Overexpression of Pendrin in Intercalated Cells Produces Chloride-Sensitive Hypertension


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ABSTRACT

Inherited and acquired disorders that enhance the activity of transporters mediating renal tubular Na⁺ reabsorption are well established causes of hypertension. It is unclear, however, whether primary activation of an Na⁺-independent chloride transporter in the kidney can also play a pathogenic role in this disease. Here, mice overexpressing the chloride transporter pendrin in intercalated cells of the distal nephron (TgB1-hPDS mice) displayed increased renal absorption of chloride. Compared with normal mice, these transgenic mice exhibited a delayed increase in urinary NaCl and ultimately, developed hypertension when exposed to a high-salt diet. Administering the same sodium intake as NaHCO₃ instead of NaCl did not significantly alter BP, indicating that the hypertension in the transgenic mice was chloride-sensitive. Moreover, excessive chloride absorption by pendrin drove parallel absorption of sodium through the epithelial sodium channel ENaC and the sodium-driven chloride/bicarbonate exchanger (Ndcbe), despite an appropriate downregulation of these sodium transporters in response to the expanded vascular volume and hypertension. In summary, chloride transport in the distal nephron can play a primary role in driving NaCl transport in this part of the kidney, and a primary abnormality in renal chloride transport can provoke arterial hypertension. Thus, we conclude that the chloride/bicarbonate exchanger pendrin plays a major role in controlling net NaCl absorption, thereby influencing BP under conditions of high salt intake.

Hypertension is one of the most common human diseases in industrialized countries. Among the different environmental and genetic factors predisposing individuals to hypertension, chronic exposure to high dietary Na⁺ intake has emerged as one of the most important risk factors. However, the molecular mechanisms underlying salt-sensitive hypertension are still poorly understood in the majority of patients. Although it is widely assumed that all pressor effects of dietary NaCl depend on its Na⁺ content, several studies have drawn attention to the fact that the sodium has to be in the form

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of NaCl to produce an increase in BP. In 1929, Berghoff and Geraci reported that BP increased in seven individuals on a high NaCl intake but not a high NaHCO₃ intake, an observation subsequently confirmed by Morgan. In humans or animals with salt-sensitive hypertension, selective dietary loading of Na⁺ without Cl⁻ has repeatedly failed to induce an increase in BP. Likewise, in hypertensive and normotensive subjects, substitution of dietary NaCl with equimolar NaHCO₃ leads to a reduction of BP, further suggesting a modulating effect of dietary chloride on BP. Surprisingly, although it is widely accepted that excessive sodium reabsorption can lead to hypertension, the potential role of primary activation of renal chloride transport in the pathogenesis of hypertension is poorly understood.

It was recently shown that Cl⁻ absorption in the collecting duct occurs through intercalated cells and requires the presence of the Cl⁻/HCO₃⁻ exchanger pendrin (Pds/Slc26a4). We subsequently showed that the functional coupling of pendrin together with the Na⁺-dependent Cl⁻/HCO₃⁻ exchanger (Ndcbe/Slc4a8) results in electroneutral, thiazide-sensitive NaCl absorption, and hence, mimicks the NaCl cotransporter (NCC) of the distal convoluted tubule. Although the importance of this system in vascular volume regulation is suspected based on the observation that pendrin disruption impairs normal renal NaCl balance or protects against mineralocorticoid-induced hypertension, its potential pathogenic role as a primary determinant of hypertension is unknown.

Thus, to investigate the potential primary role of enhanced pendrin activity in the pathogenesis of salt-sensitive hypertension, we have created a mouse model overexpressing pendrin in the intercalated cells of the aldosterone-sensitive distal nephron. We show that pendrin overexpression results in increased chloride absorption that confers on mice markedly salt-sensitive elevation of BP. Interestingly, the pressor effect of high salt intake was strictly chloride-dependent and occurred despite appropriate downregulation of the sodium transporters in the aldosterone-sensitive distal nephron. We conclude from these experiments that a primary abnormality of renal chloride reabsorption can lead to NaCl-sensitive hypertension.

RESULTS

Generation of a Mouse Model Overexpressing Pendrin in the Intercalated Cells

A 6.9-kb fragment corresponding to the human V-ATPase B1-subunit gene (ATP6V1B1 gene; GenBank accession no. NM_039350) has been successfully used to drive expression of both enhanced green fluorescent protein (EGFP) and the Cre-recombinase (Cre) into renal intercalated cells. Importantly, preliminary experiments revealed that the promoter is likely to be insensitive to a high-salt diet (Supplemental Figure 1). We used this promoter fragment to drive the expression of human pendrin (hPDS/SLC26A4) cDNA in intercalated cells of the connecting tubule and the cortical collecting duct (CCD; detailed methods of the transgene construction and genotyping can be found in Supplemental Material and Supplemental Table 1). The transgene includes the 5'-flanking region of the ATP6V1B1 gene extending to but excluding the endogenous translational start codon, the human SLC26A4 cDNA with its own translational start site, and the SV40 late region polyadenylation signal, and it is referred to as B1-hPDS (Figure 1A). The rationale for using the human instead of the mouse cDNA is to be able to distinguish the expression of the transgene from the expression of the endogenous pendrin gene. Approximately 110 live pups were analyzed for the presence of the B1-hPDS transgene by performing PCR analysis of tail DNA (Figure 1B), and 65 pups were found to be positive for transgene integration. However, only one founder transmitted the transgene in a Mendelian fashion and therefore, was used to generate animals for this study. The mice that were used in the present study were F6. Mendelian transmission of the transgene and Southern blot analyses (Supplemental Figure 2) were compatible with a single site of integration. Figure 1C shows that high levels of the human pendrin transcript were detected by RT-PCR in the renal cortex and medulla of transgenic B1-hPDS (TgB1-hPDS) mice, whereas it was not present in the wild-type littermates. We previously reported that, beyond expression in renal intercalated cells, our ATP6V1B1 promoter fragment was able to drive expression of EGFP in epididymis, uterus, small and large intestines, and nonciliated airway epithelial cells and Cre-recombinase in the brain. Thus, we tested expression of the transgene in these different tissues (Figure 1D). No hPDS transcript was detected in tissues isolated from wild-type mice, whereas in tissues isolated from TgB1-hPDS mice, hPDS transcript was detected at high levels in uterus and epididymis.

hPDS transcript was also detectable in lungs and brain, but no significant level of hPDS transcript was detected in small or large intestine or liver.

