Effect of renal denervation on prenatal programming of hypertension and renal tubular transporter abundance

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Dagan A, Kwon HM, Dwarakanath V, Baum M. Effect of renal denervation on prenatal programming of hypertension and renal tubular transporter abundance. Am J Physiol Renal Physiol 295: F29–F34, 2008.—Prenatal glucocorticoids are often administered to pregnant women to accelerate pulmonary maturation. We have demonstrated that administration of dexamethasone during specific periods of pregnancy in the rat causes hypertension in the offspring when they are studied as adults. The purpose of the present study was to determine whether the hypertension due to prenatal dexamethasone was mediated by renal nerves. We administered dexamethasone to rats daily for 4 days between days 15 and 18 of gestation. Rats underwent bilateral renal denervation or sham operation at 6 wk of age, and blood pressure was measured at 8 wk of age. Prenatal dexamethasone in the sham operation group resulted in an increase in blood pressure compared with vehicle-treated sham controls (134 ± 3 vs. 145 ± 5 mmHg, P < 0.05). Renal denervation did not affect blood pressure significantly in the prenatal vehicle-treated control group but resulted in normalization in blood pressure in the prenatal dexamethasone group and (130 ± 3 and 128 ± 5 mmHg, respectively). Prenatal dexamethasone increased type 3 Na+/H+ exchanger (NHE3), Na+/K+-2Cl– cotransporter (NKCC2), and Na+/Cl– cotransporter (NCC), but not α-, β-, and γ-epithelial Na+ channel (ENaC) protein abundance compared with controls. The increase in NHE3, NKCC2, and NCC protein abundance by prenatal dexamethasone was not seen in 8-wk-old rats 2 wk after renal denervation. Renal denervation did not affect NHE3, NKCC2, and NCC protein abundance in prenatal vehicle-treated animals. This study is consistent with renal nerves playing a role in mediating the hypertension by prenatal programming by dexamethasone.

thiazide-sensitive cotransporter; type 3 Na+/H+ exchanger; bumetanide-sensitive cotransporter

SMALL-FOR-GESTATIONAL-AGE neonates are at higher risk for developing hypertension as adults (9, 10, 26, 32). The cause for the elevated blood pressure remains elusive. Factors such as increased sodium transport (14, 34), an altered renin-angiotensin system (1, 31, 36), and low nephron number (17–19, 33) have been suggested as the predominant factor leading to the hypertension due to prenatal insults causing small for gestational age offspring.

Glucocorticoids are often administered to premature infants to accelerate pulmonary maturation (5). Prenatal administration of prednisone throughout gestation, a treatment once used to prevent premature delivery in humans, results in infants that are small for their gestational age (43). In animal models, prenatal administration of glucocorticoids results in a reduction in birth weight in rodents as well (20, 22, 35, 43). We have previously shown that there is a window during the gestation of the rat when prenatal administration of dexamethasone causes hypertension when the offspring are studied as adults. Injection of dexamethasone at 0.2 mg/kg, a comparable dose administered to humans, causes hypertension if administered for two doses between days 15 and 18 of gestation. In this study of prenatal programming by glucocorticoids, there was a 20–30% reduction in the number of glomeruli in the dexamethasone-exposed offspring, but there was no change in the glomerular filtration rate (GFR) compared with vehicle controls (39). There is increasing evidence that there is a lack of correlation between the reduction in nephron number with prenatal programming and hypertension (39, 46) as well as in other models of hypertension (15).

Studies have provided evidence that the prenatal programming-mediated hypertension may be the result of an increase in renal sodium transport (14, 22, 34, 36). Adult rats whose mothers ingested a low-protein diet had an increase in renal α1- and β1-Na+/K+-ATPase mRNA abundance, and Na+/K+–2Cl– cotransporter (NKCC2) and thiazide-sensitive cotransport (NCC) protein abundance compared with rats whose mothers were fed a normal-protein diet (14, 34). We have recently shown that prenatal administration of dexamethasone results in offspring with an increase in proximal tubule volume absorption, Na+/H+ exchanger activity and brush-border membrane type 3 Na+/H+ exchanger (NHE3) protein abundance compared with controls (22).

