location of AQP4 in the basolateral membrane of a subpopulation of ependymal cells in the SFO. Inset B shows the expression of AQP4 in astrocyte membranes in the SON. SFO, subfornical organ; MnPo, median preoptic nucleus; OVLT, organum vasculosum of the lamina terminalis; PVN, paraventricular nucleus (magnocellular); SON, supraoptic nucleus; ME, median eminence; Pit, pituitary; 3V, third ventricle; CSF, cerebrospinal fluid.

Since AQP4 is a selective water channel, being impermeable to [^{14}C]urea, [^{14}C]sucrose and charged particles [10], its presence in the SON strengthens the case for suggesting that cells in the magnocellular nuclei may be osmosensitive rather than sodium sensitive [24]. This may not be true for the SFO, where AQP4 distribution is restricted to the basolateral membrane of ependymal cells lining the ventricles. It is possible that the CVOs may not be primarily osmosensitive, but that transmembrane water movement may be driven by an ionic gradient established with the cerebrospinal fluid (CSF). Thus, osmosensitive neurones in the SFO may respond to the ionic composition of the CSF, possibly a 'permissive' CSF sodium concentration [22]. This mechanism may not be confined to the SFO, but may be a common feature of the other CVOs, including the OVLT and AP. The AP has a chequered association with osmoregulation [25], and the presence of AQP4 in the rat AP may represent a mechanism for osmotic interaction with volume regulated AVP secretion.

The neuronal–glial interactions suggested by the discovery of AQP4 may not be confined to the soma of magnocellular neurones. The presence of AQP4 in astrocytes in the median eminence [18] suggests that osmotic modulation may also occur with neurosecretory axons. AQP4, or a related aquaporin, may yet be discovered in the posterior pituitary.

6. Conclusion

The recent discovery of the membrane bound water channel, AQP4, in brain areas concerned with central osmoreception may have finally identified the mechanism conferring osmosensitivity on Verney's 'vesicular osmometers'. Should this role for AQP4 be confirmed, the glial-specific expression represents a serious challenge to the concept of intrinsic neuronal osmosensitivity. The osmotic stimulation of neuronal elements of the 'osmoreceptor complex' may be indirect, being me-

diated by an interaction with neighbouring osmosensitive astrocytes and ependymal cells. Although more research is required to underline and extend these initial findings, particularly in relation to the regulation of AQP4 expression, our understanding of central osmoreception may be about to undergo a significant change. Astrocytes in the magnocellular nuclei and ependymal cells in the circumventricular organs may correspond to Verney's 'vesicular osmometers'.

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