Chronic renal injury-induced hypertension alters renal NHE3 distribution and abundance

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Yang, Li E., Huiqin Zhong, Patrick K. K. Leong, Anjana Perianayagam, Vito M. Campese, and Alicia A. McDonough. Chronic renal injury-induced hypertension alters renal NHE3 distribution and abundance. Am J Physiol Renal Physiol 284: F1056–F1065, 2003. First published January 28, 2003; 10.1152/ajprenal.00317.2002.—Renal cortical phenol injection provokes acute sympathetic nervous system-dependent hypertension and a shift of proximal tubule Na+/H+ exchanger isoform 3 (NHE3) and Na+-P, co-transporter type 2 (NaPi2) to apical microvilli. This study aimed to determine whether proximal tubule (PT) Na+-cotransporter redistribution persists chronically and whether the pool sizes of renal Na+ transporters are altered. At 5 wk after a 50-μl 10% phenol injection, blood pressure is elevated: 154 ± 8 vs. 113 ± 11 mmHg after saline injection. Cortical membranes were fractionated into three “windows” enriched in apical brush border (WI), mixed apical and intermicrovillar cleft (WII), and intracellular membranes (WIII). NHE3 relative distribution in these windows, assessed by immunoblots and expressed as %total, remained shifted to apical from intracellular membranes (WI: 25.3 ± 3 in phenol vs. 12.7 ± 3% in saline and WII: 9.1 ± 1.3 phenol vs. 18.9 ± 3% in saline). NaPi2 and dipeptidyl-peptidase IV also remained shifted to cellular membranes (WI: 25.3 ± 3 in phenol vs. 11.3 ± 2 in saline). NHE3 relative abundance was altered, decreasing to 0.56 ± 0.06. The results demonstrate that phenol injury provokes a persistent shift of PT NHE3 and NaPi2 to the apical microvilli, along with a 44% decrease in total NHE3, evidence for an escape mechanism that would counteract the redistribution of a larger fraction of NHE3 to the apical surface by normalizing the total amount of NHE3 in apical membranes.

A model of neurogenic hypertension provoked by intrarenal injection of phenol into the cortex of a pole of one kidney was recently developed by Ye and Campese (8, 33, 34). In this model, a 50-μl 10% phenol injection causes a rapid elevation of blood pressure, preceded by a rise in norepinephrine secretion from the posterior hypothalamus and an increase in renal sympathetic nervous system activity. Renal denervation before phenol injection prevents the sympathetic nervous system activation as well as the rise in blood pressure. These results are consistent with the interpretation that this phenol 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 the point when it is just a microscopic scar. The cellular and molecular bases for the hypertension are not clearly understood. One potential contributor is activation of sodium and volume reabsorption mediated by renal efferent sympathetic nerve activity.

A dynamic relationship between blood pressure and renal sodium reabsorption is responsible, at least in part, for the blood pressure set point (15). Increases in sodium transport can be responsible for the generation and maintenance of hypertension, whereas decreases in sodium transport may be evidence of homeostatic compensation for elevated blood pressure. For example, an experimental increase in blood pressure acutely decreases proximal tubule sodium reabsorption, which both increases NaCl delivery at the macula densa, a transglomerular feedback signal to normalize renal blood flow and glomerular filtration rate, and causes pressure-natriuresis that reduces extracellular volume, which in turn counteracts the hypertension (6, 9, 10). In contrast, if renal sodium reabsorption is elevated due to excess production of an antinatriuretic (e.g., aldosterone) (30) or to a mutated epithelial sodium channel (ENaC; Liddle’s syndrome), then extracellular volume increases and blood pressure rises. The secondary hypertension depresses sodium reabsorption at sites along the nephron, for example, the thiazide-sensitive Na+-Cl cotransporter (NCC) (30), to match sodium excretion to sodium intake, a pressure-natriuresis variant known as “escape.” Although these phenomena are well established as important for the maintenance of extracellular volume and blood pres-
sure, many questions remain regarding the molecular mechanisms responsible for regulation of sodium transporters along the nephron in compensating for hypertension or in generating and maintaining hypertension.

