Kidney and Colon Electrolyte Transport in CHIF Knockout Mice

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Key Words
Glucocorticoid • Electrolyte metabolism • Ion transport • Amiloride • ENaC• Na⁺-K⁺-ATPase • FXYD

Abstract
Corticosteroid hormone induced factor (CHIF) is a small epithelial-specific protein regulated by aldosterone and K⁺ intake. It is a member of the FXYD family of single span transmembrane proteins involved in the regulation of ion transport. Recent data have suggested that CHIF interacts with the α subunit of the Na⁺-K⁺-ATPase and increases the pump’s affinity to cell Na⁺. CHIF knockout (KO) mice have mild renal phenotype under low Na⁺ or high K⁺ diets. The present study further characterizes kidney electrolyte metabolism in CHIF KO mice and describes abnormalities in the colonic ion transport function. Kidney: KO mice were not compromised in salt and water balance under resting conditions. Fractional excretions (FE) of Na⁺ and K⁺ were normal and the animals had no deficit in the adaptation to low Na⁺ or high K⁺ intake. Glucocorticoid treatment did not unmask any difference. The effects of amiloride on Na⁺ absorption were not different at any treatment protocol. In contrast, FE_K⁺ was reduced by 35% in KO mice under low Na⁺ intake. Colon: Amiloride inhibitable Na⁺ absorption was reduced in distal colon by 42%, 54% and 58% under control conditions, glucocorticoid treatment and low Na⁺ intake, respectively. Also, the cAMP dependent ion transport was significantly diminished. Forskolin induced equivalent short circuit current (I'_{sc}) was reduced by 41%, 32% and 58%, under control conditions, high K⁺, and low Na⁺ intake, respectively. The present findings support a role of CHIF as an indirect modulator of several different ion transport mechanisms and are consistent with regulation of the Na⁺-K⁺-ATPase as the common denominator.

Introduction
Corticosteroid hormone induced factor (CHIF) is a small (6.5 kDa) single span membrane protein cloned as an epithelial-specific dexamethasone-induced mRNA [1]. It is expressed in the basolateral membrane of kidney collecting duct and distal colon surface cells and can not be detected in any other epithelial and non-epithelial tissue [2, 3]. CHIF protein and mRNA are induced by
corticosteroids, Na⁺ deprivation and K⁺ loading [2, 4-6]. The responses of kidney and colon to corticosteroids and electrolyte stress are somewhat different. Thus, a low Na⁺ diet and aldosterone induce CHIF mRNA in the colon but not in the kidney [4]. CHIF protein however is elevated by low Na⁺ intake both in the kidney and colon [2, 6]. K⁺ loading too elevates CHIF protein and this effect is much stronger in the colon than the kidney [2, 6].

CHIF is a member of a new gene family of transmembrane proteins named after the invariant sequence FXYD in their extracellular domain [7]. This group includes proteins like phospholemman (FXYD1) [8], the γ-subunit of the Na⁺-K⁺-ATPase (FXYD2) [9], and Mat-8 (FXYD3) [10] thought to be involved in the regulation or mediation of ion transport. Previous studies have reported that expressing phospholemman, the γ subunit of Na⁺-K⁺-ATPase, Mat-8 and CHIF in Xenopus oocytes evokes various channel activities [1, 10-12]. The physiological relevance of these observations is yet unclear. More recently, evidence was provided that CHIF, the γ subunit of Na⁺-K⁺-ATPase, phospholemman, and FXYD7 interact with the Na⁺-K⁺-ATPase and modulate the pump kinetics [13-18]. The four proteins are specifically co-immunoprecipitated with the α subunit of Na⁺-K⁺-ATPase and their expression in Xenopus oocytes or mammalian cells alters pump properties. In particular, CHIF was found to increase the affinity of the pump to cell Na⁺ by ~2 folds expected to considerably elevate the pumping rate under limiting intracellular Na⁺ activity [13, 14]. Thus, FXYD proteins appear to be tissue specific auxiliary subunits of the Na⁺-K⁺-ATPase whose function is to adopt the pump kinetics to the physiological needs of different tissues of physiological state. It is yet unknown whether modulation of the pump kinetics is the only function of CHIF and other FXYD proteins.

