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 in 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 heart, large arteries, and pituitary gland. Angiotensin II receptor mRNAs were also detected in target organs of the renin-angiotensin system such as the kidney, adrenal gland, liver, heart, large arteries, and pituitary gland. Moreover, depending on the experimental conditions, AT\textsubscript{1} 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 fetus are of the AT\textsubscript{1} type.\textsuperscript{11} A physiological function for the AT\textsubscript{2} 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 adrenal glomerulosa tissue.\textsuperscript{13} Both of these cDNAs have a high degree of nucleotidic 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 (Ifka-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...
AT1B-Xba I in lane 2) and sense (AT1A-Sal I in lane 3) probes, a band preceded by a smear is detected. The band has the expected size (approximately 2 kb) and corresponds to complete transcripts. The smear corresponds to shorter, incomplete transcripts. These shorter probe molecules are complementary to the 3' end of the cDNA, a region having the lowest homology between the AT1A and AT1B subtypes.

paraffin according to our routine procedure. Transverse and sagittal 5-μm sections were cut and mounted on 3-aminopropytriethoxysilane (Aldrich Chemical Co)-coated glass slides. All animal procedures were performed in accordance with administrative guidelines.

The clone pCa18b containing a 2.3-kb cDNA of the rat AT1A receptor (a gift from Dr K. Bernstein) was digested with HindIII and Not I, and the insert was subcloned in the unique Xba I site of an expression vector derived from pECE15 by insertion of unique BsmI, Nru I, and Not I restriction sites into the polylinker. This construction was subsequently digested by Xba I, and the insert was subcloned in the unique Xba I site of the Bluescript KS plasmid (Stratagene, La Jolla, Calif). The Bluescript plasmid was used to synthesize the riboprobes. The cDNA was linearized by BamHI, a restriction site found in the multiple cloning site upstream to the T7 promoter of the plasmid, or Hpa I, a restriction site in the 5' region of the cDNA at position 131. Transcription in vitro was performed in the presence of 35S-UTP (Amersham, Les Ulis, France) by T7 or T3 RNA polymerase (Boehringer, Calif). The Bluescript plasmid was used to synthesize the riboprobe. The cDNA was linearized by BamHI, a restriction site located just upstream to the T7 promoter of the plasmid, or Hpa I, a restriction site in the 5' region of the cDNA at position 131. Transcription in vitro was performed in the presence of 35S-UTP (Amersham, Les Ulis, France) by T7 or T3 RNA polymerase (Boehringer, Mannheim, Germany) to synthesize the sense or antisense riboprobe, respectively.

A pCDNA II plasmid (Invitrogen, Oxon, UK) containing a 2.2-kb cDNA of the rat AT1B receptor (clone RAG6D4.60, a gift from Dr K. Sandberg) was used to prepare the AT1B riboprobe. The cDNA was linearized by Xba I, a restriction site located just upstream to the T7 promoter of the pCDNA II plasmid, and transcribed in vitro by Sph I in the presence of 35S-UTP to synthesize an antisense probe.

The AT1A and AT1B cRNA probes should correspond to the full-length cDNAs and include the coding and noncoding sequences of the cDNAs. However, the length of the probe was verified on a 0.8% agarose gel (Fig 1), which showed a band at 2 kb preceded by a smear extending toward smaller molecular weights. This smear corresponds to short transcripts, which are the product of an incomplete transcription.

Fig 1. Autoradiograph shows an agarose gel of the three AT1 receptor riboprobes. For the antisense (AT1A-Hpa I in lane 1 and AT1B-Xba I in lane 2) and sense (AT1A-BamHI in lane 3) probes, a band preceded by a smear is detected. The band has the expected size (approximately 2 kb) and corresponds to complete transcripts. The smear corresponds to shorter, incomplete transcripts. These shorter probe molecules are complementary to the 3' end of the cDNA, a region having the lowest homology between the AT1A and AT1B subtypes.

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 showed a clear signal in the zona glomerulosa and the capsule (Fig 2C), with low-level labeling in the inner layers of the cortex. AT1B mRNA was strictly localized to the zona glomerulosa and absent in the capsule (Fig 2D). The cells in the adrenal medulla were positive with the AT1A probe but not the AT1B.

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
Figure 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.

In the aortic artery, AT1A mRNA was 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 subpopulation, 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 mesenchymal 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 mesenchymes (subepidermal layers and perichondrium). Previous 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.6-7 However, with the use of nonpeptidic antagonists, these receptors had been characterized as belonging to type 2, whose functions remain speculative.11 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 Northern 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. By 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.19 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.14 Because it is not
known whether the probes used in that study were strictly specific for AT$_{1A}$ or were cross-hybridized with AT$_{1B}$ 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 AT$_{1A}$ and AT$_{1B}$ mRNAs (glomeruli of the kidney), the predominance of AT$_{1B}$ (zona glomerulosa of the adrenal gland), or the exclusive presence of AT$_{1A}$ (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 AT$_{1A}$ and AT$_{1B}$ 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 AT$_{1A}$ cDNA, which displayed only a low-level signal when hybridized with the AT$_{1B}$ probe, although they express AT$_{1A}$ mRNA at a rate far greater than normal Ang II target cells in the animal (unpublished observations).

The presence of AT$_{1A}$ and AT$_{1B}$ 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 AT$_{1A}$ 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 AT$_{1B}$ 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 AT$_{1B}$ receptor subtype. AT$_{1A}$ 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, AT$_{1A}$ mRNA is abundant in the pituitary gland of the fetus, whereas this organ is known to be rich in AT$_{1B}$ mRNA in the adult rat. This demonstrates a change in the expression of the specific AT$_{1}$ 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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