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Proximal tubule-specific deletion of the Na^+/H^+ exchanger 3 promotes the pressure natriuresis response and lowers blood pressure in mice

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Abstract

The present study directly tested the hypothesis that deletion of the Na^+/H^+ exchanger 3 (NHE3) selectively in the proximal tubules of the kidney lowers basal blood pressure by increasing the pressure natriuresis response in mice. Adult male and female, age-matched wild-type littermates (WT) and proximal tubule-specific NHE3-knockout mice (PT-*Nhe3*$^{-/-}$) (n=6–16 per group) were studied for 1) basal phenotypes of electrolytes and pH, blood pressure, and kidney function, 2) the pressure natriuresis response using the mesenteric, celiac and abdominal arterial occlusion technique, and 3) the natriuretic responses to acute saline expansion (0.9% NaCl, 10% body wt., i.p.) or 2-week of 2% NaCl diet. Under basal conditions, PT-*Nhe3*$^{-/-}$ mice showed significantly lower systolic, diastolic, and mean arterial blood pressure (p<0.01) than WT mice (p<0.01). PT-*Nhe3*$^{-/-}$ mice also exhibited significantly greater diuretic (p<0.01) and natriuretic responses than WT mice (p<0.01), without altering 24 h fecal Na^+ excretion, plasma pH, Na^+, and bicarbonate levels. In response to increased renal perfusion pressure by 30 mmHg, the pressure natriuresis response increased 5-fold in WT mice (p<0.01), but it increased 8-fold in PT-*Nhe3*$^{-/-}$ mice (p<0.01). In response to 10% acute saline expansion or 2-week 2% NaCl diet, more pronounced natriuretic responses were demonstrated in PT-*Nhe3*$^{-/-}$ than WT mice (p<0.01). Our results support the scientific premise and physiological relevance that NHE3 in the proximal tubules plays an essential role in maintaining basal blood pressure homeostasis, and genetic deletion of NHE3

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selectively in the proximal tubules of the kidney lowers blood pressure by increasing the pressure natriuretic response.

Summary

This study used a novel mouse model to demonstrate for the 1st time that deletion of NHE3 selectively in the proximal tubules of the kidney significantly increases the pressure-natriuresis response and natriuretic responses to salt and volume expansion, and lowers blood pressure in mice. The results suggest that NHE3 in the proximal tubules may be a therapeutically target to control blood pressure in hypertension by promoting the pressure-natriuresis response.

Keywords

Hypertension; kidney; NHE3; pressure natriuresis; proximal tubule-specific NHE3 knockout; saline expansion; sodium and glucose cotransporter 2

Introduction

It is well-established that hypertension, especially essential hypertension, is a multifactorial disorder involving genetic, neural, humoral, environmental, and life style factors. Although the role for each of these factors in the physiological regulation of blood pressure and the development of hypertension have been extensively investigated, their respective contributions and mechanisms of actions remain incompletely understood.[1,2] For example, over 50 common genetic variants or loci have been identified to be associated with the blood pressure regulation or hypertension, but each of these variants reportedly contributes to only about ~1 mmHg for systolic and ~0.5 mmHg for diastolic pressure in humans.[3–5] High salt diet is another most commonly referred factor contributing to hypertension in humans and animal models of salt-sensitive hypertension.[1,2,6] The roles of increased renal sympathetic nerve activity in hypertension have also been extensively investigated lately,[7,8] but a large prospective, randomized, blinded, sham-controlled SYMPLICITY HTN-3 trial of using renal denervation to treat uncontrolled and/or resistant hypertensive patients provides inconclusive evidence.[9] Clearly, further studies are required to uncover additional factors or new mechanisms underlying basal blood pressure homeostasis and the pathogenesis of hypertension.

The kidney plays a key role in maintaining basal blood pressure and preventing the development of hypertension through a highly converged mechanism, i.e., the pressure natriuresis response.[10,11] The pressure natriuresis response is a central element of the overall feedback mechanism for long-term blood pressure control, in which an acute increase in arterial pressure will lead to an increase in urinary Na^+ excretion, thereby restoring blood pressure to normal.[10,12] Various physical and humoral factors, ranging from renal interstitial hydrostatic pressure,[12,13] 20-HETE, dopamine,[14] or nitric oxide (NO),[15] have been studied extensively to determine their roles in the pressure natriuresis response, but the underlying mechanisms are far from well understood. The Na^+/H^+ exchanger 3 (NHE3) is a ~85 kDa protein encoded by the SLC9A3 gene and is directly and indirectly responsible for reabsorbing ~70% of filtered Na^+ in the proximal tubules.[16–18] The upregulation of NHE3 in

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the proximal tubules and impairment of the pressure natriuresis response have been implicated in angiotensin II-induced hypertension and in spontaneously hypertensive rats.[19–21] However, whether NHE3 in the proximal tubules is directly involved in maintaining basal blood pressure homeostasis and regulating the pressure natriuresis response has not been studied using the genetically modified, proximal tubule-specific NHE3 knockout mouse model.

In the present study, we directly tested the hypothesis that genetic deletion of NHE3 selectively in the proximal tubules of the kidney significantly increases the pressure natriuresis response and lowers basal blood pressure in mice. The hypothesis was tested using adult male and female wild-type littlemates and mutant mice with proximal tubule-specific deletion of NHE3 with the sglt2-Cre/NHE3^{loxlox} approach.[22] Our results provide unequivocal new evidence that NHE3 in the proximal tubules of the kidney plays a key role in regulating the pressure and natriuresis response and maintaining basal blood pressure homeostasis in mice.

Methods

All data, analytic methods, and study materials will be made available to other researchers by contacting Jia L. Zhuo, M.D., Ph.D., at jzhuo@umc.edu. Additional detailed methods and materials are provided in the ONLINE SUPPLEMENT.

Animals

All animal studies were performed in accordance with the Guide for Care and Use of Laboratory Animals (National Institute of Health) and approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

Proximal tubule-specific Nhe3$^{-/-}$ mice (PT-Nhe3$^{-/-}$): Homozygous PT-*Nhe3*$^{-/-}$ mice were bred and genotyped using the sglt2-Cre/NHE3^{loxlox} approach as described by Li et al. (Suppl. Fig. S1) [22] and Rubera et al.[23] Age- and body wt.-matched male and female NHE3^{loxlox} and PT-*Nhe3*$^{-/-}$ mice (NHE3$^{loxlox/sglt2\text{-}Cre}$) were used in the present study.

