Increased Expression of the Sodium Transporter BSC-1 in Spontaneously Hypertensive Rats

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ABSTRACT

The purpose of this study was to compare the expression of BSC-1 (bumetanide-sensitive Na⁺-K⁺-2Cl⁻ cotransporter) in kidneys of spontaneously hypertensive rats (SHR) versus Wistar-Kyoto (WKY) rats by immunoblotting and reverse transcription-polymerase chain reaction. To determine the specificity of any observed changes in BSC-1 expression, we also compared expression of the thiazide sensitive Na⁺-Cl⁻ cotransporter (TSC), the type-3 Na⁺-H⁺ exchanger (NHE-3), Na⁺-K⁺-ATPase-α₁, the inwardly rectifying K⁺ channel (ROMK-1), the type-1 Na⁺-HCO₃⁻ cotransporter (NBC-1), aquaporin-1, and aquaporin-2. Analyses were performed on outer cortex, outer medulla, and inner medulla. BSC-1 protein was detected in outer medulla and was markedly (6-fold) higher in SHR. TSC protein was detected in the cortex and was not overexpressed in SHR. Aquaporin-1 protein was detected in all three regions and was not overexpressed in SHR. Aquaporin-2 and ROMK-1 proteins were detected in all three regions, but were moderately elevated (2-fold) only in the SHR inner medulla. Na⁺-K⁺-ATPase and NHE-3 proteins were detected in all three regions. Na⁺-K⁺-ATPase-α₁ was modestly (25%) increased in SHR outer and inner medulla, whereas NHE-3 was moderately (2-fold) increased in the SHR cortex and inner medulla. NBC-1 protein was detected only in the cortex and was higher (2-fold) in SHR. mRNA levels of BSC-1, aquaporin-2, and ROMK-1 were not elevated in SHR, indicating a post-translational mechanism of protein overexpression. High-dose furosemide increased fractional sodium excretion more in SHR than WKY (3-fold). We conclude that increased expression of BSC-1, and to a lesser extent, aquaporin-2, ROMK-1, NHE-3, and NBC-1 may contribute to the pathogenesis of hypertension in the SHR.

The kidneys play an important role in regulating blood pressure by controlling sodium balance (Guyton, 1989, 1991). Although many systems can influence blood pressure in the short term, the long-term blood pressure setting ultimately depends on renal sodium excretion. In this regard, transporters in the apical and basolateral membranes of epithelial cells in nephrons are responsible for sodium reabsorption and fluid balance and are therefore important candidates for involvement in the development of hypertension (Su and Menon, 2001).

The results of renal transplantation experiments in genetically hypertensive and normotensive rat strains are consistent with the concept that the kidneys regulate long-term levels of arterial blood pressure, and a defect in the kidney is importantly involved in the pathogenesis of genetic hypertension (Rettig et al., 1990; Frey et al., 2000; Grisk et al., 2002). Moreover, studies using isolated perfused kidneys from spontaneously hypertensive rats (SHR) reveal an intrinsic renal abnormality in Na⁺ excretion that may contribute to the maintenance of hypertension in SHR (Heckmann et al., 1989). Furthermore, examination of the pressure-natriuresis relationship and the effect of furosemide (an inhibitor of the bumetanide-sensitive Na⁺-K⁺-2Cl⁻ cotransporter, also called BSC-1 or NKCC2) on this relationship demonstrates a resetting of the pressure-natriuresis process in SHR by a mechanism involving, in part, BSC-1 (Raine et al., 1984).

BSC-1 in the thick ascending limb of Henle’s loop mediates reabsorption of approximately 25% of the filtered Na⁺ load and is selectively inhibited by loop diuretics (Russell, 2000; Shankar and Brater, 2003). Because BSC-1 is the principal apical Na⁺ entry pathway in the thick ascending limb of Henle, it is a prime candidate for long-term dysregulation of arterial blood pressure. In support of this concept, recent studies demonstrate that enhanced expression of BSC-1 in the thick ascending limb causes sodium retention in rats.

