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The aquaporin family of water channels in kidney: An update on physiology and pathophysiology of aquaporin-2

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The aquaporin family of water channels in kidney: An update on physiology and pathophysiology of aquaporin-2. The long-standing problem of membrane water transport has been advanced by the recognition of a new family of water transport proteins, the "aquaporins" [1–3]. Not surprisingly, water transport is a major process in kidney physiology, and the biology of aquaporins is most thoroughly understood in that organ. We reviewed in detail the status of aquaporins in the kidney only one year ago [4], but the subsequent progress has dictated the need for an update. This seems especially appropriate in honor of the 100th birthday of Homer Smith, the pioneer whose foresight initiated this field.

Vasopressin plays a key role in the regulation of water metabolism. Under normal conditions the kidney collecting duct is extremely sensitive to vasopressin. Thus, during extreme antidiuresis as a consequence of high vasopressin levels, water excretion can be reduced by two order of magnitudes relative to water excretion in conditions with low vasopressin levels, without affecting the rate of solute excretion. Two fundamentally different mechanisms allow for this regulation, and they are both based on the modulation of collecting duct water permeability: short-term, regulatory, and long-term, adaptive, mechanisms. Both appear to involve aquaporin-2 water channels and vasopressin. Recent studies have also underscored the importance of aquaporin-2 in the pathophysiology of diabetes insipidus. This review focuses on long-term regulation of aquaporin-2 and its involvement in the pathophysiology of nephrogenic diabetes insipidus.

Vasopressin-mediated expression of aquaporin-2 (long-term regulation)

For many years it has been recognized that maximal urinary concentrating ability can be conditioned by long-term alterations in water intake [5]. We have proposed that this long-term conditioning effect is due in part to adaptations in the renal collecting duct that increase the osmotic water permeability independent of the short-term action of vasopressin [5, 6]. In support of this view, we have demonstrated that restriction of fluid intake in rats for 24 hours or more results in a sustained increase in water permeability in isolated perfused IMCD segments despite the absence of AVP in the peritubular bath [6]. In contrast, the urea permeability of the IMCD segments was not increased, suggesting that the effect was selective for collecting duct water permeability. An adaptational increase in IMCD water permeability in response to water restriction has been confirmed in several studies [7–9]. Recent studies have focused on the mechanism of this adaptation. Prior to our analysis using specific antibodies against aquaporin-2 (AQP2), we conducted freeze-fracture electron microscopy in isolated perfused tubules and demonstrated that IMCDs from water-restricted rats both manifest a high vasopressin-independent water permeability and contained a large number of characteristic intramembrane particle clusters in the apical plasma membrane [7]. These clusters are believed to contain vasopressin regulated water channels [10]. Thus, we reached the preliminary conclusion that long-term conditioning of collecting duct water permeability was likely to be due to the presence in the apical plasma membrane of the same type of water channels that are regulated acutely by vasopressin. With the availability of specific polyclonal antibodies to AQP2 we were able to test this hypothesis directly.

AQP2 [11] is localized predominantly in the apical plasma membrane and in subapical vesicles in kidney collecting duct principal cells [12]. Using immunoblotting and immunocytochemistry, we have demonstrated that water restriction in rats for 24 hours or more results in a marked increase in the quantity of AQP2 protein in cortical and inner medullary collecting duct principal cells [12]. This has subsequently been confirmed by use of immunoblotting, immuno-ELISA and Northern blotting techniques [13–15]. An increase in AQP2 expression was noted both in the apical plasma membrane and in intracellular vesicles, indicating that the adaptation is due to an overall up-regulation of AQP2 expression. Experiments in Brattleboro rats, which manifest an absolute lack of circulating vasopressin, showed that vasopressin infusions via osmotic minipumps for five days result in a marked increase in AQP2 expression in both inner medullary and cortical collecting ducts (Fig. 1 [16]). Quantitative immunoblotting demonstrated a threefold increase in the amount of AQP2 protein in the inner medulla and a parallel threefold increase in the osmotic water permeability of isolated perfused collecting ducts from these animals (Fig. 2) [16]. This provided direct evidence that AQP2 expression is regulated by vasopressin (directly or indirectly) and that AQP2 is the predominant vasopressin regulated water channel. Deen et al, in an elegant study [17] found mutations in the AQP2 gene (resulting in non-functional water channels) in patients suffering from severe primary nephrogenic

