Responses of proximal tubule sodium transporters to acute injury-induced hypertension

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Submitted 10 April 2002; accepted in final form 10 September 2002

Yang, Li E., Patrick K. K. Leong, Shaohua Ye, Vito M. Campese, and Alicia A. McDonough. Responses of proximal tubule sodium transporters to acute injury-induced hypertension. Am J Physiol Renal Physiol 284: F313–F322, 2003.—Renal injury-induced by phenol injection activates renal sympathetic afferent pathways, increases norepinephrine release from the posterior hypothalamus, activates renal efferent pathways, and provokes a rapid and persistent hypertension. This study aimed to determine whether phenol injury provoked a redistribution of proximal Na+/H+ exchangers from internal stores to the apical cell surface mediated by sympathetic activation, a response that could contribute to generation or maintenance of hypertension. Anesthetized rats were cannulated for arterial blood pressure tracing and saline infusion and then 50 μl 10% phenol or saline was injected into one renal cortex (n = 7 each). Fifty minutes after injection, kidneys were removed and renal cortex membranes from injected kidneys were fractionated on sorbitol gradients and pooled into three windows (WI–WIII) that contained enriched apical brush border (WI); mixed apical, intermicrovillar cleft and dense apical tubules (WII); and intracellular membranes (WIII). Na+ transporter distributions were determined by immunoblot and expressed as percentage of total in gradient. Acute phenol injury increased blood pressure 20–30 mmHg and led to redistribution of Na+/H+ exchanger type 3 (NHE3) out of WII (from 22.79 ± 4.75 to 10.79 ± 2.01% of total) to WI (13.07 ± 1.97 to 27.15 ± 4.08%), Na+/H- cotransporter 2 out of WII (68.72 ± 1.95 to 59.76 ± 2.21%) into WI (9.5 ± 1.62 to 87.15 ± 1.45%), and a similar realignment of dipeptidyl-peptidase IV immunoreactivity and alkaline phosphatase activity to WI. Renal denervation before the renal injury prevents the SNS activation and the subsequent rise in blood pressure (44). Thus renal injury activates renal afferent pathways, increases norepinephrine release from the posterior hypothalamus, activates renal efferent pathways, and raises blood pressure. Interestingly, the hypertension becomes established and persists long after the site of injury recedes to a microscopic scar and is reversed by removal of the injured kidney or renal denervation (43). The cellular and molecular bases for the hypertension are not understood. One plausible mechanism is that the renal efferent sympathetic nerve activity may stimulate Na+ and volume reabsorption and contribute to hypertension.

There is a dynamic relationship between blood pressure and renal Na+ reabsorption that is responsible for the blood pressure set point. A decrease in Na+ transport can be a homeostatic compensation to elevated blood pressure; an experimental increase in blood pressure acutely decreases proximal tubule Na+ reabsorption, which both increases NaCl at the macula densa, a transforming growth factor signal to normalize renal blood flow (RBF) and glomerular filtration rate (GFR), and causes a pressure natriuresis that reduces extracellular volume, which counteracts the hypertension (8, 13, 14). In contrast, inappropriately elevated Na+ transport, due to either excess production of an antinatriuretic substance (e.g., aldosterone) (40) or an activated Na+ transporter (Liddle’s syndrome) (33), can generate and maintain hypertension. Both responses can occur together; if renal Na+ reabsorption is elevated, then blood pressure increases and induces a pressure-natriuresis variant known as “escape” in which Na+ reabsorption is depressed at sites along the nephron not primarily affected by the excess hormone or Na+ transport, a response that balances Na+ excretion to Na+ intake.

A RAT MODEL OF NEUROGENIC hypertension provoked by a renal injury was recently developed by Campese and colleagues (10, 43, 44). In this model, injection of 50 μl 10% phenol causes a rapid elevation of blood pressure, which is preceded by a rise in norepinephrine secretion from the posterior hypothalamus and an increase in renal sympathetic nervous system (SNS) activity. Renal denervation before the renal injury prevents the SNS activation and the subsequent rise in blood pressure (44). Thus renal injury activates renal afferent pathways, increases norepinephrine release from the posterior hypothalamus, activates renal efferent pathways, and raises blood pressure. Interestingly, the hypertension becomes established and persists long after the site of injury recedes to a microscopic scar and is reversed by removal of the injured kidney or renal denervation (43). The cellular and molecular bases for the hypertension are not understood. One plausible mechanism is that the renal efferent sympathetic nerve activity may stimulate Na+ and volume reabsorption and contribute to hypertension.