Global Slc9a3$^{-/-}$ (Nhe3$^{-/-}$) mice: Adult male wild-type (*Slc9a3*$^{+/+}$ or *Nhe3*$^{+/+}$) and mutant mice with global deletion of NHE3 (*Slc9a3*$^{-/-}$ or *Nhe3*$^{-/-}$) were bred and genotyped as described by Dr. Gary E. Shull of the University of Cincinnati (Suppl. Fig. S2).[16,24–26]

Basal systolic, diastolic, and mean arterial blood pressure phenotypes of wild-type and mutant *Nhe3*$^{-/-}$ and PT-*Nhe3*$^{-/-}$ mice—The noninvasive tail-cuff technique (Visitech, NC) was first used to screen the systolic blood pressure phenotype in conscious male and female wild-type and mutant PT-*Nhe3*$^{-/-}$ mice. The direct implanted telemetry technique (Data Science) was then used to measure basal systolic, diastolic and mean arterial pressure, as described previously [19,25,26]

Basal renal cortical and medullary transporter protein expression profiles in wild-type and PT-*Nhe3*$^{-/-}$ mice—Western blot analysis was used to determine sglt2, Na$^+$/K$^+$-ATPase α1, Na$^+$/HCO_3^-, aquaporin 1 and 2, NKCC2, and ENaC α protein expression

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in the cortex and medulla of male and female wild-type and PT-*Nhe3*$^{-/-}$ mice, respectively. [19,25,27]

Intestinal and renal structural phenotypes of wild-type, *Nhe3*$^{-/-}$ and PT-*Nhe3*$^{-/-}$ mice—The digestive system and kidney structural phenotypes were compared histologically, whereas intestinal absorptive function was compared between wild-type, global *Nhe3*$^{-/-}$ and PT-*Nhe3*$^{-/-}$ mice. [25,26]

The pressure natriuresis response in anesthetized wild-type and PT-*Nhe3*$^{-/-}$ mice—The pressure natriuresis response was studied in adult male and female, age-matched wild-type and PT-*Nhe3*$^{-/-}$ mice (n=10–13 per group) using the gold-standard, mesenteric, celiac and abdominal arterial artery occlusion technique. [13,28] The procedures are described in details in the online only Data Supplement (Suppl. Fig. S3).

The acute natriuretic response to acute saline or water volume expansion in conscious wild-type and PT-*Nhe3*$^{-/-}$ mice—The acute natriuretic response to intraperitoneal injection (i.p.) of saline (0.9% NaCl) or water (H_2O), 10% body wt., was compared in three groups of adult male and female wild-type (n=10) and PT-*Nhe3*$^{-/-}$ mice (n=13), respectively.

The chronic natriuretic response to 2% NaCl in wild-type and PT-*Nhe3*$^{-/-}$ mice—The systolic blood pressure and chronic natriuretic responses to treatment with 2% NaCl diet were determined and compared using the tail-cuff and metabolic cage techniques at basal, and 1, 3, 7 and 14 days after animals were treated with 2% NaCl diet. [29,30]

Statistical Analysis

All results are presented as mean ± SEM. One-way ANOVA was first used to compare the differences in the same parameters for more than two groups of mice. If the *p* value was less than 0.05, a post-hoc Newman-Keul multiple comparison test or Student's unpaired t test was performed to compare two different group means of the same parameters.

Results

Proximal tubule-specific PT-*Nhe3*$^{-/-}$ mice do not show abnormal intestinal structural and absorptive phenotypes of global *Nhe3*$^{-/-}$ mice

Figure 1 directly compared the gross structural (**A**-**C**) and absorptive phenotypes (**D**-**F**) of the digestive system including gut weight (**D**), 24 h fecal Na^+ excretion (**E**), and cecal fluid accumulation (**F**) between adult male wild-type, global *Nhe3*$^{-/-}$ mice and PT-*Nhe3*$^{-/-}$ mice. Compared with wild-type mice (**A**), all segments of small and large intestines in *Nhe3*$^{-/-}$ mice were significantly enlarged and elongated, especially the extremely enlarged cecal segment with very thin wall and striking accumulation of intestinal fluid (**B**). No similar abnormal intestinal structural phenotype was observed in PT-*Nhe3*$^{-/-}$ mice (**C**). Overall gut weight more than doubled (**D**), whereas 24 h fecal Na^+ excretion (**E**) and cecal fluid accumulation (**F**) were about 10-fold higher in global *Nhe3*$^{-/-}$ mice than in either wild-type or PT-*Nhe3*$^{-/-}$ mice. There were no differences in structural and absorptive phenotypes in

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small intestines between wild-type and PT-*Nhe3*$^{-/-}$ mice (Fig. 1). Similarly, there were no significant differences in renal structures at the anatomical, light (**S4A-B**) and electron microscopic levels including mitochondria (**C-D**) and brush border microvilli (**E-F**) between wild-type and PT-*Nhe3*$^{-/-}$ mice.

Proximal tubule-specific deletion of NHE3 decreases proximal tubule Na^+ reabsorption and increases natriuretic response without altering plasma pH, Na^+, bicarbonate, and hematocrit in PT-*Nhe3*$^{-/-}$ mice

Figure 2 summarizes and compares basal renal functional phenotypes of adult male wild-type and PT-*Nhe3*$^{-/-}$ mice. There were no significant differences in basal plasma pH, Na^+, bicarbonate, and hematocrit levels between male wild-type and PT-*Nhe3*$^{-/-}$ mice (*n.s.*) (S5). 24 h food intake slightly, but not significantly, increased in PT-*Nhe3*$^{-/-}$ mice (S6). Compared with wild-type mice, whole-kidney glomerular filtration rate (GFR) (**2A**) and fractional proximal tubule Na^+ reabsorption (**2B**), as estimated by fractional lithium reabsorption, decreased significantly in PT-*Nhe3*$^{-/-}$ mice. However, 24 h urine (**2C**) and urinary Na^+ (**2D**) and K^+ excretion (**2E**) were significantly increased in PT-*Nhe3*$^{-/-}$ mice without altering urine osmolality (**2F**).

Proximal tubule-specific deletion of NHE3 decreases basal blood pressure in PT-*Nhe3*$^{-/-}$ mice

Basal systolic, diastolic, and mean arterial blood pressure, as measured by the telemetry technique, were about 12 ± 3 mmHg lower in male and female PT-*Nhe3*$^{-/-}$ mice throughout the 7-day period ($p<0.01$ vs. wild-types) (Fig. 3). However, there were no significant sex differences in systolic **(3A vs. 3D)**, diastolic **(3B vs. 3E)**, and mean **(3C vs. 3F)** arterial pressure between male **(3A-3C)** and female **(3D-3F)** wild-type and PT-*Nhe3*$^{-/-}$ mice (*n.s.*). Mean intra-arterial blood pressure was significantly lower in male PT-*Nhe3*$^{-/-}$ mice than wild-type mice (**S8**).

