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Postnatal modulation of prenatally programmed hypertension by dietary Na and ACE inhibition

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Submitted 11 May 2004; accepted in final form 20 September 2004

Manning, Jennifer, and V. Matti Vehaskari. Postnatal modulation of prenatally programmed hypertension by dietary Na and ACE inhibition. Am J Physiol Regul Integr Comp Physiol 288: R80–R84, 2005. First published September 30, 2004; doi:10.1152/ajpregu.00309.2004.—Adult hypertension in the rat can be programmed experimentally by changes in intrauterine environment. The offspring typically do not become hypertensive until 6 to 8 wk of age, and recent evidence suggests that renal dysfunction may participate in the pathogenesis. The present study was based on the hypothesis that the window for programming extends to the postnatal period in the rat. Adult hypertension was induced by maternal low-protein diet during the second half of gestation. After being weaned at 3 wk, the offspring were exposed to one of the following regimens for the subsequent 3 wk: 1) low-Na diet, 2) standard Na diet, 3) high-Na diet, and 4) standard Na diet with enalapril. The pups were followed for 10 wk after discontinuation of the treatments. The brief exposure to low-Na diet or enalapril totally prevented the development of hypertension and the effect lasted throughout the observation period. The development of hyperreninemia, present in the standard Na group at 16 wk of age, was abolished in the low-Na and enalapril groups. Conversely, 3-wk exposure to high-Na diet increased the severity of the later hypertension and did not prevent the hyperreninemia. The findings suggest that there is a period of susceptibility during which prenatally programmed hypertension can be modulated postnatally, possibly coinciding with a critical stage in renal maturation.

fetal origins of adult disease; kidney ontogeny; renin-angiotensin system; aldosterone

PROGRAMMING OF HYPERTENSION is the most extensively studied example of prenatal programming. Both epidemiological data (2, 4, 5, 15) and experimental studies (13, 20, 27, 30) have provided strong evidence that the in utero environment can modify adult blood pressure (BP). In experimental animals, maternal protein (13, 27, 30) and calorie (28) restriction, and maternal glucocorticoid treatment (6, 23), have been used to modify adult blood pressure (BP). In experimental animals, maternal protein (13, 27, 30) and calorie (28) restriction, and maternal glucocorticoid treatment (6, 23), have been used to program adult hypertension in the offspring. The target of the programming in the fetus has not been unequivocally identified, but most recent evidence has implicated the fetal kidney. It has been postulated that altered Na handling by the kidney leads to expansion of extracellular volume and hypertension (18). Documented abnormalities in the kidney have included decreased number of nephrons (27, 30), upregulation of Na transporters (19), and alterations in the renin-angiotensin system (RAS) (13, 20, 27, 28, 30).

Limited information is available on the extent of the window of susceptibility to prenatally programmed hypertension. In the rat, some reports have shown a very wide window (13), whereas a recent study reported that exposure only during the second half of pregnancy resulted in adult hypertension in the offspring (31). It is not known if the window abruptly closes at birth. There are several examples of environmental effect in early postnatal life on other adult characteristics. Behavior (16), sympathoadrenal activity (32), lipid metabolism (17), and endocrine functions (22), for instance, have been shown to be modulated by neonatal environment.

Because functional maturation of the rat kidney continues well into the postnatal life, even after new nephron formation ceases by 10 days of age (14), we hypothesized that prenatal programming of hypertension could be modified postnatally. Because Na balance and the RAS may play a role in the pathogenesis of the hypertension, we chose changes in dietary Na and pharmacological manipulation of the RAS as tools to test the hypothesis. The long-term effects of short-term postnatal exposure to a low- and a high-Na diet as well as to angiotensin-converting enzyme (ACE) inhibition on prenatally programmed hypertension were examined. The results indicate that a 3-wk postnatal exposure to a low-Na diet or ACE inhibitor has a long-lasting suppressive effect on the development of hypertension.

