ANG II in median preoptic nucleus and pressor responses to CSF sodium and high sodium intake in SHR

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Budzikowski, Adam S., and Frans H. H. Leenen. ANG II in median preoptic nucleus and pressor responses to CSF sodium and high sodium intake in SHR. Am J Physiol Heart Circ Physiol 281: H1210–H1216, 2001.—Pressor responses to increases in cerebrospinal fluid (CSF) sodium in Wistar rats and to high salt intake in spontaneously hypertensive rats (SHR) involve both brain ouabainlike activity ("ouabain") and the brain renin-angiotensin system (RAS). Because some of the effects of "ouabain" are mediated by the median preoptic nucleus (MnPO) and this nucleus contains all elements of the RAS, the present study assessed possible interactions of "ouabain" and ANG II in this nucleus. In conscious Wistar rats, injection of ANG II into the MnPO significantly increased mean arterial pressure (MAP) and heart rate (HR). This response was not affected by pretreatment with a subpressor dose of ouabain. MAP and HR increases by ouabain in the MnPO were significantly attenuated by MnPO pretreatment with losartan. In Wistar rats, losartan in the MnPO also abolished pressor and HR responses to intracerebroventricular 0.3 M NaCl and attenuated MAP and HR responses to intracerebroventricular ouabain. Five weeks of a high-salt diet in SHRs resulted in exacerbated hypertension and increased responses to air-jet stress and intracerebroventricular guanabenz. Losartan injected into the MnPO reversed the salt-sensitive component of the hypertension and normalized the depressor response to guanabenz but did not change responses to air-jet stress. We conclude that in the MnPO, ANG II via AT1 receptors mediates cardiovascular responses to an acute increase in CSF sodium as well as the chronic pressor responses to high sodium intake in SHR.

ouabain; sympathetic activity; guanabenz; losartan; renin-angiotensin system

Hemodynamic and Renal Sympathetic Nerve Activity (RSNA) responses to acute and chronic increases in cerebrospinal fluid (CSF) sodium can be prevented by blockade of either brain ouabainlike activity ("ouabain") using intracerebroventricular administration of antibody Fab fragments that bind ouabain and brain "ouabain" with high affinity, or by blockade of the brain renin-angiotensin system (RAS) using intracerebroventricular administration of the AT1-receptor antagonist losartan (9, 12). In spontaneously hypertensive rats (SHR), high dietary salt intake causes increased "ouabain" in the hypothalamus, enhancement of sympathoexcitatory responses to air-jet stress, decreased sympathoinhibition as assessed by intracerebroventricular injection of the α2-agonist guanabenz, and exacerbation of hypertension (10, 15). In this model, intracerebroventricular administration of either Fab fragments or losartan prevents the enhanced sympathoexcitatory responses, decreased sympathoinhibition, and increased blood pressure (BP) induced by a high-salt diet (10, 11). Moreover, cardiovascular responses to intracerebroventricular injection of ouabain and ANG II can be prevented by intracerebroventricular pretreatment with losartan (12), whereas intracerebroventricular-injected Fab fragments block the effects of ouabain but not ANG II (12). We therefore proposed that sympathoexcitatory and pressor responses to an increase in CSF sodium in normotensive rats and to a high-salt diet in SHR are mediated by increases in brain "ouabain" and subsequent activation of the brain RAS (9, 12).

The exact localization of "ouabain"-containing fibers and sites of action in the brain have not yet been well established. One of the areas in which "ouabain" exerts its actions is the median preoptic nucleus (MnPO). Administration of Fab fragments into the MnPO prevents cardiovascular responses to intracerebroventricular infusion of hypertonic saline and reverses salt-sensitive hypertension in SHR (4). The MnPO also contains ANG II receptors and angiotensin-containing fibers, and a functional role for ANG II in the MnPO in cardiovascular regulation has been demonstrated (7, 16, 21, 25). Therefore, the purpose of the present study was to assess whether "ouabain" interacts with ANG II in the MnPO during the cardiovascular responses to an increase in CSF sodium in normotensive rats and to high sodium intake in SHR. In particular, we assessed whether 1) cardiovascular effects of intracerebroventricular and MnPO injections of ouabain could be prevented by MnPO pretreatment with losartan, 2) losartan in the MnPO blocks hemodynamic responses to acute increases in CSF sodium, and 3) injection of losartan into the MnPO could reverse increased sympathoexcitatory, decreased sympathoinhibition, and increased BP in SHR on a high-salt diet.

