Differential Expression of \( \text{AT}_1 \) Receptors in the Pituitary and Adrenal Gland of SHR and WKY

Olaf Jöhren, Claudia Golsch, Andreas Dendorfer, Fatimunnisa Qadri, Walter Häuser, Peter Dominiak

Abstract—The renin-angiotensin (ANG) system has been implicated in the development of hypertension in spontaneously hypertensive rats (SHR). Because SHR are more susceptible to stress than normotensive Wistar-Kyoto rats (WKY), we measured the mRNA expression of \( \text{AT}_{1A} \), \( \text{AT}_{1B} \), and \( \text{AT}_2 \) receptors in the hypothalamo-pituitary-adrenal (stress) axis of male SHR in comparison to age-matched WKY at prehypertensive (3 to 4 weeks), developing (7 to 8 weeks), and established (12 to 13 weeks) stages of hypertension. \( \text{AT}_{1A} \) receptor mRNA was mainly expressed in the hypothalamus and adrenal gland. \( \text{AT}_{1B} \) receptor mRNA was detected in the pituitary and adrenal gland. \( \text{AT}_2 \) receptor mRNA was prominent only in the adrenal gland. When compared with WKY, SHR showed increased \( \text{AT}_{1A} \) receptor mRNA levels in the pituitary gland at all ages in contrast to reduced pituitary \( \text{AT}_{1B} \) receptor mRNA levels. In the adrenal gland of SHR, \( \text{AT}_{1B} \) receptor mRNA levels were decreased at the hypertensive stages when compared with WKY. The reduced expression of adrenal \( \text{AT}_{1B} \) receptor mRNA was localized selectively in the zona glomerulosa by in situ hybridization. No differences were observed between WKY and SHR in the expression of hypothalamic ANG receptors. ANG significantly increased plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone in dexamethasone-treated SHR but not in WKY. The aldosterone response to ANG was similar in SHR and WKY. Our results suggest a differential gene expression of \( \text{AT}_{1A} \) and \( \text{AT}_{1B} \) receptors in the hypothalamo-pituitary-adrenal axis of SHR and normotensive WKY and imply the participation of \( \text{AT}_1 \) receptors in an exaggerated endocrine stress response of SHR to ANG. (Hypertension. 2003;41:984-990.)

Key Words: receptors, angiotensin \( \mathbb{R} \) rats, spontaneously hypertensive \( \mathbb{R} \) pituitary gland \( \mathbb{R} \) adrenal gland

Angiotensin II (ANG), the main active peptide of the renin-angiotensin system, acts at 2 distinct receptor subtypes, namely type 1 (\( \text{AT}_1 \)) and type 2 (\( \text{AT}_2 \)) receptors. The role of \( \text{AT}_1 \) receptors in the regulation of cardiovascular functions, fluid homeostasis, and hormone release is well established, and several \( \text{AT}_1 \) receptor antagonists are used for the treatment of hypertension.\(^1\) In rodents, 2 pharmacologically similar \( \text{AT}_1 \) receptor isoforms, \( \text{AT}_{1A} \) and \( \text{AT}_{1B} \), exist, which are encoded by 2 distinct genes and are expressed and regulated differentially.\(^2\)–\(^5\) High levels of \( \text{AT}_{1A} \) receptor mRNA are present in the rodent brain, kidney, and vasculature, whereas \( \text{AT}_{1B} \) receptor mRNA is mainly expressed in the pituitary and adrenal glands.\(^6\)–\(^10\) Although widely expressed in fetal and young rats,\(^11\) in adult rats, the expression of \( \text{AT}_2 \) receptors is low and restricted to certain organs, such as the brain and adrenal gland.\(^1\)\(^\text{\textsuperscript{12}}\) The expression of all ANG receptor subtypes in the hypothalamo-pituitary-adrenal (HPA) axis, which is activated in response to stress, as well as the regulation of ANG receptors by restraint stress\(^13\)–\(^15\) implies important roles of the ANG system in the regulation of the activity of the HPA axis. Accordingly, ANG has been shown to regulate corticotropin-releasing factor, adrenocorticotropic hormone (ACTH), and corticosterone synthesis and secretion.\(^16\)–\(^18\)