This laboratory has investigated the proximal tubule sodium transporter responses during experimental acute hypertension induced by increasing peripheral resistance as well as in the spontaneously hypertensive rat (SHR). In both models, there was a retraction of Na+/H+ exchangers (NHE3) and Na+-P- cotransporters (NaPi) from the apical brush border to the intermicrovillar cleft and subapical endosomes, as demonstrated by both subcellular fractionation and confocal microscopy (21, 36, 37). In addition, there was a decrease in basolateral Na-K-ATPase activity with the onset of hypertension in both models (21, 37). Recently, we analyzed the acute response (30 min) to phenol injury in the rat cortex and discovered that NHE3 and NaPi redistributed from intracellular membranes to the apical microvilli, mediated by sympathetic nervous system activation, a response that could contribute to increased sodium/volume status (32). Motivated by these findings, we aimed to determine the chronic (5 wk) effects of phenol renal injury on proximal tubule sodium transporter distribution, namely, whether the proximal tubule sodium transporters would maintain a redistribution to the apical membranes or whether the hypertension would drive a retraction of proximal tubule sodium transporters from the apical microvilli as seen in the increased peripheral resistance and SHR models. It has been reported that the kidney can escape from certain sodium-retention disorders, such as hyperaldosterone states, by downregulating renal sodium transporters, such as the NaCl transporter of the distal tubule, to counteract the sodium retention (30).

Therefore, in the present study we looked for evidence of escape during chronic phenol injury-induced hypertension by examining the total pool size of renal sodium transporters along the nephron. The results demonstrated that the redistribution of NHE3 and NaPi2 to apical microvilli and Na-K-ATPase activity to the plasma membranes persists for 5 wk after phenol injury and a decrease in cortical NHE3 abundance as evidence for a coincident escape mechanism in the same region of the nephron.

EXPERIMENTAL PROCEDURES

Animal preparation. Experiments were performed in male Sprague-Dawley (SD) rats (280–320 g body wt) that had free access to food and water. After anesthesia with an intramuscular injection of ketamine (Fort Dodge Laboratories) and xylazine (1:1, vol/vol, Miles), the left kidney was exposed via a dorsal incision, 50 μl of 10% phenol or saline were injected into the lower pole of the renal cortex, the incision was sutured closed, and the rats were returned to the vivarium, where they had free access to food and water. After 5 wk, rats were anesthetized as above and placed on a thermostatically controlled warming table to maintain body temperature at 37°C. Polyethylene catheters were placed into the carotid artery to record blood pressure. In one set, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (35 mg/kg).