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ABBREVIATIONS: SHR, spontaneously hypertensive rat(s); WKY, Wistar-Kyoto; RT-PCR, reverse transcription-polymerase chain reaction; PBS, phosphate-buffered saline; BSC-1, bumetanide-sensitive Na⁺-K⁺-2Cl⁻ cotransporter; TSC, thiazide sensitive Na⁺-Cl⁻ cotransporter; AQP-1 or -2, aquaporin-1 or -2; NBC-1, type-1 Na⁺-HCO₃⁻ cotransporter; NHE-3, type-3 Na⁺-H⁺ exchanger; ROMK-1, inwardly rectifying K⁺ channel; MABP, mean arterial blood pressure; HR, heart rate; RBF, renal blood flow; UV, urine volume; GFR, glomerular filtration rate.
with congestive heart failure (Nielsen et al., 1997). Moreover, BSC-1 is up-regulated in rats with small to moderate myocardial infarctions (Nogae et al., 2000), dehydration, and cardiac failure (Marumo et al., 1998). Finally, mutations in the NKCC2 gene, which encodes BSC-1 presumably by inducing a loss of function of the transporter, result in Bartter’s syndrome, an inherited disease characterized by hypokalemic metabolic alkalosis, hypercalciuria, salt wasting, and volume depletion resulting in hypotension (Simon et al., 1996; Vargas-Poussou et al., 1998). Clearly, alterations in BSC-1 activity can influence long-term levels of arterial blood pressure.

Because BSC-1 influences arterial blood pressure, it is conceivable that increases in BSC-1 activity and/or expression contribute to genetic hypertension. The expression of BSC-1 in SHR has not been previously examined, but it can be hypothesized that changes in BSC-1 expression may play a critical role in the development of altered sodium handling in SHR thereby contributing to the pathogenesis of genetic hypertension. Accordingly, in the present study, we determined the expression of BSC-1 protein and mRNA in the outer cortex, inner stripe of outer medulla, and inner medulla of kidneys obtained from both SHR and Wistar-Kyoto (WKY) normotensive rats. To determine the specificity of any observed changes in BSC-1 expression, we also compared protein expression of the thiazide sensitive Na\(^+\)-Cl\(^-\) co-transporter (TSC), the type-3 Na\(^+\)-H\(^+\) exchanger (NHE-3), Na\(^+\)-K\(^+\)-ATPase\(\alpha\_1\), the inwardly rectifying K\(^+\) channel (ROMK-1), the type-1 Na\(^+\)-HCO\(_3\)^\(^-\) cotransporter (NBC-1), aquaporin-1, and aquaporin-2. Finally, we observed a marked increase in the expression of BSC-1 protein in the inner stripe of the outer medulla of SHR, we also compared the acute effects of the loop diuretic furosemide on hemodynamics and renal function in SHR versus WKY.

### Materials and Methods

**Animals.** Male WKY rats (11–13 weeks of age) and age-matched SHR were obtained from Taconic Farms (Germantown, NY). Rats were allowed to acclimate to the University of Pittsburgh Animal Facility for at least 1 week before initiation of the experimental protocols. Protocols were approved by the Institutional Animal Care and Use Committee. Animals were divided into two groups: one to be used for the immunoblotting and RT-PCR experiments and the other group for the furosemide infusion study.

**Kidney Dissection and Tissue Preparation for Immunoblotting.** Blood pressures in SHR and WKY rats were measured as described below; following which, kidneys were rapidly excised and washed in ice-cold PBS. The left kidneys were dissected to obtain outer cortex, inner stripe of outer medulla, and inner medulla, and the dissected tissues were homogenized in lysis buffer containing Tris-HCl, 2% SDS, glycerol, phenylmethylsulfonyl fluoride, and protease inhibitors. Protein concentrations were measured using the BCA protein assay. Whole homogenates from the cortex, outer medulla, and inner medulla were used to study the specific regional expression of the different proteins.