Spontaneously hypertensive rats (SHR) show an impaired response and habituation to various forms of stress such as immobilization and exposure to heat or open field, and they differ in their neuroendocrine reaction to stress from normotensive Wistar-Kyoto rats (WKY).\(^19\)–\(^23\) The ANG system has been implicated in the impaired stress response of SHR.\(^24\)–\(^27\) There are several studies showing differences in the numbers of binding sites for radiolabeled ANG between SHR and WKY in the kidney, vasculature, heart, pituitary, adrenal gland, and brain, including the hypothalamus and neuronal cultures.\(^28\)–\(^32\) Because \( \text{AT}_{1A} \) and \( \text{AT}_{1B} \) receptors are pharmacologically indistinguishable, the distinct expression of these \( \text{AT}_1 \) receptor isoforms was not addressed in these studies. Using a different approach, Iwai et al\(^33\) could not detect differences of \( \text{AT}_{1A} \) mRNA levels in the brain and adrenal gland between WKY and SHR, whereas Raizada et al\(^34\) found increased \( \text{AT}_{1A} \) and \( \text{AT}_{1B} \) receptor mRNA levels in the hypothalamus of SHR compared with WKY. Furthermore, \( \text{AT}_{1B} \) receptor mRNA appeared to be upregulated in the heart ventricle of SHR.\(^3\) Nevertheless, thus far, no information is available regarding the gene expression of \( \text{AT}_{1A} \) and \( \text{AT}_{1B} \) receptor subtypes in the pituitary and of \( \text{AT}_{1B} \) receptors in the adrenal gland of SHR in comparison to WKY. Therefore, we
analyzed the mRNA expression of AT\textsubscript{1A}, AT\textsubscript{1B}, and AT\textsubscript{2} receptor subtypes in the hypothalamus, pituitary, and adrenal gland of SHR at different developmental stages of hypertension in comparison with age-matched normotensive WKY.

**Methods**

**Animals and Treatments**

Male SHR and age-matched WKY at 3 different postnatal ages corresponding to the prehypertensive (3 to 4 weeks), developing (7 to 8 weeks), and established (12 to 13 weeks) stages of hypertension were purchased from Charles-River. Rats were kept under controlled conditions with a 12-hour/12-hour dark/light cycle and ad libitum access to standard diet and water. For RNA analysis, rats were anesthetized with pentobarbital (50 mg/kg IP) and killed by decapitation. Tissues were removed immediately, frozen in isopentane (−30°C), and stored at −80°C until use.

To measure the effect of ANG on adrenal hormones, 12-week-old male SHR and WKY were treated with a single intraperitoneal injection of dexamethasone (100 μg) to suppress ANG-induced ACTH release.\textsuperscript{35} Three hours after injection, rats were anesthetized with 100 mg/kg IP methohexitol, and the right femoral artery was catheterized. Anesthesia was continued with 5 mg IA pentobarbital; 1 or 10 μg ANG was infused over a period of 30 minutes, and 50 μL blood was withdrawn for hormone assays. All animal protocols complied with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Ministerium für Umwelt, Natur und Forsten of Schleswig-Holstein, Germany (animal protocol 9/A37/01).

**RNA Isolation and cDNA Synthesis**

Hypothalami were dissected according to Palkovits and Brownstein.\textsuperscript{36} The brains were adapted to −10°C, and coronal cuts were made at 900 μm (at the optic chiasm) and 4800 μm (just posterior of the mammillary nucleus) posterior of the bregma. The hypothalamic slice was turned on its posterior surface and cut laterally directly before the amygdala and dorsally just underneath the anterior commissure.

Hypothalami and pituitary and adrenal glands were homogenized in the presence of guanidinium isothiocyanate. Total RNA was isolated with the use of silica gel–based spin columns and treated with DNase I (RNeasy Kit, Qiagen GmbH). By performing PCR in control samples in which the reverse transcriptase was omitted, possible contamination with genomic DNA was monitored. As a control for RNA integrity and successful cDNA synthesis, the β-actin mRNA expression of each sample was analyzed by RT-PCR.

**Semiquantitative PCR**

AT\textsubscript{1} receptor primers spanning identical regions of the AT\textsubscript{1A} and AT\textsubscript{1B} receptor cDNAs were used.\textsuperscript{3,37} According to Ruan et al.,\textsuperscript{38} the amplified AT\textsubscript{1A} and AT\textsubscript{1B} receptor cDNA was identified by restriction with EcoRI (Figure 1A). AT\textsubscript{2} receptor and β-actin specific sense and antisense primers were designed on the basis of the published cDNA sequences.\textsuperscript{3,37} All primers (Table) were constructed with the use of Primer3 software by S. Rozen and H.J. Skaltsky (http://www-genome.wi.mit.edu/genome_software/other/primer3.html) and were obtained from Life Technologies GmbH (Karlsruhe, Germany).