Homogenization and subcellular fractionation. The procedure for subcellular fractionation of renal cortical membranes has been described previously (38, 39). In brief, the noninjected right kidney was cooled in situ by flushing with cold PBS and then excised. The renal cortices and medullas were dissected, homogenized 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)) with a TissueMizer (Tekmar Instruments), and centrifuged at 2,000 g for 10 min; the pellet was rehomogenized and centrifuged, and the low-speed supernatants (S₀) were pooled. The cortical S₀ was loaded between two hypotonic sorbitol gradients and centrifuged at 100,000 g. In some analyses, one-half the protein was also assayed to verify linearity of the detection system. Samples were denatured in SDS-PAGE sample buffer for 30 min at 37°C, resolved on 7.5% SDS-polyacrylamide gels according to Laemmli (20), and transferred to polyvinylidene difluoride membranes (Millipore Immobilon-P). Polyclonal antisera to NHE3 [NHE3-C00; McDonough laboratory (31)] and to NaPi2 (J. Biber and H. Murer, University of Zürich, Zurich, Switzerland) were used at 1:2,000 dilution. Polyclonal antisera to dipeptidyl peptidase IV (DPPIV; M. Farquhar, Univ. of California at San Diego) were used at 1:1,000 dilution. A monoclonal antibody specific for Na-K-ATPase α-subunit (4B6.6) (M. Kassahun, Yale Univ.) was used at 1:200 dilution. Polyclonal anti-Na-K-ATPase β-subunit (McDonough laboratory) and a polyclonal antisera to NaCl transporter (TSCo; D. Ellison, Oregon Health and Science Univ.) were used at 1:500 dilution. Monoclonal anti-Na-K-2Cl transporter antibody (T4; C. Lytle, Univ. of California at Riverside) and polyclonal anti-NHERF1 antibody (R-1046; E. Weinman, Univ. of Maryland School of Medicine) were used at 1:3,000 dilution. Polyclonal antisera to ENaC α- and β-subunits (Chemicon) were used at 1:1,000 dilution. Except for α- and β-ENaC, all blots were incubated with Alexa 680-labeled goat anti-rabbit (Molecular Probes, Eugene, OR) or goat anti-rabbit IRDye800 or goat anti-mouse IRDye800 secondary antibody (both from LI-COR, Lincoln, NE), detected with an Odyssey Infrared Imaging System (LI-COR), and quantitated using the accompanying LI-COR software. α- and β-ENaC were detected with the enhanced chemiluminescence (ECL) kit (Amersham Pharmacia Biotech), and autoradiographic signals were quantified with a Bio-Rad imaging densitometer with Molecular Analyst software. Multiple exposures of autoradiograms were analyzed to ensure that signals were within the linear range of the film.
Indirect immunofluorescence. Immunofluorescence analysis was conducted as described in detail previously (31). In brief, the kidney contralateral to the saline or phenol injection was fixed in situ (without perfusion of fixative to avoid changing renal perfusion pressure), cut in half on a microtome, and postfixed in periodate-lysine-paraformaldehyde, incubated overnight in 30% sucrose in PBS, embedded in Tissue-Tek optimal cutting temperature compound (Sakura Finetek, Torrance, CA), and frozen in liquid nitrogen for 5-μm cryosectioning. Sections were incubated with 1% SDS in PBS for 4 min for antigen retrieval (7), SDS was removed by washing in PBS, and then sections were blocked with 1% bovine serum albumin in PBS. Double labeling was performed by incubating with polyclonal antisera NHE3-C00 and a monoclonal antibody against villin (Immunotech, Chicago, IL) and then detected with a mixture of FITC-conjugated goat anti-rabbit (Cappel Research Products, Durham, NC) and Alexa 568-conjugated goat anti-mouse ( Molecular Probes), as described previously (31). Slides were viewed with a Nikon PCM Quantitative Measuring High-Performance Confocal System equipped with filters for both FITC and TRITC 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).

**Assays.** Na⁺-K⁺-ATPase activity was measured by the potassium-dependent p-nitrophenyl phosphatase (K⁺-pNPPase) reaction (27), alkaline phosphatase activity was measured as described (25), and protein concentration was assessed by two-tailed Student's t-test for paired samples. Data are expressed as means ± SE. Differences were regarded significant at P < 0.05. In cellular fractionation assays, two-way ANOVA was applied to determine whether there was a significant effect of treatment on the overall pattern. If significance was established, the location of the difference in the pattern was assessed by two-tailed Student’s t-test for paired samples. Differences in total cell sodium transporters were assessed by two-tailed Student’s t-test for paired samples.

**RESULTS**

**Effects of intrarenal phenol injection on systolic arterial pressure.** Ye et al. (33, 34) have reported that a limited renal injury through an intrarenal injection of 50 μl of 10% phenol caused an immediate and permanent form of neurogenic hypertension. In this study, we independently verified this finding in a different laboratory. Five weeks after 50 μl of 10% phenol or saline were administered in the lower pole of one renal cortex, arterial blood pressure was measured in ketamine- and xylazine-anesthetized rats (n = 6) via arterial cannulation, and systolic blood pressure was significantly elevated in the phenol-injected rats (129 ± 3 mmHg) compared with the saline-injected rats (115 ± 3 mmHg). In a subgroup of the ketamine/xylazine-anesthetized rats (n = 3), blood pressure was measured by tail cuff before injection and 5 wk thereafter in conscious rats: phenol injection increased systolic pressure from 123 ± 1 to 157 ± 2 mmHg, whereas injection of 50 μl saline did not change blood pressure. Because we and others have previously reported that blood pressure measured in anesthetized SHRs is higher with pentobarbital sodium compared with ketamine/xylazine (22, 37), we also checked systolic arterial pressure in pentobarbital sodium-anesthetized rats (n = 3) 5 wk after phenol injection (rats not used in further experiments) and found that the measured blood pressure was indeed higher than that in the ketamine/xylazine group (154 ± 8 vs. 113 ± 11 mmHg in phenol-injected vs. saline-injected rats). This may be attributed to the observation that xylazine, a centrally acting α₂-adrenergic agonist, promotes urinary sodium excretion by a renal nerve-dependent pathway (24). These measurements confirm the previous report that a single phenol injection provokes persistent hypertension (33) and that the measured blood pressure is higher with pentobarbital sodium anesthesia compared with ketamine/xylazine.