We next examined the expression of the hPDS protein by immunohistochemistry. However, all the different antipendrin antibodies available to us recognize only the rodent orthologs of pendrin. Thus, a new rabbit polyclonal antibody was raised against the synthetic peptide APGGRESEPPQLPEYS (corresponding to amino acids 3–18 of the N-terminal end of human pendrin protein) (Supplemental Material). Preliminary experiments showed that, although this antibody was recognizing human pendrin, it also crossreacted with the murine ortholog (Supplemental Figure 3). We used this antibody to localize both endogenous (murine) and transgenic (human) pendrin on kidney sections from TgB1-hPDS and control mice (Figure 1, E and F). As previously shown by others, pendrin expression in wild-type mice was detected exclusively in β- or non-α, non-β intercalated cells, which were identified by the coexpression of the V H⁺-ATPase but not the anion exchanger 1 (AE1). By contrast, pendrin staining was also detected in α-intercalated cells (V H⁺-ATPase and AE1-positive) of the renal cortex and medulla in TgB1-hPDS mice. No staining was detected in principle cells of the collecting duct or the...
Figure 1. Generation and characterization of transgenic mouse model overexpressing human pendrin in the renal intercalated cells. (A) Schematic of the transgene (B1-hPDS) containing ~6.9 kb from the 5'-flanking region of the ATP6V1B1 gene extending to but excluding the endogenous translational start codon, the human SLC26A4 cDNA (with its own translational start site; indicated by arrow), and the SV40 late region polyadenylation signal. Positions of the primers used for genotyping or RNA expression (quantitative PCR) are shown. (B) Normal mouse DNA spiked with 0, 1, 10, 10^2, 10^3, 10^4, 10^5, and 10^6 copies per cell equivalent of the purified transgene DNA (left) was amplified by PCR using primers specific for the hB1-EGFP transgene. PCR genotyping (right) of tail DNA from different pups (TgB1-hPDS-1 to -4); in this example, mice 1 and 4 are hemizygous for the B1-hPDS transgene, whereas mice 2 and 3 do not carry the B1-hPDS transgene, (i.e., wild-type littermates). The intensity of the PCR product from mice 1 and 4 compared with normal DNA spiked with variable amounts of the purified transgene indicates that the transgenics have an almost 1000 copies per cell equivalent of the transgene. (C) Quantitative RT-PCR analyses of the human pendrin transcript in the renal cortex and medulla from TgB1-hPDS and...
connecting tubule. Ectopic expression of hPDS in α-intercalated cells was expected, because our B1 promoter fragment was shown to drive expression of EGFP and Cre in both intercalated cell subtypes. Noteworthy, the expression of the transgene had no particular impact on AE1 or the V  H+ -ATPase cellular or subcellular localization.

In our first B1-EGFP transgenic mouse model, we noted that EGFP expression was slightly stronger in the medulla than in the cortex. In the present study, the transgene expression also looked stronger in the renal medulla than in the cortex (Figure 1, E and F). However, immunohistochemistry is not a good technique for quantitation, and we were not able to check the level of expression of the transgene by Western blot, because our antibody was not suitable for this technique. Thus, we next checked pendrin activity in the renal cortex by measuring intracellular pHi changes consecutive to manipulation of luminal Cl− concentration in intercalated cells in cortical collecting ducts isolated from TgB1-hPDS and control mice. Basolateral Cl−/HCO3− activity was not assessed in these experiments. Figure 1G shows that apical Cl−/HCO3− exchange activity was much higher in intercalated cells from TgB1-hPDS transgenic mice than wild-type littermates, showing that our transgenic strategy for overexpressing pendrin is effective in cortical intercalated cells.

**Pendrin Overexpression Stimulates Chloride Absorption by the Renal Tubule**

To determine whether the B1-hPDS transgene expression promotes excessive chloride absorption, cortical collecting ducts were isolated from either TgB1-hPDS transgensics or wild-type littermates. All animals were fed a normal salt diet (i.e., 0.3% Na+ administered as NaCl). Transepithelial fluxes of Na+ (JNa), Cl− (JCl), and K+ (JK) and transepithelial voltage (Vte) were measured as described previously. Figure 2, A and B shows that, although no transport activity was detectable in CCDs isolated from control mice, tubules from TgB1-hPDS mice exhibited net NaCl absorption. The magnitudes of Na+ and Cl− fluxes were very similar to those measured in wild-type mice maintained on a low-salt diet (figure 1 in ref. 12). CCDs from TgB1-hPDS mice did not develop a net negative Vte and did not secrete K+ (Figure 2, C and D). The latter findings suggested that epithelial Na+ channel (ENaC) activity is impaired as a consequence of excessive electroneutral NaCl absorption. However, under normal conditions, ENaC is mostly active in the connecting tubule, which is not accessible to the microperfusion. Thus, to assess the effects of the transgene on ENaC activity, we next tested whether ENaC-dependent Na+ absorption and K+ secretion are inhibited in TgB1-hPDS mice by monitoring the effects of an acute injection of amiloride (1.45 mg/kg body wt) on urinary excretion of Na+ and K+. As shown in Figure 2, E and F, amiloride injection increased urine Na+ excretion and decreased K+ excretion similarly in the TgB1-hPDS mice and wild-type littermates. Taken together, these results indicate that, although the B1-hPDS transgene promotes electroneutral NaCl absorption, it does not inhibit ENaC.

**Increased Electroneutral NaCl Absorption in the Distal Nephron Does Not Lead to High Serum K+ Concentration or Metabolic Acidosis but Provokes Chloride-Sensitive Hypertension**

Phenotypical analyses of TgB1-hPDS mice and wild-type litters are summarized in Supplemental Table 2. Increased electronegic chloride absorption in the CCD, also known as a chloride shunt, has been proposed to decrease the transepithelial voltage; thereby, it impairs tubular K+ secretion and provokes metabolic acidosis and hyperkalemia. However, TgB1-hPDS mice exhibit net NaCl absorption in the CCD, but in the absence of any Vte, they did not exhibit high serum K+ concentration. Another question was whether overexpression of pendrin can favor the development of acidosis. In fact, we initially anticipated that ectopic expression of hPDS in α-intercalated cells would short circuit acid secretion and thereby, lead to distal tubular acidosis. Thus, to determine whether TgB1-hPDS mice display hyperchloremic metabolic acidosis, we performed blood gas analyses in TgB1-hPDS mice and wild-type litters. These analyses showed that both TgB1-hPDS mice and wild-type litters fed a normal salt diet had normal blood acid–base parameters and did not display an alkaline urine pH, indicating the absence of significant bicarbonaturia. Because the effects of the transgene might have been, at least partly, masked by some compensatory mechanisms, the same analyses were again performed after feeding the mice for 2 weeks with a high-salt (3% Na+ administered as NaCl) diet. This maneuver is expected to increase urinary chloride delivery to the CCD and hence, accelerate Cl−/HCO3− exchange through the transgene. However, as shown in Supplemental Table 2, serum K+ concentration and acid–base status of TgB1-hPDS mice remained normal and identical to those levels observed in wild-type litters.
Effect of amiloride injection on urinary Na+ excretion and unpaired CCDs isolated from TgB1-hPDS and WT mice fed a normal salt diet. 1108 Journal of the American Society of Nephrology J Am Soc Nephrol absorption. (C) Vte is the transepithelial voltage. (D) Jk is the rate of K+ secretion. Statistical significance is assessed by two-tailed unpaired t test; n=7 in each group. *P<0.05 versus control group. Effect of amiloride injection on urinary Na+ excretion and unpaired CCDs isolated from TgB1-hPDS and WT mice fed a normal salt diet. (A) JNa is the rate of Na+ absorption. (B) JCl is the rate of Cl− absorption. (C) Vte is the transepithelial voltage. (D) Jk is the rate of K+ secretion. Statistical significance is assessed by two-tailed unpaired t test; n=7 in each group. *P<0.05 versus control group. Effect of amiloride injection on urinary Na+ excretion and K+ transepithelial fluxes and transepithelial voltage in CCDs isolated from TgB1-hPDS and WT mice fed a normal salt diet. (A) JNa is the rate of Na+ absorption. (B) JCl is the rate of Cl− absorption. (C) Vte is the transepithelial voltage. (D) Jk is the rate of K+ secretion. Statistical significance is assessed by two-tailed unpaired t test; n=7 in each group. *P<0.05 versus control group. Effect of amiloride injection on urinary Na+ excretion and K+ transepithelial fluxes and transepithelial voltage in CCDs isolated from TgB1-hPDS and WT mice fed a normal salt diet. (A) JNa is the rate of Na+ absorption. (B) JCl is the rate of Cl− absorption. (C) Vte is the transepithelial voltage. (D) Jk is the rate of K+ secretion. Statistical significance is assessed by two-tailed unpaired t test; n=7 in each group. *P<0.05 versus control group.

