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alphaENaC-mediated lithium absorption promotes nephrogenic diabetes insipidus.

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ABSTRACT

Lithium-induced nephrogenic diabetes insipidus (NDI) is associated with severe polyuria, aquaporin-2 (AQP2) downregulation and cellular remodelling of the collecting duct (CD). Several studies have hypothesized that a likely candidate for lithium entry is the amiloride-sensitive epithelial sodium channel (ENaC). To test whether ENaC is involved in lithium absorption, transgenic mice deficient of αENaC specifically in the CD (KO mice) and littermate controls were subjected to chronic lithium treatment. This resulted in a marked increase in water intake in controls, but not in KO mice. The lithium-treated controls also exhibited polyuria and reduced urine osmolality, which was not observed in the KO mice. AQP2 protein levels were decreased in cortex/outer medulla and inner medulla (IM) from lithium-treated controls. In KO mice, only AQP2 levels in IM were partly decreased. Immunohistochemistry showed decreased AQP2 labeling in CD of lithium-treated controls compared to lithium-treated KO mice, whereas no changes were observed in connecting tubule (CNT). Lithium induced an increased H^+-ATPase expression in IM of the controls, but not in the KO mice. No differences in blood lithium concentrations were observed between the KO and control mice. Thus, the absence of functional ENaC in CD protects the mice from lithium-induced NDI. Apparently, the CNT is able to escape the lithium toxicity. In conclusion our data supports the idea that ENaC-mediated lithium-entry into the CD principal cells is a crucial step within the pathogenesis of lithium-induced NDI.

INTRODUCTION

NDI is characterized by the inability of the kidney to concentrate urine in response to vasopressin. The disease is most commonly acquired and often occurs as a side effect in humans subjected to different drug treatments, e.g., lithium therapy. Lithium, which is a frequently used drug against manic-depressive illness, can cause NDI in up to 20-40% of the patients taking the medication.[1] Chronic lithium treatment of rats results in severe downregulation of AQP2 and AQP3 protein levels in parallel with extensive polyuria.[2,3] Moreover, lithium causes a remodeling of the rat kidney CD, which includes a decreased fraction of principal cells and an increased fraction of intercalated cells.[4,5] These deleterious effects on the CD are proposed to be linked to lithium accumulation within the principal cells. Several studies suggested that a likely candidate for lithium entry is ENaC, which is present in the late distal convoluted tubule (DCT) cells, the CNT cells and the CD principal cells.[6,7] ENaC is a heteromultimeric protein composed of three subunits – α, β and γ.[8] ENaC has a higher permeability for lithium than for sodium.[9,10] Moreover, amiloride - a specific blocker of ENaC - has been shown to reduce lithium uptake in ENaC-expressing renal cells.[11] and to block reabsorption in the distal nephron of sodium-depleted rats.[12] Recent studies also showed that amiloride partially prevents development of lithium-NDI in rats[11,13] and partially restores the urinary concentrating ability in patients on lithium therapy.[14] To possibly provide definitive proof whether ENaC is involved in the absorption of lithium, we have taken advantage of transgenic mice deficient of αENaC specifically in the CD while leaving ENaC expression in the late DCT and CNT intact.[15] Under salt restriction, whole-cell voltage clamp of principal cells of the cortical CD (CCD) showed no detectable ENaC activity, whereas large amiloride-sensitive currents were observed in the CCD of controls[15] and in the CNT/DCT of both KO and controls.[16] Despite the loss of ENaC activity in CD, the animals survived well and were able to maintain sodium and potassium balance, even when challenged by one week salt restriction, 23 hours water deprivation, or 2 days potassium

loading.[15] We have followed these transgenic mice and their littermate controls during chronic lithium treatment.

RESULTS

Lithium does not induce polyuria and polydipsia in CD-specific αENaC KO mice

Chronic lithium treatment induced increased water intake in the control animals, which was not seen in KO mice (Fig 1A). As early as day 4 after starting lithium treatment, water intake increased in the lithium-treated controls, whereas it remained at the same level throughout the entire study in the lithium-treated KO mice. After 5 days of lithium treatment there was a highly significant difference in water intake between the lithium-treated controls and the three other groups, which continued the remaining days of the diet. In contrast, water intake of the lithium-treated KO mice was not significantly different from the untreated control and KO mice (Fig 1A). The urine output of the lithium-treated controls was significantly higher compared to the three other groups (Fig 1B). In parallel, the urine osmolality was significantly lower in the lithium-treated controls compared to the other groups (Fig 1C). In contrast, there were no significant differences in urine output and urine osmolality between the lithium-treated KO mice and the untreated control and KO mice. Thus, lithium did not cause polyuria and polydipsia in the lithium-treated KO mice which was seen in the lithium-treated controls.