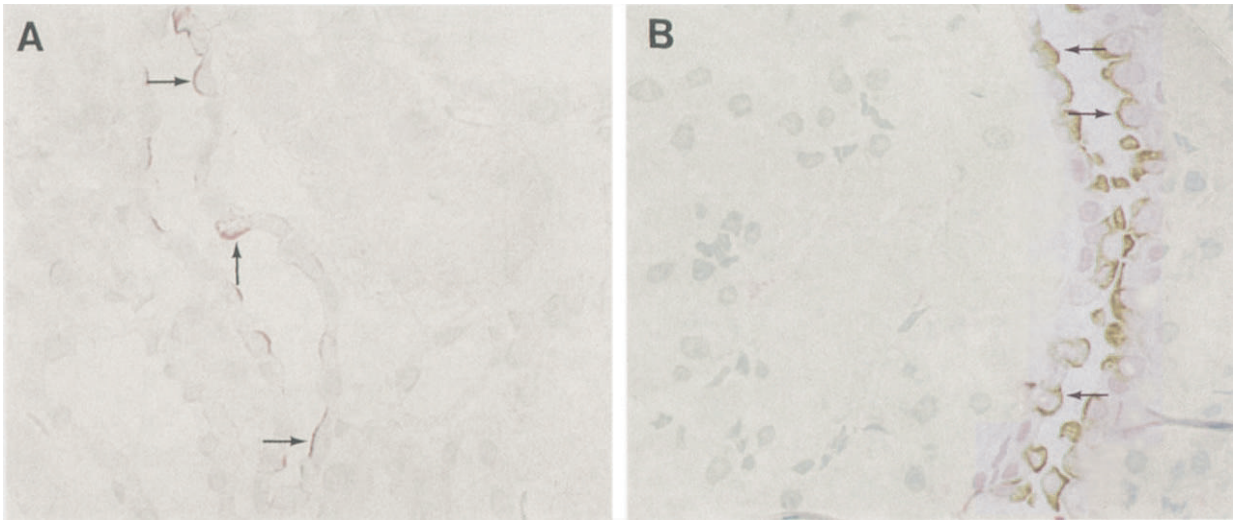

Fig. 1. *Effect of long-term infusion of vasopressin.* Brattleboro rats were infused for five days with vehicle (**A**) or AVP (**B**) in minipumps. AQP2 labeling is exclusively associated with the apical domains of cortical collecting duct principal cells (arrows). The labeling was significantly increased after vasopressin treatment. Reproduction of this figure in color is made possible by a grant from Leica.

