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# The Brain Renin-Angiotensin System Contributes to the Hypertension in Mice Containing Both the Human Renin and Human Angiotensinogen Transgenes

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**Abstract**—We have previously shown that mice transgenic for both the human renin and human angiotensinogen genes (RA+) exhibit appropriate tissue- and cell-specific expression of both transgenes, have 4-fold higher plasma angiotensin II (AII) levels, and are chronically hypertensive. However, the relative contribution of circulating and tissue-derived AII in causing hypertension in these animals is not known. We hypothesized that the brain renin-angiotensin system contributes to the elevated blood pressure in this model. To address this hypothesis, mean arterial pressure (MAP) and heart rate were measured in conscious, unrestrained mice after they were instrumented with intracerebroventricular cannulae and carotid arterial and jugular vein catheters. Intracerebroventricular administration of the selective AII type 1 (AT-1) receptor antagonist losartan (10  $\mu$ g, 1  $\mu$ L) caused a significantly greater peak fall in MAP in RA+ mice than in nontransgenic RA- controls ( $-29 \pm 4$  versus  $-4 \pm 2$  mm Hg,  $P < 0.01$ ). To explore the mechanism of a central renin-angiotensin system-dependent hypertension in RA+ mice, we determined the relative depressor responses to intravenous administration of the ganglionic blocking agent hexamethonium (5 mg/kg) or an arginine vasopressin (AVP) V<sub>1</sub> receptor antagonist (AVPX, 10  $\mu$ g/kg). Hexamethonium caused equal lowering of MAP in RA+ mice and controls ( $-46 \pm 3$  versus  $-52 \pm 3$ ,  $P > 0.05$ ), whereas AVPX caused a significantly greater fall in MAP in RA+ compared with RA- mice ( $-24 \pm 2$  versus  $-6 \pm 1$ ,  $P < 0.01$ ). Consistent with this was the observation that circulating AVP was 3-fold higher in RA+ mice than in control mice. These results suggest that increased activation of central AT-1 receptors, perhaps those located at sites involved in AVP release from the posterior pituitary gland, plays a role in the hypertension in RA+ mice. Furthermore, our finding that both human transgenes are expressed in brain regions of RA+ mice known to be involved in cardiovascular regulation raises the possibility that augmented local production of AII and increased activation of AT-1 receptors at these sites is involved. (*Circ Res.* 1998;83:1047-1058.)

**Key Words:** transgenic mice ■ angiotensin ■ blood pressure ■ genetics ■ pharmacology

It is well established that blood-borne angiotensin II (AII), the effector peptide of the renin-angiotensin system (RAS), plays a major role in the regulation of arterial blood pressure (BP) and volume homeostasis through its interaction with specific AII receptors present in vascular smooth muscle, kidney, and adrenal gland. Stimulation of these peripheral AII receptors leads to elevations in BP through increased vascular resistance, cardiac output, sodium reabsorption, and blood volume.<sup>1</sup> However, in addition to these well-known actions at peripheral sites, AII also contributes to BP and volume regulation through its receptor-mediated effects on neurons located within the central nervous system (CNS). Indeed, central administration of AII causes increases in BP, antidiuresis, drinking, and salt appetite.<sup>2</sup> This, along with evidence that all components of the RAS are present in the brain, including a predominant mapping of AII receptors to princi-

pal cardiovascular control regions, has led to the hypothesis that an intrinsic brain RAS exists and plays an important role in cardiovascular function.

The finding that the pressor response to central AII is attenuated by systemic administration of either an arginine vasopressin (AVP) V<sub>1</sub> antagonist<sup>3</sup> or agents that block sympathetic neural transmission or adrenergic receptors<sup>3,4</sup> suggests that activation of both the sympathetic nervous system and AVP secretion is involved in mediating the hemodynamic effects of central AII. Indeed, neuronal AII type 1 (AT-1) receptors are densely localized in brain regions controlling sympathetic and AVP outflow, including brain stem sites, such as the ventrolateral medulla<sup>5</sup> and the nucleus tractus solitarius,<sup>6</sup> and hypothalamic nuclei, such as the paraventricular nucleus (PVN).<sup>7,8</sup> Microinjection of AII into such AT-1 receptor-rich sites causes dose-dependent pressor responses.

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From the Departments of Internal Medicine and Physiology & Biophysics (R.L.D., C.D.S.), Department of Anatomy and Cell Biology (G.Y., M.D.C.), and Department of Psychology (T.G.B., A.K.J.), The University of Iowa College of Medicine, Iowa City, Iowa.

Correspondence to Curt D. Sigmund, PhD, Director, Transgenic Animal Facility, Departments of Internal Medicine, University of Iowa College of Medicine, 2191 Medical Laboratory (ML), Iowa City, IA 52242. E mail curt-sigmund@uiowa.edu

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es.<sup>9–11</sup> Moreover, intracerebroventricular (ICV) pretreatment with the selective AT-1 receptor antagonist losartan prevents not only the ICV AII-mediated increase in BP, but also the concomitant increase in AVP secretion and sympathetic activity.<sup>12</sup> Central AII-mediated increases in AVP release are also attenuated by inhibition of brain AT-1 receptor gene expression.<sup>13</sup>

In addition to these intrinsic brain sites, AII is also an important mediator at cardiovascular control centers located at the blood-brain interface. Neurons of the circumventricular organs (CVOs) such as the subfornical organ (SFO) and the area postrema (AP) are not only rich in AT-1 receptors, but are extremely sensitive to AII.<sup>7,8,14–16</sup> Losartan-sensitive pressor responses are elicited with very low doses of AII microinjected into both SFO and AP.<sup>14–16</sup> Moreover, application of AII antagonists or lesions of these CVOs attenuates or abolishes the pressor response to central or systemic administration of AII.<sup>17,18</sup> Activation of AT-1 receptors in both intrinsic brain regions and in CVOs by centrally and systemically derived AII is thought to modulate normal processing of sensory homeostatic information, as well as neurohumoral outflow. Abnormal activation of these central RAS pathways may lead to pathological regulation of BP and volume homeostasis. Indeed, the brain RAS has been implicated in several types of experimental and genetic hypertension.<sup>19–22</sup>

To examine the effects of lifelong overexpression of the RAS on complex cardiovascular function, we have developed a transgenic mouse model containing both the human renin (*HREN*) and human angiotensinogen (*HAGT*) transgenes (RA+). We have reported that these mice exhibit chronic hypertension and AII-mediated resetting of baroreflex control of heart rate (HR).<sup>23</sup> In addition to elevated plasma AII levels (approximately 4-fold), there is appropriate widespread tissue- and cell-specific expression of the transgenes.<sup>23–26</sup> In the present studies, we wanted to examine the possibility that the brain RAS, either through its activation at intrinsic parenchymal sites or at the blood-brain interface, contributes to the hypertension exhibited by this model. More specifically, we examined the role of central AT-1 receptors, as well as the relative contribution of sympathetic nerve activity and AVP secretion, in the maintenance of elevated baseline BP in conscious RA+ mice. In addition, because one potential mechanism of a brain RAS-mediated alteration in BP in this model is via expression of the human transgenes in brain with potential overproduction of AII locally, we explored whether *HREN* and *HAGT* mRNA are present in brain of RA+ mice.

## Materials and Methods

### Experimental Mice and Husbandry

Transgenic mice carrying both the *HREN* (R+) and *HAGT* (A+) genes were generated by breeding heterozygous *HREN* transgenic mice and heterozygous *HAGT* transgenic mice, as described previously.<sup>23–25</sup> The tissue- and cell-specific expression exhibited by both transgenes in single- and double-transgenic mice has been previously described in detail.<sup>23–27</sup> Double-transgenic mice were identified by polymerase chain reaction (PCR) of genomic DNA purified from tail biopsy samples using *HREN*- and *HAGT*-specific primer sets as described.<sup>24,25</sup> Our previous data showed that because of the species specificity of the enzymatic reaction between renin and angiotensinogen, transgenic mice containing 1 human transgene, but not both

(R+/A- or R-/A+), are phenotypically indistinguishable from nontransgenic mice (R-/A-) with regard to plasma renin activity, plasma AII levels, and BP.<sup>23</sup> We have confirmed this finding in the present studies, demonstrating that there is no significant difference between R+/A-, R-/A+, and R-/A- control animals in each study ( $P > 0.05$ ). Therefore, only 2 experimental groups are distinguished in the present studies: double-transgenic mice having both *HREN* and *HAGT* (designated RA+) and control animals containing either no transgenes or 1 transgene (designated RA-).

