Distribution of Type 1 Angiotensin II Receptor Subtype Messenger RNAs in the Rat Fetus

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Abstract The localization of the two type 1 angiotensin II receptor subtype (AT\textsubscript{1A} and AT\textsubscript{1B}) messenger RNAs in the 19-day-old rat fetus was studied by situ hybridization. AT\textsubscript{1} receptor mRNAs were detected in target organs of the renin-angiotensin system such as the kidney, adrenal gland, liver, heart, large arteries, and pituitary gland. In addition, angiotensin II receptors were present in specialized mesenchymal cells surrounding the cartilage, in the pericardium, in the lung, and in the undifferentiated mesenchymal tissue. The AT\textsubscript{1A} subtype was predominant in all tissues and organs except the adrenal cortex and glomeruli in the kidney, which expressed both AT\textsubscript{1A} and AT\textsubscript{1B} mRNAs. The widespread distribution of AT\textsubscript{1} receptors in tissues and organs involved in hydromineral equilibrium and blood pressure regulation shows that during fetal development angiotensin II may already act as a regulator of the cardiovascular system. An effect on cellular differentiation and/or proliferation via AT\textsubscript{1} receptors is also suggested by their location in several mesenchymes. (Hypertension. 1994;23:137-141.)

Key Words • receptors, angiotensin • in situ hybridization • renin-angiotensin system • pituitary gland

The octapeptide angiotensin II (Ang II) exerts many physiological actions in the cardiovascular, endocrine, and central and peripheral nervous systems. Ang II maintains circulatory homeostasis by various mechanisms including vasoconstriction, stimulation of mineralocorticoid secretion in adrenal glands, and regulation of renal sodium transport. Moreover, depending on the experimental conditions, Ang II may have a hypertrophic effect on its target cells, such as murine mesangial cells\textsuperscript{1} and rat vascular smooth muscle cells in culture,\textsuperscript{2} or it could be mitogenic as in fetal mesangial cells, which have intrinsically different growth properties.\textsuperscript{3} Recent experiments have also shown that Ang II could be mitogenic in the absence of growth factors.\textsuperscript{4}

Autoradiographic studies have shown a high transient expression of Ang II receptors in mouse,\textsuperscript{5} rat,\textsuperscript{6,7} and primate fetuses,\textsuperscript{8} particularly in the undifferentiated mesenchymes under the skin, around skeletal muscles and cartilage, and within the tongue. Differentiated tissues and organs such as skeletal muscles, retina, adrenal gland, choroid plexus, liver, lung, and kidney were also positive for radiolabeled Ang II. However, the use of nonpeptide antagonists (losartan and PD123177) that distinguish between the two known types of Ang II receptors, type 1 (AT\textsubscript{1}) and type 2 (AT\textsubscript{2}),\textsuperscript{9,10} has shown that most of these binding sites in the rat fetuses are of the AT\textsubscript{1} type.\textsuperscript{11} A physiological function for the AT\textsubscript{1} receptor is unknown, but its abundance in the fetus has led to hypotheses about its role in fetal growth and differentiation.\textsuperscript{11} Because of the large excess of AT\textsubscript{1} over AT\textsubscript{2} receptors in fetal tissues and the lack of cellular resolution, autoradiographic binding studies have not allowed a precise localization of the AT\textsubscript{1} receptor. This receptor type mediates all the known effects of Ang II on water and salt homeostasis and blood pressure.

A cDNA encoding an AT\textsubscript{1} receptor has been cloned and sequenced from rat aortic cells.\textsuperscript{12} More recently, another cDNA has been cloned and sequenced from rat adrenal glucocorticoid tissue.\textsuperscript{13} Both of these cDNAs have a high degree of nucleotide sequence identity in the coding region (91%) and a lower identity in the 5' and 3' noncoding regions (approximately 60%). These receptor subtypes are commonly referred to as AT\textsubscript{1A} and AT\textsubscript{1B}, respectively. To date, few pharmacologic differences have been reported between these two subtypes.\textsuperscript{13} However, their differential tissue distribution and their differences in the 5' regulatory regions point to probable functional specificities. In the present study, in situ hybridization with cRNA probes prepared from AT\textsubscript{1A} and AT\textsubscript{1B} cDNAs was used to describe the tissue location of the two AT\textsubscript{1} receptor subtype mRNAs in the 19-day-old rat fetus. This methodology detects even a low level of AT\textsubscript{1} receptor mRNA with a resolution at the cellular level and without interference of massive amounts of AT\textsubscript{1} present in the same tissues.

The widespread distribution of the AT\textsubscript{1} receptor mRNA in previously known and unknown target tissues for Ang II lends support to an early role of this peptide hormone as a regulator of the cardiovascular system and also possibly as a growth factor during fetal development.\textsuperscript{14} The comparison at the cellular level of AT\textsubscript{1A} and AT\textsubscript{1B} distribution may help to clarify further the regulation and biologic functions of these two receptor subtypes.

