Effect of a perinatal high-salt diet on blood pressure control mechanisms in young Sprague-Dawley rats

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Swenson, Steven J., Robert C. Speth, and James P. Porter. Effect of a perinatal high-salt diet on blood pressure control mechanisms in young Sprague-Dawley rats. Am J Physiol Regul Integr Comp Physiol 286: R764–R770, 2004. First published January 8, 2004; 10.1152/ajpregu.00492.2003.—In the present investigation we sought to determine if a perinatal high-salt treatment affects blood pressure at an early age (30 days), and if so, to determine the mechanisms responsible for the hypertension. Pregnant dams were given an 8% NaCl diet [high-salt (HS) rats] during the final one-third of gestation and throughout the suckling period. After weaning, the pups continued to receive the high-salt diet until testing at age 30 days. Control groups received a normal-salt diet (NS rats). Blockade of brain AT1 receptors with intracerebroventricular losartan decreased MAP in HS but not NS rats. Blockade of α-adrenergic receptors with intravenous phentolamine or ganglionic transmission with intravenous chlorisondamine produced a greater decrease in MAP in HS rats. Baroreflex control of heart rate was assessed using a four-parameter logistics function. The mid-range MAP (p50) was significantly increased in the HS rats. No other baroreflex parameters were affected. Specific binding of [125I]-[Sar1,Ile8]ANG II to AT1 receptors was increased in the subfornical organ (SFO) of the HS rats. Expression of AT1a receptor mRNA was greater in both SFO and PVN of the HS rats. These data suggest that even at an early age, Sprague-Dawley rats treated with a perinatal high-salt diet are hypertensive. The elevated blood pressure appears to be caused by increased sympathetic nervous activity, resulting, in part, from increased brain AT1 receptor activation.

AT1 receptor; brain; subfornical organ; hypertension; sympathetic nervous system
increase sympathetic nervous system activity (27), and the hypertension induced by a high-salt diet may involve increased sympathetic outflow to the cardiovascular system. Oparil et al. (22) found that salt sensitivity in the spontaneously hypertensive rat (SHR) was associated with changes in the sympathetic nervous system. With a high-salt diet, there was decreased activity of noradrenergic sympathoinhibitory neurons in the anterior hypothalamic area, thereby increasing the activity of sympathetic preganglionic neurons in the spinal cord. The hypertension in Dahl S rats on a high-salt diet has also been shown to involve increased sympathetic nervous activity (33). Whether a similar alteration occurs in young Sprague-Dawley rats exposed to high salt from birth is unknown.

Second, brain ANG II has been shown to decrease baroreceptor sensitivity (26), and several studies have linked a high-salt diet to altered baroreflex function. While the arterial baroreflex has been classically viewed as the minute-to-minute regulator of blood pressure, there is evidence that it may play a role in long-term blood pressure regulation under conditions of altered dietary salt intake (23). Gordon and Mark (5) proposed that a primary defect of Dahl S rats is an impairment of baroreceptor function even before they develop hypertension when challenged by a high-salt diet. Furthermore, it has also been shown that dietary salt loading for 5 wk in adult Sprague-Dawley rats produced a less responsive baroreflex in response to baroreceptor loading by phenylephrine (19). In such situations, the baroreflex impairment would favor an increase in blood pressure and could be a contributing factor to the development of hypertension. We hypothesized that a similar blunting of the baroreflex by perinatal high salt could explain the hypertension observed by Contreras et al. (4).

The results of the present study show that young rats exposed to a high-salt diet from 5 to 7 days before birth to 30 days after birth are already hypertensive at this age. The increased arterial pressure appears to be mediated by increased sympathetic outflow, in part due to an increase in ANG II activity in brain areas accessible by intracerebroventricular losartan. Increased ANG II binding in the SFO may mediate the increased ANG II receptor activation. Portions of this work have been presented in abstract form (32).

