Molecular Analysis of the Structure and Function of the Angiotensin II Type 1 Receptor

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The renin-angiotensin system hormone angiotensin II (Ang II) plays a central role in the pathophysiology of hypertension, cardiac hypertrophy, congestive heart failure, and coronary heart disease. Two distinct subtypes of Ang II receptor, type 1 (AT1) and type 2 (AT2), have been identified, and both have been shown to belong to the G-protein-coupled receptor superfamily (GPCRs). The recent Human Genome Project has revealed more than 1,000 transmembrane (TM) receptors that belong to this superfamily, and it has been estimated that 50% of all clinically used medicines modulate GPCRs activity. Recently, there have been many new insights regarding Ang II receptors and other GPCRs, such as on homo- and hetero-oligomerization, constitutive activation, movement of TM helices, internalization, desensitization and phosphorylation, trafficking, nuclear localization, intracellular protein-induced receptor activation, and receptor-associated proteins. Although AT1 receptor antagonists which prevent Ang II-induced signaling are already clinically available, we here summarize new findings regarding their structure and function, and the possibility of new therapeutic strategies for targeting Ang II receptors through molecular biological techniques.

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**Key Words:** angiotensin II type 1 receptor, G-protein-coupled receptors, structure and function

Introduction

Recently, there have been many new insights regarding the functions of members of the G-protein-coupled receptor superfamily (GPCRs), such as on homo- and hetero-oligomerization (1), trafficking (2), nuclear localization (3), constitutive activity or spontaneous activity in the absence of an agonist (4, 5), intracellular protein-induced receptor activation (6), and receptor-associated proteins (7).

Two distinct subtypes of angiotensin II (Ang II) receptors, type 1 (AT1) and type 2 (AT2), have been identified, and both have been shown to belong to the GPCRs (8). The idea that AT1 and AT2 receptors exert antagonistic activity is supported by the observation of Ang II-induced cell growth and antigrowth effects, respectively (9, 10). The renin-angiotensin system hormone Ang II, which is potentially cleaved from Ang I by the angiotensin converting enzyme and chymase (11), plays a central role as a major regulator of blood pressure, electrolyte balance, and endocrine function related to cardiovascular disease. Most of its physiological effects are mediated through the AT1 receptor. As for mediating the diverse effects of Ang II receptors, the AT1 receptor undergoes rapid internalization and desensitization upon agonist stimulation (12), whereas the AT2 receptor does not internalize and desensitize, and has constitutive activity (13, 14). AbdAlla et al. reported that the AT1 receptor and bradykinin B2 receptor hetero-oligomer showed enhanced G-protein activation (15). In contrast, the AT1/AT2 receptor hetero-oligomer inhibits AT1 signals, indicating
that the AT2 receptor is an AT1 receptor-antagonist (16).

Although the receptor structure-function relationships vary markedly, changes in seven transmembrane (TM) helical structures on GPCRs are essential for signal transduction. The activation of GPCRs has been proposed to involve a common pattern of movement of TM helices that is likely conserved in all GPCRs (17, 18). Structural changes brought about by TM movement for regulation and activation of the receptor play critical roles in receptor-induced signaling.

AT1 receptor antagonists that prevent Ang II-induced signaling are already clinically available (19–21). Based on molecular biological studies, we here summarize new findings on the structure and function of Ang II receptors and possible new therapeutic strategies for targeting these receptors.

Mode of Interaction between Ang II and AT1 Receptor

The human AT1 receptor contains 359 amino acids and has a molecular mass of 41 kDa. Rat and mouse AT1 receptors also contain 359 amino acids. Figure 1 shows a revised secondary-structure model of rat AT1 receptor based on the structure of bovine rhodopsin (22). The AT1 receptor is only 34% identical to the AT2 receptor and rhodopsin.

Site-directed mutagenesis of recombinantly expressed AT1 receptor combined with the modification of Ang II has led to the identification of several contacts (Fig. 1) between Ang II and the AT1 receptor (23–33). Two salt bridges, one between the Ang II side-chain Arg2 and the AT1 residue Asp281 and the other between Ang II α-COOH and the AT1 residue Lys199, are important for docking the hormone to the receptor (23–27). These salt-bridge interactions do not play a role in AT1 receptor activation. We have shown that two interactions, one between Phe8 of Ang II and His256 in the AT1 receptor (29) and the other between Ang II Tyr4 and Asn111, are necessary to activate the receptor (28, 32, 33).

