Acute arterial hypertension inhibits proximal tubular fluid reabsorption in normotensive rat but not in SHR.

By

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Running Title : hypertension, proximal tubular flow

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Abstract

The effect of acute arterial hypertension on proximal tubular fluid reabsorption was investigated in Sprague-Dawley rats and spontaneously hypertensive rats (SHR) by measuring proximal tubular flow with a non-obstructive optical method. Under control conditions, spontaneous tubular flow was oscillating at 0.02-0.03 Hz in Sprague-Dawley rats. Acute hypertension induced an immediate increase of mean tubular flow (50% increase after 20 min of hypertension) and augmentation of oscillatory amplitude. Acute hypertension did not alter single nephron blood flow as measured by laser Doppler velocimetry (n= 12), suggesting that the increase of tubular flow was due to inhibition of reabsorption but not increase of filtration. By contrast, spontaneous tubular flow was fluctuating aperiodically in SHR. Acute hypertension did not induce a continuous increase of tubular flow nor an increase in amplitude of fluctuations (n=15). When apical Na\(^+\)/H\(^+\) exchanger activity of proximal tubule was monitored, acute hypertension did not alter the activity in SHR (n=8), while similar procedure had been shown to inhibit apical Na\(^+\)/H\(^+\) exchanger activity of proximal tubules by more than 40% in Sprague-Dawley rats. These observations suggest that acute hypertension inhibits proximal tubular fluid reabsorption by inhibiting apical Na\(^+\)/H\(^+\) exchanger activity in Sprague-Dawley rats, and that this mechanism is impaired in SHR.

Keywords: laser Doppler velocimetry, Na\(^+\)/H\(^+\) exchangers, oscillations, tubuloglomerular feedback.
**Introduction**

Acute arterial hypertension induced by increasing total peripheral resistance with aortic clamps triggers a reversible inhibition of apical Na\(^+/\)H\(^+\) exchange activity in proximal tubules of Sprague-Dawley rats (32). Apical Na\(^+/\)H\(^+\) exchangers are responsible for ninety percent of NaCl transcellular reabsorption and 70% of NaHCO\(_3\) reabsorption in proximal tubule(1). Inhibition of apical Na\(^+/\)H\(^+\) exchange activity by acute hypertension is likely to increase proximal tubular flow because of the reduction in NaCl and NaHCO\(_3\) reabsorption. However, conventional method of measuring tubular flow by collecting tubular fluid interrupts ambient tubular flow, which might increase glomerular filtration via tubuloglomerular feedback. To circumvent this complication, a non-obstructive optical method was used in the present study to monitor directly the changes of spontaneous proximal tubular flow during the induction of acute hypertension. Moreover, single nephron blood flow on efferent arteriole was monitored with a non-invasive laser Doppler velocimetry device(23, 29), as to delineate whether the increase of tubular flow induced by acute hypertension is due to the inhibition of tubular fluid reabsorption or the increase of glomerular filtration.

Acute hypertension induced inhibition of apical Na\(^+/\)H\(^+\) exchange activity in proximal tubules of Sprague-Dawley rats (32) is associated with a redistribution of apical Na\(^+/\)H\(^+\) exchanger isoform 3 (NHE3) from brush border to intermicrovillar cleft region(31, 33, 34). However, acute hypertension provokes NHE3 trafficking only in pre-hypertensive young SHR (5 week old) but not in
hypertensive adult SHR (12 week old) (19, 31). It has been suggested that the chronic increase of arterial pressure saturates the trafficking mechanism of NHE3 in adult SHR. If the phenomenon of NHE3 trafficking is casually related to the regulation of apical Na⁺/H⁺ exchanger activity in proximal tubules, acute hypertension will not inhibit apical Na⁺/H⁺ exchanger activity and tubular fluid reabsorption in adult SHR. These hypotheses were tested by monitoring the changes of apical Na⁺/H⁺ exchanger activity and tubular flow in proximal tubules of adult SHR before and after induction of acute hypertension. Apical Na⁺/H⁺ exchangers activity in proximal tubule was measured by the initial rate of acidification during luminal Na⁺ removal using BCECF as an intracellular pH probe (32). The results from the present study indicate that acute arterial hypertension triggers an immediate and continuous increase in mean proximal tubular flow in Sprague-Dawley rats but not in SHR, and that apical Na⁺/H⁺ exchanger activity in SHR proximal tubule is not sensitive to acute increase of arterial pressure.

