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[REVIEW]

Angiotensin II Receptor Subtypes: Their Distribution, Signaling Pathways, and Physiological Functions

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ABSTRACT—Angiotensin II (Ang II) exhibits a variety of physiological actions, related mainly to the regulation of blood pressure and fluid osmolarity. Recent identification of the multiple types of the Ang II receptors raises the possibility that Ang II has other unknown functions. The Ang II type 1 receptor (AT_1) mediates most of the known physiological functions of Ang II, whereas the type 2 receptor (AT_2)-mediated functions remain unclear. AT_2 is particularly interesting because it is expressed abundantly in fetal tissues and in cells undergoing apoptosis. AT_1 and AT_2 exhibit unique signaling pathways among the superfamily of seven membrane-spanning receptors: *i.e.* the coupling of AT_1 to the Janus kinase-signal transducers and activators of transcription pathway and the coupling of AT_2 to phosphatase activation. Also, the two subtypes induce several opposite intracellular events. AT_1 mediates activation of Ca^{2+} channels and inhibition of K^+ channels, whereas AT_2 induces inhibition of Ca^{2+} channels and activation of K^+ channels. Therefore, it is of great importance to compare the two receptor subtypes with respect to their distribution, signaling pathways, and physiological functions.

Angiotensin II (Ang II), an effector peptide in the renin-angiotensin system (RAS), exhibits a variety of biological actions, related mainly to the regulation of blood pressure and fluid osmolarity (Peach, 1977; Bottari *et al.*, 1993). In recent years, Ang II has been drawing considerable attention because of the following reasons:

1) Involvement of Ang II in the development of cardiovascular diseases such as cardiac hypertrophy and atherosclerosis as well as hypertension (Powell *et al.*, 1989; Paul and Ganten, 1992; Susic and Frohlich, 1993; Bottari *et al.*, 1993). The RAS is now a major target for the development of the drugs aimed at preventing these diseases.

2) Identification of multiple Ang II receptor subtypes. Ang II receptors are separated into at least four subtypes, named AT_1, AT_2, AT_3, and AT_4 (Miyazaki *et al.*, 1988; Braszko *et al.*, 1988; Sasaki *et al.*, 1991; Murphy *et al.*, 1991; Harding *et al.*, 1992; Chaki and Inagami, 1993; Mukoyama *et al.*, 1993; Kambayashi *et al.*, 1993) although the designation AT_3 is not widely recognized. This finding is very important because the existence of receptor subtypes raises the possibility that the RAS has other novel physiological functions. The abundant expression of AT_2 in fetal tissues and in cells undergoing apoptosis is of particular interest (Pucell *et al.*, 1991; Grady *et al.*, 1991; Mukoyama *et al.*, 1993; Kambayashi *et al.*, 1993; Tanaka *et al.*, 1995; Kakuchi *et al.*, 1995; Kobayashi *et al.*, 1995; Yamada *et al.*, 1996).

3) The AT_1 receptor-induced direct activation of the Janus kinase (JAK)-signal transducers and activators of transcription (STAT) pathway, known as the signaling pathway used by cytokine receptors such as those for interleukines and interferons (Marrero *et al.*, 1995). This is the first example among the superfamily of seven membrane-spanning receptors.

Studies on AT_1 and AT_2 have been preceding those on other Ang II receptor subtypes as the cDNAs and genes for AT_1 and AT_2 have been cloned, and because of the development of their selective antagonists. Thus, this minireview aims to introduce the reader to the current topics concerning AT_1 and AT_2, especially focusing on a comparison of the two receptor subtypes with respect to their distribution, signaling pathways, and physiological functions.

ANG II IN THE RENIN-ANGIOTENSIN SYSTEM AND ANG II RECEPTOR SUBTYPES

As illustrated in Fig. 1, renin, an aspartyl proteinase, acts on its specific substrate, angiotensinogen, to produce the decapeptide angiotensin I. Under the influence of the