The urinary sodium and potassium concentration was significantly lower in the lithium-treated controls, but not in the lithium-treated KO mice (Table 1). The urinary excretion of sodium and potassium was however not changed in any of the two groups after lithium exposure and there were no changes in the fractional sodium excretion between the four groups (Table 1). Furthermore, serum concentrations of sodium, potassium, urea and creatinine and serum osmolality were unaltered in the two groups after lithium exposure (Table 1). The urinary lithium concentration was

significantly lower in the lithium-treated controls compared to the lithium-treated KO mice, whereas the urinary lithium excretion was not different between the control and the KO group (Table 1). There were no differences in lithium concentration in the blood between the two lithum-treated groups and no changes in lithium clearance and in fractional excretion of lithium were observed (Table 1). There was no significant difference in food intake between the two groups during the diet and at the end of the diet no significant changes in body weight was observed (Table 1).

Effect of lithium treatment on AQP2 and H^+-ATPase abundance in CD-specific αENaC KO mice

It has previously been shown that lithium treatment of rats causes a dramatic decrease in AQP2 abundance.[2,4] To investigate whether the effect was abolished in the lithium-treated KO mice, Western blotting was performed of samples from cortex/OM and IM (Fig 2). Consistent with the previous results, chronic lithium treatment resulted in a severe and significant downregulation of AQP2 in both IM (Fig 2A) and cortex/OM (Fig 2B). In the cortex/OM of lithium-treated KO mice, AQP2 was significant higher compared with the lithium-treated controls, and no significant difference was observed when compared with the untreated KO mice. Thus, lithium treatment did not affect total cortical and outer medullary AQP2 expression in the KO mice. In IM, AQP2 abundance was higher in the lithium-treated KO mice compared to the lithium-treated controls although not statistically significant. A significant lower AQP2 expression was seen in the lithium-treated KO mice compared to the untreated KO mice. Thus, lithium treatment appears to have some effect on AQP2 expression in the IM of KO mice.

Western blot analysis further showed no significant differences in H^+-ATPase abundance in the cortex/OM between the lithium-treated controls and the other groups (Fig 2C). In contrast the H^+-

ATPase expression was significantly increased in the IM of the lithium-treated controls compared to the other three groups (Fig 2D). Moreover, no differences between the lithium-treated and untreated KO mice were observed. Thus, lithium treatment did not cause changes in the expression of H^+-ATPase in the IM of KO mice in contrast to the control mice.

Decreased AQP2 labeling in the collecting duct of lithium-treated control mice compared to lithium-treated KO mice

To further investigate AQP2 labeling in the lithium-treated controls and the lithium-treated KO mice, we performed immunolabeling on kidney sections from these two groups. In the CD of lithium-treated KO mice, a strong AQP2 labeling was seen in IM, ISOM and cortex (Fig 3A-C), whereas in the CD of the lithium-treated controls the AQP2 labeling was markedly reduced (Fig 3E-G). Thus, lithium did not cause a downregulation of AQP2 expression in the CD of the KO mice. In the CNT there were no major changes in AQP2 labeling between the two groups (Fig 3D and H) indicating that lithium does not affect AQP2 levels in this segment.

Effect of lithium on the density of H^+-ATPase positive cells in control and KO mice

Immunohistochemistry revealed a slight increased density of H^+-ATPase positive cells in the proximal part of IM and in the ISOM of the lithium-treated controls compared to the lithium-treated KO mice (Fig 4A-B and E-F). Moreover, H^+-ATPase positive cells localized next to each other were seen in the lithium-treated controls. In the CCD of the lithium-treated control mice, H^+-ATPase positive cells were only occasionally localized next to each other and in general no major changes in the density of H^+-ATPase positive cells was observed in this segment between the two groups (Fig 4C and G). In the CNT and DCT the density of H^+-ATPase positive cells was not different (Fig 4D and H). Cellular counting of H^+-ATPase positive cells in the CNT and DCT

revealed no significant changes in the fraction of H^+-ATPase positive cells between the two groups (36 ± 1.4% in control vs 37 ± 1.0% in KO, n=3 in each group, ns). Moreover, multiple H^+-ATPase positive cells next to each other were not observed in these segments. Thus, lithium causes a slight increased density of intercalated cells in the IM and ISOM of control mice, but not in KO mice. Moreover, lithium does not appear to have an effect on the density of intercalated cells in the DCT/CNT.

DISCUSSION

In this study, we show that the absence of functional ENaC in the CD protects the mice from developing lithium-induced NDI. The data provides genetic evidence that ENaC is involved in lithium entry into CD principal cells and thus plays a crucial role in the pathogenesis of lithium-induced NDI. Interestingly, the lithium-treated KO mice did not show signs of NDI suggesting that the CNT is able to escape the lithium toxicity.