**Immunoblot detection of NHE3 distribution in phenol injury-induced chronic hypertension.** NHE is the major route for apical sodium entry across the proximal tubule, and NHE3 is responsible for virtually all Na⁺-H⁺ exchange activity in this region (1, 4). We recently discovered that the acute hypertension established 30 min after intrarenal injection of 50 μl of 10% phenol is associated with redistribution of NHE3 immunoreactivity from intermediate-density membranes enriched in markers of intermicrovillar cleft and endosomal pools to lower density membranes enriched in apical brush-border microvillar markers, a response that may contribute to the generation of phenol injury-induced hypertension and a response blocked by prior renal denervation (32). Our previous studies in a model of chronic hypertension found that as chronic hypertension developed with age in the SHR, NHE3 redistributed in the opposite direction: from lower density membranes enriched in markers of apical microvilli to higher density membranes enriched in markers of the intermicrovillar cleft and endosomes, a response also verified by confocal microscopy (36), which provides evidence for a homeostatic compensation to the developing hypertension (21). These disparate findings stimulated us to examine whether NHE3 would remain shifted to the apical membranes, as evidenced in the acute response to phenol injury, or would retrace to the intermicrovillar membranes, as evidenced in the chronic hypertensive SHR. NHE3 distribution was studied in the contralateral kidney 5 wk after phenol or saline injection. Figure 1A shows representative immunoblots of NHE3 in the renal cortical membranes fractionated into the three defined windows (W1 is enriched in apical brush-border markers alkaline phosphatase, DPPIV, and NHE3; WII contains most of the intermicrovillar cleft marker megalin as well as the apical markers; and WIII is enriched in megalin as well as the endosomal marker rab 5a and the lysosomal marker β-hexosaminidase) (31, 37). Because a constant volume, rather than protein, of each window was analyzed, the total immunoreactivity in the saline-vs.-phenol samples is not expected to be identical. The differences in total NHE3 are analyzed subsequently. The results indicate that the phenol injection-induced shift of NHE3 out of WIII into W1 were at 30 min...
persists for 5 wk (Fig. 1B): WI NHE3, expressed as %total NHE3 in the gradient, contains 25.3 ± 3% in the phenol group and 12.7 ± 2.7% in the saline group; WII NHE3 is unchanged, 65.6 ± 2.3% in the phenol group and 68.4 ± 1.9% in the saline group; WIII NHE3 contains 9.1 ± 1.4% in the phenol group and 18.9 ± 3.4% in the saline group. These results indicate that there is a persistent signal for net traffic of sodium transporters to the surface in the chronic hypertensive phenol-injected group compared with saline-injected controls. The pattern is distinct from the internalization of NHE3 that was observed in the chronic hypertensive phenol-injected group compared with saline-injected rats, as evidenced by immunoblotting and discussed in Fig. 7. This technique provides visual confirmation that NHE3 is not distributed out of the villi and is internalized to endosomes during phenol injury-induced chronic hypertension as reported in the chronic hypertension in both SHRs and Goldblatt 2K1C (36) and in acute hypertension by increasing peripheral resistance (31, 36).