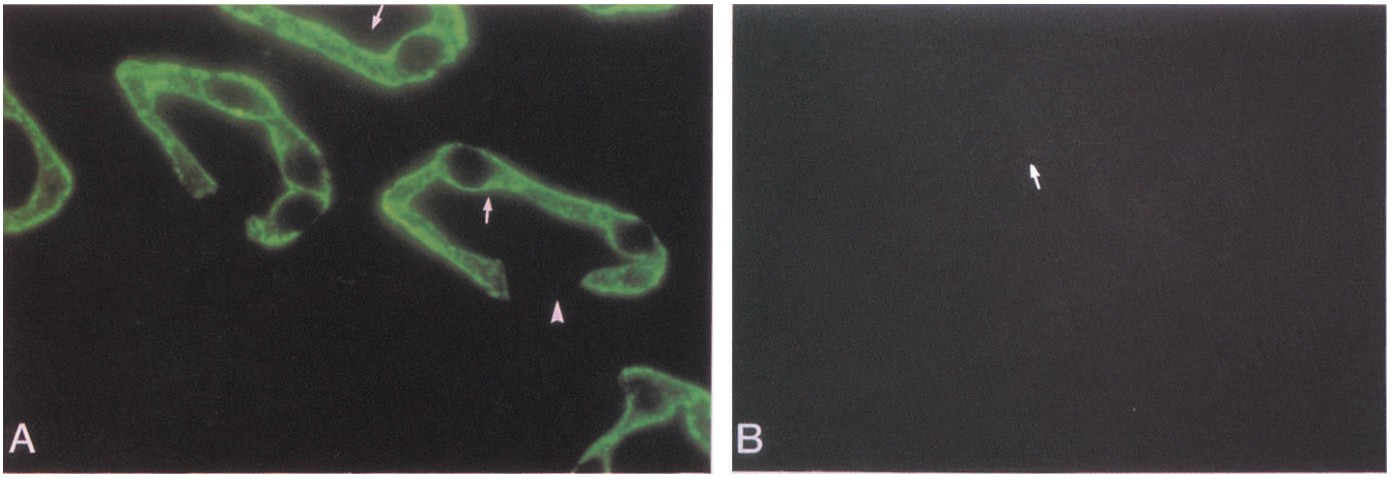

Fig. 4. *Effect of lithium on AQP2 expression: immunofluorescence microscopy of AQP2- labeling in rat inner medulla.* (**A**) In control animals, there is conspicuous labeling of collecting duct principal cells (arrows), whereas intercalated cells are unlabeled (arrowhead). (**B**) Lithium treatment for 25 days virtually abolishes AQP2-labeling (arrow). Adapted from [22]. Reproduction of this figure in color is made possible by a grant from Leica.

diabetes insipidus (NDI). Later Sasaki and associates demonstrated that treatment of rats with vasopressin receptor blockers resulted in reduced expression of aquaporin-2, providing further evidence that the expression of AQP2 is tightly regulated by vasopressin [13]. The above studies unequivocally demonstrate that AQP2 is essential for renal concentrating ability.

Recent studies have demonstrated that thirsting rats strongly up-regulate the levels of AQP2 mRNA in their renal medullas [18, 19], suggesting that increases in AQP2 protein levels may be due to transcriptional regulation. It is not presently known whether the increase in AQP2 mRNA is due to increased transcription of the AQP2 gene or to increased stability of AQP2 mRNA in thirsted animals. However, cloning of the 5′-flanking region of the AQP2 gene has revealed a putative cAMP-regulatory element (CRE), which may be involved in the vasopressin-induced increases in AQP2 transcription rate [20].

Aquaporin-2 and acquired forms of nephrogenic diabetes insipidus

The studies in Brattleboro rats, which revealed low levels of AQP2 in association with extreme polyuria [16], and the demonstration of mutated non-functional AQP2 in human NDI patients [17], triggered efforts to examine the role of AQP2 expression in certain acquired forms of nephrogenic diabetes insipidus (NDI). Acquired NDI is much more common than the relatively rare primary forms of DI [21], and the most common form is lithium-induced NDI. Lithium, used as drug for treatment of manic-depressive illness, is prescribed to about 0.1% of the population,