**Materials and Methods**

*Animal preparation.* Experiments were performed in male Sprague-Dawley rats, (250-300 g body weight) and 12-week old SHR. All rats were purchased from Harlan. The rats had free access to food and tap water before the experiments. Anesthesia was induced by placing each rat in a chamber containing 5% halothane administered in 25% oxygen and 75% nitrogen through a Fluotec Mark-3 vaporizer. A tracheostomy was performed and the rats were placed on a servo-controlled heated operating table, which maintained body temperature at
37°C. The tracheostomy tube was connected to a small animal respirator (Harvard model 683) adjusted to maintain arterial blood pH between 7.35 and 7.45 with a mixture of 25% oxygen-75% nitrogen. Tidal volume ranged from 1.9 to 2.5 ml, depending on body weight, with a frequency of 57-60 breaths per minute. The final concentration of halothane needed to maintain sufficient anesthesia was approximately 1%. A polyethylene catheter (PE 50) was placed in the right jugular vein for infusions. After a priming dose of smooth muscle vasorelaxant (Pancuronium, 1mg/Kg BW) in 1 ml 0.9% saline, a continuous infusion of Pancuronium (1mg/Kg/Hr) in 0.9% saline was given at 20 µl/min. 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 was left intact. Arterial pressure was measured in the left carotid artery with a Statham-Gould P23dB pressure transducer connected to a transducer amplifier (TBM4, WPI).

Induction of acute hypertension. An acute increase in blood pressure was induced by increasing total peripheral vascular resistance, as described by Roman and Cowley(22). Adjustable arterial clamps (Vestavia, AL) were placed on superior mesenteric and celiac arteries, and on abdominal aorta caudal to the left renal artery. Peripheral vascular resistance was increased by tightening the arterial clamps.

Measurement of tubular flow. A bolus of lissamine green dye was injected intravenously to identify early segments of proximal tubules. Proximal tubules were selected for observation only if they had a long segment (> 1mm) that ran on the surface, which permitted insertion of perfusion pipettes. Proximal tubular
flow was measured optically by a method developed by Chou and Marsh (5, 18). A micropipette (1-3 µm outer diameter) was filled with synthetic proximal tubular fluid containing 1% solution of Rhodamine-isothiocyanate 20S-labeled dextran (MW 17,200, Sigma), and was inserted into a proximal convoluted tubule. Injection was driven by a triggered pneumatic picopump (PV830, WPI). Injection frequency was set at 15 pulse/min (0.25 Hz) with a injection pressure of 20-40 lb/in² and injection duration of 5-10 ms. The volume of each injection pulse was ~ 15 pl under these conditions (12). Fluorescence was excited with a green He-Ne laser (1mW, 534 nm, Melles Griot) aimed at the renal surface, and detected with an intensified CCD video camera (IC-300, PTI) through a long pass of filter at 560 nm. The output from the camera was displayed on a 12” video monitor, and was recorded on 1.5” video tape with a video recorder (Sony V0-9600) and a frame code generator (Sony, FCG-700) for off-line analysis. Arterial pressure was recorded on the sound track of videotape. The field rate was 60 Hz.

To determine the velocity of the fluid stream, the composite video signal was digitized into 512x 480-pixel array at 8-bit resolution by a Matrox IP-8 image processing board housed on a desktop computer and displayed on a 15” monitor. The imaging board allows two sampling windows of variable size to be placed independently on the digitized image. 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. The transit time delay for the passage of the fluorescent dextran bolus between the 2 sampling sites was calculated for each pulse with a cross
correlation routine. The distance separating the 2 sampling windows and the tubule diameter were measured on the digitized image. The fluid velocity was calculated by dividing the distance with the time delay. The tubular fluid flow was calculated as the product of the velocity and the cross section area.