PCR was performed as described previously.\textsuperscript{41} Five microliters of first-strand cDNA was incubated in the presence of 10 mmol/L Tris-HCl, pH 9.0, 50 mmol/L KCl, 0.1% Triton X-100, 1.5 mmol/L MgCl\textsubscript{2}, 0.2 mmol/L dNTPs, 0.5 mmol/L sense and antisense primers, and 1.2 U DNA Polymerase (DyNAzyme II, Biometra GmbH) in a final volume of 50 μL. The PCR conditions were 28 to 32 cycles of denaturation for 1 minute at 95°C, annealing for 30 seconds at 56°C, and extension for 60 seconds at 72°C. The specificity of the amplified AT\textsubscript{1A} and AT\textsubscript{1B} receptor cDNA was confirmed by restriction with Alu I, which cuts the AT\textsubscript{1A} receptor cDNA to 20-, 54-, and 154-bp fragments and the AT\textsubscript{1B} receptor cDNA to 103-bp and 208-bp fragments (Figure 1A). EcoRI I selectively cuts the AT\textsubscript{1A} receptor cDNA to 128- and 183-bp fragments and leaves the AT\textsubscript{1B} receptor cDNA intact (Figure 1A). AT\textsubscript{2} receptor cDNA was restricted with Ssp I to 26-, 92-, and 131-bp fragments.

For semiquantitative analysis of AT\textsubscript{1A} and AT\textsubscript{2} receptor mRNA levels, agarose gels were digitized and analyzed densitometrically with Scion Image for Windows (Scion Co), which is based on the public domain NIH Image program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image). To ensure amplification within the exponential phase of the PCR, the cycle numbers used for AT\textsubscript{1A} and AT\textsubscript{2} receptor and β-actin cDNA amplification were optimized for each primer pair and tissue by kinetic analysis (22 to 40 cycles). Depending on the primers and tissues, cycle numbers

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**Table:** Nucleotide Sequences of PCR Primer

<table>
<thead>
<tr>
<th>Name</th>
<th>Orientation</th>
<th>Sequence</th>
<th>Product Size</th>
<th>GeneBank Accession No.</th>
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<tbody>
<tr>
<td>AT\textsubscript{1}</td>
<td>Sense</td>
<td>5′-CCAAAGTCACCTGCATCATC-3′</td>
<td>305</td>
<td>RRR1AIIR and RATAGTIIR*</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5′-CAAGATCGGACCAATTTACTCTC-3′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT\textsubscript{2}</td>
<td>Sense</td>
<td>5′-AGGTGTTAGGGTCAGAGCG-3′</td>
<td>380</td>
<td>RACCCYB‡</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5′-GCTGCGTAAACCCCTGAT-3′</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The sequence of primers is identical for the AT\textsubscript{1A} and AT\textsubscript{1B} receptors.\textsuperscript{3,37} †Kambayashi et al, 1993.\textsuperscript{39} ‡Nudel et al, 1983.\textsuperscript{40}
between 28 and 32 were found to produce exponential amplification of PCR products. The mRNA levels of β-actin were used as internal standard to normalize AT1 and AT2 receptor mRNA levels. 41

cDNA Cloning and In Vitro Transcription of Riboprobes

Adrenal cDNA was amplified by PCR with the following AT1B receptor specific primers, which amplify a part of the 3’-noncoding region without significant homology to the AT1A receptor cDNA: sense primer 5'-GTGAGTGAGGCTTTGCAAA-3’ and antisense primer 5'-GACATTATTCAGGCAAGCTG-3’, spanning nucleotides 1208 to 1896 of the published AT1B receptor cDNA. 3 The amplified AT1A receptor cDNA was subcloned into the PCR-II Vector with the use of a TA-cloning kit (Invitrogen), and its identity was confirmed by nucleotide sequencing (MWG Biotech).

AT1B receptor-specific antisense and sense (control) RNA probes were labeled by in vitro transcription in the presence of [35 S]UTP (800 Ci/mmol, Amersham-Pharmacia), 0.5 μg of the linearized plasmid, and T3 or T7 RNA polymerase. Labeled RNA-probes were separated from unincorporated [35 S]UTP by centrifugation through Sephadex G-50 microcolumns (Amersham-Pharmacia).

