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REVIEW

Insights Into the Activation and Inhibition of Angiotensin II Type 1 Receptor in the Mechanically Loaded Heart

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In the heart, mechanical load is a crucial regulator of myocardial structure and function; however, mechanical overload is a pathogenesis or comorbidity existing in a variety of heart diseases, such as hypertension, aortic regurgitation and myocardial infarction. Mechanical overload can be generally differentiated into 2 types, pressure overload (PO) and volume overload (VO), causing concentric and eccentric cardiac hypertrophy, respectively. The angiotensin II (AngII) type 1 receptor (AT1-R) is a 7 transmembrane G protein-coupled receptor that plays a critical role in load-induced cardiac hypertrophy. Early studies revealed the involvement of autocrine/paracrine mechanisms through stretch-induced release of AngII. Recent conceptually inspiring studies unraveled that the AT1-R could be also directly activated by mechanical stress. The activated AT1-R initiates intricate intracellular signaling pathways through G protein-dependent and G protein-independent mechanisms. AT1-R blocker (ARB) antagonizes the activation of AT1-R to regress cardiac remodeling. Some ARBs show properties of inverse agonism and arrestin-biased agonism at the AT1-R, which are potential therapeutic targets for the treatment of load-induced cardiac hypertrophy. This review summarizes the progress in the understanding of ligand- and mechanical stress-dependent activation of AT1-R, highlights recent data that investigate the role of AT1-R in the differentiation of PO- and VO-induced cardiac hypertrophy, and discusses the clinical relevance of inverse agonism and biased agonism of AT1-R ligands. (*Circ J* 2014; **78:** 1283–1289)

Key Words: Angiotensin II type 1 receptor; Cardiomyocyte hypertrophy; Inverse agonism; Mechanical stress

Angiotensin II (AngII) Type 1 Receptor (AT1-R) Plays a Central Role in the Loaded Heart

The renin-angiotensin system (RAS) is crucially involved in cardiovascular homeostasis and the development of cardiovascular diseases.[1,2] AngII, an octapeptide hormone, is the primary bioactive molecule of the RAS.[2,3] AngII mediates its multifarious effects through 4 AngII receptors (AT1-R, AT2-R, AT3-R and AT4-R).[4] In the cardiovascular system, the physiological and pathological actions of AngII are predominantly through AT1-R.[5] AT1-R is a typical member of the G protein-coupled receptor (GPCR) family. It is structurally characterized by an amino (N)-terminal extracellular domain, 7 transmembrane (TM)-spanning α-helices comprising 3 intracellular and 3 extracellular loops, and a carboxyl (C)-terminal intracellular tail domain.[6] The AT1-R expressed in humans and rodents contains 359 amino acids and has a molecular mass of 41 kDa. Human AT1-R is approximately 95% identical to that of rodents.[3,7] Although most species, including humans, only have 1 subtype of AT1-R, rodents express 2 subtypes (AT1A-R and AT1B-R).[3] It is believed that AT1A-R is required for vascular and hemodynamic responses to AngII,[8] whereas AT1B-R is more mechanosensitive.[9] AngII and mechanical stress can exert agonistic action on the AT1-R to stimulate intracellular effector systems and induce cardiac hypertrophy.[5]

AngII Mediates the Activation of AT1-R in the Stressed Heart

The AT1-R plays a crucial role in load-induced cardiac hypertrophy.[1] AngII is canonically generated by angiotensin-converting enzyme (ACE), but other AngII-forming enzymes (kallikrein, trypsin, tonin, cathepsin G) also generate AngII.[3] It is known that AngII can activate the AT1-R to induce pro-hypertrophic effects, because AngII in vitro is sufficient to cause cardiomyocyte hypertrophy,[10] and AngII infusion in vivo causes cardiac hypertrophy through AT1-R signaling, independent of blood pressure elevation.[11] In the heart, AngII is stored in the secretory granules of cardiomyocytes, as well as existing at a low level in the microcirculation. Many pathological stresses, including mechanical stretch, induce secretion of the stored AngII.[10] The systemically and locally generated AngII binds to the AT1-R and changes its conformational structure into an active formation, stimulates diverse intracellular signaling via $Ga_{q/11}$ protein, tyrosine protein kinase and arrestin proteins, elicits the immediate-early genes (eg, *c-fos*, *c-jun*, *jun B*, *Egr-*