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METHODS

Animals

Experiments in protocols 1–5 were performed in adult male Wistar rats purchased from Charles River (Montreal, Quebec, Canada). In protocol 6, young (3.5 wk) SHR and Wistar-Kyoto (WKY) rats (Taconic Farms; Germantown, NY) were used. The rats were housed under standard conditions (12:12-h light-dark cycle and ambient temperature 23°C) and received standard laboratory chow or special diet and tap water ad libitum. After arrival from the supplier, the rats were allowed up to 1 wk to become accustomed to the new environment. All experimental procedures were approved and carried out in accordance with the guidelines of the University of Ottawa Animal Care Committee for the use and care of laboratory animals.

Surgical Procedures

Implantation of guide tubes. Rats were anesthetized with pentobarbital sodium (65 mg/kg ip). The dorsal surface of the skull was exposed and the skull was leveled between the bregma and lambda. Appropriate holes were drilled, and guide cannula(s) (0.7 mm diameter) were implanted intracerebroventricularly (in the lateral cerebral ventricle) and above the MnPO. The stereotaxic coordinates for the MnPO tube were 0.4 mm caudal to the bregma, 1 mm lateral to the midline, and 5.4 mm ventral to the surface of the skull at an angle of 10° in the coronal plane (4, 20). The tip of the tube remained 1 mm above the MnPO. The intracerebroventricular tube was implanted 0.4 mm caudal to the bregma, 1.4 mm lateral to the midline, and 3 mm ventral from the surface of the skull. The tubes were secured to the skull with two jeweler’s screws and acrylic cement and were sealed with stainless steel obturator(s), and the rats were injected with penicillin G (30,000 IU im). Seven days were allowed for recovery from this major surgery.

Cannulation of vessels. While the rats were anesthetized with halothane (2% halothane in 100% oxygen), Tygon catheters (Norton Performance Plastics; Akron, OH) filled with heparin (1,000 U/ml in 0.9% NaCl) were implanted in the abdominal aorta via a femoral artery and in the inferior vena cava via a femoral vein. The opposite end of each catheter was sealed, tunneled subcutaneously, exteriorized in the intrascapular region, and secured to the skin. Hemodynamic studies started 24 h after recovery from this minor surgery. BP and heart rate (HR) were recorded using a Data Science International system (St. Paul, MN). In brief, the BP signal was transformed with a Statham transducer, amplified, and fed to the computer. Dataquest software allowed for on-line analysis of the BP signal and storage of data. Except for air-stress data (when momentary changes in BP and HR were used), individual measurements represent average values for BP and HR from 10-s periods.

MnPO Injections

MnPO injections were performed as described previously (4). In brief, the MnPO injection cannula (0.25 mm diameter) was inserted into the guide tube, and the rats were allowed 30–60 min to stabilize. Subsequently, the injection cannula was advanced to its final position whereby the lower tip extended 1 mm below the end of the guide tube. The MnPO injections were then performed as described in specific protocols. The active compounds were administered over a period of 20 s, and 2 min later the cannula was carefully removed. To ensure that the injection was successful, flow of the injectate was monitored by the movement of a small air bubble that separated the injected solution from a vehicle in the catheter. All compounds were dissolved in artificial cerebrospinal fluid [aCSF (23)], and the injected volume was kept at 250 nl to limit spillage to surrounding areas and destruction of the MnPO. At the end of day 1 studies, the arterial catheter was flushed with heparinized saline and sealed, the guide-tube styles were replaced, and the rats were returned to their home room.