It has recently been shown that a reduction in placental perfusion in rats resulted in offspring that were small for their gestational age (2). When measured as adults, the small-for-gestational age offspring had a higher blood pressure compared with controls. The hypertension was normalized by renal denervation (2). The purposes of this study were to examine whether renal denervation normalizes blood pressure in the prenatal dexamethasone model of prenatal programming and whether the hypertension mediated by prenatal dexamethasone is associated with an increase in renal transporters that mediate sodium transport along the nephron. Finally, we examined whether denervation affected renal transporter protein abundance upregulated by prenatal dexamethasone.

METHODS

Animals. Pregnant Sprague-Dawley rats, purchased from Harlan, were delivered to our institution at 13 days of gestation. Dexamethasone (0.2 mg/kg) or vehicle was administered by intraperitoneal

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injection once daily on days 15–18 of gestation. We have previously shown that prenatal dexamethasone during this point in gestation results in postnatal hypertension at 8 wk of age (22, 38, 39). Rats from multiple litters were utilized in each protocol, and only male rats were studied to decrease variability and because they tend to have more severe hypertension due to prenatal programming (38, 39, 45). All experimental procedures conformed to the APS Guiding Principles in the Care and Use of Animals and the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center.

Renal norepinephrine content. Rats were anesthetized using intraperitoneal Inactin (10 mg/100 g body wt). A midabdominal incision was then performed, and the left kidney was dissected from surrounding tissue and frozen in situ using a clamp cooled in liquid nitrogen. The kidney was then submerged in liquid nitrogen. A portion of the frozen kidney was weighed and submerged in 5 ml of 0.01 N HCl and stored at −80°C. At the time of assay, the kidney was thawed on ice and homogenized at 4°C. The homogenate was centrifuged at 1,000 g for 15 min. Ten to thirty microliters of supernatant was used to assess norepinephrine content using a radioimmunoassay kit (ALPCO Diagnostics, Salem, NH).

Bilateral renal denervation. To determine whether renal nerves were an important factor in mediating the hypertension by prenatal exposure to dexamethasone, some prenatal dexamethasone-treated and some prenatal vehicle-treated rats underwent bilateral renal denervation. Renal denervation was performed at 6 wk of age. The rats were anesthetized using ketamine (2–3 mg/100 g body wt) and xylazine (2–3 mg/100 g body wt) in our laboratory, as previously described (41). Surgery was performed on a servo-controlled heated table to maintain the rat’s body temperature at 37°C. A ventral midline abdominal incision was used to expose both kidneys under a dissecting microscope. The renal arterial adventitia was stripped, and phenol (10%) was applied circumferentially three times to each renal artery to denervate the kidney. The muscle was sutured with a 3-0 silk suture, and the skin was closed using staples. Prenatal dexamethasone-treated sham and prenatal vehicle-treated sham rats received an operation where the abdominal cavity was opened, but the renal nerves were not manipulated. All rats received buprenorphine (5 µg/100 g body wt) by an intramuscular injection after surgery and the following day to reduce postsurgical pain. The denervation was verified by measuring the norepinephrine content in the kidney 2 wk after denervation at the time of study. There was ~90% reduction in norepinephrine (50.8 ± 5.6 vs. 4.4 ± 1.2 ng/g kidney wt, P < 0.001).

Blood pressure measurements. Eight-week-old rats were trained for 4 days by placing them in a lucite restraining tube for 15 min, and a tail blood pressure cuff was inflated and deflated periodically. Blood pressures were measured on day 5 using an IITC model 179 blood pressure analyzer (Woodland Hills, CA) (22, 38, 39). There were at least six recordings for each rat. The mean of all readings was considered as the systolic blood pressure for that rat.