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Our laboratory previously investigated the molecular mechanisms responsible for the decrease in proximal tubule Na reabsorption during an experimental 5-min increase in blood pressure and discovered that there is a rapid retraction of Na+/H+ exchangers [Na+/H+ exchanger type 3 (NHE3)] and Na+/P, cotransporters (NaPi) from the apical brush border to intermucronillar cleft and subapical endosomes, demonstrated by both subcellular fractionation and confocal microscopy, as well as a decrease in basolateral Na+/K+ ATPase activity (42, 47). Motivated by these findings, we aimed to test the hypothesis that acute phenol injury, via activation of sympathetic efferents, increases proximal tubule Na+ transport by recruiting Na+ transporters from subapical pools to the brush border, contributing to the genesis of the hypertension.

There is support for this hypothesis from in vitro studies on adrenergic regulation of proximal Na+ transporters (3, 4, 34). The results of this study support the hypothesis that acute phenol injury provokes redistribution of proximal tubule NHE3 from subapical endosomal pools to apical brush border and that the response is mediated by sympathetic stimulation and may contribute to the generation and persistence of phenol injury-induced hypertension.

**METHODS**

**Animal preparation.** Experiments were performed on male Sprague-Dawley rats (300–350 g body wt) that had free access to food and water before the experiment. Rats were anesthetized intramuscularly with ketamine (Fort Dodge Laboratories) and xylazine (1:1, vol/vol; Miles) and then anesthetized intramuscularly with ketamine (Fort Dodge Sprague-Dawley rats (300–350 g body wt) that had free access to food and water before the experiment. Rats were anesthetized intramuscularly with ketamine (Fort Dodge Laboratories) and xylazine (1:1, vol/vol; Miles) and then placed on a thermostatically controlled warming table to maintain body temperature at 37°C. Each animal was cooled in situ by flushing with cold PBS and then excised. The renal cortex was dissected (injured area, ~2-mm diameter, was cut off and discarded), homogenized with a Tissuemizer (Tekmar Instruments) in isolation buffer (5% sorbitol, 0.5 mM disodium EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 9 μg/ml aprotinin, and 5 mM histidine-imidazole buffer (pH 7.5)), and centrifuged at 2,000 g for 10 min. Twelve fractions were collected from the top, diluted with isolation buffer, pelleted by centrifugation (250,000 g for 1.5 h), resuspended in 1 ml isolation buffer, and stored at −80°C, pending assay.

**Immunoblot analysis and antibodies.** A constant volume of sample from each gradient fraction was denatured in SDSPAGE sample buffer for 30 min at 37°C, resolved on the same 7.5% SDS polyacrylamide gels according to Laemmli (20), and transferred to polyvinylidene difluoride membranes (Millipore Immobilon-P). Selected samples on each blot were run at one-half the volume or protein to assure that the sample was in the linear range of detection, and multiple exposures of autoradiograms were analyzed to ensure that signals were within the linear range of the film. All blots, except for NaPi2 analysis, were detected with the ECL enhanced chemiluminescence kit (Amersham Pharmacia Biotech), and autoradiographic signals were quantified with a Bio-Rad imaging densitometer with Molecular Analyst software. For NHE3 detection, blots were probed with polyclonal NHE3-C00 (42) at 1:1,000 dilution. Polyclonal antisera to dipeptidyl-peptidase IV (DPPIV) were generously provided by M. Farquhar (University of California at San Diego). For detection of Na-Pi cotransporter 2 (NaPi2), blots were incubated with polyclonal anti-NaPi2 antibody generated by Biber and Murer (University of Zürich, Zurich, Switzerland), then with Alexa 680-labeled goat-anti-rabbit secondary antibody, and then detected with an Odyssey Infrared Imaging System (LI-COR, Lincoln, NB). Each gradient and immunoblot was from a separate rat, i.e., no pooling of samples. Results were quantitated by normalizing the density in each fraction to the total sum density from all the fractions and expressed as the percentage of total immunoreactivity within each sample. These fraction-specific results were pooled into three “windows” to simplify analyses. As previously reported (42, 46), fractions 3–5 [window I (WI)] are enriched in apical brush-border markers alkaline phosphatase, DPPIV, and NHE3; fractions 6–8 (WI) contain most of the intermucronillar cleft-marker megalin (5) as well as apical markers; and fractions 9–11 (WIII) are enriched in the endosomal marker rab 5a and the lysosomal marker β-hexosaminidase as well as megalin.