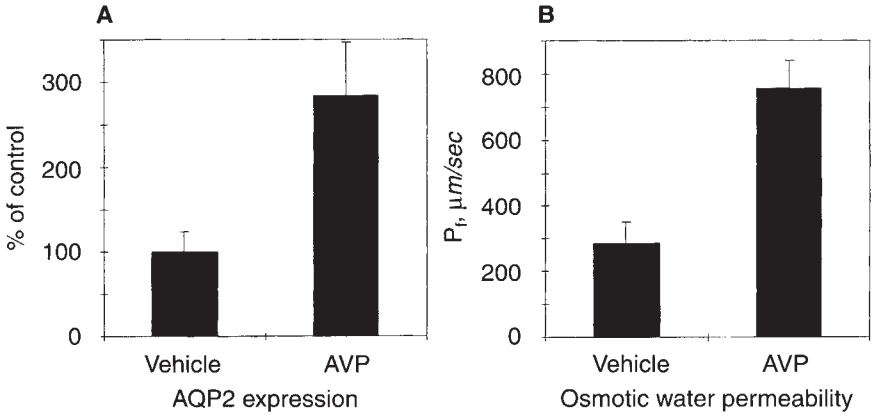

Fig. 2. *Effect of long-term infusion of vasopressin.* In Brattleboro rats infused for five days with AVP in minipumps, total AQP2 expression increased by threefold in the inner medulla (**A**). There was a parallel increase in the maximum water permeability of isolated perfused collecting ducts after acute stimulation with vasopressin (**B**). Adapted from [16].

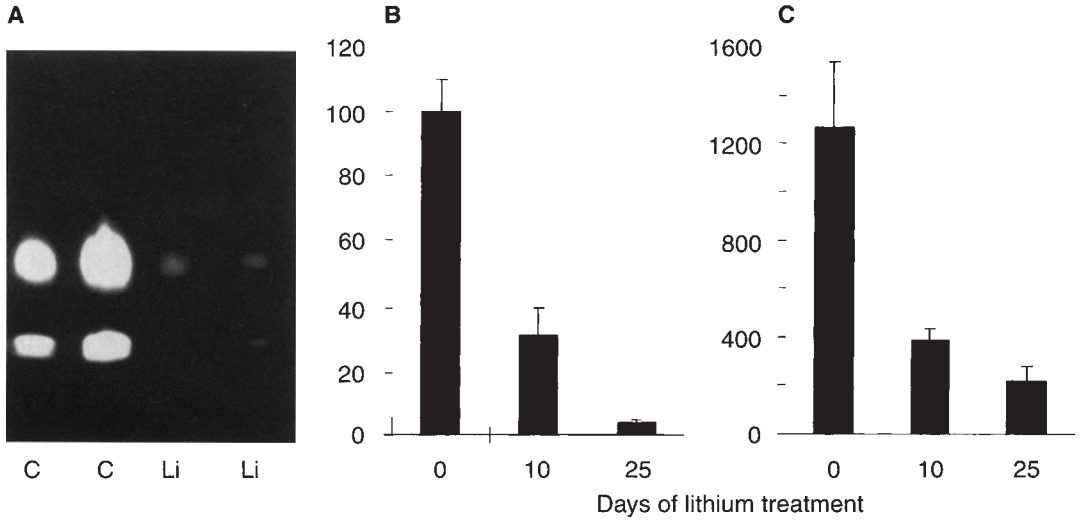

Fig. 3. *Effect of lithium on AQP2 levels and urine osmolarity.* Lithium treatment caused a dramatic decrease in AQP2 expression at 25 days, as shown by immunoblotting (**A**). Densitometry (**B**) revealed a progressive decrease in AQP2, and exceeded 95% at day 25. There was a parallel decline in urine osmolarity (**C**). Adapted from [22].

and polyuria is seen as an important side effect in about 1/5 of the patients. In a recent study [22], we found that lithium treatment of rats for 10 and 25 days was associated with a marked down-regulation of AQP2 expression to levels as low as 4% of control levels (Fig. 3 and 4). This coincided with the development of severe polyuria [16], consistent with an important role of the decreased levels of AQP2 in the development of NDI.

Clinical studies have documented a persisting defect of urinary concentrating ability and polyuria after cessation of lithium treatment. The underlying molecular basis for this has been unclear. Cessation of lithium treatment for seven days only partially restored AQP2 levels and urinary osmolality, consistent with the clinical findings of slow recovery of lithium-induced concentrating defects [22]. Thus, the slow recovery of AQP2 expression may play a profound role in long-term regulation of collecting duct water reabsorption, and hence, re-establishment of normal levels of diuresis. These studies, as well as the studies showing changes in AQP2 expression induced by changes in hydration status, may also help explain many conditions where restoration of the urinary concentrating ability lags significantly behind the correction of the primary underlying cause of polyuria/NDI [21].

