Developmental Sensitivity to High Dietary Sodium Chloride in Borderline Hypertensive Rats

Rachel A. Hunt, Diane C. Tucker

The present study compared the postweaning blood pressures and body weights of borderline hypertensive rats exposed to a high (8%) sodium chloride maternal diet either from conception to weaning or only during the weaning period with borderline hypertensive rats consistently exposed to a normal (1%) sodium chloride maternal diet. Because the effects of early sodium chloride exposure may be most evident during a subsequent challenge, rats from each group were assigned to receive either an 8% sodium chloride or a 1% sodium chloride diet from 8 to 17 weeks of age. Exposure to an 8% sodium chloride diet from conception through weaning increased the adult blood pressure of borderline hypertensive rats compared with that of controls exposed to a 1% sodium chloride diet; exposure to an 8% sodium chloride diet only during weaning did not increase blood pressure. An 8% sodium chloride diet beginning at 8 weeks of age increased systolic blood pressure. The effects of perinatal and adult exposure to high dietary sodium chloride were additive. Behavioral observations and urinary electrolyte measures confirmed that pups exposed to an 8% sodium chloride diet during weaning ingested the high-sodium chloride diet. The blood pressure and heart rate responses to autonomic nervous system ganglionic blockade were assessed at 17 weeks of age. Borderline hypertensive rats exposed to an 8% sodium chloride diet from conception through weaning showed an increased bradycardic response, but no difference in depressor response, to ganglionic blockade. These data suggest that the window of developmental sensitivity for modulation of blood pressure regulation by high dietary sodium chloride occurs during prenatal and early postnatal development. (Hypertension. 1993;22:542-550.)

KEY WORDS • blood pressure • sodium chloride • sympathetic nervous system • hypertension, sodium-dependent • rats, borderline hypertensive

During prenatal and early postnatal development, there are sensitive periods or windows of vulnerability during which environmental stimuli can modify the characteristics of the adult organism and the response to later stressors or challenges. Perinatal exposure to a high sodium chloride (NaCl) diet increases adult blood pressure in several genetic models of hypertension and in normotensive rats; however, controversy remains about the developmental window (or windows) during which adult blood pressure is affected by perinatal exposure to a high-NaCl diet. In a preliminary study, borderline hypertensive rats (BHR) exposed to high (3%) maternal dietary NaCl from conception to weaning showed higher blood pressures in adulthood than BHR exposed to low (0.12%) maternal dietary NaCl. In spontaneously hypertensive rats (SHR), exposure to high (8%) maternal dietary NaCl during gestation and/or lactation accelerated the rise in blood pressure in adulthood. Blood pressure changes in most previous studies cannot be attributed to perinatal NaCl exposure because the subjects continued to receive a high-NaCl diet after weaning. The present study determined whether the adult blood pressure or the magnitude of the pressor response to high dietary NaCl in adulthood would be increased by high maternal NaCl consumption throughout pregnancy and lactation or by exposure to a high-NaCl diet only during the weaning period.

The mechanisms through which maternal dietary NaCl affects offspring are also likely to depend on the developmental stage during which offspring are exposed. During prenatal and early postnatal life, high-NaCl exposure influences the developing offspring primarily through the mother (eg, through altered hormone levels or behavior). A maternal diet high in NaCl may increase sodium concentration in the amniotic fluid but not in the milk. During the weaning period, however, the offspring are likely to ingest the high-NaCl diet and thus be directly exposed to increased NaCl. The weaning period is hypothesized to be a period of heightened sensitivity to ingestion of a high-NaCl diet because of the ongoing maturation of the kidneys and of the systems that control fluid and electrolyte balance.

The cardiovascular control systems that are affected by early exposure to high dietary NaCl are unclear. Contreras and Oparil hypothesize that perinatal NaCl exposure increases central nervous system sensitivity to angiotensin II or vasopressin. In our previous studies,
perinatal high (3%) dietary NaCl exposure altered sympathetic nervous system control of heart rate at weaning,\textsuperscript{13} supporting previous suggestions of sympathetic mediation of the cardiovascular consequences of early exposure to high dietary NaCl.\textsuperscript{16,18} Taken together, these findings suggest that early exposure to high dietary NaCl may alter regulation of blood pressure through sympathetic, angiotensin II, and/or vasopressin control mechanisms.