**Indirect immunofluorescence.** To compare the NHE3 distribution in two models of acute hypertension (arterial constriction vs. phenol injury), blood pressure was raised to the same level in each model (20–30 mmHg above baseline as described above), and then one kidney from each animal was analyzed by confocal microscopy. The kidney contralateral to the phenol or saline injection was analyzed in this series to measured by flameless atomic absorption spectrophotometry (Perkin-Elmer 5100PC) as described previously (47).
focus on the effects of the similarly elevated blood pressure in the two models and to eliminate any potential effect of the phenol per se. Kidneys were fixed at 30–50 min in situ by placing the isolated kidney in a small plexiglass cup and bathing it in fixative (2% paraformaldehyde, 75 mM lysine, and 10 mM Na-periodate, pH 7.4 (PLP)) for 20 min. The kidneys were then removed, cut in half on a midsagittal plane, and postfixed in PLP for another 4–6 h. The fixed tissue was rinsed twice with PBS, cryoprotected by incubation overnight in 30% sucrose in PBS, embedded in TissueTek OCT Compound (Sakura Finetek, Torrance, CA), and frozen in liquid nitrogen. Cryosections (5 μM) were cut with a Microm Heidelberg ultramicrotome, transferred to Fisher Superfrost Plus-charged glass slides, and air dried. For immunofluorescence labeling, the sections were rehydrated in PBS for 10 min, followed by 10-min washing with 50 mM NH₄Cl in PBS and then with 1% SDS in PBS for 4 min for antigen retrieval (9). SDS was removed by two 5-min washes in PBS, and then sections were blocked with 1% BSA in PBS to reduce background. Double labeling was performed by incubating with polyclonal antiserum NHE3-C00 and monoclonal antibody against villin (Immunotech, Chicago, IL), incubating with polyclonal antiserum NHE3-C00 and monoclonal antibody against villin (Immunotech, Chicago, IL), both at a dilution of 1:100 in 1% BSA in PBS for 1.5 h at room temperature. After being washed for 5 min three times in PBS, the sections were incubated with a mixture of FITC-conjugated goat-anti-rabbit (Cappel Research Products, Durham, NC) and Alexa 568-conjugated goat-anti-mouse (Molecular Probes, Eugene, OR) secondary antibodies diluted 1:100 in 1% BSA in PBS for 1 h, washed three times with PBS, mounted in Prolong Antifade (Molecular Probes), and dried overnight at room temperature. Slides were viewed with a Nikon PCM quantitative measuring high-performance confocal system equipped with filters for both FITC and tetramethylrhodamine isothiocyanate fluorescence attached to a Nikon TE300 Quantum upright microscope. Images were acquired with Simple PCI C-Imaging hardware and Quantitative Measuring software and processed with Adobe PhotoDeluxe (Adobe Systems, Mountain View, CA).

Other assays. Na⁺-K⁺-ATPase activity was measured by the K⁺-dependent p-nitrophenyl phosphatase reaction (30). Standard assay was used for alkaline phosphatase activity (29). Protein concentrations were measured with a bicinechonic acid assay kit (Pierce Technology, Iselin, NJ).

Quantitation and statistical analysis. Experiments were conducted and analyzed in a pairwise fashion, that is, one phenol injected and one saline injected in 1 day. Data are expressed as means ± SE. Two-way ANOVA was applied to determine whether there was a significant effect of treatment on the overall subcellular distribution pattern. If a significance was established (P < 0.05), the location of the difference in the pattern was assessed by two-tailed Student’s t-test for paired samples, and the differences were regarded significant at P < 0.05.

RESULTS

Physiological responses to acute phenol injury. Ye et al. (43, 44) reported that acute renal injury by an intrarenal injection of 50 μl 10% phenol caused an immediate and permanent elevation in blood pressure. In this study, we verify that the effect of phenol injury could be reproduced in our laboratory with our animal preparation. Arterial blood pressure increased immediately after 50 μl of 10% phenol injection, fluctuated somewhat over the next 30 min, and then was maintained at 20–30 mmHg above baseline, an average increase from 110 ± 2.8 to 134 ± 2.4 mmHg (Fig. 1). These arterial pressures are within the autoregulatory range for GFR and RBF (13). Sham injection of 50 μl of saline caused a brief transient fluctuation in blood pressure that returned to baseline within 30 min (Fig. 1A, bottom).

