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Short Sequence-Paper

Cloning of the cDNA and the genomic DNA of the mouse angiotensin II type 2 receptor

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Abstract

Of the two major isoforms of the angiotensin II receptors, type 1 (AT_1) and type 2 (AT_2), little is known about the structure and features of AT_2. We cloned a mouse AT_2 cDNA from a mouse fetus cDNA library and an AT_2 genomic DNA from a 129SV mouse genomic DNA library. The amino acid sequence of the mouse AT_2 (363 residues) deduced from a mouse cDNA clone showed seven membrane-spanning domains. Amino acid identity of the mouse AT_2 with mouse AT_1 is 37%, and 98% with rat AT_2. The genomic DNA (4.4 kb) contained three exons and two introns and the entire coding region was contained in the third exon.

Key words: Angiotensin II; Type 2 receptor; cDNA; Genomic DNA sequence; (Mouse)

Reflecting the diverse actions of angiotensin II (Ang II), recent pharmacological studies indicated at least two isoforms of Ang II receptors, AT_1 and AT_2 [1–6]. AT_1 cDNAs have been cloned recently [7–10] and shown to mediate G-protein coupled functions such as the activation of phospholipase C, opening of Ca^{2+} channel and inhibition of adenylyl cyclase. By contrast, little is known about the structure and functions of AT_2. The use of AT_2-specific inhibitors revealed the tissue distribution of AT_2 in mesenchymal tissue of rat fetus [11], healing wound [12], certain brain regions [13] and rat adrenal medulla [4]. The lack of effects of stable GTP-analogues on the ligand-binding of AT_2 [1] and absence of ligand-induced internalization [14] suggested its unique structural feature and signaling mechanism. Very recently we have expression cloned a cDNA for rat AT_2 from a rat pheochromocytoma cell line [15] and found it to be a receptor with seven membrane-spanning domains. However, little is known about its biological functions. With the objective of clarifying its biological significance by disrupting the AT_2 gene by a homologous recombination technique, we undertook cloning of the genomic DNA of mouse AT_2 via cloning of its cDNA. In this paper, we report the nucleotide sequence of the mouse cDNA and the genomic DNA and the deduced amino acid sequence of the mouse Ang II type 2 receptor.

The cDNA for the mouse AT_2 receptor was cloned from a cDNA library prepared from two fetuses of Balb/C mice by conventional plaque hybridization with the rat AT_2 cDNA probe [15]. The nucleotide sequences were determined both in the sense and antisense directions. Of the two ATG sequences, the last one (position, 158 bp–160 bp) satisfied Kozak's rules (Fig. 1). The open reading frame between this initiation codon and the inframe termination codon, TAA (position, 1247 bp–1250 bp), consisted of 1089 bp equivalent to 363 amino acid residues. The nucleotide and amino acid sequence showed 97% and 98% sequence identity with the rat AT_2 cDNA cloned by Kambayashi et al. [15]. Hydropathy analysis according to Kyte-Doolittle indicated the presence of seven hydrophobic membrane-spanning domains as indicated by underlines, suggesting that this receptor belongs to the super family of seven membrane-spanning receptors. There is no signal sequence. The following structural features highly conserved and commonly found in