Distribution of other apical membrane proteins in phenol injury-induced hypertension. In our previous study, we demonstrated that 30 min after acute phenol injection the percentage of proximal tubule NaPi2 in WI apical microvilli increased from 9.5 ± 1.6 to 18.7 ± 1.5% and NaPi2 in WII decreased by a similar percentage. DPPIV, an NHE3-associated protein in microvilli (14), increased in WII from 19.2 ± 1.6 to 28.6 ± 2.4% of the total 30 min after phenol injection. Figure 3A summarizes the NaPi2 distribution 5 wk after phenol injection during the chronic hypertension phase. There was a significant difference in the NaPi2 distribution in the phenol- compared with saline-injected rats, indicating a shift out of WII into WI: NaPi2 in WI, expressed as %total in the gradient, was 22 ± 2.3% (phenol) vs. 11.2 ± 2.1% (saline); WII NaPi2 was 60.7 ± 1.8% (phenol) vs. 70.7 ± 2.4% (saline); and there was no change in WIII. The results indicate that NaPi2 may redistribute, as in the acute phase of hypertension (30 min after phenol injection), from the intermicrovillar cleft region and/or dense apical tubules (WII) to apical membranes (WI) during phenol injury-induced hypertension. The redistribution of the classic apical membrane protein DPPIV also persisted, similar to that of DPPIV at 30 min after phenol injection: a slight but significant increase in WI to 24.4 ± 11% of total from 18.1 ± 8.1% 5 wk after phenol injury (Fig. 3B), supporting a functional link between NHE3 and DPPIV.

We previously reported that 30 min after phenol injection the activity of the classic apical microvillar marker alkaline phosphatase increased 80% in the apical membranes in WI. Figure 4 shows alkaline phosphatase distribution and activity in the fractionated membranes from saline-and phenol-injected rats 5 wk after phenol injection. The results indicate a persistent and substantial activation of alkaline phosphatase activity during the chronic phase of phenol injury-induced hypertension: in WI, alkaline phosphatase activity was 100.9 ± 29.7% higher in phenol- compared with saline-injected rats, and WII activity was 51.4 ± 17.5% higher (no change in WIII activity).

Basolateral membrane Na-K-ATPase activity in phenol injury-induced hypertension. Our previous investigations suggest that renal cortical Na-K-ATPase activ-
ity falls as hypertension develops in both acute hypertension from arterial constriction (22, 37) and chronic hypertension, as in the developing SHR (21). However, 30 min after phenol injection we did not observe any change in Na-K-ATPase activity or distribution (32). During the chronic hypertension phase 5 wk after phenol injection (Fig. 5), Na-K-ATPase activity was significantly shifted to the basolateral membranes found in WI, perhaps mechanistically similar to the report that the sympathetic /H9252-agonist isoproterenol increases surface expression of Na-K-ATPase in cultured lung cells (2).

Profiling of sodium transporter abundance in renal cortex and medulla. To determine whether the total pool size of sodium transporters located along the nephron was altered 5 wk after phenol injection during chronic hypertension, S sub samples (homogenates subjected to a 2,000-g spin to remove poorly homogenized bits) of cortical and medullary membranes from saline- and phenol-injected rats were assayed and immunoreactivity was quantified. Figure 6 demonstrates the linearity of the infrared imaging system (LI-COR). The relative distribution of these transporters in the cortex vs. medulla was also verified: NHE3, the thiazide-sensitive NCC, and NaPi2 were enriched in the cortex, the bumetanide-sensitive Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\)-cotransporter was expressed in the medulla, and Na-K-ATPase \(\alpha_1\) and \(\beta\)-subunits were found in both the cortex and medulla, with more in the latter. Figure 7 summarizes the immunoblots of these transporters and proteins in saline- vs. phenol-injected rats. To ensure linearity, each sample was run twice, with one-half the protein loaded in the second lane. The densitometric quantitation is shown in Table 1. In the cortex, there was a remarkable decrease in NHE3 protein in phenol-induced hypertensive rats to 58.1 \(\pm\) 7.4% of that measured in the saline-injected controls. This fall in cortical NHE3 was region specific, as there was no significant change in NHE3 total abundance in the medulla. There was also a tendency toward a decrease in total renal cortical Na-K-ATPase activity (Fig. 5C), which parallels the decrease in apical NHE3 abundance, but this did not reach statistical significance and was not paralleled by a change in total Na-K-ATPase subunit pool sizes (Table 1). There were no other significant differences in immunoreactivity of the other sodium transporters or in the NHE3-associated proteins DPP4 and NHE regulatory factor (NHERF) in phenol-induced hypertension compared with saline-injected rats (Fig. 7, Table 1).

