REVIEW

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The biology of water homeostasis

Mariavittoria D'Acierno[1], Robert A. Fenton[1] and Ewout J. Hoorn[2]

[1]Department of Biomedicine, Aarhus University, Aarhus, Denmark and
[2]Department of Internal Medicine, Division of Nephrology and Transplantation, Erasmus Medical Center, University Medical Center Rotterdam, Rotterdam, The Netherlands
Correspondence to: Ewout J. Hoorn; E-mail: e.j.hoorn@erasmusmc.nl

ABSTRACT

Water homeostasis is controlled by a brain–kidney axis that consists of central osmoreceptors, synthesis and secretion of arginine vasopressin (AVP) and AVP-responsive aquaporin-2 (AQP2) water channels in kidney collecting duct principal cells that facilitate water reabsorption. In addition to AVP, thirst represents a second line of defence to maintain water balance. Water balance disorders arise because of deficiency, resistance or inappropriate secretion of AVP or disturbances in thirst sensation (hypodipsia, polydipsia). People with water balance disorders are prone to develop hyponatraemia or hypernatraemia, which expose cells to osmotic stress and activate cell volume regulation mechanisms. This review covers several recent insights that have expanded our understanding of central osmoregulation, AQP2 regulation and cell volume regulation. This includes the role of with no lysine kinase 1 (WNK1) as a putative central osmolality sensor and, more generally, as an intracellular crowding sensor that coordinates the cell volume rescue response by activating sodium and potassium cotransporters. Furthermore, several new regulators of AQP2 have been identified, including AVP-dependent AQP2 regulation (yes-associated protein, nuclear factor of activated T-cells, microRNAs) and AVP-independent AQP2 regulation (epidermal growth factor receptor, fluconazole, prostaglandin E_2). It is also becoming increasingly clear that long-term cell volume adaptation to chronic hypotonicity through release of organic osmolytes comes at the expense of compromised organ function. This potentially explains the complications of chronic hyponatraemia, including cognitive impairment, bone loss and vascular calcification. This review illustrates why these new insights derived from basic science are also relevant for developing new approaches to treat water balance disorders.

Keywords: aquaporin-2, cell volume regulation, hypernatraemia, hyponatraemia, vasopressin

CENTRAL OSMOREGULATION

Central osmoregulation is based on the body's ability to sense an increase in plasma osmolality. Specialized neurons can detect an increase in plasma osmolality (osmoreceptors) and transduce these signals as action potentials to neurosecretory neurons producing arginine vasopressin (AVP, the antidiuretic hormone). In humans, the osmotic threshold for AVP release is set at ≈280 mOsm/kg and forms the first line of defence to maintain water homeostasis. If AVP-induced water retention is insufficient to counterbalance ongoing water loss, plasma osmolality will continue to increase and activate a second line of defence, namely thirst. Several brain areas interact to integrate the processes of osmosensing, vasopressin synthesis and thirst (Fig. 1). Two of the circumventricular organs, the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT), express osmoreceptors and are further characterized by highly permeable capillaries. SFO and OVLT neurons connect with the paraventricular nucleus (PVN) and supraoptic nucleus (SON), which in turn connect with the posterior pituitary, where AVP is released into the circulation (Fig. 1). Thirst is also processed by the SFO and OVLT as well as the median preoptic nucleus (MnPO), which operates as an integratory structure for thirst stimulation and suppression [1]. The ability to stimulate or suppress thirst is further determined by SFO, OVLT and MnPO neurons expressing neural nitric oxide synthase (stimulatory) or glucagon-like peptide 1 receptors (GLP1Rs, inhibitory) [2]. In addition, cholecystokinin-producing excitatory neurons in the SFO are involved in persistent or transient suppression of water intake [3]. Finally, OVLT neurons respond to both extracellular NaCl concentrations and angiotensin II to stimulate thirst [4]. Central osmoregulation also integrates cues from the external environment. A gut–brain axis that derives from the oropharyngeal area and involves the vagus nerve provides the signal for satiation to $MnPO^{GLP1R}$ neurons during normal drinking (Fig. 1) [5]. In addition to plasma osmolality and thirst, the plasma sodium concentration is also sensed by the SFO through an atypical non-inactivating Na(x) channel (*Scn7A*), which controls thirst and salt-intake behaviour [6].

It has long been postulated that mechanosensitive channels in osmosensing neurons detect changes in extracellular tonicity, including transient receptor potential vanilloid (TRPV) 1 and 4 [1]. However, this concept has recently been challenged by the identification of with no lysine kinase 1 (WNK1) as a central osmolality sensor [7]. In mice, neuron-specific conditional deletion of WNK1 caused hypotonic polyuria and blunted AVP release after water restriction. Neuronal pathway tracing showed that genetic deletion or pharmacological inhibition of WNK1 attenuated hyperosmolality-induced increases in action potential firing in OVLT neurons. The typical role of the WNKs is to regulate channels and transporters at the plasma membrane. WNK1 in neurons is often co-expressed with the potassium channel Kv3.1, and knockdown of Kv3.1 also produced the AVP deficiency phenotype. Therefore, WNK1 activation of Kv3.1 was proposed to form the electrophysiological basis of the SFO–OVLT to PVN–SON connection for AVP release [7].