BHR rats have a moderate genetic predisposition to hypertension and a demonstrated sensitivity to high dietary NaCl,\textsuperscript{19} providing a model well suited to testing the consequences of early experience.\textsuperscript{3,20} The present study examined postweaning blood pressure and body weight of BHR rats exposed to high (8%) dietary NaCl either from conception to weaning or only during the weaning period. Because the effects of early NaCl exposure may be most evident during a subsequent challenge, rats fed both a high (8%) NaCl and a normal (1%) NaCl diet in adulthood were studied. In addition, autonomic contributions to baseline adult blood pressure were assessed.

**Methods**

**Animals**

Male BHR were used as subjects in the present studies. BHR were produced in our animal colony by mating salt-sensitive SHR (SHR-S; IBU\textsuperscript{3} colony\textsuperscript{21}) dams and normotensive Wistar-Kyoto (WKY) sires purchased from Taconic Farms (Germantown, NY). Within 24 hours of parturition, litters of BHR were culled to a maximum of 10 pups, retaining all male pups. Our animal colony was maintained on a 12-hour light-dark cycle with lights on at 7 AM. Throughout the studies, food and tap water were available to the animals ad libitum. The experimental protocols were approved by the University of Alabama at Birmingham (UAB) Institutional Animal Care and Use Committee. The UAB animal facilities are fully accredited by the American Association of Laboratory Animal Care.

**Experiment 1: Pups' Ingestion of High-NaCl Diet During Weaning**

**Procedures.** BHR litters (\(n=6\) to 8 pups per litter) were randomly assigned either to receive an 1% NaCl (Agway Prolab 3000 R-M-H Chow) diet from conception through weaning (\(n=2\) litters) or to begin receiving an 8% NaCl pelleted diet (ICN Biochemicals, Cleveland, Ohio) at 14 days of age (\(n=2\) litters). Behavioral observations of feeding, drinking, and nursing bouts were made for 1-minute periods every 5 minutes for 15 minutes twice daily when BHR litters were 14 to 30 days of age (a total of 60 observation periods per litter). Observations were made between 9 and 10 AM and 3 and 4 PM. The number of pups observed eating, drinking, or nursing during each observation period was recorded; the numbers were standardized to an eight-pup litter size. Interrater reliability was determined by using two observers for part of the observations. Urine samples were collected from a subset of pups by gently stroking the pups' anogenital region with a sable brush and collecting the urine in microfuge tubes. Samples were collected on days 18, 22, and 30 and stored at \(-20^\circ\text{C}\) for later determination of urinary sodium ion [Na\textsuperscript{+}] and potassium ion [K\textsuperscript{+}] concentrations by flame photometry.

**Experiment 2: Consequences of Early Exposure to High-NaCl Diet**

**Procedures.** BHR litters were randomly assigned to one of three preweaning dietary conditions: (1) SHR-S dams (\(n=13\) litters) receiving the 8% NaCl diet (ICN Biochemicals) throughout pregnancy and lactation (until 4 weeks after parturition), (2) SHR-S dams (\(n=12\) litters) receiving the 1% NaCl diet (Agway Prolab 3000 R-M-H Chow) during gestation and lactation and the 8% NaCl diet during the pups' natural weaning period (ie, from 2 to 4 weeks after parturition), and (3) SHR-S dams (\(n=12\) litters) receiving the 1% NaCl diet (Agway Prolab 3000 R-M-H Chow) throughout pregnancy and lactation (until 4 weeks after parturition).