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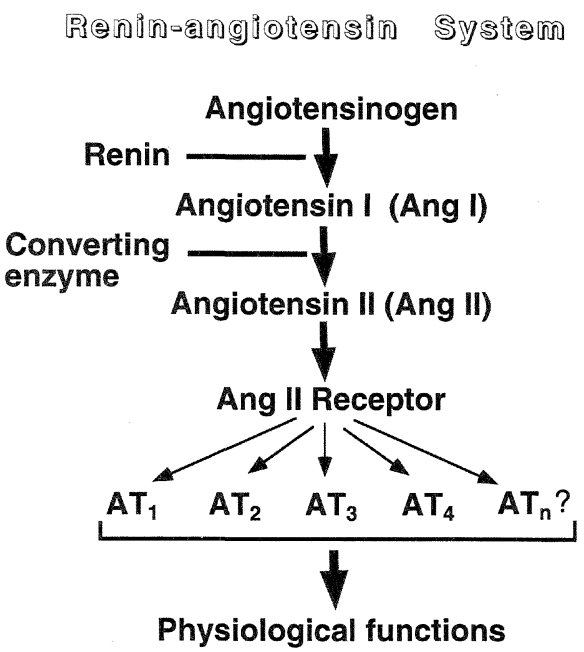

Fig. 1. Scheme of the renin-angiotensin system.

converting enzyme, angiotensin I, in turn, is converted into the octapeptide Ang II. Ang II elicits a number of physiological functions by binding to its specific receptors on the surface of target tissues. Ang II receptors are now separated into at least four subtypes, designated AT_1, AT_2, AT_3, and AT_4 (Miyazaki *et al.*, 1988; Braszko *et al.*, 1988; Sasaki *et al.*, 1991; Murphy *et al.*, 1991; Harding *et al.*, 1992; Chaki and Inagami, 1993; Mukoyama *et al.*, 1993; Kambayashi *et al.*, 1993) although the designation AT_3 is not widely recognized.

The AT_1 and AT_2 receptors are easily distinguished based upon the binding characteristics of respective subtype-selective antagonists, such as Dup753 for AT_1 and PD123319 for AT_2 (Bottari *et al.*, 1993). These subtypes also react differently to the sulfhydryl reagent dithiothreitol (DTT) and the nonhydrolyzable GTP analogue guanosine 5'-3-*O*-(thio) trisphosphate (GTPγS). In fact, we identified novel Ang II receptors in the bovine ovary eight years ago, corresponding to AT_2 according to the present nomenclature, based upon their sensitivity to DTT (Miyazaki *et al.*, 1988): *e.g.* DTT increased the Ang II binding affinity for AT_2, whereas the reagent reversely reduced the affinity for AT_1. The reagent GTPγS attenuates Ang II binding to AT_1, but does not affect Ang II binding to AT_2.

The AT_3 receptor, identified in differentiated Neuro-2A cells, does not have an affinity for either AT_1 or AT_2-selective antagonists (Chaki and Inagami, 1993). In contrast to these subtypes, AT_4 exhibits a high affinity for the hexapeptide fragment Ang II(3-8), AIV, but not for Ang II (Braszko *et al.*, 1988; Harding *et al.*, 1992). In addition, the heptapeptide Ang II(1-7) may have its own specific receptor, which is distinct from AT_1, AT_2, AT_3, and AT_4. Although these multiple populations of Ang II receptors exist, AT_1 mediates most, if not all, of the well-known functions of Ang II, such as smooth muscle contraction, stimulation of aldosterone release from the adrenal cortex, stimulation of the heart rate and cardiac contractility, and inhibition of renin release from the renal cortex (Bottari *et al.*, 1993). In contrast, the physiological functions of the other Ang II receptor subtypes have not yet been determined.

DISTRIBUTION OF AT_1 AND AT_2

The AT_1 receptor is widely distributed in tissues that are mainly related to the maintenance of blood pressure, and electrolyte and fluid homeostasis, in both adult and fetal tissues (Balla *et al.*, 1991; Bottari *et al.*, 1993). In addition to the tissues described above (vasculature, adrenal cortex, heart, and kidney cortex), this subtype is present in other tissues including the brain (such as hypothalamus and subfornical organ), anterior and posterior pituitaries, liver, testis, and ovary. On the other hand, AT_2 exhibits widespread and abundant expression in fetal tissues including the skin, tongue, brain, intestine, stomach, kidney, and connective tissue (Grady *et al.*, 1991; Millan *et al.*, 1991; Viswanathan and Saavedra, 1992; Bottari *et al.*, 1993; Mukoyama *et al.*, 1993; Kambayashi *et al.*, 1993; Kakuchi *et al.*, 1995). In these tissues AT_2 is mainly located in the undifferentiated mesenchyme; *e.g.* the mesenchyme of the submucosal layers of the intestine and stomach, and the mesenchyme near the nephrogenic area of superficial cortex in the kidney. The existence of AT_2 is detected by day 11 and reaches a maximum between day 19-21 in fetuses. Interestingly, its expression decreases dramatically and rapidly after birth.

