Abstract. Acute hypertension rapidly inhibits proximal tubule (PT) Na,K-ATPase activity and sodium reabsorption 30 to 40%, increasing sodium and volume delivery to the thick ascending loop of Henle (TALH) and macula densa, providing the error signal for tubuloglomerular feedback. The hypothesis was tested in rats that an acute increase in sodium and volume delivery to the TALH would acutely increase outer medulla Na,K-ATPase activity. Flow to the TALH was increased by either (1) elevating BP (102 to 160 mmHg) for 5 min by constricting arteries (hypertension) or (2) inhibiting PT sodium and volume reabsorption with the carbonic anhydrase inhibitor benzolamide: 2 mg/kg in 300 mM NaHCO₃ at 50 μl/min for 5 to 7 min. Both stimuli increased urine output and lithium clearance three- to four-fold and increased basolateral Na,K-ATPase activity about 40%. In homogenates, acute hypertension increased medullary Na,K-ATPase activity from 20 ± 3.5 to 27 ± 6.4 μmol Pi/mg protein per h while decreasing renal cortex activity from 10.9 ± 0.9 to 6.5 ± 0.7. Hypertension and benzolamide also doubled medullary alkaline phosphatase activity. As chronic hypertension develops in the young spontaneously hypertensive rat, medullary Na,K-ATPase activity similarly increases. In conclusion, there is a rapid activation of medullary Na,K-ATPase activity during acute hypertension that can be explained by the increase in sodium and volume flow to the region independent of hypertension. That is, the glomerulotubular balance response in the loop of Henle is accompanied by increased Na,K-ATPase activity. The rapid, downstream shift in Na,K-ATPase activity during acute hypertension contributes the driving force for activating TGF (by inhibition in the PT) and minimizes changes in distal sodium delivery (by activation in the TALH).

Arterial pressure fluctuates in normal animals, but highly efficient autoregulation prevents any measurable changes in renal blood flow (RBF) or GFR. This autoregulation is mediated in part by tubuloglomerular feedback (TGF), specifically, an increase in afferent arteriolar resistance in response to increased delivery of NaCl to the macula densa. In other words, normal fluctuations in BP provoke fluctuations in NaCl reabsorption proximal to the macula densa, which change afferent arteriolar resistance to normalize RBF and GFR (1,2).

Chou and Marsh (3,4) addressed the conceptual difficulty in understanding how acute hypertension could lead to increased NaCl delivery to the macula densa in the absence of a change in GFR or NaCl filtration rate. Using micropuncture of rat renal tubules, they discovered that acute hypertension rapidly inhibits proximal tubule (PT) volume reabsorption, increasing end PT fluid velocity 40%, which could provide the error signal for TGF, and verified that the response occurred in the absence of a measurable change in GFR. An acute decrease in salt and volume reabsorption in the PT in the face of constant GFR can be due to either (1) a change in activity per ion transporter in the plasma membrane (apical or basolateral) or (2) redistribution of ion transporters from surface to internal stores or both. We have been addressing these cellular issues and have determined that 5-min acute hypertension increases volume flow out of the PT, redistributes apical Na/H exchanger (NHE-3) and Na-Pi cotransporter (NaPi2) from the villi to intermicrovillar cleft and subapical endosomal stores, and inhibits basolateral Na,K-ATPase activity (5–7).

Another conceptual difficulty in understanding the generation of the TGF error signal at the macula densa is that the thick ascending limb of the loop of Henle (TALH) has a load-dependent response to reabsorb more salt when more is delivered, which is thought to be important in normalizing salt delivery to the distal nephron. This issue was also addressed by Chou and Marsh (3) who demonstrated that the 40% increase in flow leaving the PT during acute hypertension was reduced to a 10% increase in volume entering the early distal tubule and that the 35% increase in Cl⁻ load leaving the PT became a 35% increase in Cl⁻ delivery to the macula densa and early distal tubule over what was delivered in the control period. Volume and Cl⁻ reabsorption rates between the late proximal and early distal collection sites, estimated from their measurements, increased 86% and 34%, respectively, during acute hypertension, which provides evidence for a downstream shift in salt and volume reabsorption from the PT to the loop of Henle during