When litters were 4 weeks of age, the systolic blood pressure of all male rats was measured by the tail-cuff method, and body weight was determined. After these measurements, BHR were weaned, ear-tagged for identification, and housed in plastic cages (20\(\times\)25\(\times\)45 cm) with a maximum of three animals per cage. All BHR received the 1% NaCl diet (Agway Prolab R-M-H 1000 Chow) from 4 to 8 weeks of age.

At 8 weeks, half of the male BHR from each litter were randomly assigned to either begin receiving the 8% NaCl diet or to continue receiving the 1% NaCl diet. BHR continued to receive this postweaning diet throughout the remainder of the study. All diets contained 0.90% to 1% calcium. Potassium content was 1.3% in the 8% NaCl diet and 0.95% and 0.70% in the Agway R-M-H 3000 and 1000 diets, respectively. Body weight was determined biweekly beginning at 2 weeks.

**Indirect blood pressure measurements.** Systolic pressure was measured indirectly from the tail artery using a photoelectric sensor. Signals were amplified (model 29 amplifier, IITC, Woodland Hills, Calif) and recorded on a polygraph (model 7D, Grass Instruments, Quincy, Mass). Animals were placed in Plexiglas restrainers but were neither heated nor anesthetized before the recording. Systolic pressure was calculated to be the mean of three artifact-free recordings made while the animal was calm. Systolic pressure was determined biweekly beginning at 4 weeks and ending at 16 weeks of age.

**Direct blood pressure measurements.** Between 5 and 7 days after the body weight and indirect blood pressure measurements at 16 weeks, each animal was anesthetized with a mixture of ketamine and xylazine (100 mg/kg and 7.5 mg/kg IP, respectively), and the femoral artery was cannulated with polyethylene (PE-50) tubing for blood pressure and heart rate determination. The femoral vein was cannulated with PE-50 tubing for drug administration. Both cannulas were filled with heparinized saline (200 U/mL), plugged, and tunneled subcutaneously to exit between the scapulae. Animals were placed individually in plastic cages (20\(\times\)25\(\times\)45 cm) and allowed approximately 24 hours to recover from surgery before testing.

On the day of testing, the arterial cannula was connected to a pressure transducer (model CP-0, Century Technology, Inglewood, Calif) and flushed with heparinized saline (20 U/mL). Blood pressure and heart rate were recorded on a polygraph from awake and unrestrained animals in their home cages. Animals
were allowed to habituate for approximately 45 minutes before the onset of recording. Samples of blood pressure and heart rate were taken every 5 minutes for 20 minutes. The mean of these five samples provided the baseline blood pressure and heart rate. Heart rate was determined by counting arterial pressure pulses recorded during a 20-second period and multiplying that value by 3. Pulse pressures were at least 35 mm Hg for all rats that contributed data.

After baseline measurements, autonomic nervous system control of adult blood pressure and heart rate was assessed. Specifically, chlorisondamine (2.5 mg/kg IV, CIBA-GEIGY, Suffern, NY), a ganglion blocking agent, was given as an intravenous bolus to infer the autonomic (largely sympathetic) contribution to basal blood pressure and heart rate. Sampling of blood pressure and heart rate after autonomic blockade was made 20 minutes after chlorisondamine. This dose and time point were determined in preliminary and previous studies to provide a maximal, stable blockade of blood pressure and heart rate responses to infused phenylephrine.

Data reduction and analysis. Because different animals contributed data at each time point in experiment 1, differences in urinary [Na\(^+\)] and [K\(^+\)] and the urinary [Na\(^+\)]/[K\(^+\)] ratios were analyzed with separate analysis of variance (ANOVA) for each age (ie, at 18, 22, and 30 days of age); to protect the a level, a criterion of P<.017 was set for all other analyses. An a level of P<.0001 was set for three analyses) was set for these analyses. In data collected across time, the covariances between adjacent measures are nearly always more highly correlated than temporally distant measures. Profile analysis also tests whether the overall level differs among groups. Differences in body weight and systolic pressure at 8 to 16 weeks were examined using separate profile analyses with preweaning and adult diet as the between-groups factor. Profile analysis indicated that the overall level differs among groups without violating the compound symmetry assumption (ie, that the covariances between all measures are equal). In data collected across time, the covariances between adjacent measures are nearly always more highly correlated than temporally distant measures.