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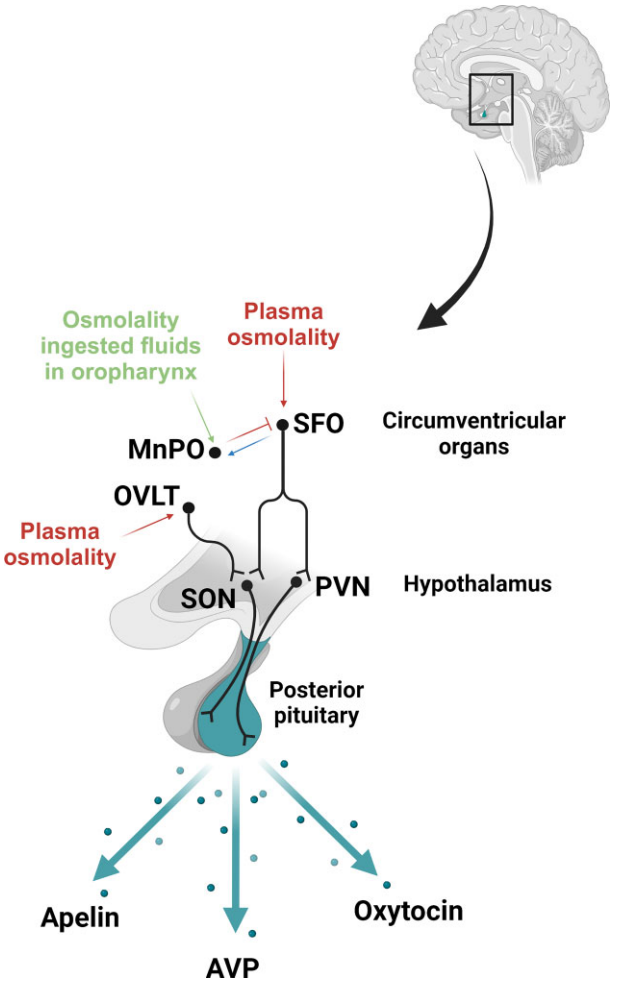

Figure 1: Central osmoregulation. The brain regions involved in osmosensing, synthesis and secretion of AVP and thirst perception. Osmosensing neurons in the SFO and OVLT, both circumventricular organs, are capable of detecting an increase in plasma osmolality. The SFO interacts with the MnPO, which also receives signals from the oropharynx (fluid volume). SFO and OVLT neurons connect with the SON and PVN in the hypothalamus. The SON and PVN express magnocellular neurons that synthesize and secrete the hormones of the posterior pituitary, including AVP, apelin and oxytocin. Created with BioRender.com.

Disturbances in the physiological release or kidney response to AVP leads to water balance disorders, which are either classified as AVP deficiency/resistance (previously diabetes insipidus) or the syndrome of inappropriate antidiuresis (SIAD; Fig. 2). Furthermore, lack of thirst (primary hypodipsia) or increased thirst sensation (primary polydipsia) also lead to water balance disorders. Depending on whether water balance disorders cause water loss or water retention, people will be prone to develop hypernatraemia or hyponatraemia. However, if thirst is intact and water intake is appropriately adjusted, people with water balance disorders remain normonatraemic. There is also a physiological mechanism to counteract the effects of AVP through the neurovasoactive peptide apelin [8]. Apelin colocalizes with AVP in magnocellular vasopressinergic neurons, but has opposite effects and causes aquaresis instead of antidiuresis. The apelin receptor is expressed in the hypothalamus and collecting duct (CD) principal cells. Activation of the apelin receptor suppresses central AVP release but also counteracts the AVP stimulation of aquaporin-2 (AQP2) by inhibiting adenylate cyclase and cyclic adenosine monophosphate (cAMP) in the kidney CD [9]. A recent study demonstrated that an agonist of the apelin receptor can stimulate aquaresis and improve hyponatraemia in a rat model of AVP-induced water retention [9].

AVP deficiency can be caused by several disorders that affect central osmoregulation, including hereditary causes, neurosurgery, neurotrauma, neurovascular disorders and infiltrative disorders. Disorders causing AVP deficiency may also impair oxytocin secretion, owing to the close anatomical proximity of AVP- and oxytocin-producing neurons (Fig. 1). The possibility that patients with AVP deficiency also have oxytocin deficiency was recently tested by using a 3,4-methylenedioxymethamphetamine (MDMA) provocation test [10]. The MDMA provocation test caused a robust increase in plasma oxytocin in healthy subjects (77 to 659 pg/ml), but only a slight increase in patients with AVP deficiency (60 to 66 pg/ml). The increase in oxytocin produced the expected prosocial, empathic and anxiolytic effects in healthy subjects but not in the patients with AVP deficiency. Whether patients with AVP deficiency benefit from oxytocin replacement is currently being investigated (ClincalTrials.gov NCT06036004).

Primary hypodipsia is usually caused by hypothalamic disorders, and this can also be accompanied by AVP deficiency (adipsic diabetes insipidus). Of interest, autoimmune causes of hypodipsia have been reported with antibody formation against the SFO or specifically against the sodium channel Na(x) [11, 12]. A disorder of increased thirst sensation is primary polydipsia, in which thirst is experienced in the absence of hyperosmolality. The presence of thirst-suppressing $MnPO^{GLP1R}$ neurons raised the possibility that GLP1R agonists not only reduce appetite, but also thirst sensation. If so, GLP1R agonists could reduce thirst in patients with primary polydipsia. This hypothesis was tested in a randomized clinical trial in which 34 patients with primary polydipsia were treated with the GLP1R agonist dulaglutide or placebo [13]. Dulaglutide reduced thirst perception and decreased fluid intake by ≈0.5 L and urine output by ≈1 L, but it did not modify brain regions linked to thirst regulation on functional magnetic resonance imaging (MRI).