Total protein and brush-border membrane isolation. Rats were studied at 8 wk of age, 2 wk after renal denervation or sham operation. The decapsulated kidney harvested as above and placed in sucrose buffer that contained 250 mM sucrose and 10 mM triethanolamine containing 1 µl/ml of protease inhibitor cocktail (Sigma St. Louis, MO) and 100 µg/ml phenylmethylsulfonyl fluoride (Calbiochem, La Jolla, CA) (34). The tissue was homogenized with 15 strokes of a polytetrafluoroethylene glass homogenizer at 4°C. The homogenate was centrifuged at 1,000 g for 15 min. The protein of the supernatant was estimated using the Bradford method with bovine albumin as the standard (16).

Brush-border membranes were prepared by placing the kidney in ice-cold PBS. The cortex was dissected and put in isolation buffer that

- FIG. 1. Effect of renal denervation on systolic blood pressure in male rats exposed to prenatal dexamethasone. Blood pressure was measured in trained 8-wk-old rats by tail cuff. Prenatal dexamethasone-treated male sham rats had a higher blood pressure than offspring of vehicle-treated sham rats, similar to what we have shown previously (22). Renal denervation (dener) did not affect the blood pressure in vehicle (veh)-treated rats; however, rats that were exposed to prenatal dexamethasone (dex) had blood pressures that were comparable to the vehicle sham group.

- FIG. 2. Renal norepinephrine content in 3- and 8-wk-old male rats exposed to prenatal dexamethasone. Renal norepinephrine content, assessed in kidneys that were snap frozen in vivo, in 3-wk-old prehypertensive male rats was significantly higher in rats whose mothers were exposed to prenatal dexamethasone than those whose mothers were treated with vehicle. Interestingly, in male rats at 8 wk of age, a time when the rats are hypertensive, there was no difference in renal norepinephrine content between the prenatal vehicle and dexamethasone groups. The data in 8-wk-old rats were confirmed in sham-operated vehicle and sham-operated dexamethasone-treated rats. The vehicle-treated rats had a norepinephrine content of 55.3 ± 10.1 (n = 8) vs. 45.7 ± 3.7 (n = 7) ng/g kidney wt. NS, not significant.
contained 300 mM mannitol, 16 mM HEPES, and 5 mM EDTA, titrated to pH 7.4 with Tris. The isolation buffer contained (1/100 ml) of protease inhibitor cocktail (Sigma St. Louis MO) and 100 μg/ml phenylmethylsulfonyl fluoride (Calbiochem). Brush-border membrane vesicles were isolated using magnesium precipitation and differential centrifugation as previously described by our laboratory (12, 44).

SDS-PAGE and immunoblotting. Whole kidney protein samples (50 μg) were denatured at 65°C for 15 min before loading on an 8% polyacrylamide gel as previously described (12). Brush-border membranes (50 μg) were incubated at 37°C for 15 min and loaded on a 7.5% polyacrylamide gel. Proteins were separated using SDS-PAGE and were then transferred to polyvinylidene difluoride membrane at 300–400 mA for 1 h at 4°C. The membranes were blocked with Blotto (5% nonfat dry milk and 0.05% Tween 20 in PBS) for 1 h before incubation with the primary antibody followed by incubation with the primary antibody at 4°C overnight. The primary polyclonal antibodies to NKCC2, used at a 1:1,500 dilution, and Na\(^+\)/H\(^+\) thiazide-sensitive cotransporter (NCC), used at a 1:600 dilution, were raised in rabbits (30). The polyclonal rabbit antibodies to the rat ENaC subunits were a gift from Dr. L. Palmer at Weill Medical College of Cornell University (25). The antibody to NHE3 was a rabbit polyclonal antibody to rat NHE3 used at a 1:750 dilution (a gift of O. W. Moe at UT Southwestern) (3). All membranes were also probed with an antibody to β-actin at a 1:7,500 dilution to ensure equal loading (Sigma Biochemicals and Reagents). After incubation, the blots were washed extensively with Blotto, and donkey anti-rabbit secondary antibodies conjugated to horseradish peroxidase were added at a 1:10,000 dilution (Santa Cruz Biotechnology, Santa Cruz, CA). Bound antibody was detected by chemiluminescence and quantitated using densitometry.

