Tissue-Specific Expression of Type 1 Angiotensin II Receptor Subtypes
An In Situ Hybridization Study

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Abstract The angiotensin II type 1 (AT\textsubscript{1}) receptor in murine species exists as two isoforms (AT\textsubscript{1A} and AT\textsubscript{1B}) encoded by two different genes. Both subtypes have a 9/10 homology in the coding sequence of their mRNA. We examined organs of adult rats (liver, pituitary gland, adrenal gland, kidney, heart, and lung) to study the differential expression of these two genes in target tissues for angiotensin II. AT\textsubscript{1A} and AT\textsubscript{1B} mRNAs were detected by in situ hybridization using specific riboprobes for the 3' noncoding region of the mRNAs that have the lowest homology (approximately 6/10). Only AT\textsubscript{1A} was expressed in the liver, heart, and lung, and only AT\textsubscript{1B} was expressed in the anterior pituitary, where most cells were positive. In the adrenal gland, AT\textsubscript{1A} mRNA was detected in the zona glomerulosa and medulla and AT\textsubscript{1B} in the glomerulosa. In the kidney, AT\textsubscript{1A} mRNA was the predominant isoform (mesangial and juxtaglomerular cells, proximal tubules, vasa recta, and interstitial cells), but AT\textsubscript{1B} was also detected in mesangial and juxtaglomerular cells and in the renal pelvis. The results of this in situ detection suggest a tissue-selective regulation of AT\textsubscript{1A} and AT\textsubscript{1B} mRNAs. This tissue-specific regulation may constitute a prerequisite condition if the two angiotensin II receptor subtypes, which are pharmacologically similar, are to selectively modulate the various effects of angiotensin II in the different target tissues. (Hypertension. 1994;24:531-537.)

Key Words • angiotensin II • rat • liver • in situ hybridization • kidney • adrenal gland • pituitary gland • heart

Angiotensin II (Ang II) receptors establish the connection between the extracellular hormonal message and the cascade of intracellular events triggered by the peptidic hormone corresponding to various effects in different target cells. Among other effects, Ang II induces aldosterone secretion in adrenal glomerulosa cells, vasoconstriction in vascular smooth muscle cells, and salt reabsorption in proximal tubule cells of the kidney as well as upregulates angiotensinogen secretion in hepatocytes. These effects, which all contribute at various levels to the regulation of blood pressure and hydromineral homeostasis, underscore the broad range of Ang II functions.

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Two Ang II receptor types can be distinguished pharmacologically: type 1 (AT\textsubscript{1}), which has a high affinity for the nonpeptidic antagonist losartan (DuP 753), and type 2 (AT\textsubscript{2}), which has a high affinity for the blockers PD 123177 and CGP 42112. The signaling and functions of AT\textsubscript{2} are still debated, contrary to AT\textsubscript{1}, which is coupled by G proteins to phosphatidylinositol-phosphate metabolism. AT\textsubscript{1} mediates all the known effects of Ang II in target organs (see reviews on AT\textsubscript{1} in References 1 and 2). Based on the pharmacological differences of these two receptor types, several studies by in situ binding on tissue sections have established the tissue distribution of the types in the kidney,\textsuperscript{3-6} adrenal gland,\textsuperscript{7,8} heart,\textsuperscript{9,10} brain,\textsuperscript{8,21} and rat fetus.\textsuperscript{12,13}

In the rat, two cDNAs coding for two AT\textsubscript{1} receptor subtypes have been cloned and sequenced.\textsuperscript{14-18} AT\textsubscript{1A} and AT\textsubscript{1B} (also called AT\textsubscript{3} in Reference 15) cDNAs share a 9/10 sequence identity in their coding sequence, which is reduced to 6/10 in their 5' and 3' untranslated regions.\textsuperscript{15} There are no known pharmacological or functional differences between the two receptor subtypes, but preliminary results indicate that they are expressed in different tissues and cells where they can play a specific role for Ang II action.