MATERIALS AND METHODS

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Brigham Young University. Experiments were performed using Sprague-Dawley rats subjected to the following high-salt protocol. Pregnant dams (14–16 days) were purchased from Harlan Sprague Dawley (Indianapolis, IN). Upon arrival, they were randomly assigned to a normal-salt diet (NS rats, n = 7) (0.7%, Harlan Teklad, TD 96329) or a high-salt diet (HS rats, n = 7) (8%, Harlan Teklad, TD 92012). All rats drank water ad libitum. When pups were born, litters were culled to 10 pups (without regard to sex). Dams continued to receive the same diet as their mother until the end of the experiment. The relationship between MAP and HR was determined every 30 min from 1 to 2 h for stabilization were allowed. Once resting MAP had been determined, a bolus intravenous injection of the nonselective α-adrenergic receptor antagonist phenolamine (4 mg/kg, Sigma) was given in one group (NS, n = 4; HS, n = 5). The dose of phenolamine was chosen to produce an intermediate fall in blood pressure (compared with the chlorsondamine, see below). MAP was again determined after the injection. Absolute decrease in MAP after phenolamine injection was divided by resting MAP before phenolamine injection and then multiplied by 100%. After the experiment, dye was used to verify placement of the cannula.

Effect of α-adrenergic receptor or ganglionic blockade on resting MAP. Two additional groups of rats were first instrumented with arterial and venous catheters as explained above. After 1 day of recovery, the arterial catheter was connected to a pressure transducer, and 30 min to 1 h of stabilization were allowed. Once resting MAP had been determined, a bolus intravenous injection of the nonselective α-adrenergic receptor antagonist phenolamine (4 mg/kg, Sigma) was given in one group (NS, n = 10; HS, n = 8) a similar procedure was followed except the ganglionic blocker chlorsondamine (5 mg/kg) was given intravenously.

Baroreflex testing. In separate groups of rats (NS, n = 14; HS, n = 19), baroreflex control of heart rate (HR) was tested on postnatal day 30 while rats were conscious and freely moving. The arterial catheter was connected to a pressure transducer, and 30 min to 1 h of stabilization were allowed before baroreflex testing. The baroreflex was assessed by delivering intravenous ramp infusions of phenylephrine (PE) (Sigma, 0.83 µg/min gradually increased to 10 µg/min) and sodium nitroprusside (SNP) (Sigma, 2.3 µg/min gradually increased to 23 µg/min). HR was continually monitored throughout the experiment. The relationship between MAP and HR was determined every 15 s (4–7 time points) for each infusion of PE and SNP.

The HR vs. MAP data for each individual rat were fitted to a four-parameter logistics curve $y = p4 + p1/[1 + e^{(p2-p3)/p2}]$ (SigmaPlot, Jandel), where $y$ is HR, $x$ is MAP, $p1$ is the HR range, $p2$ is the gain coefficient, $p3$ is the MAP corresponding to the to the midpoint over the range of HR (MAPmid), and $p4$ is the minimum HR (16). Mean values were calculated for each of the four parameters (NS means and HS means calculated separately), and composite curves were created for NS and HS rats.

Plasma renin activity assay. Plasma was obtained from some NS (n = 7) and HS (n = 10) rats immediately after baroreflex testing or after treatment with intravenous chlorsondamine or intracerebroventricular losartan. The rats were anesthetized with ketamine-acepromazine, and within 10 min, blood (1 ml) was collected through the arterial catheter and placed in heparinized tubes. Plasma renin activity (PRA) was determined using an ANG I $^{125}$I radioimmunoassay kit (Perkin Elmer Life Sciences, Boston, MA, NEA104). The manufacturer’s instructions were followed with slight modifications to allow for smaller volumes (25).

In vitro ANG II binding assay. To avoid confounding effects, the brains used for in vitro ANG II binding and in situ hybridization (see
All relative quantitation of in situ hybridization data was performed using Scion Image for Windows (Scion) and scanned images of SFO and PVN. The entire SFO or PVN for each section (4–6 consecutive) was outlined as the region of interest, and the optical density (OD) times area (in pixels) was calculated. Background was determined from an area adjacent to the SFO or PVN and was subtracted from the value for each section. The values from the consecutive sections were then averaged. Within each batch, the averaged OD times area for NS rats was standardized to 1. All HS values were adjusted accordingly. The adjusted values from each batch were then combined for statistical analysis.