To demonstrate that effect of inhibiting apical Na\textsuperscript{+}/H\textsuperscript{+} exchanger activity on proximal tubular flow without activating tubuloglomerular feedback loop, tubuloglomerular feedback was suppressed by furosemide (10 mg/Kg in bolus, plus 3 mg/Kg/hr intravenous infusion). EIPA (ethylisopropyl amiloride, a potent inhibitor of Na\textsuperscript{+}/H\textsuperscript{+} exchangers) was then perfused luminally (0.8 mM, 10 nl/min) proximal to the dextran injecting pipette with a microperfusion pump (WPI, FL).

**Measurement of single nephron blood flow.** The single nephron blood flow in surface efferent arteriole was monitored with a laser Doppler velocimetry device specifically modified to work on the kidney surface non-invasively. Details of the modifications in the optics and signal processing on the Blood Perfusion Monitor (BPM 400A, Vasomedics) have been described elsewhere(23). In brief, a He-Ne laser (633 nm wavelength) was coupled into a single-mode optical fiber with a 4 \(\mu\)m core size and focused onto the kidney surface using a GRIN-rod len (Newport, CA). The arrangement yields a working distance of approximately 4 mm with a spot size of 20 \(\mu\)m. A variable attenuator was used to adjust the light intensity such that only enough light was used to obtain a good signal. The Doppler-shifted signal was picked up with another optical fiber and directed to the central processing unit of the laser Doppler device. Both transmit and receive fibers were held in a clamp mounted on a micromanipulator. The clamp
maintained a constant angle between the two fibers. Doppler-shifted frequencies were acquired from the back panel outputs of the blood perfusion monitor and digitized with an A/D converter (Data Translation, model 2801) at 9 Hz. The Doppler shift frequency was used as a relative measure of efferent arteriolar blood flow. Spontaneous variation of efferent arteriolar blood flow was measured in a single efferent arteriole by focusing the He-Ne laser spot onto the welling point (star vessel) on the renal surface. For measurements of mean single nephron blood flow, the Doppler-shifted frequency was averaged over a period of 10 minutes before and after acute hypertension was induced.

Measurement of apical $\text{Na}^+/$$\text{H}^+$ exchanger activity. Apical $\text{Na}^+/$$\text{H}^+$ exchanger activity in proximal tubule was measured by the initial rate of acidification ($dp\text{pH}/dt$) during luminal $\text{Na}^+$ removal as described previously(32). In brief, a proximal tubule that ran on the surface (> 1mm) and permitted retrograde and orthograde perfusion was first identified. The tubule was perfused with a pH sensitive fluorescence dye BCECF/AM (50 $\mu\text{g}/\text{ml}$, Molecular Probes, 10 nl/min) in synthetic tubular fluid for 25 min with a micropipette (3-5 $\mu\text{m}$ outer diameter) coupled to a microperfusion pump. Experiments started after a waiting period of 30 min for deesterification. Intracellular pH ($p\text{H}_i$) of proximal tubule was measured ratiometrically at 2 Hz by exciting BCECF at 435 nm and 500 nm with two ultraviolet (UV) nitrogen-pulsed lasers coupled to tunable dye control modules (Laser Science), and collecting emission at 530 nm via a bandpass filter (bandwidth of 25 nm) with a photomultiplier. Luminal $\text{Na}^+$ removal was achieved by retrograde perfusion of $\text{Na}^+$-free solution at 60 nl/min for 1 min. Change in $p\text{H}_i$
during luminal Na$^+$ removal was determined in the same tubule in pre-hypertensive control period, 10 and 20 min after induced acute hypertension. \( dpH_i/dt \) was estimated by nonlinear regression using the statistical package of BMDP (9). Only one tubule was studied from each rat. Calibration of pH$_i$ was performed in the same tubule at the end of each experiment using the high-potassium nigericin technique(25, 30).