METHODS

Sprague-Dawley rats (Harlan, Indianapolis, IN) were used for all experiments. The study protocol was approved by the Institutional Animal Care and Use Committees of the Research Institute for Children and Louisiana State University Health Sciences Center. The experimental timed pregnant rats arrived at our facility on day 10 of pregnancy and were housed in a temperature-controlled 12:12-h light-dark environment. After a 48-h acclimatization period, they were placed on a low-protein diet (6% protein by weight, Purina Mills Test Diets, Richmond, VA) to induce prenatally programmed hypertension in the offspring as previously described (27). The dams were allowed to deliver. No premature deliveries or immediate postnatal abnormalities in the pups were observed. The birth weights of the pups from protein-restricted pregnancies were ~15% lower than those from normal pregnancies, consistent with our previous observations (20, 27). After birth, all dams were on standard 20% protein diet and were allowed to nurse their own offspring until weaning at 21 days of age.

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At being weaned, the offspring, all of whom were programmed to become hypertensive as adults, were randomly divided into the following experimental groups: 1) NS, standard salt diet (0.2% Na by weight); 2) LS, low-salt diet (0.03% Na); 3) HS, high-salt diet (3% Na); and 4) E, standard Na diet/Enalapril. Each of the first three groups consisted of 20 animals (10 females and 10 males); group E consisted of 16 animals (8 females and 8 males). Pups in each group were drawn from four to six different litters. All diets were isocaloric and contained standard 20% protein by weight. The diets were purchased from Purina Mills Test Diets. All animals had free access to water. The offspring in group E received enalapril, 100 mg/l in the drinking water. The sodium-modified diets and enalapril treatment were continued only for 3 wk, from age 3 wk to 6 wk, after which all animals were on standard 20% protein and 0.2% Na rodent chow. BPs in the experimental groups were monitored at 2-wk intervals beginning at 8 wk of age until death at 16 wk of age. Oscillometric tail cuff method (Kent Scientific, Litchfield, CT), as previously described by us (27), was used for the determinations. The rats were trained to remain calm in a temperature-controlled Plexiglas restrainer during the measurements. A mean of four to six readings was recorded at each session. For comparisons at 16 wk of age, BP was also measured in a contemporary group of 16-wk-old normal rats from the same vendor, housed in our facility on standard 20% protein and 0.2% sodium diet.

Blood was obtained from each animal at the end of the study, at 16 wk of age, directly from the abdominal aorta during euthanasia with pentobarbital sodium. If present, bladder urine was obtained by direct bladder puncture at the same time. Plasma renin activity (PRA) and plasma aldosterone concentrations were measured by radioimmunoassay (GammaCoat Plasma Renin Activity, Incstar, Stillwater, MN, and Coat-A-Count Aldosterone, Diagnostic Products, Los Angeles, CA, respectively). Plasma creatinine and electrolyte concentrations were measured by a standard dye-binding method.

Results are expressed as means ± SE. Statistical comparisons are done with one-way ANOVA with Dunnett’s multiple comparison test or by two-way ANOVA where appropriate. A P value of <0.05 is considered statistically significant.

RESULTS

Weight gain. As expected, male rats were heavier than female rats at all time points except at 4 wk of age. In the female offspring, there were no weight differences between the experimental groups at the end of the treatment period at 6 wk of age (Fig. 1A). Between 8 and 10 wk of age, however, the weight gain in the HS rats fell behind that of the other groups and they remained slightly lighter than the control NS group throughout the rest of the follow-up period. Group E had a slightly higher mean weight than the NS group from 10 to 16 wk of age (P < 0.01).

BP. In agreement with our previous reports on the same model of prenatally programmed hypertension (20, 27), no BP differences were observed between males and females; the BP data measurements of both sexes are therefore combined for analysis. Figure 2 illustrates the overall systolic BP profiles in
the experimental groups from 8 to 16 wk of life. The NS rats became progressively more hypertensive as previously reported by us (20). Remarkably, there was only a slight rise in BP in the LS and E groups with age throughout the observation period, and at 16 wk of age their BP readings were identical to those of normal control rats of the same age (Fig. 2). Beginning at 10 wk of age, the mean systolic BP in the HS group was higher than that of the NS group (Fig. 2). The BP difference between the two normotensive groups (LS and E) and the two hypertensive groups (NS and HS) widened with age. The effects of both age and treatment group were highly significant when analyzed by repeated-measures ANOVA (P < 0.001).

Renal function, PRA, and plasma aldosterone concentration. All determinations were done at the time of the death, at 16 wk of age, and compared with normal control rats of the same age. Plasma creatinine and electrolyte concentrations were without significant differences between the groups or sexes (Table 1). (The concentrations of all electrolytes were relatively low, perhaps due to the fact that the animals received only water during the urine collection for the final 24 h before death.) Terminal bladder urine protein:creatinine ratio in male rats was 4.56 ± 3.59 (n = 5) in LS, 4.21 ± 3.13 (n = 7) in NS, and 15.41 ± 23.86 (n = 6) in HS; the differences were not statistically significant. We were unable to obtain urine in a sufficient number of female rats for statistical analysis.

