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Biphasic changes of epithelial sodium channel abundance and trafficking in common bile duct ligation-induced liver cirrhosis

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We hypothesize that dysregulation of the epithelial sodium channel (ENaC) may be responsible for the increased sodium retention in liver cirrhosis. Liver cirrhosis was induced by common bile duct ligation (CBDL). We examined the abundance of ENaC subunits and type 2 isoform of 11β-hydroxysteroid dehydrogenase (11βHSD2) in the kidney by immunoblotting and immunohistochemistry at 6 or 8 weeks after operation. At 6 weeks, cirrhotic rats had developed ascites and displayed a positive sodium balance. The urinary sodium excretion and fractional excretion of sodium were decreased, while plasma aldosterone was unchanged. The abundance of ENaC subunits was not changed in the cortex and outer stripe of the outer medulla (OSOM). In contrast, immunoperoxidase microscopy revealed an increased apical targeting of α-, β- and γENaC in late distal convoluted tubule, connecting tubule and collecting duct. Moreover, 11βHSD2 abundance was decreased in the cortex/OSOM and inner stripe of the outer medulla. At 8 weeks, urinary sodium excretion and fractional excretion of sodium were not changed, while the plasma aldosterone level was decreased. The expression of ENaC subunits was decreased in the cortex/OSOM. Immunoperoxidase microscopy confirmed decreased expression of ENaC subunits, whereas subcellular localization was not changed. These results suggest that increased apical targeting of ENaC subunits and diminished abundance of 11βHSD2 may contribute to promote sodium retention in the sodium-retaining stage of liver cirrhosis (at 6 weeks). The subsequent decreased expression and reduced targeting of ENaC subunits may play a role in promoting sodium excretion in the later stage of liver cirrhosis (at 8 weeks).

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Liver cirrhosis is a chronic disease with marked progressive changes in systemic and renal hemodynamics. Initially, liver cirrhosis patients have peripheral vasodilatation and increased cardiac output but do not have clinical signs of fluid retention (the compensated state). During the late decompensated state, liver cirrhosis is associated with sodium retention, edema and ascites. Sodium retention is the most frequent renal functional abnormality in patients and animal models with liver cirrhosis.[1] However, the mechanism for the sodium retention is still incompletely understood and the molecular basis remains undefined.

As sodium retention is usually associated with a marked activation of the renin–aldosterone system and can be reversed by spironolactone, a specific antagonist of the tubular effect of aldosterone,[2] it has been suggested that hyperaldosteronism is among the most important mechanisms involved in the avid sodium retention in patients with decompensated liver cirrhosis. However, evidence provided by several studies indicates that the extent of sodium retention and potassium loss cannot be explained completely by the elevated aldosterone concentrations in cirrhotic patients, and renal sodium retention precedes ascites formation.[2–4] Previous findings indicate that aldosterone may be normal in a significant proportion of patients with ascites.[5] Although plasma aldosterone levels are not increased, it is well known that treatment with mineralocorticoid receptor (MR) antagonists can prevent sodium retention in animal models of cirrhosis[2,6] and, clinically, aldosterone antagonists are the established first choice in the treatment of cirrhotic ascites and edema.[7] It has therefore been suggested that the sensitivity of the collecting duct to aldosterone is increased in liver cirrhosis. Consistent with this, the activity of type 2 11β-hydroxysteroid dehydrogenase (11βHSD2), an

enzyme which normally protects the MR from stimulation by glucocorticoids, is significantly decreased in microdissected cortical collecting tubules from rats with liver cirrhosis induced by common bile duct ligation (CBDL).[8] The occupancy of MR by glucocorticoid hormone will lead to permanent maximal sodium retention. If this mechanism is operative in rats with liver cirrhosis, collecting duct sodium reabsorption might well be stimulated even in the presence of normal plasma levels of aldosterone.

As the epithelial sodium channel (ENaC) mediates the major sodium transport in the collecting duct[9] and both protein abundance and apical membrane targeting of ENaC are regulated by hormones such as aldosterone[10] and vasopressin,[11] we speculate that altered expression and/or apical membrane targeting of ENaC subunits (α, β and γ) may account for the increased sodium absorption in the distal nephron and the collecting ducts in liver cirrhosis. The purpose of this study was therefore to investigate whether CBDL-induced decompensated liver cirrhosis in rats is associated with altered regulation of ENaC subunit protein abundance and/or changes in the segmental and subcellular localization.

