FROM FINCH TO FISH TO MAN: ROLE OF AQUAPORINS IN BODY FLUID AND BRAIN WATER REGULATION

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Abstract—Charles Darwin, in his Origin of the Species, noted that different species of finches on the Galapagos Islands had adapted their beak size based on where they sought their food. Homer Smith, in his book From Fish to Philosopher, discussed the evolution of the nephron from a single conduit in salt water vertebrates, to nephrons with large glomerular capillaries and proximal and distal tubules in fresh water vertebrates, to smaller glomerular capillaries in amphibians, to nephrons with loops of Henle to allow for urinary concentration and dilution in mammals. The kidney with its million nephrons has emerged as the vital organ for regulating body fluid composition and volume. With the recent discovery of aquaporin water channels, our understanding of volume regulation has been greatly enhanced. This article reviews current knowledge regarding: 1) the unifying hypothesis of body fluid volume regulation; 2) brain aquaporins and their role in pathophysiologic states; and 3) function and regulation of renal aquaporins in the syndrome of inappropriate antidiuretic hormone secretion (SIADH).

Key words: water metabolism, unifying hypothesis of body fluid volume regulation, aquaporin, brain, SIADH.

Charles Darwin (1839), in his Origin of the Species, noted that different species of finches on the Galapagos Islands had adapted their beak size based on where they sought their food. In From Fish to Philosopher, Homer Smith (1953) suggested that the concentrating capacity of the mammalian kidney contributed to the evolution of various biologic species including man. Specifically, the early protovertebrates resided in a salt-water environment whose composition was similar to that of their own extracellular fluid. Therefore, these animals could ingest salt water freely without altering the composition of their milieu interieur (Bernard, 1885). As early vertebrates migrated into freshwater streams, the development of a more water-impermeable integument was necessary to avoid body fluid dilution by the hypoosmotic fresh water environment. Thus a vascular tuft or primitive glomerulus developed, enabling the fish to filter excess fluid from the blood. The subsequent development of the proximal and distal tubules in vertebrates was seminal for preservation of sodium and excretion of excess solute-free water, respectively. The kidney with its million nephrons has emerged as the vital organ for regulation of body fluid composition and volume. Our understanding of the regulation of water metabolism has been greatly advanced by the recent discovery of aquaporin water channels (Preston et al., 1992). These water channels have been localized to several organs in the body, including kidney and brain. This article reviews current knowledge regarding: 1) the unifying hypothesis of body fluid volume regulation; 2) brain aquaporins and their role in pathophysiologic states; and 3) function and regulation of renal aquaporins in the syndrome of inappropriate antidiuretic hormone secretion (SIADH).

UNIFYING HYPOTHESIS OF BODY FLUID VOLUME REGULATION

Body fluid volume regulation by the kidney relies on the complex interaction of numerous factors (Table 1). Clinically maladaptive responses can occur when extrarenal factors override the “innate wisdom” of the kidney (Sterling, 1909). For example, in patients with cardiac failure or liver disease and in pregnant women, the normal kidney continues to retain sodium and water despite expanded blood, plasma, and extracellular fluid (ECF) volumes. Such fluid retention may ultimately lead to pulmonary congestion, ascites, or peripheral edema. Cardiac output cannot provide the sole afferent signal for the kidney to regulate sodium and water balance, since the normal kidney may retain excessive amounts of sodium and water when cardiac output is low, e.g. low output cardiac failure, or high, e.g. cirrhosis or pregnancy.

The efferent limb of renal sodium and water regulation may also act in an apparently detrimental manner. For example, excessive amounts of sodium and water can be retained in patients with cardiac failure or liver disease prior to any fall in glomerular filtration rate (GFR). Moreover, positive sodium and water balances occur during normal pregnancy in the presence of a 30–50% increase in GFR. Verney (1947) delineated the exquisite sensitivity of hypothalamic osmoreceptors in regulating the secretion of the antidiuretic hormone, arginine vasopressin (AVP), showing altered renal water excretion with changes in...
plasma osmolality of only 1–2%. Yet patients with severe cardiac failure and hepatic cirrhosis frequently have excessive water retention leading to profound hyponatremia. The sodium retaining hormone, aldosterone, may be elevated in heart failure and cirrhosis but, in contrast to normal individuals who demonstrate escape from the renal sodium retaining effect of aldosterone, this escape does not occur in patients with advanced cardiac or liver failure. A deficiency of natriuretic hormones also does not explain the sodium and water retention in heart failure and cirrhosis since both atrial natriuretic peptide (ANP) and brain natriuretic peptide have been shown to be increased in patients with cardiac failure and cirrhosis. Resistance to the natriuretic and diuretic action of ANP has also been shown to occur in association with cardiac failure and cirrhosis (Cody et al., 1986).