We next tested the effects of pendrin overexpression on renal adaptation to a high-salt diet. Figure 3, A and B shows that animals from both genotypes exhibited the same systolic BP and urinary excretion of Na+ and Cl− when fed a normal salt diet (0.3% Na+). When animals were switched to a high-salt diet (3% Na+), urinary excretion of both Na+ and Cl− dramatically increased in both groups. However, these adaptive changes were significantly delayed in TgB1-hPDS mice compared with wild-type littermates. Urinary aldosterone was not different between genotypes in animals fed a normal salt diet, and they decreased similarly to nearly undetectable levels when animals were fed a high-salt diet (Figure 3C). The high-salt diet led to a significant rise in systolic BP in TgB1-hPDS mice, whereas the wild-type littermates experienced no change in BP (Figure 3D). Almost the same values of BP were obtained when recorded by radiotelemetry (Supplemental Figure 4); accordingly, all subsequent BP measurements were performed using the tail-cuff method. Monitoring of heart rate detected a significant decrease in heart rate in both genotypes fed a high-salt diet but did not reveal any difference between genotype (Supplemental Figure 4).

We recently described that pendrin works in tandem with Ndcbe to mediate electroneutral and thiazide-sensitive NaCl absorption. Pech et al. reported that pendrin is required for normal ENaC activity. Thus, to determine whether pendrin overexpression secondarily drives Na+ absorption by Ndcbe, ENaC, or both molecules, we tested the effects of acute injections of either amiloride (1.45 mg/kg) or hydrochlorothiazide (50 mg/kg). These experiments were performed in mice fed a high-salt diet, because the hypertensive phenotype observed in the TgB1-hPDS mice was only apparent in this situation. Figure 4 shows that the response to amiloride was increased in TgB1-hPDS mice compared with wild-type littermates, indicating that ENaC activity is increased in this model; the response to hydrochlorothiazide (HCTZ) was also greater in TgB1-hPDS mice. The NCC and pendrin/Ndcbe transporters are targeted by HCTZ in vivo. However, it is unlikely that higher sensitivity to HCTZ in the TgB1-hPDS mice reflects increased activity of NCC, because we directly observed the presence of electroneutral NaCl absorption in the collecting duct (Figure 2), a nephron segment devoid of NCC. Moreover, in our model, expression of human pendrin is driven by the B1 promoter; it is not expressed in the distal convoluted tubule but exclusively expressed in the connecting tubule and collecting duct, which is evident from two previous studies using the exact same promoter to drive expression of either EGFP or Cre. Thus, these results indicate that Cl− absorption in the distal nephron is paralleled by Na+ absorption occurring by either ENaC or Ndcbe.

Pech et al. have recently reported that extracellular bicarbonate stimulates ENaC activity by increasing β- and γ-ENaC protein abundance and more importantly, promoting γ-ENaC proteolytic cleavage, a process associated with an increase in the channel activity. Conversely, pendrin knockout mice exhibit decreased ENaC activity associated with decreased β- and γ-ENaC activity and decreased γ-ENaC proteolytic activation. In the experiments shown in Figure 4, we detected increased ENaC activity in TgB1-hPDS mice fed a high-salt diet; therefore, we next assessed the profile of expression of different sodium transporters, including ENaC, along the
nephron. Unexpectedly, expression of NCC, p(T55)NCC, ENaC (both α- and γ-subunits), and Ndcbe assessed by immunoblotting of membrane fractions isolated from the renal cortex was decreased in TgB1-hPDS mice compared with wild-type littermates (Figure 5 and Supplemental Table 3). This downregulation of sodium transporters in the aldosterone-sensitive distal nephron in TgB1-hPDS mice is expected in response to the hypoaldosteronism and the salt-sensitive hypertension observed under these conditions. The proteolytic product of γ-ENaC subunit was also undetectable, which was expected in salt-loaded animals. Taken together, these results indicate that excessive chloride absorption by the B1-hPDS transgene is able to accelerate Na⁺ absorption through ENaC and Ndcbe, although their expression levels as assayed by immunoblotting were downregulated. This result suggests that primary activation of chloride reabsorption by pendrin stimulates Na reabsorption through these transporters sufficiently to overcome the observed downregulation at the level of protein expression. Possible mechanisms include changes in transporter surface expression, phosphorylation, activation state, and/or driving force.

To further show the critical role of chloride in the pathogenesis of salt-sensitive hypertension in this model, we examined the effects of chloride on BP by administering NaHCO₃ instead of NaCl as previously described.⁴,⁷ Contrasting with the marked pressor effects of high NaCl intake observed in the preceding experiments, high NaHCO₃ intake did not alter systolic BP in the TgB1-hPDS mice or wild-type littermates, which confirms that the abnormal renal handling of chloride is the primary cause of salt-sensitive hypertension in TgB1-hPDS mice (Figure 6).

**DISCUSSION**

In most human populations, sodium is almost invariably ingested as a chloride salt, and sodium ion has no pressor effects when chloride is substituted with another anion like sulfate or bicarbonate.³,⁸,⁹ The latter observation has led several groups to conclude that salt-sensitive hypertension is chloride-dependent.³,⁴,⁷ However, renal transporters that have been shown to have a clear impact on BP, like the Na/K/2Cl cotransporter type 2 or NCC,²¹ transport equimolar amounts of sodium and chloride. In the collecting duct, it was thought until recently that chloride is mostly absorbed passively because of the lumen-negative transepithelial potential difference that results from ENaC-dependent electrogenic absorption of Na⁺.²²,²³ As a consequence, most efforts have been made to dissect the mechanisms accounting for renal sodium absorption and its regulation, whereas the pathogenic effects of abnormal renal chloride transport have not been studied as extensively.