KIDNEY WATER HANDLING

In the kidney, the greatest percentage of filtered water is reabsorbed by the highly water-permeable epithelial cells of the proximal tubules and the adjacent initial portion of the long-looped descending thin limbs of Henle's loop, where the water channel AQP1 is abundant [14]. The ascending thin limbs and thick limbs of Henle's loop are relatively impermeable to water, as they do not contain apical membrane AQPs. However, the thick ascending limb does play an important role in water conservation, as it provides the single effect for the countercurrent multiplication mechanism and generates a corticomedullary osmotic gradient in the interstitium [15]. This gradient is generated by sodium transport and enhanced by urea transport in the inner medulla and provides the driving force for regulated water reabsorption by the CDs through AQP2, AQP3 and AQP4. Basal epithelial water permeability in CD principal cells is low, but the water permeability becomes very high when stimulated with AVP [14].

AVP binds to the type II vasopressin receptor (V2R) on the basolateral membrane of CD principal cells and initiates a signalling cascade that leads to the translocation of AQP2 water channels from intracellular vesicles to the apical membrane of the CD cells (Fig. 3) [14, 16]. This increase in water permeability allows water to passively move along the osmotic gradient and leave the cell through AQP3 and AQP4 on the basolateral side, ultimately being reabsorbed back into the circulation.

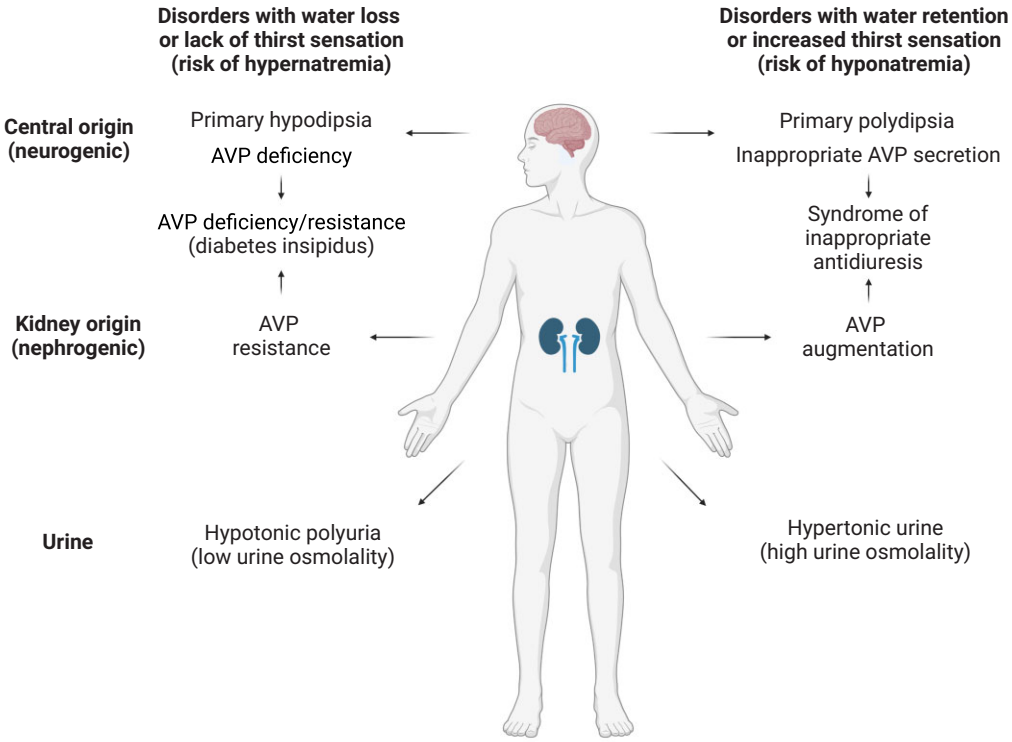

Figure 2: Water balance disorders. Water balance disorders arise as a consequence of disturbances in the secretion of or the kidneys' response to AVP or disturbances in thirst perception. The left part of the figure shows the water balance disorders primary hypodipsia, AVP deficiency and AVP resistance, which are characterized by a risk of hypernatraemia. The right part of the figure shows the water balance disorders primary polydipsia and the syndrome of inappropriate antidiuresis, which can have a central or nephrogenic origin. These water balance disorders are characterized by a risk of hyponatraemia. AVP deficiency and AVP resistance are characterized by hypotonic urine, whereas the syndrome of inappropriate antidiuresis is characterized by hypertonic urine. Created with BioRender.com.

Disturbances in the V2R–AQP2 pathway can cause AVP resistance or nephrogenic SIAD, illustrating the clinical relevance of this pathway (Fig. 2). Nephrogenic SIAD can be caused by mutations activating V2R, but can also be acquired by the use of common drugs such as sertraline, carbamazepine, haloperidol and cyclophosphamide [17]. Inhibitors of the V2R ('vaptans') can be used to induce aquaresis and treat the acquired and sometimes also nephrogenic forms of SIAD [16, 18]. V2R antagonists also decrease cAMP in principal cells. Because cAMP plays a key role in cystogenesis, V2R antagonists are now also used to attenuate kidney function decline in patients with autosomal dominant polycystic kidney disease [16].