Statistics. Comparison between two groups was performed using a Student’s t-test. Comparison between multiple groups was by analysis of variance and a post hoc Student-Newman-Keuls assessment. A P value of <0.05 was considered statistically significant.

RESULTS

Effect of prenatal dexamethasone and renal denervation on blood pressure. The results of blood pressure measurements after administration of prenatal dexamethasone or vehicle for 8-wk-old rats that received either a sham operation or renal denervation are shown in Fig. 1. As we have previously demonstrated, administration of prenatal dexamethasone resulted in a higher blood pressure than in prenatal vehicle-treated rats. Renal denervation did not affect the appearance or activity of the rats. Renal denervation did not affect the blood pressure of the vehicle-treated rats. However, denervation of the prenatal dexamethasone-treated rats lowered blood pressure to a level not different from the prenatal vehicle sham and prenatal vehicle denervation group. In other words, prenatal dexamethasone results in an elevated blood pressure that is normalized by renal denervation.

Fig. 3. Effect of prenatal dexamethasone and renal denervation on type 3 Na\(^+\)/H\(^+\) exchanger (NHE3) protein abundance. Prenatal dexamethasone resulted in an increase in NHE3 in the sham-operated group as previously shown by our laboratory when we analyzed the data by an unpaired t-test (22) but not by analysis of variance. Renal denervation at 6 wk of age resulted in a reduction in brush-border membrane vesicle NHE3 protein abundance when the rats were studied at 8 wk of age in the group that received prenatal dexamethasone.

Fig. 4. Effect of prenatal dexamethasone and renal denervation on Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter (NKCC2) protein abundance. Renal NKCC2 protein abundance was assessed in 8-wk-old rats, prenatal vehicle-treated sham-operated rats, sham-operated rats whose mothers were administered dexamethasone, prenatal vehicle-treated rats that underwent renal denervation, and prenatal dexamethasone-treated rats that underwent renal denervation. Prenatal dexamethasone resulted in an increase in NKCC2 abundance in the sham-operated group. However, this increase in NKCC2 abundance was not seen in the prenatal dexamethasone group that underwent renal denervation 2 wk earlier.

N=6

*P< 0.05 Dex Sham vs Veh Dener and Dex Denerv

NKCC2

β-actin

Veh Sham

Dex Sham

Veh Denerv

Dex Denerv

p<0.05 vs other groups n=4

Veh Shim

Dex Sham

Veh Denerv

Dex Denerv
Norepinephrine content in the kidney. To examine whether there was a difference in renal norepinephrine content, we measured norepinephrine in 3-wk- and 8-wk-old rat kidneys. As shown in Fig. 2A, the norepinephrine content, assessed per gram of kidney weight, was higher in the group that received prenatal dexamethasone. This was not true in the 8-wk-old rats, as shown in Fig. 2B, where the renal norepinephrine content was comparable in the two groups. As shown in Fig. 1, blood pressure was elevated in the group that received prenatal dexamethasone at 8 wk of age.

To examine whether this was the case at 3 wk of age, we measured blood pressure in this group as well. As we have previously shown after administration of two prenatal doses of dexamethasone (39), 3-wk-old males that received vehicle had comparable blood pressures as those that were exposed to prenatal dexamethasone (112 ± 2 vs. 106 ± 2 mmHg, respectively) (P = not significant). Thus the renal norepinephrine content was only elevated in the prehypertensive state.