To systematically evaluate the effect of the modification of Ang II side chains on binding to receptors, we synthesized several analogs of Ang II (23). The binding affinity for the AT1 receptor was affected by alanine modification at positions 2, 4, 6, and 7 ($K_d > 10 \mu\text{mol/l}$). On the other hand, the AT2 receptor had a higher binding affinity for modifications at all positions ($K_d < 10 \mu\text{mol/l}$) (34). Rather surprisingly, the affinity profile of several Ang II analogs toward the AT1 receptor was similar to the measured affinity of the constitutively active mutant AT1-N111G (in which Gly is substituted for Asn111 of the AT1 receptor) receptor (28).
at the position of Asn111 in other GPCRs, such as B2 bradykinin receptor (35), α1B adrenergic receptor (36), and β2 adrenergic receptor (37), show similar changes in ligand binding for agonists.

For G-protein activation, the cytoplasmic loop CD-segment from Tyr127 to Ile130 is important (38). In addition, the integrity of TM IV of the AT1 receptor is critical for specific G-protein selection (39).

Movement of TM Helices in the AT1 Receptor

In prototypical GPCRs, such as rhodopsin, β2-adrenergic receptor, dopamine receptor, α-adrenergic receptor, and muscarinic receptor, the results have suggested that receptor activation involves critical changes in the conformation of TM3 and TM6 (17, 40–42). Ang II binds to AT1 receptor and induces cell signaling accompanied by changes in the TM3-TM6 conformation (43). In addition, in studies using reporter cysteine accessibility mapping, we found that the interaction of TM2 and TM7 is important for TM signal transduction in the AT1 receptor (44, 45). Since the residues corresponding to Asp74 in TM2 and 298NPLFY in TM7 of the AT1 receptor are highly conserved in GPCRs, the functional significance of TM2-TM7 interaction may be common to many GPCRs.

Multiple-Step Theory for AT1 Receptor Activation

Like other GPCRs (27), the AT1 receptor is stabilized in an [R] state (inactive state) and agonist binding causes transition to the [R*] state (fully active state). In the AT1 receptor, we believe that this transition may proceed through a relaxed intermediate activated [R*WT] state (Fig. 3A) (28). Two lines of evidence support this supposition. First, constitutive AT1-N111G mutant receptor-induced high basal activity reflects the [R*WT] state, and involves partial structural changes (Fig. 3B) (28, 32, 33). Second, transition to the fully active state from this [R*WT] state can occur by the binding of Ang II analogs such as [Ile4, Ile8]Ang II to wild-type AT1 receptor can induce only some of the normally induced signals. In collaboration with Dr. Thomas, we found that [Ile4, Ile8]Ang II to wild-type AT1 receptor can induce only some of the normally induced signals. In collaboration with Dr. Thomas, we found that [Ile4, Ile8]Ang II to wild-type AT1 receptor can induce only some of the normally induced signals. In collaboration with Dr. Thomas, we found that [Ile4, Ile8]Ang II to wild-type AT1 receptor can induce only some of the normally induced signals. In collaboration with Dr. Thomas, we found that [Ile4, Ile8]Ang II to wild-type AT1 receptor can induce only some of the normally induced signals. In collaboration with Dr. Thomas, we found that [Ile4, Ile8]Ang II to wild-type AT1 receptor can induce only some of the normally induced signals.
Both signals go into the cytoplasm and lead to the \([R^\*\text{wt}]\) state. Thus, Ang II analogs can induce selective signal transduction and alter the receptor structure to the \([R^\*\text{wt}]\) state, suggesting that a selective signal blocker will be found. We hypothesize here that Ang II-induced AT1 receptor activation occurs through multiple steps. The AT1 receptor might exist in at least three different structures under equilibrium conditions on the cell membrane, and we refer to this notion as the “Multiple-step theory for AT1 receptor activation.”

