Blood Pressure Maintenance in NHE3-Deficient Mice with Transgenic Expression of NHE3 in Small Intestine

William T. Noonan¹, Alison L. Woo², Michelle L. Nieman³, Vikram Prasad², Patrick J. Schultheis⁴, Gary E. Shull², and John N. Lorenz³

Departments of ¹Genome Science, ²Molecular Genetics, Biochemistry, and Microbiology, and ³Molecular and Cellular Physiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267; and ⁴Department of Biological Sciences, Northern Kentucky University, Highland Heights, Kentucky 41099

Running Title: Blood pressure in GI-rescued NHE3 knockout

Correspondence should be addressed to:

John N. Lorenz
Department of Molecular and Cellular Physiology
University of Cincinnati College of Medicine
231 Albert Sabin Way
Cincinnati, OH  45267-0576

Telephone (513) 558-3097 FAX (513) 558-5738
email: john.lorenz@ucmail.uc.edu

Copyright © 2004 by the American Physiological Society.
ABSTRACT

NHE3 Na⁺/H⁺ exchanger knockout (Nhe3−/−) mice have severe absorptive deficits in the kidney proximal tubule and intestinal tract. The resulting hypovolemia has confounded efforts to carefully evaluate the specific effects of NHE3-deficiency on kidney function. Development of mice with transgenic expression of NHE3 in the small intestine (tgNhe3−/−) has allowed us to analyze the role of renal NHE3 in overall maintenance of blood pressure, pressure natriuresis, and autoregulation of both glomerular filtration rate (GFR) and renal blood flow (RBF).

Ambulatory blood pressure, measured by telemetry, was lower in tgNhe3−/− mice than in wild-type controls (tgNhe3+/+) when the mice were maintained on a normal NaCl diet, but was normalized when they were provided with a high NaCl intake. Furthermore, administration of the AT1-receptor blocker losartan showed that circulating angiotensin II plays a major role in maintaining blood pressure in tgNhe3−/− mice fed normal NaCl, but not in those receiving high NaCl. Clearance studies revealed a blunted pressure-natriuresis response in tgNhe3−/− mice at lower blood pressures but a robust response at higher blood pressures. Autoregulation of GFR and RBF was normal in tgNhe3−/− mice. These results show that dietary NaCl loading normalizes blood pressure in awake tgNhe3−/− mice and that alterations in NHE3 activity are not essential for normal autoregulation of GFR and RBF. Furthermore, the data strongly support the hypothesis that NHE3 plays an important role in the diuretic and natriuretic responses to increases in blood pressure, but also show that mechanisms not involving NHE3 mediate pressure natriuresis in the higher range of blood pressures studied.

KEY WORDS: telemetry, pressure natriuresis, autoregulation, Slc9a3, renal blood flow, glomerular filtration rate.
INTRODUCTION

Isoform 3 of the Na\(^+\)/H\(^+\) exchanger (NHE3) is heavily expressed in the kidney proximal tubule and in the small intestines, and is responsible for a large percentage of bulk sodium and fluid transport in both organs. Accordingly, we have previously shown that NHE3 knockout mice (Nhe3\(^{-/-}\)) mice have severe absorptive defects in both the kidney and intestine, and therefore, they are unable to fully compensate for increased renal salt loss by increasing intestinal absorption of dietary salt. In fact, attempts to improve fluid volume status of Nhe3\(^{-/-}\) mice by feeding them a high NaCl diet resulted in swelling of the intestine, severe hypovolemia and death. These mice therefore exhibit characteristics of chronic and severe volume-depletion, including low blood pressure, high levels of renin mRNA in kidney, and high serum aldosterone (15). Interestingly, we showed in a subsequent study that, although proximal tubular reabsorption was markedly reduced in Nhe3\(^{-/-}\) mice, fluid delivery to the distal tubule was normal due to reductions in glomerular filtration rate (GFR) (7). This normalization of distal fluid delivery in Nhe3\(^{-/-}\) mice raised the possibility that the proximal tubule absorptive defect itself, in the absence of an intestinal defect, might not lead to significant renal salt wasting. Thus, the co-existing intestinal absorptive defect and chronic diarrhea in Nhe3\(^{-/-}\) mice represented a major confounding factor in determining the specific effects of the loss of NHE3 in the kidney on renal Na\(^+\) conservation, GFR, and extracellular fluid-volume homeostasis.