To further support the view that reduced levels of AQP2 may be a general factor in acquired forms of NDI from a variety of causes, we examined AQP2 expression in the setting of hypokalemia or bilateral ureteral obstruction, two known causes of NDI. Potassium deprivation for 11 days resulted in a reduction in AQP2 levels in inner medullary collecting ducts to approximately 25%, in parallel with a 200 to 400% increase in urine production [23]. Thus, although not as profoundly reduced as seen after lithium therapy, a decrease in AQP2 expression was noted in association with the hypokalemia-induced polyuria. In addition a modest concentrating defect was determined in response to a period of 12 or 24 hours of thirsting, consistent with previous observations demonstrating that AQP2 is necessary for urinary concentrating ability [16, 17]. A return to a normal diet was also associated with normalization of AQP2 levels and urine output.

Polyuria after release of ureteral obstruction is often seen in patients with urological disorders. Such disorders are a frequent cause of urinary concentrating defects, so it became relevant to

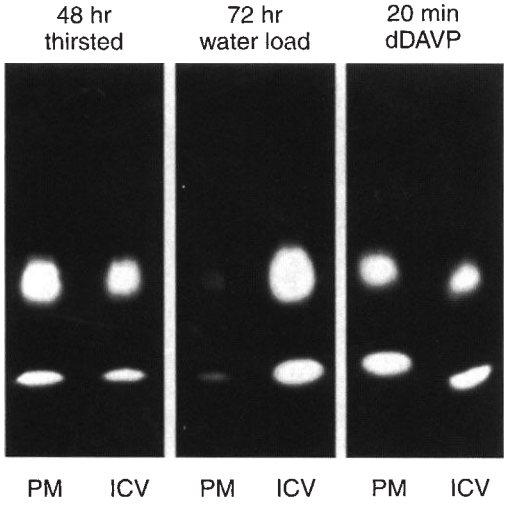

Fig. 5. *Acute effect of vasopressin is superimposed on long-term changes in AQP2 expression.* In animals thirsted for 48 hours (left panel), AQP2 was approximately equally distributed between the plasma membrane (PM) and intracellular vesicle (ICV) fractions. This ratio is similar to that seen in untreated rats, but prolonged thirsting is known to increase total AQP2 expression. Following water loading, most AQP2 is moved to the intracellular vesicles (center panel). After acute vasopressin stimulation (right panel), many water channels are moved back to the plasma membrane fraction. Thus the large reserve of AQP2 in these animals, caused by long-term conditioning, allows an enhanced response to an acute challenge. Adapted from [30].

examine AQP2 expression in such conditions. Frøkier et al examined the role of bilateral ureteral obstruction on AQP2 expression in rat kidney inner medulla [24]. AQP2 levels were already markedly reduced, to approximately 25%, after 24 hours of bilateral ureteral obstruction (that is, prior to release of obstruction). The release of obstruction was followed by development of severe polyuria. This inital postobstructive polyuria is believed to be predominantly osmotically induced. However, the polyuria persisted for several days after release of obstruction, and this is consistent with the continuing low levels of AQP2 observed. Seven days after release of obstruction, the diuresis was almost normalized when rats were allowed free access to water. However, when thirsted, the rats still displayed a significant impairment in concentrating capacity, which is consistent with the observation that AQP2 levels were only partially restored (Frøkiær, Marples, Knepper and Nielsen, unpublished data). Thus, the slow recovery in AQP2 expression may be critically involved in the slow recovery of urinary concentrating ability in various causes acquired NDI. In the three forms of NDI we examined, it appeared that there was a proportional decrease in AQP2 levels when compared to the degree of polyuria associated with the conditions, further supporting the view that there is a causative link between levels of AQP2 and polyuria in these conditions.