AT_2 is also present in adult tissues such as the adrenal, brain, ovary, and skin. In the ovary and skin its expression is strictly regulated. We recently examined quantitative changes in AT_2 during differentiation and apoptosis of rat ovarian cultured granulosa cells, which are abundant in follicles (Ohnishi *et al.*, 1994; Tanaka *et al.*, 1995). The AT_2 content was very low and did not change in the presence of follicle-stimulating hormone (FSH), a differentiation factor for these cells, but was dramatically increased in FSH-free media in a time-dependent manner (Fig. 2A). The cells cultured without FSH underwent internucleosomal DNA fragmentation characteristic of apoptosis (Fig. 2B). In addition to this *in vitro* experiment, we also confirmed that the AT_2 content was markedly increased at both the mRNA and protein levels during the development of apoptosis of granulosa cells *in vivo* by treating immature rats with pregnant mare serum gonadotropin (PMSG) (unpublished data); this treatment is known to induce follicle atresia involving apoptosis. These findings suggest that AT_2 is transiently expressed and modulates the onset and/or progression of ovarian follicle atresia during estrus cycles in adults.

Enhancement of the AT_2 content was also observed in other tissues after birth. The AT_2 expression was shown to be significantly enhanced in the rat skin during experimental wound healing (Viswanathan and Saavedra, 1992). We also

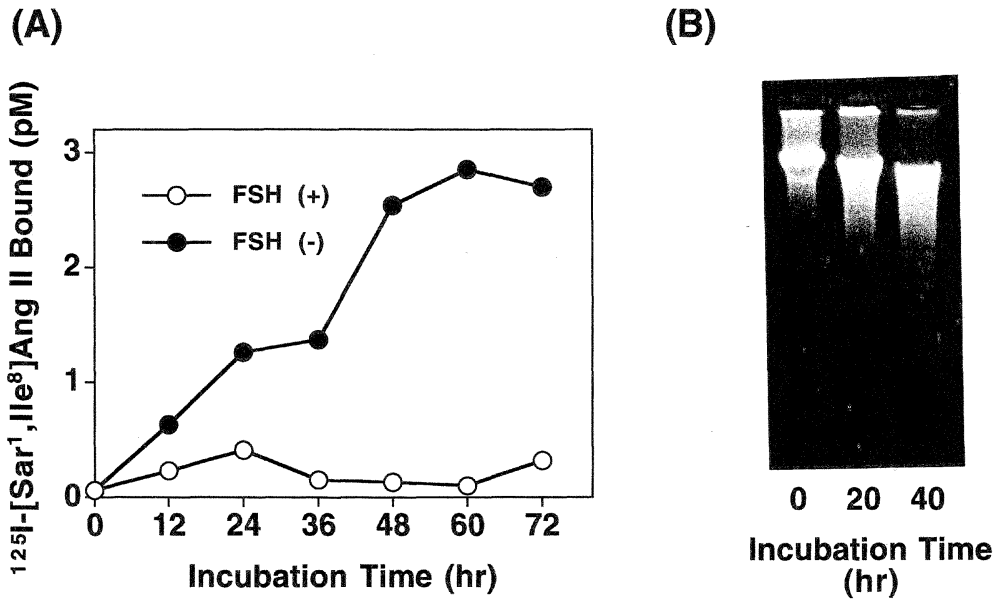

Fig. 2. Quantitative change in the AT_2 contents with or without FSH (A) and DNA fragmentation (B). (A) Follicular granulosa cells were cultured in the presence or absence of FSH for indicated periods of time. Thereafter, ^{125}I-[Sar^1, Ile^8]Ang II binding assay was performed. (B) Granulosa cells were cultured without FSH for indicated periods of time. Thereafter, genomic DNA was extracted from the cells, fractionated through 2.0% agarose gel, then the gel was stained with ethidium bromide.

found, in the hypertrophied hearts of Tsukuba hypertensive mice, which carry the human genes for renin and angiotensinogen, that the AT_2 content was markedly increased at the protein level but not at the mRNA level compared to normal mice (Fujii *et al.*, 1995).