Interestingly, a recent study challenged the long-held belief that AVP is produced solely by the neurohypophyseal system, with mouse and human kidneys being demonstrated to express *Avp* messenger RNA and provasopressin (the precursor to mature vasopressin) protein being detectable in CD epithelial cells [19]. Importantly, the production of kidney-derived AVP was found to increase in response to hypertonicity, suggesting that the kidney's autonomous production of AVP plays a role in the organ's ability to regulate water homeostasis. However, selective deletion of the AVP gene in the collecting duct did not impair appropriate concentration or dilution of the urine when challenged with water restriction, water loading, desmopressin or a V2R antagonist [20].

AVP-DEPENDENT AQP2 REGULATION

The apical plasma membrane is the rate-limiting barrier for transepithelial water transport across the CD principal cells. Acutely, AVP regulates the shuttling of AQP2 between intracellular vesicles and the apical plasma membrane and the retention of AQP2 in the membrane, which together are crucial for the magnitude of water reabsorption [16]. AVP binding to the V2R activates adenylate cyclase 6 and increases intracellular cAMP, which results in activation of a variety of protein kinases, including protein kinase A (PKA) (Fig. 3). One consequence of this cascade is enhanced phosphorylation of AQP2 on various serine/threonine residues in the AQP2 carboxyl terminus, which by various mechanisms promote the accumulation of AQP2 in the apical plasma membrane, enhancing water reabsorption. A recent study made use of 4.5× expansion microscopy to examine the cellular itinerary of AQP2 shuttling at high resolution [21]. An elevation of intracellular cAMP resulted in AQP2 translocation from large perinuclear endosomes to the apical membrane. Upon reduction in cAMP, AQP2 was endocytosed to various endosomes and lysosomes, suggesting enhanced AQP2 turnover once AVP signalling terminates. Importantly, although an increase in cAMP levels and activation of PKA are involved in facilitating the actions of V2R, studies in knockout mice and cell models have suggested that cAMP signalling pathways are not an absolute requirement for V2R-mediated AQP2 trafficking to the plasma membrane [22].

Chronically, AVP stimulation of the CD increases the abundance of CD AQPs. A recent study suggested that the transcriptional regulator Yes-associated protein (YAP) is a major node in controlling the expression of CD AQPs, with deletion of the *Yap* gene specifically in the renal tubules of mice resulting in decreased AQP2, AQP3 and AQP4 levels [23]. These effects of YAP are driven by the interaction of YAP with the transcription factors GATA2, GATA3 and nuclear factor of activated T cells 1 (NFATc1),