Data analysis. All quantitative data are presented as means ± SE. For intravenous phentolamine and chlorisondamine and intracerebroventricular losartan administration, a paired Student’s t-test was used to determine significance. In all other cases, an unpaired Student’s t-test was used. A P value < 0.05 was considered to be significant.

RESULTS

Contribution of central AT1 receptors to resting MAP. Resting MAP was measured and the contribution of central AT1 receptors to this prevailing blood pressure was assessed on postnatal day 30, 1–2 days after the rats had been instrumented with femoral arterial catheters. MAP was measured before and after a single intracerebroventricular injection of losartan. Before losartan, resting MAP was significantly higher in HS rats than NS rats (110 ± 5 vs. 96 ± 3 mmHg). In HS rats, intracerebroventricular losartan injection resulted in a significant decrease in resting MAP (Fig. 1, left). The intracerebroventricular losartan had no effect in the NS rats.

Assessment of effect of α-adrenergic receptor or ganglionic blockade on resting MAP. MAP was measured before and after α-adrenergic receptor blockade with intravenous phentolamine. Resting MAP was significantly higher in the HS rats (116.4 ± 4.2 vs. 98.3 ± 5.3 mmHg). The fall in MAP with phentolamine was also significantly greater in the HS rats (Fig. 1, right). The effect of ganglionic blockade with a single intravenous bolus injection of chlorisondamine on resting MAP was assessed in other rats. Before ganglionic blockade, resting MAP was significantly higher in HS rats than NS rats (118 ± 4 vs. 102 ± 5 mmHg). After ganglionic blockade, resting MAP was not different between groups (71 ± 4 vs. 71 ± 2 mmHg). There was a significantly greater percent decrease in resting MAP in HS rats compared with NS rats (Fig. 1, right).

Baroreflex. The resting MAP of HS rats was significantly higher than that of NS rats (Fig. 2). During baroreflex testing, the average increase in MAP with administration of PE was 44.1 ± 1.6 mmHg, and the average decrease in MAP with
administration of SNP was 42.3 ± 2.1 mmHg with no significant difference between groups (data not shown). MAP vs. HR data for each individual rat were fit to a four-parameter logistic curve. P3 (MAP_{50}) was significantly higher in HS rats than in NS rats (Fig. 2). However, none of the other baroreflex function parameters was different between the two groups.

**PRA assay.** A PRA assay was performed on plasma samples from NS (n = 7) and HS (n = 10) rats. PRA was significantly suppressed in the HS rats (0.67 ± 0.27 vs. 5.0 ± 1.7 ng ANG I/ml^{-1}h^{-1}).

In vitro ANG II binding and AT_{1a} in situ hybridization. Examples of total binding and mRNA hybridization in consecutive sections of SFO and PVN are shown in Fig. 3. 

**DISCUSSION**

The results of the present study indicate that Sprague-Dawley rats, exposed to a perinatal high-salt diet, were hypertensive by postnatal day 30. These results extend the findings of Contreras et al. (3, 4), who showed that a perinatal high-salt diet discontinued at postnatal day 30 caused hypertension at postnatal day 60 and beyond. Hence, it is possible that the rats in the study by Contreras et al. were hypertensive throughout the period from postnatal day 30 until tested at day 60, although rats were fed a 3% NaCl diet in that study vs. the 8% NaCl diet used in the present study. We used an 8% salt diet because Contreras et al. showed a graded effect of dietary salt on the development of hypertension, that is, a 1% NaCl diet raised blood pressure but not to the same extent as 3% NaCl. Since the reported increase in blood pressure with the 3% diet was modest, we hoped to produce a bigger effect by using a higher content of NaCl.