In Situ Hybridization Histochemistry

In situ hybridization was performed as described earlier. 42 Adrenal glands from SHR and WKY were sectioned at −20°C in a cryostat, and 20-μm sections were thaw-mounted alongside on aminoaalkylsilane-coated glass slides (Sigma). After fixation in 4% paraformaldehyde for 10 minutes, sections were dehydrated in ethanol and air-dried. Hybridization was performed with 1 to 2 pmol/mL labeled RNA-probes in buffer containing 50% formamide, 10% dextran sulfate, 1× Denhardt’s solution, 20 mmol/L Tris (pH 7.5), and 250 μg/mL yeast tRNA for 18 hours at 54°C. Subsequently, sections were treated with RNase A, washed in NaCl-Na citrate (SSC) buffer to a final stringency of 0.1× SSC at 65°C, dehydrated in ethanol, and exposed to Hyperfilm (Amersham-Pharmacia) along with C-14 markers DNA size marker. Film were analyzed densitometrically with Scion Image. 43

Radioimmunomasys

Plasma concentrations of ACTH, corticosterone, and aldosterone were measured with the use of 125I radioimmunomassay kits (ICN Biomedicals). For aldosterone detection, 100 μL serum was extracted twice with 2 mL ethyl acetate/hexane (3:2), evaporated, and reconstituted in 1 mL RIA buffer; 250 μL was used in the assay. For corticosterone detection, 100 μL serum was diluted 1:200 with RIA buffer and 100 μL thereof was used in the assay. For ACTH detection, 100 μL serum was used without dilution. All samples were assayed in duplicate according to the manufacturers instructions. Intra-assay and interassay variations were <6% and 10%, respectively.

Statistics

Data are presented as mean±SEM. Effects of age and genetic groups were estimated by 2-way ANOVA, with the use of GraphPad Prism Software (GraphPad Software Inc). Effects of ANG on plasma hormones were analyzed by 1-way ANOVA. For subsequent group comparisons the Bonferroni posttest was used. Probability values <0.05 were considered statistically significant.

Results

Specific Amplification of ANG Receptor Subtypes

RT-PCR with AT1 receptor–specific and AT2 receptor–specific sense and antisense primers resulted in the amplification of distinct cDNA fragments of the expected size of 311 bp and 249 bp, respectively (Figure 1). Restriction analysis of the amplified AT1 and AT2 receptor cDNA confirmed the specificity of our PCR showing the restriction fragments as predicted according to the published cDNA sequences (Figure 1). Restriction with EcoRI, which selectively cuts AT1A but not AT1B receptor cDNA, allowed the differentiation between these 2 AT1 receptor isoforms (Figure 2). In samples in which the RT was omitted and in water control reactions, no amplification products were observed after PCR. The integrity of the cDNA was confirmed by analysis of β-actin mRNA.

Distribution of ANG Receptor Subtypes in the HPA Axis

In normotensive WKY, AT1A receptor mRNA was mainly expressed in the hypothalamus and adrenal glands and was very low in the pituitary gland, whereas AT1B receptor mRNA was primarily found in the pituitary and adrenal glands (Figure 2). AT2 receptor mRNA was highly expressed in the adrenal glands and at lower levels in the hypothalamus (Figure 3). No AT2 receptor mRNA was detected in the pituitary gland.

Expression of ANG Receptor Subtypes in WKY and SHR

In the hypothalamus, no differences were detected in AT1A-, AT1B, or AT2 receptor mRNA levels between SHR and WKY at different postnatal ages corresponding to the prehyperten-
sive (3 to 4 weeks old), developing (7 to 8 weeks old), and established (12 to 13 weeks old) stages of hypertension, although there was a tendency of lower AT1A and AT1B receptor mRNA in prehypertensive SHR (Figure 4). In the pituitary gland, we detected significantly higher levels of AT1A receptor mRNA and significantly lower levels of AT1B receptor mRNA at all postnatal ages (Figure 5). In the adrenal gland, AT1a receptor mRNA levels were significantly reduced in 7- to 8- and 12- to 13-week-old SHR when compared with WKY (Figure 6). Adrenal AT2 receptor mRNA levels were high in 3- to 4-week-old rats and declined to some extent with age (Figure 6). No significant differences were found in adrenal AT1a or AT2 receptor mRNA levels between SHR and WKY at the different ages (Figure 6). On the whole, there was a tendency of reduced AT2 receptor mRNA levels in the adrenal of SHR.