All mice were fed standard mouse chow (LM-485; Teklad Premier Laboratory Diets) and water ad libitum. Care of the mice used in the experiments exceeded the standards set forth by the National Institutes of Health in their guidelines for the care and use of experimental animals. All procedures were approved by the University Animal Care and Use Committee at the University of Iowa.

## Surgical Procedures

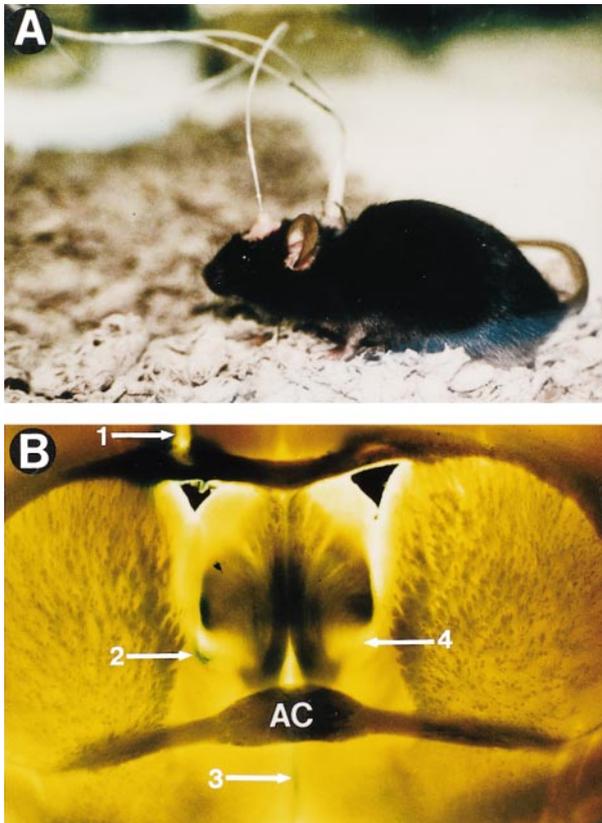
### Chronic Catheterization

At least 3 days before experiments, mice (n=35) were weighed, anesthetized with ketamine (120 mg/kg IP) and acepromazine maleate (12 mg/kg IP), and surgically instrumented with intra-arterial catheters for direct measurement of pulsatile and mean arterial pressure (MAP) and HR (with a pulse pressure-triggered cardiota-chometer) and jugular vein catheters for administration of drugs. Mice were shaved, a neck incision was made, and the left common carotid artery and right jugular vein were dissected free of fat and connective tissue. Sterile heparinized saline-filled (50 U/mL) catheters were inserted into the carotid artery (Microrenathane; Braintree Science Inc) (0.04-in outer diameter×0.025-in inner diameter, drawn over heat) and the jugular vein (polyethylene tubing, PE-10). Catheters were tunneled subcutaneously, exteriorized, and sutured in place between the scapulae as described previously.<sup>28</sup> Lines were flushed daily with dilute sterile heparinized saline (25 U/mL).

### Chronic ICV Cannulation

After catheterization, some mice (n=14) were also instrumented with ICV cannulae for microinjection of drugs into the lateral ventricles. Animals were placed in a stereotaxic apparatus (Kopf Instruments), and a guide cannula (30 gauge, 8 mm in total length, Small Parts) was implanted. The head of the mouse was aligned so the lambda-bregma plane was horizontal, and a hole was made 0.3 mm posterior and 1.0 mm lateral to the bregma (coordinates according to Paxinos and Watson,<sup>29</sup> modified for mouse). The cannula was inserted 3.0 mm below the skull surface and fixed in place with acrylic dental cement (Dentsply International, Inc) and 1 anchoring skull screw. A stylus protruding 0.5 mm past the tip of the guide cannula was inserted to maintain patency. Injections were made through a 36-gauge stainless steel injector (Small Parts) connected to tubing (PE-10, drawn over heat) fitted with a microsyringe (Hamilton). Compounds injected intraventricularly were dissolved in artificial cerebrospinal fluid (in mmol/L: NaCl 136, KCl 5.6, NaHCO<sub>3</sub> 16.2, NaH<sub>2</sub>PO<sub>4</sub> 1.2, MgCl<sub>2</sub> 1.2, and CaCl<sub>2</sub> 2.2, pH 7.4) and delivered in a 1- $\mu$ L volume over at least 30 seconds.

The coordinates for the ICV injection site were confirmed in preliminary studies (n=3), in which AII (100 ng, 1  $\mu$ L) was injected, and MAP and HR were recorded. ICV AII has been shown repeatedly in several species to elicit characteristic pressor and bradycardic responses. Indeed, this dose of AII did produce a significant pressor response and bradycardia in RA- mice in these preliminary studies and was taken as indication of a correct ICV injection site. To further confirm that the coordinates were correct, ICV AII was followed by dye injection (Evans blue dye, 0.5  $\mu$ L) into the ventricles. Mice were then anesthetized (Nembutal, 50 mg/g IP) and perfused transcardially, and the brain was removed and sectioned (400  $\mu$ m) to verify diffusion of the injected dye throughout the ventricular system (see Figure 1B). For the studies reported herein, the ICV AII-induced pressor response and bradycardia at the time of experimentation alone were used for injection site verification.



**Figure 1.** Experimental preparation. A, Photograph of a conscious and unrestrained mouse instrumented with carotid arterial and jugular vein catheters and an ICV cannula for simultaneous direct recording of arterial BP and microinjection of drugs into either the lateral ventricles or the systemic circulation. The photograph was taken during routine experimental procedures 7 days after surgery. B, Proper coordinates for ICV cannula placement were confirmed in preliminary studies in which Evans blue dye (0.5  $\mu$ L) was injected through the cannula (left ventricle), mice were perfused transcardially, and the brain was removed and sectioned (400  $\mu$ m) to verify diffusion of the dye throughout the ventricular system. Note the cannula tract (1) and staining in both lateral ventricles (2 and 4), as well as in the third ventricle (3) (arrows).

## Experimental Protocols

### Effect of ICV AII and Losartan

RA+ (n=8) and RA- (n=6) mice were surgically prepared as described above, allowed at least 3 days of recovery, and tested in the conscious, freely moving state in their home cages. Food and water were removed from the cage during testing. After connection of the arterial line to a Beckman Dynograph-coupled pressure transducer (Cobe Laboratories, Inc), connection of the venous line to a syringe, and insertion of the microinjector into the ventricular guide cannula, mice were allowed a 30- to 60-minute stabilization period before testing. A mouse instrumented and prepared for experimentation is shown in Figure 1A. Baseline pulsatile pressure, MAP, and HR were recorded for 30 minutes before injection of the first drug, and these parameters were monitored continuously throughout the experiment. First, to confirm correct placement of the ICV injector and to determine the central effect of AII in RA+ and RA- mice, cardiovascular responses to ICV AII (100 ng, 1  $\mu$ L volume) were recorded. Mice that did not respond to the central injection of AII were not used in the experiments, because it was possible that this indicated an incorrect cannula placement. Indeed, during the course of the present studies, 3 mice (RA-) were excluded based on the lack of a central AII-induced cardiovascular response (n=3). Because a lack of response to losartan would be difficult to interpret

given the possibility of incorrect cannula placement, these mice were not further tested, and no data from them are included in any of the figures.

After return of MAP and HR to baseline following AII administration (by 20 minutes), the ICV injector was removed and replaced with one for microinjection of the selective AT-1 receptor antagonist losartan. Animals were allowed another 10- to 15-minute stabilization period before ICV administration of losartan (10  $\mu$ g, 1- $\mu$ L volume). In preliminary studies, it was determined that this dose of losartan was sufficient to cause complete blockade of ICV AII (10 to 200 ng)-induced pressor and bradycardic responses in control mice (n=4). After return of MAP and HR to baseline following ICV losartan administration (by 30 minutes), 10  $\mu$ g of losartan was administered intravenously to determine whether systemic administration of this dose of the AT-1 antagonist affected cardiovascular parameters.