Proximal tubule-specific deletion of NHE3 upregulates cortical and medullary Na^+ transporter and water channel protein expression in PT-*Nhe3*$^{-/-}$ mice

In response to NHE3 deletion selectively in the proximal tubules of male and female PT-*Nhe3*$^{-/-}$ mice, the expression of the sodium and glucose cotransporter 2 (sglt2) and Na^+/K^+-ATPase α1 subunit proteins in the renal cortex was significantly increased without altering Na^+/HCO_3^- and aquaporin 1 (AQP1) expression (Fig. 4). In the renal medulla, NKCC2 protein expression was significantly decreased in both male and female PT-*Nhe3*$^{-/-}$ mice, whereas ENaC α subunit and aquaporin 2 (AQP2) protein expression was upregulated in both male and female PT-*Nhe3*$^{-/-}$ mice (Fig. 4).

Proximal tubule-specific deletion of NHE3 increases the pressure natriuresis response in PT-*Nhe3*$^{-/-}$ mice

Figure 5 shows that in response to increased renal perfusion pressure (RPP) equally by 30 ± 3 mmHg, urinary Na^+ excretion ($U_{Na}V$) increased from basal 5.55 ± 0.32 μmol/h to 29.83 ± 0.68 μmol/h in male wild-type mice ($p<0.01$, Fig. 5A). Thus the pressure natriuresis response increased 5-fold in wild-type mice ($p<0.01$). By comparison, $U_{Na}V$ response

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increased from basal 7.04 ± 0.35 μmol/h to 55.06 ± 0.52 μmol/h in male PT-*Nhe3*$^{-/-}$ mice (p<0.01). The pressure natriuresis response increased 7.8-fold in male PT-*Nhe3*$^{-/-}$ mice (p<0.01, Fig. 5A). Both Net (Δ) increase in $U_{Na}V$ (Fig. 5C) and the fractional increase in $U_{Na}V$/per h/per 10 mmHg increase in renal perfusion pressure (RPP) (Fig. 5E) were significantly greater in male PT-*Nhe3*$^{-/-}$ mice (p<0.01). The pressure natriuresis response was also greater in female PT-*Nhe3*$^{-/-}$ mice (Fig. 5B, 5D & 5F).

Proximal tubule-specific deletion of NHE3 increases the natriuresis response to acute saline expansion in PT-*Nhe3*$^{-/-}$ mice

In response to 10% of acute saline expansion, the acute natriuretic response ($U_{Na}V$) increased from basal 18.89 ± 3.96 μmol/3h to 122.24 ± 7.66 μmol/3h, or 6-fold, in male wild-type mice (p<0.01; Fig. 6A & 6D). By comparison, $U_{Na}V$ increased from basal 26.19 ± 3.86 μmol/3h to 206.52 ± 5.68 μmol/3h, or 8-fold, in male PT-*Nhe3*$^{-/-}$ mice (p<0.01; Fig. 6A & 6D). The difference in the natriuretic response to acute saline expansion between male wild-type and PT-*Nhe3*$^{-/-}$ mice is statistically significant (p<0.01). Similarly, the natriuretic response to 10% of acute saline expansion was also greater in female PT-*Nhe3*$^{-/-}$ than wild-type mice (p<0.01, Fig. 6A & 6D). However, the natriuretic response to acute water expansion was significantly smaller in male and female wild-type and PT-*Nhe3*$^{-/-}$ mice (S9).

Proximal tubule-specific deletion of NHE3 increases the natriuretic response to long-term 2% Na^+ diet in PT-*Nhe3*$^{-/-}$ mice

The daily food intake was significantly higher in both male and female PT-*Nhe3*$^{-/-}$ mice **(S6)**. In male wild-type mice, 24 h $U_{Na}V$ increased significantly in a time-dependent manner, but the natriuretic response was statistically greater in male PT-*Nhe3*$^{-/-}$ mice (p<0.01, Fig. 6B). However, systolic blood pressure was not significantly increased in wild-type or PT-*Nhe3*$^{-/-}$ mice during 2 week treatment with 2% NaCl (*n.s.*, Fig. 6C). When the administration of 2% NaCl diet was extended to 6 weeks, however, systolic blood pressure slightly increased by 5 ± 2 mmHg in male wild-type mice *(not shown)*, but not in female PT-*Nhe3*$^{-/-}$ mice. In female wild-type and PT-*Nhe3*$^{-/-}$ mice, there was no difference in 24 h natriuretic response to 2% NaCl diet (Fig. 6E), but systolic blod pressure remained significantly lower in female PT-*Nhe3*$^{-/-}$ mice (Fig. 6F).

Discussion

To the best of our knowledge, this is the 1st study to directly determine the specific role of the *Nhe3* gene in the proximal tubules of the kidney in the regulation of basal blood pressure homeostasis and the pressure natriuresis response using proximal tubule-selective NHE3 knockout mice. We demonstrated that deletion of the *Nhe3* gene selectively in the proximal tubules significantly increased 24 h urinary Na^+ excretion and decreased basal systolic, diastolic and mean arterial blood pressure in conscious as well as anesthetized adult male and female PT-*Nhe3*$^{-/-}$ mice. The lower basal blood pressure and increases in 24 h urinary Na^+ excretion were due to the direct loss of NHE3 proteins and actions as the most important Na^+/H^+ exchanger and Na^+-retaining protein in the proximal tubules. These phenotypes persist despite the fact that the expression of sglt2, Na^+/K^+-ATPase, and AQP1

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in the cortex, and ENaC α and AQP2 in the medulla were significantly upregulated (Fig. 4). This suggests that the upregulation of other Na^+ transporter and/or water channel protein expression is inadequate to fully compensate for the loss of NHE3 in the proximal tubules. More importantly, proximal tubule-specific knockout of NHE3 significantly increases the pressure natriuresis response, and natriuretic responses to acute saline expansion and chronic high Na^+ treatment especially in male PT-*Nhe3*$^{-/-}$ mice. Thus the results of the present study provide direct evidence that NHE3 in the proximal tubules physiologically promotes proximal tubule Na^+ transport and maintains basal blood pressure homeostasis in part by regulating the pressure natriuresis response, and the natriuretic response to saline expansion and high Na^+ diet.

Previous studies have shown that global deletion of NHE3 is associated with abnormal structural and absorptive phenotypes, and significant salt wasting from the digestive system.[16,22,25,26] The "kidney-selective" *tgNhe3*$^{-/-}$ mouse model, which has transgenic rescue of the *Nhe3* gene in small intestines of global *Nhe3*$^{-/-}$ mice, is unable to fully rescue the intestinal structural and absorptive phenotypes.[26,31] A recent study generated mutant mice with the whole-kidney nephron segment, panepithelial cell-specific NHE3 knockout mice to determine the role of kidney NHE3 using the Pax8-Cre/NHE3^{loxlox} approach.[32] The PT-*Nhe3*$^{-/-}$ mouse model used in the present study were different from the above-mentioned NHE3 knockout models, because we used the sglt2-Cre/NHE3^{loxlox} recombination approach, and sglt2 is primarily expressed in the early S1 and S2 proximal tubules.[22,33] Indeed, global *Nhe3*$^{-/-}$ mice showed strikingly abnormal structural and absorptive phe*n*otypes in the digestive system (Fig. 1), with significant enlargement of all intestine segments, marked accumulation of fluid in the enlarged cecum (Fig. 1B & 1F), moderate diarrhea (**S2**), and up to 10-fold increases in 24 h fecal Na^+ excretion (Fig. 1E). By comparison, none of these abnormal intestinal structural and absorptive phenotypes of global *Nhe3*$^{-/-}$ mice was observed in our male and female PT-*Nhe3*$^{-/-}$ mice (Fig. 1). Thus, the complete lack of abnormal intestinal structural and absorptive phenotypes in our PT-*Nhe3*$^{-/-}$ mice excludes the possibility of offtarget deletion of NHE3 in the digestive system.