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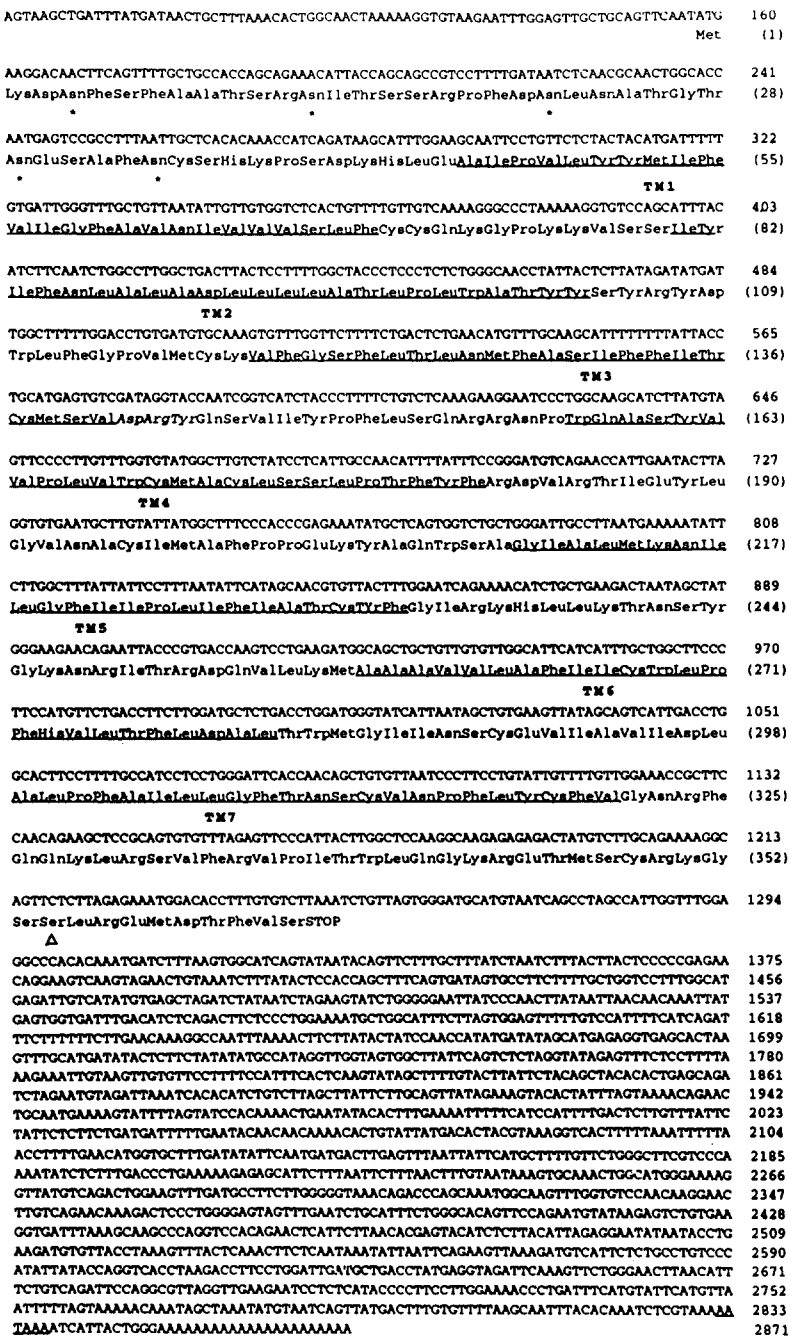

Fig. 1. The nucleotide and deduced amino acid sequences of the mouse AT_2 cDNA. The amino acid sequence deduced from the nucleotide sequence of the open reading frame is given below the corresponding DNA sequence. Putative transmembrane domains are shown by underlines (TM1 to TM7). A potential poly(A)$^+$ signal is also underlined (position 2832 bp–2837 bp). Potential N-linked glycosylation sites and possible phosphorylation site by protein kinase C are indicated by asterisks (*) and open triangle (△), respectively. Accession No. U00766.

the majority of members of this super family were also noted in the mouse AT_2 sequence, Asp^{141}-Arg^{142}-Tyr^{143} (Fig. 1, indicated in italic) immediately after the third transmembrane domain, Asp^{90} in the second transmembrane domain, and Pro^{98}, Pro^{177}, Pro^{223}, Pro^{271}, Pro^{315} in the second, fourth, fifth, sixth and seventh transmembrane domain, respectively. Other notable features are five potential N-glycosylation sites which are located exclusively in N-terminal hydrophilic region (Fig. 1, Asterisks) and short first and third cytosolic loops. There is a possible phosphorylation site by protein kinase C in C-terminal region (Fig. 1, open triangle). Important to note is only 37% amino acid homology with the mouse AT_1 [9,10], which is compatible the marked absence of functional similarity between AT_1 and AT_2. Mouse AT_2 gene also has 35% homology with rat opioid receptor and 28% homology with mouse β_2-adrenergic receptor according to the homology

