Changes in angiotensin type 1 receptor binding and angiotensin-induced pressor responses in the rostral ventrolateral medulla of angiotensinogen knockout mice

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Chen D, Hazelwood L, Walker LL, Oldfield BJ, McKinley MJ, and Allen AM. Changes in angiotensin type 1 receptor binding and angiotensin-induced pressor responses in the rostral ventrolateral medulla of angiotensinogen knockout mice. Am J Physiol Regul Integr Comp Physiol 298: R411–R418, 2010. First published November 25, 2009; doi:10.1152/ajpregu.00462.2009.—Angiotensin II (ANG II), the main circulating effector hormone of the renin-angiotensin system, is produced by enzymatic cleavage of angiotensinogen. The present study aimed to examine whether targeted deletion of the angiotensinogen gene (Agt) altered brain ANG II receptor density or responsiveness to ANG II. In vitro autoradiography was used to examine the distribution and density of angiotensin type 1 (AT1) and type 2 receptors. In most brain regions, the distribution and density of angiotensin receptors were similar in brains of Agt knockout mice (Agt−/−) and wild-type mice. In Agt−/− mice, a small increase in AT1 receptor binding was observed in the rostral ventrolateral medulla (RVLM), a region that plays a critical role in blood pressure regulation. To examine whether Agt−/− mice showed altered responses to ANG II, blood pressure responses to intravenous injection (0.01–0.1 μg/kg) or RVLM microinjection (50 pmol in 50 nl) of ANG II were recorded in anesthetized Agt−/− and wild-type mice. Intravenous injections of phenylephrine (4 μg/kg and 2 μg/kg) were also made in both groups. The magnitude of the pressor response to intravenous injections of ANG II or phenylephrine was not different between Agt−/− and wild-type mice. Microinjection of ANG II into the RVLM induced a pressor response, which was significantly smaller in Agt−/− compared with wild-type mice (+10 ± 1 vs. +23 ± 4 mmHg, respectively, \( P = 0.004 \)). Microinjection of glutamate into the RVLM (100 pmol in 10 nl) produced a robust pressor response, which was not different between Agt−/− and wild-type mice. A diminished response to ANG II microinjection in the RVLM of Agt−/− mice, despite an increased density of AT1 receptors, suggests that signal transduction pathways may be altered in RVLM neurons of Agt−/− mice, resulting in attenuated cellular excitation.

blood pressure; brain microinjection; angiotensin II; brain renin-angiotensin system

The renin-angiotensin system (RAS) plays a critical role in cardiovascular homeostasis by regulating arterial pressure (AP) and fluid electrolyte balance. The principal effector of the RAS, ANG II, plays diverse physiological roles via two main receptors, the ANG II type 1 (AT1) and the ANG II type 2 (AT2) receptor (47). Most of the classical actions of systemic ANG II are exerted via the AT1 receptor, which has a distinct distribution throughout the body, including in the brain (3, 29). The distribution of AT1 receptors is highly conserved across species from rodents to primates and humans, whereas the distribution of AT2 receptors in the adult is more variable (4).

Angiotensinogen (AGT) is the only known precursor of ANG II in vivo and is a critical component of the RAS. In humans, there is a direct link between angiotensinogen gene (Agt), AGT expression, and AP (22), while in transgenic mice, there is a linear correlation between Agt copy and AP (23). Mice in which AGT expression is absent (Agt knockout mice) have reduced blood pressure (BP) and abnormal renal development (20).

Considerable interaction occurs between different components of the RAS to regulate activity of the system. Acting via AT1 receptors, ANG II decreases renin secretion and increases AGT expression (24, 34). In addition, ANG II inhibits transcription of the AT1 receptor in vascular smooth muscle cells (25) and cardiac myocytes (9). Expression of the AT1 receptor is both upregulated and downregulated, in a tissue-dependent manner, by physiological perturbations such as altered sodium intake, which result in altered activity of the RAS (44, 45). Such intricate regulation of the activity of this system is thought to play a major role in the maintenance of cardiovascular homeostasis. In addition, altered expression of AT1 receptors is observed in the brain in many physiological and pathological states associated with activation of the RAS (31, 38). However, the precise mechanisms of how altered levels of ANG II regulate AT1 receptor expression and activity in the brain remain unclear.