Received February 19, 2001. Accepted May 22, 2001.
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1046-6673/1211-2231
Journal of the American Society of Nephrology
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acute hypertension. The aim of this study was to determine whether there are rapid increases in sodium pump activity in the outer medulla during acute hypertension, a region enriched in TALH, which are themselves enriched in Na,K-ATPase, and if so, to determine whether changes are due to the increased volume flow to the region or dependent on hypertension per se. We provide evidence that the downstream shift in sodium and volume reabsorption described by Chou and Marsh (3,4) is accompanied by a downstream shift in Na,K-ATPase activity and provide evidence that it is likely mediated by the increased volume flow to the region.

Materials and Methods

Animal Protocols

All animal experimentation described in this study was conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals and protocols approved by the USC School of Medicine. Acute experiments were performed on male Sprague-Dawley rats (300 to 350 g body wt) that had free access to food and water before the experiment. Rats were anesthetized intramuscularly with ketamine (Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (Miles, Elkhart, IN) (1:1, vol/vol) and then placed on a thermostatically controlled warming table to maintain body temperature at 37°C. Polyethylene catheters were placed into the carotid artery for monitoring BP, into the right jugular vein for infusion of 0.9% NaCl (50 ul/min during entire experimental period), and in the ureter for urine collection.

Three protocols were used in the acute studies (n = 4 to 5 rats in each group): (1) control: control sham operated; (2) acute hypertension: BP was increased 60 mmHg over basal level for 5 min by increasing total peripheral resistance by constricting superior mesenteric artery, celiac artery, and abdominal aorta below the renal artery, as adapted from the method of Roman and Cowley (8) and used in our previous studies (5–7), (3) benzolamide treatment: the carbonic anhydrase inhibitor benzolamide (obtained from R. Blantz, University of California San Diego), which inhibits PT Na+ reabsorption, was infused at 50 μl/min (2 mg/kg in 300 mM NaHCO3) over 5 to 7 min, as described previously (5, 9).

Studies were also performed in spontaneously hypertensive rats (SHR) before and after the development of chronic hypertension, as recently described (10). Four groups of male rats were compared: (1) young Sprague Dawley (YSD), 3 to 4 wk old, 79 ± 7 g body wt; (2) young SHR (YSHR), 3 to 4 wk old, 76 ± 2 g body wt; (3) adult Sprague Dawley (SD), 12 wk old, 356 ± 6 g body wt; and (4) adult SHR (SHR), 12 wk old, 286 ± 5 g body wt. In this series, all rats were anesthetized by intraperitoneal injection with Nembutal (30 mg/kg im) and cooled in situ by flushing the abdominal cavity with ice-cold phosphate-buffered saline (PBS) solution to lower temperature to a point that blocks membrane trafficking. Rats were excised, the renal outer medulla and cortex were rapidly dissected in isolation buffer (5% sorbitol, 0.5 mM Na2EDTA, 5 mM histidine-imidazole buffer with 0.2 mM phenylmethyl sulfonyl fluoride, 9 μg/ml aprotinin, pH 7.5). Outer medulla, which is enriched in TALH, and cortices, enriched in PT, were homogenized and fractionated on sorbitol density gradients and stored as described in detail previously (5,6). S, refers to the low-speed supernatant, which contains total membranes and soluble proteins that were analyzed directly or applied to the sorbitol gradients.