Methods Pregnant Sprague-Dawley rats (Iffa-Credo, L'Arbresle, France) were killed under anesthesia at day 19 of gestation, and the fetuses (eight animals from four different litters) were taken out of the uterus, decapitated, fixed in 4% buffered paraformaldehyde for 24 hours, dehydrated, and embedded in...
Results

A clear hybridization signal was observed with the antisense probes in tissues and organs known to be the target sites of Ang II in the regulation of cardiovascular homeostasis (adrenal gland, kidney, liver, large arteries, and heart) and also in other sites not directly related to the pressor effects of Ang II, such as mesenchymes, the pituitary gland, and the lung. On adjacent sections, with the sense probe, no signal was observed except for a weak uniform background labeling.

In the kidney, labeled mesenchymal cells were observed in the cortex, associated with glomeruli at different maturation stages. In the S-shaped body of the newly forming glomerulus, the mesenchymal cells that invade the immature Bowman's capsule, probably contributing to the formation of the glomerular mesangium, showed a significant signal with both AT1A and AT1B probes. In more mature glomeruli, mesangial cells were labeled (Fig 2A and 2B), whereas the outer and inner epithelial layers of the Bowman's capsule were devoid of receptor mRNAs. The hybridization signal was slightly higher, and the number of positive cells within glomeruli was also greater with AT1A (Fig 2A) than with AT1B (Fig 2B). In addition, AT1A was found in the glomeruli and other nephrogenic mesenchymal and epithelial tissues, whereas the AT1B mRNA was strictly limited to the mesangial cells within the glomeruli. In the medulla, labeled cells aligned along the epithelial tubular structures and formed figures that evoked the future vasa recta associated in vascular bundles.

In the adrenal gland, the zona glomerulosa expressed higher levels of AT1 receptor mRNAs compared with the zona fasciculata and adrenal medulla. AT1A mRNA was expressed the AT1A probe but not the AT1B. In addition, AT1A was found in the glomeruli and other nephrogenic mesenchymal and epithelial tissues, whereas the AT1B mRNA was strictly limited to the mesangial cells within the glomeruli. In the medulla, labeled cells aligned along the epithelial tubular structures and formed figures that evoked the future vasa recta associated in vascular bundles.

In the cardiac muscle, both the atria and ventricles expressed the AT1 receptor mRNA diffusely (Fig 2E), producing a signal significantly above the background observed with the sense probe (Fig 2F). The hybridization signal was particularly visible in the atrioventricular septum, the interventricular septum, and the bundle of His. The pericardium and adventitia of the root of the
aortic artery were strongly positive, in contrast to the
cardiac valves and coronary arteries, which showed only
a minimal labeling compared with the surrounding
myocardium. The only AT₁ receptor mRNA subtype
found in the heart was AT₁A.

In the liver, the predominant subtype was AT₁A
mRNA (Fig 3A), whereas AT₁B showed a weak labeling
attributable to background (Fig 3B). In the liver, three
different subpopulations of cells could be distinguished
by the intensity of labeling with the AT₁A probe (Fig
3A). A small minority of cells, with little cytoplasm and
pale stained nuclei and generally isolated or in small
groups not exceeding two or three cells, were intensely
labeled. The absence of cytological features does not
allow the identification of these cells. Another subpop-
ulation, representing more than half the cells in the
liver, displayed a moderate level of labeling. These cells
were identified as being hepatocytes by their large,
round nuclei with dispersed granulated chromatin. A
third population composed of clusters of cells with
small, dark nuclei had a minimal level of labeling,
corresponding to background. They probably belong to
the erythroblastic lineage.

In the lung, the alveolar epithelial lining showed very
low labeling, whereas clusters of cells within mesenchy-
mal tissue between the alveoli were rich in AT₁A mRNA
(Fig 3C). AT₁B mRNA was absent in the lung.

Large arteries in the fetus, especially the aorta and
the pulmonary and cerebral arteries, expressed AT₁A
mRNA in the tunica media and tunica adventitia (Fig
3D) and were devoid of AT₁B mRNA. In addition, AT₁A
mRNA but not AT₁B was detected in the pituitary gland
(Fig 3E and 3F), perichondrium, adipose tissue, and
smooth muscle of the esophagus but not that of the
intestine and in specialized mesenchymes in different
parts of the fetus.

Discussion

The present in situ hybridization study localized AT₁
receptor mRNAs in several organs of the 19-day-old rat
fetus such as the adrenal gland, kidney, liver, heart, and
large arteries, which have regulatory functions on blood
pressure in the adult animal. Other sites not apparently
linked to the pressor effect of Ang II have also been
identified: pituitary gland, lung, and specialized mesen-
chymes (subepidermal layers and perichondrium). Pre-
vious binding studies on tissue sections, evaluated after
autoradiographic exposure, had shown the presence of
large amounts of Ang II binding sites in the 19-day-old
rat fetus. However, with the use of nonpeptidic
antagonists, these receptors had been characterized as
belonging to type 2, whose functions remain specula-
tive. We now describe the cellular localization of the
two AT₁ receptor subtypes in the 19-day-old rat fetus
and demonstrate that they are expressed with a tissue
distribution partially overlapping that of AT₂ binding
sites.