Functional studies were performed in conscious rats to assess the contribution of central AT_{1} receptors and the sympathetic nervous system to the increased MAP. A portion of the elevation in MAP was due to increased activation of central AT_{1} receptors, at least those receptors accessible by intracerebroventricular losartan. Yang et al. (37) found that AT_{1} receptor antagonists injected into the anterior hypothalamic area (AHA) of spontaneously hypertensive rats (SHRs) caused a decrease in MAP, and this depressor response was more pronounced when SHRs were given a high-salt diet. There was no depressor response to AHA injection in control Wistar-Kyoto rats (WKY) fed either a normal- or high-salt diet. This same study also reported an enhanced AT_{1}-mediated pressor response to injection of ANG II into the AHA of SHRs compared with SHRs fed a normal-salt diet. Others showed that DOCA-salt-induced hypertension in Wistar rats was normalized by intracerebroventricular losartan injection (24). The hypertension in Dahl S rats on a high-salt diet can also be reduced by intracerebroventricular injection of captoril or losartan (9, 36). Li et al. (17) found that inhibition of the expression of AT_{1} receptors in the PVN abolished the hypertensive response to dietary NaCl in mREN-2 rats, a recently developed model of sodium-sensitive hypertension. The present study showed that if young Sprague-Dawley rats are treated with perinatal high salt, they too exhibit hypertension due to activation of central ANG II mechanisms. The decrease in MAP with losartan was −7 mmHg in the HS rats, which accounted for only half the approximate 14 mmHg difference in resting blood pressure between HS and NS rats. Hence, other factors also contributed to the hypertension.

Increased sympathetic outflow contributed to the hypertension in the HS rats. Partial blockade of α-adrenergic receptors with phenolamine produced a greater fall in MAP in the HS rats compared with the NS rats. Likewise, complete blockade of ganglionic transmission with chlorisondamine also produced a greater decrease in MAP in the HS rats. The entire difference in resting MAP (16 mmHg in these groups) was abolished by the ganglionic blockade, which suggests that increased sympathetic activity was completely responsible for the hypertension. Since ANG II has an effect in the brain to increase sympathetic outflow (27), it is likely that some, but not all, of the hypertensive effects of this peptide involved stimulation of sympathetic neurotransmission.

Dietary sodium-induced sympathetic hyperactivity has been shown to contribute to hypertension in both SHR and Dahl S rats (22, 33). Huang et al. (10) showed that intracerebroventricular infusion of sodium-rich artificial cerebrospinal fluid...
caused increases in MAP, HR, and renal sympathetic nerve activity in Wistar, Dahl S, and Dahl salt-resistant (Dahl R) rats. These changes were more pronounced in Dahl S vs. Wistar or Dahl R rats and more pronounced in young Dahl S rats than in old Dahl S rats. These data suggest that young salt-sensitive animals develop a higher degree of dietary sodium-induced sympathetic hyperactivity than do their older counterparts. This may be an important factor in the efficacy of a perinatal high-salt diet in producing hypertension in young Sprague-Dawley rats.

Both a high-salt diet and increased brain ANG II have been reported to blunt baroreflex function (19, 27). In our rats, the MAP$_{50}$ (p3) was increased in HS rats. This rightward shift of the baroreflex curve without a change in gain suggests that a simple resetting of the baroreflex to a higher MAP may have occurred. Because rapid adaptation to new MAP levels is a well-known characteristic of the baroreflex (15), this result is not surprising. Because the gain coefficient (p2) was not different between groups (baroreflex sensitivity was unchanged), we conclude that changes in baroreflex function were likely secondary to the increase in blood pressure and not a primary cause of the observed hypertension in HS rats.

The results of the PRA assay indicate that the peripheral RAS was suppressed in HS rats, consistent with previous research indicating that high-salt diets decrease renin activity (7). It is recognized that the anesthesia used may have increased PRA in both groups. Nevertheless, the difference between the two groups was clearly evident. Although not measured, it is likely that blood levels of ANG II were also low in the rats eating the high-salt diet. Circulating ANG II has been shown to influence both ANG II receptor number and ANG II receptor affinity in the brain, although regulation appears to be region specific (34). It is not known to what degree the differences in AT$_1$ receptor expression and binding (see below) observed in the present study were due to differences in peripheral vs. central ANG II levels.