Recently, a CHIF knockout (KO) mouse has been generated by targeted gene disruption. Under normal conditions, the null mutated animals did not express an overt phenotype except for about 15% reduction in body weight. Challenging metabolism by high K⁺ or low Na⁺ intake revealed a transiently increased diuresis and glomerular filtration rate (GFR) [6].

This study describes a more detailed examination of the kidney CHIF KO phenotype under control, hormonal stimulation and electrolyte stress conditions, and reports effects of CHIF on electrolyte transport across the distal colon epithelium. It appears that the null mutation of CHIF is fully compensated in the kidney under resting conditions, while electrolyte transport in the distal colon shows clear impairment in amiloride-inhibitable Na⁺ absorption and forskolin induced Cl⁻ secretion. These phenotypes are consistent with regulation of the Na⁺-K⁺-ATPase by CHIF.

**Materials and Methods**

**Animal treatment**

CHIF KO mice were generated by a targeted disruption of the CHIF gene, as described previously [6]. Adult animals of either sex were used in this study. All animal experimentation described in this manuscript was conducted according to the German law for the care and use of laboratory animals. Unless stated otherwise, animals had free access to drinking water. Regulation of Na⁺ and K⁺ balance was challenged by the following treatments: 1. Mice were fed a low sodium diet (Na⁺ 0.15 g/kg, K⁺ 7 g/kg, C1036, Altromin Germany) for 14 days; 2. Mice were injected subcutaneous (s.c.) with triamcinolone (Delpicort®) 40 mg/kg body weight (bw) 72 h, 48 h and 24 h before the experiment; 3. Mice were fed a high potassium diet (Na⁺ 2.4 g/kg, K⁺ 80 g/kg, Altromin, Germany) for 10 days. One control group was kept on standard diet (Na⁺ 2.4 g/kg, K⁺ 7 g/kg, C1000, Altromin, Germany). On the day of the experiment, a urine sample was obtained, amiloride 5 mg/kg bw was injected s.c., and a second urine sample as well as a blood sample from the tail vein were obtained 2.5 hours later. Afterwards, mice were then anaesthetized (ketamine/xylazine, 100/5 mg/kg bw, i.p.), thoracotomy was performed to obtain a blood sample from the right atrium, and the colon was excised and prepared as previously described [19].

**Ussing chamber experiments**

An Ussing chamber modified for very small tissue samples was used [20]. In brief, colonic tissue (mucosa and submucosa) was set up in a heated chamber perfused by a modified Ringer solution. Transepithelial voltage \( V_\text{te} \) was measured and the voltage deflection \( \Delta V_\text{te} \) caused by current injections (1.5 µA; 1s/10s) across the epithelium served to calculate transepithelial resistance \( R_\text{te} \). The equivalent short circuit current \( I'_\text{sc} \) was calculated according to \( I'_\text{sc}=V_\text{te}/R_\text{te} \). Amiloride 50 µmol/l was added to the luminal side to block luminal ENaC channels. Carbachol 100 µmol/l on the basolateral side served to activate Ca²⁺ regulated K⁺ channels. Forskolin 5 µmol/l from the basolateral side was used to stimulate cAMP dependent Cl⁻ secretion [21].