We hypothesized that reduced expression of AGT would significantly increase the expression of ANG II receptors and that, as a consequence, the response to administration of exogenous ANG II would also be enhanced. In the present study, we tested this hypothesis by examining the distribution and density of AT1 or AT2 receptors in key sites within the brain of Agt knockout and wild-type mice. We also examined the cardiovascular responses of these mice to microinjection of ANG II in the rostral ventrolateral medulla (RVLM), as well as intravenous injection of different doses of ANG II and phenylephrine.

MATERIALS AND METHODS

General Preparations

All experiments were performed in accordance with the National Health and Medical Research Council of Australia, Code of
Practice for the Care and Use of Animals for Scientific Purposes, and were approved by the University of Melbourne Animal Ethics and Experimentation Committee. Experiments were performed on adult male mice of the C57BL6 strain, in which the coding region for the Agt gene had been deleted by homologous recombination (Agt\(^{−/−}\)). These heterozygous mice, from a line (Agt\(^{++}\)) generated by H. S. Kim and colleagues (23), were bred at the Howard Florey Institute to derive homozygous Agt\(^{+/+}\) (wild-type) and Agt\(^{−/−}\) (knockout) mice. All mice were genotyped, as described previously, prior to experimentation (30). The Agt\(^{−/−}\) mice used in this study showed general characteristics that are consistent with previous observations. As observed by others, only a small percentage of Agt\(^{−/−}\) mice survive past weaning (23, 36). To overcome this, Agt\(^{−/−}\) pups in this study were given subcutaneous injections of warm isotonic saline (0.15 mol/l NaCl) during the first week of life (30). Despite attempts to use littermate homozygous Agt\(^{++}\) and Agt\(^{−/−}\) mice, the reduced survival of the Agt\(^{−/−}\) mice meant this was not always possible, and a small difference in the mean ages of the populations occurred (Agt\(^{+/+}\) 7 ± 1 mo vs. Agt\(^{−/−}\) 5 ± 1 mo). Mice were maintained under 12:12-h light-dark cycle with ad libitum access to standard chow and water.

**Localization of Receptor Binding Sites**

The distribution of angiotensin binding sites was mapped in the brain of littermate Agt\(^{+/+}\) (n = 3) and Agt\(^{−/−}\) (n = 3) mice using standard methods (27). Power calculations using standard deviations derived from previously published data from this laboratory (3, 21, 27), an expected change of 20% in mean levels, power of 0.8 and α of 0.05 were used to calculate sample size. Examining a minimum number of mice enabled all sections to be incubated at once, thus removing the need to normalize data and control for interassay variability. Briefly, the mice were deeply anesthetized, by inhalation of isoflurane in an enclosed container, and decapitated. The brains were removed, frozen in isopentane on dry ice and 20-μm coronal sections cut on a cryostat. Slide-mounted sections were incubated using standard solutions containing \(^{125}\)I-[Sar\(^{1}\), Ile\(^{8}\)] ANG II as radioligand (Prosearch International Australia Pty Ltd, Vic, Australia). Either 1 μM angiotensin II (to determine non-specific binding; Auspep, Vic, Australia), 1 μM candesartan (to displace binding from AT\(_{1}\) receptors; gift from Astra Hässlé, AB, Sweden) or 10 μM PD123319 (to displace binding from AT\(_{2}\) receptors; Sigma-Aldrich, St. Louis, MO) were added to the incubation media to define receptor types. After washing, then drying, the sections were apposed to X-ray film (UM-MAC HC, FujiFilm, Tokyo, Japan) for 14–21 days in X-ray cassettes that also contained standards of known radioactivity. Radioligand binding densities were quantitated using Scion Image 4.0.2 (Scion, Frederick, MD) with densities calculated as dpm/mm\(^2\). Alternate sections were stained with thionin to enable identification of brain nuclei for quantitation. The atlas of Franklin and Paxinos (14) was used to assist in determining the boundaries of the anatomical structures included in these measurements. Measurements in the organum vasculosum of the lamina terminalis (OVLT) and area postrema were taken from the entire nucleus in the middle of their rostro-caudal extent. The median preoptic nucleus was measured at the level of the anterior commissure from both dorsal and ventral parts. The hypothalamic paraventricular nucleus (PVN) was measured at the level of the magnocellular neurons. The nucleus of the solitary tract (NTS) and inferior olivary nucleus (IO) were measured at the mid-rostro-caudal extent of the area postrema. The RVLM was defined as a wedge-shaped region with its apex at the ventral edge of the compact formation of the nucleus ambiguous running to points on the ventral brain surface 1 and 1.5 mm lateral to the midline. In the rostro-caudal plane, we measured within 200–400 μm from the caudal boundary of the facial motor nucleus.