To provide a morphological basis to putative distinct cellular functions for each of the two receptor subtypes, we undertook the present in situ hybridization study to detect AT\textsubscript{1A} and AT\textsubscript{1B} mRNAs. We anticipated that the identification of the cells expressing AT\textsubscript{1A} and/or AT\textsubscript{1B} in known target organs for Ang II could help to correlate one receptor subtype with the functional responses of the target cells in which it is expressed.

In a previous study, we described the distribution of the AT\textsubscript{1} receptor in the rat kidney, identified with the use of an AT\textsubscript{1A} riboprobe.\textsuperscript{19} Other in situ hybridization studies have also detected AT\textsubscript{1} receptor mRNA in adult kidney\textsuperscript{20} and brain,\textsuperscript{21} in fetal liver and kidney,\textsuperscript{22} and in various organs of the rat fetus.\textsuperscript{23} We now extend our previous observations to a systematic in situ hybridization comparison between AT\textsubscript{1A} and AT\textsubscript{1B} in organs involved in the regulation of blood pressure: liver, pituitary gland, adrenal gland, kidney, heart, and lung.

Methods

Tissues
Female and male rats (Sprague-Dawley, Iffa Credo) under chloroform anesthesia were perfused with 4% paraformalde-
hyde in phosphate-buffered saline. The organs were dissected out and postfixed in the same fixative solution for 6 to 24 hours according to their size. In some cases, the organs were freshly dissected without prior perfusion and fixed by immersion in the paraformaldehyde solution. In the present study, the kidney and adrenal gland from nine animals, heart and pituitary gland from seven animals, liver from five animals, and lungs from four animals were fixed, processed, and examined. At the end of the fixation process, the organs were washed in 70% ethanol (>20 hours), dehydrated, and embedded in paraffin following standard techniques. Sections (5 µm thick) were mounted on silanated histological slides. The slides were stored at room temperature without special precaution for up to 1 year before being used for in situ hybridization. All animal procedures were performed in accordance with administrative guidelines.

Probes
The preparation of radiolabeled riboprobes has been described in detail.23,24 The cDNA templates for AT1A (derived from clone pCa18b, a gift from Dr K. Bernstein) and for AT1B (derived from clone RAG6D4.60, a gift from Dr K. Sandberg) include the noncoding and coding sequences that are 6/10 and 9/10 homologous between the two subtypes, respectively.15 Depending on the enzyme used for linearization of the plasmid before transcription, either a long probe including both the coding and noncoding sequences or a short probe including only the 3' noncoding sequences was synthesized. The AT1A probes were obtained by in vitro transcription of clone pCa18b after subcloning in a Bluescript plasmid (Stratagene). With the use of the T3 promoter after linearization by Hpa I or Ase I, a long (2.3-kb) or short (0.7-kb) antisense probe was synthesized, respectively. A long sense probe was synthesized for negative control using the T7 promoter after linearization by BamHI. The AT1B probes were obtained by transcription of clone RAG6D4.60 after subcloning and linearization by Xba I or HincII to synthesize a long (2.2-kb) or short (0.6-kb) antisense probe, respectively, using the SP6 promoter. Approximately 1 µg of each cDNA template was used for transcription, which was carried out with 80 to 120 µCi of [35S]UTP (Amersham). After purification of the probes by phenol-chloroform extraction and ethanol precipitation, the transcription products were dissolved in 100 µL TE (10 mmol/L Tris-[hydroxymethyl]aminomethane and 1 mmol/L ethylenedinitrilotetraacetic acid, pH 7.8) with 20 mmol/L dithiothreitol. The specific radioactivity of the probes was approximately 2X10^6 cpm/µL, thus showing that nearly all 35S-UTP molecules had been incorporated in the probes. The size of the probes was controlled by agarose gel electrophoresis. It has been shown previously that long probes also contain a high proportion of short incomplete transcripts corresponding to the 3' noncoding sequences.23 The relative excess of short transcripts in these long probes may explain the very limited cross-hybridization of each of these probes to the other subtype mRNA, since the 3' end of the cDNA has the lowest degree of homology. Short probes display almost only the band at 0.6 to 0.7 kb.24