For each animal, urine was collected for 20 min before the phenol or saline injection and for 20 min

| Fig. 1. Physiological responses to acute phenol injury. A: representative blood pressure traces before and after phenol (top) or saline (bottom) injection. B: summary of systolic arterial pressure before and 30 min after injection, recorded from carotid artery. BP, blood pressure. Values are means ± SE; n = 7 for each group. *P < 0.05 vs. basal period by paired Student's t-test. C: urine output collected over 20-min intervals before and after 30-min phenol injection, expressed as urine weight in μg/min. D: endogenous lithium clearance (CLi) calculated as urinary lithium concentration × urine output × plasma lithium concentration⁻¹ (μl/min). ○, Data from individual rats; ●, mean values; n = 9 for each group.
between 30 and 50 min after the injection. Figure 1, C and D, summarizes the effects of intrarenal phenol injection on urine output and endogenous lithium clearance ($C_{Li}$), a measure of volume flow from the proximal tubule (39). The mean values were elevated slightly, although not statistically significantly: mean urine output was 10.3 ± 3.3 before and 18.5 ± 3.0 µg/min 30–50 min after phenol injection and $C_{Li}$ was 60.65 ± 11.1 before and 95.12 ± 12.8 µl/min after phenol injection. In comparison, acutely raising blood pressure by arterial constriction causes a marked pressure diuresis with consistent three- to fourfold increases in $C_{Li}$ and urinary output (42, 47).

NHE3 redistribution in response to phenol injury. Na$^+$/H$^+$ exchanger is the major route for apical Na$^+$ entry across the proximal tubule, and NHE3 is responsible for virtually all the Na$^+$/H$^+$ exchange activity in this region (2, 6). Our previous studies using confocal microscopy established that acute hypertension due to arterial constriction is associated with a rapid redistribution of NHE3 immunoreactivity out of the brush-border microvilli to intermicrovillar cleft and endosomal membranes (42); the same conclusion was reached when analyzed by subcellular fractionation, NHE3 redistributed from lower-density membranes enriched in markers of apical microvilli to higher-density membranes enriched in markers of intermicrovillar cleft, dense apical tubules, and endosomes (42, 47). These same techniques were applied to the kidneys after acute phenol injection to test the hypothesis that NHE3 would redistribute into the brush border in response to the increased sympathetic efferent activity; the alternative hypothesis was that the NHE3 would retract out of the brush border in response to the hypertension per se, identical to the response to arterial constriction. Representative immunoblots of NHE3 in 12 gradient fractions from saline- and phenol-injected kidneys are shown in Fig. 2A. Samples were pooled into three windows to simplify analyses, as described in METHODS. After phenol injection, a significant fraction of NHE3 (expressed as percentage of total in the gradient) shifts out of WIII into WI (Fig. 2B): NHE3 in WI increased from 13.07 ± 1.97 to 27.15 ± 4.08% of total, NHE3 in WI remained unchanged (63.7 ± 3.53 to 61.45 ± 2.15%), and NHE3 in WIII decreased from 22.79 ± 4.75 to 10.79 ± 2.01% after phenol injury ($P < 0.05$ vs. saline, assessed by ANOVA and followed by paired Student’s $t$-test). These results support the hypothesis that phenol injury provokes a redistribution of NHE3 from intracellular and intermicrovillar membrane pools to the apical microvilli, a response that could favor increased salt and water transport and the generation and persistence of hypertension after phenol injury.

NHE3 distribution in denervation vs. denervation followed by phenol injection. Renal injury activates renal afferent pathways, increases norepinephrine release from the posterior hypothalamus, activates renal efferent pathways, and raises blood pressure. To test the hypothesis that the activation of renal efferent pathways provokes the apical redistribution of NHE3, we performed two sets of experiments in which the left kidney was denervated before phenol injection. As previously shown by Ye et al. (44), we found that denervation of the left renal nerves before the phenol injection into the left kidney prevented the increase in blood pressure (not shown). As shown in Fig. 2, denervation per se did not affect the distribution pattern of NHE3 compared with the saline-injected kidney; however, denervation before phenol injection did prevent the redistribution of NHE3 to WI (Fig. 2C). This provides direct evidence that the NHE3 redistribution to apical brush border in phenol injury (Fig. 2, A and B) is associated with the activated renal efferent pathways and not to the local effects of the injected phenol.
**NHE3 distribution in phenol injury vs. arterial constriction-induced hypertension.** The change in the density gradient distribution pattern of NHE3 after phenol injection-induced hypertension is reciprocal to that seen with acute hypertension due to arterial constriction, providing evidence for bidirectional regulation of NHE3 between apical microvilli and intermicrovillar/subapical membranes. In our previous study of the arterial constriction model of acute hypertension, blood pressure was raised 50–70 mmHg (42, 47), whereas after phenol injection blood pressure increased 20–30 mmHg, which allows for the possibility that the distinct responses were a function of the different levels of hypertension. NHE3 redistribution responses were re-examined by immunocytochemistry after blood pressure was increased by 20–30 mmHg in both the phenol-induced and the arterial-constriction models of hypertension. Thirty minutes after saline or phenol injection or arterial constriction, kidneys were fixed in situ for another 20 min, as described in METHODS. Double labeling was performed on cryosections harvested from each of the three groups. NHE3 was imaged with polyclonal NHE3-C00 with FITC-conjugated anti-rabbit secondary, and villin, the actin bundling protein localized to the microvilli, was imaged with monoclonal anti-villin with Alexa 568-conjugated anti-mouse secondary antibody. We previously demonstrated that the subcellular distribution of villin was unaltered during acute hypertension, so it provides a consistent background marker for the microvilli as the NHE3 redistributes (47). In saline-injected rats, the staining of NHE3 is restricted to the brush border, as evidenced by colocalization with staining for villin (Fig. 3, top). When blood pressure is increased 20–30 mmHg by arterial constriction hypertension (Fig. 3, bottom), NHE3 moves out of the apical brush-border microvilli, leaving the tops of the villi stained red with anti-villin, NHE3 is detected in the intermicrovillar cleft region where it coincides with villin (Fig. 3, yellow, arrow), and NHE3 appears in subapical vesicles where it does not overlap villin (Fig. 3, green, arrowhead). This response is indistinguishable from that observed when blood pressure is increased 50–60 mmHg by arterial constriction. After phenol injection associated with a 20–30 mmHg hypertension, NHE3 remained colocalized with villin staining (Fig. 3, middle). This technique was not sensitive enough to detect the low levels of subapical NHE3 at baseline blood pressure, thus no redistribution was evident, but the finding provides strong visual confirmation that NHE3 was not inter-

