Phenol injury-induced hypertension stimulates proximal tubule Na\(^+/\)H\(^+\) exchanger activity

Patrick K. K. Leong,1 Li E. Yang,1 Carol S. Landon,2 Alicia A. McDonough,1 and Kay-Pong Yip2

1Department of Physiology and Biophysics, University of Southern California Keck School of Medicine, Los Angeles, California; and 2Department of Physiology and Biophysics, University of South Florida, Tampa, Florida

Submitted 3 October 2005; accepted in final form 27 December 2005

In the acute renal injury neurogenic hypertension model developed by Campose and co-workers (4, 28), an injection of 50 μl 10% phenol to the lower pole of the renal cortex causes an increase in blood pressure within 30 min of injection. This hypertension is preceded by a rise in norepinephrine secretion which provokes acute hypertension that persists for weeks. We have previously shown with membrane fractionation that phenol injury caused a redistribution of the main proximal tubule (PT) apical transporter NHE3 (Na\(^+/\)H\(^+\) exchanger isoform 3) to low density membranes enriched in apical microvilli. The aim of this study was to determine whether phenol injury increases PT apical Na\(^+/\)H\(^+\) exchanger (NHE) activity. NHE activity was measured in vivo as the initial rate of change in intracellular pH (dpHi/dt) during luminal Na\(^+\) removal in PT preloaded with the pH-sensitive fluorescence dye BCECF. Injection of 50 μl 10% phenol increased blood pressure from 113 ± 5.2 to 130 ± 4.6 mmHg without changing glomerular filtration rate or urine output. NHE activity increased 2.6-fold by 70 min after phenol injury. The increase of NHE activity was accompanied with an increase of tubular reabsorption. Total NHE activity/NHE3 protein in cortical brush-border membrane (BBM) vesicles, measured by acridine orange quench and immunoblot, respectively, was unchanged by phenol injury. In conclusion, acute phenol injury provokes coincident increases in PT apical NHE activity, redistribution of NHE3 into low density apical membranes, and hypertension. The increase in NHE activity may contribute to the lack of pressure-diuresis and the maintenance of chronic hypertension in this model.

sympathetic nervous system; sodium/hydrogen exchanger isoform 3; glomerular filtration rate; proximal tubular flow

IN THE ACUTE RENAL INJURY neurogenic hypertension model developed by Campose and co-workers (4, 28), an injection of 50 μl 10% phenol to the lower pole of the renal cortex causes an increase in blood pressure within 30 min of injection. This hypertension is preceded by a rise in norepinephrine secretion from posterior hypothalamus and an increase in renal sympathetic nerve activity (RSNA) (29). This injury-induced hypertension persists for at least 5 wk, when the injury site is only a microscopic scar (28). Removal of the injured kidney or renal denervation (28) reverses and/or prevents this hypertension demonstrating that this is renal neurogenic hypertension.

In this phenol injury model, we have established that the density distribution of the main proximal tubule (PT) sodium transporters [Na\(^+/\)H\(^+\) exchanger isoform 3 (NHE3) and Na\(^+\)-Pi\(^-\) cotransporter type 2 (NaPi2)], determined by membrane fractionation, are shifted to lower density membranes enriched in microvillus markers within 30 min of phenol injection (26) indicating redistribution of Na\(^+\) transporters from unidentified pools to the microvilli. The redistribution and ensuing hypertension is prevented by prior renal denervation, indicating that the response is due to RSNA and not phenol per se (25). Activation of adrenergic receptors has been shown to stimulate Na\(^+/\)H\(^+\) exchanger activity in isolated PT cells (17, 18, 21) but this phenomenon has yet to be demonstrated in vivo. The aim of the present study was to determine whether an acute increase in PT Na\(^+/\)H\(^+\) exchanger activity could be detected in vivo corresponding to the enrichment of NHE3 in microvilli enriched membranes during the neurogenic phenol-injury hypertension. We found that phenol injury induces a rapid increase in PT Na\(^+/\)H\(^+\) exchanger activity. This response may contribute to the blunted pressure natriuresis seen in this model vs. the decrease in PT reabsorption that accompanies acute hypertension in other models (23, 32).