The magnitude of the effects of AII and losartan on MAP and HR were calculated as the difference between the mean of multiple MAP and HR samplings taken before drug administration and the mean of multiple samplings taken during the maximal drug-induced changes in the cardiovascular parameters.

### Effect of Blockade of the Sympathetic Nervous System and Peripheral AVP $V_1$ Receptors

In separate groups of animals, the effects of blockade of sympathetic ganglionic transmission with hexamethonium (HEX, 5 mg/kg IV) (RA+, n=6; RA-, n=6) and of antagonism of peripheral AVP  $V_1$  receptors (AVPX, Manning Compound [d(CH<sub>2</sub>)<sub>5</sub>Tyr<sup>2</sup>(ME)Arg<sup>8</sup>]-vasopressin, 10  $\mu$ g/kg IV; RA+, n=5; RA-, n=4) on baseline MAP were determined. Mice were surgically prepared with arterial and venous catheters and allowed 3 days of recovery, and MAP was measured as described above. Baseline BP was measured for at least 30 minutes before injection of either HEX or AVPX and was continuously monitored throughout drug-induced responses and return to baseline. The choice of these doses of HEX and AVPX were based on preliminary studies in RA- mice (n=4). Adequate ganglionic blockade was indicated by the absence of a sodium nitroprusside (8  $\mu$ g/kg IV)-induced reflex tachycardia in HEX-treated mice. In the same animals but on the following day, blockade of AVP (5 to 100 ng/kg IV)-induced pressor responses in mice pretreated with this dose of the  $V_1$  antagonist was taken to indicate blockade of peripheral  $V_1$  receptors. Again, the data in the figures reflect the difference between the mean of multiple samplings of MAP before and during peak drug-induced changes in MAP.

### Plasma AVP Levels

To determine baseline levels of AVP in plasma, separate groups of RA+ (n=3) and RA- mice (n=3) were decapitated, and trunk blood (0.5 to 1.0 mL) was collected in chilled tubes containing heparin. Samples were stored on ice for a maximum of 20 minutes before centrifugation (1600g for 15 minutes) at 4°C. Plasma (0.25 mL) was collected and stored at -80°C until processed. Plasma proteins were initially precipitated with cold acetone. The acetone was extracted with cold petroleum ether, the phases were separated by centrifugation, and the ether phase was discarded. The lower phase containing the AVP was taken to dryness and stored at -20°C until the assay was performed.

Levels of AVP in plasma extracts were determined by radioimmunoassay using an AVP antibody generated and generously provided by W.K. Samson.<sup>30</sup> Briefly, antibodies to synthetic AVP (Peninsula Laboratories) conjugated to bovine thyroglobulin (Sigma) using carboimide were raised in adult male rabbits (New Zealand White, Hickory Hill Farms). Antibody and cold standards (Peninsula Labs) or sample extracts (diluted in acidic saline) were preincubated overnight and <sup>125</sup>I AVP (New England Nuclear, Boston, Mass) added on the second day. After an additional overnight incubation, the antibody-bound AVP was precipitated by the polyethylene glycol separation technique (PEG 8000, 12.5%). AVP assay buffer used to dilute antibody and tracer consisted of 0.05 mol/L Tris, pH 8.0, 0.01 mol/L Na<sub>2</sub>-EDTA, and 0.01% NaN<sub>3</sub> containing 0.1% BSA.

## Analysis of Nucleic Acids

### RNase Protection Assay

To determine whether *HAGT* mRNA was expressed in brain tissue in the double-transgenic mice, RNase protection assay was performed on brain sections and kidneys from RA+ and nontransgenic controls. We have previously demonstrated a high level of *HAGT* expression in kidneys of RA+ mice; therefore, we used renal tissue as a positive control.<sup>23</sup> Mice were killed by CO<sub>2</sub> asphyxiation, and tissues were removed. Kidneys were frozen immediately, whereas brains were dissected into regions before freezing. Cortex, diencephalon, brain stem, and cerebellum were carefully dissected from separated fore-brain and hindbrain tissue. Total RNA was isolated from tissues by homogenization in guanidine isothiocyanate followed by phenol emulsion extraction at pH 4.0 as described previously.<sup>23</sup> Samples of RNA (20 μg) were hybridized with single-stranded labeled antisense RNA probes transcribed by SP6 polymerase using the Maxiscript Kit (Ambion, Inc). The template for the *HAGT* probe was a partial PCR-amplified cDNA derived from exon 2 of the *HAGT* gene (coordinates 302 to 811), and a partial mouse GAPDH cDNA (Ambion, Inc, Austin, Tex) was the template used to generate a probe employed as a control for sample loading. RNase protection was performed as described by the manufacturer, and products were separated on 5% acrylamide urea gels and visualized via autoradiography. Fragments of 539 and 300 bp were expected for protected *HAGT* and mouse GAPDH mRNA, respectively.

### Reverse Transcriptase PCR

To determine whether *HREN* mRNA was present in brain of double-transgenic mice, we used reverse transcriptase (RT)-PCR. Brains were collected, specific regions were dissected, and tissues and total RNA were extracted from RA+ mice; nontransgenic controls were obtained as described above, except that whole brain tissue was used. Kidney RNA was again used as an internal positive control, because we have shown previously that *HREN* mRNA is expressed at a high level in renal tissue of RA+ mice.<sup>23</sup> We treated 20 μg of brain and kidney RNA with DNase and subjected it to RT-PCR as described in detail previously.<sup>31</sup> RT was left out of control reactions, and 2 μL of the RT reaction was used for PCR amplification. PCR products were visualized by ethidium bromide staining after agarose gel electrophoresis. A 720-bp fragment was expected using the following primers: 5'-GACACTGGTTCGT-CCAATG-3' and 5'-CTGGTGAGCGTGTATTCT-3'.

### In Situ Hybridization

Mice were killed by CO<sub>2</sub> asphyxiation, and the brain was removed, immersed in 30% dextrose, and frozen in dry ice. Frozen sections were cut 8 μm thick on a Reichert-Jung cryostat and were fixed in 4% paraformaldehyde for 15 minutes. Prehybridization was performed by washing the tissue sections with 3× PBS (in mmol/L: NaCl 137, KCl 2.7, 4.3 Na<sub>2</sub>HPO<sub>4</sub> 4.3, and KH<sub>2</sub>PO<sub>4</sub> 1.4) and 1× PBS. Pronase E treatment was used to increase target RNA accessibility and was inactivated by washing in glycine solution (2 mg/mL in 1× PBS) and 1× PBS. Tissue sections were treated with acetic anhydride (0.25%) to decrease nonspecific electrostatic binding of probe, rinsed in 2× SSC, and dehydrated. An antisense RNA probe colabeled with [<sup>3</sup>H]UTP and [<sup>3</sup>H]CTP was generated by in vitro transcription and hydrolyzed as described.<sup>25</sup> Hybridization was in 300 mmol/L NaCl, 10 mmol/L Tris-Cl, 1 mmol/L EDTA, 50% formamide, 1× Denhardt's solution, and 10% dextran sulfate at 60°C for 16 hours. Sections were treated with RNase A (0.002%) and RNase T1 (500 U) for 30 minutes to remove nonhybridized RNAs and washed. Autoradiography was as previously described.<sup>25</sup>

### Statistics

Data are expressed as mean±SEM. SEM was determined by the formula (EMS/n)<sup>1/2</sup>, where EMS is the error mean square term from the ANOVA, and n is the number of mice per group. Data were analyzed by repeated-measures ANOVA<sup>32</sup> followed by the Student modified *t* test with Bonferroni correction for multiple comparisons between means using the modified EMS term from the ANOVA.<sup>33</sup>

## Baseline MAP and HR in R/A- and R/A+ Mice

Group	Before ICV AII		Before ICV Losartan	
	MAP, mm Hg	HR, bpm	MAP, mm Hg	HR, bpm
RA- (n=6)	119±3	632±20	121±5	614±24
RA+ (n=8)	156±4*	594±23	158±5*	590±29

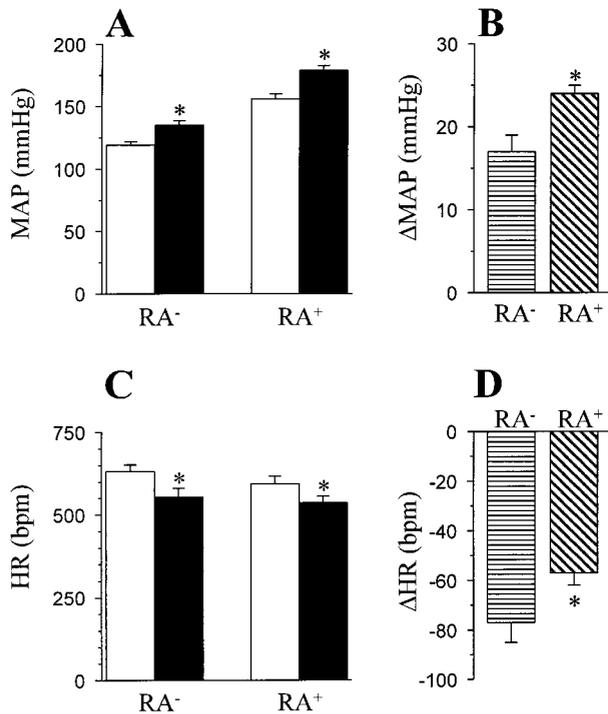
\**P*<0.01 R/A+ vs R/A-.