Experimental Protocols: Employing Separate Groups of Rats

Protocols 1 and 2: dose response to ouabain and ANG II in MnPO. Two separate sets of conscious Wistar rats (n = 7 rats/set) instrumented with MnPO guide tubes and arterial catheters were allowed to stabilize for 30 min. Increasing doses of ouabain (50, 100, 200, and 400 ng; Sigma; St. Louis, MO) for protocol 1 and increasing doses of ANG II (5, 10, and 20 ng; Sigma) for protocol 2 were then injected into the MnPO while BP and HR were recorded. Before each of the injections, enough recovery time (up to 30 min) was allowed for the hemodynamic parameters to return to resting values.

Protocol 3: interaction of ouabain and ANG II in the MnPO. This experiment was designed to determine whether ouabain sensitizes MnPO neurons to ANG II. Conscious, normotensive Wistar rats (n = 8 rats) equipped with MnPO guide tubes and arterial catheters were pretreated in the MnPO with either a subpressor dose of ouabain (50 ng) or aCSF. To minimize sequence effects, ouabain and aCSF injections were randomized between days 1 and 2. Ten minutes later, a pressor dose of ANG II (10 ng) was injected into the MnPO, and BP and HR responses were recorded.

Protocol 4: blockade of ouabain action in the MnPO by losartan. Conscious, normotensive Wistar rats (n = 7 rats) were instrumented with MnPO guide tubes and arterial catheters. After stabilization, rats were pretreated with either aCSF or losartan (5 μg) into the MnPO. The latter dose represents 25% of an effective intracerebroventricular dose (12). To minimize sequence effects, losartan and aCSF injections were randomized between days 1 and 2. Ten minutes after pretreatment, rats were injected with a pressor dose of ouabain (200 ng) into the MnPO. BP and HR were monitored continuously.

Protocol 5: blockade of cardiovascular responses to intracerebroventricular hypertonic saline and intracerebroventricular ouabain by losartan in the MnPO. Conscious, normotensive Wistar rats (n = 8 rats) instrumented with arterial and venous catheters and MnPO and intracerebroventricular guide tubes were used. After the stabilization period, rats were injected with either aCSF or losartan (5 μg) into the MnPO (randomized between days 1 and 2 to minimize sequence effects). Ten minutes later, the V1 antagonist [d(CH2)₂Tyr(Me)AVP; 30 μg/kg in 0.2 ml of 0.9% NaCl iv; Sigma] was given. Five minutes after intravenous injection of the vasopressin antagonist, hypertonic saline (0.3 M NaCl, 3.8 μl/min, as used in Ref. 12) was infused intracerebroventricularly for 10 min. After the pressor response to intracerebroventricular hypertonic saline had subsided (~20 min), the rats received an intracerebroventricular injection of ouabain (0.6 μg in 5 μl of aCSF, as used in Ref. 12). The V1 antagonist was given to exclude effects of endogenous vasopressin, which is increased by intracerebroventricular hypertonic saline.

To determine cardiovascular responses to volume injection into the cerebroventricular system, a separate series of experiments was performed in Wistar rats implanted with intracerebroventricularly guide tubes and arterial catheters. aCSF (3.8 μl/min over a 10-min period) was infused intracerebroven-
RESULTS

Hemodynamic Effects of Ouabain and ANG II in MnPO

Injection of aCSF into the MnPO caused only minor hemodynamic effects, whereas both ouabain and ANG II significantly increased MAP and HR. These responses to ouabain became evident within several minutes and lasted for 20–30 min (see Fig. 1) with the threshold for a pressor response to ouabain being 50–100 ng (see Table 1). Pressor responses to ANG II developed within 10–20 s and lasted 2–4 min (data not shown). The threshold for a pressor response to ANG II was ≤5 ng (see Table 1).

Interaction of Ouabain and ANG II in MnPO

After pretreatment with aCSF in the MnPO, 10 ng of ANG II injected into the MnPO significantly increased both MAP and HR. Pretreatment in the MnPO with a threshold pressor dose of ouabain (50 ng) did not affect the responses to ANG II throughout the observation period. Maximal responses are shown in Fig. 2. Comparison of responses to ANG II after pretreatment with aCSF on day 1 versus day 2 showed similar responses (for MAP, +10 ± 1 vs. +9 ± 3 mmHg) indicating the absence of a sequence effect.
Blockade of Ouabain Action in MnPO by Losartan

In the control experiment (aCSF pretreatment into the MnPO), 200 ng of ouabain into the MnPO caused gradual long-lasting increases in MAP and HR which were similar to the responses in protocol 1 (see Fig. 1 and Table 1). Pretreatment with losartan did not change baseline hemodynamics but significantly attenuated the initial pressor response and abolished the increase in HR to injection of ouabain into the MnPO (see Fig. 1).