METHODS

Animal preparation and surgical protocols. Experiments were carried out in accordance with guidelines for the care and use of research animals. All experiments were performed under protocols approved by the Institutional Animal Care and Use Committee at the University of South Florida, in accordance with Public Health Service Policy on Human Care and Use of Laboratory Animals. Experiments were performed on male Sprague-Dawley rats (200–300 g body wt) maintained under diurnal light conditions with free access to food and water. All rats were anesthetized with 0.2 ml pentobarbital sodium (50 mg/ml ip; Abbott Laboratories) per 100 g body wt and body temperature maintained at 37°C on a thermostatically controlled operating table. A tracheostomy was performed and the tube was connected to a small animal respirator (Harvard model 683) with a mixture of 25% oxygen-75% nitrogen to maintain arterial blood pH between 7.35 and 7.45. Tidal volume was adjusted between 1.9 and 2.5 ml at 57–60 breaths/min. Polyethylene catheters (PE-50) were placed into the carotid artery to monitor blood pressure and into the jugular vein to infuse 0.9% saline (20 μl/min) to maintain euvolesia. The left kidney was exposed through a flank incision, immobilized with a Lucite ring, and superfused with saline preheated at 37°C. The renal capsule remained intact during all in vivo renal measurements. Acute renal injury was induced by injection of 50 μl 10% phenol in saline into the cortical lower pole of the left kidney with a Hamilton syringe (MICROLITER digital syringes #705; 50 μl; DS80500). Sham-operated animals were injected with 0.9% saline. Urine was collected from ureter catheter over 10-min intervals and volume determined gravimetrically. Glomerular filtration rate (GFR) was measured as clearance of FITC-inulin as described (16, 19).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
In vivo measurement of apical Na\(^+\)/H\(^+\) exchanger activity. PT apical Na\(^+\)/H\(^+\) exchanger activity was determined in vivo by measuring the initial rate of change of intracellular pH (pHi) during luminal Na\(^+\) removal by retrograde perfusion (80 nl/min), as described in detail previously (32). Early segments of PT that ran entirely on the surface were identified and their flow direction determined by intraluminal bolus injection of Lissamine Green dye. The lumen of selected tubules was loaded with a pH-sensitive fluorescent dye BCECF/AM (50 µg/ml, Molecular Probes) in synthetic tubular fluid at 10 nl/min for 20 min with a micropipette (3- to 5-µm outer diameter) coupled to a microperfusion pump. Measurements began after a waiting period of >30 min to allow for deesterification of the dye. Acute renal injury was induced as described above only once in each rat and only one PT from each rat was studied. Calibration of pHi was performed in the same tubule at the end of each experiment using the high-potassium nigericin technique (24, 31).

A Zeiss Ultrafluar objective (×10, numeric aperture 0.25) mounted on a Leitz Stophot compound microscope equipped with epifluorescence was used for excitation and for detection of emission from the kidney surface. Two computer-controlled pulsed nitrogen lasers (emission λ: 337 nm; pulse duration: 3 ns; Laser Science) were coupled to corresponding tunable dye control modules to provide excitation at 435 and 500 nm for ratiometric measurement of pHi. Mounted directly to the dye control modules were adjustable fiber-optic mounts with collimators that couple the light into a fused silica optical fiber with a core size of 400 µm. The two optical fibers combined into a single transmission fiber with a core size of 600 µm. The transmission fiber was coupled to a fused silica focusing lens that passed the light to a dichroic mirror (510 DCLP, Omega Optical) and focused the lasers, through the objective of the Leitz microscope, onto the selected PT. The size of the focused laser spot, although adjustable via a diaphragm in front of the focusing lens, was routinely set at 100 µm\(^2\). Fluorescence emission from the kidney surface was collected through the dichroic mirror and a band-pass filter (530/25 nm, Omega Optical). The intensity of emission from each excitation pulse was collected with a Hamamatsu photomultiplier sequentially, and the current in the photomultiplier (sampled at 380 KHz for 39.5 µs) was integrated numerically and stored digitally. The emission ratio (500/435) was calculated after background subtraction at each wavelength.