Thus, several dilemmas must be resolved in order to understand body fluid composition and volume regulation by the kidney. Upon these observations, we have proposed our unifying hypothesis of body fluid volume regulation (Schrier, 1988a,b, 1990, 1992; Schrier and Abraham, 1999; Schrier and Ecder, 2001).

The majority (85%) of circulating blood resides on the venous side of the circulation whereas only an estimated 15% of intravascular blood is in the arterial circulation. In this context excessive sodium and water retention could occur and expand total blood and ECF volume if the kidney responded primarily to the integrity of the arterial circulation. Specifically, renal salt and water retention could occur and expand the venous side of the circulation and increase total blood volume even as a reduced cardiac output caused underfilling of the arterial circulation (Fig. 1). The neurohumoral activation in response to low cardiac output increases systemic vascular resistance through activation of the sympathetic nervous system and renin–angiotensin–aldosterone system (RAAS), enhances sodium retention via the RAAS, and increases renal water retention through the nonosmotic release of AVP. In vivo neurophysiological studies of vasopressin neurons in the supraoptic nuclei have shown that a single neuron can respond to either an osmotic or a nonosmotic baroreceptor-mediated stimulus (e.g. hypotension; Kannan and Yagi, 1978). In fact, the competition between such stimuli as occurs in the presence of hypoosmolality, a suppressor of AVP release, and a nonosmotic stimulus of AVP release may be the most common mechanism for the so-called reset osmostat. This attempt to maintain arterial integrity may ultimately contribute to increased morbidity, and in this context, higher plasma renin activity, aldosterone, norepinephrine, and AVP concentrations have been associated with worse clinical outcomes in cardiac failure (Cohn et al., 1984; Hensen et al., 1991; Lee and Packer, 1984; Watkins et al., 1976).

Such a sequence of events, however, would not explain the sodium and water retention which occurs in states of increased cardiac output as seen in cirrhosis and pregnancy. Thus, we have proposed that despite an increase in blood volume, arterial underfilling could also occur when the amount of blood in the arterial circulation is inadequate to fill the vasodilated intravascular compartment (Schrier, 1988b; Schrier et al., 1988; Schrier and Briner, 1991; Fig. 2). Such peripheral arterial vasodilation would be accompanied by increased systemic vascular resistance and renal sodium and water retention as described above for low cardiac output states. The effect of decreased cardiac output and peripheral arterial vasodilation on water metabolism is illustrated in Fig. 3.

**Table 1. Body fluid volume regulation**

<table>
<thead>
<tr>
<th>Afferent limb</th>
<th>Efferent limb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total blood volume</td>
<td>GFR</td>
</tr>
<tr>
<td>Total plasma volume</td>
<td>Vasopressin</td>
</tr>
<tr>
<td>Total ECF Volume</td>
<td>Aldosterone</td>
</tr>
<tr>
<td>Cardiac output</td>
<td>ANP</td>
</tr>
</tbody>
</table>

**Fig. 1.** Sequence of events in which reduced cardiac output initiates renal sodium and water retention.
AVP and aquaporins

Water retention with associated hyponatremia is a common finding in patients with advanced cardiac failure and cirrhosis and in pregnant women. For many years the mechanism(s) of hyponatremia in these subjects was controversial because an increase in plasma AVP by bioassay could not be consistently detected. Thus, intrarenal mechanisms including enhanced proximal tubular fluid reabsorption with resultant decreased delivery of fluid to the distal diluting segment of the nephron was proposed. However, experimental studies of low output cardiac failure due to inferior vena caval constriction demonstrated a profound antidiuresis which was abolished in hypophysectomized, glucocorticoid-replaced animals (Anderson et al., 1974). These results led to the hypothesis that baroreceptor-mediated nonosmotic release of AVP was involved in hyponatremic heart failure, but was undetectable by bioassay. In this regard, the nonosmotic release of AVP was found to be associated with activation of the neurohumoral axis as occurs with arterial underfilling. This hypothesis could not be accepted or rejected until the radioimmunoassay (RIA) for measurement of AVP was perfected and vasopressin V2 antagonists were developed. In a series of 37 heart failure patients with hyponatremia and hypomosmolality of a degree that would suppress plasma AVP to undetectable levels in normal subjects, plasma AVP concentrations were still detectable by RIA in all but seven patients (Szatalowicz et al., 1981). This experiment provided the first clinical evidence for a potential role of nonosmotic AVP release in the hyponatremia associated with cardiac failure. Further studies in an experimental rat model of heart failure secondary to coronary artery ligation demonstrated an increase in the mRNA for vasopressin in the hypothalamus (Kim et al., 1990) and a profound water diuresis with the administration of a non-peptide, orally active vasopressin V2 receptor antagonist (Xu et al., 1997). Subsequent studies have confirmed a role for the nonosmotic release of AVP in water retention in cirrhosis (Bichet
et al., 1982; Kim et al., 1993; Fujita et al., 1995) and in pregnancy (Ohara et al., 1998).

