Altered Regulation of Renal Aquaporins and Sodium Transporters in Experimental Chronic Renal Failure

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Chronic renal failure, which has an increased single nephron glomerular filtration rate in remnant kidney, is known to cause characteristic structural alterations in renal tubule epithelia in association with impaired urinary concentration and deranged urinary sodium excretion. This mini-review will deal with the changes in the renal expression of aquaporins (AQPs) and sodium transporters for elucidating the underlying cellular and molecular mechanisms for the urinary abnormalities of decreased urinary concentration and increased urinary sodium excretion.

Key words : Kidney Failure, Chronic, Aqauporins, Membrane transport proteins

Decreased expression of AQP2 in the collecting duct

A major function of the kidney is to regulate body water and sodium balance. This function is achieved and finely regulated by a number of cellular and molecular processes, including tubular reabsorption of water and sodium through renal tubular water channel proteins (aquaporins : AQPs) and/or sodium transporters[1-5]. Since water can slowly diffuse through lipid bilayers, all biological membranes exhibit some degree of water permeability[6]. Nevertheless, specialized membrane water transport molecules must exist in renal tubules that have distinctively high water permeability, hence this is crucial for maintaining the fluid reabsorption and the urinary concentration mechanism. Urinary concentration requires 1) establishment and maintenance of a hypertonic medullary interstitium and 2) vasopressin-regulated water transport across the collecting duct epithelium for osmotic equilibration[7]. Thus, a defect in any of these mechanisms would be predicted to be associated with urinary concentrating defects. There was a marked reduction in the expression of the two collecting duct water channels AQP2 (Fig. 1) and AQP3 in rats with CRF induced by remnant kidney model (5/6 nephrectomy), indicating a defect in collecting duct water reabsorption[8, 9]. This is consistent with previous functional studies. In CRF, micropuncture and microcatheterization studies have indicated that the impaired urinary concentrating ability may, at least partly, be caused by the impairment of vasopressin-stimulated water reabsorption in the collecting duct[10, 11]. Consistent with this, patients with end-stage renal disease have urine which remains hypotonic to plasma despite the administration of supramaximal doses of vasopressin[12, 13]. This vasopressin-resistant hyposthenuria specifically implies abnormalities in collecting duct water reabsorption in patients with CRF. Fine et al[14] observed that isolated and perfused cortical collecting ducts dissected from remnant kidneys of severely uremic rabbits exhibited a defect in the response to vasopressin. This was demonstrated both as decreased water flux (Jv) and

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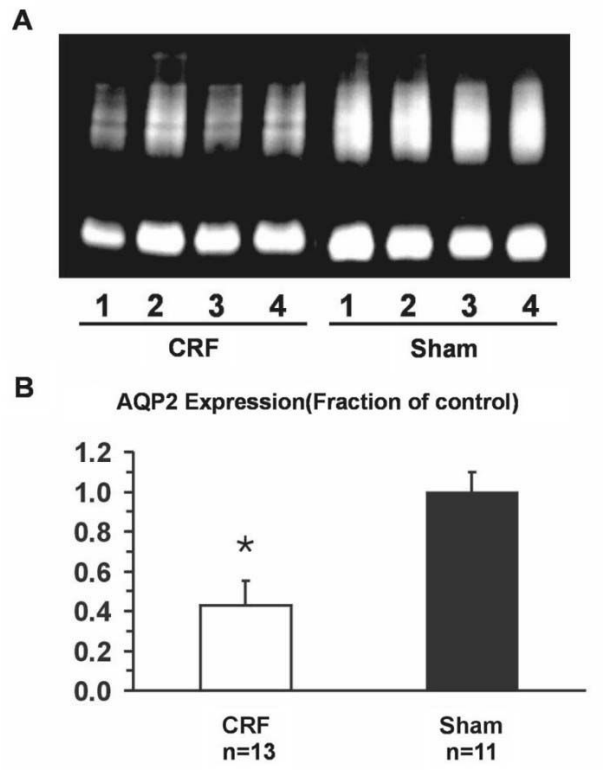

Fig. 1. *Immunoblot of membrane fractions of total kidneys from CRF and sham operated rats. A) The immunoblot was reacted with affinity purified anti-AQP2 and reveal 29 kDa and 35-50 kDa AQP2 bands, representing non-glycosylated and glycosylated forms of AQP2 (densities were 66±10% in CRF rats and 100±5% in sham operated rats, respectively, $p<0.05$). B) Densitometric analysis of all samples from CRF and sham operated rats (corrected according to loading and loading fraction of total kidney mass) reveals a marked decrease in AQP2 expression from 100±11% in sham operated controls (n=11) to 43±12% in CRF rats (n=13). $^*p<0.05$.*

decreased adenylate cyclase activity[14]. Importantly, they also showed that the cyclic AMP analogue 8-bromo-cAMP failed to induce a normal hydrosmotic response in the cortical collecting duct from remnant kidneys. Moreover, RT-PCR of total RNA from the inner medulla of CRF rat kidneys revealed a virtual absence of vasopressin V2-receptor mRNA[15]. Thus, these studies provide firm evidence for significant defects in the collecting duct. The observation of reduced AQP2 and AQP3 expression including demonstration of a marked reduction of AQP2 in the apical plasma membrane (i.e., the active site) may explain these functional defects at the molecular level.