**Fractional excretions**

Fractional excretions of Na⁺ and K⁺ (FE\(_{\text{Na⁺}}\) and FE\(_{\text{K⁺}}\)) were calculated from concentrations of creatinine and electrolytes in plasma (p) and urine (u) according to the equation \( \text{FE}_{\text{Na⁺}}=\frac{\text{Na}^+_{(p)}-\text{Na}^+_{(u)}}{\text{Creatinine}_{(p)}-\text{Creatinine}_{(u)}} \). Urine samples were obtained before and 2.5 hours after injection of amiloride. Blood samples were drawn from tail veins for electrolyte measurements, and directly from the right atrium for creatinine measurements. Electrolyte concentrations were measured using standard flame photometry. Creatinine was measured using an enzymatic assay (Crea-Plus, Roche

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Table 1. Plasma electrolytes and fractional excretions before (control) and after glucocorticoid (glu) pretreatment in CHIF knockout and wild type mice. CHIF knockout mice show normal plasma electrolyte concentrations under control conditions. Amiloride (5 mg/kg bw, s.c.) inhibits renal Na+ absorption and the driving force for K+ secretion. This in turn reduces FE$_{\text{Na}^+}$. The effect of amiloride on FE$_{\text{Na}^+}$ or FE$_{\text{K}^+}$ is reflected by ΔFE$_{\text{Amil}}$.

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>CHIF knockout control</th>
<th>CHIF knockout glu</th>
<th>wild type control</th>
<th>wild type glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>141.2±0.54 (38)</td>
<td>137.46±2.37 (7)</td>
<td>141.97±0.9 (16)</td>
<td>141.88±0.88 (8)</td>
</tr>
<tr>
<td>K$^+$</td>
<td>6.3±0.14 (34)</td>
<td>6.35±0.5 (7)</td>
<td>6.51±0.15 (27)</td>
<td>6.19±0.59 (8)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>51.5±0.78 (32)</td>
<td>49.5±0.79 (7)</td>
<td>53.6±0.72 (28)</td>
<td>55.1±1.47 (7)</td>
</tr>
<tr>
<td>[Crea]$_p$</td>
<td>9.19±0.42 (10)</td>
<td>6.19±0.55 (7) *</td>
<td>8.62±0.43 (8)</td>
<td>7.29±0.97 (8)</td>
</tr>
<tr>
<td>FE$_{\text{Na}}$ (%)</td>
<td>0.26±0.02 (30)</td>
<td>0.31±0.11 % (7)</td>
<td>0.18±0.03 (14)</td>
<td>0.33±0.07 % (8)</td>
</tr>
<tr>
<td>FE$<em>{\text{Na}}$$</em>{\text{Amil}}$ (%)</td>
<td>0.89±0.08 (30)</td>
<td>0.75±0.14 % (6)</td>
<td>0.87±0.12 (13)</td>
<td>0.83±0.12 % (8)</td>
</tr>
<tr>
<td>ΔFE Na$_{\text{Amil}}$</td>
<td>0.67±0.08 (29)</td>
<td>0.42±0.15 % (6)</td>
<td>0.71±0.13 (13)</td>
<td>0.50±0.11 % (8)</td>
</tr>
<tr>
<td>FE$_{\text{K}}$ (%)</td>
<td>13.32±1.07 (30)</td>
<td>10.85±1.77 % (7)</td>
<td>11.84±0.80 (25)</td>
<td>13.66±3.15 % (8)</td>
</tr>
<tr>
<td>FE$<em>{\text{K}}$$</em>{\text{Amil}}$ (%)</td>
<td>2.80±0.35 (30)</td>
<td>2.12±0.59 % (6)</td>
<td>2.82±0.39 (23)</td>
<td>2.58±0.12 % (8)</td>
</tr>
<tr>
<td>ΔFE K$_{\text{Amil}}$</td>
<td>-10.6±1.08 (29)</td>
<td>-7.71±1.57 % (6)</td>
<td>-8.85±1.03 (23)</td>
<td>-11.08±2.36 % (8)</td>
</tr>
</tbody>
</table>

Diagnostics, Mannheim, D), which is highly robust with regard to mouse plasma and urine chromogens [19].

Water deprivation
Urinary osmolality, fractional excretions and urine creatinine were measured during and after 21 hours of water deprivation. Urine osmolality was measured using a semi-micro-osmometer (Knauer, Berlin, D) after 20 fold dilution of the respective urine samples.