The studies described above revealed that there was an identical decrease in AQP2 levels both in inner medullary collecting ducts both after hypokalemia and lithium treatment. This indicates that interstitial tonicity may not be important in the regulation of AQP2 expression. This is further substantiated by the lack of major changes in AQP2 expression after 24 hours of furosemide treatment (Marples et al, unpublished observations) and the failure of five-day furosemide treatment to blunt the the upregulatory response to AVP infusion [25]. Thus, there is considerable evidence to support the view that interstitial tonicity does not have a major influence in inducing AQP2 expression. However, Sasaki and associates recently demonstrated that hypertonicity itself increases the promotor activity of human AQP2 gene using a CAT assay after transfection of OMCD cells with the construct [26]. Thus an effect of tonicity cannot be ruled out completely, although the whole animal experiments suggest that tonicity plays only a minor role.

Recently, Apostol et al demonstrated an 87% decrease in AQP2 in puromycin aminonucleoside (PAN) nephrosis in association with a marked decrease in the urinary concentrating capacity (but no polyuria). Thus, changes in AQP2 expression may also be of particular importance in conditions having both dilutional and concentrating defects as seen in nephrotic syndromes and in conditions with hyponatremia (see below). The down-regulation during PAN nephrosis was seen despite markedly elevated levels of vasopressin. Also, bilateral ureteral obstruction has been shown to be associated with increased levels of vasopressin. Thus, the direct mechanism for the down-regulation of AQP2 remains to be established in the above described conditions. Although vasopressin directly induces expression of AQP2 [16], as described above, changes in hydration status of normal rats also significantly alter the levels of AQP2 expression [12, 13]. However, it remains to be documented whether thirsting/water loading-induced changes are mediated entirely via changes in vasopressin levels/sensitivity, or whether vasopressin-independent mechanisms may also have a role. In this context, Marples et al [22] found that thirsting of lithium treated rats induced a considerably greater increase in AQP2 expression than did seven days of dDAVP treatment (which effectively increased urine osmolality). In contrast, thirsting was not very effective in inducing a redistribution of AQP2 to the apical plasma membrane, while dDAVP was effective. This suggests that the thirsting-induced increase in AQP2 may be at least partly due to a mechanism other than that stimulated by vasopressin. Further studies are necessary to clarify these issues.

Sasaki et al demonstrated that AQP2 can be detected in human urine [27], and Kanno et al recently exploited this observation by examining the urinary excretion of AQP2 in normal individuals and in patients suffering from NDI [28]. Although the cellular mechanism for AQP2 excretion remain unknown, these studies indicate that normal individuals had significantly increased excretion of AQP2 in response to vasopressin treatment, whereas X-linked NDI and central DI patients did not. Thus, reduced levels of AQP2 are also seen in humans suffering from various forms of DI, extending the initial observations by Deen et al [17].

Vasopressin regulated translocation of Aquaporin-2 (short-term regulation): Superimposed effect of long-term regulation

In vitro and *in vivo* studies documented a translocation of AQP2 from intracellular vesicles to the apical plasma membrane, which was paralleled by an increase in water reabsorption [29] and urine osmolarity [30]. Two other groups have recently reported similar results [31, 32]. Phosphorylation of AQP2 directly by PKA had only a marginal effect on water conductance [33], or no effect at all [34]. Thus, translocation of AQP2 appears to be the major cellular mechanism by which vasopressin induces an increase in collecting duct water permeability and hence water conservation, supporting the "shuttle hypothesis" as originally proposed by Wade, Stetson