## Results

Double-transgenic mice containing the *HREN* and *HAGT* transgenes were previously generated to examine the effects of lifelong overexpression of the endocrine and tissue RAS. We previously reported that these mice exhibit appropriate tissue-specific expression of the transgenes and had chronically elevated plasma AII levels and hypertension.<sup>23</sup> However, the relative roles of circulating and tissue-derived AII in causing the elevated BP in these animals is not known. Given the increasing evidence that the brain RAS is functionally important in both normal and pathological regulation of BP, we hypothesized that overactivity of the brain RAS may contribute to the hypertension in RA+ mice. We reasoned that both increased activation of CVO sites by elevated plasma AII and enhanced local brain AII-mediated activation of intrinsic brain cardiovascular control regions were potential mechanisms in the pathogenesis of hypertension in these animals. Consequently, we compared the cardiovascular effects of blockade of the central RAS in the double-transgenic and control mice. To consider the possibility that sites inside the blood-brain barrier may be involved in this RAS-dependent hypertension, we needed to determine whether both transgenes are expressed in brain tissue of RA+ mice. Finally, the pressor mechanism through which overactivity of a central RAS may be causing elevated BP in these mice was investigated.

### Effects of ICV Losartan and AII on MAP and HR

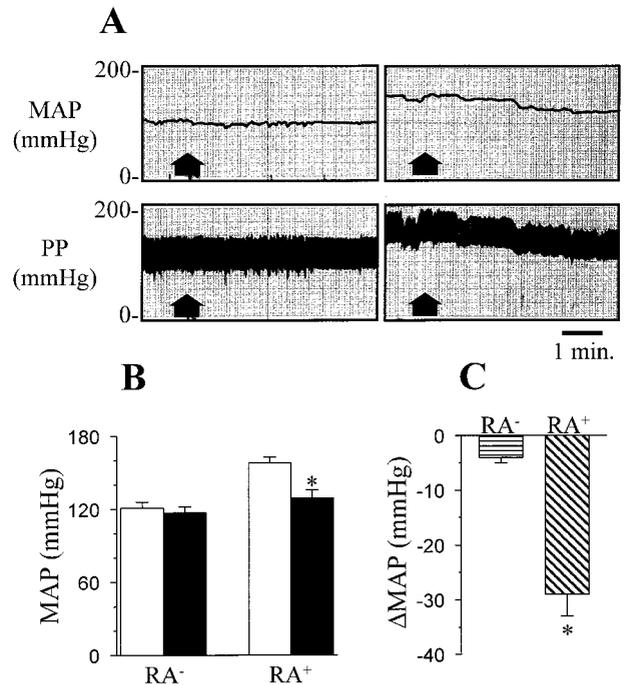
Mice containing both the *HREN* and *HAGT* transgenes (RA+) and mice containing either 1 or no transgenes (RA-) were identified by PCR analysis of tail DNA samples. To determine whether the central RAS is important in the maintenance of elevated baseline BP in the double-transgenic mice, the selective AT-1 receptor antagonist losartan was administered intraventricularly in conscious, unrestrained RA+ (n=8) and RA- (n=6) mice. We examined the effects of central losartan on BP, because AT-1 is the predominant AII receptor subtype in cardiovascular control regions in both intrinsic and CVO sites.<sup>5-8</sup> Before losartan, AII was administered ICV to check for correct ICV cannula placement. An AII-mediated pressor and bradycardic response was required for continuation of the losartan experiment. Additionally, we also wanted to compare the central AII-elicited cardiovascular responses in these 2 groups of mice. Resting MAP and HR values before ICV administration of both AII and losartan are summarized in the Table. Baseline MAP was significantly elevated in RA+ before any experimental treatment. No difference in HR between RA+ and RA- was observed. These findings are consistent with our previously reported data in RA+ mice.<sup>23</sup> After ICV AII administration, MAP and



**Figure 2.** Response to ICV AII. A summary of the effects of AII (100 ng, 1  $\mu$ L) administered ICV on MAP (A) and HR (C) in conscious RA<sup>-</sup> (n=6) and RA<sup>+</sup> (n=8) mice. MAP and HR values before ( $\square$ ) and after ( $\blacksquare$ ) AII administration are shown. A comparison of the ICV AII-induced absolute change in MAP ( $\Delta$ MAP; B) and HR ( $\Delta$ HR; D) in the groups is also shown. \* $P$ <0.05, pre- vs post-AII (A and C) and RA<sup>-</sup> vs RA<sup>+</sup> (B and D).

HR returned to pre-AII levels in both groups before administration of losartan.

A summary and comparison of the peak AII-induced cardiovascular responses in RA<sup>+</sup> and RA<sup>-</sup> mice is shown in Figure 2. Central AII (100 ng, 1  $\mu$ L) produced a transient (15 to 20 minutes) pressor response and bradycardia (latency 30 to 60 seconds) in both groups of animals. The pattern and time course of this hemodynamic response is similar to that produced by AII administered intraventricularly at this dose in other species.<sup>3,34,35</sup> ICV AII caused significant increases in MAP (panels A and B) and reductions in HR (panels C and D) compared with baseline in both groups. The absolute changes in MAP and HR after AII administration were marginally greater ( $P=0.048$  and  $P=0.046$  for MAP and HR, respectively) in RA<sup>+</sup> mice compared with controls. However, when calculated as a percentage change, the AII-induced pressor and bradycardic responses were not significantly different in these 2 groups (MAP, 15% $\pm$ 1 versus 14% $\pm$ 2; HR, -10% $\pm$ 2 versus -12% $\pm$ 1 [for RA<sup>+</sup> versus RA<sup>-</sup>, respectively;  $P$ >0.05]). Because of the well-known effects of central AII in modulating baroreflex function, we also compared the relative reduction in HR accompanying the pressor response produced by this dose of ICV AII in RA<sup>+</sup> and RA<sup>-</sup> mice. Indeed, RA<sup>+</sup> mice had a significantly reduced bradycardic response to a given AII-elicited pressor response compared with controls ( $\Delta$ HR/ $\Delta$ MAP,  $-2.4\pm 0.2$  versus  $-4.8\pm 0.6$  bpm/mm Hg [ $P=0.01$ ]; % $\Delta$ HR/% $\Delta$ MAP,  $-0.6\pm 0.04$  versus  $-0.9\pm 0.16$  [ $P=0.043$ ]). Although this is



**Figure 3.** Response to ICV losartan. A, Representative examples of the effects of losartan (10  $\mu$ g, 1  $\mu$ L) administered ICV on MAP and pulsatile pressure (PP) in conscious, unrestrained RA<sup>-</sup> (left) and RA<sup>+</sup> (right) mice. Arrows indicate time of injection. B and C, Summary of the effects of the selective AT-1 receptor antagonist losartan (10  $\mu$ g, 1  $\mu$ L) administered ICV on MAP (B) in conscious RA<sup>-</sup> (n=6) and RA<sup>+</sup> (n=8) mice. MAP values before ( $\square$ ) and after ( $\blacksquare$ ) losartan administration are shown. A comparison of the ICV losartan-induced absolute change in MAP ( $\Delta$ MAP, C) between the groups is also shown. \* $P$ <0.01, pre- vs postlosartan (B) or  $P$ <0.001, RA<sup>-</sup> vs RA<sup>+</sup> (C).

not a complete analysis of the relationship between the change in HR versus the change in MAP over a range of doses of central AII, these results suggest that centrally induced baroreflex responses may be blunted in RA<sup>+</sup> mice. Whether this diminished baroreflex-mediated bradycardia in RA<sup>+</sup> mice is specific to central AII-elicited pressor responses, or if other centrally administered pressor agents would induce a similarly blunted reflex HR response in these mice, is not known.