We previously showed PRA to be high at this age in our model of prenatally programmed hypertension without gender differences (20), and this was confirmed in the present study; both hypertensive groups, NS and HS, had increased PRA values as shown in Fig. 3. Early Na restriction or enalapril treatment abolished the hyperreninemia. Plasma aldosterone levels at 16 wk of age are depicted in Fig. 4. Early high-Na diet increased the severity of later hypertension. Our results with RAS inhibition are similar to those reported by Sherman and Langley-Evans (25, 26); to our knowledge, the effects of early dietary Na manipulations have not been previously described.

We showed that the prenatally programmed hypertension becomes progressively more pronounced with age and results in a shortened life span (27). Analysis of human data also suggests that the effect of birth weight on BP is the greatest in the oldest age groups (15). A similar trend is evident in the present series even with the relatively short follow-up period, and the early exposure to high-Na diet seems to further accelerate the process.

The results show that prenatally programmed adult hypertension in the rat can be modified by a short exposure to low-Na diet or to ACE inhibition in early life. Both exposures appeared to totally abolish the development of hypertension, although longer follow-up would be necessary to determine if the effect is life long. In addition, exposure to high-Na diet increased the severity of later hypertension. Our results with RAS inhibition are similar to those reported by Sherman and Langley-Evans (25, 26); to our knowledge, the effects of early dietary Na manipulations have not been previously described.

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The mechanism by which either manipulation prevents the development of hypertension is not clear. Experimental prenatal hypertension is associated with reduced total nephron count (12, 27, 30), which may be important in the pathogenesis. Because nephrogenesis in the rat is complete by the end of the second postnatal week (14), the effect of low-Na diet or ACE inhibition could not be through influence on new nephron

**DISCUSSION**

The present study was based on the hypothesis that early postnatal factors may modify prenatally programmed hypertension. Because Na balance and the RAS are hypothesized to play a role in prenatally programmed hypertension, we chose to manipulate early-life Na balance and RAS in rats programmed to develop adult hypertension in later life. Changing dietary Na intake was not practical before weaning, and immediate postnatal pharmacological ACE inhibition may lead to major disruption in renal architecture (7); for these reasons, the

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Table 1. Plasma creatinine and electrolyte concentrations at 16 wk of age

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Cr, mg/dl</th>
<th>Na, mmol/l</th>
<th>K, mmol/l</th>
<th>Cl, mmol/l</th>
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<tr>
<td>Low Na, m</td>
<td>0.37±0.03</td>
<td>131±1</td>
<td>3.2±0.1</td>
<td>103±2</td>
</tr>
<tr>
<td>Low Na, f</td>
<td>0.40±0.03</td>
<td>129±2</td>
<td>3.1±0.2</td>
<td>100±2</td>
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<tr>
<td>Sd Na, m</td>
<td>0.45±0.03</td>
<td>132±1</td>
<td>3.0±0.1</td>
<td>104±2</td>
</tr>
<tr>
<td>Sd Na, f</td>
<td>0.45±0.03</td>
<td>130±2</td>
<td>2.9±0.2</td>
<td>102±1</td>
</tr>
<tr>
<td>High Na, m</td>
<td>0.42±0.03</td>
<td>127±2</td>
<td>3.3±0.2</td>
<td>98±2</td>
</tr>
<tr>
<td>High Na, f</td>
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<td>125±3</td>
<td>3.0±0.3</td>
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<tr>
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<tr>
<td>Enalapril, f</td>
<td>0.40±0.03</td>
<td>127±1</td>
<td>3.2±0.3</td>
<td>98±1</td>
</tr>
</tbody>
</table>

Values are means ± SE; m, male; f, female; Sd, standard.

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**Fig. 3.** Plasma renin activity (PRA) in the experimental groups compared with the mean of control rats (horizontal line) at 16 wk of age. Column designations as in Fig. 2. Statistically significant differences from control rats indicated. *\(P < 0.01\), NS, standard salt diet (0.2% Na by weight); LS, low-salt diet (0.03% Na); HS, high-salt diet (3% Na); and E, standard Na diet + enalapril.