In the adult rat, the presence of AT₁A and AT₁B
mRNAs has been shown in various organs by North-
ern blot or polymerase chain reaction. However, these
studies could not identify the type of cells expressing
one or the other or both AT₁ receptor subtypes. In in
situ hybridization with the same AT₁A cRNA probe used
in the present work, we showed the expression of this
AT₁ receptor mRNA in mesangial and juxtaglomerular
cells. In the fetus and neonate, a recent study reported
the presence of AT₁ receptor mRNAs in the rat kidney
and liver during the perinatal period. Because it is not

Fig 2. Photomicrographs show in situ hybridization of AT₁ receptor mRNAs in kidney (A and B), adrenal cortex (C and D), and heart
(E and F) of rat fetus, with antisense cRNA probes for AT₁A (A, C, and E) or AT₁B (B and D) and sense probe for AT₁A (F). In kidney, AT₁A
is detected in mesangial cells within the glomeruli and also in mesenchymal and tubular cells outside (A), whereas AT₁B is detected
exclusively in mesangial cells (B). In adrenal gland, AT₁A is detected in the capsule and zona glomerulosa (C), and AT₁B exclusively in
the zona glomerulosa (D). In heart, cardiomyocytes express only AT₁A mRNA (E); the sense probe produces minimal labeling (F).
Exposure time, 8 weeks; x500.
FIG 3. Photomicrographs show in situ hybridization of AT₁ receptor mRNAs in liver (A and B), lung (C), aorta (D), and pituitary gland (E and F) of rat fetus with cRNA probes for AT₁A (A, C, D, and E) or AT₁B (B and F). In liver, AT₁A mRNA is detected in hepatocytes (characterized by large, clear nuclei) and not in cells of the erythroblastic lineage (characterized by small, dark nuclei), whereas AT₁B is not detected (B). In lung, clusters of mesenchymal cells adjacent to the alveolar epithelium express AT₁A mRNA (C). In aorta, the adventitia and media express AT₁A mRNA (D). In pituitary gland, small clusters of cells are labeled with the AT₁A probe (E) but not the AT₁B probe (F). Exposure time, 8 weeks; ×500.

known whether the probes used in that study were strictly specific for AT₁A or were cross-hybridized with AT₁B mRNA, the signal obtained could be attributed to either one or both receptor subtype mRNAs. Our study demonstrates the existence of three different types of distribution: almost similar levels of AT₁A and AT₁B mRNAs (glomeruli of the kidney), the predominance of AT₁B (zona glomerulosa of the adrenal gland), or the exclusive presence of AT₁A (lung, liver, and the pituitary gland).

Although the two cDNAs from which our cRNA probes were transcribed share a high degree of homology in the coding regions, there appears to be very limited cross-hybridization under our experimental conditions. This is shown by the differential distribution of AT₁A and AT₁B mRNAs in the kidney (Fig 2A and 2B), adrenal gland (Fig 2C and 2D), liver (Fig 3A and 3B), and pituitary gland (Fig 3E and 3F). The specificity of the two riboprobes can be explained by the fact that although they are obtained after transcription of the entire cDNA, they contain a large proportion of short transcripts as shown by the presence of a smear in the autoradiography of the gel (Fig 1). These short transcripts corresponding to the 3' noncoding regions lie close to the initiation site of transcription and show an identity of less than 62%, insufficient for cross-hybridization under our conditions. This is also confirmed in CHO cells transfected with the AT₁A cDNA, which displayed only a low-level signal when hybridized with the AT₁B probe, although they express AT₁A mRNA at a rate far greater than normal Ang II target cells in the animal (unpublished observations).

The presence of AT₁A and AT₁B mRNAs in the 19-day-old fetus suggests that Ang II plays an important role in the regulation of blood pressure during fetal development. The widespread distribution of AT₁A mRNA in organs and tissues such as the adrenal gland, kidney, liver, heart, lung, and pituitary gland and in the differentiating mesenchymes suggests that this receptor subtype may be predominant in mediating the effects of Ang II in the rat fetus. The restricted distribution in the fetus of AT₁B mRNA to the zona glomerulosa of the adrenal gland and kidney suggests that Ang II may be involved in water and salt homeostasis through the AT₁B receptor subtype. AT₁A might rather act as a mediator of growth and/or differentiation in tissues, such as the lung, heart, and mesenchymes, although more specific functions, such as the feedback control of angiotensinogen expression in the liver, are also probable.

Interestingly, AT₁A mRNA is abundant in the pituitary gland of the fetus, whereas this organ is known to be rich in AT₁B mRNA in the adult rat. This demonstrates a change in the expression of the specific AT₁ receptor subtype occurring after birth. Thus, our study of the two subtypes of the Ang II receptors in the 19-day-old rat fetus demonstrates the differential prenatal and postnatal expression and regulation of these receptors. The cause of this differential level of mRNA of the two receptor subtypes can be attributed not only to the dissimilarity of the nucleotide sequence between the two mRNAs but also to other developmentally regulated cytoplasmic or nuclear transcriptional factors. The specific pattern of spatiotemporal expression of the two Ang II receptor subtypes in the fetus and adult rat
may be the basis of functional specificities that remain to be elucidated.

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References

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