To assess these issues, we developed a transgenic mouse in which NHE3 is expressed in the small intestine via the intestinal fatty acid binding protein (IFABP) promoter and crossed it with Nhe3\(^{-/-}\) mice (16). Both dietary Na\(^+\)-restriction and Na\(^+\)-loading were better tolerated in transgenic Nhe3\(^{-/-}\) (tgNhe3\(^{-/-}\)) mice than in non-transgenic Nhe3\(^{-/-}\) mice (ntgNhe3\(^{-/-}\)), and salt loading led to a substantial reduction of aldosterone levels in tgNhe3\(^{-/-}\) mice, indicating a partial
correction of the extracellular fluid-volume deficit. In spite of their improved ability to absorb dietary salt, tg$\text{Nhe}3^{-/-}$ mice remained mildly hypotensive under anesthesia and had reduced GFR, suggesting that alterations in renal hemodynamics may be a regulated compensatory response to the proximal tubule transport deficit. Finally, since there is recent evidence suggesting that NHE3 may play a central role in the phenomenon of pressure-natriuresis as well as tubuloglomerular feedback (TGF) regulation of renal blood flow and GFR (8, 10, 17, 18), it is possible that animals lacking proximal tubular NHE3 may demonstrate appreciable derangements in both pressure natriuresis and autoregulatory behavior.

The goals of the present study were two-fold. First, we sought to evaluate, using radiotelemetry, whether specific renal NHE3 deficiency results in altered ambulatory blood pressure. We found that when provided a sufficiently high salt diet, the tg$\text{Nhe}3^{-/-}$ mice had the same blood pressure as wild type mice. Secondly, we examined pressure natriuresis and autoregulatory behavior in tg$\text{Nhe}3^{-/-}$ mice in order to determine whether alterations in NHE3-dependent Na$^+$ transport in the proximal tubule are an absolute requirement for these homeostatic responses. The data showed that while autoregulation of renal blood flow and GFR was largely normal in tg$\text{Nhe}3^{-/-}$ mice, the pressure-natriuresis relationship was blunted, especially in the lower range of blood pressure.

**METHODS**

*Animals.* Transgenic mice in which the intestinal fatty acid binding protein (IFABP) promoter was used to drive expression of NHE3 in the small intestine (16) were obtained from an established colony. These IFABP/NHE3 transgenic mice were backcrossed for two to three generations with $\text{Nhe}3^{+/-}$ mice, also from an established colony (15), to produce $\text{Nhe}3^{+/+}$ and $\text{Nhe}3^{-/-}$ mice harboring the IFABP/NHE3 transgene (tg$\text{Nhe}3^{+/+}$ and tg$\text{Nhe}3^{-/-}$). $\text{Nhe}3^{-/-}$ mice
without the IFABP/NHE3 transgene (ntgNhe3−/−) were also used in these experiments.

Genotyping was performed by PCR of tail biopsies as described (16). Experiments were performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee at the University of Cincinnati.