**Fig. 4.** Plasma aldosterone concentration in the experimental groups compared with the mean of control rats (horizontal line) at 16 wk of age. Column designations as in Fig. 2. Statistically significant difference from control rats indicated. *\(P < 0.05\).
formation. However, functional maturation of the kidney continues after nephrogenesis is completed; postnatal manipulations may conceivably affect the maturation process and thereby permanently “reprogram” renal Na handling. We previously showed postnatal upregulation of distal nephron Na transporters (19) and ANG II type 1 receptor (28) at 4 wk of age in the kidney in our model of prenatally programmed hypertension. Because the upregulation precedes the development of hypertension, it may participate in the pathogenesis and could be the target of postnatal modulation. The concept of a defined window of susceptibility during maturation is further supported by the finding that when given in later life, beginning at 23 wk of age, short-term ACE inhibition did not have a permanent effect on prenatally programmed hypertension (11).

Our study did not address the issue of whether the effect of enalapril could have been due to nonspecific antihypertensive or vasodilatory action rather than specific to RAS inhibition. The fact that the drug was administered only during the prehypertensive period suggests that the effect was not through a BP-lowering action. Moreover, the study by Sherman and Langley-Evans (26) showed that the effect could not be duplicated by a calcium channel blocker, suggesting a specific action through inhibition of RAS or the bradykinin pathway. Racasan et al. (24) reported that in the spontaneously hypertensive rat (SHR), renal arteriopathy progressed despite early amelioration of hypertension. Because renal histology was not available in the present study, it is not known whether the prevention of hypertension resulted in renoprotection.

Systemic PRA is not increased in our model until after hypertension is manifest (20), and the hyperreninemia may therefore be a secondary consequence of a primary pathophysiology such as intrarenal vascular injury and ischemia. This is supported by the finding that when hypertension was prevented by low-Na diet or enalapril, the later increase in PRA was also abolished. Plasma aldosterone concentration did not differ from control values in the NS group and was not affected by low-Na diet or enalapril, suggesting that changes in plasma aldosterone did not mediate the ameliorating effect on hypertension. We previously showed plasma aldosterone levels to be increased at 4 and 8 wk of life in our model (27). The present results in the NS group suggest that the hyperaldosteronism later spontaneously resolves. Although high-Na intake is expected to suppress aldosterone secretion during the exposure, aldosterone concentration in the HS group was increased at 16 wk of age, 10 wk after the discontinuation of the diet. The increase may reflect a role for aldosterone in the incremental worsening of prenatally programmed hypertension in the HS group, but elucidating the mechanism will require further study. Females in the HS group exhibited slower weight gain than females in the other groups, and male HS animals showed a similar but not statistically significant trend. Our previous study also reported declining weight gain with progression of the hypertension (20), and we speculate that the higher BP may have caused decreased food intake.

It is notable that a postnatal window for modifying the development of hypertension may also be present in genetically determined hypertension in rats, although to a lesser extent. Cross-fostering of SHR pups by normotensive Wistar-Kyoto dams appears to permanently ameliorate, although not totally abolish, the hypertension (21). The effect has been suggested to be due to differences in milk intake (8) and may be mediated through modification of tubular responsiveness to ANG II (9). The development of hypertension in SHR is also ameliorated by early-life ACE inhibition (3, 10). These results, suggesting a role for dietary factors and the RAS, are similar to the present study on prenatally programmed hypertension, indicating that the postnatal window for modulating future adult BP profile may not be unique to any single experimental model. There are, however, also differences between the models; the SHR is not hyperreninemic, and early ACE inhibition does not appear to have a lasting effect on PRA in SHR (3).

In summary, our results illustrate that prenatally programmed hypertension can be modified by early-life dietary Na content or ACE inhibition. Remarkably, the effect appeared to be a lasting one. Whether similar mechanisms are operative in humans who possess relatively mature kidneys at birth is not known. It has, however, been speculated based on epidemiological studies that early-life nutrition and weight gain participate in concert with prenatal factors to program the ultimate adult BP profile (1).

GRANTS
Support for this study was provided by National Heart, Lung, and Blood Institute Grant 1 ROI-HL-66158 and by contract HEF(2001–06)-07 from Health Excellence Fund of Louisiana Board of Regents.

REFERENCES