Solutions
All substances were purchased either from Sigma (Deisenhofen, Germany) or from Merck (Darmstadt, Germany), and were of analytical grade. The normal Ussing chamber perfusion solution contained (in mmol/l): NaCl 145, K$_2$HPO$_4$ 1.6, KH$_2$PO$_4$ 0.4, Ca-gluconate 1.3, MgCl$_2$ 1, D-glucose 5. The preservation solution for colon epithelium contained: NaCl 127, KCl 5, MgCl$_2$ 1, glucose 5, Na-pyruvate 5, CaCl$_2$ 1.25, Na-HEPES 10. All solutions for Ussing chamber experiments contained in addition indomethacin 10$^{-6}$ mol/l.

Statistics
Data are shown as original recordings and as mean values ± SEM from n observations. Paired as well as unpaired Student’s t-test was used as appropriate. A P value of <0.05 was accepted to indicate statistical significance.

Results
Kidney function
A) Resting conditions and glucocorticoid treatment. CHIF KO mice were viable and fertile and did not show any apparent phenotype. Assessment under standard conditions and after glucocorticoid pretreatment could not reveal any major defect in salt and water balance. Plasma values for Na$^+$, K$^+$, haematocrit and creatinine are shown in table 1. Electrolyte transport in the whole kidney was assessed by the measurement of fractional excretions (FE) of Na$^+$ and K$^+$, and calculated values did not differ between WT and KO mice. Electrolyte transport via epithelial Na$^+$ channels was estimated by examining the effect of amiloride on FE$_{\text{Na}^+}$ and FE$_{\text{K}^+}$. Also for the amiloride inhibitable part of Na$^+$ and K$^+$ excretion there was no significant difference between KO and wild type (WT) animals.

In another series of experiments we tested whether glucocorticoid injections could unmask any difference in electrolyte balance or fractional excretions between the genotypes (table 1). Still there was no difference for these parameters except for plasma creatinine, which decreased in KO mice but not in WT animals.

B) Sodium restriction. Sodium restriction significantly reduced FE$_{\text{Na}^+}$ in both KO and WT mice (figure 1). This increase in sodium absorption was sufficient to maintain normal plasma electrolyte concentrations in both genotypes (table 2). Interestingly, the absolute effect of amiloride on FE$_{\text{Na}^+}$ (i.e. ΔFE$_{\text{Na}^+}$) was not altered by the low sodium diet suggesting that amiloride sensitive Na$^+$ transport is active under control conditions already and cannot be further activated. Additional Na$^+$ transport mechanisms seem to play a major role in increased Na$^+$ absorption under Na$^+$ restriction. In addition, CHIF does not seem to be essential for the overall Na$^+$ homeostasis.

Sodium restriction did not alter FE$_{\text{K}^+}$ in WT mice. However, it led to a significant decrease in FE$_{\text{K}^+}$ in KO mice (figure 2). This decrease was not coupled to a compromised ENaC function in KO compared to WT since the amiloride inhibitable part was even larger in KO mice. This finding would suggest a role of CHIF in

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K+ secretion independent of ion channel mediated K+ recycling during Na+ absorption via ENaC.

C) K+ loading. To further examine the role of CHIF for K+ excretion, mice were fed a diet containing about ten times the normal K+ load. 22% of mice in the KO group versus 36% in the WT group died during this treatment. All mice showed a weight loss by 8.7±0.9 g (n=12) in KO and by 6.3±0.8 g (n=9) in WT mice. This weight loss could in part be attributed to dehydration, which is reflected by the increased haematocrit values (table 3). Surviving mice were hyperkalaemic, again with no difference between KO and WT mice. Figure 3 summarizes the values for FEK+ (A, B) and FENa+ (C, D) and the effects of amiloride under high K+ and resting conditions. A strong increase both in FEK+ and in AFEK+ (i.e. total and ENaC mediated) reflects the enhanced K+ excretion both via amiloride inhibitable and other mechanisms along the nephron. However, KO mice did not show any impairment in K+ excretion at either

Table 2. Haematocrit, plasma electrolyte and creatinine concentrations before (control) and after sodium restriction (Na+↓) in CHIF knockout and wild type mice. No significant effect on plasma electrolytes in either genotype. Mean ± SEM (n).