**Telemetric blood pressure recording.** Experiments were performed in 8-18 week old tgNhe3+/+ (n=12, 8 male, 4 female), tgNhe3−/− (n=8, 4 male 4 female), and ntgNhe3−/− mice (n=7, 5 male, 2 female). Continuous ambulatory blood pressure recordings were made using the TA11PA-C20 pressure transmitter (Data Sciences International, St. Paul, MN). Each transmitter was calibrated prior to implantation by applying known pressure steps to the transducer and recording the output. The output signal from each Model RPC-1 receiver was channeled to a R11CPA Pressure Analog Adaptor, and recorded and analyzed using a PowerLab system (ADInstruments, Colorado Springs, CO). Transmitters were implanted using carotid artery cannulation and subcutaneous transmitter placement under isoflurane anesthesia as described (1). Following implantation, mice were returned to their home cages for monitoring, and were allowed to recover for 5 days prior to data collection. The mice were synchronized to a 12:12 hour light-dark schedule with lights on at 7:00 A.M, and maintained on a normal salt diet (1% NaCl). Blood pressure was monitored in one-minute episodes at five-minute intervals. To evaluate the blood pressure on different levels of salt intake, drinking water was replaced with 0.9% NaCl. To evaluate the contribution of the renin-angiotensin system to the maintenance of blood pressure in the different groups of animals, pressure was recorded continuously for one hour prior to and following i.p. injection of losartan at 10 µg/gram of bodyweight (gBW). We found that this dose of losartan completely blocked the hypertensive response produced by i.p. injection of 40 ng/gBW angiotensin II (unpublished observation).
**Western analysis of AT1 receptor expression.** Age- and gender-matched tg\(Nhe3^{+/+}\) and \(tgNhe3^{-/-}\) were maintained on either a normal (1% NaCl) or a high salt (5% NaCl) diet for 5 days with free access to food and water (\(n=4\) for each of the 4 groups). Mice were then euthanized by CO\(_2\) asphyxiation, the kidneys removed and quick frozen in liquid N\(_2\), and stored at -80ºC. The kidneys were homogenized using a Polytron in chilled homogenization buffer (in mM: 20 HEPES (pH 7.5), 10 KCl, 1 EDTA, 2 DTT, 0.2% NP-40, 10% glycerol, protease inhibitors phosphatase inhibitors). Proteins were allowed to solubilize over ice for 2 hrs after which aliquots were quick-frozen in liquid N\(_2\). Protein concentration was estimated by the Bradford method (Pierce). Proteins (4-8 µg/lane) were resolved by reducing, discontinuous SDS-polyacrylamide gel electrophoresis (8.5%) and transferred to a nitrocellulose membrane by established procedures. Membranes were blocked in 5% Non-fat dry milk and probed with the rabbit anti-Angiotensin II type 1 Receptor antibody (RDI-ANGIO2RXabr, Research Diagnostics, N.J.) at a dilution of 1 µg/ml. Bound primary antibody was revealed by HRP-conjugated anti-rabbit IgG antibody (KPL, Inc. MD) and chemiluminescence was developed using the SuperSignal West Pico Reagent (Pierce). Bands were detected on X-ray films and quantitated by densitometry, using β-actin as a loading control.

**Autoregulation and pressure natriuresis.** Transgenic \(Nhe3^{+/+}\) (\(n=8\), 5 male, 3 female) and \(Nhe3^{-/-}\) mice (\(n=6\), 3 male, 3 female) aged 9-15 weeks were provided saline to drink for 3 days prior to experimentation in order to assure that both groups were relatively volume and salt replete (16). On the day of the experiment, mice were anesthetized with ketamine (50 µg/g body wt) and inactin (100 µg/g body weight) and surgically instrumented for clearance measurements as described previously (7). In addition, the left renal artery was exposed via a flank incision, and fitted with a Transonics flow probe (Model 0.5SB, Transonics Systems, Ithaca, NY). Ligatures
were also loosely placed around the mesenteric and celiac arteries, and the abdominal aorta below the renal arteries to permit step increases in renal perfusion pressure according to the method described by Roman et al. (13). Immediately following surgery, mice were given an 8 µl/gBW bolus PBS containing 0.33 % FITC-inulin, 2% BSA, 1µg/ml norepinephrine, 0.5 ng/ml arginine vasopressin, 0.2 mg/ml hydrocortisone, and 0.2 µg/ml aldosterone (13). This was followed by a maintenance infusion of the same solution at 0.45 µl/min/g body weight, and a 30 min equilibration period. Renal function was then determined over three consecutive 20-minute clearance periods in which the renal perfusion pressure was elevated over baseline (period 1) in a step-wise fashion by first tying the mesenteric and celiac ligatures (period 2), and then the aortic ligature (period 3). After tying each set of ligatures, blood pressure was allowed to re-stabilize for 5 minutes before beginning the next clearance period. At the midpoint of each urine collection, an arterial blood sample (60 µl) was obtained for determination of plasma FITC-inulin (6) and electrolyte concentration, and donor blood was administered to replace the lost volume after each sample was obtained. Plasma and urine Na\(^+\) and K\(^+\) concentrations were determined using a Corning 480 Flame Photometer (Medfield, MA). Hemodynamic data was recorded and analyzed using a PowerLab system.