However, it was not until 1992 that the molecular mechanisms underlying AVP-mediated water reabsorption were elucidated. The Nobel Prize-winning discovery by Peter Agre and associates of the first water channel aquaporin-1 (AQP1) in the red blood cell, proximal tubule, and descending limb of Henle’s loop initiated an important new era in the understanding of renal water excretion (Preston et al., 1992). The AQP1 gene was localized on chromosome 7 and was identical to the gene for the Colton blood group. In both null patients for the Colton blood group (King et al., 2001) and AQP1 knockout mice (Ma et al., 1998), a urinary concentrating defect was observed. Sasaki and associates in Japan then cloned AQP2 in the rat collecting duct (Fushimi et al., 1993). It was subsequently demonstrated that AVP exerted short and long term effects on AQP2 (Nielsen et al., 1995). The short term effect involved a cascade initiated by AVP binding to its V2 receptor on the basolateral membrane of the collecting duct principal cell, with subsequent activation of adenylyl cyclase, cyclic AMP, protein kinase A, and phosphorylation of the C-terminus of AQP2. This latter event induced trafficking of AQP2 from cytosolic storage vesicles to the apical membrane allowing reabsorption of water from lumen to cell. Deletion of serine-256 on the C-terminus of AQP2 was shown to block AQP2 trafficking to the apical membrane (Fushimi et al., 1997). Suppression of AVP was shown to induce endocytic retrieval of AQP2 to the cytosolic vesicles. The long term effect of AVP leads to upregulation of AQP2 protein and involves the cyclic AMP response element on the promoter of the AQP2 gene (DiGiovanni et al., 1994).

On this background, it was found that 3–6% of apical AQP2 during AVP exposure could be detected in the urine by Western blot (Rai et al., 1997). Subsequently, it has been shown that urinary AQP2 can also be assessed by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) methodology (Saito et al., 1997b; Umenishi et al., 2002) and may ultimately be a useful tool in the evaluation of patients. Patients with cardiac failure and cirrhosis (Fukuma et al., 1996; Pedersen et al., 2003) and in pregnant women (Buemi et al., 1998) have been shown that urinary AQP2 can also be assessed by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) methodology (Saito et al., 1997b; Umenishi et al., 2002). Similarly, urinary AQP2 excretion has been shown to be increased in patients with cirrhosis (Fukuma et al., 1996; Pedersen et al., 2003; Ivarsen et al., 2003) and in pregnant women (Buemi et al., 2001). Despite improvements in water balance, an effect of vasopressin V2 antagonists on mortality in cardiac failure and cirrhotic patients has yet to be demonstrated.

Aquaporins and the brain

Water homeostasis in the brain is of central physiologic and clinical importance. Cerebral edema may produce increased intracranial pressure with associated impairment in cerebral perfusion. Such cerebral ischemia may lead to herniation and death. This process is critical in the pathophysiology of brain trauma, infarction, neoplasm, and local or systemic infection or inflammation. Recent data suggest that AQP water channels may provide a key route for water movement in the brain (Venero et al., 2001; Papadopoulos et al., 2002; Amiry-Moghaddam and Ottersen, 2003). More than 10 different mammalian aquaporins have been identified to date, and at least seven are known to be present in the rodent brain (Table 2; Chen et al., 2004).