**Electrophoresis and Immunoblotting.** Proteins were solubilized at 60°C for 15 min in Laemmli sample buffer. SDS-polyacrylamide gel electrophoresis was performed on gradient polyacrylamide gels (4–12%) loaded with 20 μg of protein per lane. For immunoblotting, proteins were transferred electrophoretically to polyvinylidene difluoride membranes. Membranes were blocked in 5% milk for 2 h, probed overnight at 4°C with the respective primary antibodies in PBS containing 1% milk: BSC-1 (1:2,000), TSC (1:600), aquaporin-1 (AQP-1; 1:2,000) and aquaporin-2 (AQP-2; 1:2,000), NBC-1 (1:1,100), NHE-3 (1:1,100), Na\(^+\)-K\(^+\)-ATPase\(\alpha\), (1:5,000), and ROMK-1 (1:1,000). Membranes were probed with β-actin (1:10,000; Sigma-Aldrich, St. Louis, MO) for 1 h to determine loading efficiency. BSC-1, TSC, AQP-1, and AQP-2 were the kind gift of Dr. M. A. Knepper (National Institutes of Health). All other primary antibodies were from Chemicon International (Temecula, CA). All antibodies were found to be highly specific for the protein of interest, and their specificity has been extensively characterized. Subsequently, membranes were exposed to a secondary horseradish peroxidase conjugated donkey anti-rabbit polyclonal antibody (1:5,000; Pierce Biotechnology Inc., Rockford, IL) in PBS containing 1% milk for 1 h at room temperature. Bound antibodies were visualized using a luminol-based enhanced chemiluminescence substrate (Supersignal West Dura Extended Duration Substrate; Pierce Biotechnology Inc.) before exposure to X-ray film (Kodak 165-1579; Eastman Kodak Co., Rochester, NY). Densitometric analysis was performed using ImageQuant TL (Amersham Biosciences Inc., Piscataway, NJ), and band densities were normalized to β-actin.

**RNA Isolation and RT-PCR.** The right kidneys were used for RNA isolation. Kidneys were dissected to obtain outer cortex, inner stripe of outer medulla, and inner medulla. RNA was isolated from the dissected tissues using TRIzol reagent (Invitrogen, Carlsbad, CA) as per the manufacturer’s instructions. By using the primer sequences listed in Table 1, RNA (0.5 μg) was reverse transcribed and amplified using a Titanium One-step RT-PCR kit (BD Biosciences Clontech, Palo Alto, CA). Each PCR cycle (total 30 cycles) consisted of denaturing at 94°C for 30 s, annealing at 64°C for 30 s, and extension at 68°C for 60 s. RT-PCR products were separated on a 1.2% agarose gel and visualized by incorporating ethidium bromide in the gel. Densitometric analysis was performed using ImageQuant TL, and band densities were normalized to β-actin.

**Effects of Furosemide in SHR and WKY Rats.** Acute renal and hemodynamic responses to the loop diuretic furosemide (Sigma-Aldrich) at bolus doses of 3 and 50 mg/kg were measured in SHR and WKY. Each rat was anesthetized with pentobarbital (45 mg/kg; Sigma/RBI, Natick, MA) and placed on an isothermal pad. Temperature was monitored with a rectal probe thermometer and kept at 37°C with a heat lamp. A short section of polyethylene tubing (PE-240) was placed in the trachea to facilitate respiration. The left carotid artery was exposed and cannulated with PE-50 tubing for blood sample collections and for mean arterial blood pressure (MABP) and heart rate (HR) measurements via a digital blood pressure analyzer (Micro-Med, Inc., Louisville, KY). A PE-50 catheter was placed in the left jugular vein for infusion of [\(^{14}\)C]inulin (0.5 μCi bolus and 0.055 μCi/100 μl of 0.9% saline/min; PerkinElmer Life and Analytical Sciences, Boston, MA). A PE-20 catheter was also inserted.

### Table 1

<table>
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<tr>
<th>Accession Number</th>
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<td>BSC-1</td>
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<td></td>
<td></td>
<td>Reverse: GAACTGGGAGAGTGTCACAAC 2676</td>
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<tr>
<td>ROMK-1</td>
<td>AF081365</td>
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<td>421</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse: ACCTTGGGTTCAGAGAGGTACA 1725</td>
<td></td>
</tr>
<tr>
<td>AQP-2</td>
<td>NM01209</td>
<td>Forward: AAGAGAAAGAGAGAGGGAGGGA 46</td>
<td>753</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse: GGGGAACAGCAGGATTTGAG 798</td>
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into the jugular vein to administer bolus doses of furosemide. An incision was made in the rat’s abdomen, and a PE-10 catheter was placed in the left and right ureters to facilitate collection of urine. A flow probe (model 1RB; Transonic Systems, Inc., Ithaca, NY) was placed on the left renal artery for determination of renal blood flow (RBF).