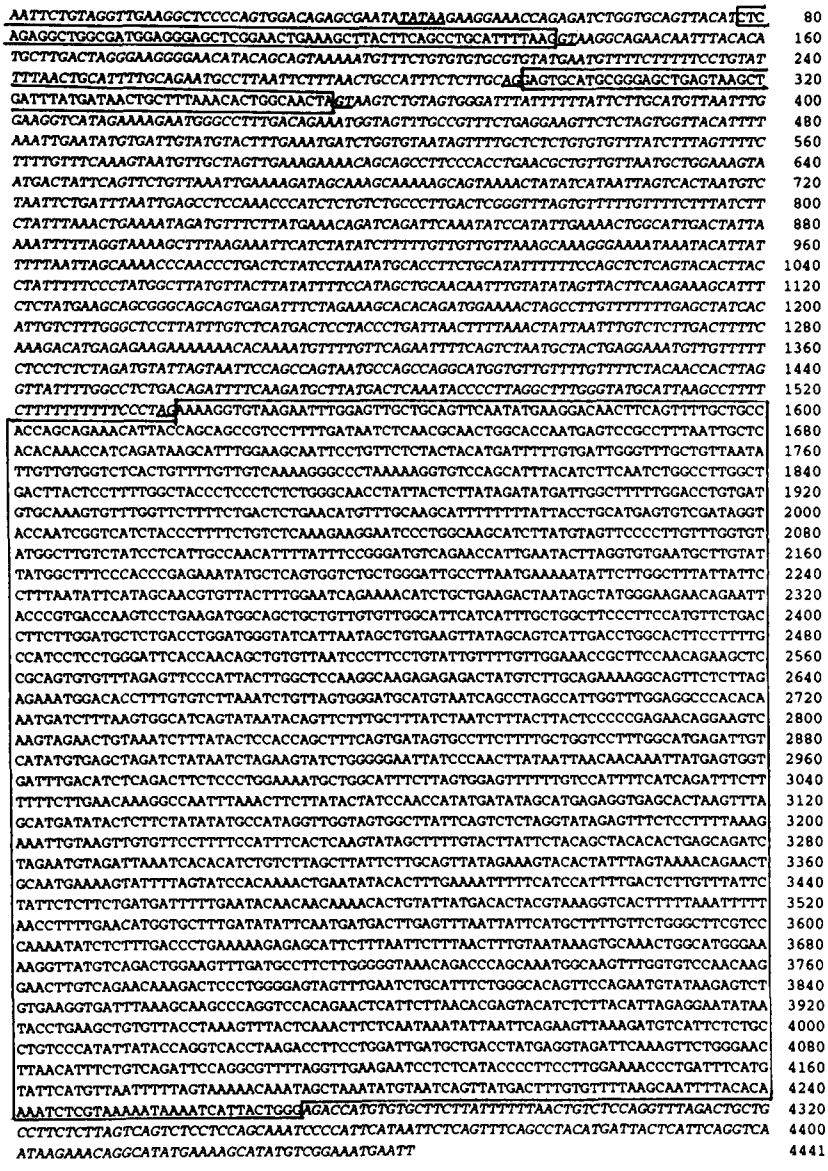

Fig. 2. The nucleotide sequence of the mouse AT_2 genomic DNA. Three exons are indicated by boxes. A putative TATAA box (position 42 bp–46 bp) and exon-intron boundary sequences (GT and AG) are underlined. Accession No. U00768.

search of GenBank data base. Homologous residues are mainly located in transmembrane portion. On the other hand, between rat and mouse AT_2, only four amino acid residues were different out of the total 363 residues. DNA containing mouse AT_2 was cloned from 129SV mouse genomic library. The entire nucleotide sequence of the 4.4 kb *Eco*RI fragment was determined both in the sense and antisense directions (Fig. 2). Comparison with the nucleotide sequence of the AT_2 cDNA revealed that the 4.4 kb genomic DNA contained the entire transcribable sequence. Deduced from the 5′ end of the AT_2 cDNA clone, there is a putative TATAA box 36 bp upstream of the first exon (position, 42 bp–46 bp). Two short exons (61 bp for the first exon and 60 bp for the second exon) were followed by a long third exon containing 2733 bp. Each of the two intervening sequences contained the 5′ boundary sequence gt and 3′ boundary sequence ag. Open reading frame in the third exon was uninterrupted by any intron as is often found in many seven membrane-spanning receptor genes.

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