Here, we show that overexpression of pendrin leads to chloride-dependent hypertension. Because the trangene hPDS was detectable by PCR in the brain, we cannot rule out the possibility that overexpression of pendrin in the central nervous system did also alter central regulation of BP. However, the expression of hPDS in the brain was marginal compared with the kidney. We also did not detect differences in heart rate between TgB1-hPDS and control mice that might have revealed some dysregulation of the autonomous nervous system. Importantly, we show that renal overexpression of pendrin drives excessive renal chloride reclamation, which in turn, is responsible for chloride-sensitive hypertension. Moreover, we show that, despite normal downregulation of all the different
sodium transporters in the aldosterone-sensitive distal nephron, excessive chloride absorption by transgenic pendrin is still able to drive parallel sodium absorption. This important finding challenges the classic paradigm assuming that sodium transport is always primary and secondarily drives chloride as the accompanying anion. Rather, these studies support the possibility that a primary increase in chloride transport can serve as the primary event driving NaCl absorption in the distal nephron. It was previously shown that mice with pendrin disruption do not increase vascular volume and do not raise BP in response to administration of deoxycorticosterone pivalate, a mineralocorticoid that is expected to maximally stimulate sodium absorption by ENaC.13 We now show that a primary increase in pendrin activity can cause hypertension, even when aldosterone is decreased. The fact that primary changes in chloride transport can drive NaCl absorption also has important therapeutic implications, because our results suggest that pendrin might be a very interesting target to modulate NaCl absorption by the distal nephron; pendrin blockers could, therefore, represent a new class of antihypertensive compounds.

From the study of two independent mouse models of pseudohypoaldosteronism type II,25,26 it has been concluded that increased electroneutral NaCl absorption by NCC is the main mechanism leading to this syndrome. Functional coupling of pendrin and Ndcbe mediates NCC-like activity.12

**Figure 4.** Effects of amiloride and hydrochlorothiazide injections in Tg^{B1-hPDS} and WT mice fed a high-salt diet. Urinary (A) Na\(^+\) and (B) K\(^+\) excretion after amiloride injection in Tg^{B1-hPDS} and WT mice. Urinary (C) Na\(^+\) and (D) K\(^+\) excretion after HCTZ injection in Tg^{B1-hPDS} and WT mice. Mice on a high-salt diet (3% Na\(^+\)/8% NaCl in food) for 2 weeks were subcutaneously injected with amiloride (1.45 mg/kg body wt), HCTZ (50 mg/kg body wt), or vehicle. Urines from Tg^{B1-hPDS} and WT mice were collected over 2 days from 9 AM to 3 PM; the first-day injections were in vehicle only (white bars), whereas the diuretics were injected at 9 AM on the following day (black bars). The concentration of excreted Na\(^+\) and K\(^+\) (millimolar) were normalized to the urinary concentration of creatinine (millimolar) to minimize the effects of incomplete urine sampling over such short periods. Values are the mean \pm SEM from seven determinations in each group. Statistical significance was tested versus time controls by paired t test. *P<0.05, **P<0.01.

**Figure 5.** Protein abundance of critical sodium transporters along the nephron in Tg^{B1-hPDS} and WT mice fed a high-salt diet. (A) Immunoblots of membrane fractions from the renal cortex extracted from seven Tg^{B1-hPDS} and seven WT mice fed a high-salt diet for 2 weeks. Each lane was loaded with 10–15 \(\mu\)g protein. Immunoblots were probed with the different primary antibodies as indicated. (B) Densitometric analyses of data were expressed as percentages of control \(i.e.,\) percent of control. Mean control values and SEMs for each respective immunoblot are provided in Supplemental Table 3. Bars represent mean \pm SEM. Statistical significance is assessed by two-tailed unpaired t test; \(n=7\) in each group. *P<0.05, **P<0.01 versus controls.
Moreover, this transport system is expressed in the nephron segments where ENaC-dependent K⁺ and H⁺ secretion occurs. Thus, we hypothesized that upregulation of this transport system might decrease ENaC activity and hence, might impair K⁺ and H⁺ secretion even more than NCC upregulation. However, our present results clearly indicate that this hypothesis is not the case. The absence of effects on potassium and acid–base homeostasis cannot be attributed to a failure of our transgenic strategy, because we clearly show that the B1-hPDS transgene was able to increase apical Cl⁻/HCO₃⁻ exchange activity in the cortical collecting duct. Moreover, we were able to show that the transgene accounts for excessive renal NaCl absorption, and our TgB1-hPDS mice displayed salt-sensitive hypertension. Nevertheless, our results are in line with two recent studies that reported that increased NCC activity is not sufficient to cause Gordon’s syndrome.27,28

In summary, our study solidifies the concept of chloride-sensitive hypertension by showing that a primary enhancement of renal chloride transport by overexpression of pendrin can lead to salt-sensitive hypertension. Thus, we conclude that the chloride/bicarbonate exchanger pendrin has a major role in controlling net NaCl absorption and hence, BP in the setting of high salt intake.

CONCISE METHODS

Animals
All the experimental procedures have been reviewed and approved by the local ethics committee from the University Pierre et Marie Curie, and they were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication No.93–23, revised 1985).

Metabolic Studies
All experiments were performed using age- and sex-matched TgB1-hPDS mice and wild-type littermate (3–5 months). For urine collection, mice were housed in metabolic cages (Techniplast, France). Mice were given deionized water ad libitum and pair-fed with standard laboratory chow containing 0.3% sodium (INRA, France). They were allowed to adapt for 3–5 days in the cages. At steady state, urine collection was performed daily under mineral oil in the urine collector for electrolyte measurements. Mice were then switched to a high-salt diet (3% Na⁺), either as NaCl or NaHCO₃ (INRA, France). After the switch, urine was collected every 24 hours.

All biochemical and hormonal analyses were performed using standard methods as detailed in Supplemental Material.

BP Measurements in Conscious Mice
Mice were fed normal or high-salt diet for at least 2 weeks. Systolic BP was measured using a computerized tail-cuff system after 2 weeks of daily training, which has been described elsewhere.29 Then, at least 10 measurements were performed every day for at least 7 consecutive days. Only the last 4 days were kept for analyses. If the variability of the measurements made in a single day exceeded the SD by more than 20%, the day was discarded and replaced by an additional day of measurement. Moreover, to ensure that it was the case in our study, BPs were also measured by radiotelemetry in mice fed either a normal or high-salt diet in a pilot experiment (Supplemental Material). Similar values were obtained using both techniques (compare Figure 4 with Supplemental Figure 3).