AQP4 is the predominant and the first described water channel in the brain. AQP4 exists as two isoforms, differing at their N-termini, because of translation initiation at the first methionine (M1, 323 aa) or the second methionine (M23, 301 aa; Jung et al., 1994; Lu et al., 1996). Both isoforms are present in brain, but M23 is at least three-fold more abundant (Neely et al., 1999, 2001). Endogenous AQP4 typically exists as a tetramer containing M1 and M23 subunits. The water permeabilities of M1 and M23 are reportedly similar, and any functional differences are not yet known (Jung et al., 1994; Neely et al., 1999). AQP4 is normally present within brain parenchyma and is particularly concentrated in astrocyte end-feet surrounding brain capillaries in the rat (Frigeri et al., 1995; Nielsen et al., 1997a). AQP4 is also abundant in astrocyte processes forming the glia limitans at brain surfaces, ependymal cells lining brain ventricles, and Müller cells facing the vitreous body and retinal blood vessels (Nagelhus et al., 1998). Upregulation of AQP4 in perivascular membranes of astrocyte end-feet has been implicated in neurological disorders, including acute hyponatremia, postischemic injury, epileptic seizures, and brain tumors (Vajda et al., 2002; Amiry-Moghaddam et al., 2003a,b). Consistent with these findings, AQP4 null mice demonstrate an approximately 50% decrease in cerebral edema and improved neurological outcome following water intoxication or focal cerebral ischemia (Manley et al., 2000) as well as increased seizure threshold and latency to generalized seizure (Binder et al., 2004). These findings suggest that water movement via AQP4 may modulate intrinsic brain excitability. Thus, inhibition or modulation of AQP4 by specific pharmacological agents may represent a novel target for protection against cerebral edema or epilepsy. It is important to note, however, that ischemic brain injury has been shown to be associated with loss of AQP4 from perivascular membranes in the post-ischemic phase (Amiry-Moghaddam et al., 2003a). This is likely beneficial in prevention of further cerebral edema but may also limit resolution of such edema. This finding has important therapeutic implications for the future development and use of AQP4 inhibitors.

AQP1 is restricted to the choroid plexus of the lateral ventricles and probably facilitates cerebrospinal fluid (CSF) secretion (Nielsen et al., 1993). AQP1 null mice have been shown to have impaired CSF production as compared with wild-type mice (Oshio et al., 2003). Although not normally found within brain parenchyma, AQP1 is extensively expressed within the cytoplasm of primary
malignant brain tumors such as astrocytomas, suggesting a possible role for AQP1 in the generation of tumor-associated edema (Saadoun et al., 2002). It has also been suggested that selective inhibition of AQP1 might be useful for treatment of communicating hydrocephalus (Griesdale and Honey, 2004).

AQP9 is found on astrocytic processes and cell bodies (Tsukaguchi et al., 1998) and is also upregulated in response to transient focal brain ischemia (Badaut et al., 2002). The functional role of AQP9 in the brain is not well understood. However, this water channel belongs to a subfamily of aquaporins that also facilitate glycerol transport, and it has been suggested that AQP9 may have a role in energy metabolism in the brain (Amiry-Moghaddam and Ottersen, 2003). The clinical significance of interactions between AQP1, AQP4, and AQP9 in CNS pathophysiology has not been delineated.

Aquaporins-3, -5, -8, and -11 have also been identified in rat brain at low abundance by Northern blot and reverse transcription-polymerase chain reaction techniques. However, the physiologic role of these AQPs remains to be determined (Badaut et al., 2002).

These data provide valuable information regarding the role of aquaporin water channels in the brain in normal and pathologic states. Brain edema is a major contributor to morbidity and mortality in a wide variety of CNS disorders such as head injury, brain tumors, ischemic stroke, and infection. Further studies are needed to characterize the function and regulation of these brain aquaporins in health and disease. Moreover, additional investigation is needed to determine if inhibition of these aquaporins may provide a novel approach for therapy of CNS pathology.