The 1st key finding of the present study is to demonstrate an important role of proximal tubule NHE3 in maintaining basal blood pressure homeostasis. In the kidney, nearly 80% −90% of NHE3 is expressed in the proximal tubules alone.[34,35] NHE3 is the most important Na^+/H^+ exchanger or antiporter in the proximal tubules, and is directly and indirectly responsible for up to 70% of Na^+ in the proximal tubules.[18] However, apart from in vivo evidence derived from global *Nhe3*$^{-/-}$ mice, [16,36] inhibition of NHE3 by S3226 in rats,[37] or whole-kidney tubular pan-epithelial NHE3 deletion,[32] the direct role of proximal tubule NHE3 in the blood pressure regulation has not been studied previously. Li et al. previously reported a small, but not significant, decrease in systolic blood pressure in a small number of these animals as measured by the indirect tail-cuff technique.[22] In the present study, we measured basal systolic, diastolic, and mean arterial blood pressure using the direct implanted telemetry approach in conscious, as well as the intra-arterial catheter in anesthetized, male and female wild-type and PT-*Nhe3*$^{-/-}$ mice weekly from 12 weeks to one-year old (see figure S8). Basal systolic (Fig. 3A & 3D), diastolic (Fig. 3B & 3E) and mean arterial blood pressure (Fig. 3C & F) were consistently about 12 to 15 mmHg lower in

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adult male and female, age-matched, PT-*Nhe3*$^{-/-}$ mice. This level of basal blood pressure in PT-*Nhe3*$^{-/-}$ mice in the present study is statistically similar to basal levels of blood pressure previously reported in adult male, age-matched, global *Nhe3*$^{-/-}$[16,36] or tg*Nhe3*$^{-/-}$ mice with transgenic rescue of the *Nhe3* gene in small intestines.[26,31] In a recent study, Fenton et al. reported that deletion of NHE3 throughout all renal tubules using the Pax8-Cre/NHE3^{loxlox} approach also significantly decreased systolic blood pressure from about 100 mmHg to about 80 mmHg in anesthetized NHE3^{loxloxCre} mice.[32] The decrease in basal blood pressure in adult male and female PT-*Nhe3*$^{-/-}$ mice in the present study is most likely due to significant diuretic and natriuretic responses (Fig. 2C & 2D), due to the decrease of the whole-kidney fractional proximal tubule Na^+ reabsorption as a direct result of NHE3 deletion selectively in the proximal tubules (Fig. 2B). This finding is supported by a previous study that showed significant decreases in net fluid (J_V) and HCO_3^- absorption (J_{HCO3^-}) in microperfused proximal tubules of PT-*Nhe3*$^{-/-}$ mice.[22] A small but significant decrease in the whole-kidney GFR in PT-*Nhe3*$^{-/-}$ mice (Fig. 2A) may suggest an increased tubuloglomerular feedback response due to increased Na^+ and fluid delivery from the end of the proximal tubule.

The 2nd key finding of the present study is that we uncovered a novel role of NHE3 in the proximal tubules of the kidney as a new modulator of the pressure natriuresis response in male and female PT-*Nhe3*$^{-/-}$ mice (Fig. 5A & 5B). Whether NHE3 in the proximal tubules of the kidney plays a role in the pressure natriuresis response has not been studied directly previously due to the lack of proximal tubule-specific NHE3 knockout animal models. This finding is highly significant and relevant to the normal blood pressure regulation, because the pressure natriuresis response is the central element of the overall feedback mechanism for long-term blood pressure control, in which an acute increase in arterial pressure will lead to an increase in urinary Na^+ excretion, thereby restoring blood pressure to normal.[10,12] Guyton believed that the kidney, through the pressure natriuresis response, plays a key role in long-term regulation of basal blood pressure and the development of hypertension.[10] Previous studies have shown that the pressure natriuresis response was associated with inhibition of Na^+ transport in the proximal tubule,[38] increases in renal interstitial hydrostatic pressure [12] or 20-HETE in the proximal tubule, [13] activation of the AT_2 receptor by ANG III,[39,40] dopamine-mediated natriuresis,[14,41] or NO production in the kidney.[15,42] Since NHE3 in the proximal tubule is directly and indirectly responsible for reabsorbing up to 70% of filtered Na^+ load in the proximal tubule,[18] we reasoned that deletion of NHE3 and therefore its Na^+-retaining action selectively in the proximal tubule is expected to promote the pressure natriuresis response in PT-*Nhe3*$^{-/-}$ mice. Indeed, when renal perfusion pressure was raised to a similar level (~30 mmHg) by occluding mesenteric and celiac arteries and/or the abdominal aorta below the renal arteries,[43] the pressure natriuresis response (or the urinary Na^+ excretion response) increased 5-fold in male wild-type mice, whereas the same response increased almost 8-fold in male (Fig. 5A) and female PT-*Nhe3*$^{-/-}$ mice (Fig. 5B). Both net $U_{Na}V$ increase (Fig. 5C & 5D) and the increase in $U_{Na}V$ with every 10 mmHg elevated (Fig. 5E & 5F) were significantly higher in male and female PT-*Nhe3*$^{-/-}$ mice than male and female wild-type mice. These results suggest that to excrete the same amount of Na^+, the blood pressure set-point is significantly lower in PT-*Nhe3*$^{-/-}$ mice than in wild-type mice.

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The 3rd key finding of the present study is that deletion of NHE3 selectively in the proximal tubules of the kidney significantly augments the natriuretic responses to acute and long-term salt expansion in male and female PT-*Nhe3*$^{-/-}$ mice. In response to 10% acute saline expansion, the natriuretic response increased 5.6-fold in male wild-type mice, while it increased 8.6-fold in male PT-*Nhe3*$^{-/-}$ mice (Fig. 6). The increase in this natriuretic response in PT-*Nhe3*$^{-/-}$ mice is Na^+-specific, since a similar 10% water expansion led to a much smaller natriuretic response in these mice (**S9**). Furthermore, in response to 2% NaCl diet for 2 weeks, our data show that at any given time point, male PT-*Nhe3*$^{-/-}$ mice excreted significantly more Na^+ than wild-type mice, (Fig. 6). However, we found no significant increase in systolic blood pressure in both male and female wild-type and PT-*Nhe3*$^{-/-}$ mice after 2 week treatment with 2% NaCl (Fig. 6). A longer than 2-week treatment with 2% or higher NaCl diet in these mice may be necessary to elicit the difference in the blood pressure response in PT-*Nhe3*$^{-/-}$ mice.