Infusions of saline and \(^{14}\text{C}\)inulin were initiated, and following a 2-h stabilization period, a urine sample and midpoint blood sample were collected during a 30-min baseline clearance period. MABP, HR, and RBF were recorded at 5-min intervals and averaged. A bolus dose of furosemide (3 mg/kg) was administered, and a 10-min stabilization period was allowed, following which, MABP, HR, and RBF were recorded, and a urine sample and midpoint blood sample were collected during an additional 30-min clearance period. Another bolus dose of furosemide (50 mg/kg) was administered and the above procedure repeated.

Rats were euthanized and the left kidneys were weighed. Urine volume (UV) was determined gravimetrically for each of the collection periods, and samples were analyzed for \(^{14}\text{C}\)inulin radioactivity (model 2500TR liquid scintillation analyzer; Packard Instrument Company, Downers Grove, IL) and sodium/potassium concentrations (model IL943 flame photometer; Instrumentation Laboratory, Lexington, MA). Renal clearance of \(^{14}\text{C}\)inulin was used as an estimate of glomerular filtration rate (GFR). The RBF, GFR, UV, and excretion rates of sodium (\(\text{UNaV}\)) and potassium (\(\text{UKV}\)) were corrected to kidney weight measured in grams.

Statistical Analysis. All data are presented as mean ± S.E.M. Comparisons between groups were made by unpaired \(t\) test. Group comparisons for the furosemide study were performed using analysis of variance and Fisher’s least significant difference test. \(p\) values <0.05 were considered significant.

Results

Expression of BSC-1 and TSC Proteins. BSC-1 and TSC are sodium transporters that are expressed predominantly in the apical membranes of the thick ascending limbs and distal tubules, respectively. Semiquantitative immunoblotting showed that the expression of BSC-1 protein was found to be significantly higher in the outer medulla of SHR compared with WKY (6-fold, \(p < 0.05\); Fig. 1). We could not detect expression of BSC-1 in the outer cortex and inner medulla of either group. In contrast to BSC-1, TSC protein was expressed primarily in the outer cortex, and the expression in SHR versus WKY was similar (Fig. 1).

Expression of NHE-3, Na\(^+\)-K\(^+\)-ATPase-\(\alpha_1\), and NBC-1 Proteins. Previous studies have shown that expression of NHE-3 in the proximal tubule and Na\(^+\)-K\(^+\)-ATPase in the collecting duct are up-regulated in the SHR kidney (Gesek and Schoolwerth, 1991; Tsuruya et al., 1991). Semiquantitative immunoblotting revealed expression of NHE-3 protein in the outer cortex, outer medulla, and inner medulla with significantly higher expression in the cortex and inner medulla of SHR kidneys (2-fold, \(p < 0.05\); Fig. 2). Na\(^+\)-K\(^+\)-ATPase-\(\alpha_1\) protein expression was detected in the outer cortex and outer and inner medulla, and Na\(^+\)-K\(^+\)-ATPase-\(\alpha_1\) protein expression was significantly, albeit modestly (approximately 25%), higher in the outer and inner medulla of the SHR compared with WKY (\(p < 0.05\); Fig. 2). We also detected expression of NBC-1 protein, which is localized to basolateral membranes of proximal tubules, in the outer cortex, but not outer or inner medulla, and found the expression of NBC-1 protein to be higher in the outer cortex of the SHR compared with WKY (2-fold, \(p < 0.05\); Fig. 3).