Localization of AT1B Receptor mRNA in the Adrenal Gland of WKY and SHR

The AT1B receptor mRNA expression in the adrenal gland of WKY and SHR was localized exclusively in the zona glomerulosa by in situ hybridization with specific AT1B receptor antisense RNA probes (Figure 7). Hybridization with control sense RNA probes resulted in a very low background signal (not shown). Semiquantitative analysis demonstrated reduced AT1B receptor mRNA levels in the zona glomerulosa of 12- to 13-week-old but not in 7- to 8-week-old SHR when compared with WKY (Figure 7).

Effect of ANG on Plasma Hormones in Dexamethasone-Treated WKY and SHR

Infusion of a low dose of ANG (1 μg) over a period of 30 minutes had no effect on plasma levels of ACTH, corticosterone, and aldosterone in dexamethasone-treated WKY or SHR (Figure 8). However, a high dose of ANG (10 μg) significantly increased plasma levels of ACTH and corticosterone in dexamethasone-treated SHR but not in dexamethasone-treated WKY (Figures 8A and 8B). Plasma levels of aldosterone were equally increased in both WKY and SHR after infusion of 10 μg ANG (Figure 8C).

Discussion

The current results demonstrate for the first time the differential mRNA expression of AT1a and AT1b receptors in the pituitary and adrenal glands of SHR compared with WKY. The changes in pituitary and adrenal mRNA levels of AT1a and AT1b receptors in SHR are accompanied by an increased responsiveness of the HPA axis to ANG in dexamethasone-suppressed animals.

ANG regulates blood pressure by regulating the vascular tone and fluid homeostasis, which are controlled by the stimulation of aldosterone, norepinephrine, and vasopressin release, effects mediated by AT1 receptors. Little is known about the relative physiological roles of the 2 AT1 receptor isoforms that are found in rodents. Recent results in AT1a and AT1b receptor-deficient mice have shown the involvement of central AT1a receptors in the regulation of blood pressure and fluid homeostasis and that of central AT1b receptors in drinking behavior. Because AT1a and AT1b receptors cannot be distinguished pharmacologically or by immunohistochemistry, we assessed the mRNA expression of these AT1 receptor isoforms by semiquantitative RT-PCR combined with receptor isoform-specific restriction of the PCR products. Using this method, we confirmed the tissue-specific distribution of AT1a, AT1b, and AT2 receptor mRNA and their high expression in the HPA axis.

In the pituitary gland of SHR, we found AT1a receptor mRNA levels notably increased compared with WKY, whereas AT1b receptor mRNA levels were decreased, and these differences were evident at a young age, before the onset of hypertension. Reduced ANG binding sites in the anterior pituitary of SHR as described elsewhere may reflect this decrease in AT1b receptor expression. However, since in normotensive adult rats pituitary AT1a receptor mRNA levels are usually very low or undetectable, the significant
mRNA expression of pituitary AT$_{1A}$ receptors in adult SHR in the current study also suggests specific ANG-related functional changes of the pituitary gland in SHR. Although AT$_{1B}$ receptors appear to be mainly expressed in prolactin-producing pituitary cells and to a lower degree in ACTH-producing cells, the pituitary cells expressing AT$_{1A}$ receptors were not identified thus far, and considerations about possible functions of pituitary AT$_{1A}$ receptors in SHR are only speculative at this point. ANG was shown to directly affect pituitary prolactin, ACTH, and growth hormone release, and these effects appear to be mediated differentially by AT$_{1A}$ and AT$_{1B}$ receptors. For example, ANG may directly stimulate prolactin release from the pituitary gland by acting on AT$_{1B}$ receptors and inhibit pituitary prolactin indirectly by stimulating dopamine release through central AT$_{1A}$ receptors. Earlier studies on the pituitary hormonal system revealed higher basal prolactin in SHR and enhanced growth hormone but not prolactin secretion from pituitary cells after ANG in SHR. Thus, reduced pituitary mRNA levels of AT$_{1B}$ receptors in SHR may reflect adaptive changes to high prolactin levels caused by other secretagogues. We found elevated plasma levels of ACTH and corticosterone in response to ANG in dexamethasone-treated SHR but not in dexamethasone-treated WKY. Since this increased responsiveness to ANG is associated with an increased mRNA expression of AT$_{1A}$ receptors and a decreased mRNA expression of AT$_{1B}$ receptors in the pituitary, the pituitary AT$_{1A}$ receptor may account for the ANG-induced elevation of plasma ACTH and a consequent increase of plasma corticosterone in SHR. Interestingly, restraint stress results in a similar shift of pituitary AT$_{1A}$ and AT$_{1B}$ receptor expression. Thus, the increased pituitary AT$_{1A}$ receptors may make SHR more susceptible to stress. However, the detailed physiological role of the pituitary AT$_{1A}$ receptors in SHR remains unclear and awaits further clarification.