*Spectral Analysis of time series.* Time series of variations in tubular flow were sampled at 0.25 Hz for spectral analysis using Fast Fourier Transform. Each time series with about 300 points was distributed into 4 overlap segments with 50% overlap, to reduce the variance of the power spectrum. Each segment was subjected to linear trend removal, and was operated on by a cosine window function to minimize leakage. Normalized power spectral density was calculated by averaging the power spectral density from all segments derived from a given time series (28). To determine the change in the magnitude of oscillations due to tubuloglomerular feedback before and after acute hypertension, the normalized power spectral density was integrated from 0.01 Hz to 0.06 Hz and compared.

*Solutions.* Synthetic proximal tubular fluid used contains (in mM) 127 NaCl, 25 NaHCO$_3$, 3 KCl, 1 MgSO$_4$$\cdot$7H$_2$O, 1 K$_2$HPO$_4$, 5 urea and 1.8 CaCl$_2$. Sodium was replaced with choline in Na$^+$-free synthetic proximal tubular fluid. The calibration solution was composed of (in mM) 140 KCl, 2 K$_2$HPO$_4$, 1 MgSO$_4$$\cdot$7H$_2$O, 1 CaCl$_2$, 10 glucose, 10 HEPES, and 10 µM nigericin. pH of the calibration solution was adjusted to 6.8, 7.2, and 7.6 using N-methyl-glucamine.
**Statistics.** Paired or unpaired Students t-test, regression analysis were used wherever applicable. Results were reported as mean ± SE.

**Results**

Under control conditions, spontaneous proximal tubular flow in Sprague-Dawley rat was under continuous oscillation at a frequency of around 0.03 Hz (Fig.1A) as observed in tubular pressure (12, 28). The oscillations are due to the operation of tubuloglomerular feedback, in which the intrinsic time delays and nonlinearities within the feedback loop result in periodic variations of pre-glomerular vascular resistance(12, 13). The periodicity in tubular flow was clearly discerned as a single peak in the power spectrum derived from the same time series (Fig.1B). The changes of flow in response to acute hypertension of a single nephron are shown in Fig.2A. Tightening of arterial clamps induced an acute hypertension of 27 ± 3 mmHg (n=12) increase in mean arterial pressure. It was associated with an immediate increase in the magnitude of oscillations and followed by a gradual increase of mean tubular flow. The increase of flow was continuous and long lasting. The mean tubular flow was increased by 50% after 20 min (Fig.3), as indicated by the regression line with a significantly positive slope (regression coefficient 0.0034 ± 0.00003 s⁻¹, p<0.05, n=12). There was no such increase in the mean tubular flow in the timed control (regression coefficient -0.00004 ± 0.00003 s⁻¹, n=5). The mean normalized power spectral density between 0.01-0.06 Hz (integrated area between 0.01-0.06 Hz in the power spectra) was increased by 46 ±16% (from 0.53 ± 0.06 to 0.74 ± 0.06, p<.05, n=12) after hypertension was induced. Therefore, both the mean tubular flow and
amplitude of oscillations in tubular flow were increased during acute hypertension.

It was previously shown that acute hypertension inhibited apical \( \text{Na}^+/\text{H}^+ \) exchangers activity in proximal tubules of Sprague-Dawley rat (32). To demonstrate that inhibition of apical \( \text{Na}^+/\text{H}^+ \) exchanger activity might account for the increase in proximal tubular flow in acute hypertension, EIPA (0.8 mM, 10 nl/min) was perfused luminally proximal to the dextran injecting pipette with tubuloglomerular feedback suppressed by intravenous infusion of furosemide. Suppression of tubuloglomerular feedback was necessary because luminal EIPA application resulted in an increase of [NaCl] at macula densa, which reduced glomerular filtration via tubuloglomerular feedback. Fig.4 is the changes of tubular flow of a single tubule when synthetic tubular fluid (with and without EIPA) was perfused at a site proximal to dextran injection site. Intraluminal perfusion of synthetic tubular fluid (10 nl/min) increased tubular flow by 33 \( \pm \) 9\% (n=10) compared to pre-perfusion baseline, while EIPA containing solution increased tubular flow by 80 \( \pm \) 10\% (n=10). These observations indicate that inhibition of apical \( \text{Na}^+/\text{H}^+ \) exchanger activity increases proximal tubular flow in free-flow nephron.

