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). In HS rats, mean arterial pressure (MAP) was significantly increased (110 ± 5 vs. 96 ± 3 mmHg) compared with 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 (p3) 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 subformical 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; subformical organ; hypertension; sympathetic nervous system

Sprague-Dawley rats are usually considered to be salt resistant, that is, increased dietary salt intake does not lead to increased arterial pressure (6, 13). However, studies by Contreras et al. (3, 4) found that Sprague-Dawley rats exposed to a perinatal high-salt diet (mothers were given a high-salt diet (3% NaCl) throughout pregnancy and lactation, and their offspring were given the same high-salt diet from weaning at postnatal day 21 to postnatal day 30) had significantly elevated mean arterial pressure (MAP) of 7–14 mmHg in adulthood. These data suggest that, during development, the level of dietary salt can permanently alter adult cardiovascular regulatory mechanisms in Sprague-Dawley rats. However, mechanisms for this salt-induced elevation in blood pressure have not been investigated. Furthermore, it is not known if the high-salt-induced hypertension appeared before adulthood. The aims of the present study were to 1) determine if Sprague-Dawley rats exposed to a perinatal high-salt protocol are already hypertensive by postnatal day 30, and 2) to examine potential mechanisms for the elevated blood pressure. The use of this salt-resistant strain is ideal because it raises the possibility of early-life dietary salt as a potential mechanism for increased blood pressure in animals with presumably no genetic predisposition for hypertension.

Increased activity of the brain renin-angiotensin system (RAS) and/or increased brain angiotensin receptor sensitivity could play a role in the hypertensive response. All of the components of the RAS as well as both ANG II receptor subtypes have been found in the brain, suggesting the existence of a local brain RAS, functioning separately from the peripheral RAS. An immunohistochemical study by Lind et al. (18) found ANG II immunoreactivity in the magnocellular and parvocellular hypothalamic paraventricular nucleus (PVN), the supraoptic nucleus, the suprachiasmatic nucleus, and the subformical organ (SFO). Zhenhui and Ferguson (38) found that, in Sprague-Dawley rats, SFO efferents projecting to the PVN used ANG II as a neurotransmitter and that its effects were mediated by AT1 receptors. Receptor autoradiography studies have shown that SFO and PVN have high densities of the AT1 ANG II receptor subtype (28). The brain RAS has been implicated in the pathogenesis of some forms of salt-induced hypertension. For example, intracerebroventricular infusion of 0.3 M NaCl caused sympathoexcitatory and pressor effects in Wistar rats, and these effects were blocked by concomitant administration of intracerebroventricular losartan, an AT1 receptor antagonist (8). Also, in Dahl salt-sensitive (Dahl S) rats on a high-salt diet, peripheral administration of irbesartan, a lipophilic AT1 receptor antagonist, was effective in reducing hypertension only to the extent that central AT1 receptor blockade was induced (16). Recently, it has been shown that as early as 1 wk after starting a high-salt diet, Dahl S rats exhibited increased binding of ANG II in forebrain sites such as the SFO and PVN (36). Dietary salt loading in adult male Sprague-Dawley rats increased preproangiotensinogen mRNA in the anterior hypothalamus (29). Hence, a tonic increase in activity of the RAS produced by a high-salt diet during critical developmental periods of the Sprague-Dawley rat could potentially play a role in the development and/or maintenance of hypertension in this strain that is typically not considered to be salt sensitive.

An increase in activity of the RAS in the brain, if present, could contribute to hypertension through at least two mechanisms. First, increases in brain ANG II with concomitantly increased brain ANG II receptor stimulation are thought to

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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. 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 their respective diets until pups were weaned at postnatal day 21. After weaning, pups continued to receive the same diet as their mother until the end of the experiment on postnatal day 30. In total 56 (28 female, 22 male, 6 undetermined) NS pups and 55 (28 female, 22 male, 5 undetermined) HS pups were used.

Preparatory surgery was performed on postnatal days 28 or 29. All rats received a femoral arterial catheter to measure MAP. Rats assigned to baroreflex measurement and ganglionic blockade protocols were also instrumented with femoral venous catheters for administration of drugs. Rats assigned to receive intracerebroventricular losartan were instrumented with an intracerebroventricular cannula (see below for specific surgical materials and procedures). One to 2 days were allowed for recovery from surgery.

Contribution of central AT1 receptors to resting MAP. A group of rats (NS, n = 7; HS, n = 6) was first instrumented with a femoral arterial catheter as explained above. While still under anesthesia, rats were instrumented with an intracerebroventricular guide cannula (23-gauge; Plastics One, Roanoke, VA). Each cannula was implanted so as to end 1 mm dorsal to the lateral ventricle and was anchored in place using small screws and cranioplastic cement. A dummy cannula was inserted into the guide cannula until the time of the experiment.