Background emission was collected from a segment of neighboring PT that was not infused with BCECF/AM. The initial rate of change (20–25 s) of pHi (dpHi/df) induced by luminal removal of Na\(^+\) was calculated by fitting the time course of decrease in pHi into a first-order differential equation with a non-linear algorithm statistical package as described previously (32). Emission ratio was acquired at 2 to 5 Hz and the duration of each laser pulse was set at 3 ns. The time delay between the 435- and 500-nm excitation pulses was 4.1 ms. All software used for coordinating the firing of the lasers, sampling the output of photomultiplier and arterial blood pressure, and displaying of these variables on a monitor was written in the C programming language.

Measurement of PT flow. PT flow was measured by a method developed by Chou and Marsh (7, 23) as described previously. This technique measures the propagation velocity of fluorescent dextran, which is injected periodically into the ambient tubular flow with a micropipette. In brief, a bolus of lissamine green dye was injected intravenously to identify early segments of PT. A micropipette (2–3 µm outer diameter) filled with synthetic PT fluid (127 mM NaCl, 25 mM NaHCO\(_3\), 3 mM KCl, 1 mM MgSO\(_4\)·7H\(_2\)O, 1 mM K\(_2\)HPO\(_4\), 5 mM urea, and 1.8 mM CaCl\(_2\)) containing 1% solution of rhodamine-isothiocyanate 20S-labeled dextran (MW 17,200, Sigma) was then inserted into the proximal convoluted tubule. Injection was driven by a triggered pneumatic picopump (PV830, WPI). Injection frequency was set at 15 pulses/min (0.25 Hz) with a injection pressure of 20 lb/in\(^2\) and injection duration of 5–10 ms. Fluorescent dextran was excited with a green He-Ne laser (1 mW, 534 nm, Melles Griot) aimed at the renal surface, and the emission was detected with an intensified CCD video camera (IC-300, PTI) through a long pass filter at 560 nm.

To determine the velocity of the fluid stream, the composite video signal was digitized into 512 × 480-pixel array at 8-bit resolution by a Matrox IP-8 image processing board. A pair of sampling window was positioned downstream to the dextran injection site in the digitized images. The board returned two digital signals at 60 Hz. Each signal is proportional to the light intensity in the area defined by the sampling windows. The upstream signal served as template for the downstream signal of the same site. The transit time delay for the passage of the fluorescent dextran bolus between the two sampling windows was calculated for each pulse with a cross correlation routine. The distance separating the two sampling windows and the tube diameter were measured on the digitized image. Fluid velocity was calculated by dividing the distance by the time delay. Tubular fluid flow was calculated as the product of the velocity and the cross-sectional area.

Measurement of Na\(^+\)/H\(^+\) exchanger activity in brush-border membranes. Brush-border membranes (BBMs) were prepared from rat renal cortices as described by Biber and coworkers (2). In brief, at the end of the acute renal injury protocol, kidneys were cooled in situ by flushing with cold PBS to block membrane trafficking then excised and all subsequent steps conducted on ice or at 4°C. Renal cortex from the injected kidney was dissected after removal of the injured area (~2 mm in diameter) and homogenized with a Polytron (Brinkmann Instruments) at setting 5 for 2 min in 12 ml isolation buffer (2), brought to 15 mM MgSO\(_4\) for 15 min, centrifuged at 2,400 g for 15 min, then the supernatant was centrifuged at 30,000 g for 30 min. The resulting pellet was resuspended in 6 ml of isolation buffer and 6 ml of bidistilled water, homogenized with four strokes of a motor-driven Potter-Elvehjem glass Teflon homogenizer, treated with 15 mM MgSO\(_4\) and membranes were pelleted as above. The BBM-enriched pellet was resuspended in 3 ml of sorbitol buffer (5% sorbitol, 0.5 mM Na\(_2\)EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 9 µg/ml aprotinin, and 5 mM histidine-imidazole, pH 7.5) and frozen in aliquots at −80°C until assayed. Membrane marker mapping revealed that the BBM-enriched pellet prepared using the current protocol also carried other low-density membrane markers such as the basolaterally located Na\(^+\)–K\(^+\)–ATPase α1-subunit (data not shown). The presence of basolateral membranes in our BBM preparation, however, should not impact our analysis because NHE3 is primarily apically located.