![Fig. 3. Effect of acute hypertension induced by 50 μl 10% phenol injection vs. arterial constriction on NHE3 subcellular distribution. NHE3 was detected in renal proximal tubules from rats with saline injection (top), phenol injection with an accompanying 20- to 30-mmHg increase in blood pressure (middle), or arterial constriction with an accompanying 20- to 30-mmHg increase in blood pressure (bottom). Kidneys were fixed in situ with 2% paraformaldehyde, 75 mM lysine, and 10 mM Na-periodate, pH 7.4, during the last 20 min. Sections were double labeled with polyclonal anti-rabbit NHE3-C00 antibody and then FITC-conjugated anti-rabbit secondary antibody, and with monoclonal anti-villin antibody and then Alexa 568-conjugated anti-mouse secondary antibody. NHE3 staining is green (arrowhead), villin staining is red, and overlapping NHE3 and villin appears yellow (arrow). Bar = 10 μm.](http://ajprenal.physiology.org/)

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nalized during phenol injury-induced hypertension. This comparison demonstrates that although blood pressure was increased 20–30 mmHg over baseline in both animal models, NHE3 is not internalized from apical membranes during phenol injury-induced hypertension as it is during arterial constriction hypertension.

**Effect of acute phenol injury on the distributions of other apical membrane proteins.** To determine the specificity of the redistribution of NHE3 during acute phenol injury, the distribution of NaPi2 was investigated. NaPi2 is mainly expressed in the proximal tubule apical brush border. NaPi2 has been shown to move from apical membranes to intracellular membranes in response to acute hypertension due to arterial constriction and during a high-Pi diet (24, 47). Figure 4A shows representative immunoblots of NaPi2 and summarized data expressed as percentage of total in the three windows from saline- vs. phenol-injected rats. In phenol-injected rats, NaPi2 increased in WI from 9.5 ± 1.62 to 18.7 ± 1.45% of total, NaPi2 decreased in WII from 68.72 ± 1.95 to 59.76 ± 2.21% of total, and there was no change in WIII (P < 0.05 vs. saline, assessed by ANOVA and followed by paired Student’s t-test). The results indicate that NaPi2 may, similarly to NHE3, move from intermicrovillar cleft region and/or dense apical tubules (WII) to apical membranes (WI) during phenol injury-induced hypertension.

The distribution of two additional classic apical membrane proteins also changed in a manner similar to that of NHE3 and NaPi2 after phenol injury. DPPIV, detected by immunoblot at 105 kDa, increased in WI from 19.21 ± 1.59 to 28.58 ± 2.36% of total during phenol injury (Fig. 4B). Alkaline phosphatase activity (percentage of total) increased in WI from 15.96 ± 0.87 to 28.6 ± 2.36%, whereas activity in WII decreased from 60.47 ± 1.76 to 52.73 ± 1.73% and WIII decreased from 19.89 ± 1.5 to 15.14 ± 1.3%, evidence for redistribution from intracellular and intermicrovillar cleft regions to the brush border along with NHE3 and NaPi2 (Fig. 5A). For both the DPPIV and the alkaline phosphatase shifts, P < 0.05 vs. saline, as assessed by ANOVA and followed by paired Student’s t-test.