Hybridization
The technique of in situ hybridization used has been described.25 Briefly, the deparaffinized sections were postfixed in paraformaldehyde, digested by proteinase K to facilitate access of the probes to the mRNAs, acetylated to reduce the background, and finally dehydrated and fixed by immersion in the mounting on silanated histological slides. The sections were stored at room temperature without special precaution for up to 1 year before being used for in situ hybridization. All animal procedures were performed in accordance with administrative guidelines.

Results
The AT1A and AT1B probes revealed a distribution of mRNAs characteristic for each of the two subtypes. Only organs known as targets for Ang II were examined. The brain was not studied.

Liver
The AT1A probe produced a uniform labeling in all or most hepatocytes (Fig 1A). There was no apparent regionalization of the signal between different parts of the liver. With the AT1B probe (Fig 1B), the signal was minimal and its intensity comparable to the sense probe (not shown). The capsule of the liver was weakly labeled with the AT1A probe. There was no indication of labeling in cell types other than hepatocytes.

Pituitary
With the AT1A probe, the signal was minimal in the three lobes of the pituitary: anterior (Fig 1C), intermediate (Fig 1E), and posterior (not shown). With the AT1B probe, a strong signal was detected in a numerically important subpopulation of cells of the anterior lobe (Fig 1D), whereas the intermediate (Fig 1F) and posterior (not shown) lobes displayed the same intensity of labeling as the control sense probe.

Adrenal Glands
AT1A mRNA was detected with the highest intensity in the zona glomerulosa of the adrenal cortex (Fig 2A), but a weaker signal was also visible in two other cortical zones: zona fasciculata and zona reticularis. In addition, a few interstitial cells were strongly labeled in the zona reticularis. The chromaffin cells of the adrenal medulla were labeled with AT1A, with some clusters of cells more intensely labeled than others. Whenever observed in a section among chromaffin cells, very large, ganglion-type cells showed an intense AT1A signal (Fig 2D). The capsule of the adrenal gland was weakly labeled.

AT1B mRNA in the adrenal cortex was detected in the zona glomerulosa (Fig 2B), with a signal intensity higher than that of AT1A. No other cell type in the cortex or medulla (Fig 2E) was positive with AT1B. No labeling was observed in the capsule. The sense probe produced only a very weak background signal in all adrenal layers (Fig 2C).
Kidney

With the AT\textsubscript{1A} probe, a hybridization signal was observed in mesangial cells within glomeruli, in juxtaglomerular cells (Fig 3A), in epithelial cells of the proximal tubule, in the vasa recta (Fig 3C), and occasionally in a few interstitial cells of the kidney cortex. The same distribution has been described in a previous study using the only AT\textsubscript{1A} probe available at that time, ie, the long AT\textsubscript{1A} probe.\textsuperscript{19} A clearly different localization of the hybridization signal was obtained with the AT\textsubscript{1B} probe. Only mesangial cells and, to a lower level, juxtaglomerular cells were positive (Fig 3B). Under the same technical conditions (two sections placed on the same slide and receiving the same amount of radiolabeled probe), mesangial cells were more intensely labeled with AT\textsubscript{1B} than AT\textsubscript{1A} probe; the converse was true for juxtaglomerular cells. Vasa recta (Fig 3D), epithelial cells of the proximal tubule, and interstitial cells were negative with the AT\textsubscript{1B} probe.

The smooth muscle cells of the renal pelvis showed a weak signal with the AT\textsubscript{1B} probe only, a noteworthy observation because in vascular smooth muscles of the kidney arteries, large or small, no labeling was detected with either of the antisense probes (not shown).