THE SIGNALING PATHWAYS OF AT_1 AND AT_2

Both AT_1 and AT_2 belong to the superfamily of seven membrane-spanning receptors. The AT_1 receptor associates with the Gq and Gi families of GTP-binding proteins (G protein), whereas AT_2, in part, couples with the Gi family (Ohnishi *et al.*, 1992; Bottari *et al.*, 1992; Kang *et al.*, 1994, 1995; Shibata *et al.*, 1996). Figure 3 illustrates the signaling pathways used by each receptor subtype.

Ang II binding to AT_1 leads to the activation of phospholipase C_β (PLC_β) with a subsequent increase in the intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$), and the inhibition of adenylate cyclase activity. In recent years, growth factors such as vasopressin, bombesin, and endothelin, which interact with G-protein coupled receptors, have been shown to induce the rapid tyrosine phosphorylation of various substrates involved in cell proliferation in a manner similar to tyrosine kinase-coupled receptors such as epidermal growthn factor (EGF) and platelet derived growth factor (PDGF) receptors (Zachary *et al.*, 1991). Several investigators demonstrated that Ang II also exhibits a cell growth promoting activity, and stimulates the tyrosine phosphorylation of proteins such as PLCγ1, Src, focal adhesion kinase (FAK), paxillin, and Src homologous and collagen (SHC) via AT_1 in different kinds of cells including vascular smooth muscle cells, cardiac fibroblast cells, and liver epithelial cells (Huckle *et al.*, 1992; Marrero *et al.*, 1994; Schorb *et al.*, 1994; Leduc and Meloche, 1995). When we introduced the recombinant AT_1 into NIH3T3 (a mouse fibroblast cell line) and PC12 cells (a rat pheochromocytoma cell line), which exhibited no and extremely low Ang II binding activity, respectively, these transfected cells underwent Ang II-dependent DNA synthesis. These findings suggest that AT_1 primarily has a cell growth promoting activity. However, at present, the Ang II-evoked pathway leading to tyrosine phosphorylation is not completely understood.

One of the major recent topics in the field of intracellular signaling pathways as well as the RAS is the finding that AT_1 may directly stimulate the JAK-STAT pathway used by cytokine receptors (Marrero *et al.*, 1995). That is, Ang II binding to AT_1 induced the rapid tyrosine phosphorylation of JAK2 and Tyk2, and their activation, resulting in the tyrosine phosphorylation of the JAK family substrates STAT1 and STAT2, in rat aortic smooth muscle cells. In addition, JAK2 co-precipitates with AT_1, suggesting that AT_1 may directly interact with JAK2 like cytokine receptors bind to JAK family proteins.

The signaling pathway of AT_2 is still far from being completely understood, although its cDNA and gene have recently been cloned. Table 1 compares AT_2-induced intracellular events with those of AT_1. Interestingly, each receptor subtype induces opposite events. For example, AT_1 activates protein tyrosine kinases (*e.g.* FAK and JAK) and serine/threonine kinases (*e.g.* protein kinase C and calcium/calmodulin kinase II) (Huckle *et al.*, 1992; Bottari *et al.*, 1993; Marrero *et al.*, 1994; Schorb *et al.*, 1994; Leduc and Meloche, 1995; Marrero *et al.*, 1995), whereas AT_2 activates protein tyrosine phosphatase and serine/threonine phosphatase