**Effect of acute phenol injury on basolateral membrane Na⁺-K⁺-ATPase.** We previously determined that renal cortical Na⁺-K⁺-ATPase was inhibited in response to arterial constriction hypertension (26), so we investigated whether Na⁺-K⁺-ATPase activity was altered during phenol injury-induced hypertension. The subcellular distribution of Na⁺-K⁺-ATPase activity in renal cortex (Fig. 5B) indicates a peak of Na⁺-K⁺-ATPase activity in WI that did not change in activity or distribution pattern after acute phenol injury.

**DISCUSSION**

Abundant evidence has accumulated supporting a role of increased SNS activity in renal injury-induced hypertension. Campese and colleagues (10, 43, 44) developed a rat model of renal injury-induced hypertension. Injection of the ANG II AT₁ receptor agonist caused an injection of 50 μl 10% phenol to the lower pole of one kidney, which leads to an immediate elevation of norepinephrine secretion from posterior hypothalamus and a rise in blood pressure. They also measured an increase in plasma norepinephrine level and renal sympathetic activity recorded directly from renal nerves after phenol injection. The injury per se is not sufficient to provoke the hypertension, because renal denervation before phenol injection prevents the increase in blood pressure and norepinephrine secretion from posterior hypothalamus (44). Recent results from the same laboratory indicate that afferent impulses triggered by the phenol injury activate ANG II formation in brain nuclei, which inhibits IL-1β and nNOS, leading to activation of central and peripheral SNS activity. Injection of the ANG II AT₁ receptor agonist...
Fig. 5. Alkaline phosphatase and \(\text{Na}^+\)-\(\text{K}^+\)-\text{ATPase} activity distributions in phenol injury-induced hypertension. A: alkaline phosphatase, expressed as percentage of total activity in all three windows, redistributes to low-density membranes in response to 50 \(\mu\)l 10% phenol injection. \(B\): \(\text{Na}^+\)-\(\text{K}^+\)-\text{ATPase} activity, assessed as \(\text{K}^+\)-dependent \(p\)-nitrophenyl phosphatase did not change in phenol injection-induced hypertension. Values are means ± SE; \(n = 7\) in each group. *\(P < 0.05\) vs. saline, assessed by ANOVA and followed by paired Student’s \(t\)-test.

Acute phenol injection provoked a rapid redistribution of NHE3 and NaPi2 to the apical microvilli, a response that may contribute to the generation or maintenance of hypertension in this model.

Formal analysis of the molecular mechanisms of SNS activation of \(\text{Na}^+\) transport in vivo have not been previously conducted or reported; however, there are a number of studies on the in vitro effects of norepinephrine on \(\text{Na}^+\)/\(\text{H}^+\) exchangers. The proximal tubule contains numerous \(\alpha\)-adrenergic receptor binding sites (19, 37, 38) and conditions that increase receptor number or postreceptor components responsible for \(\alpha_{1A}\)- and \(\alpha_{1H}\)-adrenergic-mediated \(\text{Na}^+\) reabsorption in proximal tubule can contribute to \(\text{Na}^+\) retention and elevated blood pressure (19). Liu and colleagues (22, 23) found that proximal nephron \(\text{Na}^+\)/\(\text{H}^+\) exchange transport activity is increased by activation of \(\alpha_{1A}\) and \(\alpha_{1H}\)-adrenergic receptor subtypes facilitated by the MAPK signaling pathway. The findings of the present study provide the first direct in vivo evidence that SNS stimulation activates apical \(\text{Na}^+\)/\(\text{H}^+\) exchange activity by increasing NHE3 transporters at the apical surface, a response that can be prevented by renal denervation. The increase in apical NHE3 may be accomplished by decreasing endocytosis or increasing exocytosis from intracellular stores, or both. Because the subapical endosomal pool of NHE3 is difficult to detect at baseline blood pressures by confocal microscopy, it is quite plausible that the apical NHE3 and NaPi2 accumulate due to depressed endocytosis.