Statistics. Statistical analysis was performed by analysis of variance (ANOVA), using a single factor or mixed factorial design with repeated measures on the second factor. Individual contrasts were used to compare individual group means when needed. Analysis of covariance (ANCOVA) was used to analyze pressure-natriuresis and autoregulation, with pressure as the covariate. Data are presented as means ± SEM, and statistical significance was regarded as \( P < 0.05 \).
RESULTS

Twenty four-hour blood pressure traces from \textit{tgNhe3}^{+/+} (n=12), \textit{tgNhe3}^{-/-} (n=8), and \textit{ntgNhe3}^{-/-} (n=7) mice maintained on a normal salt diet are shown in the top panel of Fig. 1. Confirming our earlier observations (15), \textit{ntgNhe3}^{-/-} mice had markedly lower blood pressure throughout the day compared to wild type mice. In contrast, \textit{tgNhe3}^{-/-} mice had significantly higher blood pressure compared to those without the transgene (P<0.05). However, blood pressure in these transgenic NHE3 knockouts was still lower than that of wild type mice. In several experiments, we also compared blood pressure in wild type mice with and without the transgene, and found no difference (data not shown). When provided isotonic saline to drink in order to elevate NaCl intake, blood pressure in \textit{tgNhe3}^{-/-} mice was completely normalized compared to \textit{tgNhe3}^{+/+} controls (Fig. 1, lower panel), and \textit{ntgNhe3}^{-/-} mice did not survive. Interestingly, we observed that \textit{tgNhe3}^{-/-} mice consumed almost twice as much saline as did \textit{tgNhe3}^{+/+} mice (12.8 ± 1.4 ml/day vs. 6.5 ± 0.4 ml/ day), indicating that they have a substantially greater salt appetite. Since our previous studies have indicated that intestinal absorption of NaCl by \textit{tgNhe3}^{-/-} mice is ~50% as efficient as that of \textit{tgNhe3}^{+/+} mice, we can surmise that actual gastrointestinal Na$^+$ absorption was reasonably similar between the two groups when provided saline to drink (16).

We have previously reported that blood pressure was 20-30 mmHg lower in anesthetized \textit{tgNhe3}^{-/-} mice compared to wild type controls regardless of diet (16). To evaluate the extent to which the renin-angiotensin system contributes to the normalization of blood pressure in the conscious \textit{tgNhe3}^{-/-} mice, we measured the blood pressure response to intraperitoneal injection of the AT1 receptor blocker losartan during conditions of normal salt intake and high salt intake. As shown in Fig. 2, blood pressure decreased modestly in wild type animals and the response
was not influenced by diet. In ntgNhe3⁻/⁻ mice on normal salt intake, blood pressure decreased dramatically in response to losartan suggesting that their blood pressure was largely dependent on the activity of the renin-angiotensin system. These mice did not tolerate the switch to high salt intake. tgNhe3⁻/⁻ mice also showed a large blood pressure decrement in response to losartan when they were on a normal salt intake, but when provided with high salt, which was well tolerated, the blood pressure response to losartan was markedly blunted such that the observed decrease was similar to that seen in wild type animals.

These data, and those from our previous study (16), suggest that components of the renin-angiotensin-aldosterone axis are largely normalized in tgNhe3⁻/⁻ mice with high NaCl intake. However, to determine whether there may be differences in AT1 receptor levels between the two genotypes, we used Western analysis to evaluate the level of AT1 receptor expression in tgNhe3⁻/⁻ and tgNhe3⁺/⁺ mice fed normal and high NaCl diets. The results show that AT1 receptor expression is not different between the two genotypes regardless of diet (Fig. 3). The four sets of samples (2 genotypes x 2 diets) were also analyzed together on a single blot (not shown). Densitometric measurements comparing AT1 receptor to β-actin indicated that high NaCl intake caused a small increase in receptor expression, consistent with a previous report (14). The increase in AT1 receptor expression was similar in both genotypes.

