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Molecular Mechanism of Attenuated Inverse Agonism of ARBs for Active-State of AT1 receptor

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ABSTRACT

Typically AngII the octapeptide hormone produced by the renin angiotensin system binds to angiotensin II type 1 receptor (AT1R) and activates its functions which can be competitively inhibited by AT1R blockers (ARBs). However several studies have demonstrated ligand-independently activated AT1R in clinical setting such as mechanical stretch and auto-antibodies as well as receptor mutations. Clinically used ARBs prevent ligand-independent activation of the AT1R by inverse agonistic effect with variable efficacies. Ligand-independent transition of AT1R to activated state is known to attenuate inverse agonistic efficacy of the ARBs but the molecular mechanism is unknown. Therefore, identifying the molecular basis of reduced inverse agonist efficacy of ARBs for the active-state of the AT1R provided a fundamental insight for application of ARBs in treatment of diseases as well as for future drug development. Since AT1R is an extensively studied member of G-protein coupled receptor superfamily encoded in human genome the new regulatory mechanisms of inverse agonist function we describe is relevant to disorders caused by other members of this superfamily. In this review, we focus on the molecular mechanism of attenuated inverse agonism of the ARBs.

INTRODUCTION

The angiotensin II (Ang II) type 1 receptor (AT1R) belongs to G-protein coupled receptor (GPCR) superfamily. The AT1R regulates blood pressure, body-fluid homeostasis and plays vital roles in cardiovascular and renal pathophysiology. Binding of the octapeptide hormone Ang II is traditionally known to activate the AT1R. However, recent studies have demonstrated that mechanical stress and AT1R-directed autoantibodies can activate AT1R in absence of classical agonist-binding **(Figure1).**[1-4] Both modes of ligand-independent activation of AT1R may occur clinically as in hypertension, cardiac overload conditions or in preeclampsia which can be reduced by actions of inverse agonists such as, Candesartan[5,6] but not very efficaciously by neutral antagonist ARBs. The mechanism for such differences is unknown. Mutation-induced ligand-independent activation is known to cause conformational change in the AT1R and markedly reduce the efficacy of the inverse agonists. However, the molecular mechanism for a decrease of the inverse agonist efficacy of ARBs for activated state of AT1R was not clear until our recent study[7]. In this review, we focus on a potential molecular mechanism for attenuated inverse agonism of four biphenyl-tetrazol ARBs Losartan, EXP3174, Valsartan and Irbesartan **(Figure 2)** for activated-state AT1R.

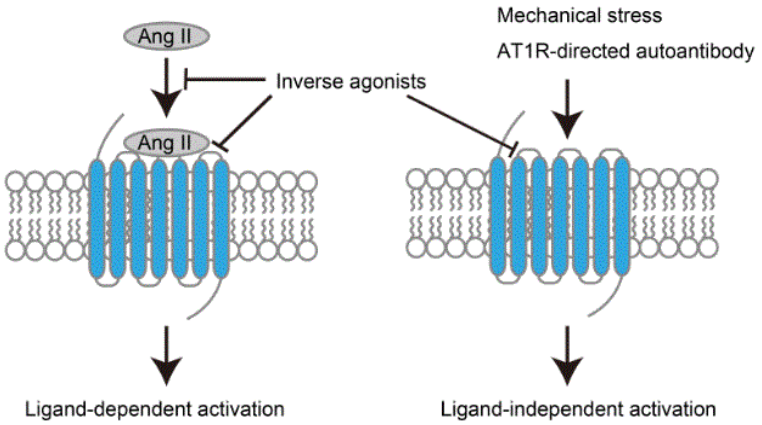

Figure 1. Ligand-dependent and ligand-independent activation of the AT1R. Ang II binds to the AT1R and causes ligand-dependent AT1R activation. On the other hand, mechanical stress and AT1R-directed autoantibody cause ligand-independent AT1R activation. Inverse agonists block not only Ang II binding and ligand-dependent activation (indicated by two different arrows on the left side, I assume) but also ligand-independent AT1R activation.

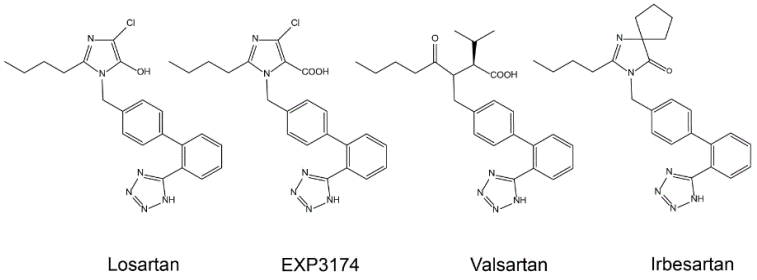

Figure 2. The chemical structures of Losartan, EXP3174, Valsartan and Irbesartan. All four biphenyl-tetrazole group ARBs share a structure with biphenyl tetrazole.