Effect of prenatal dexamethasone and renal denervation on brush-border membrane vesicle NHE3 protein abundance. We had previously shown that prenatal dexamethasone increased NHE3 protein abundance and proximal tubule Na\(^+\)/H\(^+\) exchanger activity (22). Using an unpaired t-test, these findings were verified in these studies of sham-operated rats. However, this increase was not statistically significant using analysis of variance. Renal denervation resulted in a decrease in NHE3 protein abundance in the prenatal dexamethasone-treated group (Fig. 3).

Effect of prenatal dexamethasone and renal denervation on NKCC2 protein abundance. In the next experiment, we examined the effect of prenatal dexamethasone on NKCC2 abundance. As shown in Fig. 4, rats whose mothers were treated with prenatal dexamethasone had an increase in NKCC2 abundance. The increase in NKCC2 abundance was totally abrogated by renal denervation.

Effect of prenatal dexamethasone and renal denervation on NCC protein abundance. The effect of prenatal dexamethasone on NCC is shown in Fig. 5. Prenatal administration of dexamethasone increased NCC protein abundance in 8-wk-old rats. Renal denervation did not significantly decrease NCC abundance in the prenatal vehicle-treated group, but denervation resulted in a decrease in NCC abundance in the prenatal dexamethasone group to levels comparable to sham operated prenatal vehicle-treated controls.

Effect of prenatal dexamethasone and renal denervation on ENaC subunit abundance. In the last series of experiments, we examined whether prenatal dexamethasone affected ENaC protein abundance. Neither α-, β-, or γ-ENaC subunits were affected by prenatal dexamethasone or renal denervation. These results are shown in Table 1 and Fig. 6.

**DISCUSSION**

Our study supports the hypothesis that dysregulation of the renal sympathetic nerves contributes to elevated blood pressure.

Table 1. Effect of prenatal dexamethasone and renal denervation on ENaC subunit abundance

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Sham</th>
<th>Dexamethasone Sham</th>
<th>Vehicle Denervated</th>
<th>Dexamethasone Denervated</th>
<th>P Value</th>
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<tr>
<td>α-ENaC</td>
<td>0.6±0.1</td>
<td>0.5±0.1</td>
<td>0.5±0.1</td>
<td>0.3±0.1</td>
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<tr>
<td>β-ENaC</td>
<td>0.7±0.1</td>
<td>0.6±0.1</td>
<td>0.6±0.1</td>
<td>0.5±0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>γ-ENaC</td>
<td>0.28±0.1</td>
<td>0.43±0.1</td>
<td>0.5±0.1</td>
<td>0.5±0.1</td>
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Values are means ± SE. ENaC, epithelial sodium channel.

Fig. 5. Effect of prenatal dexamethasone and renal denervation on thiazide-sensitive Na\(^+\)/Cl\(^-\) cotransporter (NCC) protein abundance. NCC protein abundance was assessed by immunoblotting in 8-wk-old rats, prenatal vehicle-treated rats, prenatal dexamethasone-treated sham-operated adult rats, and those that received denervation. Prenatal dexamethasone programmed an increase in NCC protein abundance compared with vehicle controls. This increase in NCC by prenatal dexamethasone was no longer present in rats that received renal denervation 2 wk before study.

Fig. 6. Effect of prenatal dexamethasone and renal denervation on epithelial sodium channel (ENaC) subunit abundance. α-, β-, and γ-ENaC protein abundance was assessed in 8-wk-old rats by immunoblotting. Shown are α-, β-, and γ-ENaC protein abundance in adult animals whose mothers receive either vehicle or dexamethasone. Some animals were denervated 2 wk before study. There was no effect of renal denervation or prenatal dexamethasone on ENaC subunit protein abundance.
in the dexamethasone programming of hypertension. This study demonstrates higher renal norepinephrine content in kidneys from rats whose mothers were treated with prenatal dexamethasone in the prehypertensive state but not when they are hypertensive. Renal sympathetic denervation normalized the systolic blood pressure in rats whose mothers were administered prenatal dexamethasone but did not affect prenatal vehicle-treated rats. Renal NHE3, NKCC2, and NCC protein abundance were elevated in 8-wk-old adults exposed in utero to dexamethasone compared with in utero vehicle-treated rats. Renal denervation at 6 wk of age resulted in a decrease in NHE3, NKCC2, and NCC protein abundance to the level in vehicle-treated sham rats. ENaC protein abundance was unaffected by prenatal dexamethasone and renal denervation.