After return of cardiovascular parameters to baseline following AII, losartan was administered. Typical examples of the ICV losartan-induced changes in cardiovascular parameters in RA<sup>+</sup> and RA<sup>-</sup> mice are shown in Figure 3A. Losartan (10  $\mu$ g, 1  $\mu$ L) caused slight reductions in HR and MAP in RA<sup>-</sup> mice, whereas a marked depressor response was observed in RA<sup>+</sup> mice (latency 1 to 2 minutes). A small tachycardia accompanying the losartan-induced depressor response was also observed in RA<sup>+</sup> mice. These responses were sustained for 20 to 30 minutes. ICV losartan-induced changes in MAP in both groups are summarized in Figure 3B and 3C. Central AT-1 receptor antagonism caused an approximately 30 mm Hg fall in MAP in RA<sup>+</sup> mice, reducing MAP in these animals to nearly baseline MAP values in controls. In contrast, intravenous administration of this dose of losartan had no effect on MAP in RA<sup>+</sup> mice (data not shown). Baseline MAP in RA<sup>-</sup> mice was not significantly altered by

either ICV or intravenous losartan. These results suggest that there is an exaggerated contribution of central AT-1 receptors to the maintenance of the elevated BP in the double-transgenic mice, and this cannot be attributed to systemic effects of losartan because of partial leakage of it into the circulation. In contrast, there is not a significant role for central AII/AT-1 receptors in the regulation of baseline BP in the controls. Widely variable HR responses to ICV losartan were observed in both RA+ ( $590 \pm 29$  pre- versus  $621 \pm 33$  postlosartan administration,  $\Delta\text{HR } 31 \pm 18$  bpm,  $P > 0.05$ ) and RA- ( $614 \pm 24$  pre- versus  $600 \pm 26$  postlosartan administration,  $\Delta\text{HR } -13 \pm 19$  bpm,  $P > 0.05$ ). This variable effect of ICV losartan on HR is consistent with data reported in other species.<sup>4,34</sup>

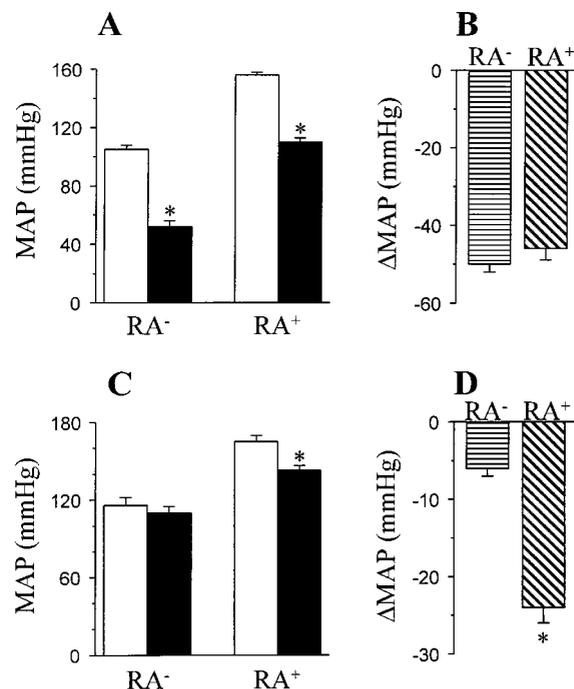
### Effects of Intravenous HEX and AVP V<sub>1</sub> Antagonism on BP

Evidence for an enhanced role of the brain RAS in RA+ mice in these initial experiments prompted us to investigate the pressor systems that may be involved in mediating this effect. Because the major mechanisms of central AII effects on BP are via activation of the sympathetic nervous system and/or AVP secretion from the posterior pituitary, we wanted to determine the relative contribution of these 2 pressor mechanisms to the maintenance of elevated BP in RA+ mice. We hypothesized that the effects of one or both of these systems may be elevated in RA+ mice as a result of enhanced central RAS activity. As an index of sympathetic nerve activity, the depressor responses to intravenous administration of the ganglionic blocking agent HEX were compared in RA+ ( $n=6$ ) and RA- ( $n=6$ ) mice. Similarly, the role of circulating AVP was determined by examining the intravenous AVPX-induced depressor responses in RA+ ( $n=5$ ) and RA- ( $n=4$ ) mice.

A summary of the effects of AVPX and HEX on MAP is shown in Figure 4. Baseline MAP was elevated to similar levels in both RA+ groups receiving either HEX (panels A and B) or AVPX (panels C and D). This level of hypertension is similar to that observed in mice in the present study receiving ICV AII and losartan, and also to that previously reported.<sup>23</sup> Ganglionic blockade with HEX caused equivalent depressor responses in RA+ and control mice, reducing MAP by about 50 mm Hg in both groups. In contrast, AVPX caused a significantly greater reduction in MAP in RA+ mice. No significant change in MAP was elicited by intravenous AVPX in RA- mice. These results suggest that the basal hemodynamic tone associated with neural activity is not enhanced in RA+ mice, whereas the contribution of AVP to baseline BP is augmented in these animals.

### Plasma Vasopressin Levels

To determine whether the exaggerated depressor effect of the AVP V<sub>1</sub> antagonist in RA+ mice could be accounted for at least partially by increased levels of circulating AVP, plasma was collected from RA+ ( $n=3$ ) and RA- ( $n=3$ ) mice and evaluated by radioimmunoassay. RA+ had an approximately 3-fold ( $2.92 \pm 0.44$ ) elevation in basal plasma AVP levels compared with controls ( $P < 0.001$ ). These results suggest that

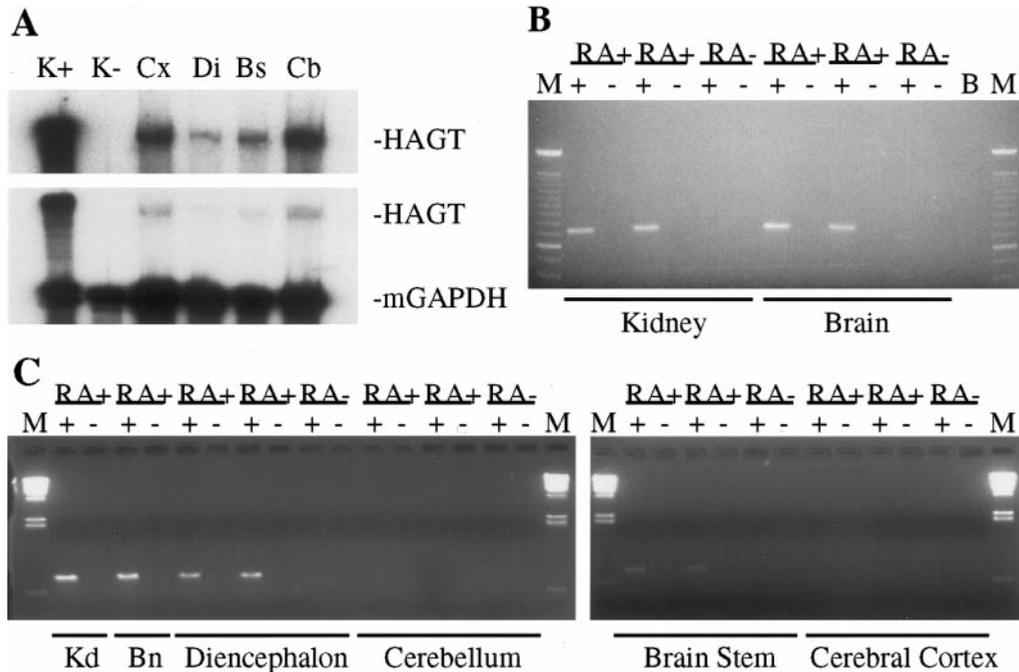


**Figure 4.** Response to HEX and AVPX. Summary of the effects of blockade of sympathetic nerve activity with HEX (5 mg/kg IV; A and B) or antagonism of vasopressin V<sub>1</sub> receptors (AVPX, 10  $\mu\text{g/kg IV}$ ; C and D) on MAP in conscious RA- (HEX,  $n=6$ ; AVPX,  $n=4$ ) and RA+ (HEX,  $n=6$ ; AVPX,  $n=5$ ) mice. MAP values before ( $\square$ ) and after ( $\blacksquare$ ) HEX (A) and AVPX (C) administration are shown. HEX- and AVPX-induced changes in MAP are compared for RA- and RA+ in B and D. \* $P < 0.01$ , pre- vs post-HEX or AVPX treatment (A and C) or  $P < 0.001$ , RA- vs RA+ (B and D).

enhanced levels of circulating AVP, perhaps mediated by overactivation of central AII pathways involved in the secretion of this hormone, may play a role in the chronic hypertension exhibited by RA+ mice. However, the vascular reactivity to AVP in RA+ mice is not known, and therefore enhanced vasoconstrictor effects of AVP cannot be ruled out as a possible mechanism of the augmented depressor effect of the V<sub>1</sub> antagonist in these animals.