Na,K-ATPase and Enzymatic Marker Measurements

Na+–K+–ATPase activity was measured by two methods. The K+-dependent p-nitrophenyl phosphatase reaction (K+-pNPPase, expressed as μmol Pi/mg protein per h) (13) was used to measure sodium pump activity in membrane fractions. A constant volume of each fraction from experimental and paired control samples was assayed under identical conditions, and activity was expressed as the pNPPase activity measured in each fraction divided by the total protein recovered in the 12 fractions (to correct for differences in the amount of protein applied to the two gradients). In addition, the distribution of pNPPase activity in the gradient was expressed as the pNPPase activity in each fraction as percentage of the total activity measured in all 12 fractions. Ouabain-sensitive Na,K-ATPase activity (expressed as μmol Pi/mg protein per h) was measured in S, samples after deoxycholic acid (0.1%) permeabilization, as described previously (14) under Vmax conditions with the modification that samples were preincubated with 2.5 mM ouabain for 30 min. Standard assays used for protein concentration (15) and alkaline phosphatase activity (16) have been implemented in related studies (5,6).

Immunoblot Analysis and Antibodies

A constant amount of S, protein from control and treated samples or a constant volume of sample from each gradient fraction was prepared in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (final concentration: 2% SDS, 1% β-mercaptoethanol, 0.25 mM Na2EDTA, and 2.5 mM H3PO4-HPO4 buffer, pH 7.0), incubated for 30 min at 37°C, resolved on 7.5% SDS polyacrylamide gels, and transferred to polyvinylidene fluoride membranes according to standard methods. The antibody incubation protocol has been detailed previously (5,6). Three anti-Na,K-ATPase α1 subunit monoclonal antibodies were used: McK1 (provided by K. Sweadner, Harvard University) (17), 464.6 (also known as 6H) (provided by M. Kasgarian, Yale University) (18), and Fam05 (provided by D. Fambrough, Johns Hopkins University) (19), and FPβ1, a rabbit
anti-rat β1 polyclonal generated in this lab and used previously in related studies (5,6). Antibody-antigen complexes were detected with 

\[ ^{125}I \] labeled Protein A (ICN) after incubation with rabbit anti-mouse secondary antibody where appropriate. The resulting autoradiographic signals were quantified with a Bio-Rad imaging densitometer with Molecular Analyst software (Hercules, CA). Multiple amounts of protein or volumes of density gradient fractions were analyzed, and multiple exposures of autoradiograms were analyzed to ensure that signals were within the linear range of the film.

**Statistical Analyses**

Data are expressed as mean ± SEM. For analysis of density gradient fractions, ANOVA was applied to determine whether there was a significant effect of treatment on the overall fractionation pattern of a given parameter. For \( S_n \) samples, effect of treatment was assessed by two-tailed t test for paired samples, and differences were regarded significant at \( P < 0.05 \).

**Results**

**Physiologic Responses**

Figure 1 summarizes the physiologic responses to acute hypertension. A 60-mmHg increase in arterial pressure induced a 3.9 ± 0.6-fold increase in urine output and a 3.3 ± 0.8-fold increase in the endogenous lithium clearance (C Li ), consistent with an increase in volume flow out of the PT (11,12). Benzolamide was given to increase volume flow out of the PT without raising BP. Benzolamide inhibits luminal membrane carbonic anhydrase (CA) and reduces PT sodium reabsorption by secondary inhibition of Na\(^+\)/H\(^+\) exchangers. It is specific for luminal CA because it is very membrane-insoluble (9,20).

Features common to benzolamide treatment and acute hypertension include depressed PT solute and volume reabsorption, increased renal hydrostatic pressure, increased flow through the loop of Henle and past the macula densa (3,4,9,21). However, these two protocols have distinct effects on GFR: a 60-mmHg increase in BP does not change GFR (3,4), and benzolamide treatment decreases GFR 25 to 30% (9,21). Figure 1 summarizes the effect of benzolamide infusion with a protocol that replaces sufficient volume and NaHCO\(_3\) to maintain euvolemia and serum HCO\(_3\) and which did not change arterial pressure (not shown). Urine output increased 3.0 ± 0.4-fold and C Li increased 4.0 ± 0.7-fold. That the benzolamide regime increases flow out of the PT (C Li ) and increases urine output to the same extent as the 5-min acute hypertension protocol suggests that the acute pressure diuresis can be accounted for by a decrease in proximal tubule sodium and volume reabsorption.