Experiment 1

Behavioral observations. Pups were observed eating the pelleted diets beginning at 21 to 22 days of age, with eating behavior observed at least as frequently in litters supplied with an 8% NaCl diet as in litters given a 1% NaCl diet (Fig 1). Nursing behavior was the most frequent behavior among 19- to 20-day-old pups and was observed until 25 to 26 days of age, consistent with previous studies of the natural weaning process. Nursing behavior was twice as frequent among 19- to 20-day-old pups given an 8% NaCl diet compared with those given a 1% NaCl diet. Drinking of water was observed beginning at 21 to 22 days of age and was more frequent among pups given an 8% NaCl diet (Fig 1). These behavioral observations suggest that pups will ingest an 8% NaCl diet during weaning and that pups in litters given an 8% NaCl diet may ingest more fluid, initially by nursing and then by drinking water.

Urinary electrolytes. BHR exposed to an 8% NaCl diet from 14 to 30 days of age had significantly lower urinary [K\(^+\)] by day 18 than BHR exposed to a 1% NaCl diet [F(1,4) = 19.71, P<.01; see Table 1]. Urinary [Na\(^+\)] did not differ between groups on day 18 [F(1,4) = 1.71, P=.05] but was somewhat increased on day 22 [F(1,4) = 13.19, P<.02] with a significant increase by day 30 [F(1,4) = 130.6, P<.0001]. Differences in urine [Na\(^+\)] and [K\(^+\)] between groups were maintained through day 30. At all ages, pups from litters given an 8% NaCl diet showed some increase in the ratio of [Na\(^+\)]/[K\(^+\)] in the urine compared with pups from litters fed a 1% NaCl diet [F(1,4) = 13.77, P<.10 at 18 days; F(1,4) = 7.82, P<.05 at 22 days; and F(1,4) = 4.26, P<.0001] at 30 days of age. Urine volume was not measured in the present study, making it impossible to determine the actual amount of Na\(^+\) and K\(^+\) excreted by pups. By 30 days of age, pups being weaned onto an 8% NaCl diet showed a fourfold increase in urinary [Na\(^+\)] and nearly a 20-fold increase in the ratio of urinary [Na\(^+\)]/[K\(^+\)] compared with pups being weaned onto a 1% NaCl diet, consistent with increased Na\(^+\) excretion. Together, the behavioral observation and urinary electrolyte data support the conclusion that pups were exposed to the high-NaCl diet provided during weaning.

Experiment 2

Body weights. By 2 weeks of age, the body weight of BHR exposed to an 8% NaCl diet throughout the perinatal period was only 67% of the weight of control pups exposed to a 1% NaCl diet [ie, 15.6 versus 23.3 g; F(2,32) = 30.57, P<.0001], and this difference was sustained throughout the perinatal period [F(2,32) = 32.10, P<.0001; see Fig 2]. Profile analysis indicated that exposure to an 8% NaCl maternal diet significantly altered the growth profile of pups between 2 and 8 weeks of age [F(6,60) = 11.84, P<.0001; see Fig 2]. The maximal suppression in body weight was observed at 4 weeks of age. At 4 weeks of age, the body weight of pups exposed to 8% maternal dietary NaCl from conception to weaning was 52% that of pups exposed to a 1% NaCl maternal diet. Even after 4 weeks on a 1% NaCl diet (ie,
FIG 1. Line graphs show incidence of eating, nursing, and drinking behaviors observed among litters of borderline hypertensive rats (BHR) being fed a diet containing 1% NaCl (n=2 litters) or 8% NaCl (n=2 litters). Data are expressed as number of pups observed engaged in the behavior per 5-minute observation period. Because the number of pups per litter varied between six and eight, the data were standardized to an eight-pup litter. The observations suggest that pups offered an 8% NaCl diet during weaning ingest the diet and consume more fluid, initially by nursing and later by drinking water.

at 8 weeks of age), the body weight of pups exposed to an 8% NaCl maternal diet from conception through weaning was suppressed by 25% compared with 1% NaCl-exposed controls. Exposure to an 8% NaCl diet only during the weaning period reduced body weights (approximately 20% at 4, 6, and 8 weeks), indicating that exposure to high-NaCl diet during weaning did not affect the developing BHR (see Fig 2).