**Measurement of BP and HR**

For physiological studies, mice were initially anesthetized by inhalation of isoflurane, followed by intraperitoneal injection of a mixture of urethane (0.75 mg/g ip) and chloralose (0.05 mg/g ip). A stable surgical plane of anesthesia was determined by the loss of the pedal withdrawal reflex to a strong noxious stimulus and loss of the corneal reflex. Suplemental doses of urethane (75 μg/g ip) were administered if necessary. Mice were tracheotomized and artificially ventilated (UGO Basile mouse ventilator 28025; Comerio, VA, Italy) with room air. The left carotid artery was cannulated for BP measurement with heat stretched polyethylene tubing (0.96 mm OD, 0.58 mm ID), as previously reported (26). This cannula was filled with sterile saline containing heparin (50 IU/ml) to prevent clotting. The catheter was connected to a BP transducer (Neurolog System Digitimer, Hertfordshire, England) and the signal amplified (Neurolog System Digitimer, Hertfordshire, England), digitized (2-kHz sampling rate), and stored on computer (MacLab, Takoma Park, MD) for later analysis. Mean arterial pressure (MAP) and heart rate (HR) were derived from this signal. Rectal temperature was maintained at 37°C with a heating pad (ATC1000 Animal Temperature Controller, World Precision Instruments, Sarasota, FL). In preliminary experiments, blood samples (0.1 ml) were taken from the carotid artery to measure arterial blood gases and pH. Ventilation parameters (stroke volume: 0.3–0.4 ml, and rate: 135–150 strokes/min) were then determined to maintain arterial PCO\(_2\) at ~4.5%.

**Microinjections into the RVLM**

After instrumentation, Agt\(^{+/+}\) (n = 9) and Agt\(^{−/−}\) (n = 8) mice were placed in a stereotaxic apparatus (Benchmark Stereotoxic Instruments, MyNeurolab, St. Louis, MO) in a prone position. The position of the head was adjusted, so that the skull surface was horizontal between lambda and bregma. The occipital bone overlying the cerebellum was carefully removed while keeping the lambdoid suture intact. The lambdoid and midline sutures served as reference points for rostrocaudal and lateral coordinates, respectively. Microinjections were made to the RVLM in the region of the following coordinates: 1.2 mm lateral to the midline, 1.7 mm caudal to lambda, and 5.3 mm ventral to the dorsal surface of the brain. Microinjections were made through single-barrel glass micropipettes pulled from capillary tubing (World Precision Instruments, Sarasota, FL), with tip diameter 30–50 μm. Solutions were ejected using pressurized nitrogen gas delivered via a pneumatic pressure device (Model SYS-PV820; World Precision Instruments) and volumes determined by observation of movement of the fluid meniscus using a monocular microscope fitted with an eye-piece graticule (Cole-Parmer, Vernon Hills, IL). Microinjections of L-glutamate (100 pmol: 10 nl of 10 mM solution) were made to functionally identify the RVLM. The RVLM was defined as the site where L-glutamate elicited a prompt increase in AP of 10 mmHg or greater. Once localized, a new pipette was inserted at these coordinates and ANG II injected unilaterally (50 pmol: 50 nl of 1 mM solution; Auspep, Vic, Australia). The ANG II solution contained a 1% concentration of rhodamine-labeled microspheres (Molecular Probes, Eugene, OR) for histological verification of the injection site. The response to microinjection of vehicle (saline) in the RVLM or injections of ANG II outside the RVLM was also tested in some animals at the end of RVLM microinjections. In two wild-type mice, the response to microinjection of ANG II was assessed after microinjection of the AT\(_{1}\) receptor antagonist, candesartan (50 nl of a 10 mM solution), in the RVLM.