Alexander et al. (2) showed that reduced uterine perfusion in the rat starting at 14 days of gestation resulted in neonates that were small for their gestational age. Blood pressure measured in male offspring at 12 wk of age was elevated in the rats that were the product of reduced uterine perfusion compared with sham-operated controls. Renal denervation at 10 wk normalized the blood pressure at 12 wk in rats that were the product of mothers with reduced uterine perfusion to control levels. Denervation did not affect the blood pressure in intrauterine sham-operated controls. Similar findings by this group were demonstrated if the denervation occurred at 4 wk and the blood pressure was measured at 6 wk (37). Our results using a different model of intrauterine growth retardation support the fact that prenatal programming of hypertension is mediated, at least in part, by renal nerves.

Sympathetic nerve activity correlates with renal venous norepinephrine content (23). As an index of norepinephrine exposure to the renal tubules, we measured norepinephrine content of kidneys that were snap frozen in situ. Our results show that the offspring of pregnant rats that received prenatal dexamethasone had an increase in renal norepinephrine content at 3 wk of age, an age before they became hypertensive. The norepinephrine content was not different from control rats at 8 wk, suggesting the renal norepinephrine levels may be involved in the generation of, but not in the maintenance of, hypertension.

Renal nerves play an important role in regulating renal sodium reabsorption (24). All segments of the nephron are innervated (6–8, 24). Increased renal nerve activity or increases in basolateral catecholamine concentration increase proximal tubule, thick ascending limb, and distal convoluted tubule sodium reabsorption (4, 11, 13, 21, 27, 40, 42, 47). However, renal nerves or catecholamines do not increase sodium absorption in the collecting tubule (28, 29). This is consistent with the fact that the increase in NHE3, NKCC2, and NCC protein abundance by prenatal dexamethasone was normalized by renal denervation but ENaC subunit protein abundance was unaffected.

Previously, Manning et al. (35) demonstrated that feeding pregnant rats a low-protein diet from 12 days of gestation to the end of gestation caused intrauterine growth retardation in the neonates and an increase in blood pressure when the rats were studied as adults. They subsequently showed that there was an increase in NKCC2 and NCC mRNA and protein abundance, but no change in NHE3 or ENaC abundance. This study with prenatal dexamethasone confirms that prenatal programming is associated with an upregulation of NKCC2 and NCC protein abundance. Unlike the dietary protein-deprivation model, we have previously demonstrated that prenatal dexamethasone is associated with an increase in brush-border membrane NHE3 protein abundance (22). While these studies show an increase in transporter protein abundance from a kidney homogenate, this does not necessarily mean that there is an increase in transport in the nephron segment where that transporter resides. For there to be an increase in transport, the transporter must be on the apical membrane and there must be a parallel upregulation of other transporters including the Na+/K+ ATPase on the basolateral membrane. To date only an increase proximal tubule sodium absorption and Na+/H+ exchanger activity has been demonstrated in offspring of prenatal programming by dexamethasone (22).

The current study extends the previous studies in that the offspring of the rats administered dexamethasone not only had a higher NHE3, NKCC2, and NCC protein abundance but that this was completely abrogated by renal denervation. Thus we speculate that the hypertension mediated by prenatal programming is, at least in part, mediated by sympathetic nerves increasing blood pressure by increasing sodium transport in the proximal tubule, thick ascending limb, and distal convoluted tubule.

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GRANTS

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