### Expression of HREN and HAGT Transgenes in Brain

We wanted to determine whether one possible mechanism of a brain RAS-mediated increase in BP in RA+ mice is via expression of the human transgenes in intrinsic brain tissue, with potentially increased local AII production. To accomplish this, RNase protection assay and RT-PCR were performed to detect HAGT and HREN mRNA, respectively, in brain and kidney of RA+ and RA- mice (Figure 5). Kidney RNA was used as a positive control in both assays, because we have previously shown high-level expression of both HREN and HAGT in this tissue.<sup>23-25</sup> The HAGT transgene was expressed in all brain regions examined, including cortex, diencephalon, brain stem, and cerebellum in RA+ mice (Figure 5A). Even with a 3-day exposure (Figure 5A, top panel), no HAGT expression was evident in nontransgenic controls (K-), demonstrating the specificity and sensitivity of the assay.



**Figure 5.** Expression of *HREN* and *HAGT* in brain. A, Representative RNase protection assay performed to detect *HAGT* mRNA in brain regions including cortex (Cx), diencephalon (Di), brain stem (Bs), and cerebellum (Cb) of a RA+ mouse. Kidneys from the RA+ mouse (K+) and from a nontransgenic control (K-) were included as controls. Each lane contained a GAPDH control probe and a *HAGT* probe. The position of the protection products obtained with each probe is indicated. Exposure times are 3 days and 1 day for the top and bottom panels, respectively. B, Expression of *HREN* mRNA in kidney (left portion) and brain (right portion) of RA+ and RA- mice as detected by RT-PCR. Reactions were performed with (+) and without (-) RT. The primers used amplified a 720-bp fragment in RT-treated kidney and brain samples of RA+ mice. C, Expression of *HREN* mRNA in whole brain and in brain regions including diencephalon, cortex, brain stem, and cerebellum of RA+ and RA- mice as detected by RT-PCR. Kidney from an RA+ mouse was included as a positive control. Reactions were performed with (+) and without (-) RT. The primers used amplified a 720-bp fragment in RT-treated kidney, whole brain, diencephalon, and brain stem samples of RA+ mice. M, marker; B, blank.

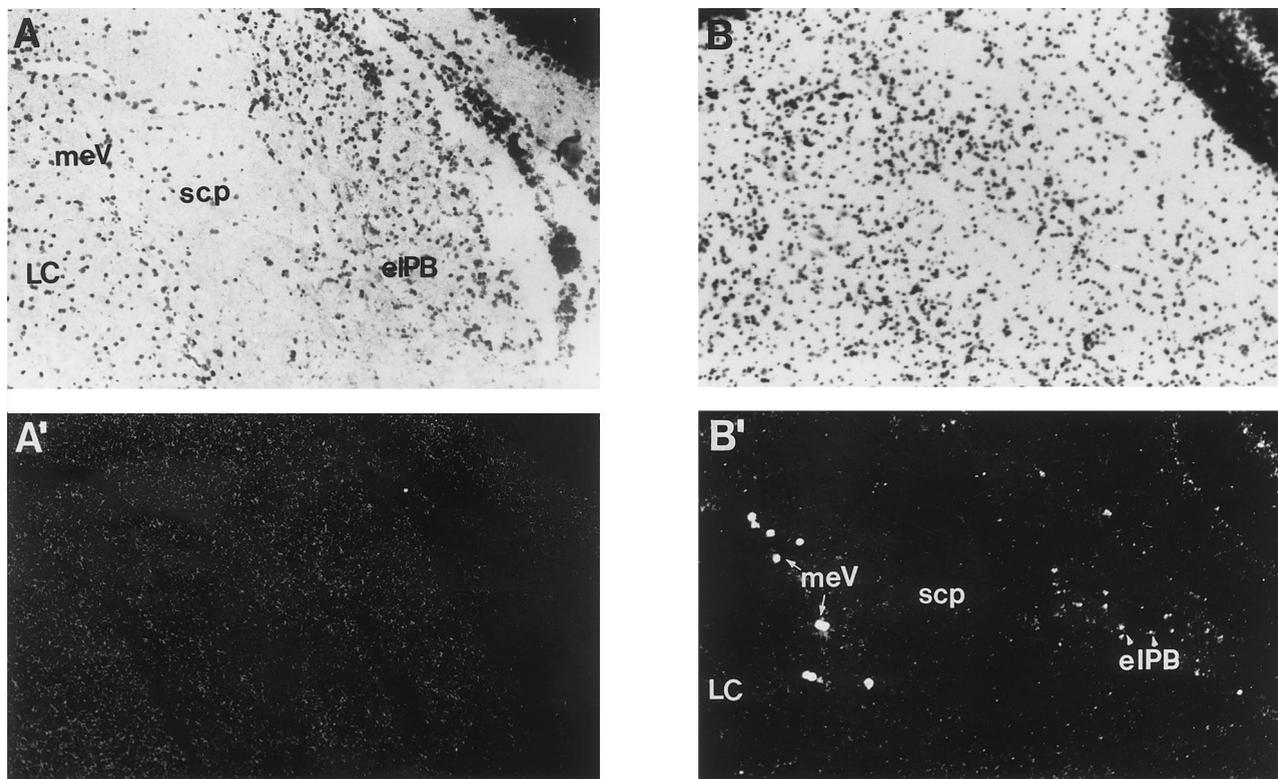
In addition, we have performed in situ hybridization analysis to elucidate the cellular localization of *HAGT* expression. Neuronal expression of *HAGT* was shown in restricted areas of the brain, including brain stem nuclei such as the parabrachial nucleus (PBN) and the mesencephalic nucleus of the trigeminal (meV) (Figure 6), as well as the SFO (Figure 7). *HAGT* mRNA was also detected in neurons of the PVN and in astrocytes in cortex, diencephalon, brain stem, and cerebellum in transgenic mice (data not shown). No in situ hybridization signal was detected on sections hybridized to a sense probe.

The precision and sensitivity of the RT-PCR assay were used to examine expression of the *HREN* transgene in brain of RA+ mice (Figure 5B and 5C). Specific RT-PCR products of the predicted size (720 bp) were detected in whole brain, diencephalon, and brain stem, but not in cortex and cerebellum, of RA+ mice. As expected, RT-PCR of RA+ kidney RNA also resulted in the predicted 720-bp product. The integrity of the RNA samples subjected to the assay was confirmed by our RT-PCR detection of *HAGT* in each of the 4 brain regions, confirming our RNase protection results (data not shown). No RT-PCR products were evident either in reactions in which RT was omitted or in brain or kidney RNA from nontransgenic controls. The low level of expression of *HREN* in brain precluded us from determining the precise cellular localization of this transgene in these regions by in situ hybridization.