CD-specific αENaC KO mice are protected from development of NDI

In control mice, lithium treatment caused severe polyuria, low urine osmolality and high water intake consistent with an impairment of urinary concentration and thus NDI.[2-4,17] In contrast, lithium did not have an effect on the urinary concentrating ability in mice which lack αENaC in the CD. Our results are consistent with a recent rat study where co-treatment with lithium and amiloride attenuated the lithium-NDI[11,13] and with the fact that amiloride administration ameliorates polyuria in patients on long-term lithium treatment.[18] Furthermore, amiloride prevents lithium-induced AQP2 downregulation in mCCDc11 cells.[11] We have also previously used lithium protection as a positive control for experiments dealing with the role of ENaC in thiazolidinedione-induced fluid-retention.[19]

The higher AQP2 expression in the CD of the lithium-treated KO mice compared to the lithium-treated controls is consistent with the absence of a urinary concentrating defect in the CD of the KO mice. However, western blot analysis showed that lithium did have an effect on AQP2 levels in IM of the KO mice as seen by a significant lower AQP2 abundance in the lithium-treated KO mice compared to the untreated KO mice. This suggests that the lithium effect on AQP2 levels in the IMCD principal cells is not only via an ENaC-mediated entry. Simultaneously administration of amiloride and lithium to rats has also been shown not to fully restore AQP2 levels in the IM.[11] This effect may be mediated by PGE2, because lithium increases cyclooxygenase 2 (COX2) expression in and PGE2 release from medullary interstitial cells,[20] and it is known that PGE2 can inhibit the effect of vasopressin on water permeability in the CD.[21,22] The decreased AQP2 expression is likely not related to a decrease in interstitial osmolality, since furosemide-induced changes in medullary tonicity did not affect AQP2 levels in rat IM.[23] Finally we cannot exclude that some residual ENaC activity remains in the IM of the KO mice,[19] but this is unlikely to play a physiologically role. The AQP2 downregulation in the IMCD was not correlated with changes in urine output and osmolality in the KO mice. Thus the water reabsorption ability did not appear to be impaired. A possibility is that lithium did not affect AQP2 trafficking and that a substantial amount of AQP2 remained in the apical plasma membrane to sustain water reabsorption. We previously showed that although cytoplasmic AQP2 is markedly reduced in rat IM after chronic lithium treatment, some AQP2 still remains in the apical membrane.[4]

Lithium administration was associated with a slightly higher density of intercalated cells (H^+-ATPase positive cells) in the IM and ISOM of the control mice. The increased density of intercalated cells was however less pronounced compared to previous studies in rats.[4,11,17] The phenomenon was not seen in KO mice showing that lithium had no effect on the intercalated cells

in these mice. Thus, lithium-induced changes of the intercalated cells require the action of lithium on the principal cells.

Western blotting also revealed an upregulation of H^+-ATPase protein levels in the IM of the lithium-treated control mice as shown in other studies in rats and is likely due to an increase in the density of intercalated cells.[11,17] We did not observe changes in H^+-ATPase abundance in OM/cortex in contrast to previously shown in lithium-treated rats[11,17] suggesting that the effect of lithium on the intercalated cells in mice is not as dramatic as in rats.

There was no significant change in plasma lithium concentration between the lithium-treated groups and the plasma lithium concentration of 0.6 mM in the lithium-treated control mice corresponds to studies in rats using the same protocol.[3]

ENaC-mediated lithium entry

Under normal conditions, i.e. in sodium-replete conditions, renal lithium clearance is used as a measurement of tubular fluid delivery from the proximal tubule. The lithium clearance technique assumes that transcellular transport of lithium does not occur in the distal tubule and the CD. Previous studies have shown that amiloride-sensitive lithium reabsorption only takes place in the distal nephron during conditions with sodium depletion (low sodium diet).[12,24] In the present study we showed that lithium-treated mice receiving a normal sodium containing diet develop NDI and that the absence of ENaC in the CD prevents the development of NDI. Thus, we show that ENaC-mediated lithium reabsorption does occur in the CD in mice on a normal salt diet.

Lithium treatment is known to be associated with natriuresis and experiments with rats using the same lithium protocol as in the present study (with the same sodium intake in lithium-treated and control rats) have shown that the rate of urinary sodium excretion is increased in lithium-treated animals compared to controls.[3] In the present study we did not observe a difference in urinary

sodium excretion between the four mice groups. In another study by Nielsen et al, rats on lithium diet consuming the same amount of sodium as the controls also had no differences in urinary sodium excretion rates, but these rats had a marked increase in the fractional excretion of sodium.[25] However, no differences in FE-Na^+ were observed in this study either. Thus there may be differences in the lithium-induced sodium handling in mice and rats.

Lithium escape in the CNT

Mice with ENaC inactivated in the CD did not respond on the lithium treatment suggesting that the CNT is able to escape the deleterious effects of lithium. This is in spite of the fact that the CNT cells contain ENaC and therefore should be expected to respond to lithium exposure. The CNT exhibit the same components for water transport as the CD. AQP2 is expressed in both the CNT and the CD although the abundance is lower along the CNT.[26,27] Vasopressin-mediated regulation of AQP2 expression and trafficking occurs in rat CNT and in mouse and rat the V_2-receptor is equally expressed in the CNT and CCD.[27,28] Vasopressin also increases adenylate cyclase activity in rat CNT.[29]