DISCUSSION

Ye and Campese (8, 33, 34) have characterized many features of this phenol injury model that support a role for central and renal sympathetic nervous system
activation in the genesis and maintenance of hypertension. In brief, the injection of 50 µl 10% phenol into the cortex of one kidney leads to an immediate elevation of norepinephrine secretion from the posterior hypothalamus, a rise in blood pressure, and an increase in plasma norepinephrine level. Renal denervation before phenol injection prevents the increase in both blood pressure and norepinephrine secretion from posterior hypothalamus. Sympathetic activity recorded directly from renal nerves increases after phenol injection, and the α-adrenergic-receptor blocker phentolamine normalizes blood pressure (35). Five weeks after phenol injection, the site of injection is reduced to a microscopic scar, yet hypertension and elevated norepinephrine secretion from posterior hypothalamus persist; ablation of the injured kidney at 4 wk normalizes blood pressure, perhaps due to elimination of the renal afferent impulses (8). These findings all support a role for central and renal SNS activation and α-adrenergic-receptor activation in the genesis and maintenance of hypertension induced by phenol injury. These results complement the evidence supporting a role of SNS activation in the pathogenesis of hypertension induced by renal diseases, including chronic renal failure (CRF) (5, 11, 34).

Our laboratory recently investigated the acute effects of phenol injury on renal sodium transport (32). Thirty minutes after phenol injection, NHE3 and NaPi2 were redistributed from the intermicrovillar cleft and intracellular membranes to the apical microvilli and apical alkaline phosphatase activity doubled. Additionally, the responses were prevented by prior denervation. These findings provide the first in vivo evidence that SNS stimulation activates proximal tubule apical sodium entry by recruiting NHE3 and NaPi2 transporters to the apical surface, responses that may contribute to the generation and maintenance of elevated blood pressure by hampering the pressure-natriuresis response. The results of this study demonstrate that a single injection of phenol into one kidney can cause permanent hypertension associated with a persistent redistribution of renal cortical NHE3, NaPi2, and Na-K-ATPase to the plasma mem-

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Fig. 3. Na+/Pi cotransporter type 2 (NaPi2) and dipeptidyl peptidase IV (DPPIV) redistribute to low-density membranes during phenol injury-induced chronic hypertension. NaPi2 and DPPIV were prepared and assayed as described in Fig. 1. A: typical immunoblots of NaPi2 from saline- vs. phenol-injected rats and summary of NaPi2 distribution in 3 windows expressed as the percentage of the total signal in all 3 windows. B: typical immunobLOTS of DPPIV from saline- vs. phenol-injected rats and summary of DPPIV distribution in 3 windows expressed as the percentage of the total signal in all 3 windows. Values are means ± SE; n = 5/group. *P < 0.05 vs. saline, assessed by ANOVA and followed by paired Student’ s t-test.

Fig. 4. Alkaline phosphatase distribution and activity in phenol injury-induced chronic hypertension 5 wk after injection of 50 µl of 10% phenol vs. 50 µl of saline. A: alkaline phosphatase activity (µmol Pi·h¹·mg¹) was increased in windows I and II. B: alkaline phosphatase distribution (%total) was unchanged. Values are means ± SE; n = 6/group. *P < 0.05 vs. saline, assessed by ANOVA and followed by paired Student’ s t-test.
brane and an increase in alkaline phosphatase activity, rather than a return to the basal pre-phenol injection pattern or a change in the pattern observed in other models of chronic hypertension (SHR, 2K1C), where NHE3 is retracted out of the microvilli (see below). In addition, this study demonstrates a significant decrease in NHE3 pool size in the renal cortex 5 wk after phenol injury, an escape phenomenon that would counter the effect of redistributing a larger fraction of sodium transporters to the apical cell surface.