We next evaluated the pressure-natriuresis relationship in NHE3 knockout and wild type mice harboring the transgene. Mice of both genotypes were given saline to drink for 3 days prior to the acute experiment in order to improve the volume status of the knockout. Baseline values are given in Table 1. Since body weight and kidney weight were not different between the two groups of mice, data were not normalized. Plasma Na⁺ concentration was not different between the two groups, but plasma K⁺ was slightly elevated in the tgNhe3⁻/⁻ compared to
tgNhe3+/+ mice. Despite our current finding that conscious blood pressure was similar between the groups when drinking saline, we observed that anesthetized blood pressure was lower in tgNhe3−/− mice compared to tgNhe3+/+ mice (79±3 vs 94±4 mmHg), and this is consistent with our previous report (16). This finding perhaps suggests that in spite of nearly complete normalization of the renin-angiotensin-aldosterone axis, there may be some degree of volume depletion remaining in the tgNhe3−/− mice, that is normally compensated by the sympathetic nervous system and revealed by anesthesia. The lower anesthetized pressure in the tgNhe3−/− was associated with lower values of urine flow rate and Na+ excretion, as well as GFR and filtered load of Na+ and K+ (Table 1). Pressure-natriuresis/diuresis relationships are shown in Fig. 4. In response to step-increases in perfusion pressure, urine flow increased in both groups of animals (P<0.01), and while the magnitude of the increase appeared to be slightly blunted in tgNhe3−/− mice, the difference was not significant (interaction P = 0.10). Absolute and fractional urinary Na+ excretion also increased in response to step-increases in perfusion pressure in both groups (P<0.01), but the magnitude of the increase was significantly blunted in the tgNhe3−/− mice compared to tgNhe3+/+ (interaction P < 0.05). With both absolute and fractional changes, it was apparent that the pressure-natriuresis relationships were markedly blunted at lower pressure, and much less effected at the higher pressures.

We also measured renal blood flow (RBF) and GFR in these experiments to evaluate the autoregulatory efficiency in kidneys lacking NHE3, and results are illustrated in Fig. 5. Consistent with our previous findings (16), GFR was significantly reduced in the tgNhe3−/− mice under baseline conditions and at increased levels of perfusion pressure (P < 0.04). In response to step-increases in perfusion pressure, GFR (top panel) did not increase in either group indicating efficient autoregulation of filtration rate in the absence of renal expression of NHE3. Likewise,
renal blood flow was autoregulated well in both groups of animals, since RBF did not change in response to changes in renal perfusion pressure in either tgNhe3^{−/−} or tgNhe3^{+/+} mice. Renal blood flow and renal vascular resistance (RVR) (calculated as the ratio of blood pressure to blood flow) were not different between the two groups over the entire range of perfusion pressure (Fig. 5, middle and lower panel). RVR increased comparably in both tgNhe3^{−/−} and tgNhe3^{+/+} mice in response to increases in perfusion pressure, reflecting the efficient autoregulation of blood flow in both groups of animals.

**DISCUSSION**

In our initial report of NHE3 knockout mice with transgenic expression of NHE3 in the small intestine, we found that tgNhe3^{−/−} mice demonstrated a markedly improved phenotype compared to non-transgenic Nhe3^{+/−} mice, especially with regards to their ability to tolerate increases and decreases in dietary salt intake (16). Unlike their non-rescued counterparts, which died when placed on a high salt diet, tgNhe3^{−/−} mice fed a 5% NaCl diet increased their renal Na⁺ excretion (which approximates intestinal absorption) to a level almost 20 times greater than when they are fed a 1% NaCl diet, and ~2.5 times greater than in tgNhe3^{+/+} mice fed a 1% NaCl diet. Since these changes in salt intake were associated with marked decreases in both serum aldosterone and renin mRNA expression in the kidney, as well as increases in anesthetized blood pressure, it was concluded that NaCl loading can dramatically improve the volume status of GI-rescued NHE3-deficient mice. However, since blood pressure measurements had been performed under anesthesia, it remained unclear whether provision of excess NaCl could fully normalize blood pressure in awake mice. The experiments reported here using telemetry are important, therefore, in that they demonstrate that tgNhe3^{−/−} mice can normalize their ambulatory
blood pressure when provided with sufficient dietary salt. On a normal NaCl diet, \( \text{tg}Nhe3^{-/-}\) mice had a higher blood pressure than the non-transgenic knockouts, but their pressure was still lower than in wild type mice. However, when provided with saline to drink, \( \text{tg}Nhe3^{-/-}\) mice drank twice as much as their wild-type counterparts, resulting in awake blood pressures that were not different from that of the wild type. Together, these data suggest that actual gastrointestinal absorption of NaCl is substantially normalized in GI-rescued NHE3 knockouts when drinking isotonic saline.