and Lewis [35]. Vasopressin binds to heterotrimeric G-protein-coupled vasopressin receptors (V2-receptors [36], which activate adenylyl cyclase to produce cAMP serving as a second messenger [37]. The cellular effects of cAMP involve activation of protein kinase A, which phosphorylates various proteins [38], presumably also regulatory proteins, which in turn result in an increase in the water permeability. However, PKA effects may also directly or indirectly induce increased expression, providing a direct link between long-term and short-term effects. To test if long-term regulatory mechanisms may influence the short-term effects of vasopressin we used different pretreatment protocols including thirsting and water loading of rats [30]. The results revealed that changes in hydration status influenced markedly the magnitude of the vasopressin-induced shift in AQP2 localization. For example, the response to dDAVP was markedly enhanced after initial dehydration (to increase AQP2 levels) followed by water loading (to maximize intracellular localization). After this manipulation, the dDAVP-mediated response increased plasma membrane levels of AQP2 by several-fold (Fig. 5) [30]. These data indicate that short-term and long-term mechanisms are superimposed.

Expression in kidney during fetal development

Expression of aquaporins during fetal developement is hypothesized to be critical for the development of specific organ functions, such as in kidney and lung. AQP1 is first identified in circulating red cells at the third postnatal day, which correlates directly with acquisition of high osmotic water permeability [39]. Coincident with AQP1 expression in rat red cells after birth, a marked increase in AQP1 expression in rat kidney proximal tubules and thin descending limbs was demonstrated [39] at the time of development of renal concentrating ability. Induction of AQP2 during fetal development follows a similar time course, although AQP2 appears to be expressed at low levels earlier in development [32, 40]. Urinary concentrating capacity increased markedly during development from postnatal day 10 to day 40, and concomitantly a marked increase in AQP2 mRNA and AQP2 protein (3.5-fold) was seen in this period [40]. Moreover, Yasui et al found a marked effect of betamethasone, which increases both urinary osmolality, renal AQP2 mRNA (2-fold) and AQP2 protein (7-fold), [40]. This was only seen in infant rats; no increase was observed in 40-day-old rats. The mechanism for the long-term induction of AQP2 by corticosteroids has not been established, although the time courses appear to suggest an indirect effect of betamethasone. An important role of glucocorticoids for aquaporin expression and organ development, was also concluded by King et al. In lung, a marked increase in AQP1 was observed in the perinatal period, and betamethasone treatment markedly increased AQP1 levels, indicating a role of AQP1 in the development of lung in preparation for air breathing [41].

AQP2 expression in hyponatremic states with water retention

As described [42], conditions with hyponatremia can be categorized, according to the state of the extracellular fluid (ECF) volume. An important group consists of disorders in which there is a large excess of ECF, with the development of ascites, as seen in association with cardiac failure, cirrhosis, and nephrotic syndrome. In these conditions, water retention is associated with increased release of AVP, and this would be expected to increase AQP2 levels in the collecting duct via the long-term regulatory mechanisms discussed above. In turn, the increased AQP2 levels may contribute to the impairment in the ability of the kidney to excrete water. Preliminary evidence to support this comes from recent findings of Asahina et al [43], observing an increase in AQP2 protein levels in the inner medulla of cirrhotic rats. In other conditions, in which ECF volume is reduced (for example due to excessive diuretic use or osmotic diuresis) increased levels of vasopressin, and thus AQP2, would be expected. Preliminary observations in streptozotocin treated rats with diabetes mellitus reveals a significant upregulation of AQP2 in kidney inner medulla (Marples, Rasch, Knepper and Nielsen, unpublished observations).

Further studies will undoubtedly focus on changes of aquaporin expression in various pathological conditions including congestive heart failure, cirrhosis, nephrotic syndrome, hydronephrosis, cerebral edema, etc., and such studies may reveal essential information about the physiology and pathophysiology of AQPs.

Conclusions

We have attempted here to summarize the recent studies which aimed at clarifying the long-term processes by which AQP2 is regulated in the collecting duct. The molecular explanations for poorly understood clinical disorders of renal collecting duct are emerging. As is often the case in physiology, the details are extremely complex and yet the basic principles are simple. This is certainly true for the aquaporins.

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