Table 3. Haematocrit, plasma electrolyte and creatinine concentrations before (control) and after potassium loading (K+↑) in CHIF knockout and wild type mice. Potassium loading causes significant hyperkalaemia in both knockout and wild type mice. Mean ± SEM (n) * p < 0,05 compared with control conditions. ✦ significant effect vs control.

Fig. 1. Fractional excretion of Na+ (FENa+)(%) and effect of amiloride under control conditions (standard diet) and low Na+ diet (low Na+ diet) in CHIF knockout (CHIF KO) and wild type mice. ✦ significant effect of amiloride; ✧ significant difference between control and low Na+ diet. No difference between CHIF knockout and wild type mice. Mean ± SEM (n).

Fig. 2. Fractional excretion of K+ (FEK+)(%) and effect of amiloride under control conditions (standard diet) and low Na+ diet (low Na+ diet) in CHIF knockout (CHIF KO) and wild type mice. ✦ significant effect of amiloride; ✧ significant difference between control and low Na+ diet. ✦✧ difference between CHIF knockout and wild type mice. Sodium restriction does not alter FEK+ in wildtype mice, but significantly lowers FEK+ in CHIF KO mice. The absolute effect of amiloride on FEK+ (AFEK+(Amil)) is not affected by sodium restriction and not different between genotypes. Mean ± SEM (n).

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localization when compared to WT mice. While FE $\text{Na}^+$ did not change by K$^+$ loading ∆FENa$^+$ increased in both genotypes and tended even to be larger in KO animals.

Water deprivation

The original observation of a transiently increased diuresis in KO mice after challenge of Na$^+$ and K$^+$ metabolism prompted us to examine the ability to concentrate urine. This effect has been interpreted as an indirect consequence of altered ion transport rather than being due to differences in water conductivity. In our study, no clear differences in urine concentration were seen (figure 4). In the period of 21h we observed a transient rise in urine osmolality up to around 3500 mosmol/kg. This was followed by a decrease in FENa$^+$, FE$\text{K}^+$ and osmolality reflecting the ability to conserve water by conserving salt. While the diminished excretion of Na$^+$ and K$^+$ might explain the fall in urine osmolality towards the end of the 21 h period, the continuous rise in urine creatinine shows the continuing concentration of urine, which is significantly higher in KO mice.

Transepithelial ion transport in colon

The pattern of expression of CHIF suggests a role not only for renal, but also for intestinal ion transport. We examined epithelial ion transport in the distal colon by the Ussing chamber technique. An original trace is shown in figure 5. Our data show that CHIF KO mice have an impaired colonic electrogenic Na$^+$ absorption and Cl$^-$ secretion: Under standard conditions, $I'_\text{sc}$ was significantly smaller in KO mice when compared to WT mice (figure 6). This difference appears to be entirely due to a diminished electrogenic Na$^+$ absorption in KO mice, as luminal amiloride almost completely inhibits $I'_\text{sc}$ in both genotypes (figures 5 and 6).

Glucocorticoid pre-treatment, K$^+$ loading, and Na$^+$ restriction, increased $V_\text{w}$ and $I'_\text{sc}$ in both genotypes (figure 7). Thus, despite the observed difference in electrogenic Na$^+$ absorption under resting conditions, KO mice can still respond to corticosteroid stimulation or electrolyte stress. However, the stimulation of electrogenic Na$^+$ absorption in CHIF KO mice is significantly smaller than in WT mice. Interestingly, K$^+$ loading resulted in the highest stimulation of amiloride inhibitable Na$^+$ absorption in the colon. The large scatter of the data in this treatment...
Fig. 4. Urinary concentrating ability and fractional excretions of Na⁺ (FE_{\text{Na⁺}}) and K⁺ (FE_{\text{K⁺}}) during water restriction in CHIF knockout (KO) and wild type (Wt) mice. Mice were deprived of drinking water for 21 hours. Urine samples were taken at 0, 12, 15, 18 and 21 hours and urine osmolality (A), urine creatinine concentration (B), FE_{\text{Na⁺}} (C) and FE_{\text{K⁺}} (D) were measured. Mean ± SEM (n), ¥ significant difference between genotypes.