Perspectives

In summary, this was the 1st study to determine a direct cause and effect relationship between NHE3 in the proximal tubules of the kidney and the physiological pressure natriuresis response using a genetically modified mouse model with proximal tubule-specific knockout of NHE3. We demonstrated that genetic deletion of NHE3 selectively in the proximal tubules of the kidney promotes the pressure natriuresis response, lowers basal blood pressure, and augments acute and chronic natriuretic responses to saline expansion in male and female PT-*Nhe3*$^{-/-}$ mice. These results support a key role of NHE3 in the proximal tubule in maintaining basal blood pressure homeostasis, and have important physiological relevance in basal blood pressure control and potentially clinical implications in hypertension. Since increased natriuretic responses and lower blood pressure, as a result of proximal tubule-specific deletion or inhibition of NHE3, are not associated with abnormal intestinal structural and absorptive phenotypes, renal structures, or abnormal plasma pH, Na^+, bicarbonate and hematocrit, NHE3 in the proximal tubules of the kidney may serve as a potential therapeutic target in blood pressure control and hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

What Is New?

- The Na^+/H^+ exchanger 3 (NHE3) in the proximal tubules of the kidney has been implicated in the pressure-natriuresis response and its resetting in hypertension, but the direct cause and effect relationship between NHE3 in the proximal tubules and the pressure-natriuresis relationship has never been investigated previously.
- This is the 1st study to use genetically modified mouse model with deletion of NHE3 selectively in the proximal tubules to directly test the hypothesis that the deletion of NHE3 selectively in the proximal tubules promotes the pressure-natriuresis response and lowers basal blood pressure.

What Is Relevant?

- The pressure natriuresis response in the kidney plays a central role in maintain normal blood pressure homeostasis, and its resetting to high pressures contributes to the development of most, if not all, forms of hypertension in humans.
- NHE3 in the proximal tubules of the kidney may be a new pharmacological target to control blood pressure in hypertension by promoting the pressure-natriuresis response.

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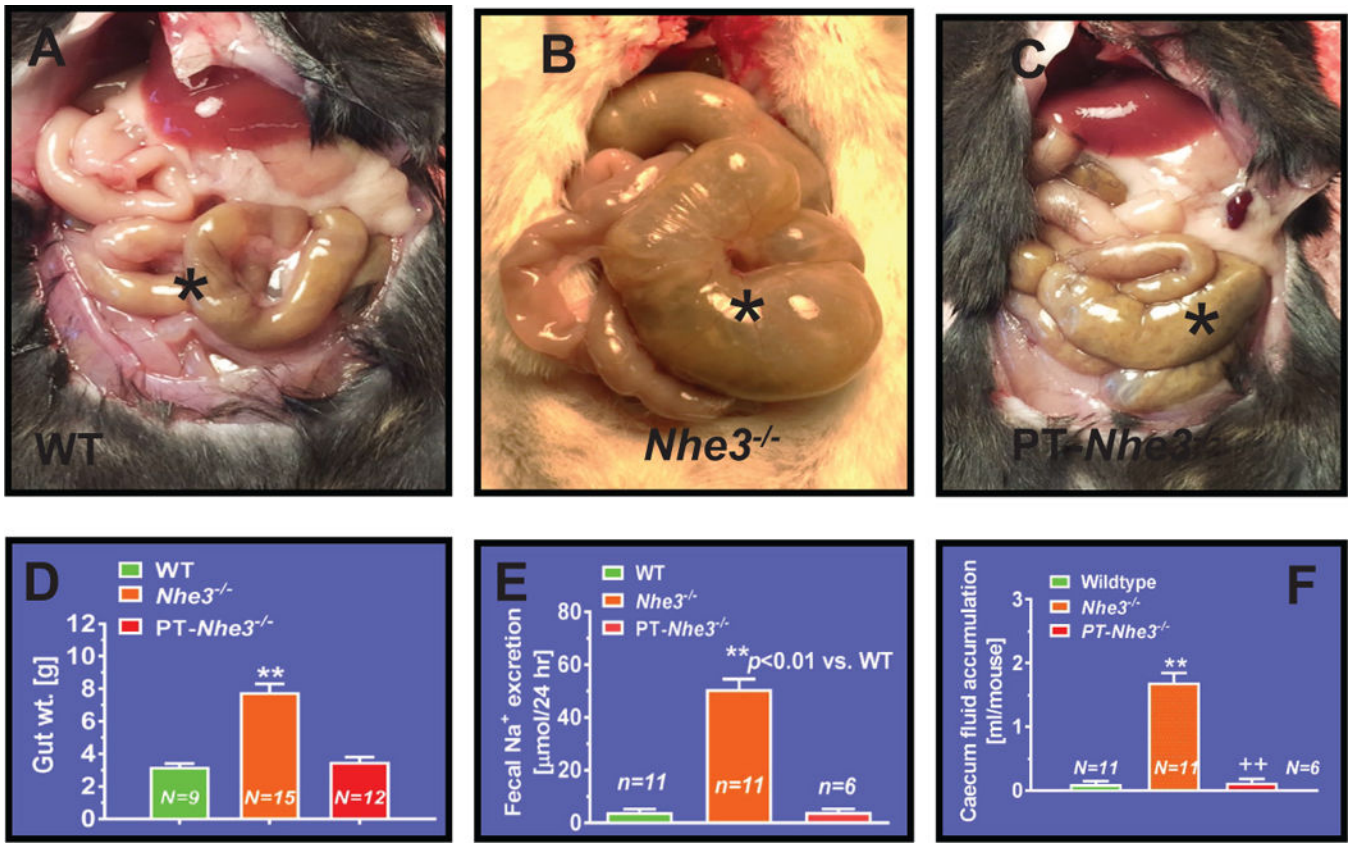

Figure 1.

Proximal tubule-specific deletion of NHE3 does not significantly alter the intestinal structural and absorptive phenotypes in PT-*Nhe3*$^{-/-}$ mice. **A:** a representative normal caecum segment between small and large intestines in a wild-type mouse (*). **B:** a representative cecum segment between small and large intestines in a global *Nhe3*$^{-/-}$ mouse, showing the extremely enlarged cecum segment accumulated with a large volume of fluid content inside (*). **C:** a representative cecum segment between small and large intestines in a PT-*Nhe3*$^{-/-}$ mouse, showing the lack of enlarged cecum segment and no accumulation of a large volume of fluid content in the cecum segment (*). **D:** the overall gut weight more than doubled in global *Nhe3*$^{-/-}$ mice than wild-type and PT-*Nhe3*$^{-/-}$ mice (p<0.01). **E:** 24 h fecal Na^+ excretion was ~10-time higher in global *Nhe3*$^{-/-}$ mice than wild-type and PT-*Nhe3*$^{-/-}$ mice (p<0.01). **F:** accumulation of fluid content in the cecum segment was ~10 times higher in global *Nhe3*$^{-/-}$ mice than wild-type and PT-*Nhe3*$^{-/-}$ mice (p<0.01). There were no differences in the overall gut weight and 24 h fecal Na^+ excretion between wild-type and PT-*Nhe3*$^{-/-}$ mice.