Expression of ROMK-1 Protein. ROMK-1 in the apical membranes of the thick ascending limb allows for apical K\(^+\) recycling for the efficient functioning of BSC-1, and ROMK-1 in the distal convoluted tubule and collecting duct participates in K\(^+\) secretion. Semiquantitative immunoblotting detected expression of ROMK-1 protein in the outer cortex and outer and inner medulla, which is consistent with its localization. Although ROMK-1 has been originally cited as being a 45 kDa protein (Ecelbarger et al., 2001), under our experimental conditions, the band of interest was found to be around 85 kDa which is

![Fig. 1. Expression of BSC-1 and TSC in the kidney outer cortex, inner strip of outer medulla, and inner medulla of SHR and WKY rats. In panel A, each lane was loaded with 20 μg of protein from a different rat. Blots were probed with rabbit anti-BSC-1, anti-TSC, and mouse anti-β-actin antibodies. Panel B summarizes densitometric analysis of BSC-1 and TSC normalized to β-actin. Densitometric analysis revealed that expression of BSC-1 was significantly higher in the outer medulla of SHR, whereas expression of TSC in the cortex was similar in SHR and WKY. * \(p < 0.05\) compared with WKY. Values represent means ± S.E.M. for seven observations.](image-url)
approximately double the size of the ROMK monomer and is believed to represent homodimeric and/or heterodimeric complexes formed by ROMK isoforms (Xu et al., 1997). Expression of ROMK-1 protein in the outer cortex and outer medulla was similar between both groups. However, expression of ROMK-1 in the inner medulla was significantly higher in the SHR compared with WKY (2-fold, \( p < 0.05 \); Fig. 3).

Expression of AQP-1 and AQP-2 Proteins. Several studies have demonstrated altered expression and apical targeting of aquaporins in water balance disorders (Nielsen et al., 1997; Bickel et al., 2002). AQP-1 and AQP-2 protein expression was detected in the outer cortex and outer and inner medulla. The expression of AQP-2 was significantly higher in the inner medulla, but not outer cortex or outer...
medulla, of the SHR compared with WKY (2-fold, \( p < 0.05 \)); Fig. 4). The expression of AQP-1 between both groups was similar in all three kidney regions (Fig. 4).

**Expression of BSC-1, AQP-2, and ROMK-1 mRNA.** To determine whether the increases in BSC-1, AQP-2, and ROMK-1 protein expression were accompanied by similar increases in mRNA expression, we examined the mRNA expression for these proteins in outer cortex, outer medulla, and inner medulla using RT-PCR. RT-PCR demonstrated that levels of BSC-1, ROMK-1, and AQP-2 mRNA in both groups were similar (Fig. 5), suggesting that post-transcriptional events are likely to be responsible for the increase in the expression of these proteins.

**Effects of BSC-1 Inhibition.** As a measure of the relative activity of the BSC-1 transporter, we measured the response to low and high doses of furosemide (3 and 50 mg/kg, respectively) in WKY and SHR. Administration of furosemide lowered the mean arterial blood pressure significantly and resulted in normalization of blood pressure in SHR (Fig. 6, A and B). The effects of furosemide on renal blood flow (Fig. 6, C and D) and renal vascular resistance (Fig. 7, A and B) in WKY and SHR were similar; however, the reduction in glomerular filtration rate (Fig. 7D) was greater in SHR compared with WKY with both doses of furosemide (44.4 and 69.6% versus 20.6 and 49.7%, respectively). In contrast, diuretic responses to both low and high doses of furosemide (Fig. 8B) were augmented in WKY (1099 and 1043%) compared with SHR (740 and 904%). Furosemide also increased absolute (Fig. 8, C and D) and fractional (Fig. 9, A and B) sodium excretion in the SHR and WKY. However, the percentage increase in absolute and fractional sodium excretion with 50 mg/kg furosemide was higher in the SHR compared with WKY (1350% versus 766%, respectively).

**Discussion**

The present study was based on the concept that the pathogenesis of sustained hypertension involves the kidneys. Altered renal sodium handling in the SHR has been previously reported (Roman and Cowley, 1985; Firth et al., 1989); how-
ever, the factors contributing to this effect are unknown. We have shown through semiquantitative immunoblotting that the expression of sodium transporters BSC-1, Na\(^+\)-K\(^+\)-ATPase-\(\alpha_1\), NHE-3, NBC-1, the potassium channel ROMK-1, and the water channel AQP-2 are elevated in the SHR, suggesting that the pathophysiology of altered renal excretory function in genetic hypertension may involve alterations in several transporters located along the nephron. TSC protein expression was not changed suggesting that the distal convoluted tubule sodium transport mediated by TSC is not affected in this genetic model of hypertension.