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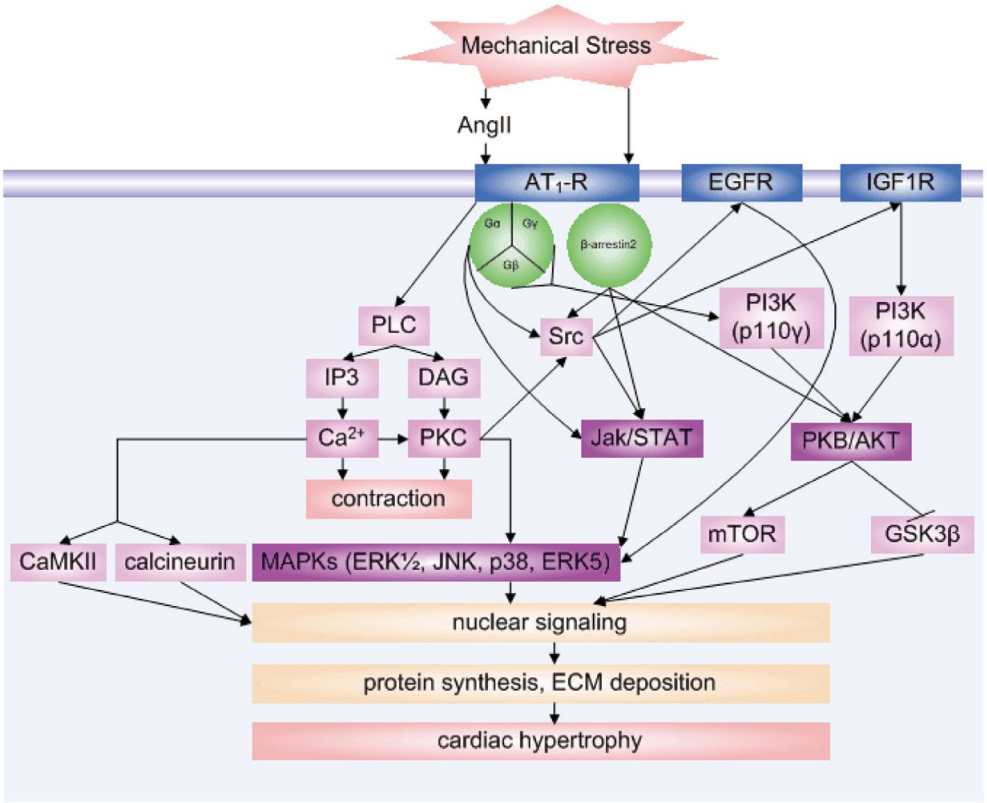

Figure 1. Angiotensin II (AngII) type 1 receptor (AT1-R) is activated through AngII and directly by mechanical stretch in the mechanically loaded heart. Activation of AT1-R triggers downstream intracellular signaling pathways that coordinate the cardiac hypertrophic response. CaMKII, calmodulin dependent kinase II; DAG, diacylglycerol; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; GSK3β, glycogen synthase kinase-3β; IGF1R, insulin-like growth factor-1 receptor; IP3, inositol-1,4,5-trisphosphate; Jak, Janus family kinase; JNK, c-Jun N-terminal kinase; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; STAT, signal transducers and activators of transcription.

1, *c-myc*) and the later-induced fetal-type genes (eg, atrial natriuretic factor and skeletal α-actin), and then induces protein synthesis and cardiomyocyte hypertrophy[2,10,12,13] (**Figure 1**).