Changes in single nephron blood flow induced by acute hypertension were monitored in a separate experiment. Single nephron blood flow in Sprague Dawley rat was found to be oscillatory (Fig. 5A) as previously reported (29). However, there was no significant difference between the mean single nephron blood flow before and after hypertension induction (Fig. 6), as indicated by the
regression line with a slope not different from zero (regression coefficient 0.002±0.1 mmHg\(^{-1}\), n=12). These observations suggest that single nephron is well autoregulated during acute hypertension, and that increase of proximal tubular flow is most likely the consequence of inhibition in Na\(^+\)/H\(^+\) exchanger-dependent reabsorption but not increase of glomerular filtration.

Spontaneous proximal tubular flow in SHR was not oscillating periodically but fluctuated aperiodically (Fig.1C). The aperiodicity was reflected in a broadband distribution of power spectral density (Fig. 1D). The changes of tubular flow in response to acute hypertension in a SHR are shown in Fig.2B. There was an immediate transient increase in tubular flow but no augmentation in the magnitude of fluctuations. Moreover, there was no significant continuous increase of the mean tubular flow after induction of hypertension (Fig. 7), as indicated by the regression line had a slope (regression coefficient \(-3.7\times10^{-5} \pm 2.2 \times10^{-5} \text{ s}^{-1}\), n=15) not different from zero. There was also no hypertension-induced increase in the mean power spectral density at 0.01-0.06 Hz frequency band (\(-3 \pm 7\%\), n=15). Despite lacking continuous increase of tubular flow, single nephron blood flow was autoregulated in SHR. The regression line had a slope (regression coefficient \(-0.003 \pm 0.002 \text{ mmHg}\(^{-1}\), n=7) not different from zero (Fig.6). Acute hypertension induced in SHR was similar to that in Sprague-Dawley rats with an averaged increase in mean arterial pressure of 28 ± 6 mmHg (n=15).

Apical Na\(^+\)/H\(^+\) exchange activity in proximal tubule of SHR was measured by the initial rate of acidification \((d\text{pH}/dt)\) during luminal Na\(^+\) removal. Luminal
Na⁺ removal induced an immediate intracellular acidification in proximal tubule because of inhibition of Na⁺ dependent H⁺ exchange on apical membrane (Fig.8). To test whether acute hypertension inhibited apical Na⁺/H⁺ exchanger activity, \( \frac{dpH}{dt} \) was determined in pre-hypertensive control period, 10 and 20 min after induction of acute hypertension in the same tubule. Data were summarized in Table 1. There was no significant difference in \( \frac{dpH}{dt} \) before and after hypertension was induced (n=8). These results were dramatically different from those obtained in Sprague Dawley rats, in which acute hypertension significantly reduced \( \frac{dpH}{dt} \) by more than 40% (Fig.5 in (32)). These observations indicate that apical Na⁺/H⁺ exchange activity in SHR is not sensitive to acute increase of arterial pressure.

**Discussion**

Chou and Marsh (5-7) were the first to show that acute arterial hypertension inhibits proximal tubular reabsorption and increases in proximal tubular flow in Sprague-Dawley rats. The sampling intervals in their studies were in the order of minutes (5, 7). There was a delay of 90 s for the first measurement of flow after hypertension was induced. In the present study the preparation was optimized to measure proximal tubular flow at 0.25 Hz digitally, and there was no interruption in flow measurement when arterial pressure was raised. Secured adjustable arterial clamps were used to increase peripheral vascular resistance to minimize kidney movement. Injection pipette was inserted into proximal tubule parallel to the tubular flow. Intraluminal injection of fluorescent dextran was driven at 0.25 Hz via a pneumatic pico-pump. These modifications enabled
continuous intraluminal injection of fluorescent dextran and continuous sampling of fluid velocity throughout the experiment. The improved sampling strategy revealed two new features of proximal tubular flow in response to acute hypertension. The increase of flow was immediate and continuous, and the magnitude of TGF-mediated oscillations in tubular flow was amplified by hypertension.