Total Na\(^+\)/H\(^+\) exchanger activity was measured in BBM vesicles in vitro using the acidine orange (AO) quench method as described previously (25). In brief, BBMs were diluted in loading solution (25), pelleted at 200,000 g and resuspended in loading solution to 15 mg protein/ml (BCA, Pierce Technology). After a 2-h preincubation at 25°C, the assay was initiated with the addition of a 10-µl aliquot of the BBM suspension to a cuvette containing 2 ml of Na\(^+\)-free reaction medium (25) in the presence of 1 µM of valinomycin. The amount of AO trapped as AOH in the BBM vesicles, where its fluorescence was quenched, is a function of the H\(^+\) influx via the Na\(^+\)/H\(^+\) exchanger. After 1 min incubation, sodium gluconate concentration was brought to 25 mM which initiated coupled Na\(^+\) influx and H\(^+\) efflux via Na\(^+\)/H\(^+\) exchangers that led to a concomitant AO efflux. The rate of Na\(^+\)/H\(^+\) exchange was calculated from the slope of the initial 2.5-s rate of appearance of AO fluorescence, expressed as arbitrary fluorescence unit change per second per milligram protein, assayed in triplicate for each sample.

Subcellular fractionation. To determine the subcellular density distribution pattern of NHE3 and NHERF-1 (NHE regulatory factor 1) kidneys were removed at 50–60 min after phenol (or saline) injection and the total cortical tissue homogenate [supernatant of homogenized kidney cortices after low-speed (3,000 g) centrifugation] was subjected to centrifugation at 100,000 g in a hyperbolic sorbitol gradient and cortical membranes collected into 12 fractions, all as described in detail previously (33, 34).
Immunoblot analysis. To determine protein distribution in density gradients, a constant volume of each membrane fraction was assayed and expressed as the percentage of the total in all 12 fractions for each gradient. To determine protein abundance in BBM, a constant amount of membrane protein was assayed after establishing that the signals were in the linear range of detection. In all cases, the membrane samples were denatured in SDS-PAGE sample buffer for 30 min at 37°C, resolved on 7.5% SDS-PAGE mini-gels (Bio-Rad), and transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore) according to standard methods. Membrane blots were probed with the following polyclonal antibodies: anti-DPPIV (1:1,000; M. Farquhar, UCSD), anti-myosin VI (1:2,000; T. Hasson, UCSD), anti-NaPi2 (1:2,000; McDonough Laboratory), anti-NHE3 (1:2,000; McDonough Laboratory), anti-NHE3 (AB3085, 1:1,000; Chemicon International), and anti-NHERF (1:3,000; E. Weinman, Univ. of Maryland). All primary antibodies were detected with Alexa 680-labeled goat anti-rabbit secondary antibody (Molecular Probes). The density of the protein bands was quantitated using the Odyssey Infra-red Imaging System and quantitation software (LI-COR).

Immunofluorescence. Kidneys were fixed in situ with PLP fixative and prepared for indirect immunofluorescence analysis as previously described (27). Sections were dual labeled with polyclonal anti-NHE3 and monoclonal anti-villin (1:100) in 1% BSA/PBS 1.5 h, 25°C then washed, and 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 (1:100) in 1% BSA/PBS 1 h, washed, mounted and dried overnight, all as described (27). 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.