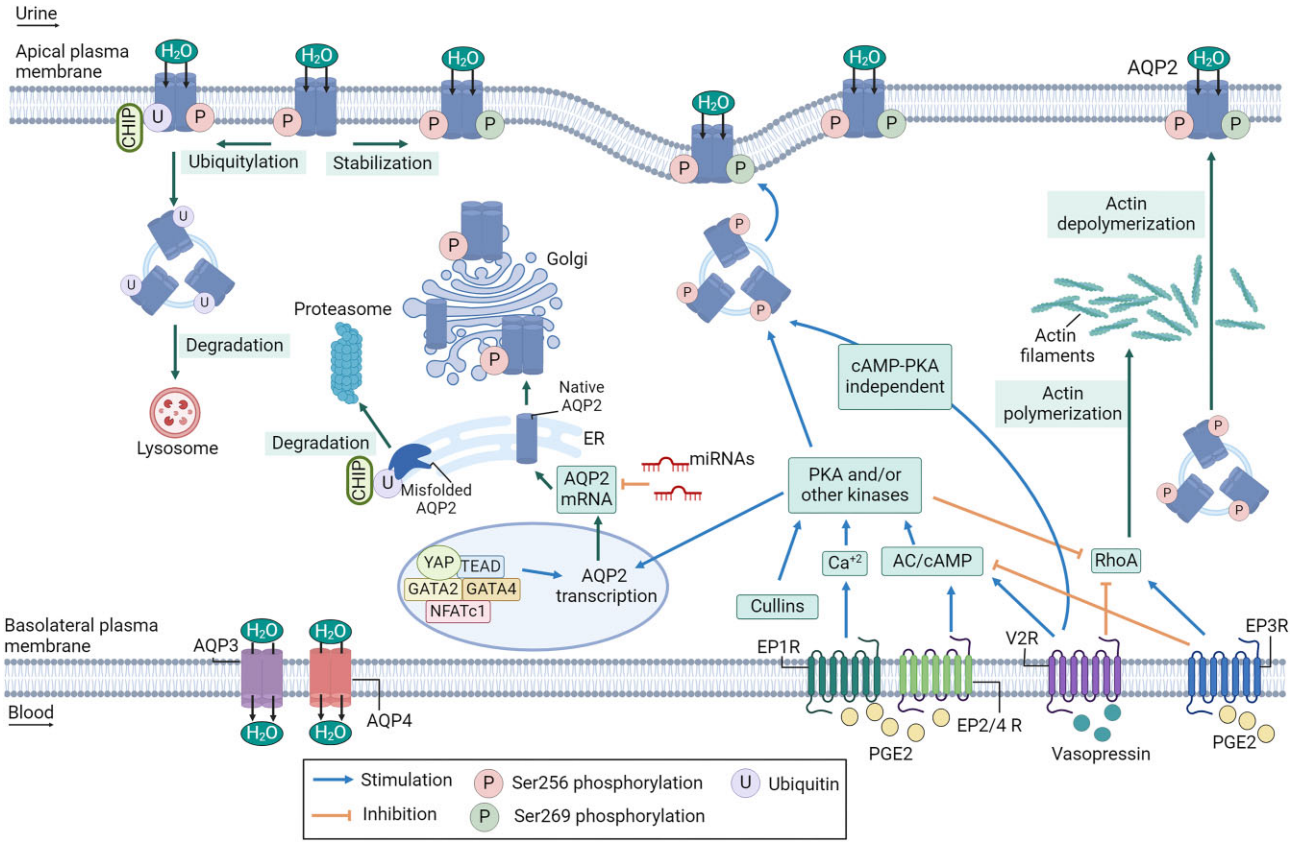

Figure 3: Modulators of AQP2 trafficking. The figure illustrates some of the key pathways involved in the regulation of AQP2 in collecting duct principal cells. In the canonical pathway, AVP activation of V2R results in greater cAMP levels, increased phosphorylation of AQP2 at Ser256 by PKA and translocation of AQP2 from intracellular vesicles to the apical membrane. Further phosphorylation of AQP2 at Ser269 increases AQP2 retention on the apical plasma membrane. cAMP-independent pathways are also important, and AQP2 can be phosphorylated by other kinases such as AMP-activated protein kinase (AMPK). AVP also modulates Rho-dependent remodelling of the actin cytoskeleton, which aids in AQP2 vesicle translocation. PGE_2 alone acts through EP2 and EP4 receptors to enhance cAMP levels and AQP2 membrane accumulation. However, AVP stimulates expression of EP1 and/or EP3 receptors. Together EP1 and EP3 inhibit AQP2 translocation by stimulating RhoA and the formation of F-actin, alongside retrieval of AQP2 from the plasma membrane. In the absence of AVP, the ubiquitin E3 ligase CHIP promotes AQP2 ubiquitylation, internalization and lysosomal degradation. At a genetic level, the transcription cofactor YAP, through a complex consisting of GATA2, GATA3, NFATc1 and YAP/TEA domain transcription factor (TEAD) are critical for AQP2 transcription, which can be enhanced by AVP. The AQP2 gene can also be regulated by epigenetic factors because of alterations in principal cell miRNAs. Created with BioRender.com.

which ultimately influences *Aqp2*, *Aqp3* and *Aqp4* gene transcription (Fig. 3). A role for another NFAT, NFAT5, to modulate *Aqp2* gene expression was also recently demonstrated using mice with principal cell-specific deletion of this transcription factor [24]. NFAT5-deficient mice ($NFAT5^{PC\text{-}KO}$) had significantly higher water intake and their 24-hour urine volume was almost 10-fold greater than controls, effects that were concomitant with reduced abundance of AQP2 and other water channels or transporters. When challenged with AVP or water restriction, $NFAT5^{PC\text{-}KO}$ mice were unable to concentrate their urine, confirming AVP resistance. In contrast, in mice with principal cell–specific deletion of E74-like factor 5 (ELF5), another transcription factor proposed to regulate AQP2, no clear differences in AQP2 abundance were observed and there were only mild changes in renal water handling. These findings indicate that while ELF5 plays a minor role in water homeostasis, a YAP–NFAT5 axis is a major regulator of *Aqp2* gene transcription.

Another recent study has unveiled an additional layer of post-transcriptional regulation of the *Aqp2* gene mediated by microRNAs (miRNAs) (Fig. 3). CD-specific deletion of the miRNA biogenesis enzyme Dicer in mice led to a severe reduction in kidney AQP2 and AQP4 levels, and the mice suffered from AVP resistance [25]. Further analysis revealed significant changes in the expression of 31 miRNAs predicted to target key epigenetic regulators and transcription factors involved in AQP2 expression, including *Gata2*, *Gata3* and *Elf3*. Three specific miRNAs (miR-7688-5p, miR-8114 and miR-409-3p) were demonstrated to be important for modulating the epigenetic machinery, leading to decreased RNA polymerase II association at the *Aqp2* gene promoter.

In addition to enhancing AQP2 transcription and translation, AVP limits the degree of AQP2 degradation. Although these effects are multifactorial, a recent study suggests that members of the Cullin-RING ubiquitin E3 ligases play a vital role in mediating some of the effects of AVP to increase AQP2 abundance in a mechanism dependent on AQP2 phosphorylation [26]. Another E3 ubiquitin ligase important for AQP2 biogenesis is the C-terminus of Hsp70 interacting protein (CHIP), which ubiquitylates AQP2 to regulate its abundance and hence modifies renal water handling (Fig. 3) [27].