Mechanical Stress Activates AT1-R Without the Involvement of AngII

The aforementioned stretch-AngII-AT1-R connection has long been viewed as a solid doctrine. However, a growing body of studies have challenged the paradigm and presented conceptually novel findings. Almost 2 decades ago, Yamazaki et al found that AngII only partly mediated mechanical stretch-induced cardiomyocyte hypertrophy, implying that there were other mechanisms of inducing cardiac hypertrophy.[14] Later, several studies reported some differences between AngII- and mechanical stretch-induced signaling pathways (*c-fos*, some RAS components) in cardiomyocytes in vitro.[15,16] Moreover, subpressor dose of AngII increased left ventricular (LV) mass in wild-type (WT) mice but not in *AT1A-R* knockout (KO) mice, whereas pressure overload (PO) fully induced LV hypertrophy in both KO and WT mice.[17]

This evidence presented a looming challenge: can the AT1-R be directly activated by mechanical stress? To solve the enigma, we first measured AngII concentration in the culture medium, but did not find a significant increase in AngII concentration after stretch. Coincidently, we observed that a neutralizing antibody of AngII abolished the AngII- but not stretch-induced activation of extracellular signal-regulated protein kinases (ERKs). Those findings suggested that locally generated AngII played a marginal role in the full activation of the AT1-R after stretch in cardiomyocytes.[1] We then found neither mechanical stretch nor AngII activated ERKs in human embryonic kidney 293 (HEK293) cells showing no detectable AT1-R, but both of them activated ERKs in HEK293 cells with WT mouse AT1-R. Moreover, pretreatment with candesartan, an AT1-R blocker (ARB), inhibited the activation of ERKs induced not only by AngII but also by mechanical stress.[1] Intriguingly, (Sar^1,Ile^8)-AngII, a competitive inhibitor for AngII, abrogated AngII-induced but not stretch-induced ERK activation in HEK293 cells expressing AT1-R.[1] We further imposed mechanical stretch and AngII on HEK293 cells expressing a mutant AT1-R whose binding site Lys^{199} was replaced by glutamine, and found that AngII did not activate ERKs in such cells but mechanical stretch did. Those findings strongly implied a role of the AT1-R as a mechanoreceptor without AngII.[1] To further confirm that notion, stretch was imposed to activate ERKs in cardiomyocytes from angiotensinogen-deficient ($ATG^{-/-}$) mice, in which AngII was not detected. In addition, 2-week PO in the heart by transverse aorta constriction (TAC) induced sig-