Comparing the acute and chronic proximal tubule responses to hypertension of distinct origins illustrates that an alteration in renal sodium transport may be causal with regard to some varieties of hypertension and compensatory in other varieties. The proximal tubule sodium transporter responses in the phenol-induced hypertension model are quite distinct from that seen in the SHR. In young prehypertensive SHRs, NHE3 and Na-K-ATPase activity in renal cortex are higher vs. in age-matched Wistar-Kyoto (WKY) (16, 26) or SD rats (21), suggesting that elevated sodium transport may contribute to the development of hypertension. These differences in activity disappear in adult SHRs with established hypertension vs. WKY or SD rats (13, 21). Biochemical (21) and confocal immunofluorescence (36) studies reveal that NHE3 is localized to the apical brush border in young prehypertensive SHRs and then redistributes to the intermicrovillar cleft and subapical membranes as hypertension becomes established in adult SHRs, mimicking the redistribution in SD rats challenged by acute hypertension (37, 38). NHE3 is similarly retracted in the Goldblatt 2K1C model (36). The retraction of NHE3 as hypertension develops (acutely or chronically) is likely a homeostatic compensation to normalize salt and water balance as well as a key mechanism to stimulate transglomerular feedback. The proximal tubule responses in the phenol-induced hypertension model are the opposite to that seen in the SHR. Specifically, the NHE3 distribution patterns are nearly indistinguishable between the acute phase (30 min) of phenol injury and chronic phase of hypertension (5 wk), demonstrating a persistent shift of NHE3 to apical microvilli from internal membranes: in the acute phase, the NHE3 percentage in WI is 27.2 ± 4.1 (phenol) vs. 13.1 ± 2% (saline), that in WII is unchanged, and in WIII is 10.8 ± 2.7% (saline); after 5 wk, the percentage in WI is 25.3 ± 3 (phenol) vs. 12.7 ± 2.7% (saline), that in WII is unchanged, and in WIII is to 9.1 ± 1.4 (phenol) vs. 18.9 ± 3.4% (saline).

**Fig. 5.** Na-K-ATPase activity and distribution in phenol injury-induced chronic hypertension 5 wk after injection of 50 μl of 10% phenol vs. 50 μl of saline. A: Na-K-ATPase activity, assessed as K⁺-dependent ß-nitrophenyl phosphatase (K⁺-ßNPPase). B: Na-K-ATPase distribution (%total). C: total Na-K-ATPase activity in cortical and medullary membranes. Values are means ± SE; n = 6/group. *P < 0.05 vs. saline, assessed by ANOVA and followed by paired Student’s t-test.

**Fig. 6.** Detection of sodium transporter proteins in renal cortical and medullary membranes using LI-COR infrared imaging system. Sodium transporters were detected over a range of protein loading amounts to define linear range for detection and quantitation. NHE3 at 83 kDa, NaPi2 at 82 kDa, and thiazide-sensitive Na⁺-Cl⁻ cotransporter (NCC) at 160 kDa are enriched in the cortex; Na⁺-K⁺-2Cl⁻ cotransporter (NKCC) at 160 kDa is detected in medulla; and Na-K-ATPase (NKA) α1-subunit at 190 kDa and Na-K-ATPase β1-subunit at 50 kDa are detected in both regions and are relatively enriched in the medulla.

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NaPi2 and DPPIV distribution patterns, as well as alkaline phosphatase activation, are also similar in acute and chronic phases of phenol-induced hypertension. The fact that the percentages of NHE3, NaPi2, and DPPIV and the activity of alkaline phosphatase in the microvilli are persistently increased goes along with the observed persistence of SNS stimulation. In summary, the responses of proximal tubule transporters to the chronic hypertension in the phenol injury model are the opposite to those seen in the chronic hypertension of the SHR and Goldblatt 2K1C models. We speculate that the signals driving the internalization of NHE3 and NaPi2 during hypertension per se are overridden by opposing signals, most likely the activated SNS, which drive transporters to the apical membrane, associated with a significant blunting of the pressure-induced diuresis and natriuresis.