**Table 2. Aquaporins in the brain**

<table>
<thead>
<tr>
<th>Aquaporin (Abbreviation)</th>
<th>Number of Amino Acids</th>
<th>Gene Locus</th>
<th>Localization</th>
<th>Subcellular Distribution</th>
<th>Function</th>
<th>Effect of Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP1 (CHIP28)</td>
<td>269</td>
<td>7p14</td>
<td>Apical membrane of choroid plexus epithelial cells, renal proximal tubule and descending thin limb, erythrocytes, cornea, capillary endothelia (except brain)</td>
<td>APM/BLM</td>
<td>Constitutively expressed water channel, facilitating &quot;isotonic&quot; fluid movement</td>
<td>Impaired urinary concentration in mice and humans</td>
</tr>
<tr>
<td>AQP3 (GLIP)</td>
<td>285</td>
<td>9p13</td>
<td>Glial and neuronal cells, principal cell of renal collecting duct, colon, various epithelial cells</td>
<td>BLM</td>
<td>Water channel (also glycerol permeable)</td>
<td>Moderate polyuria with near normal urinary concentrating capacity</td>
</tr>
<tr>
<td>AQP4 (MIWC)</td>
<td>262</td>
<td>M1: 323 M23: 301</td>
<td>18q11.2–q12.1 Astrocytic end-feet in contact with brain vessels and ependymal cells, principal cell of renal collecting duct, inner ear, skeletal muscle, gastric parietal cells, and various epithelial cells</td>
<td>BLM</td>
<td>Water channel</td>
<td>Modulator of brain edema in mice and mild polyuria</td>
</tr>
<tr>
<td>AQP5</td>
<td>282</td>
<td>12q13</td>
<td>Astrocytes and ependymal cells, lacrimal glands, cornea, salivary glands, lung, trachea</td>
<td>APM/IVC</td>
<td>Water channel, facilitating production of isotonic secretions</td>
<td>Diminished and viscous saliva in mice</td>
</tr>
<tr>
<td>AQP8</td>
<td>263</td>
<td>16p12</td>
<td>Glial and neuronal cells, renal cortex and medulla, testis, epididymis, pancreas, liver, colon, heart, placenta</td>
<td>ICV</td>
<td>Water channel</td>
<td></td>
</tr>
<tr>
<td>AQP9</td>
<td>295</td>
<td>15q22.1–q22.2</td>
<td>Astrocytic processes and cell bodies and ependymal cells, kidney, liver, leukocytes, lung, spleen, epididymis, testis</td>
<td>APM/PM</td>
<td>Channel for water and small uncharged molecules?</td>
<td></td>
</tr>
<tr>
<td>AQP11</td>
<td>271</td>
<td>11q13.4</td>
<td>Brain, kidney, pancreas</td>
<td>Polycystic kidneys in mice</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

APM, apical plasma membrane; BLM, basolateral plasma membrane; CHIP, channel forming integral protein; GLIP, glycerol-transporting integral protein; ICV, intracellular vesicles; MIWC, mercury-insensitive water channel; PM, plasma membrane. Most of the brain aquaporins have been cloned from several species (human, rat, and mouse).

**SIADH**

Hyponatremia is a common electrolyte disorder in the setting of CNS disease and is often attributed to the syndrome of inappropriate secretion of antidiuretic hormone (SIADH). This syndrome is characterized by a sustained elevation of plasma AVP concentration with inappropriately concentrated urine, increased urine sodium concentration, and euvoolemia (Berl and Schrier, 2002). Fortunately, the degree of hyponatremia is limited by a process called "vasopressin escape," which is associated with a sudden increase in urine volume and decrease in urine osmolality.
independent of circulating AVP levels (Levinsky et al., 1959). In rats receiving a liquid diet and dDAVP, a V2 receptor-selective agonist of vasopressin, it has been shown that the onset of vasopressin escape coincides temporally with a marked reduction in renal AQP2 mRNA and protein expression in the collecting ducts (Ecelbarger et al., 1997). Further studies examining the mechanisms of AQP2 signaling have shown that vasopressin escape is associated with a decrease in vasopressin V2 receptor mRNA expression and binding as well as a decrease in cyclic AMP production in response to acute dDAVP administration in collecting duct suspensions in this animal model (Tian et al., 2000; Murase et al., 1998; Ecelbarger et al., 1998). Moreover, several ion transporters involved in distal sodium reabsorption, including the thiazide-sensitive NaCl cotransporter of the distal convoluted tubule, the α-subunit of the epithelial sodium channel (ENaC) of the collecting duct, and the 70-kDa band of the γ-subunit of ENaC, have been shown to be increased in this animal model of vasopressin escape (Ecelbarger et al., 2001). It has been proposed that such increased distal nephron sodium reabsorption might attenuate the hyponatremia associated with SIADH.

Consistent with these observations is the finding that administration of a vasopressin V2 receptor antagonist was associated with increased urine volume, decreased urine osmolality, and resolution of hyponatremia and hypoosmolality in a rat model of SIADH (Fujisawa et al., 1993). Such drugs have also been shown to acutely improve hyponatremia in patients with SIADH (Saito et al., 1997a), and studies of chronic administration of vasopressin V2 receptor antagonists in this condition are currently under way.

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