**Response of Renal Outer Medulla Na,K-ATPase to Acute Hypertension**

We aimed to determine whether the increased solute and volume flow out of the PT during acute hypertension would increase Na,K-ATPase activity in TALH. We and others have demonstrated that Na,K-ATPase activity and abundance is highest in the TALH and very low in other tubular elements present in the outer medulla (22,23). The outer medulla is anatomically enriched in TALH; therefore, we use outer medullary Na,K-ATPase activity to estimate changes in TALH Na,K-ATPase activity. After 5-min acute hypertension, renal outer medulla membranes were fractionated and assayed for Na,K-ATPase activity by the pNPPase activity assay (Figure 2A). The broad peak of pNPPase activity, indicative of basolateral membranes, is located between fraction 6 to 9. Activity was significantly increased after acute hypertension (\( P = 0.03 \) by ANOVA). The 5-min stimulus is not long enough to increase the total pool size of sodium pumps; therefore, this change represents an increase in enzymatic activity per transporter. The distribution pattern of the pNPPase activity was not altered by acute hypertension (Figure 2B), indicating that the increase in activity occurs throughout the fractionated membranes, rather than by activation in a discrete region of the gradient. To assess the possibility that the increase in pNPPase activity was accompanied by a redistribution of pumps from one region of the gradient to another, the relative abundance of the sodium pump α1 catalytic and β1 glycoprotein subunit immunoreactivity was measured in a single paired set in which the increase in pNPPase activity was the largest. As shown in Figure 2C, the control α1 and β1 subunits had very similar distribution patterns that mimicked that of % pNPPase activity, focused in fraction 5 to 10. With acute hypertension, there was no evidence for a shift of α1 or β1 subunits into the peak activity fractions. α1 was detected with two monoclonal antibodies that recognize distinct epitopes: McK1 was made

**Figure 1.** Physiologic responses to raising BP for 5 min by constricting celiac and superior mesenteric arteries and abdominal aorta (acute hypertension) or to infusing 2 mg/kg benzolamide at a rate of 50 \( \mu \)l/min in 300 mM NaHCO\(_3\), for 5 to 7 min (benzolamide). Urine output was collected over 5-min intervals before and after the change in BP or benzolamide infusion and measured gravimetrically. Endogenous lithium clearance was calculated for the same 5-min intervals (urine [Li\(^+\)] × urine output rate divided by plasma [Li\(^+\)]). Both urine output and lithium clearance are displayed as the fold increase after acute hypertension (\( n = 5 \)) or benzolamide infusion (\( n = 4 \)) compared with the pre-stimulus control from the same animal. All comparisons were statistically significant (\( P < 0.05 \)); data are expressed as mean ± SE. Paired controls did not show any significant change in urine output or Li\(^+\) clearance after sham intervention.
against a peptide in the N-terminus (17), and Famα5 recognizes an epitope in the central portion of the α1 subunit (19).

The previous observation that acute hypertension caused a rapid decrease in PT and renal cortical Na,K-ATPase activity (5, 6) coupled with the current observation that it causes a rapid increase in outer medullary Na,K-ATPase activity illustrates that there are region-specific changes in Na,K-ATPase activity. This leads to the prediction that within a given kidney exposed to acute hypertension, the ratio of Na,K-ATPase activity in medulla to cortex will increase from what is measured at baseline. This was tested in So fractions (the low-speed supernatants containing both membranes and soluble proteins). Ouabain-sensitive Na,K-ATPase activity, assayed under V_max conditions, increased significantly in medulla to 1.27 ± 0.04-fold above paired controls (P = 0.04) and decreased significantly in cortex to 0.60 ± 0.04-fold below paired control (P = 0.004) (Figure 3). The ratio of activity in medulla relative to cortex increased from 1.81 ± 0.33 in controls to 3.88 ± 1.15 after 5-min hypertension, evidence for a simultaneous decrease driving force for sodium reabsorption in the cortex and an increase driving force for sodium reabsorption in the medulla.