**Inverse Agonist and the Possibility of Constitutive Activity of the Wild-Type AT1 Receptor**

Wild-type GPCRs, such as \(\alpha_{1B}\) adrenergic receptor, \(\beta_2\) adrenergic receptor, 5-HT1 receptor, and AT2 receptor, display “constitutive activity”—i.e., spontaneous activity in the absence of an agonist (4, 5, 13). Histamine 3 receptor also has constitutive activity, and an inverse agonist has been found (47). Although it is not known whether the wild-type AT1 receptor has constitutive activity, we propose that it may have such activity based on our results with an AT1-N111G mutant receptor-expressing cell model. We found that the activity of the AT1-N111G mutant receptor induced G1 arrest and hypertrophy, and this function was dependent on the amount of receptor present (48). Occupancy by Ang II induces distinct intracellular signals, along with decreased cell size and increased cell number (Fig. 4A). Expression of the wild-type AT1 receptor produced a phenotype that may be similar to that produced by the expression of AT1-N111G receptor. There may be some mechanism by which the Ang II/AT1 receptor system causes a reversible transition between hyperplasia and hypertrophy (Fig. 4B), and thus the constitutive activity of AT1 receptor might induce hypertrophy.

If the AT1 receptor does have constitutive activity, we can use such activity to classify many AT1 receptor antagonists. For example, Exp 3174, an AT1 receptor antagonist (49), but not losartan, was an inverse agonist for inositol phosphate (IP) production against the constitutive activity of the AT1-N111G mutant receptor (Miura S and Karnik SS, unpublished).
published data; Fig. 5A). Thus, Exp 3174, but not losartan, showed inverse agonism for the AT1-N111G mutant receptor, and [R[^N111G]] changed to [R N111G] (Fig. 5B). It is useful to classify clinically available AT1 receptor antagonists with respect to their ability to block basal AT1 receptor function—e.g., as neutral antagonists, partially inverse agonists, or inverse agonists.

**Homo- and Hetero-Oligomerization of the AT1 Receptor**

Recent studies on GPCRs have found that β2-adrenergic, muscarinic, dopamine D2, and opioid receptors undergo homo-oligomerization following agonist stimulation (Fig. 6) (50–53). The presence of homo-oligomerization suggests that it may be possible to modulate receptor function through intermolecular interactions. Although the AT1 receptor oligomerization was agonist-independent and altered AT1 receptor-mediated responses (15, 54), inhibition of the homo-oligomerization of AT1 receptors through the disruption of intermolecular interactions may make it possible to block receptor function.

Hetero-oligomerization refers strictly to interactions between different GPCRs. AbdAlla et al. reported that AT1 receptor and bradykinin B2 receptor hetero-oligomerization showed enhanced G-protein activation (15). Since many different studies have demonstrated that AT2 receptor-induced signaling can counteract the function of AT1 receptors (8), and since AT2 receptor is an antagonist for AT1 receptor signaling through the hetero-oligomerized AT2 receptor and AT1 receptor (16), hetero-oligomerization might explain why the functions of AT1 and AT2 receptors are antagonistic. Interestingly, the detected AT1/AT2 receptor hetero-oligomerization leading to AT1 receptor signal inhibition was independent of the binding of Ang II to AT2 receptor (16), and AT2 receptor has constitutive activity (11), suggesting that the constitutive activity of AT2 receptor itself might induce hetero-oligomerization independent of Ang II stimulation. Therefore, overexpression of AT2 receptor may induce AT1 receptor antagonism through hetero-oligomerization. In addition, a new drug may be discovered that resembles a dimeric ligand formed by two monovalent ligands. Such a ligand would bind and block or stimulate the function of the hetero-oligomerized receptor (Fig. 6) (1).

**Conclusions**

We have summarized new findings on the structure and function of Ang II receptor activation. AT1 receptor antagonists which prevent Ang II-induced signaling are clinically available. It may be possible to create new therapeutic strategies for inhibiting AT1 receptor function, such as a cell signal selection by Ang II analog, a classification of AT1 receptor antagonists, overexpression of AT2 receptor-induced AT1 receptor antagonism, inhibition of homo-oligomerization, or the discovery of a dimeric ligand. A good understanding of the molecular-level structure and function of GPCRs, including Ang II receptors, may lead to the discovery of better therapeutic agents.

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