In our study we did not observe differences in AQP2 levels in the CNT of the lithium-treated control and KO mice and the density of intercalated cells did not appear to be increased in the two groups. Moreover, AQP2 protein levels in OM/cortex were only decreased in the lithium-treated control mice and not in the lithium-treated KO mice. Consistently, lithium causes AQP2 downregulation mainly in the CCD and to a lesser extent in CNT of rats.[30]

Differences in ENaC expression between CNT and CCD were observed in rats subjected to lithium diet with fixed sodium intake (but not with free access to sodium).[25] In these animals, β- and γENaC downregulation was observed in CCD and OMCD, but not in CNT. In contrast, increased apical labeling of all three ENaC subunits was observed in this segment. This was explained by possible

compensatory mechanisms occurring in CNT to limit the renal sodium loss observed in these animals.[25]

The difference in lithium sensitivity between the CNT and CD could be explained by differences in lithium delivery to the CNT and CD resulting in differences in the luminal lithium concentration along the CNT and the CD. It is also possible that the lithium intracellular concentration rises to toxic levels only in the CD due to a different exit pathway for lithium either through the basolateral or the apical plasma membrane in the CNT cells and CD principal cells. Yet another possibility is that the expression or the sensitivity to an intracellular target for lithium is different in the CNT and the CD. A potentital intracellular target for lithium is glycogen synthase kinase 3β (GSK3β). Lithium has been shown to inhibit GSK3 activity in the kidney[20] and proteomic studies of IMCD isolated from lithium-treated rats revealed an upregulation of the phosphorylated inactive form of GSK3β.[31] Moreover, an impaired response to vasopressin has recently been shown in CD-specific GSK3β KO mice suggesting a potential important role of GSK3β in the vasopressin-mediated water reabsorption.[32] A recent study showed equal expression of the GSK3β transcript in mouse DCT/CNT and CD.[33] However, a potential different sensitivity to e.g. phosphorylation and thus activity of the GSK3β protein may exist in the two segments.

CONCISE METHODS

Experimental protocols

Treatment of transgenic mice:

Lithium chloride was solubilized in water and the solution was added to food to yield a lithium concentration of 40 mmol/kg of dry food as previously described.[3] CD-specific αENaC KO mice ($Scnn1a^{lox/lox}$//HoxB7:Cre, KO mice) and the corresponding control mice ($Scnn1a^{lox/lox}$) (2-4 and 12 months old) were given lithium containing diet for 24-25 days. In parallel, CD-specific αENaC KO

mice and control mice were given a normal diet for 24 days. All mice were placed in individual conventional cages and the water intake was measured daily. From day 22 to day 24 of the experiment all mice were placed in metabolic cages to measure urine output and osmolality. During the entire experiment mice had free access to food and water. At the end of the experiment blood was collected from the eye or from the aorta. The experiment was performed three times and data was pooled.

Urine and plasma analysis

Osmolarity, sodium and potassium concentration were analyzed at the Laboratoire Central de Chimie Clinique, Centre Hospitalier Universitaire Vaudoise (CHUV), Switzerland or at Department of Clinical Biochemistry, Skejby University Hospital, Denmark. Lithium concentration was measured at Service of Nephrology, CHUV, Switzerland or at Department of Anatomy, Aarhus University, Denmark.

Western blot analysis

The kidneys were dissected into cortex/outer medulla (OM) and IM and homogenized in dissecting buffer as previously described.[4] The homogenates were centrifuged for 15 min at 4^{o}C. The total protein concentration was measured (Pierce BCA protein assay reagent kit, Pierce, Rockford, IL). To confirm equal loading of protein, an initial gel was stained with Coomassie Brilliant blue. SDS-PAGE was performed on 12.5% polyacrylamide gels (Pierce, Rockford, IL). After transfer of proteins by electroelution to nitrocellulose membranes, blots were blocked with 5% milk in PBS-T (80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, 0.1% Tween 20, pH 7.5) for 1 hr and incubated with anti-AQP2 antibody (7661 AP, 1:1000) or anti-H^+-ATPase (7659 AP, 1:1000).[5] The labeling was visualized with a peroxidase-conjugated secondary antibody using an enhanced

chemiluminescence system (Amersham Pharmacia Biotech, UK). The labeling density was quantitated using Quantity One software (Bio Rad laboratories Ltd, Hertfordshire, UK).

Immunohistochemistry

Kidneys from lithium-treated KO mice (n=3) and lithium-treated control mice (n=3) were fixed by intravascular perfusion with 3% paraformaldehyde in 0.1 M phosphate buffer and subjected to paraffin embedding and sectioning (2 μm thick sections).

Sections were incubated overnight at 4ºC with rabbit polyclonal AQP2 antibody (7661AP, 1:6,000 dilution) or rabbit polyclonal H^+-ATPase antibody (H7659AP, 1:1,000 and 1:2,000 dilution),[5] followed by incubation with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody. Labeling was visualized using 3,3'-diaminobenzidine staining.[4]

Quantification of H^+-ATPase positive cells in CNT/DCT

Cellular counting was performed on kidney sections from lithium-treated KO and lithium-treated control mice labeled with H^+-ATPase antibody. Counting was performed on electronic images taken with an x25 objective. The number of H^+-ATPase positive and negative cells with a distinct nucleus were counted in the CNT and the DCT of lithium-treated KO mice (1751 cells, n=3) and in the CNT/DCT of lithium-treated control mice (1732 cells, n=3). The fraction of H^+-ATPase positive cells was calculated from the number of H^+-ATPase positive cells divided by the total number of cells counted for each animal.