Because some investigators have found that the dose response of phosphorylation of Na,K-ATPase α1 at Ser18 in kidney cortex slices is associated with the dose response of inhibition of Na,K-ATPase activity (23, 24) while others are not (17). The So samples were also probed with two antibodies that recognize different epitopes on Na,K-ATPase α1: McK1, a monoclonal that loses its epitope recognition when the protein kinase C (PKC) site on Ser18 is phosphorylated (17) and 464.6 (also known as 6H), which has not been reported to be sensitive to this phosphorylation (24). We tested whether the recognition with McK1 would decrease in cortex and/or increase in medulla during acute hypertension when constant amounts of So protein were compared. The results, which are summarized as arbitrary densitometry units normalized to the mean control value = 1.0 in Table 1, show that there was no statistically detectable difference in the immunoreactivity of the samples between control and pressure with either anti-α1 antibody. These are the same samples in which there was a

tase (pNPPase) activity corrected for total protein in the gradient (described in Materials and Methods), was significantly increased by acute hypertension (group difference, P = 0.03). (B) The distribution of Na,K-ATPase activity, expressed as percentage of the total Na,K-ATPase activity in the gradient, was unaltered by hypertension. (C) The relative distribution of Na,K-ATPase α and β subunits was analyzed in the paired experiment where there was the greatest increase in Na,K-ATPase activity. A constant volume of fractions 2 to 12 (8 μl) was resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), blotted, probed with α1-specific (McK1) and β1-specific (FPβ1) antibodies, and quantitated by scanning densitometry. Subunit-relative immunoreactivity is expressed as the percentage of the total signal in all the fractions. (D) Autoradiograms of blots of constant volumes of fractions 2 to 12 (8 μl) as well as 1/2 volume fractions 7 to 8 (4 μl) probed with anti-α1 McK1, anti-α1 Famα5, and anti-β1 FPβ1. Differences in total signal between control and hypertension samples results from the differences in total protein applied and recovered from the gradients.

Figure 2. Effects of 5-min acute hypertension on Na,K-ATPase in membrane fractions of renal medulla. Two groups were compared: control sham treated and acute hypertension (n = 5 each). (A) Na,K-ATPase–specific activity, measured as p-nitrophenyl phospho-
significant difference in Na,K-ATPase activity, the results suggest that either acute hypertension does not provoke changes in the phosphorylation state of Ser18 or that such a small fraction of the pumps in the So fraction are altered at Ser18 that the effect is masked by the unaffected pumps. This finding confirms our previous finding of no difference in α1 total abundance in renal cortex after acute hypertension using McK1 (before it was known that the antibody reacted only with unphosphorylated pumps) (5). The immunoblot results in Figure 2C and 2D also support this conclusion; the density distribution pattern of α1 detected with either McK1 or Fama5, which recognizes that the cytoplasmic loop of Na,K-ATPase α1 was not altered by acute hypertension.

During acute hypertension, solute and volume delivery to the TALH are increased. To test the hypothesis that the change in Na,K-ATPase activity in the outer medulla is secondary to the increased volume flow to the region and independent of the hypertension per se, benzolamide was infused (2 mg/kg in 300 mM NaHCO3 at 50 μl/min for 5 to 8 min) as discussed under Physiologic Responses. We have previously demonstrated that benzolamide infusion does not have any effect on pNPPase activity or distribution in the cortex (5). As summarized in Figure 4A, pNPPase activity increased significantly in outer medulla after benzolamide infusion (P = 0.0002 by ANOVA), and the overall distribution pattern of pNPPase activity was not significantly altered (Figure 4B). The response of outer medulla Na,K-ATPase activity to benzolamide (Figure 2A) is statistically indistinguishable from the response to acute hypertension (P = 0.77 by ANOVA). From this finding, we conclude that the increase in medullary activity during acute hypertension can be attributed, in part or in whole, to the increase in volume flow to the region, secondary to decreased sodium and volume reabsorption in the PT.