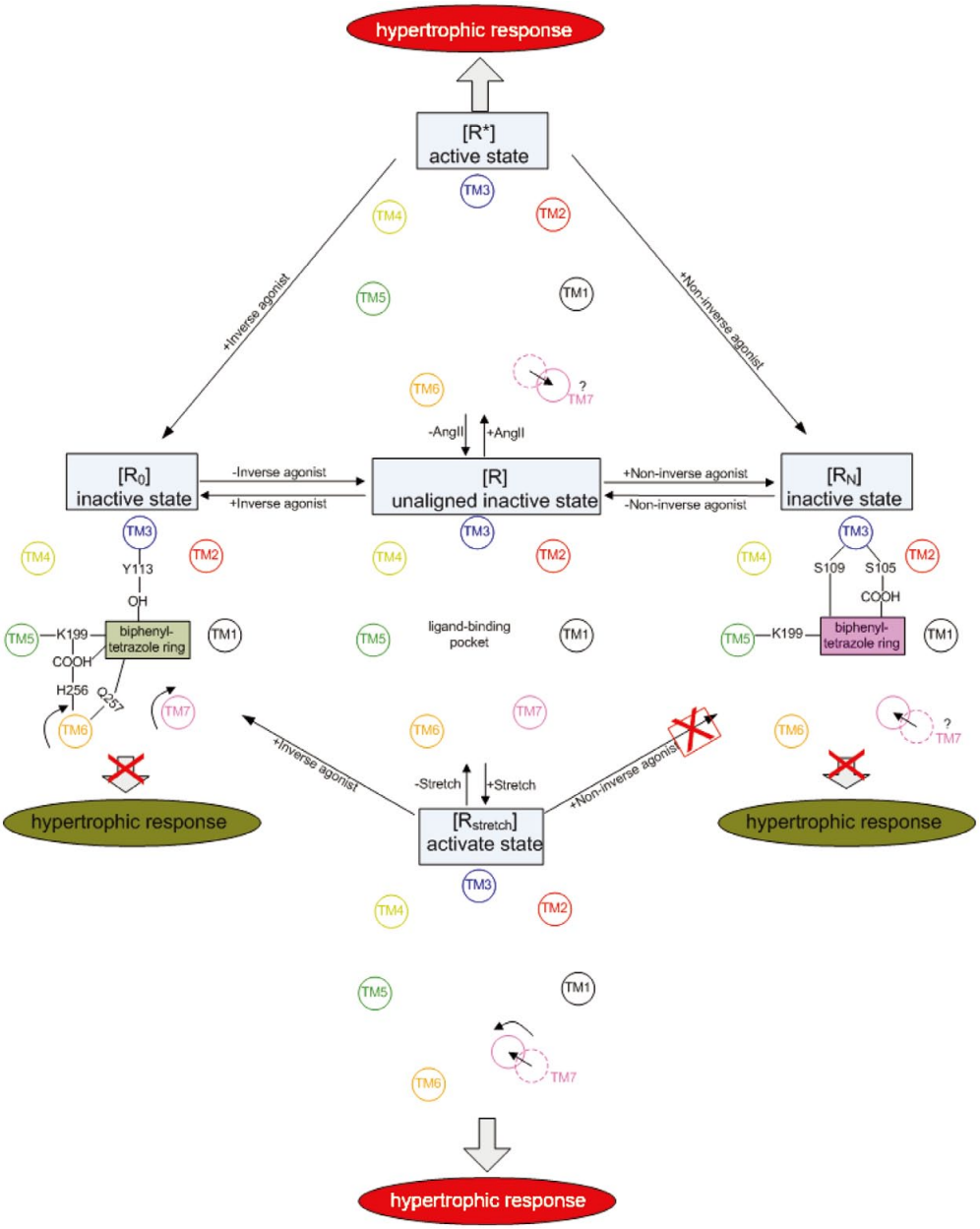

Figure 2. Conformational changes of the angiotensin II (AngII) type 1 receptor (AT1-R). [R] is an unaligned inactive state (basal state). [R_0] is an inactive state stabilized by an inverse agonist. [R_N] is another inactive state stabilized by a non-inverse agonist. [R*] is an active state stabilized by agonist AngII. [$R_{stretch}$] is another active state stabilized by mechanical stretch, without the involvement of AngII. An inverse agonist can forcibly induce a distinct transition from [R] to [R_0], and prevent a shift of equilibrium to [$R_{stretch}$] or [R*]. TM, transmembrane domain.

nificant LV hypertrophy in *ATG*$^{-/-}$ mice. In contrast, candesartan pretreatment markedly suppressed the activation of ERKs and cardiac hypertrophy in vivo.[1] Collectively, the findings presented compelling evidence that the AT1-R is activated by mechanical stress without the involvement of AngII.

The AT1-R is the first recognized mechanosensitive GPCR.[1,13] Activation of several other GPCRs, such as the receptors of endothelin-1 (ET-1) and isoproterenol, also induces cardiomyocyte hypertrophy.[14] Nevertheless, mechanical stretch did not significantly activate ERKs in COS7 cells expressing either the ET-1 type A receptor or the β2-adrenoceptor.[1] Those results suggested that mechanical stretch-induced activation is not a general phenomenon but specific to only some GPCRs, including the AT1-R. The mechanism for the distinctive property of the AT1-R remains unknown, but is probably related to the diversity in the expression and structure of the GPCRs.

There are several possibilities for how the AT1-R directly perceives mechanical stress. First, cell membrane tension directly causes thinning of the membrane lipid bilayer. To avoid hydrophobic mismatch and to rectify the lateral pressure pro-

file, the conformation of the AT1-R changes.[5] Second, mechanical stretch may activate specific stress sensors, which subsequently activate the AT1-R. It is known that some ion channels, integrins and muscle LIM protein are stress sensors that can be involved in load-induced cardiac hypertrophy.[1,8]

Different Active Conformations of AT1-R Under Mechanical stress and AngII Effects