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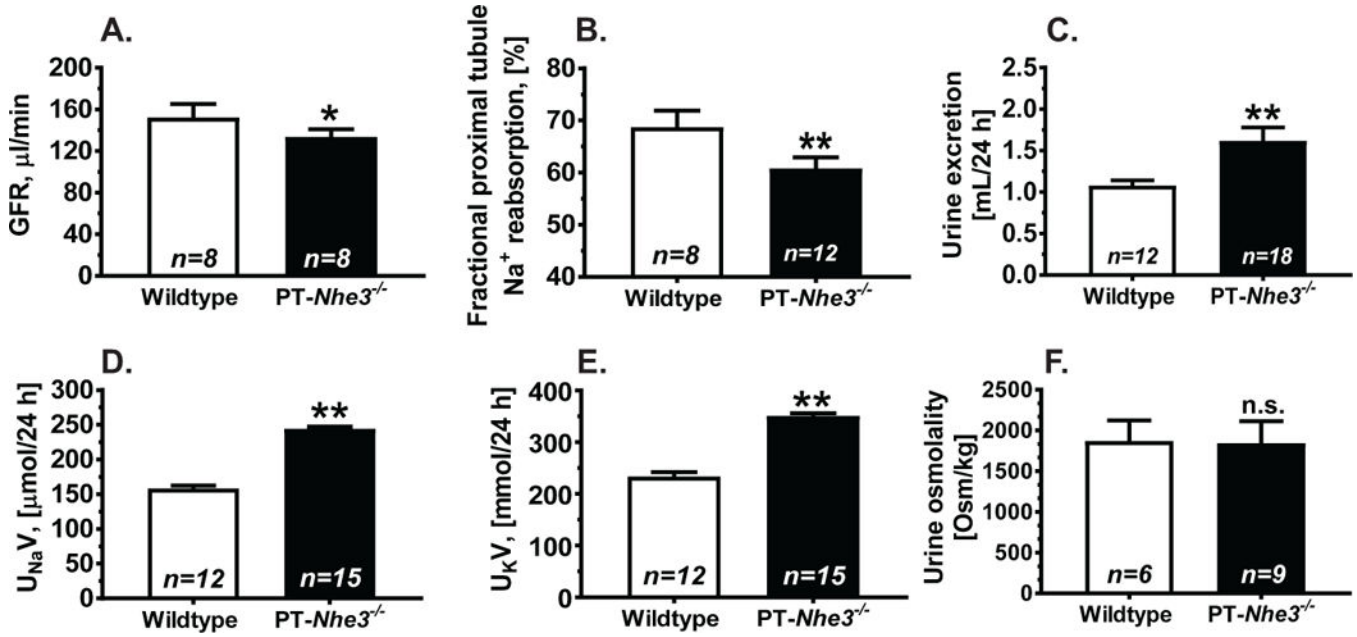

Figure 2.

Systematic comparisons of plasma pH, Na^+, bicarbonate, hematocrit, food intake, glomerular filtration rate (GFR), fractional proximal tubule Na^+ reabsorption, and 24 h urinary Na^+ excretion between male wild-type and PT-*Nhe3*$^{-/-}$ mice. Please note that GFR was lower whereas 24 h urine and urinary Na^+, K^+ excretion were higher in PT-*Nhe3*$^{-/-}$ mice, without significantly altering plasma pH, Na^+, bicarbonate, hematocrit, and urine osmolality (**S9**). *p<0.05, or **p<0.01 vs. wild-type.

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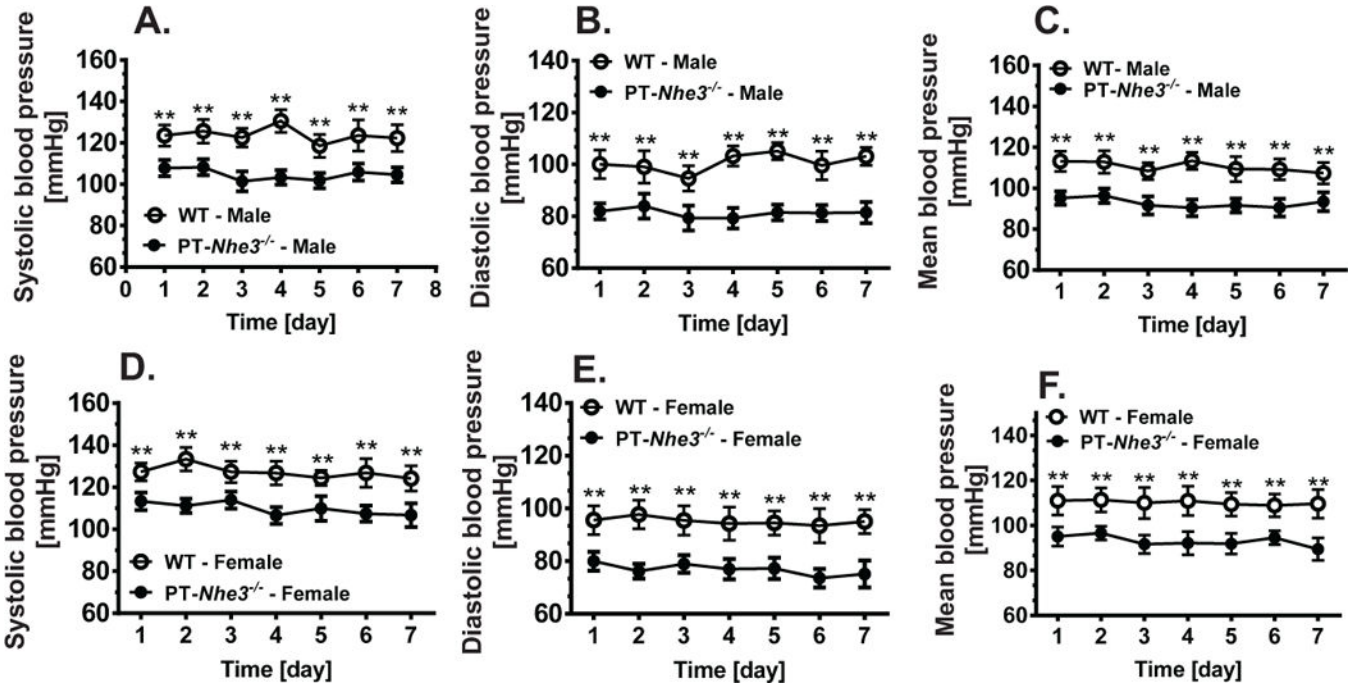

Figure 3.