### Discussion

We have previously reported that mice expressing both the *HREN* and *HAGT* transgenes are chronically hypertensive.<sup>23</sup> However, the precise mechanism(s) by which lifelong overexpression of the RAS in this model results in hypertension is not known. Although RA+ mice have elevated plasma AII levels compared with controls (approximately 4-fold), enhanced AII-mediated direct peripheral vasoconstriction alone cannot account for the hypertension, because acute systemic administration of the angiotensin-converting enzyme inhibitor enalapril did not normalize BP in these mice.<sup>23</sup> Moreover, because both the *HREN* and *HAGT* transgenes are expressed appropriately in extrarenal and extrahepatic tissues, respectively,<sup>24,25</sup> we hypothesized that activation of an intrinsic tissue RAS may play a role in the maintenance of elevated BP in these animals. Indeed, increasing evidence indicates a role for individual tissue RAS in regulating cardiovascular function, and in particular, there is substantial evidence for the existence and functional importance of a brain RAS in both normal and pathological regulation of BP.<sup>36</sup> Therefore, we examined the possibility that an overactive brain RAS contributes to the hypertension exhibited in this model.

The present study demonstrates that there is an enhanced role for central AT-1 receptors in the maintenance of elevated BP in mice containing both the *HREN* and *HAGT* transgenes. At a dose that had no effect on BP when given peripherally, ICV injection of the selective AT-1 receptor antagonist

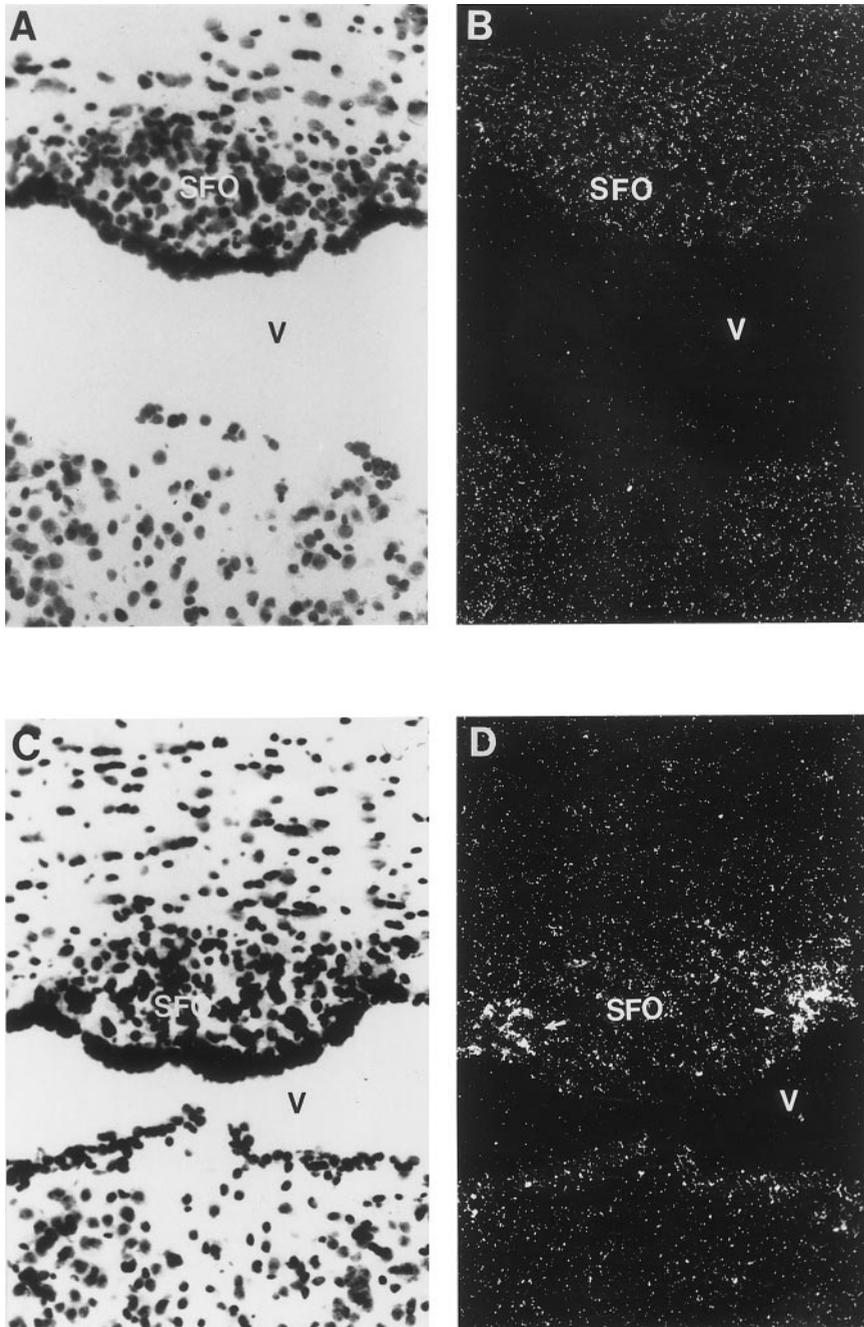


**Figure 6.** Cell-type specific expression of the *HAGT* gene in the brain stem of transgenic mice. In situ hybridization analysis was performed on PBN of *HAGT* transgenic mice. Sections were hybridized with a  $^3\text{H}$ -labeled sense (A and A') or antisense (B and B') RNA probe. A and B are bright-field images, whereas A' and B' are the dark-field images of the same fields. LC indicates Locus ceruleus; scp, superior cerebellar peduncle; and eIPB, external lateral PBN. Magnification,  $\times 20$ .

losartan reduced BP in the double-transgenic mice to levels nearly equivalent to that of baseline in control animals. In contrast, we showed that central AT-1 receptors, at least those accessed by ICV administration of losartan, do not play a significant role in the regulation of baseline BP in normal mice. These results are consistent with previous reports that blockade of the formation or action of central AII does not alter baseline BP in normotensive rats.<sup>20,21,37</sup> To examine the possibility that the exaggerated activation of central AT-1 receptors in RA+ mice results in altered sympathetic nerve and/or AVP outflow from the CNS, we determined the relative contribution of each of these pressor systems to BP in double-transgenic mice and controls. The finding that the depressor response to the  $V_1$  antagonist was significantly greater in RA+ mice compared with controls suggests an enhanced contribution of circulating AVP to vascular tone in this model. In contrast, vascular tone associated with sympathetic nerve activity is equivalent in RA+ and controls. An increased role for circulating AVP in BP regulation in RA+ mice was also supported by the approximately 3-fold greater levels of plasma AVP in these mice. Taken together, these results suggest that an enhanced AII-mediated activation of CNS AT-1 receptors, perhaps within brain regions involved in AVP secretion, plays a role in the chronic hypertension exhibited by mice with lifelong overexpression of the RAS.

Although the present study does not establish a direct cause-and-effect relationship between increased activity of the brain RAS and exaggerated release of AVP from the posterior pituitary, there is considerable evidence of the

central RAS-AVP axis link and, therefore, several potential CNS pathways that could be involved in exaggerated AT-1-mediated release of AVP and hypertension in this model. There is considerable evidence that AII-mediated activation of both intrinsic brain sites and regions at the blood-brain interface elicit increases in BP and secretion of AVP from the posterior pituitary. Increased AII in either the systemic circulation or within the cerebrospinal fluid is known to activate AT-1 receptor-rich CVOs such as the SFO and organum vasculosum lamina terminalis, causing pressor responses and AVP release.<sup>10,17,38-42</sup> Indeed, the AII-containing neural network among these CVOs and AVP-synthesizing magnocellular hypothalamic neurons, such as the supraoptic nucleus (SON) and the PVN, is necessary for this response pattern. AII-activated efferent projections from the SFO directly to the SON and PVN have been identified,<sup>39-41</sup> and AII-mediated induction of cFos immunoreactivity in the SON and PVN was completely prevented by ablation of the SON.<sup>43</sup> The brain stem CVO AP is also rich in AT-1 receptors and is activated by circulating AII, although the mechanism by which BP is increased is primarily activation of brain stem sites, leading to increases in sympathetic outflow.<sup>44,45</sup> Brain sites that do not interact with blood-borne AII but are rich in AT-1 receptors, are involved in BP regulation, and contain neural pathways associated with AVP secretion include the SON and PVN themselves, the anterior hypothalamic area, and brain stem nuclei such as the nucleus tractus solitarius and ventrolateral medulla.<sup>2,37,38</sup> It is thought that these intrinsic regions interact with AII produced locally within the brain.