Active transcellular reabsorption in proximal tubule is mainly mediated by Na\(^+\)/H\(^+\) exchangers(1). Acute hypertension induces a reversible inhibition of apical Na\(^+\)/H\(^+\) exchanger activity in intact proximal tubule(32), but it is not known whether inhibition of apical Na\(^+\)/H\(^+\) exchange activity will be manifested as increases in tubular flow. In the absence of intact tubuloglomerular feedback, intraluminal perfusion of synthetic tubular fluid proximal to the fluorescent dextran injection site triggered an increase of tubular flow as expected (Fig. 4). Inclusion of EIPA in luminal perfusate induced an additional increase in proximal tubular flow. These observations suggest that inhibition of apical Na\(^+\)/H\(^+\) exchanger activity may account for increase of proximal tubular flow in acute hypertension. Paracellular back leak of fluid through gap junctions in proximal tubules due to increase of renal interstitial pressure has been suggested as an alternative mechanism for inhibiting tubular reabsorption in pressure natriuresis (10). The contribution of this mechanism to the increase of tubular flow in acute hypertension is negligible because similar increase in tubular flow was observed in decapsulated kidneys (data not shown). Decapsulation is a procedure known
to block the increase of renal interstitial hydrostatic pressure when renal perfusion pressure is increased (15).

Previous studies indicated that whole kidney renal blood flow, glomerular filtration rate, and single nephron efferent blood flow were well autoregulated when arterial pressure was acutely raised (6, 29). A mixture of norepinephrine, aldosterone, vasopressin, and hydrocortisone was intravenously infused to provide a steady hormonal environment for kidneys in these studies (5-7, 29). No kidney sensitive hormone was used in the present study in order to avoid potential effects from these exogenous hormones on sodium transporters. Autoregulation of single nephron efferent blood flow was still observed. Therefore increase of proximal flow induced by acute hypertension is mostly likely due to inhibition of tubular reabsorption but not an increase in filtration.

Oscillations of tubular flow were first reported by Holstein-Rathlou and Marsh (12). They showed that proximal tubular pressure, proximal tubular flow, and Cl⁻ concentration in distal tubule are all oscillating at the same frequency but with a phase lag in the same nephron. These oscillations are due to the operation of tubuloglomerular feedback in regulating afferent arteriolar vascular resistance, and its activity is in general confined to 0.02-0.05 Hz as assessed by power spectral analysis (13, 14, 20, 27). Mathematical simulation predicted that the amplitude of tubuloglomerular feedback-mediated oscillations is augmented when tubular flow rate is moderately increased, because of shifting the operation point to the steepest part in the feedback curve (11). Intraluminal fluid perfusion at Loop of Henle has been shown to trigger tubular pressure oscillations (17).
Therefore, amplification of tubular flow during acute hypertension is most likely the consequence of increase in tubular flow, which shifts the operation point of tubuloglomerular feedback in the feedback curve. The potential effects of oscillations on tubuloglomerular feedback regulation of fluid and sodium delivery to distal tubules have been examined mathematically by Layton et al. (16, 20). They suggested that tubuloglomerular feedback regulatory ability is decreased when oscillations occurs, resulting in enhanced distal sodium delivery and renal sodium excretion. It was speculated that apical sodium uptake in distal nephron might not directly track the oscillations in luminal sodium concentration. As a result, the time-average sodium uptake is decreased while time-average luminal sodium concentration is increased (16). Therefore, the amplification of oscillatory magnitude in tubular flow by hypertension could be a hemodynamic response with natriuretic and diuretic consequence.

In SHR, tubular flow and efferent arteriolar blood flow were fluctuating aperiodically as observed in tubular pressure (28). Fluctuations in tubular pressure, tubular flow and single nephron blood flow are most likely due to arrhythmic variations of afferent arteriolar vascular resistance. Tubular pressure in 12 week old SHR is fluctuating in random appearance but with deterministic characteristics, as measured by correlation dimension and Lyapunov exponent (28). The correlation dimension and Lyapunov exponent of tubular flow fluctuations were not determined in the present study because of the limitation in temporal resolution when measuring tubular flow. Acute hypertension triggered only a transient disturbance of tubular flow in SHR. There
was no continuous increase of tubular flow, nor augmentation of TGF mediated oscillations in tubular flow. Missing of these two components might contribute to the attenuation of pressure natriuresis observed in SHR(21).