Our previous studies in cortex demonstrated that acute hypertension also depressed the activity of the apical membrane marker alkaline phosphatase; therefore, we studied the effect of acute hypertension or benzolamide infusion on alkaline phosphatase activity in outer medulla. There is a broad peak of activity in fractions 6 to 9 (Figure 5), which was about one tenth of that measured in cortex (5,6). Nonetheless, peak alkaline phosphatase activity doubled after either acute hypertension or benzolamide (P < 0.001), which suggests that solute and volume flow to the outer medulla increases activity of this apical microvillar enzyme (Figure 5, A and B).

In a recent report, we compared the effects of acute hypertension on proximal tubule Na,K-ATPase to the effects of developing chronic hypertension in YSHR and discovered that there was a similar decrease in sodium pump activity in both situations (10); these results are redrawn in Figure 6A. Specifically, Na,K-ATPase activity in prehypertensive YSHR is significantly higher than that in YSD rats, and as hypertension develops, activity of the SHR falls to that seen in theagematched SD. In this study, we tested the hypothesis that the stimulation of outer medullary Na,K-ATPase activity during acute hypertension is mimicked during the development of chronic hypertension, secondary to the fall in PT sodium transport. Figure 6A summarizes experiments that were conducted simultaneously on sets of one each of the four groups: YSHR, YSD, SHR, SD. Outer medulla Na,K-ATPase activity was more than 30% lower in the YSHR compared with YSD, and as hypertension developed in SHR, Na,K-ATPase activity in the outer medulla increased significantly to a level not different from that in the age matched SD; there was no age-related change in Na,K-ATPase activity in SD in cortex or cortex activity fell to 0.60 ± 0.04 of paired control.

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Protein expression of \( \alpha_1 \) in this set of samples is summarized in Figure 6B. There appears to be a near doubling of \( \alpha_1 \) protein abundance in both SHR and SD between 3 and 12 wk of age. The change was statistically significant in adult compared with young SHR (\( P = 0.04 \)) but not SD (\( P = 0.09 \)) because of the wide variability in the increase with age. Nonetheless, the changes in \( \alpha_1 \)-protein levels are greater than the changes in activity during this period in the four sets that were assayed, which suggests inactive or less active \( \alpha_1 \) subunits in the medullae of 12-wk animals. Like Na,K-ATPase activity, as hypertension developed in the SHR, alkaline phosphatase activity in the outer medulla increased 70% from 0.84 ± 0.24 (YSSH) to 1.44 ± 0.29 (SHR) \( \mu \)mol Pi/h per mg. These findings demonstrate that for the specific case of SHR, the changes in sodium transporters that occur during chronic hypertension mimic those seen during acute hypertension in SD: a downstream shift in sodium reabsorption and Na,K-ATPase activity from PT to TALH. The physiologic impact in both cases would be to limit the increase in delivery of sodium chloride and volume to the distal nephron.

Discussion

The observation that there are rapid reciprocal changes in cortex versus medulla Na,K-ATPase activity during acute hypertension illustrates that there is a dynamic continuous fluctuation in the Na,K-ATPase activity ratio in cortex versus medulla with normal BP fluctuations. The results of the benzolamide series indicate that fluctuations in medullary activity will likely occur in response to any stimulus that increases volume flow out of the proximal tubule, independent of arterial pressure. We make the assumption that cortex sodium pump activity approximates that in the proximal tubule while outer medulla activity approximates that in the TALH. The region-specific changes in activity deserve further comment because each region contains overlapping tubular elements: there are
TALH of cortical nephrons in the cortex, and proximal straight 
tubules (PST) in the outer medulla. This regional “cross-
contamination” likely leads to an underestimation of the 
changes that actually occur in PT versus TALH during acute 
hypertension. The contribution of the PST to the activity of the 
outer medulla is predicted to be very minor because the Na,K-
ATPase subunit abundance and activity in this region is 
roughly one tenth of that in the TALH (22). We did not attempt 
to obtain purified populations of tubules because the steps 
toward purification, including collagenase digestion at 37°C 
and incubation with oxygenation, would likely reverse the 
changes provoked by acute hypertension.