MOLECULAR MECHANISM OF INVERSE AGONISM OF THE BIPHENYL-TETRAZOLE GROUP ARBS FOR AT1R IN GROUND STATE

Since the crystal structure of the AT1R was not available, previous studies analyzed molecular model of ligand/AT1R complexes based on crystal structure of other GPCRs such as bovine rhodopsin, β2-adrenergic receptor and CXCR4.[8-13] However, crystal structure of the human AT1R bound to the biphenyl-tetrazole group ARB ZD7155[14] was recently solved. This landmark achievement allowed us to analyze docking models of Losartan, EXP3174, Valsartan and Irbesartan in the AT1R based on determined structure[7]. The ARB/AT1R complexes indicate that the interactions of the four biphenyl-tetrazole group ARBs with the AT1R are completely different from previously proposed the ARB-AT1R interactions. Canonical ARB binding pocket of AT1R consists of interacting residues of trans membrane (TM)-helices I-VII as well as mainly from second extracellular loop (ECL2). The tetrazole group, a common acidic moiety present in all four biphenyl-tetrazole group ARBs, interacts with Arg167 in ECL2. The complex structures suggest that flexible side chain of Lys199 in TM5 retains some conformational heterogeneity in AT1R, that the amino group of this residue can form salt bridges with acidic moieties of ARBs or participate in water-mediated interactions with biphenyl scaffold in ARBs. The imidazole ring of Losartan and EXP3174 and equivalent substituents in Irbesartan interact with Trp84 in TM2 and floor of the ligand pocket including residues Tyr292 in TM7 and Asn295 in TM7, The short alkyl tails of four biphenyl-tetrazole group ARBs interact with Tyr35 in TM1, and the biphenyl rings of four biphenyl-tetrazole group ARBs interact with Val108 in TM3 and Ser109 in TM3 as well as with Trp253 in TM6 and Gln257 in TM6. In addition, all four biphenyl-tetrazole group ARBs may hydrophobically interact with the residues, Tyr113 in TM3, Phe182 in ECL2, Tyr184 in ECL2 and His256 in TM6.

The crystal structure of human AT1R demonstrated that a hydrogen bond (H-bond) between Asn111 in TM3 and Asn295 in TM7 stabilize the AT1R in an inactive state[14]. Activation of the AT1R disrupts this H-bond leading Asn295 in TM7 to interact with the conserved Asp74 in TM2 and forms the Asn46-Asp74-Asn295 H-bond network. This H-bond network in active state involves additional residues, Trp253 in TM6 from the "toggle-switch" motif[15,16], Phe77 in TM2, Val108 in TM3, Ile288 in TM7 and Tyr292 in TM7 and Asn298 in TM7 from the NPxxY motif. [13,14]Thus, the network of interacting residues around Asn111 in TM3 and Asn295 in TM7 play an essential role in AT1R activation, probably by relaying the conformational changes in the ligand-binding pocket to the cytoplasmic domain coupling to the G proteins. This network may also impact the inter-helical interactions required for the binding and functional properties of four biphenyl-tetrazole group ARBs as well consequent inactivation of the AT1R. We propose that the tight interaction of the four biphenyl-tetrazole group ARBs with a set of residues Ser109 in TM3, Phe182 in ECL2, Gln257 in TM6, Tyr292 in TM7 and Asn295 in TM7 constrains this network, thereby leading to stabilize inactive state of the receptor- and results in potent inverse agonism in the ground state of the AT1R. All residues except for Phe182 in ECL2 involved in inverse agonism of four biphenyl-tetrazole group ARB in AT1R are conserved at equivalent position in many GPCRs, implying that this may

be a general mechanism for inverse agonism of the GPCRs. On the other hand the role of Phe182 in ECL2 in the inverse agonism of four biphenyl-tetrazole group ARBs may be unique to AT1R but seems to be supported by previous functional studies[17,18] and by the X-ray structure of AT1R.[14]

Molecular mechanism of attenuated inverse agonism of the biphenyl-tetrazole group ARBs for activated state AT1R

