Upregulation of renal BSC1 and TSC in prenatally programmed hypertension

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Manning, Jennifer, Kathleen Beutler, Mark A. Knepper, and V. Matti Vehaskari. Upregulation of renal BSC1 and TSC in prenatally programmed hypertension. Am J Physiol Renal Physiol 283: F202–F206, 2002. First published February 5, 2002; 10.1152/ajprenal.00358.2001.—Prenatal factors, especially intrauterine growth retardation, have been shown to correlate with the risk of essential hypertension in adult life, but the mechanisms are unknown. An experimental model of prenatal programming of hypertension in the rat, induced by a maternal low-protein diet during pregnancy, was employed to study the role of renal Na reabsorption in the pathogenesis. The abundance of the apical Na transporter type III Na/H exchanger (NHE3), bumetanide-sensitive Na-K-2Cl cotransporter (BSC1), thiazide-sensitive Na-Cl cotransporter (TSC), and the amiloride-sensitive epithelial Na channel (ENaC) was determined by semiquantitative immunoblotting in kidneys from the offspring at 4 wk of age, before hypertension became manifest. There were no significant differences between the experimental and control rats in the abundance of NHE3 or any of the ENaC subunits. In contrast, the quantity of BSC1 in the experimental group was increased to 302% of control (P < 0.001) and that of TSC to 157% of control (P < 0.05). Determination of specific mRNA levels by ELISA-linked RT-PCR revealed a significantly increased BSC1 mRNA at 1 day (P < 0.01), 4 wk (P < 0.01), and 8 wk (P < 0.001) of age, and a significantly increased TSC mRNA at 4 wk of age (P < 0.05) in the experimental group. The results suggest that prenatal programming of hypertension involves transcriptional upregulation of Na transport in thick ascending limb and distal convoluted tubule.

sodium transport; thick ascending limb; distal convoluted tubule; essential hypertension; pressure natriuresis; bumetanide-sensitive sodium-potassium-2 chloride cotransporter; thiazide-sensitive sodium-chloride cotransporter

METHODS

Animal model. As previously described by our laboratory (20, 26), timed-pregnant Sprague-Dawley rats were placed on a 6% protein (low protein; LP) or an isocaloric 20% protein (control) diet from the gestational age of 12 days through the remainder of the pregnancy (21.5 days). We have previously shown this protocol to result in a proportionate 15–20% reduction in kidney and body weights at birth (26). After birth, the pups were nursed by their own mothers, who were on standard 20% protein rat chow, and weaned to the same standard chow at 4 wk of age. Systolic blood pressures were measured oscillometrically by the tail-cuff method (Kent Scien...
entific, Litchfield, CT). The rats were trained to remain calm in a warmed restrainer during the measurement. The mean of four to six readings was recorded at each session. Offspring, taken randomly from different litters, were killed at 1 day, 4 wk, and 8 wk of age with a lethal dose of pentobarbital sodium; kidneys were rapidly removed without perfusion before the heart stopped. One kidney was used for total RNA isolation and the other for protein isolation. Fetal kidneys were obtained on gestational day 19; one kidney from six to eight pups from each pregnancy was pooled for RNA isolation.

Protein quantification. Semiquantitative immunoblotting, as previously described in detail (13), was carried out using previously characterized antibodies to type III Na-H exchanger (NHE3), BSC1, TSC, as well as α-, β-, and γ-subunits of the amiloride-sensitive epithelial Na channel (ENaC) (10, 12, 14, 21). Briefly, total protein was isolated from whole kidneys by homogenization in an ice-cold isolation solution of 250 mM sucrose, 10 mM triethanolamine, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride. Final protein concentration was adjusted to 2 μg/ml, and the samples were solubilized in Laemmli sample buffer. Importantly, for each set of samples, an initial gel was stained with Coomassie blue to confirm equal loading among samples (13). SDS-PAGE was performed on polyacrylamide gels, and the bands were transferred to nitrocellulose membranes, probed overnight at 4°C with the respective primary antibodies, and then incubated with secondary antibody (horseradish peroxidase-conjugated donkey anti-rabbit IgG; Pierce no. 31458). The bands were visualized by chemiluminescence (LumiGLO, Kirkegaard and Perry Laboratories, Gaithersburg, MD), exposed to X-ray film, and quantified by laser densitometry (model PDS1-P90, Molecular Dynamics).