AVP-INDEPENDENT AQP2 REGULATION

In addition to AVP-V2R signalling, a variety of alternative mechanisms that modulate AQP2 localization and function have been

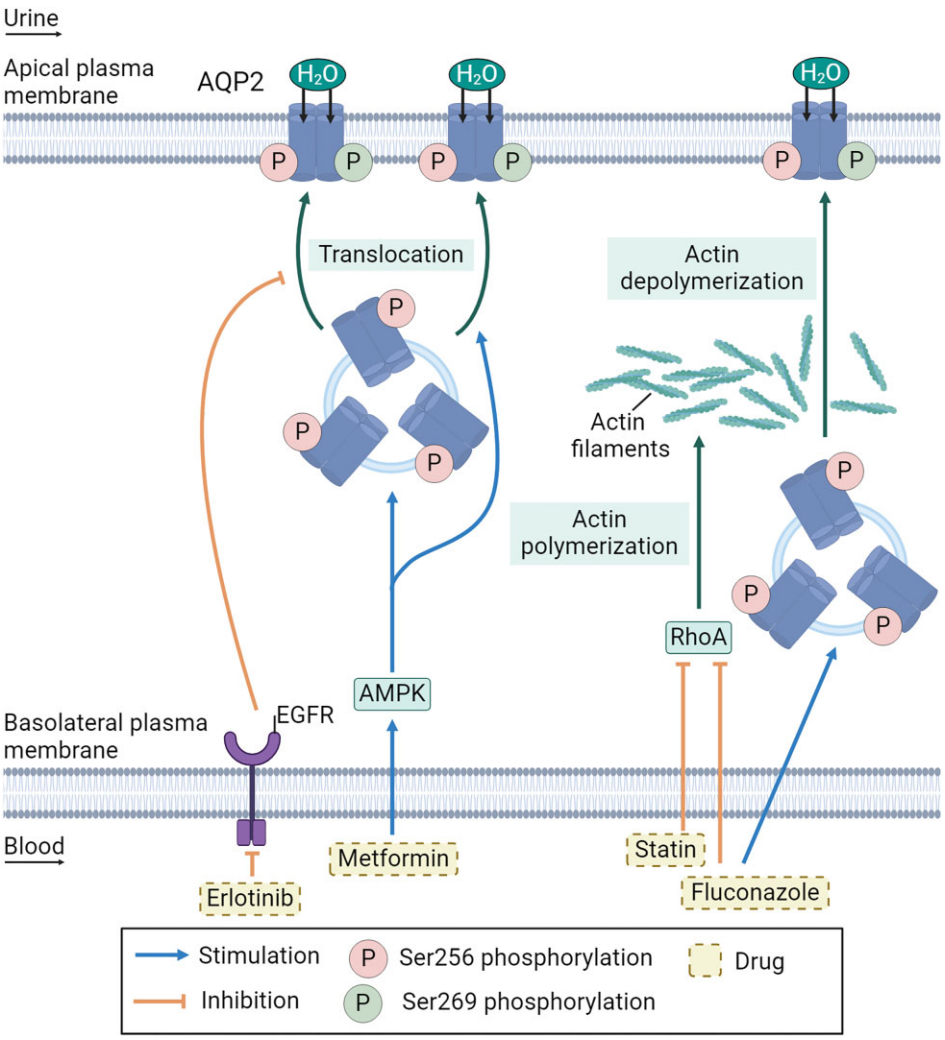

Figure 4: Vasopressin-independent modulators of aquaporin-2 trafficking. The figure illustrates some of the regulators of AQP2 plasma membrane targeting that are independent of the V2R and/or cAMP pathway. The EGFR inhibitor erlotinib enhanced AQP2 plasma membrane targeting and improved urine concentration in mice with lithium-induced AVP resistance. Metformin activates AMP-activated protein kinase (AMPK), leading to the phosphorylation of AQP2 at Ser256, increased AQP2 plasma membrane targeting and enhanced urine concentration in rats with tolvaptan-induced AVP resistance and in V2R knockout mice. Statins and fluconazole facilitate AQP2 plasma membrane targeting by inhibiting RhoA activity and actin polymerization. Created with BioRender.com.

recently uncovered that provide insights into potential therapeutic targets for disorders associated with impaired water balance [15] (Fig. 4). The role of the epidermal growth factor receptor (EGFR) in water homeostasis was investigated using erlotinib, an EGFR inhibitor used clinically to treat lung cancer [28]. Erlotinib increased AQP2 accumulation at the plasma membrane of kidney principal cells. In a mouse model of lithium-induced AVP resistance, erlotinib treatment led to a reduction in urine volume and an increase in urine osmolality, accompanied by an increase in membrane-localized AQP2. The effects of EGFR inhibition on AQP2 trafficking were independent of the canonical AVP–cAMP–PKA pathway, but crosstalk between EGFR and AVP signalling is likely, as the EGFR ligand EGF could antagonize AVP-induced phosphorylation of AQP2.

The antifungal drug fluconazole also promotes AQP2 accumulation in the plasma membrane of CD principal cells independent of the normal AVP signalling pathway [29], as demonstrated by enhanced osmotic water reabsorption across isolated CD epithelia and reduced urine output in mice treated with a V2R blocker. Fluconazole effects are proposed to be through modulation of AQP2 phosphorylation, ubiquitylation and inhibition of the small GTPase RhoA, which normally act as barriers to AQP2 vesicle trafficking and plasma membrane insertion (Fig. 4). Fluconazole is currently being tested in patients with congenital AVP resistance (EudraCT 2020-002204-38).