Statistical analysis

Results are presented as mean ± SE. Data were analyzed by one-way ANOVA and unpaired *t* test. P values <0.05 were considered statistically significant.

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DISCLOSURES

None

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FIGURE LEGENDS

Figure 1: Water intake (A), urine output (B) and urine osmolality (C) were measured in lithium-treated control mice (n= 12, black), lithium-treated KO mice (n=11, white), untreated control mice (n=8, bricks) and untreated KO mice (n=7, hexagons). After 4 days of lithium treatment the water intake was significantly increased in the lithium-treated control mice compared to the lithium-treated KO mice and after 5 days there was a highly significant difference between the lithium-treated control mice and the three other groups (A). There were no difference in water intake between the lithium-treated KO mice, the untreated control mice and the untreated KO mice during the entire diet (A). At the end of the diet the urine output was significantly increased and the urine osmolality significantly decreased in the lithium-treated control mice compared to the three other groups (B-C), whereas no differences were observed between the lithium-treated KO mice, the untreated control mice and the untreated KO mice (B-C). (At day 1, n=6, 6, 3, 3; at day 5-6 and 18-19, n=9, 8, 8, 7; at day 8-9, n=11, 11, 8, 7). *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Figure 2: Western blot and corresponding densiometric analysis of AQP2 (A-B) and H^+-ATPase expression (C-D) in cortex/OM (A and D) and IM (B and D) of untreated control mice (control), untreated CD-specific αENaC KO mice (ENaC KO), lithium-treated control mice (Li-control) and lithium-treated CD-specific αENaC KO mice (Li-ENaC KO). In cortex/OM, AQP2 protein was significantly reduced in Li-control compared to the other three groups, whereas AQP2 levels in the Li-ENaC KO was not different from ENaC KO (A). AQP2 protein in IM was significantly lower in Li-control compared to the two untreated groups, but not compared to Li-ENaC KO (B). The expression of AQP2 in the IM of Li-ENaC KO was significant lower compared to ENaC KO (B).
No differences in H^+-ATPase protein were observed between the Li-control and the other groups in cortex/OM (C). In the IM H^+-ATPase was significant higher in the Li-control compared to all three

other groups, whereas the expression in the Li-ENaC KO was not significantly different from the untreated groups (D). *p<0.05, **p<0.01, ***p<0.001.

Figure 3: Immunohistochemistry using whole kidney sections from lithium-treated CD-specific αENaC KO mice (A-D) and lithium-treated control mice (E-H). Sections were incubated with anti-AQP2 antibody. In the IM (A and E), ISOM (B and F) and in the CCD (C and G) AQP2 labeling was markedly reduced in the lithium-treated control mice (E-G) compared to the lithium-treated CD-specific αENaC KO mice (A-C). In the CNT there were no changes in AQP2 labeling between the two groups (D and H).

Figure 4: Immunohistochemistry using whole kidney sections from lithium-treated CD-specific αENaC KO mice (A-D) and lithium-treated control mice (E-H). Sections were incubated with anti-H^+-ATPase antibody. The density of H^+-ATPase-positive cells was slightly increased in the IM and ISOM of the lithium-treated control mice (E and F) compared to the lithium-treated CD-specific αENaC KO mice (A-B). In the CCD (C and G) and in the CNT (D-H) there were no major changes in the density of H^+-ATPase-positive cells.

Table 1. *Urinary and blood measurements from control and KO mice on a normal diet or a Li-diet*