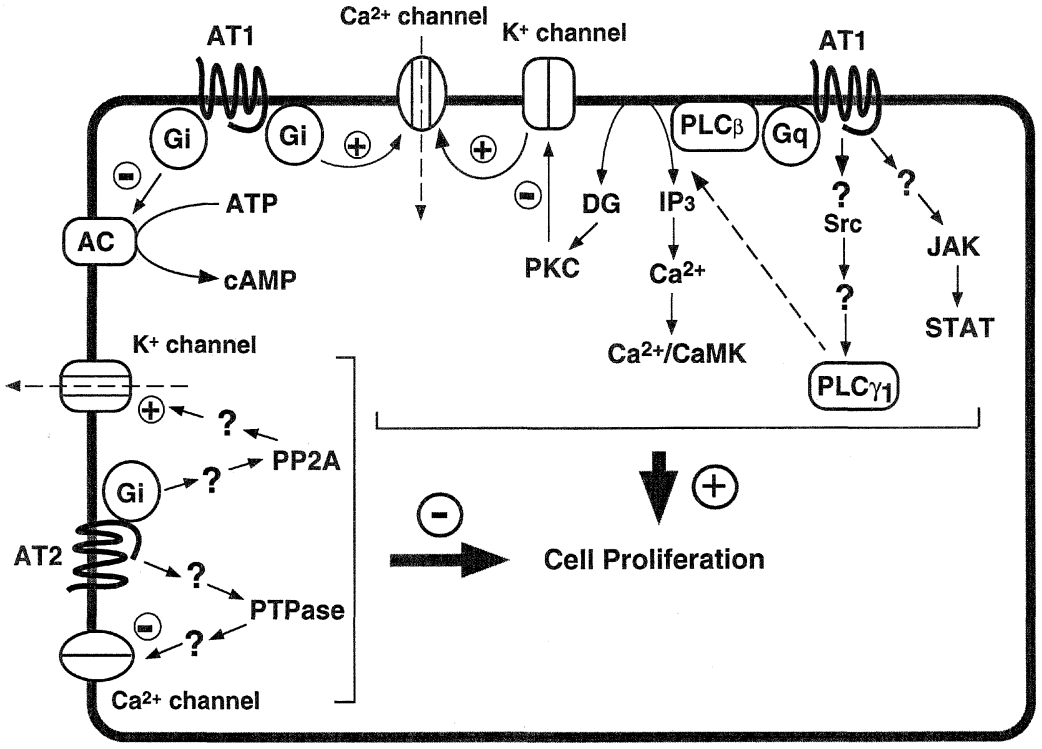

Fig. 3. AT_1- and AT_2-mediated intracellular signaling pathways. AT1, angiotensin II type 1 receptor; AT2, angiotensin II type 2 receptor; AC, adenylate cyclase; PLCβ, phospholipase Cβ; PLCγ, phospholipase Cγ; IP_3, inositol-1,4,5-triphosphate; DG, diacylglycerol; CaMK, calcium/calmodulin dependent protein kinase; PTPase, phosphotyrosin phosphatase; PP2A, phosphoprotein phosphatase 2A.

Table 1. AT1 and AT2 induced activation and inhibition of signaling fators

AT_1	AT_2
activation	activation
Phospholipase C	Tyr phosphatase
Ser/Thr kinase (PKC, Ca^{2+}/CaM kinase II)	Ser/Thr phosphatase (PP2A)
Tyr kinase (JAK, FAK)	K^+ channel
Phospholipase D	
Phospholipase A_2	
Ca^{2+} channel (L-, T-type)	
inhibition	inhibition
K^+ channel	Ca^{2+} channel (T-type)
Adenylate cyclase	Guanylate cyclase

(Bottari *et al.*, 1992; Kang *et al.*, 1994, 1995; Buisson *et al.*, 1995). Also, AT_1 activates Ca^{2+} channels (L-type and T-type) and inhibits K^+ channels, whereas AT_2 inhibits Ca^{2+} channels (T-type) and activates K^+ channels (the delayed rectifier K^+ current) (Ohnishi *et al.*, 1992; Bottari *et al.*, 1993; Kang *et al.*, 1994, 1995; Buisson *et al.*, 1995). Activation of K^+ channels through AT_2 is known to be mediated by Gi proteins and serine/threonine phosphatase (PP2A), although the pathways that connect PP2A with Gi and K^+ channels remain unclear (Kang *et al.*, 1994, 1995). Activation of AT_2 inhibits T-type Ca^{2+} channels via protein tyrosine phosphatase (Buisson *et al.*, 1995). In this pathway G proteins other than Gi and Go seem to be involved in because activation of the channels was blocked by guanosine 5'-*O*-2-(thio)diphosphate ($GDP_\beta S$) but not by pertussis toxin. These findings indicate the presence of multiple signaling pathways mediated by AT_2.

As AT_1 and AT_2 have opposite effects, we speculate that AT_2 may inhibit cell growth. In fact, AT_2 inhibited proliferation of bFGF-stimulated coronary endothelial cells (Stoll *et al.*, 1995). In our study, when the recombinant AT_2 was introduced into NIH3T3 and PC12 cells, which exhibited no and extremely low Ang II binding activity, respectively, these transfected cells underwent Ang II-dependent inhibition of serum-induced DNA synthesis. These data suggest that AT_2 primarily has an anti-proliferative activity. To date, among the superfamily of seven membrane-spanning receptors only the dopamine D_3 and somatostatin type 1 and type 2 receptors as well as AT_2 are known to have an anti-proliferative effect (Florio *et al.*, 1992; Buscail *et al.*, 1994).