Blockade of Cardiovascular Responses to Intracerebroventricular Hypertonic Saline and Ouabain by Losartan in MnPO

Infusion of aCSF caused small, nonsignificant changes in MAP and HR; however, intracerebroventricular infusion of hypertonic saline significantly increased MAP and HR. Losartan injected into the MnPO prevented these responses such that responses were similar to those in the volume-control experiment (Fig. 3A). Bolus intracerebroventricular injection of aCSF did not change baseline hemodynamics, whereas 0.6 μg of ouabain injected intracerebroventricularly significantly increased both MAP and HR. These effects were significantly attenuated by losartan injected into the MnPO (Fig. 3B).

Reversal of Salt-Sensitive Hypertension in SHR by Blockade of AT₁ Receptors in MnPO

High dietary salt intake for 5 wk resulted in a significant exacerbation of hypertension (see Table 2) and increased responses to air-jet stress and intracerebroventricular guanabenz in SHR but had no effects in WKY rats. In SHR with a high salt intake, losartan in the MnPO significantly decreased the BP within 20 min after the injection; maximal responses are shown in Fig. 4. HR remained unchanged (data not shown). In SHR on a high-salt diet, losartan normalized the depressor and reduced the HR response to intracerebroventricular injection of guanabenz but did not affect responses to air-jet stress (see Fig. 5). In WKY rats on a regular diet or with high salt intake and in SHR on a regular diet, losartan did not affect baseline hemodynamic responses (see Fig. 4) to intracerebroventricular

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Table 1. MAP and HR responses to aCSF, ANG II, or ouabain at increasing doses injected into MnPO of normotensive Wistar rats

<table>
<thead>
<tr>
<th></th>
<th>aCSF</th>
<th>ANG II, ng</th>
<th></th>
<th>Ouabain, ng</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>0.2 ± 0.1</td>
<td>8 ± 1*</td>
<td>10 ± 2*</td>
<td>13 ± 3*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>1 ± 8</td>
<td>58 ± 12*</td>
<td>44 ± 7*</td>
<td>54 ± 8*</td>
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</tbody>
</table>

Values are means ± SE; n = 7 rats per group. aCSF, artificial cerebrospinal fluid; MnPO, median preoptic nucleus; MAP, mean arterial pressure; HR, heart rate. Baseline MAP and HR were 103 ± 6 mmHg and 428 ± 11 beats/min, respectively. *P < 0.05 vs. response to aCSF.
injection of guanabenz or to air-jet stress (see Figs. 5 and 6).

**DISCUSSION**

The present study demonstrates the major new finding that blockade of AT1 receptors in the MnPO prevents MAP and HR responses to increases in CSF sodium in normotensive rats and reverses salt-sensitive hypertension in SHR.

MnPO neurons receive angiotensinergic synapses from the subfornical organ projection, and ANG II released in the MnPO appears to be involved in the dipsogenic and cardiovascular effects of intracerebroventricularly administered ANG II (likely through this projection; 5, 16, 24). Injections of ANG II into the MnPO cause a pressor response, and this response can be blocked by pretreatment with peptide and nonpeptide ANG II antagonists (5, 21). Losartan injected into the MnPO did not change baseline hemodynamics in conscious normotensive rats, which indicates that baseline release of ANG II (if any) in the MnPO is not high enough to exert appreciable cardiovascular effects. In contrast to this minimal role under resting conditions, ANG II in the MnPO appears to mediate increases in MAP and HR in response to increases in CSF sodium (as shown in Fig. 3). Blockade of brain "ouabain" in the MnPO also prevents entire pressor and HR responses to CSF sodium (4). This would suggest that these responses to CSF sodium are dependent on both ANG II and "ouabain" in the MnPO. Pretreat-