Immunofluorescence Studies and Immunoblot Analyses
Kidney sections were triple labeled with a rabbit polyclonal human pendrin antibody (diluted 1:200), a guinea pig anti-AE130 (diluted 1:5000), and a chicken anti-AE1 (diluted 1:10,000). They were incubated with antibodies against NCC phospho-Thr55 antibody (residues 41–60 of human NCC phosphorylated at Thr55, HPSHLTHSTFCRMpTFGYNT, S908B; 1:275),37 anti-NCC phospho-Thr55 antibody (residues 41–60 of human NCC phosphorylated at Thr55, HPSHLTHSTFCRMpTFGYNT, S908B; 1:275),37 Protein loading was assessed on gels ran in parallel and stained with Coomassie blue.33 Blots were probed with anti α-ENaC (dilution 1:10,000), anti γ-ENaC (1:30,000), anti-αENaC (1:30,000),34 anti-Ndcbe (1:250),12 anti-NHE3 the Na/H exchange type 3 (1:1000),35 anti-NKCC2 the Na/K/2Cl cotransporter type 2 (1:5000),35 anti-NCC (1:50,000),36 and anti-NCC phospho-Thr55 antibody (residues 41–60 of human NCC phosphorylated at Thr55, HPSHLTHSTFCRMpTFGYNT, S908B; 1:275).37 Proteins were detected by chemiluminescence (ECL Kit; Amersham Biosciences). Antibodies against α- and γ-subunits of ENaC were generously donated by J. Lofving (University of Zurich, Zurich, Switzerland). Antibody against α-ENaC was raised against the N terminus of mouse α-ENaC (MLDHRAPELNLDDLIDVSNC).

RT-PCR
Total RNA was extracted using the RNeasy Kit (Qiagen). Reverse transcription was performed by using a first-strand cDNA synthesis kit.
for RT-PCR (Roche Diagnostics). Real-time PCR was performed on a LightCycler (Roche Diagnostics) with a LightCycler 480 SYBR Green I Master qPCR Kit (Roche Diagnostics). No amplification was observed in the absence of reverse transcription, which confirmed that samples were free of genomic DNA. Primer sequences and a methodology for the PCR amplification are provided in detail in Supplemental Material. Results are expressed as the mean ± SEM from six mice.

Transgene expression in different organs was assessed in one male mouse and one female mouse as described in Figure 1D.

**In Vitro Microperfusion of Isolated Collecting Ducts**

CCDs were isolated and microperfused in vitro as described by Burg et al. Intracellular pH was monitored by using the pH-sensitive dye BCECF. [Na⁺], [K⁺], and [creatinine] measurements were performed by HPLC; transepithelial voltage (Vte) was measured continuously during perfusion to calculate the rate of K⁺ (JK) and Na⁺ (JNa) transport, which is described in detail in Supplemental Material.

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**DISCLOSURES**

None.

**REFERENCES**


Supplemental Informations

1- Supplemental methods

**Generation of the Tg**(B1-hPDS)** transgenics:** The human SLC26A4 cDNA was ligated into the pBluescript cloning vector containing 6.9kbp of the human ATP6V1B1 promoter\(^1\), \(^2\). The SV40 late region polyadenylation signal was cloned downstream of the cDNA. The transgene includes the 5'-flanking region of the ATP6V1B1 gene extending to but excluding the endogenous translational start codon, the human SLC26A4 cDNA, with its own translational start site, and the SV40 late region polyadenylation signal, and is referred as B1-hPDS. Transgene integrity was confirmed by restriction digest and bidirectional sequencing of ligation sites. To prepare for injection, the transgene was linearized from the vector by SalI and NotI digestion, followed by gel purification using an electroelution method and then concentrated using ElutipD columns (Whatman). The transgene was then further concentrated by ethanol precipitation and resuspended in low EDTA injection buffer (10mM tris 0.1mM EDTA). Transgene integrity was confirmed by restriction digest and bidirectional sequencing of ligation sites. Transgenic mice were created by the University of Utah transgenic mouse core facility using standard procedures as described\(^1\), \(^2\). Genotyping demonstrated that 63 pups were positive for transgene integration. One founder transmitted the transgene in a Mendelian fashion and was subsequently used for all the experiments described. The mice that were used in the present study were F6. Mendelian transmission of the transgene and southern blot analyses (Suppl. Fig S1) were compatible with a single site of integration. The transgenic founder was crossed with wild-type C57BL/6 x CBA F1 mice and the resulting hemizygous offspring were analyzed. The control mice consisted of sex-matched wild type littermates.

**Genotyping:** Mouse genomic DNA was prepared from tail tissue by standard methods. To detect the B1-hPDS transgene, PCR primers were designed to amplify across the hPDS cDNA and SV40 Polyadenylation signal sequence to provide a PCR product of 345 bp (Forward primer: 5'...
AGAGGTCAAGGTCCATTTAG-3'; Reverse primer: 5’-CAAACCACAAGCTAGAATGCAGTG-3’). PCR consisted in denaturation 95°C for 7 min followed by 40 cycles of amplification (30 sec at 95°C, 30 sec at 57°C, 30 sec at 72°C) and 5 min at 72°C.

**RNA extraction and reverse transcription:** Animals were killed and kidneys were harvested and rapidly frozen in liquid nitrogen. Snap-frozen kidneys (six kidneys for each condition) were homogenized in RLT-Buffer (Qiagen, Basel, Switzerland) supplemented with β-mercaptoethanol to a final concentration of 1%. Total RNA was extracted from 200 µl aliquots of each homogenized sample using the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. Quality and concentration of the isolated RNA preparations were analyzed by the ND-1000 spectrophotometer (NanoDrop Technologies). Total RNA samples were stored at –80°C. Each RNA sample was diluted to 100 ng/µl and 3 µl used as a template for reverse transcription using the Bioline cDNA synthesis kit (BioLine, Randolph, MA). For reverse transcription, 1 µg of RNA template were diluted in a 10-µl reaction mix that contained (final concentrations) RT buffer (1X), oligo (dT)₁₈ (2.5 µM), RNase inhibitor (0.5 U/µl), the Bioline reverse transcriptase enzyme (2.5 U/µl), dNTP mix (0.5 µM each), and RNase-free water.