Statistical analysis. All dpH/dt data are expressed as means ± 95% confidence interval. All other data are expressed as means ± SE. ANOVA was used to determine the significance of a regression and for multiple-group comparisons. If a significant difference among groups was concluded by ANOVA, further pairwise comparisons were assessed by two-tailed Student’s t-test with the application of Bonferroni’s adjustment to correct for multiple comparisons. Comparisons between two data groups were assessed directly by paired two-tailed Student’s t-test. P value <0.05 was considered significant.

RESULTS

Physiological responses to acute phenol injury. Intrarenal injection of 50 μl 10% phenol caused a transient drop in mean arterial pressure from baseline (113 ± 5.2 mmHg) followed by a significant increase in blood pressure (131 ± 4.3 mmHg) which was maintained for >50 min after phenol injection (Fig. 1A). Saline injection (50 μl) had no effect on mean arterial pressure (Fig. 1B). These findings validate the previous reports that phenol injection acutely increases blood pressure (4, 26, 28). Neither phenol nor saline injection has a significant effect on GFR which was maintained between 0.6 and 0.8 ml/min (Fig. 1C). Urine output increased in the sham injected rats from 4.6 ± 0.6 μg/min at baseline to 17.5 ± 1.5 μg/min by 50 min postinjection (Fig. 1D). This may reflect the cumulative effect of the continuous 20 μl/min saline infusion. Saline was also infused at this rate in the phenol injected rats, but the increase in urine output did not achieve statistical significance due to higher variability for this data set (Fig. 1D). In a previous study where n = 9 we also failed to observe a significant pressure diuresis after phenol injury (26). Thus pressure diuresis appears to be blunted in this neurogenic hypertension model.

Effect of phenol injury on subcellular distribution of NHE3 and NHERF-1. As demonstrated previously, acute phenol injury induced a shift in NHE3 density distribution to lower density membranes enriched in apical microvilli markers (Fig. 2A) (26). In contrast, there was no significant parallel redistribution of the Na+/H+ exchanger regulatory factor 1 (NHERF-1), postulated to be involved in tethering NHE3 to the cytoskeleton and regulating its activity (Fig. 2B) (22). The redistribution of NHE3 to the microvilli cannot be detected by confocal microscopy after phenol injection (Fig. 2C) which is not surprising given the large amount of NHE3 colocalizing with villin in the microvilli at baseline (25).

Effect of phenol injury on in vivo PT Na+/H+ exchanger activity. In vivo Na+/H+ exchanger activity, measured as the rate of acidification (dpH/dt) during luminal Na+ removal, was measured at intervals of 2 to 5 min between 0 min and 70 min after phenol or saline injection except that no Na+/H+ exchanger activity measurements were made within the first 15 min after phenol injection because of the arterial pressure fluctuations evident during this time period (Fig. 1A). Representative tracings of change of pHl over time from a single PT in response to phenol injury are shown in Fig. 3A. Luminal Na+ removal induced a rapid initial rate of acidification (dpH/dt) that recovered with the addition of luminal Na+. This initial rate of acidification was the measure of in vivo Na+/H+ exchanger activity. As shown in Fig. 3B, phenol injury induced a significant time-dependent increase in Na+/H+ exchanger activity [ANOVA, variance ratio = 13.24, F(0.05(2), 1.56 = 5.31)]. Phenol induced a sustained increase in Na+/H+ exchanger activity, as demonstrated by the significant slope of the regression line, from 0.0123 ± 0.0060 pH/s at baseline to 0.0330 ± 0.0075 pH/s by 70 min after phenol injection. In contrast, saline injection had no significant effect on in vivo Na+/H+ exchanger activity [Fig. 3C: ANOVA, variance ratio = 1.06, F(0.05(2),1.13 = 6.41)].