Basal systolic, diastolic and mean arterial blood pressure in adult male and female wild-type and PT-*Nhe3*$^{-/-}$ mice, as measured continuously for 7 days using the direct implanted telemetry technique. Please note that basal systolic, diastolic and mean arterial blood pressure were consistently about 12 ± 3 mmHg lower in male and female PT-*Nhe3*$^{-/-}$ than wild-type mice (p<0.01).

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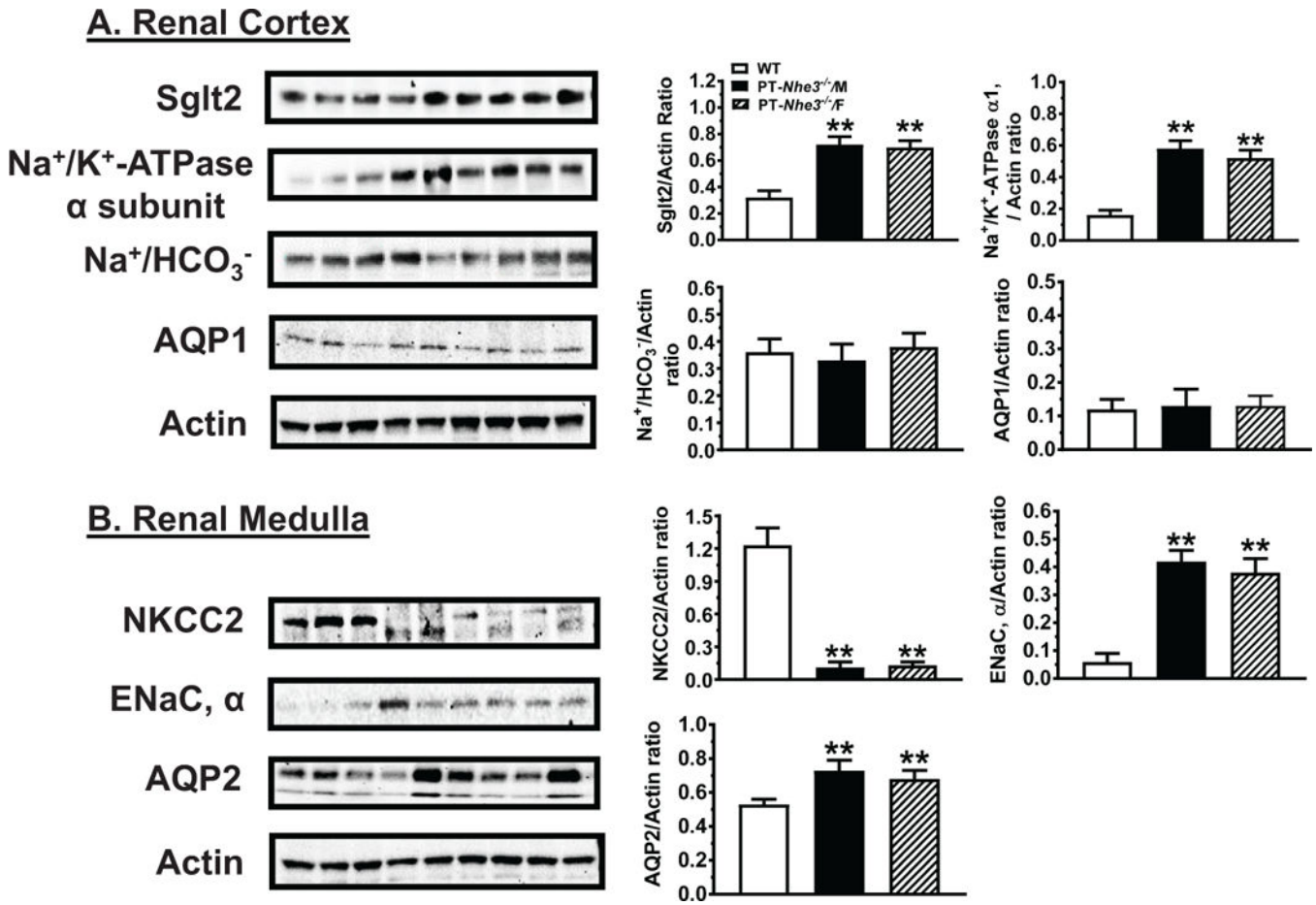

Figure 4.

Western blot analysis of key renal cortical and medullary Na^+ transporter and water channel protein expression in male and female wild-type and PT-*Nhe3*$^{-/-}$ mice. Please note that proximal tubule-specific deletion of NHE3 upregulates cortical sglt2, Na^+/K^+-ATPase α and renal medullary ENaC α and AQP2 protein expression in male and female PT-*Nhe3*$^{-/-}$ mice ($p<0.01$ vs. wild-type).