There have been many reports of the effects of norepinephrine on proximal tubule sodium transport and transporters in isolated proximal tubules. In brief, norepinephrine stimulates sodium reabsorption and ouabain-sensitive rubidium uptake and decreases intracellular sodium, evidence for an increase in plasma membrane Na-K-ATPase number or activity (12). Studies of the signaling mechanisms in isolated proximal tubule have shown that α-agonist oxymetazoline stimulation of Na-K-ATPase transport activity was prevented by either α1- or α2-receptor antagonism (17), and studies in cultured proximal tubule cells demonstrate a β2-adrenoreceptor-mediated increase in Na-K-ATPase transport activity secondary to increased apical sodium entry (29). In this study, we observed a redistribution of Na-K-ATPase activity to WI, where the peak basolateral membrane Na-K-ATPase resides (37), and did not measure stimulation of total Na-K-ATPase V_{\text{max}} activity in a membrane preparation after phenol injury-induced hypertension. These findings are in agreement with the norepinephrine studies in cell and tubules, where transport activity in the membrane was assessed. The findings are also in agreement with studies in cultured lung cells, where adrenergic agents have been shown to stimulate Na-K-ATPase via insertion of Na-K-ATPase from intracellular vesicles to the plasma membranes (2). We have previously shown that hypertension per se decreases Na-K-ATPase in the renal cortex (22, 23, 37), so it is likely that the Na-K-ATPase activity and distribution after phenol injury may be the product of the combined multiple stimuli of SNS stimulation and hypertension.

Hypertension and other renal pathologies can alter the total abundance of sodium transporters along the nephron in a pathology-dependent fashion. For example, in certain disorders of extracellular volume expansion, kidneys can escape from sodium retention by...
downregulating one or more of renal sodium transporters, thereby depressing sodium reabsorption to match sodium excretion to sodium intake. The molecular mechanisms responsible for regulation of sodium transporters along the nephron have been investigated in a number of models (30). For example, when the aldosterone level is inappropriately elevated, e.g., primary aldosteronism, the reabsptive activity of ENaCs is increased while the renal abundance of NCC is profoundly and selectively decreased. This response appears to be the chief molecular mechanism by which the kidney overcomes the sodium-retentive effect of aldosterone. Similarly, long-term pressure-natriuresis has been reported to be associated with inhibition of distal tubule sodium transporters (23). In contrast, increased Na-K-ATPase, NCC, and ENaC protein pool sizes are increased in the obese Zucker rat and may be responsible for sodium retention and hypertension (3). In the present study, phenol injury-induced chronic hypertension is associated with a 44% fall in total NHE3 abundance in the renal cortex. When one considers the fact that NHE3 distribution in W1 roughly doubles (from 12.7 ± 2.7 to 25.3 ± 3%), this 44% drop in total cortical NHE3 abundance would return the total amount of NHE3 in apical membranes (W1) close to that seen before phenol injection. Therefore, this marked reduction may be the major molecular mechanism responsible for adaptation to chronic stimulation of SNS activity and may contribute to resetting the pressure-natriuresis relationship. It remains to be determined whether this decrease in NHE3 is due to decreased synthesis or increased degradation. Proximal tubule NHE3 abundance is also reduced 50% in CRF induced by % nephrectomy, a response that may contribute to increased sodium excretion in CRF (19). Both NHERF and DPPIV have been reported to directly interact with NHE3 (14, 28). Despite the fact that the cortical NHE3 pool size decreased 44% after 5 wk, there was no detectable decrease in total DPPIV or NHERF, indicating that the decrease in NHE3 was specific and that there is not a defined stoichiometry between NHE3 and these associated proteins (which are known to have other functions in the cells). Additionally, the drop in NHE3 abundance is region specific as there was no detectable change in medullary NHE3 abundance. In addition, there was no significant change in loop or distal sodium transporter abundance in renal adaptation to the neurogenic phenol injury-induced hypertension and thus no evidence for escape regulation beyond the proximal tubule, in contrast to other models of hypertension studied (18).

In summary, the persistent redistribution of NHE3, NaPi2, and Na-K-ATPase to the plasma membranes may contribute to the generation and/or maintenance of chronic hypertension induced by phenol injury. In parallel, the decrease in total cortical NHE3 abundance in the chronic phase of phenol-induced hypertension is evidence for a coincident escape mechanism in the same region of the nephron that could counteract the effect of redistributing a larger fraction of sodium transporters to the apical cell surface.

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