Table 2. Resting MAP and HR of SHR and WKY before MnPO treatments

<table>
<thead>
<tr>
<th></th>
<th>Control Groups (aCSF Injected into MnPO)</th>
<th>Active Treatment Groups (Losartan Injected into MnPO)</th>
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<tbody>
<tr>
<td></td>
<td>RNa</td>
<td>HNa</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
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<tr>
<td>MAP, mmHg</td>
<td>122±3</td>
<td>125±4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>384±15</td>
<td>414±10</td>
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<tr>
<td>SHR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>153±4*</td>
<td>172±2*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>429±7</td>
<td>426±11</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 rats/group for SHR; n = 4 rats/group for WKY. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; RNa, regular-salt diet; HNa, high-salt diet. *P < 0.05 vs. WKY on same diet; †P < 0.05 vs. SHR on RNa diet.

Fig. 4. Maximal changes in resting MAP and HR after injections of 5 μg of losartan (Los) or aCSF control (Cont) into the MnPO of Wistar-Kyoto rats (WKY, A) or spontaneously hypertensive rats (SHR, B) on either a regular (RNa) or high (HNa)-salt diets. Values represent means ± SE (n = 7 rats/group for SHR; n = 4 rats/group for WKY). *P < 0.05 vs. baseline; †P < 0.05 as indicated.

Fig. 5. Peak MAP and HR responses to icv injections of guanabenz (A) and air-jet stress (B) after injections of 5 μg of losartan or aCSF (control) into the MnPO of SHR on either a RNa or HNa diet. Values represent means ± SE (n = 7 rats/group); *P < 0.05 HNa vs. RNa; #P < 0.05 losartan vs. control on same diet.

Ouabain and ANG II in MnPO and Responses to Intracerebroventricular Hypertonic Saline

MnPO neurons receive angiotensinergic synapses from the subfornical organ projection, and ANG II released in the MnPO appears to be involved in the dipsogenic and cardiovascular effects of intracerebroventricularly administered ANG II (likely through this projection; 5, 16, 24). Injections of ANG II into the MnPO cause a pressor response, and this response can be blocked by pretreatment with peptide and nonpeptide ANG II antagonists (5, 21). Losartan injected into the MnPO did not change baseline hemodynamics in conscious normotensive rats, which indicates that baseline release of ANG II (if any) in the MnPO is not high enough to exert appreciable cardiovascular effects. In contrast to this minimal role under resting conditions, ANG II in the MnPO appears to mediate increases in MAP and HR in response to increases in CSF sodium (as shown in Fig. 3). Blockade of brain "ouabain" in the MnPO also prevents entire pressor and HR responses to CSF sodium (4). This would suggest that these responses to CSF sodium are dependent on both ANG II and “ouabain” in the MnPO. Pretreat-
the presence of a V1 antagonist, and therefore did not injections. In addition, these studies were performed in relevant damage to the MnPO caused by repeated the MnPO were similar on to note that pressor responses to ANG II injected into the MnPO (see Fig. 2), which suggests sensitization occurs with higher doses of ouabain. Pretreatment in the MnPO with a threshold pressor stimulation in the MnPO. At least two types of interaction between ouabain and ANG II in the MnPO are possible. During increases in CSF sodium, release of brain “ouabain” in the MnPO may lower resting membrane potential and thereby sensitize neurons to ANG II and other neurotransmitters, and/or “ouabain” may cause the release of ANG II from axons or glia. Pretreatment in the MnPO with a threshold pressor dose of ouabain did not change responses to ANG II injected into the MnPO (see Fig. 2), which suggests that sensitization does not take a place and favors the concept that brain “ouabain” releases ANG II in the MnPO. However, one cannot exclude that such sensitization occurs with higher doses of ouabain.

From a methodological point of view, it is important to note that pressor responses to ANG II injected into the MnPO were similar on days 1 and 2 excluding relevant damage to the MnPO caused by repeated injections. In addition, these studies were performed in the presence of a V1 antagonist, and therefore did not assess a role of the MnPO in vasopressin responses to CSF sodium.