Heart

Cardiomyocytes were labeled with the AT\textsubscript{1A} probe (Fig 3E). Although limited differences may exist between the different parts of the heart muscle, the labeling appeared evenly distributed throughout all parts of the heart, atria, and ventricles. A quantitative study will be necessary to establish possible differences in signal intensities. No hybridization signal distinctively assignable to connective tissue cells between myocytes was observed. Cardiac valves were not labeled, whereas the outermost layers of cells around the aorta (adventitia and probably part of the media) displayed a moderate signal. No signal was observed with the AT\textsubscript{1B} probe in the heart (Fig 3F).

Lung

Because a previous study had shown the presence of AT\textsubscript{1A} mRNA in mesenchymal cells of the lung in the rat...
FIG 2. Photomicrographs show angiotensin II receptor mRNA subtypes in adrenal cortex (A, B, and C) and medulla (D and E) revealed by in situ hybridization with $^{35}$S-labeled AT$_{1A}$ (A and D) or AT$_{1B}$ (B and E) riboprobes. In the zona glomerulosa, AT$_{1A}$ (A) and AT$_{1B}$ (B) are detected; the zona fasciculata displays only a weak labeling with AT$_{1A}$ and not with AT$_{1B}$. The sense AT$_{1A}$ probe is used as negative control (C). A, B, and C are adjacent sections. In the adrenal medulla, only AT$_{1A}$ mRNA is detected at a particularly high level in ganglial cells (D). In an adjacent section, a very low AT$_{1B}$ mRNA level is detected (E). Bar=25 μm.

fetus,$^{23}$ we examined the adult lung. In the lung parenchyma, a weak labeling was observed with the AT$_{1A}$ probe. The signal was so low and the cell cytoplasm so thin that it was not possible to accurately identify the positive cells. Neither arteries nor airways were labeled. The AT$_{1B}$ probe did not label any tissue or cell in the lung.

Discussion

Binding of radiolabeled ligands to receptors detected by autoradiography in tissue sections had been previously used to localize target tissues for Ang II. However, this technique, almost limited to the macroscopic level, provided little information on the exact nature of cells involved. The subtype specificity of the receptor is another limitation of this technique. With the use of Ang II in situ binding and autoradiography, it is possible to distinguish between AT$_{1A}$ and AT$_{1B}$ receptors, but the distinction between the AT$_{1A}$ and AT$_{1B}$ subtypes is impossible because no selective pharmacological properties have yet been described. Another technique, the polymerase chain reaction after reverse transcription (RT-PCR), can discriminate between the expression of two closely related genes with a high degree of identity. Although RT-PCR is more sensitive than in situ hybridization and selective enough to characterize the two receptor subtypes, it requires the extraction of mRNAs from organs and therefore cannot easily determine the type of cells involved in expressing a definite receptor subtype.

The aim of the present study was to identify the cells that express AT$_{1}$ receptors in known target organs for Ang II. The results confirm that the sensitivity, specificity, and resolution of in situ hybridization are appropriate for demonstrating the cellular distribution of the two mRNA subtypes of AT$_{1}$ receptors. The subtype specificity of the probes was critical for distinguishing the distribution and regulation of AT$_{1A}$ from that of AT$_{1B}$ receptors. Two arguments prove this specificity. The short probes, which have a degree of identity of less than 6/10, did not cross-hybridize with the heterologous mRNA, as is evident in tissues that express exclusively or predominantly one mRNA subtype (AT$_{1A}$ in liver or AT$_{1B}$ in pituitary gland). Moreover, we have demonstrated elsewhere that there was no cross-hybridization of the AT$_{1B}$ and AT$_{1A}$ probes in established CHO cells transfected with the AT$_{1A}$ and AT$_{1B}$ cDNA, respectively.$^{24}$