Body weights for rats exposed to the 8% NaCl maternal diet from conception to weaning remained significantly lower in adulthood, regardless of the NaCl content of the adult diet [F(2,34)=16.37, P<.0001; see Table 2]. Exposure to an 8% NaCl diet beginning at 8 weeks of age also compromised body weight gain [F(4,31)=2.94, P<.04 for effect of adult diet on profile of body weights]. Specifically, the body weight gain of adult rats was significantly reduced during the first 2 weeks on an 8% NaCl diet compared with controls fed a 1% NaCl diet [ie, between 8 and 10 weeks of age, F(1,34)=5.52, P<.03]. By 14 weeks of age, the absolute body weight of rats fed an 8% NaCl diet in adulthood was significantly lower than rats fed a 1% NaCl diet [F(1,34)=7.09, P=.01].

Indirect blood pressure measurements. Profile analysis showed that BHR exposed to 8% maternal dietary NaCl throughout the perinatal period had significantly higher systolic blood pressures by 4 weeks of age than controls exposed to 1% maternal dietary NaCl [102±3 versus 88±4 mm Hg; F(2,34)=5.67, P<.008; see Fig 3]. Exposure of BHR to an 8% NaCl diet only during the weaning period did not alter systolic blood pressure compared with 1% NaCl-exposed BHR (83±5 mm Hg). Systolic blood pressures for BHR exposed to 8% maternal dietary NaCl from conception through weaning remained significantly elevated throughout the study, regardless of adult diet [F(2,37)=18.90, P<.0001; see Fig 4A].

BHR exposed to an 8% NaCl diet beginning at 8 weeks of age showed the expected increase in systolic blood pressures by 12 weeks of age [F(1,37)=8.49, P<.01 for effect of adult diet on systolic blood pressure at 12 weeks of age]. The increased systolic blood pressure in BHR fed an 8% NaCl diet in adulthood was maintained throughout the study [F(1,37)=8.33, P<.01 for the overall difference between BHR fed 1% versus 8% NaCl diet in adulthood]. Although still statistically reliable, the systolic blood pressure differences among groups decreased by 16 weeks of age due to the gradual increase in blood pressure of the 1% NaCl-fed rats. There was no significant statistical interaction between preweaning and adult exposure to an 8% NaCl diet.
TABLE 1. Urinary Sodium and Potassium Ion Concentrations In Weaning Borderline Hypertensive Rats

<table>
<thead>
<tr>
<th>Measurement</th>
<th>18 Days After Birth</th>
<th>22 Days After Birth</th>
<th>30 Days After Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, g</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% NaCl diet</td>
<td>23.3±0.4 (3)</td>
<td>35.1±1.6 (2)</td>
<td>66.0±1.7 (10)</td>
</tr>
<tr>
<td>8% NaCl diet</td>
<td>20.6±0.5 (3)*</td>
<td>25.0±0.4 (4)*</td>
<td>45.8±1.7 (6)*</td>
</tr>
<tr>
<td><strong>Urinary [Na⁺], mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% NaCl diet</td>
<td>20±7 (3)</td>
<td>137±70 (2)</td>
<td>93±18 (10)</td>
</tr>
<tr>
<td>8% NaCl diet</td>
<td>31±5 (3)</td>
<td>316±17 (4)*</td>
<td>371±8 (6)*</td>
</tr>
<tr>
<td><strong>Urinary [K⁺], mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% NaCl diet</td>
<td>88±8 (3)</td>
<td>352±80 (2)</td>
<td>297±46 (10)</td>
</tr>
<tr>
<td>8% NaCl diet</td>
<td>34±10 (3)*</td>
<td>46±8 (4)*</td>
<td>66±4 (6)*</td>
</tr>
<tr>
<td><strong>Urinary [Na⁺]/[K⁺], mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% NaCl diet</td>
<td>0.21±0.07 (3)</td>
<td>0.36±0.12 (2)</td>
<td>0.30±0.06 (10)</td>
</tr>
<tr>
<td>8% NaCl diet</td>
<td>1.01±0.20 (3)*</td>
<td>7.69±1.75 (4)*</td>
<td>5.71±0.33 (6)*</td>
</tr>
</tbody>
</table>