mRNA quantification by ELISA-linked RT-PCR. Total RNA was isolated from freshly harvested kidneys using the Ultraspec RNA system (Biotecx Laboratories), treated with DNase I (Boehringer Mannheim), and reverse transcribed as previously described (27). PCR primers (18–22 bp) for BSC1, TSC, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed to produce an amplicon length of 150–250 bp; in addition, an 18- to 22-bp biotinylated capture probe complementary to the midportion of each amplicon was synthesized. An ELISA-linked PCR kit (catalog no. 1636120, Roche) was used for quantification of the specific cDNA species, according to the manufacturer’s instructions. Briefly, GAPDH primers were added to each PCR reaction as an internal control, and a digoxigenin label was incorporated into all amplicons by including 0.02 mM digoxigenin-dUTP in the PCR mix. The labeled PCR products were then denatured and hybridized to their respective biotinylated capture probes in an aqueous solution, followed by immobilization on a streptavidin-coated microtiter plate. The bound hybrids were detected by peroxidase-conjugated anti-digoxigenin antibodies and read by a standard microplate reader. Preliminary experiments were carried out to optimize the conditions for each primer pair, including the choice of a PCR cycle number corresponding to the exponential phase of the amplification. Negative (including absence of RT) and positive (using rat poly(A) RNA) controls were always carried out simultaneously throughout the RT-PCR-ELISA procedure.

Statistical analysis. All results are expressed as means ± SE. All comparisons between the control and experimental groups were done by Student’s t-test, and statistical significance was defined as P < 0.05.

RESULTS

Weights and blood pressures. The LP offspring appeared healthy at birth but had ~15% lower birth weights than the control offspring. By 4 wk of age, the weight difference had disappeared. The systolic blood pressure of the 4-wk-old LP rats was similar to that of control rats (102 ± 2 mmHg (LP) vs. 103 ± 1 mmHg (control)), but at 8 wk of age the LP rats had developed significant hypertension (127 ± 3 vs. 107 ± 2 mmHg, respectively; P < 0.001); there were no significant differences between males and females. These findings are similar to our previous results (20, 26).

Na transport protein abundance. The abundance of apical Na transport proteins NHE3, BSC1, TSC, and of all three subunits of ENaC was determined by immunoblotting of kidneys obtained from LP and control rats at 4 wk of age. No differences were seen between males and females. The combined results of both sexes are depicted in Figs. 1 and 2. The LP and control groups did not differ significantly in the abundance of NHE3 or of any of the ENaC subunits. In contrast, there was a marked increase in both BSC1 and TSC in the LP group; BSC1 abundance was 307% of control (P < 0.001) and TSC 157% of control (P < 0.05). Immunoblotting for BSC1 and TSC in 1-day-old rats failed to consistently detect either BSC1 or TSC, presumably because of low levels of expression.

Na transport protein mRNA levels. ELISA-linked RT-PCR was used to quantify specific mRNA levels in whole kidneys obtained from fetuses on gestational day 19 and from postnatal animals at 1 day, 4 wk, and 8 wk of age. Results from both sexes were combined and normalized to the GAPDH mRNA level. As shown in

Fig. 1. Semiquantitative immunoblots of Na transporter proteins in 6 control and 6 low-protein (LP) kidneys at 4 wk of age. The abundance of bumetanide-sensitive Na-K-2Cl cotransporter (BSC1) and thiazide-sensitive Na-Cl cotransporter (TSC) was significantly increased in LP kidneys (*; see Fig. 2). ENaC, amiloride-sensitive epithelial Na channel; NHE3, type III Na/H exchanger.

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Fig. 3. BSC1 mRNA in kidneys from control (open bars) and LP (solid bars) rats determined by ELISA-linked RT-PCR on gestational day 19 (E19; n = 5 and 7, respectively), postnatal day 1 (n = 7 and 7, respectively), postnatal day 28 (n = 9 and 5, respectively), and postnatal day 56 (n = 8 and 5, respectively). Values are normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. **P < 0.01. ***P < 0.001.

DISCUSSION

The fact that the prenatal environment can modify the adult blood pressure profile is now firmly established and is supported by both epidemiological data (2, 6, 7, 19, 22) and experimental studies (18, 20, 26, 31). The mechanisms in humans are poorly understood and difficult to study. Several experimental models, including those of maternal protein (18, 26, 31) and caloric (30) restriction, as well as of maternal glucocorticoid treatment (8, 23), have been used to program adult hypertension in offspring. It appears likely that the various manipulations work through a common pathway; the experimental models may therefore reveal the mechanism(s) behind human blood pressure programming.