Effect of phenol injury on PT flow. Alteration of apical Na+/H+ exchange activity in PTs are known to be associated with changes in PT flow (6, 23, 32). Phenol injury induced a significant decrease in PT flow from 26.7 ± 3.0 nl/min before to 17.9 ± 2.0 nl/min by 10 min after phenol injection, or a 33% drop in PT flow (Fig. 4A), whereas saline injection had no significant effect on PT flow (Fig. 4C). All PT flow data were normalized to facilitate comparisons. Due to the removal of the injection micropipette during intrarenal phenol or saline injection, no PT flow data were recorded during and immediately after phenol or saline injection. The corresponding changes in normalized arterial pressure during phenol (Fig. 4B) and saline (Fig. 4D) injection were also presented.

Effect of phenol injury on in vitro BBM Na+/H+ exchanger activity. Our density distribution and in vivo activity measurements are consistent with the working hypothesis that acute phenol injury provokes redistribution of active Na+/H+ exchangers (NHE3) into microvilli which increases the apical Na+/H+ exchanger activity as demonstrated in Fig. 3. We also tested whether there was an increase in Na+/H+ exchanger activity per transporter in isolated BBM vesicles during acute phenol injury. In vitro Na+/H+ exchanger activity was measured in BBM isolated from total renal cortices of saline and phenol-injected rats as the rate of reappearance of AO from AO-loaded vesicles in response to an inwardly directed Na+ gradient (see Methods). Na+/H+ exchanger activity normalized
to sample protein was unchanged (P > 0.05) by phenol injection: 3.46 ± 0.40 fluorescent unit·s⁻¹·transporter⁻¹ in saline-injected rats and 4.89 ± 0.87 fluorescent unit·s⁻¹·transporter⁻¹ in phenol-injected rats. 

NHE3 protein abundance assayed in the same BBM samples, normalized to constant protein, did not change with phenol injection (Fig. 5) implying that the density redistribution evident in Fig. 1 occurs within domains found in the BBM. From these results we conclude that phenol injury provokes redistribution of transport competent NHE3 within domains found in the BBM samples, or that allosteric or covalent regulatory factors were not retained during the standard BBM preparation protocol and/or Na⁺/H⁺ exchanger activity assay.

Effect of phenol injury on PT transporters and regulators.

Acute phenol injury did not alter the abundance in BBM of the Na⁺-phosphate cotransporter or other PT apical membrane proteins reported to associate with and regulate NHE3 activity including myosin VI (3), DPPIV (13, 14) or NHERF-1 (5) (Fig. 5), indicating that any trafficking of these proteins induced by phenol injection occurs within this crude BBM fraction as opposed to moving into or out of the BBM.

DISCUSSION

Previous studies in this phenol injury model strongly implicate central and renal sympathetic nerve activation in the genesis and maintenance of the induced hypertension (4, 28) establishing this as a relevant model to study the effects of chronic sympathetic nervous system (SNS) activation on renal function and blood pressure. In this study, phenol injection provoked a rapid increase in blood pressure of 20 mmHg in the absence of a change in GFR, not surprising since the phenol-induced increase in arterial pressure was within the autoregulatory range for GFR in rats (6). There was a tendency for an accompanying diuresis but it was no more than that seen in the saline infused controls and likely explained by the continuous saline infusion in both groups. In this study, as described before, NHE3 density distribution, determined by density gra-
The central goal of this study was to determine whether the phenol injury-provoked redistribution of NHE3 to low density PT apical membranes was accompanied by a physiological correlate, specifically, an increase in proximal tubular Na\(^+\)/H\(^+\) exchanger activity measured in vivo. Such an increase in PT Na\(^+\) transport could both blunt the expected pressure diuresis response and contribute to the generation and maintenance of chronic hypertension in this neurogenic renal injury model of hypertension. Using dpH/dt as an index of PT apical Na\(^+\)/H\(^+\) exchanger activity in vivo, we measured a 2.6-fold increase by 70 min after phenol injection. This increase represents a sustained time-dependent increase in activity of 3.8%/min.