In the present study, we sought to further explore the potential mechanisms involved in maintaining blood pressure in the rescued NHE3 knockout mice. In order to evaluate to what extent circulating angiotensin II contributes to blood pressure maintenance in the \( \text{tg}Nhe3^{-/-}\) mice, we treated mice acutely with intraperitoneal injections of the AT1-receptor blocker losartan. In wild type mice on either normal or high salt intake (saline drinking), AT1-receptor blockade resulted in only mild decreases in pressure (10-12 mmHg), indicating that circulating levels of angiotensin II are normally low in these mice and contribute minimally to the maintenance of blood pressure. On the other hand, blood pressure in knockout mice (both rescued and non-rescued) on a normal salt diet showed a large dependence on circulating angiotensin II, since losartan markedly lowered blood pressure by 25-30 mmHg. By contrast in \( \text{tg}Nhe3^{-/-}\) mice dinking saline, losartan administration caused a much smaller decrement in blood pressure that was not significantly different from that in \( \text{tg}Nhe3^{+/+}\) mice. Western blot analyses showed that AT1 receptor protein expression is the same in \( \text{tg}Nhe3^{+/+}\) and \( \text{tg}Nhe3^{-/-}\) mice maintained on either a 1% or a 5% NaCl diet, thereby demonstrating that the differences in response to losartan were not due to differences in receptor levels. These data indicate that circulating angiotensin II
plays an important role in maintaining blood pressure in \( \text{tg}N\text{he}3^{-/-} \) mice on a normal salt diet, but much less so on high salt, where its contribution is similar to that occurring in wild-type controls.

Despite the dramatic improvement seen in \( \text{tg}N\text{he}3^{-/-} \) mice with saline loading, the observation that anesthesia nonetheless resulted in a greater blood pressure decrease in \( \text{tg}N\text{he}3^{-/-} \) than in \( \text{tg}N\text{he}3^{+/+} \) mice suggests that the absence of NHE3 specifically in the kidney resulted in a reduced renal set point for \( \text{Na}^+ \) and fluid homeostasis. In the second part of this study, therefore, we examined the effects of renal NHE3-deficiency on the pressure-natriuresis relationship in saline loaded mice. In \( \text{tg}N\text{he}3^{+/+} \) mice, stepwise increases in renal perfusion pressure produced essentially monotonic increases in urine flow and \( \text{Na}^+ \) excretion, characteristic of conventional pressure diuresis and natriuresis relationships. By contrast, in the \( \text{tg}N\text{he}3^{-/-} \) mice, the first step-increase in perfusion pressure, from approximately 80 to 115 mmHg, resulted in remarkably small increases in fluid and \( \text{Na}^+ \) excretion. Despite this blunted responses at lower pressures, the diuretic and natriuretic responses at higher pressures (i.e., above 120 mmHg) in \( \text{tg}N\text{he}3^{-/-} \) mice were robust, with a slight rightward shift and parallel slope with respect to wild type controls. These data therefore suggest that NHE3-dependent processes in the proximal tubule play an important role in mediating pressure-induced increases in \( \text{Na}^+ \) excretion at lower perfusion pressures, but not at higher pressures.

Since it has been shown that either reduced salt intake or increased aldosterone can shift the pressure natriuresis relationship to the right (3, 14), it might be argued that the rightward shift observed in \( \text{tg}N\text{he}3^{-/-} \) mice could be due to lower salt absorption in the intestine and/or elevated aldosterone. Since the exact level of gastrointestinal NaCl absorption is not known in these mice, and since our earlier studies showed that aldosterone levels are markedly reduced by high NaCl intake in the \( \text{tg}N\text{he}3^{-/-} \) mice, but not completely normalized, we cannot completely rule
out either possibility. Nevertheless, it is clear that saline loaded \( \text{tgNhe3}^{-/-} \) mice are not in a state of dietary salt depletion. Importantly, in studies by other investigators in which reduced salt consumption caused a rightward shift in the response curves, the slope of the pressure-natriuresis relationship was unaltered by dietary NaCl intake and no blunting was observed at lower blood pressures. Similarly, increased aldosterone shifts the curve to the right but does not blunt the response at lower pressures (3, 14). Therefore, while it is possible that a slightly lower level of salt absorption and/or increased serum aldosterone concentration may contribute to the rightward shift in \( \text{tgNhe3}^{-/-} \) mice, it is unlikely that they could account for the blunted slopes of the pressure-natriuresis relationship at lower pressures.

An important corollary to the blunted pressure-natriuresis response at lower pressure is the observation that at higher blood pressures the response is robust and similar to those of the wild type mice. Since this increase in urinary fluid and Na\(^+\) excretion occurs in the absence of a change in GFR or filtered Na\(^+\) load, these data clearly indicate that there are tubular Na\(^+\) transport mechanisms, not involving NHE3, that participate in pressure diuresis and natriuresis at the higher blood pressures. This is consistent with earlier studies indicating that distal mechanisms of Na\(^+\) absorption were involved in the pressure natriuresis mechanism at higher blood pressures (9, 11), but not at lower blood pressures, where more proximal Na\(^+\) transport mechanisms appear to predominate.