The number of pups contributing data at each time point is indicated in parentheses. Values are mean±SEM.

*8% NaCl diet-exposed pups different from 1% NaCl diet-exposed pups (P<.017).
†[Na⁺]/[K⁺] ratio in 1% NaCl diet was 0.27 (Agway R-M-H 3000).
‡[Na⁺]/[K⁺] ratio in 8% NaCl diet was 1.41 (ICN Biochemicals).

Direct blood pressure and heart rate measurements. Profile analysis showed no overall differences in either baseline systolic or diastolic blood pressures at 17 weeks of age (P>.20 for both systolic and diastolic blood pressure analyses; see Table 3). Baseline heart rates did not differ among groups. Table 3 presents the direct blood pressure and heart rate measurements and the responses to ganglionic blockade. Neither preweaning nor adult dietary NaCl affected the magnitude of the depressor response to chlorisondamine (P>.10 for both systolic and diastolic blood pressure analyses; see Table 3). After ganglionic blockade, BHR given an 8% NaCl diet in adulthood had significantly lower heart rates compared with BHR given a 1% NaCl diet [F(1,32)=4.96, P<.04]. Profile analysis indicated that BHR exposed to an 8% NaCl diet from conception through weaning showed an increased bradycardic response to ganglionic blockade compared with both BHR exposed to an 8% NaCl diet only during weaning and BHR exposed to a 1% NaCl diet from conception through weaning [F(2,32)=3.36, P<.05], regardless of adult diet. This suggests that exposure to an 8% NaCl diet from conception through weaning can affect autonomic nervous system control of heart rate in BHR.

Organ weights. The relative adrenal and kidney weights were expected to be increased by an 8% NaCl adult diet. Heart weight was compared with body weight to determine whether there was evidence of cardiac hypertrophy in groups with increased blood pressures.

FIG 2. Line graph shows body weight of juvenile borderline hypertensive rats (BHR), which was measured biweekly beginning at 2 weeks of age. BHR exposed to 8% dietary NaCl begun at conception (8% NaCl conception-weaning) weighed less at the first measurement (2 weeks after birth), and their growth was significantly suppressed during the period between 2 and 8 weeks of age. Exposure to an 8% NaCl diet only during the weaning period (between 2 and 4 weeks of age; 8% NaCl weaning only) significantly suppressed growth between 2 and 4 weeks, with this reduction being maintained after weaning. All rats were fed a 1% NaCl diet from 4 to 8 weeks of age. Data are mean±SEM. The number of litters that contributed to the analysis is indicated in parentheses. *Significant decrease in body weight compared with 1% NaCl-exposed rats, P<.05.
Retroperitoneal fat pads were weighed to determine whether prenatal or adult exposure to an 8% NaCl diet altered body composition (ie, decreased fat stores).

In BHR exposed to an 8% NaCl diet from conception through weaning, absolute kidney weight, heart weight, and retroperitoneal fat pad weights were decreased \[F(2,29)=5.83, P<.01; F(5,28)=6.20, P<.01; F(5,28)=8.58, P<.01, \text{respectively; see Table 4}\]. The decreases in kidney and heart weight were, however, proportional to the reduction in body weight in 8% NaCl-exposed rats compared with 1% NaCl-exposed controls (ie, the relative kidney and heart weights were not altered by preweaning dietary NaCl). The relative weight of the retroperitoneal fat pads was decreased by exposure to 8% NaCl from conception to weaning \[F(2,28)=3.60, P<.04\]. This finding is consistent with the persistent decrease in body weight observed in rats exposed to 8% NaCl from conception through weaning.