**Figure 7.** Cell type-specific expression of the *HAGT* gene in the SFO in brain of transgenic mice. In situ hybridization analysis was performed on SFO of *HAGT* transgenic mice. Serial frozen brain sections were hybridized with a  $^3\text{H}$ -labeled sense (A and B) or antisense (C and D) RNA probe. A and C are bright-field images, whereas B and D are dark-field images. V indicates ventricle. Arrows in D indicate lateral portion of SFO. Magnification,  $\times 75$ .

There are 2 possible mechanisms by which the increased AII-mediated activation of central AT-1 receptors, perhaps concomitant with enhanced AVP secretion, may occur in RA+ mice. The increased circulating AII in these animals could cause chronic increased activation of AT-1 receptors at CVOs such as the SFO, and the ICV losartan-induced lowering of BP in RA+ mice would be due mainly to blockade of the effects of systemically derived AII at AT-1 receptors located at the blood-brain interface. However, it has been reported that ICV administration of AT-1 receptor antagonists (at doses shown to block ICV AII-elicited responses) does not attenuate the hypertension produced by chronic intravenous AII infusion.<sup>46,47</sup> Only when the antagonist was administered directly into the AP was a

lowering of the BP observed, suggesting that chronic intravenous AII-hypertension does not involve activation of AT-1 receptors in intrinsic brain sites or in CVOs that are known to be penetrated by antagonists administered ICV (eg, SFO).<sup>47,48</sup> In addition to evidence that ICV administered AT-1 receptor blockers may not reach AP neurons,<sup>47,49</sup> a role for the AP in the hypertension of RA+ mice in the present study is not supported, because the mechanism of chronic intravenous AII/AP-mediated hypertension involves increased sympathetic nerve activity rather than enhanced AVP secretion.<sup>44</sup> This does not, however, rule out a possible role for the AP in the maintenance of normal BP in the control mice in this study. The finding that the AP is necessary for the full

expression of intravenous losartan-induced hypotension in normotensive rats suggests that AII-mediated activation of AP AT-1 receptors is important in tonic BP regulation.<sup>50</sup>

Alternatively, local overproduction of AII within intrinsic brain tissue of RA+ mice may stimulate AT-1 receptors at sites inside of the blood-brain barrier, leading to hypertension. If this is the case, the ICV losartan-induced reduction in BP in the RA+ mice would be due to blockade of the effects of locally derived AII. This latter possibility is supported by our findings that both the *HREN* and *HAGT* transgenes are expressed in brains of RA+ mice. Furthermore, the colocalization of expression of the transgenes in the diencephalon and the brain stem, both important cardiovascular control regions, suggests the possibility that increased AII production may result from the cleavage of *HAGT* by *HREN* in these areas. Indeed, increased AII production in either the diencephalon or the brain stem could potentially cause increased AVP release.<sup>3</sup> Our finding that *HAGT* mRNA, as detected by in situ hybridization, was found in neurons in restricted regions of the brain, including the SFO and brain stem sites such as the PBN, PVN, and meV, further suggests a possible mechanism for increased AII production in sites involved in AVP release. We are currently examining the hypothesis that an upregulation of AT-1 receptor levels in these brain regions contributes to the mechanism of hypertension in these mice.

In contrast to models of chronic intravenous AII-mediated hypertension, ICV administration of blockers of the RAS has been shown to be effective in lowering BP in genetic models of hypertension in which the brain RAS is upregulated. For example, acute or chronic ICV administration of AII receptor antagonists markedly reduced BP in the adult spontaneously hypertensive rat (SHR),<sup>12,20</sup> and chronic ICV-converting enzyme inhibitor prevented the development of hypertension in young SHRs.<sup>21</sup> Indeed, compared with normotensive Wistar-Kyoto rats, SHRs have elevated levels of brain RAS components<sup>51,52</sup> but normal plasma AII levels. Moreover, direct microinjection of losartan into the anterior hypothalamic area, an intrinsic brain site rich in AT-1 receptors with projections to PVN and SON, caused depressor responses in SHR but not WKY,<sup>37</sup> suggesting a role for endogenous AII at this intrinsic brain site in SHR. Another genetic model of hypertension characterized by elevated brain renin expression and AII levels but normal plasma AII levels, the transgenic rat containing the mouse renin *Ren-2d* gene, is also sensitive to the BP-lowering effects of losartan administered ICV.<sup>22,53</sup> Interestingly, these rats also have increased levels of AVP in several brain regions.<sup>54</sup>

Whether or not increased activation of central AT-1 receptors causes enhanced release of AVP from central pituitary sites, a role for AVP in the etiology of the hypertension in RA+ mice must be considered. Indeed, the significantly augmented depressor effect of the  $V_1$  antagonist, along with the marked elevations of plasma AVP levels in RA+ mice, suggests chronically increased AVP-mediated vasoconstriction as a possible mechanism of the elevated BP in this model. Although a role for AVP in the etiology of hypertension in humans and animal models is controversial, recent evidence suggests that chronic 4-fold elevations in plasma AVP concentrations result in sustained hypertension in rats

via a  $V_1$  receptor-mediated mechanism independent of body fluid retention.<sup>55</sup> Increases in renal medullary vascular resistance were implicated in the production of the systemic hypertension, and a role for vascular  $V_2$  receptors in opposing the vasoconstrictor effects of stimulation of  $V_1$  receptors was suggested.<sup>56</sup> In addition, the initial fluid-retaining effects of infused AVP and resulting expansion of blood volume and hypertension, with eventual "escape" from these antidiuretic and hypertensive effects via pressure diuresis mechanisms, has been described.<sup>57</sup> Neither the role of  $V_2$  receptors nor the body fluid profile of RA+ mice is known, but these are currently being investigated. Indeed, we believe that in addition to being an important model for understanding the hemodynamic effects of lifelong overexpression of the RAS, the long-term elevated levels of circulating AVP make these mice interesting from the standpoint of understanding AVP-mediated regulation of body fluid homeostasis.

We have previously investigated baroreceptor reflex function in RA+ mice elicited by peripheral administration of vasoconstrictors and vasodilators.<sup>23</sup> In these studies, we demonstrated an AII-mediated resetting of baroreflex control of HR to a higher pressure, but no alteration in gain or sensitivity of the reflex. In the present study, the decrease in HR elicited by the ICV AII-induced pressor response was significantly reduced in RA+ compared with control mice. Although not a complete profile of MAP and HR responses over a range of doses of ICV AII, these results suggest that centrally induced baroreflex responses may be blunted, at least to this dose of AII. This difference in baroreflex function with central versus peripheral administration of a pressor agent is consistent with a study by Barron et al.,<sup>58</sup> in which they report differences in HR responses elicited by ICV versus intravenous AII administration in rats. Because increases in BP initiated from the CNS can be influenced by both the buffering effects of peripheral activation of baroreceptors and direct interactions within the CNS between central baroreflex and central pressor pathways,<sup>58</sup> it is possible that the diminished HR response to the central AII-induced pressor response in this study is due to greater direct modulating actions of AII in central pressor and/or baroreflex pathways in RA+ mice. Further study is required to determine the mechanism of such a central modulatory influence of AII, as well as to determine whether this is a central AII-specific effect or whether other central pressor agents elicit a similar response in RA+ animals.

As the first report of physiological recording in conscious, unrestrained mice during administration of agents into the brain, this study demonstrates an exaggerated central RAS component in the mechanism of chronic hypertension produced by lifelong overexpression of both the *HAGT* and *HREN* transgenes. Expression of both transgenes in the brain raises the possibility that augmented local production of AII in the CNS plays an important role in the production and/or maintenance of the elevated BP in these mice. We will examine whether increased levels of AT-1 receptors in brain tissue of RA+ mice is involved in the hypertension in this model. An exaggerated contribution of plasma AVP but not sympathetic nerve activity to BP, perhaps originating from increased central AII/AT-1 stimulation of AVP-secreting

pathways, was also demonstrated in RA+ mice. Further site-specific brain lesion and microinjection studies are ongoing to elucidate the relative contribution of plasma AII and endogenous brain AII to the overactivation of central AT-1 receptors in these mice. Moreover, we believe this is a unique model for studying the role of long-term overproduction of both AII and AVP in the regulation of body fluid homeostasis. These studies illustrate the power of using an experimental strategy that combines genetic manipulation and classical physiological/pharmacological analyses in delineating determinants of BP control and hypertension in a way not possible before.

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