PHYSIOLOGICAL FUNCTIONS OF AT_1 AND AT_2

As described above, *in vitro*, AT_1 exhibits a cell proliferative activity, whereas AT_2 shows an anti-proliferation activity. The cell proliferative activity of AT_1 is thought to be involved in neointima formation in the injured rat arterial wall, which occurs due to the proliferation of smooth muscle cells (Powell *et al.*, 1989; Paul and Ganten, 1992). This is because AT_1-selective antagonists and converting enzyme inhibitors

effectively inhibit the proliferation of these cells and attenuate neointima formation. Also, Ang II stimulates cardiomyocyte hypertrophy and cardiac fibroblast hyperplasia via the cell growth promoting activity of this receptor under patho-physiological conditions (Susic and Frohlich, 1993; Paul and Ganten, 1992). On the other hand, to date, there is no direct evidence that Ang II acts as an anti-proliferative factor through AT_2 *in vivo*. However, it has recently been shown that overexpression of AT_2 induced by transfection of an AT_2 expression vector into the balloon-injured rat carotid artery attenuated neointima formation (Nakajima *et al.*, 1995). This data suggests the possibility that AT_2 may mediate anti-proliferative effects under physiological or patho-physiological conditions. In addition, the abundant expression of AT_2 during fetal and neonatal development prompted us to speculate that this subtype may contribute to not only cell growth regulation but also to cell differentiation.

The AT_1 receptor is known to mediate blood pressure maintenance. The contribution of AT_1 to this role was confirmed using AT_1-deficient mice that display chronic hypotension (Sugaya *et al.*, 1995). Based upon the opposite characteristics of AT_2 and AT_1, one would speculate that AT_2 may induce an opposite effect on the regulation of blood pressure. As expected, very recently, AT_2-deficient mice have been indicated to have significantly higher blood pressure and increased sensitivity to the presser action of Ang II (Hein *et al.*, 1995; Ichiki *et al.*, 1995). Therefore, AT_2 was found to mediate a depressor effect and antagonize the AT_1-induced presser action of Ang II. Indeed, AT_2 is present in the vasculature at low levels and abundantly expressed in the adrenal cortex, both of which play a crucial role in the regulation of blood pressure. Moreover, these mutant mice exhibited attenuated exploratory behavior and had a lower body temperature, indicating the novel AT_2-mediated functions of the RAS in the central nervous system.

We suggested that AT_2 may modulate the onset and/or progression of ovarian follicle atresia involving apoptosis during estrus cycles (Tanaka *et al.*, 1995). The relation of this receptor to apoptosis has recently been demonstrated *in vitro* using PC12W (a substrain of the PC12 cell line) and R3T3 cells (a mouse fibroblast cell line) (Yamada *et al.*, 1996). In this experiment nerve growth factor (NGF) inhibited apoptosis of PC12W cells induced by the removal of serum from the medium. Addition of Ang II overrode the anti-apoptotic effect of NGF via AT_2. The receptor also stimulated apoptosis of R3T3 cells induced by the removal of serum. Morphologic analysis by *in situ* hybridization indicated that the sites of the AT_2 expression overlapped closely with that of a specific group of cells undergoing apoptosis following nephrogenesis in the fetal kidney (Kakuchi *et al.*, 1995). These findings demonstrated that this receptor subtype may be involved in apoptosis *in vivo* in adult and fetal tissues.

Identification of the multiple types of the Ang II receptors raises the possibility that the RAS has other unknown physiological functions. It is of great importance to clarify this issue in the field of clinical science as well as basic science, because the RAS is involved in several cardiovascular diseases. Moreover, AT_1 and AT_2 exhibit unique signaling pathways among the superfamily of seven membrane-spanning receptors: *i.e.* the coupling of AT_1 to the JAK-STAT pathway and the coupling of AT_2 to phosphatase activation. Therefore, elucidation of the signaling events induced by these two types of Ang II receptors will lead to understanding novel signaling pathways mediated by seven membrane-spanning receptors.

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