Chronic exposure to an 8% NaCl diet in adulthood produced the expected increase in the relative size of the adrenals \[F(1,29)=4.56, P<.05\] and kidneys \[F(1,29)=54.16, P<.0001\]. An increased heart weight/body weight ratio was observed in BHR fed an 8% NaCl diet as adults \[F(1,28)=9.19, P<.01, \text{see Table 4}\], consistent with the increased systolic blood pressure observed in BHR fed an 8% NaCl diet as adults. As expected, decreased retroperitoneal fat pad weights paralleled the reduced body weight resulting from adult exposure to an 8% NaCl diet \[F(1,28)=32.00, P<.0001\].

**Discussion**

In the present study, exposure of BHR pups to increased maternal dietary NaCl during the prenatal and early postnatal period accelerated the postweaning increase in blood pressure and permanently reduced body weight. In contrast, when exposure to a high maternal NaCl diet was restricted to the weaning period (2 to 4 weeks of age), blood pressure was unaffected. These data argue against the weaning period as a time when developing blood pressure regulatory mechanisms are especially sensitive to high dietary NaCl. Instead, the results suggest that blood pressure regulation of BHR is sensitive to maternal NaCl intake during pregnancy and/or lactation. A similar conclusion was reached using Piebald rats. Because the duration of exposure to high maternal dietary NaCl and the developmental period of exposure were not manipulated independently in the present study, it is possible that the duration of exposure rather than the developmental period during which it occurred was the active factor. The present study did not examine the effects of a high maternal NaCl diet during gestation and lactation without also exposing the pups to high-NaCl diet during weaning. Therefore, we cannot conclude that exposure

![FIG 3. Line graph shows systolic blood pressure (BP) of male borderline hypertensive rats (BHR) measured biweekly using tail-cuff plethysmography beginning at 4 weeks of age. BHR exposed to high (8%) maternal dietary NaCl from conception through weaning showed higher systolic BPs than BHR pups exposed to an 8% NaCl diet only during the weaning period or to a 1% NaCl maternal diet throughout the preweaning period. All rats were fed a 1% NaCl diet from 4 to 8 weeks of age. Data are mean±SEM. The number of litters that contributed to the analysis is indicated in parentheses. *Significant increase in systolic blood pressure compared with 1% NaCl-exposed rats, P<.05.](http://hyper.ahajournals.org/)
to high-NaCl before weaning is sufficient to increase blood pressure. Instead, it is possible that the combination of perinatal high-NaCl and exposure to high-NaCl during the weaning period is necessary to increase adult blood pressure.

Although exposure to 8% NaCl only during the weaning period did not influence adult blood pressure, other measures indicate that the pups were affected. Body weight gain was significantly slowed by ingestion of 8% NaCl during the weaning period and remained suppressed for several months. Behavioral observations indicated that pups ingested the high-NaCl diet during weaning as frequently as a 1% NaCl diet and that high NaCl-fed pups showed more drinking behavior. High-NaCl diet during the weaning period also altered pups' urinary electrolyte concentrations. Pups from litters given an 8% NaCl diet showed increased ratios of [Na⁺]/[K⁺] in the urine compared with pups from litters fed a 1% NaCl diet. Because urine volume and flow rate were not measured, total Na⁺ and K⁺ excretion could not be calculated. However, both behavioral and urinary electrolyte data do support the conclusion that pups were exposed to high dietary NaCl during the weaning period.