RESULTS

Urinary sodium excretion and fractional excretion of sodium were decreased in rats with 6-week cirrhosis (Table 1)

We established a window at 6 weeks after bile duct ligation as a critical time point at which ascites develops in rats. At 6 weeks, CBDL-induced cirrhotic rats developed significant ascites, and exposure of the abdomen at the termination of the study showed that the cirrhotic rats had a large amount of ascites. After 6 weeks, the quantity of peritoneal fluid in cirrhotic rats was significantly greater than in controls. Plasma concentrations of alanine aminotransaminase (ALAT) and bilirubin were significantly increased in cirrhotic rats. Plasma creatinine levels were not changed, whereas renal creatinine clearance was significantly decreased in rats with CBDL. Importantly, the 24-h urinary sodium excretion and fractional excretion of sodium were significantly decreased at 6 weeks in rats with CBDL compared with controls. Consequently, cirrhotic rats displayed a positive sodium balance. The urinary Na/K ratio was markedly decreased in 6-week CBDL rats, indicating increased aldosterone effectiveness in the distal nephron. Accordingly, fractional excretion of potassium was increased. On the other hand, plasma aldosterone levels were not changed in rats with 6-week CBDL.

Table 1 | Changes in hepatic and renal function in control rats and rats with 6-week CBDL

	Control (n=7)	Cirrhosis (n=10)
Body weight (g)	312±2	295±8
Food intake (g)	16.7±0.5	13.7±1.9
Water intake (ml)	23.4±0.6	21.9±2.5
Ascites (ml)	Nondetectable	4.5±1.2**
UO (μl/min)	9.8±0.7	11.1±1.0
P_{ALAT} (U/l)	38.7±1.8	64.1±4.6**
$P_{bilurubin}$ (μmol/1)	1.8±**0.1**	104.9±5.2**
P_{cr} (μmol/1)	34.1±1.4	36.4±2.2
Ccr (ml/min)	1.51±0.06	1.11±0.12*
P_{Na} (mEq/1)	138.6±0.5	139.6±0.7
P_K (mEq/l)	5.5±0.1	4.6±0.2**
$U_{Na} \times UO$ (mmol)	1.27±0.08	0.63±0.11**
$U_K \times UO$ (mmol)	4.19±0.10	3.39±0.39
Sodium balance (mmol)	0.26±0.07	0.52±0.05*
FE_{Na} (%)	0.42±0.02	0.28±0.05*
FE_K (%)	35.5±1.0	47.6±3.6**
Urine Na/K	0.30±0.01	0.19±0.03**
$P_{aldosterone}$ (pg/ml)	339±53	357±53
U_{osm} (mosm/kgH_2O)	1826±103	1528±171
P_{osm} (mosm/kgH_2O)	298±1	298±1
U/P_{osm}	6.1±0.4	5.1±0.6

Values are expressed as mean±s.e. These values are measured at the last day of experiments (6 weeks). UO, urine output; P_{alat}, plasma alanine aminotransferase; $P_{bilurubin}$, plasma bilirubin; P_{Na}, plasma sodium; P_K, plasma potassium; P_{cr}, plasma creatinine; C_{cr}, creatinine clearance; $U_{Na} \times UO$, rate of urinary sodium excretion; $U_K \times UO$, rate of urinary potassium excretion; sodium balance, the difference between dietary sodium intake and urinary sodium excretion in the 24 h; FE_{Na}, fractional excretion of sodium in urine; FE_K, fractional excretion of potassium in urine; urine Na/K, urine sodium/potassium ratio; $P_{aldosterone}$, plasma aldosterone; U_{osm}, urine osmolality; P_{osm}, plasma osmolality. *$P<0.05$, **$P<0.01$ compared with control.

The protein abundance of ENaC subunits was not changed in the kidney cortex/outer stripe of the outer medulla in rats with 6-week CBDL

The protein abundance of αENaC was not changed in the cortex/outer stripe of the outer medulla (OSOM), while it was increased in the inner stripe of the outer medulla (ISOM) in rats with 6-week CBDL compared with controls (Figure 1). The abundance of βENaC was not changed in the cortex/OSOM and ISOM. The 85 kDa band abundance of γENaC was decreased in the cortex/OSOM (Figure 1, Table 2), but not in the ISOM. However, total γENaC abundance and 70 kDa band expression were not changed in the cortex/OSOM and ISOM. The analyses of normalized band densities are shown in Table 2.

Increased membrane targeting of ENaC subunits in rats with 6-week CBDL

In control rats, immunoperoxidase staining for the βENaC subunits showed diffuse punctuate labeling throughout the distal convoluted tubule (DCT2), connecting tubule (CNT) and collecting duct principal cells. At 6-weeks in CBDL rats, βENaC labeling was predominantly localized to the apical domains (i.e. plasma membrane and intracellular vesicles close to the membrane), with only marginal labeling of the cytoplasm corresponding to labeling of intracellular vesicles. This increased apical targeting was evident in almost all cross-sectioned tubules of DCT2 (not shown), CNT (Figure 2a and b), cortical collecting duct (CCD, Figure 2c and d) and outer medullary collecting duct (OMCD) (not shown). Moreover, immunohistochemical analysis also revealed similar changes in the subcellular distribution of γENaC in kidneys from 6-week CBDL rats. There was a marked increase in apical γENaC immunolabeling in DCT2 (not shown), CNT (Figure 2e and f), CCD (Figure 2g and h) and OMCD