There was increased $^{125}$I-SI ANG II binding in the SFO of HS rats. Binding in the PVN also tended to be higher, but the difference was not significant. Because the SFO is outside the blood-brain barrier and has access to circulating ANG II, the increase in AT$_1$ binding may have resulted from altered plasma ANG II levels or from central determinants. The fact that AT$_{1a}$ mRNA expression was also increased in the SFO of HS rats argues that the increased binding was likely due to increased receptor expression. A recent study reported that Dahl R rats given a high-salt diet for 4 wk showed increased AT$_1$ receptor binding in the PVN but not the SFO (36). This was in contrast to Dahl S rats that showed more marked increases in AT$_1$
receptor binding within 1 wk in both PVN and SFO. It was speculated that there may be two populations of AT1 receptors in the brain; those that are regulated by the salt and water status of the animal and those that modulate sympathetic nervous activity and blood pressure. Unlike Dahl R rats given a high-salt diet, our Sprague-Dawley rats given a perinatal high-salt diet were hypertensive. It could be that the intracerebroventricular losartan given in the present study lowered MAP by blocking the increased ANG II binding in the SFO. Alternatively, it could have blocked AT1 receptors at sites other than PVN that influence sympathetic activity inside the blood-brain barrier. For example, it has recently been shown that microinjection of an AT1 receptor antagonist into the rostral ventrolateral medulla of hypertensive Dahl S rats given a high-salt diet produced a marked decrease in blood pressure that was not seen in similarly treated Dahl R rats (11).

In the past, experiments using RT-PCR to study the effects of a high-salt diet on AT1 receptor regulation in the rat brain have produced conflicting results (12, 30, 31). In addition to differences in salt content, duration, and timing of high-salt diet, these conflicts may be a result of different investigators isolating mRNA from different brain areas (whole brains, decorticated brains, hypothalamus, etc.). Because RT-PCR quantifies mRNA but does not localize it, and the AT1 receptor has been shown to be regulated differently in separate brain areas (12), it would be possible to get different results depending on how much of the brain or which brain area mRNA is isolated from.

We sought to overcome this problem by using in situ hybridization. It should be noted that binding and hybridization studies were done using brains from rats that did not receive any surgical or experimental interventions before death. It is not known to what extent the expression of AT1a mRNA may have been influenced by these procedures in the physiological studies. AT1a mRNA hybridization was increased in the SFO and PVN of HS rats using semiquantitative analysis. Apparently the increased AT1 mRNA in the PVN did not translate into increased receptor protein, at least as assessed by binding studies. Charron et al. (2) showed that acute sodium deficit induced by furosemide injection caused increased AT1a mRNA expression in both the SFO and PVN, which suggests that low-salt conditions may also upregulate AT1a receptors. A low-salt diet also was reported to increase AT1 receptors in the hypothalamus/thalamus/septum but decrease AT1 receptors in the medulla (34). These different findings underscore the complexity of AT1 receptor regulation.

The present study did not investigate other components of the brain RAS. In addition to increased AT1 receptor expression, the increase in central ANG II activity could have been due to increased generation of ANG II itself. Nishimura et al. (21) showed that intracerebroventricular infusion of amidolidesensitive Na+ channel activators coupled with a high-salt diet upregulated angiotensin-converting enzyme and renin mRNA in the hypothalamus of mature Wistar rats. Similar effects in these young Sprague-Dawley rats are possible. It is not known when or how the high-salt treatment produced the hypertension in the young rats. Maternal high-salt diet does not increase sodium concentration in amniotic fluid (1), and it is not known if plasma or cerebrospinal fluid sodium concentration was increased in our pups at birth. The milk of mothers eating a high-salt diet has increased NaCl concentra-

tion (35), and therefore the suckling pups presumably had increased salt intake; this may have contributed to the changes that led to the hypertension. Further studies are needed to address specific questions about the mechanisms for transmission of the hypertension to the pups.

In summary, we have shown that a perinatal high-salt diet in Sprague-Dawley rats caused an increase in MAP at postnatal day 30. This rise in MAP is partially dependent on increased tonic activation of central AT1 receptors, possibly mediated via an increase in the number of AT1a receptors in the SFO, although increases in other components of the RAS cannot be ruled out. The hypertension is completely dependent on increased sympathetic nervous mechanisms as assessed pharmacologically. Blunting of baroreflex responsiveness does not appear to contribute to the hypertension. We did not study any rats that were allowed to grow to adulthood after the high-salt treatment but suggest that similar mechanisms are likely to explain the persistent hypertension reported in these rats by Contreras et al. (4).

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GRANTS

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REFERENCES