The mechanism by which high maternal dietary NaCl increases blood pressure has yet to be defined. In the present study, BHR exposed to an 8% NaCl diet from conception through weaning showed evidence of increased autonomic (largely sympathetic) control of heart rate but no differences in depressor response to ganglionic blockade. Available evidence suggests that high dietary NaCl is not directly transferred from the lactating mother to the offspring, although this has not been studied in SHR females. In a careful study of heterozygous Brattleboro rats, Hazon et al. showed that high-NaCl feeding somewhat increased the [Na⁺] in the amniotic fluid but not in the milk. Pups of the hypertensive parent strain of BHR (ie, SHR pups) show sodium retention and increased plasma renin activity during the weaning period. Despite these predispositions, exposure of BHR rats to increased NaCl only during weaning in the present study was not sufficient to increase blood pressure.

**Table 3. Blood Pressure and Heart Rate Recorded From Indwelling Cannulas**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>1% NaCl Adult Diet</th>
<th>8% NaCl Adult Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Conception-Weaning)</td>
<td>(Conception-Weaning)</td>
</tr>
<tr>
<td>Baseline</td>
<td>(n=6)</td>
<td>(n=7)</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>154±6</td>
<td>159±4</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>107±4</td>
<td>105±3</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>326±11</td>
<td>345±8</td>
</tr>
<tr>
<td>Chlorisondamine, 2.5 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>110±9</td>
<td>108±3</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>79±6</td>
<td>74±2</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>324±7</td>
<td>310±5</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; and bpm, beats per minute. Values are mean±SEM.
A diet high in NaCl may alter maternal levels of hormones (eg, aldosterone, angiotensin II, and arginine vasopressin) and thereby perhaps alter the fetal milieu. In addition, maternal behavior may be changed by a high-NaCl diet. Contreras recently observed that dams being fed a high-NaCl diet show increased licking of pups, primarily in the anogenital region. Lactating females recover a significant amount of fluid by stimulating micturition of pups during anogenital licking \(^{28-29}\); thus, increased anogenital licking could aid dams in maintaining fluid balance during ingestion of a high-NaCl diet. A complex interplay could thus occur among hormonal, behavioral, and other consequences of high dietary NaCl.

The observation that perinatal exposure to a high-NaCl diet permanently decreased body weight has a precedent in genetically hypertensive rats. In studies with SHR, both DiNicolantonio et al \(^{30}\) and Contreras and Oparil \(^{31}\) have reported that lower body weight persisted into adulthood after exposure to high-maternal NaCl from conception to weaning (3% and 8% NaCl diets, respectively). However, in normotensive Sprague-Dawley rats, exposure to high (3%) NaCl maternal diet during gestation and suckling did not affect body weight. \(^{31}\) Body weight gain in preweaning BHR is positively correlated with adult blood pressure. \(^{32}\) The suppressed growth after perinatal exposure to high-NaCl in the present study may have attenuated the NaCl-induced increase in blood pressure.

Direct blood pressures taken at 16 to 17 weeks of age failed to detect differences among dietary NaCl groups in baseline blood pressure. BHR exposed to an 8% NaCl maternal diet from conception to weaning reached their asymptotic blood pressure sooner than pups fed an 8% NaCl diet only during the weaning period or pups exposed to a 1% NaCl diet from conception to weaning. By 17 weeks of age, the baseline blood pressure of all groups had increased to the level of rats exposed to an 8% NaCl diet throughout perinatal development. BHR fed an 8% NaCl diet in adulthood showed an increased heart weight/body weight ratio, which is consistent with the higher systolic pressures recorded during biweekly indirect measures. In Lawler's study of BHR fed an 8% NaCl diet, an increase in blood pressures measured from an indwelling cannula was found after 5 weeks on the 8% NaCl diet (ie, at 13 weeks of age). After 5 weeks on the 8% NaCl diet in the present experiment, the separation in adult blood pressures among groups in our experiment was larger than after an additional 4 weeks on the adult diets.

In summary, the present study argues against ingestion of high (8%) NaCl diet by pups as the primary mechanism through which a high maternal NaCl diet from conception through weaning increases adult blood pressure. Instead, the data point toward pregnancy and/or lactation as periods when developing blood pressure regulatory mechanisms are sensitive to increased NaCl concentration in the maternal diet.

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