**Histological Analysis**

At the end of the experiments, mice were killed by a bolus injection of urethane, and brains were removed and fixed in 4% paraformaldehyde for 2 days. Fifty-micrometer coronal sections were cut using a vibrating microtome (Microllicer 1500E; Pelco International, Red-
Peripheral ANG II and Phenylephrine Bolus

Agr\(^{+/+}\) and Agr\(^{-/-}\) mice were prepared as described above for measurement of BP and HR. The left external jugular vein was cannulated with polyethylene tubing (0.61 mm OD, 0.28 mm ID) for the administration of ANG II and phenylephrine. The AP response to intravenous injection of a 0.1 ml bolus of ANG II (0.01, 0.05, or 0.1 \(\mu g/kg\)) was examined in Agr\(^{+/+}\) (n = 7) and Agr\(^{-/-}\) (n = 8) mice. In between each ANG II dose, saline was used to wash out residual ANG II in the catheter, and mice were allowed to recover for at least 20 min. In a subset of these mice [Agr\(^{+/+}\) (n = 5) and Agr\(^{-/-}\) (n = 4)], a 0.1 ml bolus of phenylephrine (4 \(\mu g/kg\) and 2 \(\mu g/kg\)) was also administered intravenously.

Statistical Analysis

Data are expressed as means \(\pm SE\). Student’s t-test was used to compare the receptor-binding densities in specific regions between Agr\(^{+/+}\) and Agr\(^{-/-}\) mice using GraphPad Prism (GraphPad software, San Diego, CA). Baseline MAP and HR are calculated based on 5-min averages at the start of each recording. For an analysis of the responses to RVLM microinjections, 30-s averages of MAP and HR signals were determined for the period 5 min prior to the microinjections and for at least 15 min afterwards. The peak response to each microinjection was the highest 30-s average value after microinjection and was compared with the baseline measure. Comparisons of the peak ANG II and glutamate responses were made between Agr\(^{+/+}\) and Agr\(^{-/-}\) mice with a two-way repeated-measures ANOVA followed by the Newman-Keuls multiple-comparisons test [Sigma Stat ver. 3.5 (Jandel, San Jose, CA)]. For analysis of intravenous ANG II and phenylephrine responses, 10-s averages of MAP and HR were calculated for the 1-min period prior to injection (baseline) and for at least 5 min after injection. The peak response to each injection was determined by the highest 10-s point and was compared with the baseline measure. Comparisons of the intravenous ANG II and phenylephrine AP changes were made between Agr\(^{+/+}\) and Agr\(^{-/-}\) mice within each dose by two-way ANOVA followed by Holm-Sidak multiple-comparison test (Sigma Plot 11, Jandel, San Jose, CA). Different statistical programs and post hoc tests were used in data analysis to conduct the most stringent comparisons. Statistical significance was set at \(P < 0.05\).

RESULTS

General Parameters

Wild-type (n = 14) mice were 7 \(\pm\) 1 mm of age, and Agr\(^{-/-}\) (n = 14) mice were 5 \(\pm\) 1 mm of age. The average weights of the Agr\(^{+/+}\) mice was 29 \(\pm\) 1 g and Agr\(^{-/-}\) mice was 24 \(\pm\) 1 g, (\(P < 0.05\)). Under anesthesia, the baseline BP was significantly higher in Agr\(^{+/+}\) mice compared with Agr\(^{-/-}\) mice (systolic BP: 81 \(\pm\) 4 vs. 58 \(\pm\) 3 mmHg, respectively and MAP: 64 \(\pm\) 4 mmHg vs. 39 \(\pm\) 1 mmHg, respectively, \(P < 0.0001\)) (Fig. 3A). HR was not different in Agr\(^{+/+}\) and Agr\(^{-/-}\) [519 \(\pm\) 35 bpm vs. 473 \(\pm\) 23 bpm, respectively, (not significant, N.S.]).

Receptor Distribution

The distribution of binding sites for \(^{125}\)I-[Sar\(^1\), Ile\(^8\)] ANG II was examined throughout the brain of Agr\(^{+/+}\) and Agr\(^{-/-}\) mice. A similar overall distribution was observed for each genotype, which was comparable to the distribution in the mouse brain that has been published previously (17, 21). The sites of highest density binding were in the lamina terminalis, the PVN, the NTS, area postrema, and IO. Comparison of the density of AT\(_1\) and AT\(_2\) receptors in a selection of these regions indicated that, in general, there was little difference in density between the Agr\(^{+/+}\) and Agr\(^{-/-}\) mice (Fig. 1). The exceptions to this were the NTS, where there was a small, but significant decrease in AT\(_1\) receptor binding density, with no alteration in the AT\(_2\) receptor density in the Agr\(^{-/-}\) mice; the IO, with a small increase in AT\(_2\) receptor density in the Agr\(^{-/-}\) mice; and the RVLM, with a small increase in AT\(_1\) receptor density in the Agr\(^{-/-}\) mice.