One of the main AT$_{1}$ receptor–mediated effects of ANG is the stimulation of aldosterone release from the adrenal gland. The finding that the aldosterone response to sodium depletion is not influenced in AT$_{1A}$ receptor–deficient mice suggests a major role of the AT$_{1B}$ receptor. Indeed, although AT$_{1A}$ receptors are present in all cortical layers and the medulla of the adrenal gland, we found AT$_{1B}$ receptor mRNA highly and specifically expressed in the zona glomerulosa, in accordance with earlier studies. Furthermore, we show that the reduced adrenal AT$_{1B}$ receptor mRNA levels in SHR are localized exclusively in the aldosterone-producing zona glomerulosa. Thus, our data suggested an impaired aldosterone response to ANG in SHR. However, in dexamethasone-treated WKY and SHR, we found a similar increase of plasma aldosterone after ANG treatment. Since plasma aldosterone levels appear to be increased in SHR, reduced AT$_{1B}$ receptor mRNA levels may reflect adaptive changes to reduce aldosterone release and to protect from excess aldosterone. On the other hand, changes in AT$_{1B}$ receptor mRNA levels may not translate to changes in protein levels. The dose of dexamethasone we used was shown to efficiently suppress ANG-dependent ACTH release in normotensive Sprague-Dawley rats. In contrast to dexamethasone-treated WKY, ANG still increased ACTH in dexamethasone-treated SHR. Thus in SHR, ANG-induced ACTH release may also contribute to the aldosterone release. We found no differences in the mRNA levels of adrenal AT$_{2}$ receptors between WKY and SHR. The decrease of adrenal AT$_{2}$ receptor mRNA levels with increasing age is in line with earlier observations in adrenals and many other tissues.
In the hypothalamus, we did not find differences in AT₁ receptor mRNA levels between SHR and WKY despite previous findings of an increase of AT₁ receptor mRNA in the hypothalamus and brain stem of SHR compared with WKY.34 However, our findings correspond to previous results showing that ANG binding sites were unaffected in various hypothalamic and brain stem nuclei of SHR, except for the spinal trigeminal nucleus, when compared with WKY.30 Hence, differences in AT₁ receptor mRNA might be restricted to distinct brain nuclei and therefore not accessible by our method. Nevertheless, we found significant differences in the mRNA expression of AT₁A and AT₁B receptors in the pituitary and adrenal gland of SHR compared with WKY. These differences occurred before the onset of hypertension and in the phase of developing high blood pressure. Thus, a causal role between an exaggerated stress response and an altered expression of pituitary and adrenal AT₁ receptors may exist. This correlation is further supported by our findings in SHR of an enhanced activity of the HPA axis in response to ANG. Interestingly, the regulation of pituitary and adrenal AT₁A and AT₁B receptor mRNA in SHR, as described in our current study, shows similarities with the increased pituitary AT₁A receptor and decreased pituitary and adrenal AT₁B receptor expression found in rats after immobilization stress.15 Our data suggest that the known susceptibility of SHR to stress is linked to the differential expression of pituitary and adrenal AT₁A and AT₁B receptors and support an important role of the ANG system in the regulation of the stress response in SHR.

Perspectives
In humans, enhanced stress reactivity may be involved in combination with other environmental and genetic factors in the development of hypertension.54 In contrast to rodents, a single gene encodes the human AT₁ receptor.55 However, several splicing variants of the human AT₁ receptor exist that are not only expressed differentially but are functionally different.56,57 Since the physiological response of AT₁ receptors to ANG is very similar in humans and rodents, a better understanding of divergent regulations and functions of AT₁ receptor isoforms in rodents may help to elucidate the involvement of AT₁ receptor isoforms in the development of stress-related disorders in humans, including hypertension.

Acknowledgments
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