Fig. 2. Effect of phenol injection on subcellular distribution of Na\(^+\)/H\(^+\) exchanger isoform 3 (NHE3) and NHE regulatory factor 1 (NHERF-1). Comparison of protein density distribution of NHE3 (A) and NHERF-1 (B) between saline-injected (○, n = 4) and phenol-injected (●, n = 4) rats. Immunoreactivity in each fraction is expressed as the percentage of total signal in all 12 fractions (means ± SE, n = 4, *P < 0.05 compared with corresponding control values in the same fraction). Shown below each panel are representative immunoblots from a typical experiment. C: indirect immunofluorescence microscopy of NHE3 distribution in the proximal tubule following saline (left) or phenol (right) injection. Sections were double labeled with anti-rabbit NHE3 antibody (green) and anti-villin antibody (red); overlapping staining appears yellow. Bar = 10 μm.

Fig. 3. Effect of acute phenol injury on Na\(^+\)/H\(^+\) exchanger activity measured in vivo. A: time course of change in pHi, during luminal Na\(^+\) removal in a representative proximal tubule in response to acute phenol injury: before phenol injection, 26 min after phenol injection and 53 min after phenol injection. The bottom tracing in each plot indicates the exact duration of luminal Na\(^+\) removal ("0 [Na\(^+\)]"). Calibration of pHi was performed in the same tubule at the end of each experiment using calibration solutions at pH 6.8, 7.2, and 7.6. Effect of acute phenol (B) or saline (C) injection on dpH/dt is shown. The initial rate of change of pHi (dpH/dt) was used as an index of in vivo apical Na\(^+\)/H\(^+\) exchanger activity. Values plotted at 0 min were dpH/dt measured between −10 min and 0 min (i.e., before injection). Broken lines represent the 95% confidence bands. For the phenol linear regression line: a = 0.0123; b = 0.000295; \(r^2 = 0.191\); n = 58 generated from 14 independent proximal tubules; ANOVA, Variance ratio = 13.24, \(F_{0.05(2,1,56)} = 5.31\). For the saline linear regression line: a = 0.0107; b = 0.00007; \(r^2 = 0.076\); n = 15 generated from 5 independent proximal tubules; ANOVA, Variance ratio = 1.06, \(F_{0.05(2,1,13)} = 6.41\).
Since there was no change in $dpH/dt$ in saline-injected tubules with time, the increase in $dpH/dt$ is consistent with an increase in apical Na$^+/H^+$ exchanger activity per se and not due to a decrease in intracellular buffering capacity resulting from repetitive $dpH/dt$ measurements in the same tubular segment. We are confident about the validity of this conclusion because PT flow also showed an acute phenol injury-induced decrease, indicating an increase in PT reabsorption (Fig. 4). This phenol-induced increase in PT Na$^+/H^+$ exchanger activity is consistent with the apparent redistribution of NHE3 to the lower density apical membranes (26). This finding also indicates that the response to phenol can override the typical responses to acute hypertension: redistribution of NHE3 from the top to the base of the microvilli and inhibition of PT Na$^+$ transport (8, 11, 16, 27).

The phenol-induced stimulation of in vivo Na$^+/H^+$ exchanger activity can be accounted for by either an increase in the number of transporters in the relevant membrane domain via protein trafficking and/or a change in the activity per transporter via allosteric or covalent modification. NHE3 is the primary transporter responsible for PT apical Na$^+/H^+$ exchanger activity (1, 20). We demonstrated previously (26) and validated in the present study (Fig. 2A) that phenol injection provokes rapid redistribution of NHE3 to lower density membranes containing markers of apical microvilli. This biochemical approach detects a significant shift that was not apparent by confocal microscopy examination of apical membrane NHE3, which is enriched in NHE3 at baseline (Fig. 2C). We conclude that phenol injection stimulates NHE3 redistribution from a Na$^+$ inaccessible pool to a Na$^+$ accessible pool in the microvilli, from a domain where it is inhibited to one in which it is active, or alters protein-protein association in a manner that changes density distribution and activity.