The antidiabetic drug metformin also increased the protein abundance of key transporters involved in urine concentration, including the urea transporter UT-A1 and AQP2, and restored urine osmolality in a rat model of AVP resistance treated with the V2R antagonist tolvaptan or in V2R knockout mice [30]. Similarly, the cholesterol-lowering drug simvastatin enhanced AQP2 surface expression and urinary concentration in a rat model of AVP deficiency [31]. Furthermore, a combination of fluvastatin and secretin increased AQP2 membrane expression and urine concentration in V2R knockout mice [32]. However, in mice, atorvastatin was unable to ameliorate AVP resistance induced by lithium or potassium depletion [33]. A double-blind, randomized, placebo-controlled pilot trial in patients with AVP resistance due to lithium

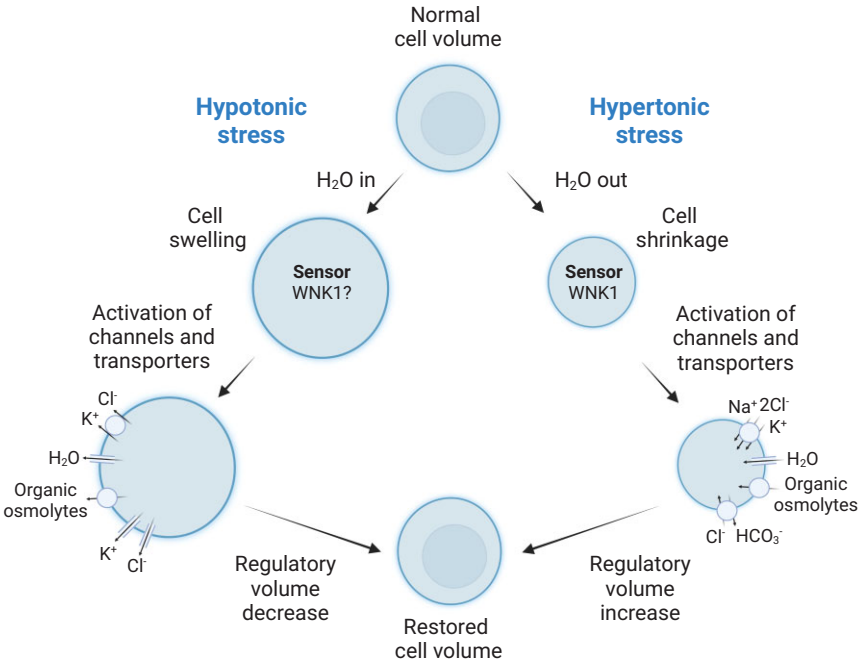

Figure 5: Cell volume regulation. The effects of hypotonic and hypertonic stress on cell volume are depicted. Cells respond to hypotonic and hypertonic stress by activating cell volume regulation mechanisms to restore cell volume. WNK1 was identified as a sensor for a reduction in cell volume [42]. It is unclear if WNK1 can also sense cell swelling. In response to sensing a change in volume, cells activate channels and transporters to restore cell volume. Transport of water and organic osmolytes plays a role in both RVD and RVI. In RVD, potassium–chloride efflux also plays an important role, whereas in RVI, NKCC1 and chloride–bicarbonate transporters are activated. It is unclear if RVD and RVI are really orchestrated by a different set of transporters or if all transporters play a role in both processes. Created with BioRender.com.

also showed no effect of atorvastatin [34]. In healthy subjects, simvastatin, but not metformin, caused a significant but modest increase in urine osmolality after water loading [35].

The effects of prostaglandin E_2 (PGE_2) on AVP-mediated CD water transport are well established, with PGE_2 antagonizing the effects of AVP, whereas in the absence of AVP, water transport is enhanced by PGE_2 (Fig. 3) [36]. The effects of PGE_2 are mediated via activation of four receptors, the prostaglandin E receptors 1–4 (EP1, EP2, EP3 and EP4). Activation of one or more of these receptors under any specific condition may be responsible for the divergent effects of PGE_2 in natriuresis and diuresis. A recent study shed light on these contrasting effects, demonstrating that PGE_2 induces changes in EP receptor subtype expression [37]. Using a cell model of the mouse cortical collecting duct (mpkCCDc14 cells), the researchers confirmed previous observations that PGE_2 alone increased AQP2 abundance, but AQP2 was decreased when PGE_2 and AVP were both present. These effects appear to be due to the ability of AVP to increase AQP2-antagonizing EP1 expression and decrease AQP2-stimulating EP4 expression. Thus these studies suggest that CD cells can adapt to receptor-mediated signalling by desensitizing receptors activating the same pathway while sensitizing receptors of alternative pathways. AVP-independent AQ2 regulation by PGE_2 is clinically relevant, as it appears to play a role in thiazide-induced hyponatraemia [38, 39] and thiazide-mediated attenuation of tolvaptan-induced polyuria [40].

CELL VOLUME REGULATION

Changes in extracellular osmolality expose cells to osmotic stress and lead to cell swelling in the setting of acute hyponatraemia or cell shrinkage in the setting of acute hypernatraemia (Fig. 5). Cells respond to hypotonic stress by activating a process called regulatory volume decrease (RVD) and to hypertonic stress by activating regulatory volume increase (RVI) [41]. WNK1 was recently identified as a molecular crowding sensor that mediates RVI [42]. Hypertonicity-induced cell shrinkage causes macromolecular crowding in the cytosol that initiates phase separation, a biophysical process that forms biomolecular condensates in the cell. WNK1 forms condensates after hypertonic stress through its intrinsically disordered C-terminal domain. This allows WNK1 to activate Ste20-related proline/alanine-rich kinase (SPAK) and oxidative stress responsive 1 (OSR1) kinase, which in turn activate sodium–potassium–chloride cotransporter 1 (NKCC1) and inhibit potassium–chloride cotransporters (KCCs). This increases the cellular influx of sodium, potassium and chloride through NKCC1 and prevents potassium efflux through KCCs—together, these processes restore cell volume. The role of WNK1 in RVI may also explain its proposed role as an osmolality sensor in the osmosensing neurons of the SFO and OVLT [7, 42] (Fig. 1).