In a recent study, we described the presence of an AT$_{1}$ mRNA in the rat kidney.$^{19}$ It is established in the present study and in Reference 23 that the probe used was AT$_{1A}$ subtype specific and could not detect AT$_{1B}$ under our hybridization conditions. This result is confirmed here by the differential distribution of the two mRNA subtypes in the kidney: AT$_{1A}$ is expressed in all target tissues for Ang II (glomeruli, proximal tubules, vasa recta, and interstitial cells), and AT$_{1B}$ is restricted to glomeruli (mesangial and juxtaglomerular cells). One of the renal effects of Ang II is vasoconstriction, and the absence of a hybridization signal in interlobular and preglomerular...
arteries is surprising. In our previous study with the AT1A probe, we had speculated that another type or subtype of Ang II receptor could be expressed in these arteries. The present study shows this is not AT1B. The only blood vessels expressing AT1 mRNA in the kidney are the afferent arterioles and vasa recta. Also, some interstitial cells seen near or along the capillaries and small arteries are positive for AT1A mRNA, but they are clearly not vascular smooth muscle cells. The discrepancy between these observations and the immunostaining of blood vessels, particularly in the kidney, obtained with an antibody to a peptidic sequence of the rat AT1 receptor can be explained if in vascular smooth muscle cells few copies of AT1 mRNA are sufficient to translate significant levels of the protein. There is, however, no clear explanation for such a lower level of mRNA compared with other neighboring cell types that show similar (or even lower) levels of the immunostained protein. This failure to localize AT1 receptor mRNA in arteries contrasts with the detection of AT1B mRNA in smooth muscles of the renal pelvis next to the renal artery, an observation that makes improbable a negative artifact specific to renal vascular smooth muscle cells.

The distribution of AT1 mRNAs we show here in blood vessels of the kidney is in good agreement with that of the ligand binding detected by autoradiography on tissue sections: a high level of binding to smooth muscles of the renal pelvis has been reported, compared with only weak or no AT1-type binding sites in renal arteries. Moreover, no hybridization signal for AT1 has been reported in arterioles of the rat kidney. One possible explanation is that the level of AT1 mRNA expression is very low in these vascular smooth muscle cells and could be detected only by RT-PCR. Finally, it is interesting to note that we could not show a significant hybridization signal in the aorta and mesenteric arteries. This was in part due to a background with the sense probe higher than in other tissues. However, if there was a specific labeling in the media and adventitia of these arteries, it was low and masked by the background, and by analogy with small arteries in various organs, including the kidney, it is probable that it remained below the threshold of detection.

The expression of AT1A mRNA in the kidney correlates well with the known functions of Ang II in that organ at various levels: regulation of glomerular filtration by mesangial cells, control of renin secretion by juxtaglomerular cells, Na+ reabsorption and HCO3- transport in proximal convoluted tubules, vasoconstriction of vasa recta, and prostaglandin secretion by inter-
Ang II exerts its roles in cardiac hypertrophy and growth modulation remain to be investigated, as do the other factors involved in the process. Although AT₂ receptors are also present in the adrenal medulla, this receptor type does not seem to take part in the regulation of catecholamine secretion (see review in Reference 2). Concerning the ganglial cells within the adrenal medulla that express both mRNA subtypes, it may not be accidental that the glomerulosa, which expresses both mRNA subtypes, is also the proliferative zone of the adrenal cortex, in addition to its secretory functions.

The presence of AT₁A mRNA in chromaffin cells of the adrenal medulla is to be paralleled with the increase in catecholamine secretion mediated by AT₁ receptors. Although AT₂ receptors are also present in the adrenal medulla, this receptor type does not seem to take part in the regulation of catecholamine secretion (see review in Reference 2). Concerning the ganglial cells within the adrenal medulla that express both mRNA subtypes, it is interesting to note that functional Ang II receptors have been demonstrated in sympathetic ganglia, and some of these receptors are of the AT₁ type. Moreover, as the sympathetic nervous system controls the secretion of epinephrine and norepinephrine by the adrenal medulla, the expression of AT₁A in these intramedullary ganglial cells may be of some physiological importance that remains to be elucidated.