In vivo studies have provided mixed results regarding adrenergic regulation of basolateral \(\text{Na}^+\)-\(\text{K}^+\)-ATPase in isolated and cultured renal proximal tubules. Norepinephrine was found to increase solute and fluid reabsorption and \(\text{Na}^+\)-\(\text{K}^+\)-\text{ATPase} activity (1, 3) in isolated proximal tubules, an effect that may be driven by increased apical \(\text{Na}^+\) entry (34). In another system, adrenergic stimulation drives exocytic insertion of \(\text{Na}^+\) pumps into the plasma membrane of cultured lung cells (4). However, Holtback et al. (18) concluded that norepinephrine has no net effect on proximal tubule \(\text{Na}^+\)-\(\text{K}^+\)-\text{ATPase} activity because of combined activation of \(\alpha\)- and \(\beta\)-adrenergic receptors in the proximal tubule. In the present study, there was no effect of phenol injection-induced hypertension on \(\text{Na}^+\)-\(\text{K}^+\)-\text{ATPase} activity in isolated membranes resolved on sorbitol gradients. There was a slight tendency to redistribute \(\text{Na}^+\)-\(\text{K}^+\)-\text{ATPase} activity from WII to the W1 basolateral membrane peak, which may reflect insertion of \(\text{Na}^+\)-\(\text{K}^+\)-\text{ATPase} from intracellular vesicles to the plasma membranes, similar to the effect of adrenergic agents in cultured lung cells (4), but establishing this will require an improved fractionation strategy or pharmacological manipulation of \(\alpha\)- vs. \(\beta\)-adrenergic receptor levels. In contrast, our laboratory previously determined that an acute increase in blood pressure by artery constriction inhibits proximal tubule \(\text{Na}^+\)-\(\text{K}^+\)-\text{ATPase} activity measured in isolated membranes (25, 47). It is possible that the opposing forces of SNS stimulation to increase activity and hypertension to decrease activity may counteract each other, an issue that could be tested by SNS stimulation in a setting of servocontrolled blood pressure. Can \(\text{Na}^+\) reabsorption
increase without a change in Na⁺-K⁺-ATPase activity? Na⁺-K⁺-ATPase activity in vivo in the tubule is very high to start with (28) and may indeed be activated by increased Na⁺ availability or adrenergic stimulation while not detected enzymatically in a V<sub>max</sub> assay in broken membranes in vitro.

Regarding the phenol injury itself, the hypertension occurs in the face of minimal renal injury (1- to 2-mm wide) (43). In this study, before cell fractionation, a small area surrounding the injection site was removed, and areas beyond this injection area appeared normal when examined by electron microscopy (43). Activation of the SNS reflex does not appear to be the result of an unspecified renal injury as lesions of the same dimensions caused by burning, administration of alkali (NaOH), acid (HCl), or methanol do not raise blood pressure (not shown). In addition, phenol injections to other sites, including the spleen and peritoneum, change blood pressure only transiently over <10 min (44).

We have previously reported that an acute increase in arterial blood pressure brought about by arterial constriction provokes a rapid decrease in proximal tubule Na⁺ reabsorption, the key to increasing NaCl at the macula densa, which activates tubuloglomerular feedback to autoregulate RBF and GFR (13, 14, 47). The accompanying natriuresis and diuresis are a compensatory response to restore elevated blood pressure toward normal levels (16). Using C<sub>Li</sub> as a measure of volume flow out of the proximal tubule, we have consistently observed that arterial constriction hypertension causes a three- to fourfold increase in C<sub>Li</sub> and urine output (42, 47). The role of proximal tubule Na⁺ reabsorption, estimated by C<sub>Li</sub> in the generation or maintenance of hypertension has been assessed by different investigators with differing results (12). This is likely because elevated Na⁺ and volume reabsorption may be causal to some varieties of hypertension, whereas decreased Na⁺ and volume reabsorption may be compensatory in other varieties of hypertension. An increase in proximal reabsorption has been demonstrated in unanesthetized spontaneously hypertensive rats (7) and in hypertensive patients (11, 27, 35). A decrease in proximal Na⁺ transport in hypertensive patients (32, 41) or no change (17, 36) has been reported as well. In the present study, hypertension caused by acute phenol injury did not cause a significant change in either urinary output or C<sub>Li</sub>, which could be explained by the combined effect of SNS activation to increase Na⁺ and volume reabsorption and elevated blood pressure, which would evoke a compensatory decrease in Na⁺ and volume reabsorption. Whether there is a significant increase in proximal tubule volume reabsorption associated with the Na⁺ transporter redistribution during phenol-induced hypertension is an important question that remains to be answered. In any case, the hypertension evoked by the acute phenol injection does not lead to the homeostatic compensation known as “pressure natriuresis” or to the three- to fourfold increase in C<sub>Li</sub> observed during arterial constriction hypertension, evidence for a significant resetting of the renal function curve (16).