Salt-Sensitive Hypertension in SHR

In SHR with regular salt intake, losartan injected into the MnPO did not change resting BP, which indicates that AT1 receptors in the MnPO are not involved in the maintenance of the genetic component of hypertension in the SHR. This observation is consistent with previous studies which indicate that hypertension in SHR on a regular-salt diet is not affected by blockade of central AT1 receptors via intracerebroventricular-injected losartan (6, 11, 14), although losartan injected into the anterior hypothalamic area does lower the BP of SHR on a regular-salt diet (27). In contrast, intracerebroventricular injection of saralasin or sarthdan did decrease BP in SHR with regular salt intake in some (18, 22) but not other (2, 3, 19) studies. This may possibly indicate a role for ANG II receptors other than AT1 receptors in central mechanisms contributing to hypertension in SHR on a regular salt diet.

Via AT1 receptors, the brain RAS contributes clearly to the salt-sensitive component of hypertension in SHR. Chronic intracerebroventricular administration of losartan prevents the increased sympathoexcitation, decreased sympathoinhibition, and exacerbated hypertension in this model (11). The present study for the first time indicates that a major part of the ANG II action in salt-sensitive hypertension in SHR is localized in the MnPO. Losartan injected into the MnPO reversed the salt-sensitive component of hypertension and normalized the decreased sympathoinhibition as measured by the response to intracerebroventricular injection of the α2-agonist guanabenz. However, the MnPO is not the only brain structure through which ANG II may exert its prohypertensive action in salt-sensitive SHR. Blockade of AT1 receptors in the anterior hypothalamic area (AHA) caused a significantly larger decrease in BP in SHR on a high-salt diet vs. SHR on a regular salt diet (27). Our results may indicate that ANG II action in the AHA depends on ANG II effects in the MnPO, because blockade of AT1 receptors in the MnPO normalizes responses to guanabenz, which presumably are AHA dependent. A direct anatomic connection linking these nuclei gives an anatomic basis for this mechanism (13). However, to what extent hyperpolarizing α2-adrenoceptors in the MnPO (1) contribute to the hemodynamic responses to intracerebroventricularly administered guanabenz cannot be determined from the present experiments.

Whereas intracerebroventricularly injected losartan normalizes the enhanced responses to air-jet stress in SHR with a high salt intake (11), losartan injected into the MnPO does not affect these enhanced responses (see Fig. 5). Similarly, blockade of “ouabain” by intracerebroventricularly injected Fab fragments (11) normalizes enhanced responses to air-jet stress but not blockade of “ouabain” in the MnPO (4). These results are consistent with the proposed concept that the effects of “ouabain” in the MnPO depend on activation of the RAS in the MnPO, but also indicate that both “ouabain” and ANG II are involved in cardiovascular responses to environmental stress in other brain nuclei, e.g., the limbic-hypothalamic circuitry (8). In addition, these findings substantiate that the effects of losartan injected into the MnPO do not depend on leakage of losartan into the ventricles, because then
attenuation of responses to air-jet stress should occur as well. The marked differences in doses of losartan used (5 μg in MnPO vs. 1 mg icv; Ref. 11) also support this conclusion.

To what extent losartan diffuses into adjacent brain tissue cannot be assessed from our experiments. This is an important limitation, and followup experiments using, e.g., 3H-labeled losartan or AT1-receptor autoradiography to assess the extent of losartan diffusion over time are clearly relevant. Extent of dye diffusion is of limited value, because the diffusion kinetics are likely different from losartan.

In conclusion, in the present study we demonstrate for the first time that ANG II via AT1 receptors in the MnPO mediates cardiovascular responses to increases in CSF sodium in normotensive Wistar rats and to high dietary salt in SHR. The primary event in the development of salt-sensitive hypertension in the SHR remains unclear, but accumulating evidence (9–12) supports the hypothesis that increased dietary salt intake (possibly through short-term increases in CSF sodium) activates brain “ouabain.” Activation of brain “ouabain” in turn increases activity of the brain RAS. Increased ANG II stimulates pathways involved in central release of arginine vasopressin (17) and other pathways leading to decreased sympathoinhibition, increased sympathoexcitation, and increased BP. For the increase in resting sympathetic tone and BP in rats on a high-salt diet, “ouabain” and ANG II in the MnPO appear to play a major role.

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