Injection Studies in Anesthetized Mice

Glutamate response. Identification of the RVLM was based upon the AP response to microinjection of the excitatory amino acid L-glutamate and histological verification of the placement of fluorescent microspheres (Fig. 2B) within the anatomical boundaries of the nucleus. Mice were excluded from the study...
if these conditions were not met. Microinjection of glutamate into the RVLM (100 pmol in 10 nl) caused a rapid pressor response that was not different in Ago+/+ and Ago−/− mice as shown in Fig. 3B (peak response of +17 ± 3 mmHg vs. +13 ± 2 mmHg, respectively, N.S.). Even when expressed as a percentage change in BP, to account for the lower basal AP of the Ago−/− mice, there was no difference in the glutamate response between the genotypes (28 ± 4% in each case). In approximately half the mice, glutamate microinjection into the RVLM also induced changes in respiratory activity with increased breathing against the cycle of the respirator (Fig. 2D). Vehicle microinjections (saline, 10 nl and 50 nl) into the RVLM had no effect on BP, HR, or respiratory parameters (data not shown).

Ang II response in RVLM. Unilateral microinjections of Ang II (50 pmol in 50 nl) were made into the RVLM in Ago−/− and Ago+/+ mice (either left or right side of the brain). Comparable responses were observed between the left and right side injections; therefore all injections sites are mapped on the one side in Fig. 2A. Microinjection of Ang II into the RVLM of Ago+/+ and Ago−/− mice caused a prompt increase in AP with no change in HR (Fig. 3A). The Ang II-induced pressor response was significantly larger in the Ago+/+ mice (+23 ± 4 mmHg) compared with the Ago−/− mice (+10 ± 1 mmHg, P = 0.004) (Fig. 3B). The difference in response remained significant when expressed as a percentage of the baseline AP (44 ± 9% and 24 ± 2% in Ago+/+ and Ago−/− mice, respectively, Student’s t-test, P < 0.05). The time for the peak Ang II pressor response to occur was similar in Ago+/+ and Ago−/− mice (83 ± 19 s vs. 75 ± 20 s, respectively, N.S.). In four (3 Ago+/+ and 1 Ago−/−) mice, where injections were histologically verified to be dorsal and rostral to the anatomical boundary of the RVLM, Ang II had no effect on AP or HR. Unlike glutamate, microinjection of Ang II did not cause any significant changes in respiratory activity when injected into the RVLM. In two wild-type mice, the response to microinjection of Ang II was assessed after microinjection of the AT1 receptor antagonist, candesartan (50 nl of a 10 mM solution). In both cases, the pressor response to Ang II was abolished (data not shown).

Intravenous Ang II response. Intravenous injection of Ang II in anesthetized Ago+/+ and Ago−/− mice caused a brisk pressor response that was not different between the genotypes (Fig. 4A). Because of the lower basal BP of the Ago−/− mice, when these changes were expressed as a percentage of basal BP, there was a significant increase in the response of the Ago−/− mice to the higher doses of Ang II [0.1 µg/kg Ang II: +105 ± 5% vs. +143 ± 8% (P < 0.05); 0.05 µg/kg Ang II: +79 ± 11% vs. +117 ± 16% (P < 0.05); 0.01 µg/kg Ang II: 45 ± 10% vs. 61 ± 11% (N.S.); Ago+/+ vs. Ago−/−, respectively].

Intravenous phenylephrine response. Intravenous injection of two different doses of phenylephrine (2 µg/kg and 4 µg/kg) in anesthetized Ago+/+ and Ago−/− mice caused similar responses in both groups of mice (Fig. 4B). When expressed as a percentage of basal BP, there was no significant difference in the responses between the genotypes [4 µg/kg phenylephrine: 80 ± 18% vs. 81 ± 16%; 2 µg/kg phenylephrine: 31 ± 8% vs. 47 ± 9%; Ago+/+ vs. Ago−/−, respectively, (N.S.)].