Fig. 5. Original Ussing chamber recording of electrogenic ion transport under current clamp conditions in distal colon of CHIF knockout and wild type mouse. Transepithelial voltage $V_{\text{te}}$ under resting conditions and after application of luminal amiloride (lu, 50µmol/l). Current pulses of 1.5 µA cause the voltage deflection $\Delta V_{\text{te}}$.

Fig. 6. Effect of amiloride (50µmol/l) on electrogenic ion transport (Ussing chamber experiments) in distal colon of CHIF knockout (KO) and wild type (Wt) mice. Equivalent short circuit current ($I_{\text{sc}}$) under control conditions is significantly smaller in KO mice ($\dagger$). This difference appears to be almost entirely due to diminished electrogenic Na⁺ absorption in KO mice. Mean ± SEM (n). ¥ significant effect of amiloride washout.

Discussion

Previous studies have suggested a role of CHIF in kidney and intestinal electrolyte transport using heterologous expression systems and phenotypic analysis of CHIF KO mice [6, 13, 14]. The current study further characterizes kidney function in CHIF KO mice under various conditions and reports impairment of colonic transport by deleting this gene.
Under normal conditions no abnormalities had been observed in any of the kidney related plasma and urine factors assessed. These include: plasma Na⁺, K⁺, creatinine and haematocrit, as well as the amiloride-inhibitable and ENaC-independent fractional excretion of Na⁺ and K⁺. No significant effect of CHIF on electrolyte balance was observed following glucocorticoid stimulation as well. The only phenotype was a reduced plasma creatinine concentration in the KO mice, which might be due to an increased GFR and urine volume reported in [6]. Interestingly, neither Na⁺ restriction nor glucocorticoid treatment had an effect on ∆FE_{Na⁺}^{(Amil)} and ∆FE_{K⁺}^{(Amil)}. This observation may be accounted for by one of three mechanisms (1) ENaC could already be stimulated under control conditions. (2) The previously documented increase in ENaC cell surface expression may be compensated by a downregulation of other transporters so that a net change in fractional excretion is not observed. (3) A strong increase of sodium absorption via other Na⁺ transporters proximal to ENaC reduces tubular sodium concentration and limits the driving force for ENaC-mediated transport. As for glucocorticoid stimulation the KO mice had lower plasma creatinine, and in this case also a somewhat reduced FE_{K⁺}^{(Amil)}, which appears to be due to a decrease in amiloride-independent K⁺ excretion. The functional relevance of this observation is not yet clear.
the null mutated animals resulting in a smaller rate of basolateral Na\(^+\)/K\(^+\) exchange. An obvious outcome would be a decrease in colonic Na\(^+\) absorption. The decrease in Cl\(^-\) secretion (in the presence of amiloride) can be secondary to a reduced K\(^+\) excretion and decreased luminal potential. Special care has been taken in these experiments to exclude technical or structural problems which may cause low electrical resistance in the KO mice. Accordingly, tissues displaying an electrical resistance below 25 Ohm*cm\(^2\) were excluded from the data averaged. The significance of the observed differences in I\(_{sc}\) is further emphasized by the fact that in both KO and WT tissues, I\(_{sc}\) could be significantly and equally enhanced by corticosteroid treatment or electrolyte stress which may cause low electrical resistance in the KO mice.

In conclusion the present study provides the first demonstration for impaired electrolyte transport in CHIF KO mice. Such phenotype is seen by in vitro distal colon measurements but not in whole animal electrolyte metabolism study. It presumably reflects a situation in which collecting duct specific phenotypes are compensated by other processes. The data is consistent with the activation of the Na\(^-\)K\(^+\)-ATPase by CHIF and does not support another major effect of this protein.

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