	Controls Li-diet	KO mice Li-diet	Controls Normal diet	KO mice Normal diet
Body weight (%)	99.0 ± 2.2^{+} (n=12)	104.2 ± 1.7 (n=11)	107.4 ± 2.3 (n=8)	105.8 ± 1.1 (n=7)
Food intake (g/g BW)	0.25 ± 0.03 (n=12)	0.22 ± 0.02 (n=11)	0.26 ± 0.03 (n=8)	0.24 ± 0.03 (n=7)
Serum-Na^+, mM	149.7 ± 0.8 (n=6)	151.4 ± 1.0 (n=5)	147.3 ± 0.7 (n=3)	147.0 ± 1.0 (n=3)
Serum-K^+, mM	4.3 ± 0.2 (n=6)	4.4 (n=2)	4.2 ± 0.4 (n=3)	4.5 (n=1)
Serum-osm, mosmol/kgH_2O	345 ± 3 (n=6)	334 ± 8 (n=3)	341 (n=2)	344 ± 4 (n=3)
Serum-urea, mM	7.7 ± 0.4 (n=6)	7.6 (n=2)	8.2 ± 0.6 (n=3)	7.4 (n=1)
Serum-creatinine, uM	27 ± 1.4 (n=9)	27 ± 2.2 (n=8)	28 ± 1.0 (n=6)	27 ± 1.1 (n=6)
Plasma/serum-Li^+, mM	0.61 ± 0.16 (n=7)	0.38 ± 0.07 (n=8)	0.75 ± 0.04 x 10^{-3} (n=3)	0.84 ± 0.06 x 10^{-3} (n=3)
U-Na^+, mmol/24 h	0.16 ± 0.03 (n=11)	0.15 ± 0.03 (n=11)	0.13 ± 0.02 (n=7)	0.14 ± 0.01 (n=7)
U-Na^+, mM	23 ± 4$^{\#\#}$ (n=11)	97 ± 13 (n=11)	91 ± 13 (n=7)	108 ± 20 (n=7)
FE-Na^+, %	0.47 ± 0.06 (n=5)	0.97 ± 0.19 (n=5)	0.77 ± 0.18 (n=3)	1.04 ± 0.22 (n=3)
U-K^+, mmol/24 h	0.54 ± 0.07 (n=11)	0.48 ± 0.07 (n=11)	0.40 ± 0.03 (n=7)	0.46 ± 0.03 (n=7)
U-K^+, mM	79 ± 16$^{\#\#}$ (n=11)	328 ± 50 (n=11)	324 ± 70 (n=7)	349 ± 62 (n=7)
C-creatinine, µl*min^{-1}*g^{-1}	9.7 ± 2.4 (n=9)	8.3 ± 3.7 (n=8)	6.0 ± 1.5 (n=6)	7.7 ± 2.3 (n=6)
U-creatinine, µM	1819 ± 541 (n=12)	4260 ± 732 (n=11)	3577 ± 500 (n=7)	4207 ± 935 (n=7)
U-Li^+, mmol/24 h	0.08 ± 0.01 (n=11)	0.05 ± 0.01 (n=12)	0.20 ± 0.02 x 10^{-3} (n=3)	0.17 ± 0.005 x 10^{-3} (n=3)

U-Li^+, mM	13.4 ± 2.5** (n=11)	35.5 ± 6.1 (n=12)	93 ± 25 x 10^{-3} (n=3)	105 ± 15 x 10^{-3} (n=3)
C- Li^+, µl*min^{-1}*g^{-1}	5.2 ± 0.9 (n=7)	4.8 ± 1.0 (n=3)	6.2 ± 0.8 (n=3)	6.3 ± 0.8 (n=3)
FE- Li^+, %	70 ± 23 (n=7)	197 ± 105 (n=8)	69 ± 1 (n=3)	51 ± 10 (n=3)

All measurements are from day 24 except the body weight, which is measured at day 21. $^+$, $p<0.05$ (controls on Li-diet vs controls on normal diet). $^{\#\#}$, $p<0.01$ (controls on a Li-diet vs three other groups). **, $p<0.01$ (controls on a Li-diet vs KO on a Li-diet). The body weight in percentage is calculated as a fraction of day 0 (beginning of the experiment).