Regarding proximal mechanisms of pressure natriuresis, in a compelling series of studies, McDonough and coworkers demonstrated that acute changes in renal perfusion pressure result in a rapid and reversible re-distribution of NHE3 from the apical plasma membrane to endosomal stores that is concomitant with a decrease in proximal tubule reabsorption (2, 10, 17, 19). These responses were found to be partially dependent on an intact and responsive renin-angiotensin
system, and it was surmised that the responses might be induced by the sustained elevation in macula densa NaCl delivery during acute hypertension (4). These investigators also showed that both acute and chronic hypertension cause the redistribution of NHE3 and they proposed, therefore, that this response is an integral part of pressure natriuresis (18). If this hypothesis were correct, then one would predict that NHE3 null mice, in which internalization of NHE3 could not serve as a natriuretic mechanism, would exhibit impaired pressure natriuresis and that the degree of impairment would indicate the relative importance of NHE3 internalization in the overall mechanism. Thus, the blunted pressure natriuresis response in $\text{tg}N\text{he3}^{-/-}$ mice are fully consistent with this hypothesis and indicate that the proposed mechanism operates at lower perfusion pressures.

In addition to a role in mediating the pressure natriuresis response, it has also been suggested that pressure-induced alterations in proximal tubule transport via NHE3 play an important role in the autoregulation of renal blood flow and glomerular filtration rate. In the studies discussed above, McDonough and Marsh and colleagues postulated that acute hypertension decreases proximal tubule reabsorption and that the resulting increase in salt and fluid delivery to Henle’s loop is sensed at the macula densa, thereby providing the error signal to increase afferent arteriole resistance via the tubuloglomerular feedback (TGF) mechanism (2, 5, 10, 12, 17). According to this hypothesis, then, changes in NHE3 transport are intimately involved in the TGF-dependent component of autoregulation. The RBF and GFR data presented here in NHE3-deficient mice, however, would argue against such a role, since autoregulatory behavior is well preserved (see Figure 5). In earlier micropuncture studies using the non-transgenic NHE3 knockout model, we showed that although proximal reabsorption is markedly reduced in NHE3 knockouts, the late proximal flow rate is normal due to compensatory changes
in single nephron GFR. In these animals then, acute increases in pressure could not further
decrease proximal reabsorption via NHE3 and the postulated error signal would therefore be
absent. Nonetheless, we found that blood flow and filtration rate are remarkably stable over a
wide range of perfusion pressures in NHE3-deficient kidneys. Given these results, it must be
concluded that, in response to acute hypertension, changes in proximal tubule Na⁺/H⁺ exchange
are not required to provide the necessary error signal for initiation of the TGF-dependent
autoregulatory component. Alternatively, it is possible that under these circumstances, TGF
does not play a critical role in mediating the autoregulatory response.

In summary, we have demonstrated that NHE3-deficient mice expressing the
IFABP/NHE3 transgene in the small intestine largely rescues the volume and pressure deficits
seen in the non-transgenic NHE3 knockout, and that dietary NaCl loading normalizes
cardiovascular function to a great extent in these mice. This mouse therefore represents an
important model for studies regarding the specific role of NHE3 in overall renal function, since
such studies can be accomplished without the confounding influences associated with severe
volume depletion and decreased blood pressure. In this regard, our data provide strong support
for the hypothesis that NHE3 plays an important role in the diuretic and natriuretic responses to
acute increases in blood pressure, and further, that the effects of pressure-induced reductions in
NHE3 activity are likely limited to the lower range of blood pressures. Our data do not support
the concept that modulation of NHE3 activity is a critical factor in the autoregulation of renal
blood flow and GFR.
ACKNOWLEDGMENT

This work was supported by National Institutes of Health Grants DK-57552 and DK-50594.
REFERENCES


**FIGURE LEGENDS**

**Figure 1.** Twenty-four hour telemetric measurements of arterial blood pressure in wild type mice expressing the IFABP/NHE3 transgene (tg\(Nhe3^{+/+}\)), nontransgenic NHE3 knockout mice (ntg\(Nhe3^{-/-}\)), and NHE3 knockout mice expressing IFABP/NHE3 (tg\(Nhe3^{-/-}\)). Pressure was recorded for 1-minute episodes at 5-minute intervals. Values represent the average after at least 5 days on each diet. Top: mice were fed a normal 1% NaCl diet and given water to drink; differences are as shown. Bottom: mice were fed a normal 1% NaCl diet and given saline to drink; values were not different between tg\(Nhe3^{+/+}\) and tg\(Nhe3^{-/-}\). High NaCl intake was not tolerated by the ntg\(Nhe3^{-/-}\) mice.