**Real-time quantitative PCR:** Primers were designed using Primer Express software from Applied Biosystems for GAPDH (Forward 5’-GCACAGTCAAGGCGAGAAT-3’; Reverse 5’-GCCTTCTCCATGGTGTTGAA-3’), human pendrin (Forward 5’-AAATCTCAAGGGGTCAGGTTCC-3’; Reverse 5’-ACATCAAGTCTTCTTCGTCCAGT-3’), mouse Atp6v1b1 (Forward 5’-ATCAATGTGCTCCCATCCCTCT-3’; Reverse 5’-AATGCAGCTTTCAGCATCTTCTTCC-3’). Primers were chosen to produce amplicons ≤150 bp that spanned intron-exon boundaries as to exclude amplifying genomic DNA. The specificity of all primers was first tested on mRNA derived from kidney. Real-time PCR was performed using a 5 ng of cDNA quantity on a LightCycler (Roche Diagnostics, Meylan, France) with LightCycler 480 SYBR Green I Master qPCR kit (Roche Diagnostics, Meylan,
France). Briefly, 2µl (2.5 ng/µl) cDNA, 0.5 µl of each primer (25 µM), 2 µl RNase free water, 5 µl SYBR Green Master mix (Roche Applied Biosystems, Meylan, France); for a final volume of 10 µl. Reaction conditions were as follows: 95°C for 10 minutes followed by 45 cycles of 95°C for 20 seconds, 62°C for 20 seconds, and 72°C for 20 seconds. All reactions were run in duplicate. A negative control was amplified in the absence of the reverse transcriptase. Cycle thresholds were recorded at within the linear range of fluorescence intensity, which was set at 0.06. Gene expression was normalized to that glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Relative expression ratios were calculated as R=2(Ct(GAPDH)-Ct(test gene)), where Ct represents the cycle number at the threshold 0.06.

Southern Blot analyses of Genomic DNA: A 894 bp fragment of the hPDS coding sequence was excised by EcoRI and served as a probe template. The probe was radiolabelled with 32P-dCTP (Hartmann Analytik, Braunschweig, Germany) and purified with Illustra ProbeQuant G-50 columns (GE Healthcare, Waukesha, WI, USA). Phenol-Chloroform purified genomic DNA (5 µg) from wild-type and transgenic B1-hPDS mice was digested with either BamHI or EcoRI (Thermo, Waltham, MA, USA), respectively, and separated on 0.8% agarose gels. Digested genomic DNA was blotted on Hybond-XL membranes (GE Healthcare, Waukesha, WI, USA) and hybridized with radiolabelled probes following standard protocols.

**Generation and characterization of a anti human pendrin antibody:** The synthetic peptide (APGGRSEPPQLPEYS) corresponding to amino-acids 3 to 18 of the N-terminal end of human pendrin protein was synthesized by the DB-BioRun Laboratory (Nantes, France), coupled to keyhole limpet hemocyanin with the Imject Maleimide Activated Immunogen Conjugation Kit (Pierce), and used to immunize rabbits to generate polyclonal antiserum (DB-BioRun Laboratory, Nantes, France). Supplemental Figure 2 shows that analyses of mouse and human kidney sections labeled with this new antibody revealed exactly the same pattern of labeling in both species that was consistent with known pendrin expression. Pre-immune serum did not yield
any labeling, and all staining was abolished when the antiserum was pre-
incubated with the immunizing peptide. Moreover, when the antiserum was
used to stain kidney sections obtained from mice with pendrin disruption\(^3\) no
signal was detected which confirmed that this antiserum is specific for
pendrin. (Supplemental Figure 1).

**Biochemical and hormonal measurements:** Urine creatinine (enzymatic
method) was measured with a Konelab 20i auto-analyzer (Thermo Electron
Corporation, Eragny Parc, France). Urinary chloride was measured with a DL
55 titrator (Mettler Toledo, Viroflay, France). Urinary Na\(^+\) and K\(^+\) were
measured by flame photometry (IL943, Instruments Laboratory, Lexington,
MA). Urine aldosterone was measured by RIA (DPC Dade Behring, La Défense, France). Blood was collected by tail incision on mice anesthetized by
peritoneal injection of a mixture (0.1ml/g body weight) of ketamine
(Imalgene\(^\circledR\), Rhône Mérieux, Lyon, France; 10%) and xylazine (Rompun\(^\circledR\),
Bayer AG, Leverkusen, Germany; 5%) and [Na\(^+\)], [K\(^+\)], hematocrit and [Cl\(^-\)]
were measured with an ABL 77 pH/blood-gas analyzer (Radiometer,
Copenhagen, Denmark). Blood gases analyses were performed by retro-
orbital punction on awake animals and pH, PCO\(_2\), and PO\(_2\) were measured
with an ABL 77 pH/blood-gas analyzer (Radiometer, Copenhagen, Denmark).
Blood bicarbonate concentration was calculated by the autoanalyzer from the
measured values using the Henderson-Hasselbach equation.

**Measurements of blood pressure by radiotelemetry:**
Animals were anaesthetized with pentobarbital (50 mg/kg) by intraperitoneal
(IP) injection. Telemetric devices were implanted in 6 wild-type and 6 Tg\(^{B1-bPDS}\)
according to the standard protocols. Briefly, catheters were inserted into
the right carotid artery and attached to a radiotransmitter (Physio Tel HD-X11;
Data Sciences International) located on the back. Mice were allowed to
recover for two to three days at which time they were placed in metabolic
cages (Hatteras Instruments, Cary, NC) and permitted to acclimate to the
cages and the gel diet for an additional five days. Blood pressure was
continuously recorded using a telemetry receiver (Physio Tel DSI receiver;
Data Sciences International).
**In vitro microperfusion of mouse CCDs:** Kidneys were removed and cut into 1 to 2 mm coronal slices that were transferred into a chilled dissection medium containing (mM): 118 NaCl, 25 NaHCO$_3$, 2.0 K$_2$HPO$_4$, 1.2 MgSO$_4$, 2.0 calcium lactate, 1.0 sodium citrate, 5.5 glucose, and 12 creatinine, pH 7.4, and gassed with 95% O$_2$-5% CO$_2$. CCD segments were isolated from corticomedullary rays under a dissecting microscope with a sharpened forceps. Because CCDs are highly heterogeneous, relatively short segments (0.45-0.6 mm) were dissected to maximize the reproducibility of the isolation procedure. **In vitro** microperfusion was performed as described by Burg et al. 4. Briefly, isolated CCDs were rapidly transferred to a 1.2 ml temperature- and environmentally-controlled chamber, mounted on an inverted microscope, and perfused and bathed initially at room temperature with dissection solution. The specimen chamber was continuously suffused with 95% O$_2$-5% CO$_2$ to maintain pH at 7.4. Once secure, the inner perfusion pipette was advanced and the tubule was opened with a slight positive pressure. The opposite end of the tubule was then pulled into a holding collection pipette. In the holding collection pipette, 2 to 3 cm of water-saturated mineral oil contributed to maintain the tubule open at a low flow rate of perfusion. The perfusing and collecting end of the segment was sealed into a guard pipette using Dow-Corning 200 dielectric fluid (Dow Corning Corp., Midland, MI). The tubules were then warmed to 37°C and equilibrated for 20 minutes while the collection rate was adjusted to a rate of 1-4 nl/min. The length of each segment was measured using an eyepiece micrometer. Because CCDs from mice are frequently unstable and collapse, measurements were conducted during the first 90 minutes of perfusion. Usually, collections from 4 periods of 15 minutes were performed in which 25 to 30 nanoliters of fluid were collected. The volume of the collections was determined under water-saturated mineral oil with calibrated volumetric pipettes. 20 nanoliters were required for [Na$^+$], [K$^+$] and [creatinine] measurements. Transepithelial voltage (V$_{te}$) was measured continuously using Ag-AgCl electrodes connected to 0.15 M NaCl-agar bridges inserted in the perfusion pipette and bathing solutions. The initial value after rewarming of the tubule was noted. Values of each period were averaged.
**Measurements of Na⁺, K⁺ and creatinine concentrations with high-pressure liquid chromatography:** Cation concentrations were measured as previously described⁵. The Dionex-500 system (Dionex DX-500, Dionex Corp., Voisins le Bretonneux, France) consisted of an AS50 autosampler, a GP50 gradient pump, an ED40 electrochemical detector (Na⁺ and K⁺), and an AD20 UV absorbance detector 220 nm (creatinine). The signal-to-noise ratio of the conductivity measurement was enhanced by employing a cation self-regenerating suppressor (CSRS-ultra, 4mm) that was set in the autosuppression recycle mode. The HPLC column consisted of a Dionex IonPac CS12 column (4 x 250 mm), equipped with a guard column CG12A, (4 x 50 mm). The mobile phase consisted of 18mM methanesulfonic acid. Tubular fluid, perfusion solution, and standard solutions were drawn under mineral oil with a calibrated pipette (about 20 nl) and transferred to a vial containing 39 µl to the mobile phase of the HPLC with LiNO₃ as an internal standard. Peaks of each measured analyte (Na⁺, K⁺, Creatinine) were adjusted with the value of the Li⁺ internal standard to limit the variations due to automatic injection. In each run of experiments, perfusion and bath solutions were tested in 4 or 5 replicates and the reproducibility of the measure was evaluated: Coefficient of variation (CV) < 0.10 for K⁺ determination, and CV <0.05 for creatinine determination. In addition, a calibration curve for each analyte was tested with correlation coefficients >0.98.