Figure 1

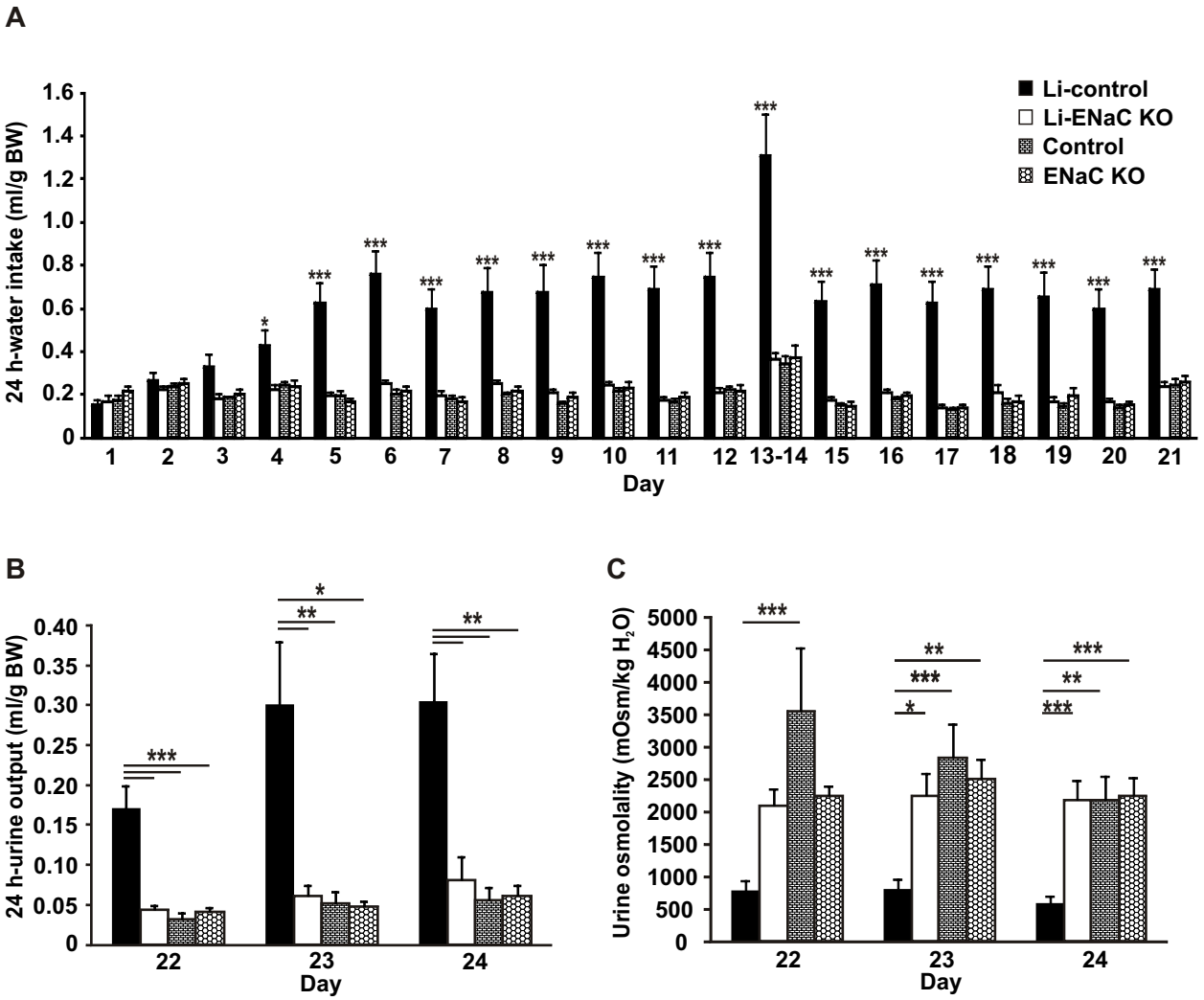
A
Li-control
Li-ENaC KO
Control
ENaC KO
24 h-water intake (ml/g BW)
Day
B
24 h-urine output (ml/g BW)
Day
C
Urine osmolality (mOsm/kg H2O)
Day

Figure 2

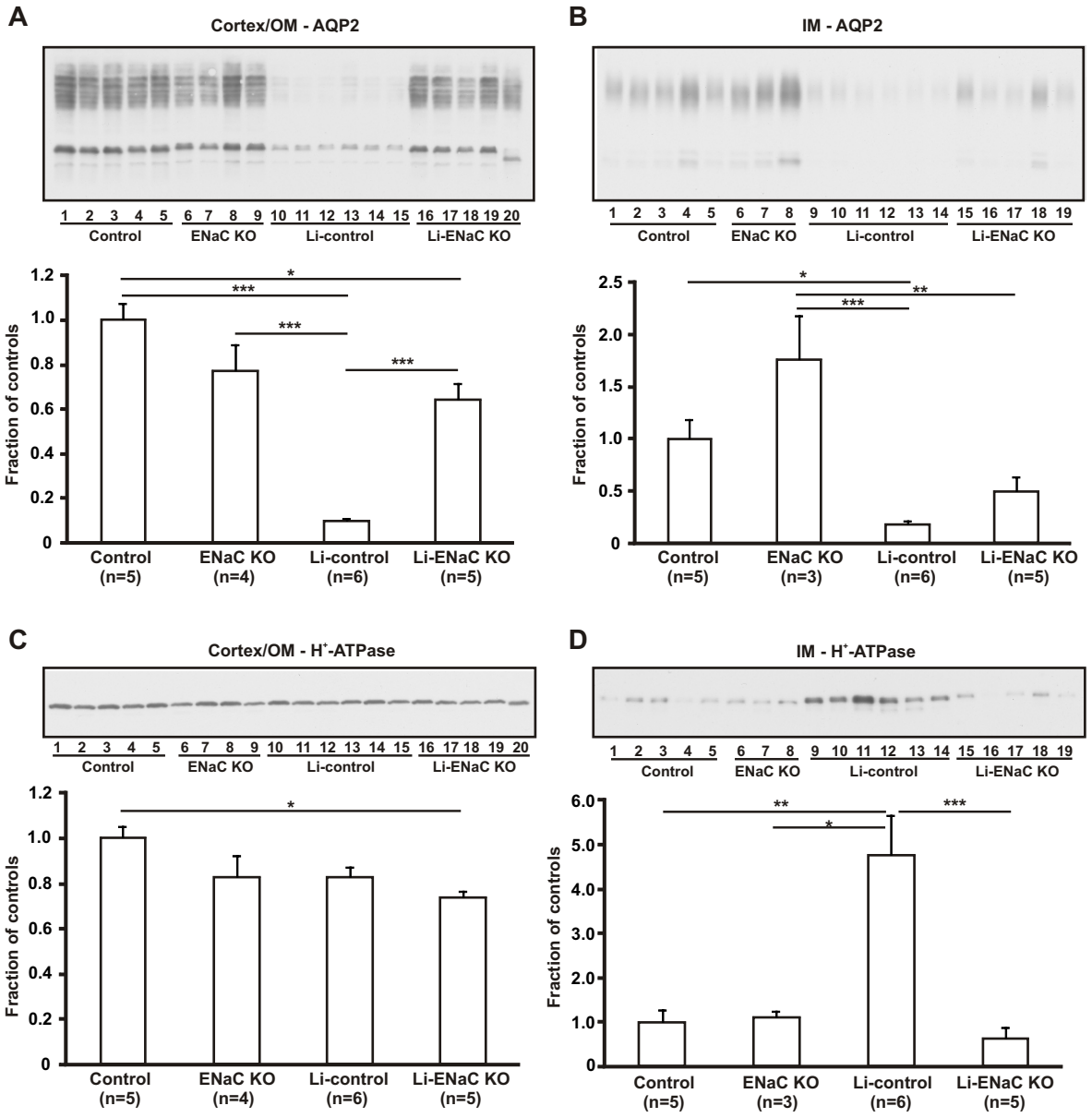
A
Cortex/OM - AQP2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Control
ENaC KO
Li-control
Li-ENaC KO
Fraction of controls
Control (n=5)
ENaC KO (n=4)
Li-control (n=6)
Li-ENaC KO (n=5)
B
IM - AQP2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Control
ENaC KO
Li-control
Li-ENaC KO
Fraction of controls
Control (n=5)
ENaC KO (n=3)
Li-control (n=6)
Li-ENaC KO (n=5)
C
Cortex/OM - H+-ATPase
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Control
ENaC KO
Li-control
Li-ENaC KO
Fraction of controls
Control (n=5)
ENaC KO (n=4)
Li-control (n=6)
Li-ENaC KO (n=5)
D
IM - H+-ATPase
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Control
ENaC KO
Li-control
Li-ENaC KO
Fraction of controls
Control (n=5)
ENaC KO (n=3)
Li-control (n=6)
Li-ENaC KO (n=5)