**Figure 2.** Change in blood pressure in response to i.p. injection of losartan (10µg/gBW) in wild type mice and in NHE3 knockout mice with and without the IFABP/NHE3 transgene (see figure 1 for abbreviations). The effects of AT1-receptor blockade were determined while mice were on a normal NaCl intake (water to drink) and on a high NaCl intake (saline to drink). Measurements were made 30 minutes after injection. The number of animals/group is the same as in figure 1. All responses to losartan were statistically significant (p<0.05). * P < 0.05 compared to tgNHE3\(^{+/+}\) mice on the same diet. † P < 0.01 compared to corresponding genotype on a normal NaCl diet.

**Figure 3.** Western blot analysis of AT1 receptor expression in kidneys from tg\(Nhe3^{+/+}\) and tg\(Nhe3^{-/-}\) mice fed 1% NaCl (top row) and 5% NaCl diets (bottom row). Adjacent lanes were loaded with either 4 or 8 µg of total protein, as indicated, and membranes probed with rabbit anti-AT1 receptor antibody. Membranes were also probed with an anti-actin antibody (Sigma) as a loading control for densitometric analysis, which revealed no significant differences in the expression of AT1 receptor between tg\(Nhe3^{+/+}\) and tg\(Nhe3^{-/-}\) on either diet.

**Figure 4.** Pressure diuresis and natriuresis in tg\(Nhe3^{+/+}\) and tg\(Nhe3^{-/-}\) mice. Changes in urine flow rate (top), Na\(^+\) excretion (middle), and fractional Na\(^+\) excretion were measured at baseline and after step increases in renal perfusion pressure produced by sequential tying of ligatures.
around 1) the celiac and mesenteric arteries, and 2) the abdominal aorta below the renal arteries. P values for the group effects and interaction are as indicated.

**Figure 5.** Glomerular filtration rate (GFR), renal blood flow (RBF) and renal vascular resistance (RVR) in tg*Nhe3*\(^{+/+}\) and tg*Nhe3*\(^{-/-}\) mice at baseline and after step increases in pressure (see figure 4 legend). P values for the group effects and interaction are as indicated.
Table 1. Body and kidney weight, plasma concentration and filtered load of Na\(^+\) and K\(^+\).

<table>
<thead>
<tr>
<th></th>
<th>tgNhe3(^{+/+})((n = 8))</th>
<th>tgNhe3(^{+/-})((n = 6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days)</td>
<td>79 ± 4</td>
<td>85 ± 5</td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td>31 ± 1</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>Kidney Weight (g)</td>
<td>0.398 ± 0.027</td>
<td>0.386 ± 0.027</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>47 ± 1</td>
<td>47 ± 2</td>
</tr>
<tr>
<td>P(_{Na}) (mEq/L)</td>
<td>164 ± 2</td>
<td>162 ± 1</td>
</tr>
<tr>
<td>P(_{K}) (mEq/L)</td>
<td>4.7 ± 0.1</td>
<td>5.3 ± 0.2*</td>
</tr>
<tr>
<td>GFR (µl/min)</td>
<td>585 ± 41</td>
<td>397 ± 29</td>
</tr>
<tr>
<td>FL(_{Na}) (µEq/min)</td>
<td>96.1 ± 7.1</td>
<td>64.1 ± 4.8*</td>
</tr>
<tr>
<td>FL(_{K}) (µEq/min)</td>
<td>2.72 ± 0.18</td>
<td>2.08 ± 0.15*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. P\(_{Na}\), P\(_{K}\), plasma Na\(^+\) and K\(^+\) concentration; GFR, glomerular filtration rate; FL\(_{Na}\), FL\(_{K}\), filtered load of Na\(^-\) and K\(^-\).
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5