After 1 day of recovery, the arterial catheter was connected to a pressure transducer, the dummy cannula was removed and replaced with an injector prefilled with losartan, and 30 min to 1 h were allowed for stabilization. Once resting MAP had been determined, a bolus intracerebroventricular injection of losartan (10 μg, 5 μl) was given over the course of 10–15 s. MAP was again determined after the injection. Absolute change in MAP after losartan injection was divided by resting MAP before losartan 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 intracerebroventricular 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 chlorisondamine, 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%. In the second group (NS, n = 10; HS, n = 8) a similar procedure was followed except the ganglionic blocker chlorisondamine (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)x] (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 (MAPp), 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 chlorisondamine 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 125I 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
below) were obtained from additional groups of rats (HS, n = 10; NS, n = 10) that did not undergo any surgery or procedures before death at age 30 days. The rats were anesthetized with ketamine-acepromazine and then decapitated. After decapitation, the brains were removed and immediately frozen in plastic molds (containing mounting medium) floated on dry ice-cold 100% ethanol. The frozen brains were kept at −96°C until subsequent processing for binding or hybridization. Consecutive coronal sections (20 μm) through the SFO and PVN were cut (1 in 3 series) with a cryostat and thaw-mounted onto slides (Superfrost Plus, Fisher). One of the series of slides was used for in situ hybridization, and the other two series were used for in vitro ANG II binding studies. Sections were processed for ANG II binding within 1 wk of cutting. On the day of the assay, slides were placed back to back and the frosted ends mounted into Peel-A-Way slide grips (1 experimental and 1 control slide per slot). Each group of slides was then preincubated for 30 min in assay buffer [150 mM NaCl, 1 mmol/l Na₂EDTA, 1 mmol/l Na₂HP0₄, 141 mg (70,000 units/g)/l bacitracin, pH of buffer adjusted to 7.1–7.2]. Slides were then incubated for 2 h in assay buffer + 500 pM [¹²⁵I-Sar¹, Ile⁸]ANG II (125I-SI ANG II, Peptide Radioiodination Service Center, Washington State University, Pullman, WA) (total binding) or assay buffer + 125I-SI ANG II + 3 μM ANG II (nonspecific binding).

After incubation, slides were swirled for 2–3 s in two consecutive 400-ml beakers filled with distilled water and then were rinsed in four consecutive Coplin jars filled with assay buffer (1 min per jar). The slides were again swirled for 2–3 s in two consecutive 400-ml beakers filled with distilled water. Slides were then laid out and dried for 2 min with an air dryer (room temperature air). Dried slides were then placed into a film cassette and exposed to autoradiographic film (Hyperfilm MP, Amersham Biosciences) for 1 day. Image analysis was performed using scanned images of the developed film and Scion Image for Windows (Scion). Optical density readings were converted to units of optical density of 125I-SI ANG II bound per gram of tissue using calibrated 125I standards (Microscales, Amersham Biosciences). Specific binding was calculated as total binding minus nonspecific binding. Binding (in fmol/g) was multiplied by the pixel area of each analyzed section in each area (SFO and PVN), and an average value was obtained.

**AT₁a in situ hybridization.** The sections used for hybridization were fixed (4% buffered paraformaldehyde) and acetylated. Hybridization, in situ, was carried out overnight at 55°C using a [³²P]UTP-labeled (~3 × 10⁶ cpm per 3-section slide) antisense riboprobe to AT₁a (640-bp insert from AT₁a 3′-untranslated region) (Dr. Terry Elton, Brigham Young University, Provo, UT). Unincorporated probe was removed by incubating the slides in RNase (14 μg/ml Sigma) for 30 min followed by washes in buffer without RNase, 1× SSC (room temperature) and 0.5× SSC (60°C). Because there were so many sections, hybridization was performed in two batches. In each batch there were equal numbers of NS and HS brains. Visualization of the hybridized probe utilized a 6-day exposure to autoradiographic film (Hyperfilm MP, Amersham Biosciences). Utilization of an AT₁a sense probe showed no detectable hybridization signal (data not shown).

All relative quantification 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 was fit to a four-parameter logistic curve. P3 (MAP50) 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 AT1a in situ hybridization.** Examples of total binding and mRNA hybridization in consecutive sections of SFO and PVN are shown in Fig. 3. 125I-SI ANG II binding in the SFO and PVN was specific for the AT1 receptor [i.e., blocked by losartan (tested in sections from only one brain, data not shown)]. There was significantly greater binding (40%) in the SFO of HS rats compared with NS rats. In the PVN, binding tended to be increased (19%) in the HS rats, but the effect was not significant. (Fig. 3, left). In situ hybridization was performed with a 35P-labeled AT1a riboprobe on coronal sections adjacent to those used for the binding study. Hybridization was observed in the SFO and the PVN of both NS and HS rats. In one HS rat, all sections showed poor hybridization for unknown reasons. Data from this rat were not included in the analysis. Also, usable sections through the SFO could not obtained in two NS rats. HS rats exhibited significantly greater relative expression of AT1a mRNA in both the SFO (28% greater) and the PVN (32% greater) (Fig. 3, right).

**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 AT1 receptors and the sympathetic nervous system to the increased MAP. A portion of the elevation in MAP was due to increased activation of central AT1 receptors, at least those receptors accessible by intracerebroventricular losartan. Yang et al. (37) found that AT1 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 AT1-mediated pressor response to injection of ANG II into the AHA of SHRs fed a high-salt diet 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 captopril or losartan (9, 36). Li et al. (17) found that inhibition of the expression of AT1 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 phentolamine 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 MAP50 (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 AT1 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 125I-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 AT1 binding may have resulted from altered plasma ANG II levels or from central determinants. The fact that AT1a 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 AT1 receptor binding in the PVN but not the SFO (36). This was in contrast to Dahl S rats that showed more marked increases in AT1.

Fig. 3. Representative examples of 125I-[Sar1,Ile8]ANG II (125I-SI ANG II) total binding (left) and AT1a receptor mRNA in situ hybridization (right) in consecutive sections through subfornical organ (SFO) or paraventricular nucleus (PVN). Images were obtained by scanning the X-ray films used to visualize the signal.
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 amiloride-sensitive 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-

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