The changes in Na,K-ATPase activity during the onset of 
chronic hypertension in the SHR mimic the responses to acute 
hypertension in the SD: in 12-wk hypertensives versus 3- to 
4-wk prehypertensives, there is a decrease in Na,K-ATPase 
activity in the cortex and an increase in the outer medulla 
(Figure 6). Garg et al. (25) did a similar comparison of YSHR 
versus WKY rats and measured lower Na,K-ATPase activity in 
TALH of YSHR and no significant differences in the adults of 
these strains. Our comparisons in the SHR versus SD rat 
complement these observations. Consistent with the pattern 
during acute hypertension, Roman et al. (28) analyzed chloride 
and volume transport along the nephron by micropuncture in 
the hypertensive Dahl salt sensitive (SS) versus salt resistant 
(SR) strains on low-salt diets where BP was slightly higher in 
the SS strain. The results revealed that in the SS strain the 
percent of the filtered Cl\(^-\) and volume reabsorbed in the PT 
was lower and the percent of Cl\(^-\) and volume reabsorbed in 
the loop of Henle was higher compared with the SR strain, phys-
ologic evidence for a downstream shift in ion and volume 
reabsorption in this strain that mimics the response to acute

![Figure 6](image-url)

**Figure 6.** Comparison of Na,K-ATPase activity and α1 subunit abundance in total membranes of renal cortex and outer medulla from young and adult Sprague Dawley (SD) and spontaneously hypertensive rats (SHR). Sets of one of each young SD and SHR and adult SD and SHR 
were collected and analyzed together (n = 4 sets). (A) Ouabain-sensitive Na,K-ATPase activity in membranes from cortex is elevated in young 
SHR compared with young SD and compared with adult SHR by paired t test (P < 0.05) while adult levels of Na,K-ATPase in SD versus SHR 
are indistinguishable, results redrawn from Magyar et al. (10). Ouabain-sensitive Na,K-ATPase activity in membranes from outer medulla is 
significantly lower in young SHR compared with young SD or adult SHR by paired t test (P < 0.05) while adult levels in SD versus SHR are 
indistinguishable. (B) Protein levels of α1 measured in 0.5 μg of homogenate protein were detected with anti-α1 464.6 and 125I-Protein A. 
Adult SHR levels of α1 were significantly elevated compared with young SHR (P < 0.05).
hypertension. Thus, the hypertension-related elevation in outer medulla Na,K-ATPase activity and abundance in the SHR and salt and water reabsorption in the Dahl SS rat are both indicative of a compensation to an increase in flow to the TALH.

An exception to the observation that an increase in BP (whether acute or chronic) is generally associated with a downstream shift in Na,K-ATPase activity and salt and water transport is seen in the young Milan hypertensive rat (MHS), where outer medulla Na,K-ATPase activity and α1 abundance is higher than in the normotensive strain (MNS), even before hypertension develops. Subsequently, activity increases significantly in both strains by 11 to 12 wk, and activity and α1 abundance are elevated in adult MHS versus MNS in both outer medulla and cortex (26,27). We suggest that a compensatory downstream shift is not observed in the MHS as hypertension develops because the lesion driving sodium reabsorption and development of hypertension may reside in the TALH itself.

We adjusted a dose of benzolamide to acutely increase volume flow out of the PT to the same extent as we observed it itself.