Discussion

Small, but statistically significant, changes in Ang II receptor density were observed in some brain nuclei in the Ago−/−
mice in this study, but the overriding conclusion is that genetic deletion of AGT expression in mice does not alter the overall distribution of AT1 or AT2 receptor binding sites. Small alterations were observed in AT2 receptor density in the IO and in AT1 receptor density in the NTS and the RVLM. Despite the elevation in AT1 receptor density in the RVLM, the pressor response to microinjection of ANG II into the RVLM was decreased in the Agr−/− mice compared with the wild-type controls. This decrease in response to ANG II is not observed in the vasculature, where the increase in BP produced by intravenous ANG II is either not altered between strains or increased in the Agr−/− when expressed as a percent change from the decreased basal BP.

The changes in AT1 receptor density occur in two medullary regions that play a key role in central regulation of cardiovascular function. The NTS, which is the primary synapse for viscerosensory afferents, showed a small decrease in AT1 receptor density, whilst the RVLM, the site of sympathetic premotor neurons, showed a small increase in AT1 receptor density. It is not clear from the current studies whether these changes are due to the loss of AGT directly or to another factor such as the altered resting AP displayed by the Agr−/− mice. Genetically modified mice lacking AGT show decreased resting AP, abnormal kidney morphology, increased body fluid turnover, and reduced body weight (23, 28, 30, 36, 45, 46). Therefore, it is possible that any of the changes we observed between the Agr+/+ and Agr−/− mice in this study could be secondary responses to these dramatic alterations in basal physiology.

Genetic deletion of components of the RAS results in substantial decreases in AP. Using tail-cuff methods in conscious mice, researchers have reported the basal systolic BP to be −120 mmHg (Agr+/+)) vs. −98 mmHg (Agr−/−) (23) and 100 mmHg (Agr+/+)) vs. 67 mmHg (Agr−/−) (46). In this study, we used a combination of urethane and chloralose in an attempt to minimize the dampening effect of anesthesia. However, clearly this does result in further decreases in BP in these mice, with a suggestion that the effect of anesthesia is greater in RAS knockout mice (Agr+/+): systolic pressure 84 mmHg; MAP 67 mmHg vs. Agr−/−: systolic pressure 58 mmHg; MAP 39 mmHg). Interestingly these BP are very similar to those reported by Gembratt et al. (15) in anesthetized mice with all three ANG II receptors deleted [MAP 62 mmHg (wild-type) vs. 34 mmHg (all ANG II receptor knockout)]. Whilst the BP in the anesthetized Agr−/− mice in this study are relatively low, the mice maintained stable BP and blood gases throughout the experiment and appeared to be well perfused.

The expression level of the AT1 receptor is a major contributor to the biological efficacy of ANG II (12); hence, altered expression of the AT1 receptor is one potential control mechanism for physiological and pathological regulation of the cardiovascular system. Modulation of AT1 receptor expression is observed in a number of tissues, including the brain, in response to both physiological challenges and pathological
conditions (10, 43). In addition to altered gene expression (12, 13), posttranscriptional regulation of the \( \text{AT}_1 \) receptor occurs (25, 41). Whilst the factors involved in this regulation are numerous, one is homologous regulation by ANG II (25, 35). Therefore, we predicted that the expression of \( \text{AT}_1 \) receptors would be altered in \( \text{Agtr}^{-/-} \) mice. As reported previously, the \( \text{Agtr}^{-/-} \) mice from the colony used in this study have no ANG II receptors in the brain of the \( \text{Agtr}^{-/-} \) mouse. Our expression of antisense specific for AGT and decrease AGT production by glia caused a 50% reduction of hypothalamic AGT levels but no significant effect on hypothalamic \( \text{AGT} \) production by glia caused a 50% reduction of hypothalamic angiotensin receptors. Two studies have reported the presence of a non-angiotensin ligand derived from brain homogenates that can bind to angiotensin receptors (8, 37). To date, the identity of this ligand has not been reported, but it is possible that a non-angiotensin ligand could be released in the absence of AGT to act on and regulate brain angiotensin receptors. Using a different approach, Schinke et al. (40) developed a transgenic rat line with decreased levels of brain AGT. This model uses the glial fibrillary acidic protein promoter to drive expression of antisense specific for AGT and decrease AGT transcription (40). The greater than 90% reduction in brain AGT production by glia caused a 50% reduction of hypothalamic ANG II levels but no significant effect on hypothalamic ANG II concentrations (19). These rats have reduced plasma vasopressin levels, decreased basal AP, altered baroreceptor reflex regulation of HR, and partial central diabetes insipidus