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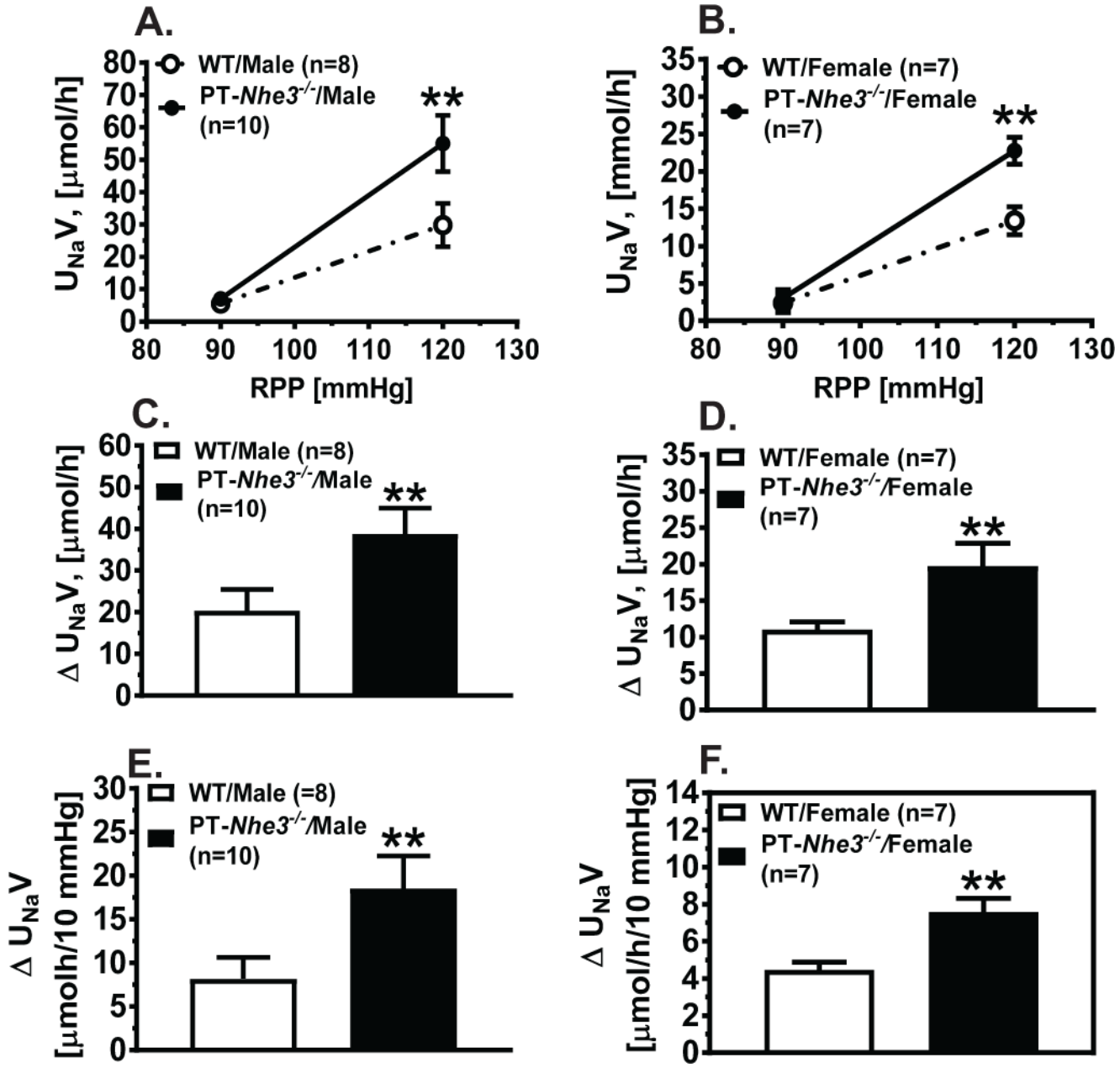

Figure 5.

The pressure-natriuresis response in male and female wild-type and PT-*Nhe3*$^{-/-}$ mice. In response to an increase of ~30 mmHg in renal perfusion pressure, the pressure-natriureiss response increased ~5-fold in male wild-type mice, whereas the response increased ~8-fold in male PT-*Nhe3*$^{-/-}$ mice (**p<0.01). Both net urinary Na^+ excretion and as a response to 10 mmHg blood pressure increase were significantly higher in male PT-*Nhe3*$^{-/-}$ mice (p<0.01; n=10) than in wild-type mice (p<0.01; n=8). A similar pattern of the pressure-natriuresis response was also observed in female PT-*Nhe3*$^{-/-}$ mice, although it was smaller than male PT-*Nhe3*$^{-/-}$ mice.

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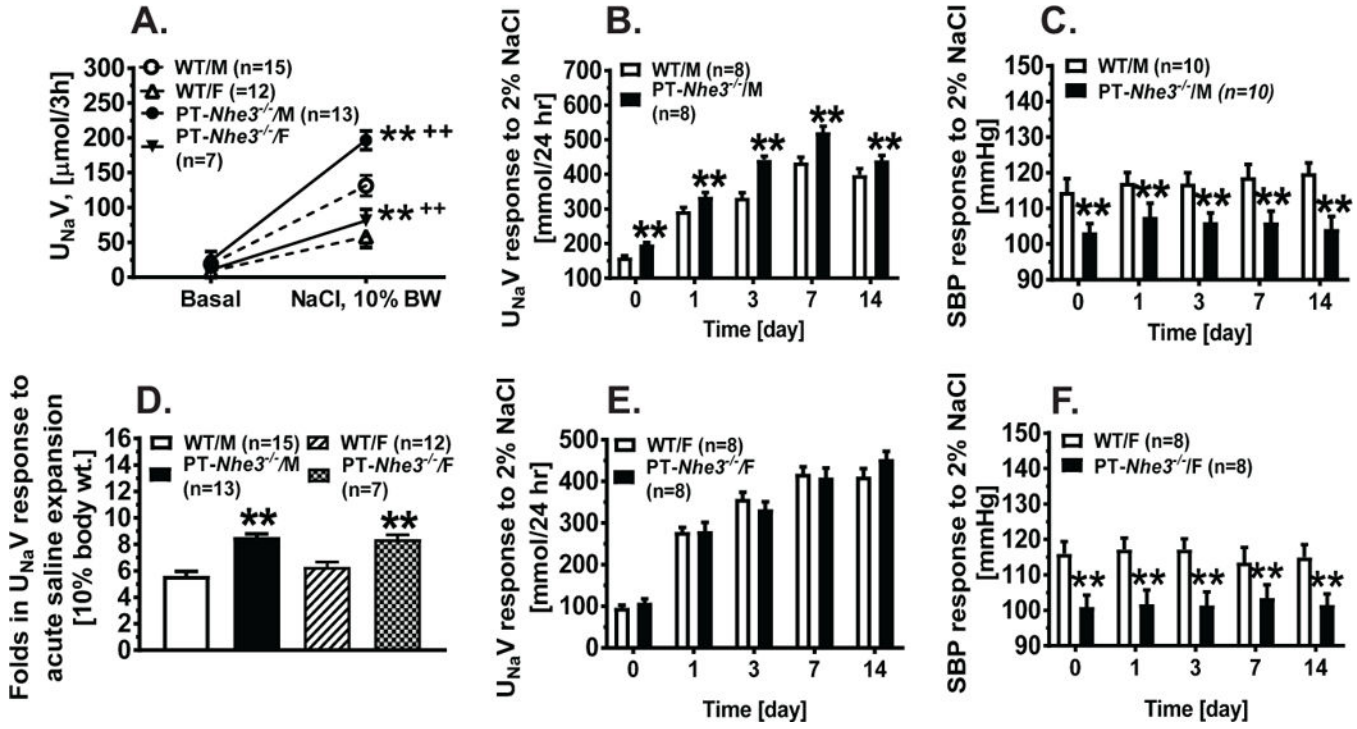

Figure 6.

The natriuretic responses to acute and long-term saline expansion in male and female wild-type and PT-*Nhe3*$^{-/-}$ mice. In response to 10% acute saline expansion, urinary Na^+ excretion increased 6-fold in wild-type mice, and ~8-fold in male PT-*Nhe3*$^{-/-}$ mice (p<0.01 vs. wild-type). In response to 2% high salt diet for 2 weeks, the natriureticesponse was significantly greater in male, but not female PT-*Nhe3*$^{-/-}$ mice (p<0.01 vs. wild-type). Systolic blood pressure was unchanhed in both male and female wild-type and PT-*Nhe3*$^{-/-}$ mice throughout 2-week 2% NaCl treatment. This suggests that thedeletion of NHE3 selectively from the proximal tubules of the kidney augments the natriuretic response to long-term high salt diet.