**Measurement of fluid absorption:** Creatinine was used as the volume marker, and therefore was added to the perfusion solutions (both perfusate and bath) at a concentration of 12 mM. The rate of fluid absorption (Jᵥ) was calculated as $J_v = (V_{perf} - V_{coll})/L$, with $V_{perf} = C_{coll}/C_{perf} \times V_{coll}$.

Crcoll and Crperf are the concentrations of creatinine in the collected fluid and perfusate, respectively. Vcoll is the collection rate at the end of the tubule. L is the length of the tubule.

**Calculation of the rate of absorption of Na and K:** For each collection, Na⁺ flux (JNa) and K⁺ flux (JK) where calculated and reported to the length of the
tubule:
\[ J_{Na} = \frac{([Na]_{perf} \times V_{perf}) - ([Na]_{coll} \times V_{coll})}{L} \]
\[ J_{K} = \frac{([K]_{perf} \times V_{perf}) - ([K]_{coll} \times V_{coll})}{L} \]

Therefore, positive values indicate net absorption, whereas negative values indicate net secretion of the ion. For each tubule, the mean of the 4 collection periods was used.

**Intracellular pH measurements on isolated microperfused tubules**

During intracellular pH measurement experiments, the average tubule length exposed to bath fluid was limited to 300 – 350 µm in order to prevent motion of the tubule.

Two solutions were used, differing in their content in chloride. The composition of the solutions were as follows; chloride-containing solution was composed of (in mM) 119 NMDG-Cl, 23 NMDG-HCO₃, 2 K₂HPO₄, 1.5 CaCl₂, 1.2 MgSO₄, 10 HEPES, and 5.5 D-glucose; chloride-free solution was composed of (in mM) 119 NMDG-gluconate, 23 NMDG-HCO₃, 2 K₂HPO₄, 7.5 Ca-gluconate, 1.2 MgSO₄, 10 HEPES, and 5.5 D-glucose. All solutions was adjusted to pH 7.40 and continuously bubbled with 95% O₂/5% CO₂.

At the beginning of all experiments, tubules were bathed and perfused with the Cl-containing solution.

To identify principal and intercalated cells, we labeled the apical membrane of intercalated cells by adding fluorescent peanut lectin (PNA, Vector Labs) to the luminal perfusate for 5 minutes and observed which cells were fluorescent. Intracellular pH in CCD cells was assessed with imaging-based, dual excitation-wavelength fluorescence microscopy with use of the fluorescent probe 2',7'-Bis- (2-Carboxyethyl)-5- (And-6)- carboxyfluorescein (BCECF, Molecular probes). Tubules were loaded for ~20 min at room temperature with 5x10⁻⁶mol/L of the acetoxymethyl ester of BCECF added to the peritubular fluid. Loading was continued until the fluorescence intensity at 440 nm excitation wavelength was at least one order of magnitude higher than background fluorescence. The loading solution was then washed out by initiation of bath flow and the tubule was equilibrated with dye-free solution for 5-10 minutes. Bath solution was delivered at a rate of 20 ml/min and warmed
to 37±0.5°C by water jacket immediately upstream to the chamber. During the fluorescence recording, the Cl-containing solution was delivered to the perfusion pipette via a chamber under an inert gas (N₂ pressure around 1 bar) connected through a manual 6-way valve. With this system, opening of the valve instantaneously activated flow of one solution. The majority of the fluid delivery to the pipette exited the rear of the pipette system through a drain port at a rate of 4 ml/min. This method resulted in a smooth and complete exchange of the luminal solution in less than 3 to 4 s as measured by the time necessary for appearance of colored dye at the tip of the perfusion pipette. After 3-minutes recording, luminal fluid was instantaneously (at the rate of 4 ml/min in the draining) replaced by the corresponding Cl-free solution for 3 minutes. Finally, the luminal solution was changed again for the Cl-containing solution.

Intracellular dye was excited alternatively every 2 seconds at 440 and 500 nm with a light-emitting diode (Optoled, Cairn Research, Faversham, UK). Emitted light was collected through a dichroïc mirror, passed through a 530 nm filter and focused onto a EM-CCD camera (iXon, Andor Technology, Belfast, Ireland) connected to a computer. The measured light intensities were digitized with 14-bit precision (16384 grey level scale) for further analysis. For each tubule, 3-4 intercalated cells were analyzed and the mean grey level was measured with the Andor IQ software (Andor Technology, Belfast, Ireland). Background fluorescence was subtracted from fluorescence intensity to obtain intensity of intracellular fluorescence.

Intracellular dye was calibrated at the end of each experiment using the high [K⁺]-nigericin technique. Tubules were perfused and bathed with a HEPES-buffered, 95-mM K⁺ solution containing 10 µM of the K⁺/H⁺ exchanger nigericin. Four different calibration solutions, titrated to pH 6.9, 7.3, 7.5 or 7.8 were used.