![Graph](https://example.com/graph.png)

**Fig. 4.** A: histograms showing the peak change in MAP (ΔMAP) in response to intravenous bolus injections of ANG II in \( \text{Agtr}^{+/+} \) mice (n = 7) and \( \text{Agtr}^{-/-} \) mice (n = 8). Data for the change in response to different doses of ANG II, 0.1 \( \mu \)g/kg (ANG II 0.1); 0.05 \( \mu \)g/kg (ANG II 0.05); and 0.01 \( \mu \)g/kg (ANG II 0.01); are shown. B: histograms showing the peak change in MAP (ΔMAP) in response to intravenous bolus injections of phenylephrine in \( \text{Agtr}^{+/+} \) mice (n = 5) and \( \text{Agtr}^{-/-} \) mice (n = 4). Data for the change in response to different doses of phenylephrine, 4 \( \mu \)g/kg (Phe 4 \( \mu \)g/kg) and (Phe 2 \( \mu \)g/kg) are shown. Solid bars are from \( \text{Agtr}^{+/+} \) mice and open bars from \( \text{Agtr}^{-/-} \) mice. Results are presented as means ± SE.
(7, 40). Receptor binding studies, using methods similar to those described in this study, demonstrated that rats with decreased brain AGT exhibit an increase in AT_1_ receptor binding in areas inside the blood-brain barrier, and a decrease in AT_1_ receptor binding in the circumventricular organs which, being outside the blood-brain barrier, are exposed to systemic ANG II (32). Interestingly, when the drinking response to intracerebroventricular administration of ANG II was tested, the antisense rats displayed an increased sensitivity compared with controls (32). Similarly, an increased AP response to microinjection of ANG II into the RVLM was observed in the transgenic rats with a decreased brain AGT content compared with their Sprague-Dawley controls (6). These differences between the observations in the current study and those published on the antisense rat are perplexing, and a plausible explanation is not apparent. The major difference between the Agt⁻/⁻ mouse and the brain AGT antisense rat is that ANG II (32). Interestingly, when the drinking response to intracerebroventricular administration of ANG II was tested, the antisense rats displayed an increased sensitivity compared with their Sprague-Dawley controls (6). These differences between the observations in the current study and those published on the antisense rat are perplexing, and a plausible explanation is not apparent. The major difference between the Agt⁻/⁻ mouse and the brain AGT antisense rat is that ANG II is completely absent in the mouse, both systemically and centrally, while the antisense rat has a brain-selective decrease in ANG II production without reduced brain ANG II levels. Clearly, the results indicate that regulation of expression and responsiveness of the RAS in the brain has considerable complexity.

We conclude that complete absence of angiotensin peptides throughout development does not dramatically alter the overall distribution of ANG II receptor binding sites in the adult brain and causes only small changes in receptor density in restricted sites. The changes in AT_1_ receptor density occurred in medullary nuclei involved in neural regulation of autonomic activity to the cardiovascular system. Thus, it is feasible that such changes were secondary to the altered arterial pressure observed in the Agt⁻/⁻ mouse. Interestingly, despite increased AT_1_ receptor density in the RVLM, a critical sympathetic vasomotor control nucleus, the response to microinjection of ANG II was diminished, suggesting impaired coupling between the receptor and intracellular transduction pathways.

Perspectives and Significance

Altered expression of components of the RAS plays an important role in the homeostatic responses to various physiological and pathological perturbations and in regulating the activity of this system. This has been demonstrated both systemically and in the brain. In some peripheral tissues, altered levels of ANG II alter transcription and translation of the AT_1_ receptor, and this could, therefore, be an important mechanism for modulation of ANG II activity. Our results in the adult mouse indicate that this may not occur in the brain, at least following chronic and complete absence of angiotensin peptides. Rather, and in distinct contrast to our hypothesis, the absence of AGT expression appears to result in downregulation of the responsiveness of the AT_1_ receptor, indicating that presence of the ligand may be required for maintenance of coupling between the receptor and its effector pathways. Our study adds to evidence that regulation of the components of the RAS is tissue specific and points to the need for further information regarding regulation of RAS components in the brain.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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