The sodium pump abundance and activity in the TALH is so high that the renal outer medulla is the standard tissue for purification of the α1 and β1 subunits for protein studies. High sodium pump activity is necessary to extract sodium from the lumen and concentrate it in the basolateral interstitium, a process critical for both urinary dilution and concentration. Even so, activity increases further when volume flow is increased, which suggests that there is a tightly regulated match between sodium delivery and ATP-driven sodium transport that would provide energy efficiency to a process that requires a high rate of ATP consumption. What potential mediators could stimulate Na,K-ATPase activity in the TALH? Only a few studies have addressed this question. The chronically elevated chloride transport in the Dahl SS versus SR rat has been attributed to differentially lower expression of cytochrome P450 4A2 and resultant lower production of 20-HETE, a known inhibitor of TALH sodium transporters (34). 20-HETE, in turn, has been shown to activate PKC in the kidney and inhibit Na,K-ATPase activity (35); therefore, turning down signaling through this cascade could stimulate Na,K-ATPase activity. Whether acute hypertension treatment turns down an inhibitory pathway and/or activates a stimulatory pathway remains to be determined. The results with benzolamide suggest that a simple increase in apical Na⁺ entry may be sufficient to drive the increase in Na,K-ATPase activity.

In the current experiments, Na,K-ATPase activity was measured enzymatically (rather than as Na⁺ or Rb⁺ transport) and the activation of sodium pump activity persisted through tissue disruption and membrane isolation, indicating an increase in the activity of the total cellular pump pool. Whether there is also a realignment of pumps from intracellular pools to the plasma membrane remains to be further investigated in a subcellular fractionation scheme that does a better job at separating different populations of membranes. The increase in V_max activity/transporter suggests covalent modification or an allosteric interaction. The study of Kiroytcheva et al. (36) demonstrates an association between the cAMP-driven increase in α1 phosphorylation and increase in Na,K-ATPase activity in the TALH, and the experiments of Li et al. (24) in renal cortical slices and tubules demonstrated an association between PKC-driven increase in α1 phosphorylation and inhibition of Na,K-ATPase activity. The direct assays of the effects of PKC phosphorylation on Na,K-ATPase activity conducted by Fenschenko and Sweadner (17) add to the complexity of interpreting these associations. After cellular Na,K-ATPase was phosphorylated by stimulation of endogenous PKC (to stoichiometry of 0.9 at Ser18), there was no effect on Na,K-ATPase V_max activity or on apparent affinity for Na⁺, which lead these investigators to conclude that any effects of PKC on Na,K-ATPase activity are lost on cell disruption and not directly due to phosphorylation of Ser18. Complementing this conclusion is that from the careful study in renal proximal tubule cells of Chibalin et al. (37) that analyzes the molecular mechanisms responsible for depressing Na,K-ATPase--medi-
ated transport during dopamine stimulation, which is mediated at least in part by PKC and accompanied by increased phosphorylation at Ser11 and Ser18. Activity and trafficking regulation of sodium pump alpha subunit were analyzed in parallel. The results demonstrate that phosphorylation of Ser18, not Ser11 mediates internalization and resultant fall in activity of plasma membrane ATPase activity and that prevention of endocytosis blocked the inhibitory effect of dopamine on activity, which supports the notion that phosphorylation of either site does not change Na,K-ATPase V max activity. In this article we do provide evidence for an increase in Na,K-ATPase V max activity that persists through membrane isolation. The results of these studies (17,37) concur with our failure to detect a change in phosphorylated α1 with the antibody sensitive to Ser18 phosphorylation (Table I and Figure 2D) during acute hypertension and suggest that other mechanisms such as covalent modification at other sites or allosteric regulation should be investigated.

Acknowledgments

This work was supported by National Institutes of Health Grants DK 34316 to AMcD, and HL 45623 to NHHR. Portions of this work were presented at the 1998 Annual Meeting of the American Society of Nephrology. YBZ was supported by a Fellowship award from the American Heart Association Greater Los Angeles Affiliate.

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