To address whether phenol injury increases Na$^+/H^+$ exchanger activity/NHE3 transporter, we isolated BBMs using the classical technique (2) and measured in vitro Na$^+/H^+$ exchanger activity by the AO quench technique. Using this BBM preparation, Fan and coworkers (12) demonstrated that parathyroid hormone (PTH) induced a time-dependent decrease in the specific activity of NHE3 in rat cortical BBMs. We did not detect a significant stimulation of specific Na$^+/H^+$ exchanger activity in BBMs after acute phenol injection, despite the facts that in vivo measurement of Na$^+/H^+$ exchanger activity reflected more than a doubling (Fig. 3) and that there was an enrichment of NHE3 in the low density apical mem-

![Fig. 4. Effect of phenol or saline injection on mean normalized proximal tubular flow (A and C) and mean arterial pressure (B and D). Arrows indicate time of phenol (50 μl 10% phenol in 0.9% saline) or saline (50 μl 0.9% saline) injection. Dotted lines are SE (phenol: $n = 5$; saline: $n = 4$).](F1548)

![Fig. 5. Effect of acute phenol or saline injection on proximal tubular proteins. Abundance of apical proteins in brush-border membrane proteins is shown. Protein abundance was detected and quantified with the Odyssey Infrared Imaging System (Li-Cor, Lincoln, NE).](F1548)
branes assessed by sorbitol density gradient fractionation (Fig. 2). In addition, we saw no evidence for a shift of NaPi2, NHE3 or proteins that are proposed to regulate NHE3 including NHERF-1, DPP4 or myosin VI into or out of the BBM after phenol injury. Taken together, our results suggest that phenol injury induces a redistribution of NHE3 between domains that are both isolated with the BBM and that interactions and/or modifications responsible for the increase in PT Na+/H+ exchanger activity detected in vivo are not preserved through the stringent divalent cation precipitation used to prepare BBM.

In the phenol-induced hypertensive model, the redistribution of the microvilli and activation of PT Na+/H+ exchanger activity is the opposite of the Na+/H+ exchanger inhibition observed in the acute hypertension or spontaneously hypertensive rat models, despite similar elevations in blood pressure. These results demonstrate that a homeostatic adjustment to acute hypertension (namely a decrease PT Na+ reabsorption) is absent in this interesting neurogenic model. We speculate that the signal(s) driving retraction of NHE3 to the base of the microvilli in other hypertensive models are overridden or prevented in the phenol-injury model by the increased RSNA that likely is responsible for elevating Na+/H+ exchanger activity. The renal sympathetic nerve is implicated in the redistribution of NHE3 and elevation of Na+/H+ exchanger activity because phenol injection increases RSNA (30) and renal denervation, which eliminates any potential sympathetic stimulation of the renal nerve and prevents the redistribution of NHE3 in the PT induced by phenol injury (26). Relevant studies in isolated or cultured PT cells (17, 18, 21) have shown that activation of adrenergic receptors stimulates NHE activity. This stimulation, rather than inhibition, of PT Na+/H+ exchanger activity associated with hypertension likely accounts for the significant blunting of the compensatory pressure-induced diuresis and natriuresis that resets the renal function curve during hypertension (15). That is, phenol injury shifts the pressure-natriuresis relationship to higher blood pressures (9, 10), which may contribute to the generation and maintenance of chronic elevated arterial pressure in this neurogenic hypertensive model. Future studies, manipulating independently renal SNS activity and blood pressure, are needed to determine the connection between the increase in renal SNS activity and hypertension in this phenol injury model.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-60501 (K.-P. Yip) and DK-34316 (A. A. McDonough), P. K. K. Leong and L. E. Yang were supported by fellowship awards from the American Heart Association, Western States Affiliate.

REFERENCES