**References**


Supplemental Figure S1. Effects of high salt diet on Atp6v1b1 mRNA.
Wild type C57BL/6 mice were fed either a normal salt (0.3% Na⁺ as NaCl salt) or high salt diet (3% Na⁺) for two weeks. RT-PCR analysis were performed using the primers shown in suppl. table S1. Results were normalized to GAPDH as described above in the method section. Results are the mean ± S.E., n = 6 in both groups. The absence of statistical difference was tested by unpaired Student’s t test.
Supplemental Figure S2. Southern Blot analyses of Genomic DNA from WT or TgB1-hPDS mice. Genomic DNA from wildtype (Wt) and transgenic mice (Tg) was digested with either BamHI or EcoRI as indicated above. Digestion with BamHI which does not cut within the transgenic construct, revealed a single band of strong intensity supportive of a single integration site. An additional genomic digest with EcoRI, which also excises the probe template, resulted in a single band corresponding to the size of the probe. Binding of the probe to fragments which include the endogenous murine Pds sequence are marked by asterisks.
Supplemental Figure S3. Characterization of a new anti human pendrin antibody. Low magnification (A) and high magnification (B) of mouse kidney sections, and high magnification (C, D) of human kidney sections labeled with the rabbit anti human pendrin antibody (red labeling), plasmic membranes were counterstained with FITC-conjugated phallloidin, and nuclei were counterstained with DAPI. The pattern of labeling was identical in human and mouse kidney, and was consistent with the known localization of pendrin in β-ICs. Staining of mouse kidney sections (E), was completely abolished when the antibody was pre-incubated with the immunizing peptide in excess (F),
and was absent when pre-immune serum was used instead of the immune serum (G). Staining was also absent in sections obtained from mice with pendrin disruption (Pds-/-) (I) compared to WT mice (H) and stained using same procedure than in panel A and B demonstrating the specificity of the signal.
Supplemental Figure S4: Systolic blood pressure and heart rate measured in WT and TgB1-hPDS mice by radio-telemetry. Mice were fed either normal (0.3% Na⁺ as NaCl) or high salt (3% Na⁺ as NaCl) for two weeks. Data are means ± S.E. of data from 5 independent mice in each group. Data were analyzed by ANOVA followed by bonferroni post hoc-test when appropriate. ** indicate p<0.01 vs. Normal salt diet.
3. Supplemental Tables

**Supplemental Table 1 : Primers used in this study**

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<tr>
<th></th>
<th>Forward primers</th>
<th>Reverse Primers</th>
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<td><strong>Mouse Gapdh</strong></td>
<td>5’-GCACAGTCAAGGCGAGAAT-3’</td>
<td>5’-GCCTTCTCCATGGGTGGA-3’</td>
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<tr>
<td><strong>primers B1-hPds (genotyping)</strong></td>
<td>5’-AGAGGTCAAGGTTCCATTTTAG-3’</td>
<td>5’-CAAACCACAATAGAATGCAGTG-3’</td>
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<td><strong>human PDS</strong></td>
<td>5’-AAATCTCAAGAGGTAAGGTTC-3’</td>
<td>5’-ACATCAAGTTCTTCCGTCAG-3’</td>
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<td><strong>Mouse Atp6v1b1</strong></td>
<td>5’-ATCAATGTGCTCCATCCCT-3’</td>
<td>5’-AATGCCTGCAGCATCTCTTT-3’</td>
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Supplemental Table 2: Physiological data from Tg^{B1-hPDS} and WT mice fed either a normal salt or a high salt diet for two weeks

Data are means ± S.E. of data obtained from 8 Tg^{B1-hPDS} and 8 WT mice. Both diet conditions are presented in same table but are from two independent series and statistical significance was only assessed vs. WT fed same diet, using a two-tailed unpaired Student’s t-test.

<table>
<thead>
<tr>
<th></th>
<th>0.8% NaCl (0.3% Na^+^)</th>
<th>8% NaCl (3% Na^+)</th>
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<tr>
<td></td>
<td>Tg^{B1-hPDS}</td>
<td>WT</td>
</tr>
<tr>
<td>Plasma</td>
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</tr>
<tr>
<td>[Na^+^], mM</td>
<td>148 ± 1</td>
<td>149 ± 1</td>
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<tr>
<td>[K^+^], mM</td>
<td>4.15 ± 0.19</td>
<td>4.03 ± 0.11</td>
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<tr>
<td>[Cl^-], mM</td>
<td>117 ± 1.3</td>
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<tr>
<td>Ht, %</td>
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<td>42 ± 0.77</td>
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<tr>
<td>blood</td>
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<tr>
<td>pH</td>
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<td>[HCO_3^-], mM</td>
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<td>pCO_2, mmHg</td>
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<td>54 ± 1.8</td>
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<tr>
<td>urine</td>
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</tr>
<tr>
<td>pH</td>
<td>5.76 ± 0.07</td>
<td>5.83 ± 0.06</td>
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Supplemental Table 3: Effect of a high salt diet on different renal transporters protein abundance. Densitometric analyzes of immunoblots of membrane fractions isolated from the renal cortex of Tg^{B1-hPDS} mice or WT mice. All animals were pair-fed a high salt diet (3%Na+ as NaCl) for one week.

<table>
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<tr>
<th></th>
<th>WT</th>
<th>KO</th>
<th>p value</th>
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<tr>
<td>NHE3</td>
<td>100 ± 9.9</td>
<td>75.5 ± 12</td>
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</tr>
<tr>
<td>NKCC2</td>
<td>100 ± 5.4</td>
<td>109.5 ± 6.7</td>
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<td>NCC</td>
<td>100 ± 8.1</td>
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<tr>
<td>pNCC</td>
<td>100 ± 16.5</td>
<td>58.0 ± 8.0</td>
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<tr>
<td>αENaC 90kDa</td>
<td>100 ± 6.5</td>
<td>75.0 ± 5.3</td>
<td>0.012*</td>
</tr>
<tr>
<td>αENaC 30kDa</td>
<td>100 ± 9.1</td>
<td>55.3 ± 5.7</td>
<td>0.001*</td>
</tr>
<tr>
<td>γENaC</td>
<td>100 ± 12.8</td>
<td>64.3 ± 7.3</td>
<td>0.032*</td>
</tr>
<tr>
<td>mPds</td>
<td>100 ± 7.0</td>
<td>86.7 ± 11.9</td>
<td>0.33</td>
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<tr>
<td>B1-H-ATPase</td>
<td>100 ± 6.2</td>
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</tr>
<tr>
<td>Ndcbe</td>
<td>100 ± 3.9</td>
<td>61.4 ± 7.2</td>
<td>0.0005*</td>
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</table>

Data are mean ± S.E and are expressed as a percentage of control values. Data obtained from 7 Tg^{B1-hPDS} mice and 7 WT mice. Statistical significance was determined by a two-tails unpaired Student’s t-test, a p value < 0.05 is considered as significant and labeled with *. 