Transition of AT1R to activated state attenuates inverse agonistic effect of four biphenyl-tetrazole ARBs through changes in specific ligand-receptor interactions. Conformational changes in the ligand binding pocket in the activated state compared to the inactive state have been identified in crystal structures of agonist-bound β2 adrenergic receptor, light-activated rhodopsin, the constitutively active rhodopsin mutant and the agonist-bound adenosine A2A receptor.[19-22] By analogy to these GPCRs, we suggest that active-state of AT1R harbors conformational changes in the ligand binding pocket. Furthermore, direct structure-function studies on AT1R have suggested both rotational and translational motion of TM2, TM3, TM5, TM6 and TM7 in the N111G-AT1R [23-26]. Based on molecular dynamics simulation studies on a constitutively active mutant N111G-AT1R, an active-state H-bond network where Asp74 in TM2 interacts with Asn46 in TM1 and Asn295 in TM7 was proposed[13]. The same study also proposed that the N111G mutation leads to hydrate the hydrophobic core and facilitate the interaction of the "toggle switch" residue, Trp253 in TM6 with Ala291 in TM7 and Leu112 in TM3[13]. All four biphenyl-tetrazole group ARBs may thus prevent stability of the Asn46-Asp74-Asn295 H-bond network and reduce hydration of the TM core through their hydrophobic characteristics. However, active-state transition in AT1R shift the ARB-AT1R interactions to a different set of residues, Val108 in TM3, Ser109 in TM3, Ala163 in TM4, Phe182 in ECL2, Lys199 in TM5, Tyr292 in TM7 and Asn295 in TM7 from the interaction of the ARBs with a set of residues Ser109 in TM3, Phe182 in ECL2, Gln257 in TM6, Tyr292 in TM7 and Asn295 in TM7 in the ground state **(Figure 3)**. We propose that change of ARB-AT1R interactions in active-state of the AT1R attenuate preventive effect for stability of the Asn46-Asp74-Asn295 H-bond network and reduce hydration of the TM core through their hydrophobic characteristics, resulting in attenuated inverse agonism of four biphenyl-tetrazole group ARBs.

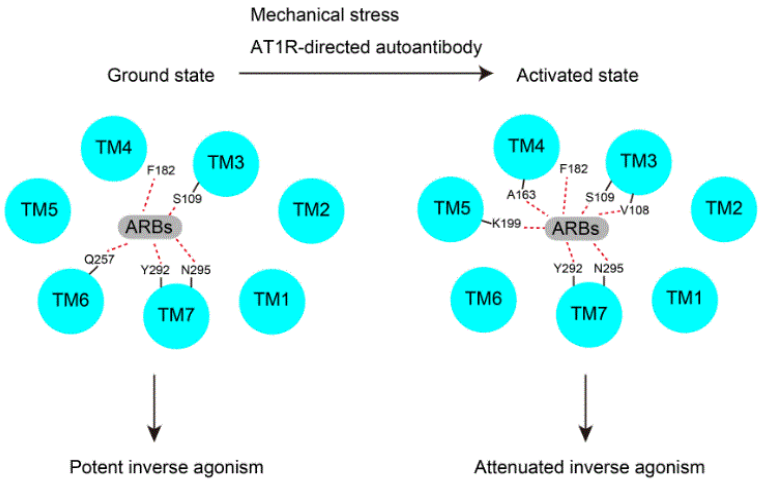

Figure 3. Difference between ARB-receptor interactions in the ground state and in the activated state of the AT1R. Seven TM are viewed from extracellular side. The interaction of the ARBs with a set of residues Ser109, Phe182, Gln257, Tyr292 and Asn295, results in potent inverse agonism in the ground state. However, ligand-independent activation of the AT1R shift the ARB-AT1R interactions to a different set of residues, Val108, Ser109, Ala163, Phe182, Lys199, Tyr292 and Asn295, resulting in attenuated inverse agonism.

CONCLUSION

The ARBs are the well-known clinically used anti-hypertension drugs. The ARBs causes therapeutic effect by not only blocking Ang II binding to AT1R but also inverse agonistic effect. Although the AT1R can be ligand-independently activated in clinical setting by mechanical stretch and auto-antibodies as well as receptor mutations, since active-state AT1R transition attenuate the inverse agonism of biphenyl-tetrazole group ARBs, commercially available ARBs may not show enough therapeutic effect for clinical settings in which ligand-independent activation of AT1R such as hypertension, preeclampsia and renal transplantation. Therefore, novel ARBs that cause potent inverse agonist efficacy for activated state AT1R than the current commercially available ARBs need to be developed. This review provides significant information for developing potent inverse agonists for active state of AT1R.

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CONFLICT OF INTEREST

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