In Sprague-Dawley rats, acute hypertension induced increase in proximal tubular flow serves as an error signal (increased NaCl delivery to macula densa) for tubuloglomerular feedback to maintain autoregulation in single nephron blood flow(6). Single nephron blood in SHR was well autoregulated during acute hypertension despite lacking a continuous increase of proximal tubular flow (Fig.6). There are several factors that might account for autoregulation in single nephron blood flow of SHR without a detectable error signal. Tubuloglomerular feedback is more sensitive to flow-dependent change of [NaCl] at distal tubule in SHR than in Sprague-Dawley rats because the gain of tubuloglomerular feedback is higher in SHR(8). Tubuloglomerular feedback-mediated nephron-to-nephron interactions as measured by stop flow pressure is three times higher in SHR than in Sprague-Dawley rats(4). KCl induced vasoconstriction on afferent arteriole of SHR propagates much further than that of Sprague-Dawley rats(3, 24). All these observations suggest that nephron-to-nephron interactions mediated by tubuloglomerular feedback as well as myogenic response are stronger in SHR than Sprague-Dawley rats. Nephron-to-nephron interactions are crucial for efficient renal blood flow autoregulation(2, 13, 27). By applying white noise forcing to renal perfusion pressure, transfer function analysis confirmed that renal blood flow autoregulation is more efficient in SHR than in Sprague-Dawley rats(3). Therefore, the magnitude of error signal required
for tubuloglomerular feedback to maintain renal autoregulation would be smaller in SHR than in Sprague-Dawley rats.

Acute hypertension provokes internalization of apical NHE3 in proximal tubules of Sprague-Dawley rats but not in 12 week old SHR (31). Internalized NHE3 in Sprague-Dawley rats was functional when assayed in vitro(26), suggesting that trafficking of NHE3 is a major mechanism to inhibit Na⁺/H⁺ exchanger activity in proximal tubules. The observation that acute hypertension inhibited apical Na⁺/H⁺ exchange activity in Sprague-Dawley rats ( Fig.5 in (32) ) but not in SHR (Fig.8) are consistent with this notion. Acute hypertension also increased proximal tubular flow in Sprague-Dawley rats (Fig.3) but not in SHR (Fig.7). Collectively these observations strongly suggest that there is a causal link between apical Na⁺/H⁺ exchange activity and proximal tubular reabsorption in acute hypertension, and that inhibition of apical Na⁺/H⁺ exchange activity might be related to internalization of NHE3.

In summary, the present study demonstrates that acute arterial hypertension triggers an immediate and continuous increase in mean proximal tubular flow in Sprague-Dawley rats but not in SHR, and that acute hypertension does not inhibit apical Na⁺/H⁺ exchanger activity in SHR proximal tubule as in Sprague-Dawley rats. These observations are consistent with the notion that Na⁺/H⁺ exchanger-dependent fluid reabsorption in SHR is insensitive to acute increase of arterial pressure, which might contribute to the attenuation of pressure natriuresis in SHR.
Acknowledgement: The study was supported by National Institute of Health Grants HL-59156 and DK-15968.
References


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Table 1. Mean blood pressure, initial $\text{pH}_i$ before luminal $\text{Na}^+$ removal, decrease in $\text{pH}_i$ and $\text{dpH}_i/\text{dt}$ during luminal $\text{Na}^+$ removal measured at 3 different time points from SHR.

<table>
<thead>
<tr>
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<th>baseline</th>
<th>10 min hypertension</th>
<th>20 min hypertension</th>
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<tr>
<td>Blood pressure, mmHg</td>
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<td>139 ± 4</td>
<td>133 ± 5</td>
</tr>
<tr>
<td>Initial $\text{pH}_i$</td>
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<td>7.27 ± .07</td>
<td>7.33 ± .09</td>
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<tr>
<td>Decrease in $\text{pH}_i$</td>
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<td>0.27 ± .06</td>
<td>0.23 ± .05</td>
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<tr>
<td>Normalized $\text{dpH}_i/\text{dt}$</td>
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<td>0.92 ± 0.09</td>
<td>0.99 ± 0.06</td>
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<tr>
<td>$n$</td>
<td>8</td>
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Values are means ± SE on $n$ tubules studied. $\text{pH}_i$, intracellular pH; $\text{dpH}_i/\text{dt}$, initial rate of change in $\text{pH}_i$. Only one tubule is used from each SHR. The mean $\text{dpH}_i/\text{dt}$ in prehypertensive baseline is $0.055 ± 0.012$ s$^{-1}$. 
Legends