Whether WNK1 also plays a central role in RVD remains to be investigated. RVD is clinically relevant, as it counteracts cell swelling induced by acute hyponatraemia. Acute hyponatraemia (development in <48 hours) causes brain cell swelling that can result in cerebral oedema and death.

AQP4 has been identified as the major water channel regulating cerebral water balance. AQP4 is expressed in the foot processes of astrocytes and mediates water permeability of the blood–brain barrier. AQP4 is a key player in brain oedema, but whether it plays a positive or negative role depends on the type of cerebral oedema. AQP4-deficient mice have improved survival compared with wild-type mice in a model of cerebral oedema caused by acute water intoxication [43]. AQP4-deficient mice also have better neurological outcomes after ischaemic stroke [43]. Hypoxia upregulates AQP4 in a calmodulin-dependent manner and calmodulin inhibition

with trifluoperazine improved hypoxia-induced cerebral oedema [44]. Hyponatraemia and hypoxia are both forms of cytotoxic cerebral oedema. In contrast, AQP4 deficiency worsens outcomes in the vasogenic and hydrocephalic forms of cerebral oedema [45, 46]. In addition to its role in cerebral water balance, AQP4 also appears to play a role in the physiological suppression of AVP neurons during hypotonicity [47]. Autoantibody formation against AQP4 causes the disease neuromyelitis optica, which can be accompanied by SIAD through inappropriate AVP secretion [48].

CONSEQUENCES OF CELL VOLUME ADAPTATION TO CHRONIC HYPONATRAEMIA

In addition to the rapid initial phase of RVI and RVD (seconds), there is a slower secondary phase (days) that involves the efflux of organic osmolytes (Fig. 5). This secondary phase mediates long-term adjustment to chronic changes in extracellular tonicity. However, this adjustment comes at the cost of an increased vulnerability to rapid correction of extracellular tonicity, especially during chronic hyponatraemia. Indeed, overcorrection of chronic hyponatraemia can give rise to a neurological disorder called osmotic demyelination, in which astrocyte death and disruption of the blood–brain barrier precedes the development of demyelination [49].

It is also becoming increasingly clear that the cellular release of organic osmolytes can contribute to the neurological complications of chronic hyponatraemia, including gait disturbances and cognitive impairment [50]. A rat model of SIAD recapitulated the neurological complications of chronic hyponatraemia and revealed long-term potentiation of hippocampal synapses [51]. Microdialysis showed that this was accompanied by elevated extracellular concentrations of glutamate. In primary astrocyte cultures, low extracellular sodium concentration prevented the cellular uptake of glutamate [51], although the hypotonicity-induced loss of taurine appears to be even greater than for glutamate [52]. Recently, the orphan G-protein coupled receptor GPR158 was shown to mediate the neuronal excitability of glycine and to a lesser extent taurine [53]. Because glycine and taurine are closely linked to cognitive and affective disorders, these neurotransmitters may also be involved in the neurological effects of chronic hyponatraemia. Importantly, the neurological effects of chronic hyponatraemia are reversible upon treatment. This was recently demonstrated in a clinical study in which 26 patients received neuropsychological and psychomotor testing and functional MRIs before and after treatment for chronic hyponatraemia (plasma sodium changes from 118 to 136 mmol/l) [54]. Most of the tests improved upon correction of hyponatraemia, with MRI studies showing decreased brain tissue volumes, neuronal activity and synchronization after correction of hyponatraemia. The volume effects were most prominent in the hippocampus.

In addition to the effects on the brain, chronic hyponatraemia also contributes to bone demineralization, osteoporosis and an increased risk of fractures [55]. This is likely caused by calcium release from bone cells, which may explain why chronic hyponatraemia is also associated with hypercalciuria and an increased risk of kidney stones [56]. In a recent study, hyponatraemia was also found to promote vascular calcification through Rac1-Akt pathway activation [57]. In vascular smooth muscle cells and aortic rings, low osmolality exacerbated calcification of the extracellular matrix through oxidative stress and osteogenic differentiation. Low osmolality also accelerated the generation and maturation of calciprotein particles, which are calcium- and phosphate-containing nanocrystals that promote vascular calcification. Finally, human autopsy specimens showed that hyponatraemia was associated with a greater area of arterial intimal calcification. These data are relevant because hyponatraemia is consistently identified as an independent contributor to increased mortality [58]. Furthermore, hyponatraemia is a relatively common electrolyte disorder in patients with chronic kidney disease and kidney failure, who are particularly prone to vascular calcification [59, 60]. In these patients, hyponatraemia is also associated with increased all-cause and cardiovascular mortality, even after kidney failure transition [59, 60]. Future studies should address whether prevention or correction of hyponatraemia improves these outcomes. Since the effects of hypotonicity on vascular smooth muscle cells are mediated by sodium–calcium exchanger 1 (NCX1), it would also be interesting to investigate if hypotonicity-induced vascular calcification can be prevented by inhibiting this transporter.

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AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and design of the work, drafted parts of the manuscript and worked to revise it critically and approved the final version.

DATA AVAILABILITY STATEMENT

No new data were generated or analysed in support of this research.

CONFLICT OF INTEREST STATEMENT

None declared.

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