In the present study, expression of BSC-1 protein was elevated in the SHR more so than any other protein examined. This finding suggests that the increase in BSC-1 expression may be the most important contributing factor to altered tubular function in SHR. In support of this conclusion, our results also demonstrate that when the response to furosemide (an inhibitor of BSC-1) is expressed as a percentage of the baseline sodium excretion (either absolute or fractional), the natriuretic response to furosemide is greater in SHR versus WKY. Thus, both our biochemical and pharmacological data are consistent with an important role of BSC-1 in the pathophysiology of hypertension in SHR. Although it is possible that ROMK-1 activity becomes rate limiting with regard to sodium reabsorption in SHR with increased BSC-1 expression, our results with furosemide indicate that the increased expression of BSC-1 is associated with increased sodium transport.

In the present study, acute administration of furosemide lowered arterial blood pressure in SHR, but not WKY. Generally, changes in renal excretory function do not alter arterial blood pressure within the time frame of the current study. However, anesthetized rats are much more sensitive to volume depletion. Therefore, it is possible that the acute reduction in blood pressure induced by furosemide in SHR was secondary to more severe volume depletion following furosemide-induced natriuresis. At any rate, it is important to note that despite the greater reduction in renal perfusion pressure (which would tend to attenuate sodium excretion), furosemide still had a greater effect on sodium excretion in SHR.

Importantly, the increased steady-state levels of BSC-1, ROMK-1, and AQP-2 proteins in the SHR were not accompanied by an increase in their respective steady-state mRNA levels, suggesting that a post-transcriptional mechanism is responsible for the overexpression of these transporters. In this regard, our findings are consistent with previous reports demonstrating that post-transcriptional mechanisms are re-

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**Fig. 6.** MABP and RBF (A and C) and percentage change in MABP and RBF (B and D) following furosemide administration to SHR and WKY rats. a, \(p < 0.05\) versus baseline; b, \(p < 0.05\) versus corresponding value in WKY. Values represent means \(\pm\) S.E.M. for six observations.
sponsible for the increase in protein expression and activity of the NHE-3 transporter as well as Na\(^+\)/K\(^+\)-ATPase in the SHR (Hayward et al., 1999; LaPointe et al., 2002). Whether higher steady-state levels of BSC-1, ROMK-1, and AQP-1 in SHR are due to greater translational efficiency or enhanced stability of the proteins or both cannot be deduced from the present study.

Our study is consistent with the idea that an intrinsic abnormality in sodium handling by the kidney contributes to the pathogenesis of hypertension in SHR. Studies in an experimental model of prenatally programmed hypertension suggest that prenatal programming of hypertension involves transcriptional up-regulation of sodium transporters BSC-1 and TSC in the thick ascending limb and distal convoluted tubule, respectively (Manning et al., 2002). Also, development of gene-targeting techniques in mice has enabled direct assessment in vivo of the roles of different apical renal Na\(^+\)/H\(^+\) transporters in the control of extracellular fluid volume and blood pressure (Takahashi et al., 2000, 2002). In this regard, gene-targeting experiments show that the most detrimental mutation is the inactivation of the NKCC2 gene, which directly affects the countercurrent urine-concentrating mechanism and triggers profound disorganization of renal tissue.

The mechanisms mediating the up-regulation of BSC-1 expression in genetic hypertension in SHR are yet to be determined. Several hormones such as vasopressin, angiotensin, prostaglandins, catecholamines, and atrial natriuretic factor may be involved in BSC-1 regulation. In this regard, our finding that the water channel AQP-2 is also up-regulated in the SHR may implicate vasopressin. Both BSC-1 and AQP-2 are vasopressin-regulated proteins, and administration of vasopressin increases sodium and water reabsorption in the thick ascending limb and the collecting ducts (Kim et al., 1999; Hasler et al., 2002). The vasopressin-induced expression of BSC-1 in the thick ascending limb may be responsible for the enhanced urinary concentrating ability associated with sustained antidiuresis. Moreover, vasopressin regulates water permeability across the collecting duct by trafficking AQP-2 from intracellular vesicles to the apical plasma membrane (Marplels et al., 1995). Thus, the increased expression of BSC-1 and aquaporin-2 could well be a vasopressin-mediated response. However, our finding that AQP-2 is only elevated in the inner medulla, not the outer medulla, weighs against this conclusion because vasopressin increases AQP-2 expression all along the medullary collecting duct.