The distinct responses to hypertension provoked by phenol injection vs. arterial constriction persist at the molecular level. Our laboratory previously studied the molecular mechanisms responsible for the decrease in proximal tubule Na⁺ reabsorption during arterial constriction hypertension and discovered there is an accompanying retraction of transport competent NHE3 as well as NaPi2 from apical brush border to intermicrovillar cleft and subapical membrane pools and inhibition of basolateral Na⁺-K⁺-ATPase (25, 42, 47). In contrast, during acute phenol injury-induced hypertension, NHE3 and NaPi2 redistribute in the opposite direction from subapical endosomes to the apical brush border. The two hypertension-dependent patterns appear remarkably distinct when analyzed by confocal microscopy. Although blood pressure was raised to the same extent (~20–30 mmHg) in both models, proximal tubule NHE3 was internalized in the arterial constriction hypertension, presumably a compensatory response, but not in the phenol injection-induced hypertension. By confocal analysis, there is no obvious shift of NHE3 from subapical stores to the microvilli. There are a variety of interpretations that could be resolved by electron microscopy analysis; NHE3 may redistribute laterally from stores in the intermicrovillar cleft to the microvilli (decreased traffic to the cleft or increased traffic to the microvilli) or redistribution from the endosomal pools (decreased internalization or increased exocytosis) is below the level of detection by immunofluorescence, because the concentration of NHE3 is too low at baseline. Perhaps SNS activation without hypertension would lead to a more obvious redistribution of NHE3 to the brush border.

Girardi et al. (15) have studied proteins that associate with proximal tubule NHE3 by coimmunoprecipitation and discovered that DPPIV associates with NHE3 predominantly in the microvillar fraction in which NHE3 is active, as opposed to the intermicrovillar cleft region, suggesting that the association may affect NHE3 surface expression and/or activity. During phenol injection-induced hypertension, we found that DPPIV was recruited to the apical membranes in WI along with NHE3, which is consistent with the findings of Girardi et al. (15) that this would indicate increased association and NHE3 activity in the microvilli. In contrast, during arterial-constriction hypertension, DPPIV is internalized along with NHE3 (47), which is also consistent with a functional link between NHE3 and DPPIV.

The pattern of redistribution of NaPi2 to WI apical-enriched membranes during phenol injury-induced hypertension was distinct from that of NHE3 redistribution to WI; NHE3 was redistributed from WIII (intermicrovillar cleft, dense apical tubules, and endosomes) and NaPi2 was redistributed from WII (apical, intermicrovillar cleft, and dense apical tubule). This finding suggests that NaPi2 is translocated from intermicrovillar cleft or dense apical tubules, not intracellular endosomes, to the apical surface. This in-
terpretation is consistent with Murer et al. (31), demonstrating that when NaPi2 is translocated from apical brush border to endosomes during parathyroid hormone treatment or high dietary P_i, it is directly routed to lysosomes for degradation, and recovery of transport activity requires de novo synthesis of NaPi2 rather than redistribution from an endosomal pool. A relevant in vivo study demonstrated that a rapid adaptive increase in renal proximal tubule apical NaPi2 abundance in response to acute administration of a low-P_i diet is independent of de novo protein synthesis mediated by microtubule-dependent translocation of presynthesized NaPi2 protein to the apical brush border (21, 24), suggesting the existence of an intracellular NaPi2 pool of limited size that could be involved in the fine adjustment of renal P_i reabsorption. Whether the NaPi2 that moved to WI is nascent NaPi2 en route to the apical membrane or from a recruitable pool was not determined.

The activity of the apical brush-border marker alkaline phosphatase is also differentially regulated by hypertension induced by phenol injury vs. arterial constriction. After phenol injection, alkaline phosphatase activity shifts to the apical membrane-enriched WI from WII and WIII. During arterial constriction, total alkaline phosphatase activity is decreased and the peak shifts out of lower density apical membranes (fractions 3–5, WI) into higher density membranes (fraction 6, WII) (47). These results suggest alkaline phosphatase may also be involved in the regulation of NHE3 and/or NaPi2 traffic through its association or dissociation with these apical transporters.

In summary, in this neurogenic hypertensive model, acute phenol injection provokes a rapid redistribution of NHE3 and NaPi2 to the apical microvilli. Renal denervation prevented the NHE3 redistribution, suggesting that SNS activation of proximal tubule Na\(^+\) transport in vivo is mediated by recruiting transporters to the apical brush border. Renal denervation also prevented the development of hypertension, suggesting that the proximal tubule response may play a role in the generation or maintenance of the phenol injury-induced hypertension. Finally, the results provide evidence for bidirectional regulation of NHE3 and NaPi2 during hypertension; transporters may be internalized consistently with a compensatory response, as observed in arterial constriction hypertension, or may be recruited to the microvilli in a fashion that would contribute to hypertension, as observed in neurogenic phenol injury-induced hypertension.

We are grateful to Michaela Mac Veigh for assistance with confocal microscopy.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-34316, fellowship support from the AHA Western States Affiliate (L. E. Yang and P. K. K. Leong), and National Institutes of Health Core Center Grant DK-48522.

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