Vasopressin-resistant polyuria and downregulation of AQP2

The finding of a vasopressin-resistant downregulation of AQP2 (and AQP3) expression in CRF rats raises the following possibilities : 1) Vasopressin resistance could, in part, be due to a potential downregulation of the V2-receptor protein. This would be consistent with the reduced vasopressin V2-receptor mRNA levels observed previously[15], 2) Inefficiency of vasopressin to increase adenylate cyclase activity and intracellular cAMP levels may also be due to potential defects in the signaling cascade distal to the V2-receptor (e.g., adenylate cyclase, PKA, etc). These two possibilities may be relevant since a cAMP-response element has been reported to be present in the AQP2 promotor[16, 17], 3) Other factors may override the cAMP-mediated control process. This may involve a "vasopressin-independent" signaling pathway, which has previously been suggested to exist [18]. Each of the above three possibilities could hypothetically be involved in the previously described "vasopressin escape"-induced downregulation of AQP2. Thus, the downregulation of AQP2 observed in rats with CRF (where plasma vasopressin levels are known to be elevated and where AQP2 expression is unchanged in response to long-term dDAVP treatment) would be consistent with a "vasopressin escape"-like downregulation of AQP2 in CRF.

Altered expression of renal sodium transporters

Rats with CRF induced by 5/6 nephrectomy had 1) extensive hypertrophy and dilatation of remaining nephrons, 2) significant azotemia, 3) significant increases in urinary sodium excretion, and 4) marked increase in urine output with an impairment of urinary concentrating ability. In association with this, there was a significant decrease in total kidney levels of the major proximal tubule sodium transporter

NHE3 (type 3 Na-H exchanger), NaPi-2 (type-2 Na-Pi cotransporter), and Na,K-ATPase in rats with CRF[9]. Immunocytochemistry confirmed that NHE3 and Na,K-ATPase levels in the proximal tubule were reduced[9]. Consistent with this, densities per nephron of NHE3, NaPi-2, and Na,K-ATPase did not increase proportionately to the extensive nephron hypertrophy, associated with the hyperfiltration in remnant nephrons. This is likely to play a significant role for the increased sodium excretion in CRF. In contrast, total kidney levels of the TAL (thick ascending limb) and DCT (distal convoluted tubule) sodium transporters NKCC2 (type-2 Na-K-2Cl cotransporter or type-1 bumetanide-sensitive Na-K-2Cl cotransporter) and NCC (thiazide-sensitive Na-Cl cotransporter) were not decreased in rats with CRF[9]. Consistent with this, the estimated densities per nephron of both NKCC2 and NCC were significantly increased, as also demonstrated by immunocytochemistry. Also, Na,K-ATPase labeling intensity was unchanged in the TAL and the DCT. Thus, expression of sodium transporters in the distal tubule is not decreased in remnant kidneys, indicating that the altered tubular handling of sodium in CRF may be caused primarily by changes in proximal tubule sodium transporter expression levels and that there appears to be a compensatory increase in sodium transporter expression in the distal tubule.

Dysregulation of sodium transporters in the proximal tubule

The proximal tubule is the site of reabsorption of approximately two thirds of the filtered NaCl that enters the tubular fluid[19]. Thus, it is evident that structural and functional adaptation of the proximal tubule in response to increased SNGFR and tubular fluid flow rate, which are known to occur in remnant kidney, may play a critical role in the changes of reabsorption and excretion of sodium and water. It has been demonstrated that the absolute sodium reabsorption in the proximal tubule is increased in parallel with the rise in SNGFR in the remnant kidney[10, 20, 21], whereas the fraction of the filtered sodium and water reabsorbed in the proximal tubule was significantly reduced[10, 22, 23].