The renin-angiotensin system also regulates expression of epithelial transporters and could be involved in up-regulation of transporter expression in SHR. Studies by several groups demonstrate that administration of angiotensin II increases BSC-1 expression in rat kidneys (Kwon et al., 2003)
and that expression of BSC-1 is reduced in mice lacking angiotensin-converting enzyme (Klein et al., 2002). Additionally, studies with angiotensin-converting enzyme inhibitors in rats with cardiac failure show that blockade of angiotensin II synthesis normalizes both BSC-1 expression and renal sodium excretion suggesting that angiotensin II influences renal sodium handling in cardiovascular disease via BSC-1 (Staahltoft et al., 2002).

Prostaglandins may also regulate renal sodium excretion in SHR. Recent studies with cyclooxygenase inhibitors show that COX-2 inhibitors increase BSC-1 expression in rat kidneys, thus implicating a role for prostaglandins in BSC-1 regulation (Fernandez-Llama et al., 1999).

Although our study demonstrates a role for BSC-1 in hypertension in the SHR, it does not rule out the involvement of other sodium transporters. Previous studies have shown that sodium reabsorption in the proximal tubule is also increased in the SHR (Biollaz et al., 1986; Firth et al., 1989). Studies also show that sodium transporters of the proximal tubule, namely the NHE-3 and Na\(^+\)/K\(^+\)-ATPase, are up-regulated in the SHR kidney (Tsuruya et al., 1991; LaPointe et al., 2002). Our data are in concordance with these findings. In addition, our studies indicate that expression of the potassium channel ROMK-1 is also higher in the inner medulla of the SHR. Thus, it appears that the pathophysiology of genetic hypertension is complex and that several transporters located along the nephron may be involved.

As noted above, previous studies clearly indicate that sodium reabsorption in the proximal tubule is increased in the SHR. Thus, it is conceivable that the observed increases in BSC-1 expression in the inner strip of the outer medulla (corresponding to the thick ascending limb) in SHR are merely secondary to increased proximal tubular transport in SHR. However, this seems unlikely because numerous studies with loop and thiazide diuretics indicate that, in normal animals and humans, changes in sodium transport in one nephron segment are always accompanied by opposite changes in sodium transport in all other nephron segments. For example, chronic inhibition of sodium transport in the thick ascending limb with loop diuretics or in the distal convoluted tubules with thiazide diuretics causes a compensatory increase in sodium reabsorption and/or transporters in other nephron segments (Ellison et al., 1989; Abdallah et al., 2001; Na et al., 2003). This phenomenon provides the basis for the synergy that is achieved when diuretics acting at different nephron segments are combined to provide sequential blockade (Flouvat et al., 1991). Therefore, it is unlikely that increases in proximal tubule transport would cause increases in BSC-1 expression in the thick ascending limb because the expected response would be a decrease, not an increase, in BSC-1 expression.

In summary, the present study demonstrates a marked increase in the expression of BSC-1 and more modest increases in the expressions of Na\(^+\)/K\(^+\)-ATPase-\(\alpha_1\), NHE-3, NBC-1, ROMK-1, and AQP-2 in the SHR kidney. Therefore, dysregulation of the steady-state levels of renal epithelial transporters may importantly contribute to the pathogenesis of hypertension in genetic hypertension.
References


Acknowledgments

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Fig. 9. Fractional sodium (FeNa) and potassium (FeK) excretion rates (A and C) and percentage change in fractional sodium and potassium excretion rates (B and D) following furosemide administration to SHR and WKY rats. a, p < 0.05 versus baseline; b, p < 0.05 versus corresponding value in WKY. Values represent means ± S.E.M. for six observations.


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