The present study was based on the hypothesis, suggested by previous work from our laboratory (20), that the target of the programming is renal Na handling by the fetus. The hypothesis is consistent with the concept that the pathogenesis of sustained hypertension involves the kidneys because a normal renal pressure-natriuresis response would correct the hypertension (11). Brenner and co-workers (3) have proposed that the fault lies in the reduced final number of nephrons, which limits filtration of Na and leads to an expansion of extracellular fluid volume. Indeed, our laboratory (26) and others (31) have documented a reduced nephron complement in the present rat model. However, the decrease in the number of glomeruli is only ~25–30%, and the total renal glomerular filtration rate is only marginally or not reduced at all due to hyperfiltration of the remaining glomeruli (20, 31). This is difficult to reconcile with the concept of reduced filtration being the sole mechanism of Na retention. The other aspect of renal Na handling, tubular reabsorption, has not been previously investigated in any of the experimental models of prenatal blood pressure programming.
The present study examined the expression of four key apical Na transport proteins. Because apical Na entry is believed to constitute the rate-limiting step in Na reabsorption in the different nephron segments, these transporters are critical for the regulation of Na balance and extracellular volume. The results show upregulation of BSC1 and TSC, the apical Na transporters of TAL and DCT, respectively, at both the mRNA and the protein level. It is likely that the upregulation reflects increased Na reabsorption in these two segments. NHE3 expression was not changed, suggesting that proximal tubule Na transport, at least the major fraction mediated by NHE3, is not affected by the prenatal programming or that NHE3 is upregulated by mechanisms not associated with altered protein abundance. Because ENaC activity is important for Na homeostasis and under hormonal (mineralocorticoid) control (21), it might be expected to be a target of programming. However, no upregulation of any of the ENaC subunit proteins was detected.

The prenatally programmed hypertension in our model develops between 4 and 8 wk of life and becomes more pronounced with age (20, 26). The upregulation of BSC1 and TSC is present before the development of hypertension, at 4 wk of age, and is therefore unlikely to be a consequence of increased blood pressure. Rather, we postulate that enhanced Na transport in TAL and DCT participates in the pathogenesis of hypertension by leading to an expansion of extracellular volume. Equally important is the finding that the Na transporters were not downregulated after the hypertension became manifest, at 8 wk of age. Wang et al. (29) have recently shown that downregulation of TSC is an important component of the pressure-natriuresis response designed to correct hypertension by increasing renal Na excretion. Therefore, prenatal programming of the Na transporters may override the normal pressure-natriuresis mechanism.

The mechanisms of the upregulation of BSC1 and TSC require further study. The pattern of Na transporter expression in the present study resembles that described in rats with 5/6 nephrectomy (15). The postulated stimulus for increased TAL and DCT Na reabsorption in the latter model was increased distal delivery of Na and fluid per nephron due to hyperfiltration of the remaining glomeruli. Upregulation of BSC1 can also be achieved by chronic saline loading of rats with normal kidneys, presumably by a similar mechanism (9). We cannot rule out a role for increased distal delivery in the transporter upregulation in our model, but we think it is unlikely to be the primary mechanism for the following reasons. First, with the modest reduction in the number of glomeruli in the present model, compared with the 83% reduction in the remnant kidney model (15), one would expect very different filtered loads and distal deliveries of Na per nephron, yet the magnitude of the transporter upregulation was similar in the two models. Second, BSC1 mRNA was already upregulated at 1 day of life, suggesting prenatal onset. An increased filtered load before birth is unlikely to be the stimulus because of the very low glomerular filtration rate of fetal kidneys (24). Conceivably, the observed upregulation could be the result of an accelerated increase in filtered load during the first day of life in the LP group, but a more plausible explanation is that the programming of the BSC1 gene expression occurs by mechanisms independent of filtered load and distal delivery of Na.

The signal(s) from mother to fetus that result in transporter upregulation are unknown. One proposed factor is fetal overexposure to maternal glucocorticoids due to decreased placental activity of the 11β-hydroxysteroid dehydrogenase type 2 enzyme (16, 25). Maturation of renal Na transport, measured as Na-K-ATPase expression, is regulated by glucocorticoids (4); abnormal glucocorticoid exposure could therefore have a direct effect on the maturing kidney. How glucocorticoid stimulation would result in a specific and permanent upregulation of Na transport in only two distal nephron segments is unclear. Aldosterone is the major regulator of distal nephron Na transport. Although aldosterone has been shown to regulate TSC (5, 14, 28), the other target, ENaC, would also be expected to be upregulated under mineralocorticoid stimulation; this was not seen in the present study.

In summary, we have documented upregulation of two critical renal Na transporters, BSC1 and TSC, in prenatally programmed hypertension, suggesting that increased Na reabsorption in TAL and DCT plays an important role in the development of hypertension.

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