Fig.1 : Spontaneous variations of tubular flow and the corresponding power spectrum from a single proximal tubule of Sprague-Dawley rat (A, B) and SHR (C, D).

Fig.2 Effects of acute hypertension in tubular flow from a single proximal tubule of Sprague-Dawley rat (A) and SHR (B). Acute hypertension was induced at time=300 s.

Fig.3. Mean normalized proximal tubular flow of Sprague-Dawley rat in response to acute hypertension (A) and the timed control (B). Acute hypertension was induced at time=0. Straight lines are regression lines. Dotted lines are standard error. Regression coefficient is $3.4 \times 10^{-4} \pm 0.26 \times 10^{-4} \text{ s}^{-1}$ for acute hypertension ($p<.05$, n= 12), and is $-0.4 \times 10^{-4} \pm 0.27 \times 10^{-4} \text{ s}^{-1}$ (n=6) for timed control. Mean increase of arterial pressure during acute hypertension is $27 \pm 3 \text{ mmHg}$ (n=12).

Fig.4 . Effect of intraluminal perfusion of artificial tubular fluid (10 nl/min) in tubular flow of a single tubule with 0.8 mM EIPA (A) and without EIPA (B) in the absence of tubuloglomerular feedback. Perfusion pipette was placed proximal to the site where flow velocity was measured. Bottom line on each graph indicates the onset of microperfusion. Intraluminal perfusion of synthetic tubular fluid increased tubular flow by $33 \pm 9\%$ (n=10), while EIPA containing solution increased tubular flow by $80 \pm 10\%$ (n = 10).
Fig. 5. Spontaneous variations of single nephron efferent blood flow in Sprague-Dawley rat (A) and SHR (B).

Fig. 6. Relationship between mean efferent arteriole blood flow and blood pressure during acute blood pressure increase in Sprague-Dawley rats and SHR. Dotted lines are regression lines. Regression coefficient is $0.002 \pm 0.1 \text{ mmHg}^{-1}$ (n=12) for Sprague-Dawley rats, and is $-0.003 \pm 0.002 \text{ mmHg}^{-1}$ (n=7) for SHR.

Fig. 7. Mean normalized proximal tubular flow of SHR in response to acute hypertension (A) and the timed control (B). Acute hypertension was induced at time=0. Straight lines are regression lines. Dotted lines are standard error. Regression coefficient is $-3.7 \times 10^{-5} \pm 2.2 \times 10^{-5} \text{ s}^{-1}$ for acute hypertension (n=15), and is $-0.54 \times 10^{-4} \pm 1.5 \times 10^{-4} \text{ s}^{-1}$ (n=6) for timed control. Mean increase of arterial pressure during acute hypertension is $28 \pm 6 \text{ mmHg}$ (n=15).

Fig. 8. Time course of changes in pH$_i$ of a SHR proximal convoluted tubule during luminal Na$^+$ removal. Control period (A) and 20 min (B) after induced acute hypertension. The bottom tracing in each plot indicates the duration of luminal Na$^+$ removal by retrograde perfusion. The corresponding mean arterial pressure are 122 and 147 mmHg, respectively.
Figure 1
Figure 2

A

B

Figure 2
Figure 3
Figure 4
Figure 5
Fractional change of mean Doppler Freq

Step increase in arterial pressure, mmHg

Figure 6
Figure 7

Normalized tubular flow against time (s) for different conditions.
**Figure 8**

A

[Graph showing Intracellular pH over time with labeled time points and 0 Na^+ condition.]

B

[Graph showing Intracellular pH over time with labeled time points and 0 Na^+ condition.]