Figure 3

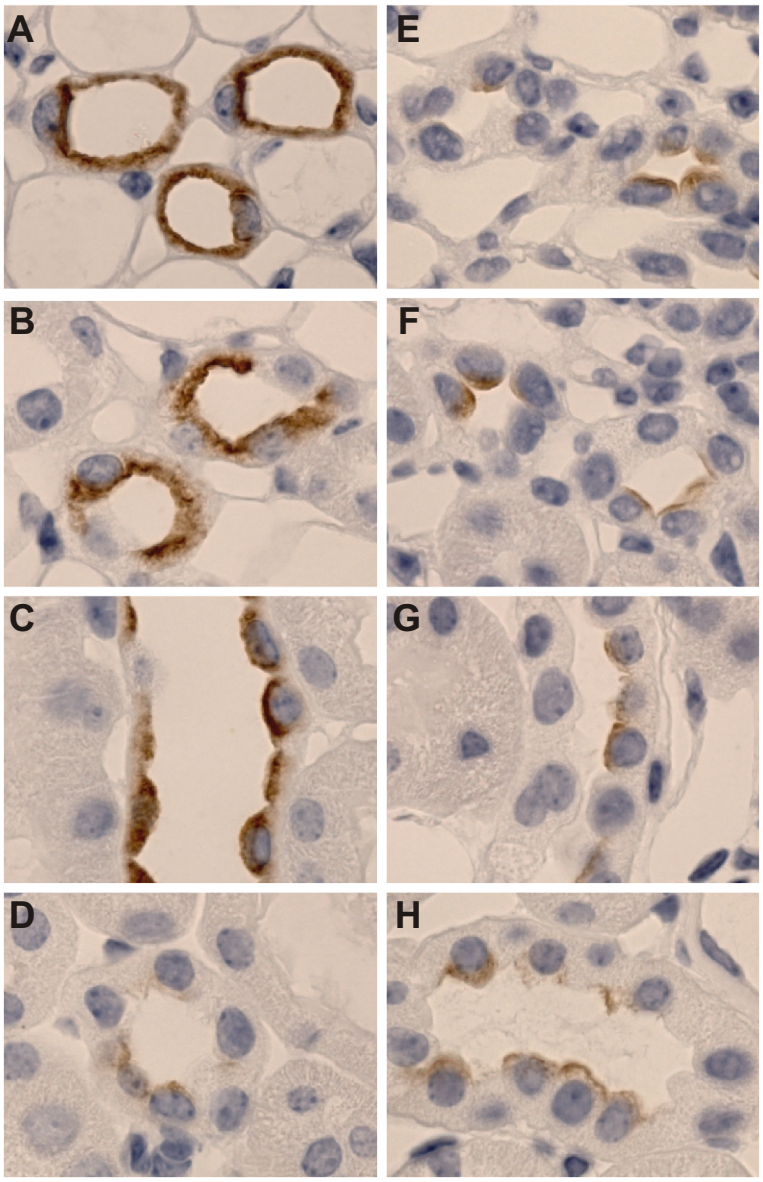

Figure 4