In hepatocytes, only AT₁A mRNA was detected by in situ hybridization, as already reported by Northern blot and RT-PCR. Therefore, this receptor subtype, among other effects, mediates the positive feedback control of Ang II on angiotensinogen secretion. As the circulating concentration of angiotensinogen may be a rate-limiting factor for Ang II production in the blood, this level of control is a critical step in the regulation of the renin-angiotensin system.

Previous studies have shown that Ang II binds to receptor sites in the cardiac muscle and has direct and indirect growth factor properties on cardiomyocytes in culture. The role of Ang II in the remodeling process and hypertrophy after heart failure, as well as the presence of Ang I–converting enzyme in the scar tissue, also strongly points to the expression of Ang II receptors in the heart. Here we show that only AT₁A mRNA is detected in cardiomyocytes in both the ventricles and atria. This result is in agreement with the presence of AT₁A mRNA detected in cardiomyocytes in the anterior pituitary by Northern blot and RT-PCR. Here we demonstrate that a large subpopulation of cells in the anterior pituitary express this mRNA at a level higher than in any other known target site for Ang II, such as mesangial cells and adrenal glomerulosa cells. In contrast to findings of the present study, Bunnemann et al., using an AT₁, probe and in situ hybridization, reported the presence of AT₁A mRNA in the intermediate lobe. This discrepancy can be explained by the insufficient resolution of their macroscopic study, which did not allow one to distinguish the thin intermediate lobe from the adjacent layers of the anterior lobe. The physiological effects of Ang II on pituitary hormone secretion, particularly on prolactin and corticotropin, and the detection of AT₁A mRNA in most anterior pituitary cells strongly suggest that an effect of Ang II is exerted directly at the pituitary level. The number of positive cells probably exceeds that of lactotrophs and corticotrophs in normal animals, and it can be postulated that Ang II has other effects on pituitary cells such as thyrotrphys (see Reference 41 for a review). A combined study of hybridization and immunohistochemistry will be necessary to further characterize these cells.

In a previous report, we showed that the pituitary of the 19-day-old rat fetus expresses only AT₁A mRNA. In contrast to other tissues and organs that express the same subtype in the fetus and adult, subtypes shift (from AT₁A to AT₁B) in the pituitary during the first week after birth to express mostly or exclusively AT₁B in the adult. The sequential expression of the two subtypes exemplifies the temporal regulation of AT₁A and AT₁B mRNA and may provide a modulated mechanism of Ang II action during development. It remains to be shown whether the same cells express both subtypes successively. It will also be interesting to verify whether AT₁A mRNA expression is resumed in the pituitary under particular physiopathological conditions.

Limited pharmacological differences have been reported between AT₁A and AT₁B. There is no present argument for translating such differences into specific effects of Ang II in target cells via two different signaling mechanisms, although limited detailed studies have been carried out. Rather, one can propose that it is the regulatory elements that confer the cell specificity to the two subtypes. For example, a regulatory factor specific for AT₁B could induce a variation of AT₁A expression level in the adrenal glomerulosa cells without affecting the expression of AT₁A in medullary cells or in other target organs. Conversely, another regulatory factor specific for AT₁A would not alter corticotropin secretion in the pituitary, which expresses only AT₁B, but would modify aldosterone secretion in adrenal glomerulosa cells. If the effects of Ang II are proportional to the number of receptor sites at the cell surface, the subtype-specific mode of regulation ensures differentiated effects in different target cells via two very similar receptor subtypes using a single ligand. This mechanism, combined with the cell specificity of the response to that ligand, would constitute a simple, versatile, and efficacious system for the animal to adjust its homeostasis.

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References


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