In the "remnant kidney model", SNGFR increases uniformly to values that are two- to threefold greater than normal[10, 24]. However, if the fraction of the filtered sodium load reclaimed by the proximal tubule is significantly decreased as suggested by many investigators[10, 22, 23, 25], it appears that changes in sodium excretion are modulated by proximal nephron segments. Indeed, a significant decrease in total kidney NHE3, NaPi-2, and Na,K-ATPase levels were observed in association with reduced labeling of NHE3 and Na,K-ATPase in the proximal tubule in rats with CRF. Moreover, estimated densities of NHE3 and Na,K-ATPase per nephron in remnant kidneys revealed a modest increase, but not significant. The hypertrophy, manifested by an increase in tubular diameter and length of the proximal tubule in remnant kidneys, appears to offer an explanation for this modest increase in densities per nephron of NHE3 and Na,K-ATPase. This may provide an explanation for the previously observed increase in absolute sodium reabsorption in the proximal tubule from CRF animals[20, 21]. However, at the same time, the inability to increase the densities per nephron of these sodium transporters proportionately to the increased SNGFR and tubular fluid flow rate may provide an explanation for 1) the previously observed reduced fractional reabsorption of sodium in the proximal tubule, 2) the glomerulotubular imbalance, and 3) the increased urinary excretion of sodium in CRF.

Dysregulation of sodium transporters in the distal tubule

The delivery of sodium and water to the distal end of the proximal tubule of superficial nephrons and to

the bend of Henle's loop of juxtamedullary nephrons is known to be increased in remnant kidneys due to an increase in the filtered load and a decrease in the fractional reabsorption of sodium and water in the proximal tubule[10]. This condition may be similar to acute and chronic loading with isotonic saline in normal rats. A large amount of evidence indicates that isotonic saline loading is associated with an elevation of SNGFR as well as a decrease in fractional proximal NaCl reabsorption[26]. Furthermore, Landwehr et al.[27] demonstrated that the increase in delivery to the loop of Henle is associated with a marked increase in NaCl absorption by the loop of Henle, presumably due to an increase in TAL NaCl absorption. Consistent with this, Ecelbarger et al.[28] demonstrated that chronic oral saline loading in rats markedly increased NKCC2 abundance in the TAL of rat kidneys. The increased densities of NKCC2 in CRF rats are also consistent with this view[9]. Moreover, it was observed that long-term oral NaCl loading results in an increase in the Na,K-ATPase activity of the medullary TAL of rats[29]. This is also consistent with our observation that the Na,K-ATPase labeling of the TAL cells of remnant kidney was similar to the sham operated controls, which contrasts the significant reduction in the labeling of the proximal tubule. This also supports the view that a marked increase in the delivery of NaCl and water to the loop of Henle may partly upregulate several sodium transporters across the TAL and DCT. Moreover, a recent finding revealing that candesartan-treated CRF rats was associated with a dramatic increase of NKCC2 compared with untreated CRF rats further support this view[30]. Thus, it could be possible that the increase in density of NKCC2 seen following 2 weeks after induction of 5/6 nephrectomy could at least partly be a consequence of a prolonged increase in the NaCl load delivered to the TAL partly due to both hyperfiltration and altered expression in the proximal sodium transporters in remnant kidneys. Other factors may also play an important role including potential increases in plasma vasopressin levels, which previously have been found to be associated with CRF induced by surgical reduction in kidney mass[31]. Consistent with this, dDAVP treatment for 7 days of rats with CRF resulted in a greater increase in NKCC2 levels than seen in response to CRF alone, indicating that dDAVP can induce an increase in NKCC2 expression in CRF rats as demonstrated previously in normal rats[32]. The NCC is expressed in the DCT of the kidney and is responsible for a large fraction of the net sodium and chloride reabsorption that occurs in the distal portion of the mammalian renal tubule[33, 34]. It was demonstrated that the DCT is an important site of action of mineralocorticoid and aldosterone upregulates the expression of NCC significantly[35]. Since plasma aldosterone levels are known to be elevated significantly[36], this is likely to play a role in increasing NCC expression in 5/6 nephrectomized rats.

Summary

1. Reduced expression of renal AQP2, AQP3 and AQP1 in chronic renal failure

There is marked reduction in the expression levels of AQP2, AQP3, and AQP1 which is associated with the urinary concentration defect in polyuric CRF rats. The reduction is resistant to long-term treatment with dDAVP consistent with NDI. Since vasopressin levels are known to be elevated in rats with CRF induced by 5/6 nephrectomy, this suggests that there may be a "vasopressin escape"-induced downregulation of AQP2 in CRF rats. The observed downregulation of the collecting duct water channels AQP2, AQP3 and the proximal nephron water channel AQP1 may provide a molecular explanation for the urinary concentration defect associated with CRF.

2. Altered expression of major renal sodium transporters in chronic renal failure

The reduction in total kidney levels of NHE3,

NaPi-2 and Na,K-ATPase with altered expression per nephron of these transporters in response to the increased SNGFR and tubular fluid flow rate may provide cellular and molecular mechanisms for the increase in urinary sodium excretion in remnant nephrons. In contrast, there are compensatory increases in NKCC2 and NCC densities and maintained Na,K-ATPase expression in TAL and DCT. This indicates a compensatory increase which may be partly due to increased blood vasopressin and aldosterone levels and increased delivery of NaCl to the distal tubule in CRF.

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