AngII binds to the AT1-R mainly by 4 unique interactions.[7] Two of the interactions are salt bridges: (1) the AngII side chain Arg^2 and the AT1-R residue Asp^{281}; (2) the AngII α-COOH and the AT1-R residue Lys^{199}. They are important for the docking of AngII towards the AT1-R, but not necessary for AT1-R activation.[7,18] The other 2 interactions, 1 located between AngII Phe^8 and AT1-R His^{256} and the other located between AngII Tyr^4 and AT1-R Asn^{111}, are necessary for AT1-R activation.[7,18] More specifically, for AngII docking, the Tyr^4:Asn^{111} interaction plays a more significant role because an amino-aromatic bonding operates at the Tyr^4 switch of AngII and a stacked rather than an amino-aromatic bonding operates at the Phe^8 switch locus.[18]

Mechanical stress induces counterclockwise rotation of the TM domain 7 (TM7). The Cys^{289} residue, originally facing in the direction of TM1, becomes accessible to the ligand-binding pocket of the AT1-R. As Ile^{288} becomes more accessible after stretch, TM7 shifts inside the ligand-binding pocket. The movements of TM7 cause intracellular signaling pathways.[5–7] In contrast, TM7 shifts away from the ligand-binding pocket while the AT1-R is partly activated by AngII.[5–7] Therefore, the active conformation of the AT1-R induced by mechanical stretch is probably different from that induced by AngII (**Figure 2**).

Downstream Signaling Pathways of the AT1-R

The activated AT1-R triggers numerous G protein-dependent and G protein-independent downstream signaling pathways. The heterotrimeric G protein comprises a Gα subunit, of which there are 4 main families (Gα_s, G$\alpha_{i/o}$, G$\alpha_{q/11}$, and G$\alpha_{12/13}$), coupled to both Gβ and Gγ subunits.[19] G$\alpha_{q/11}$ is critically involved in cardiac remodeling under mechanical stress.[19,20] Overexpression of Gα_q independently induces hypertrophy and apoptosis in cultured cardiomyocytes and transgenic mouse hearts.[21] Conversely, inhibition of G$\alpha_{q/11}$ signaling blunts or even completely prevents PO-induced cardiac hypertrophy in the heart.[22,23] The activated AT1-R via G$\alpha_{q/11}$ and G$\alpha_{12/13}$ stimulates the mitogen-activated protein kinase (MAPK) family, including ERKs (ERK1/2 and ERK5), c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38).[5,20,24] ERKs, JNK, and p38 can translocate to the nucleus to phosphorylate stress-responsive transcription factors, leading to reprogramming of cardiac gene expression as part of the hypertrophic response.[20,25] Activated ERKs promote protein synthesis that is associated with compensatory cardiac hypertrophy, whereas JNK, and p38, may act in the longer term.[5,20] The roles of JNK and p38 are still controversial.[26] G$\alpha_{q/11}$ is also coupled to phospholipase Cβ (PLCβ) for phosphatidylinositol hydrolysis, leading to formation of diacylglycerol (DAG), which activates protein kinase C (PKC), and production of inositol-1,4,5-trisphosphate (IP3), which elevates the intracellular Ca^{2+} concentration.[20] This elevation mediates hypertrophic signaling through calcineurin-NFAT activation and calmodulin-dependent kinase II (CaMKII)-histone deacetylase (HDAC) inactivation.[27,28] Specifically, calcineurin is involved in AT1-R-mediated cardiac hypertrophy induced by mechanical stress independent of AngII.[27] AT1-R-stimulated Ca^{2+} and PKC also induces their actions through the Src family of nonreceptor tyrosine kinases, resulting in receptor tyrosine kinase transactivation, Ras-ERK signal transduction, DNA translation and protein synthesis[19,29] (**Figure 1**).

G protein-independent signaling molecules are also essential for mechanical overload-mediated cardiac remodeling.[30] AT1-R activation stimulates multiple G-protein-independent signaling pathways such as activation of β-arrestins, tyrosine kinases (Jak/STAT, Src), and transactivation of epidermal growth factor receptor (EGFR) and insulin-like growth factor-1 receptor (IGF1R).[5,24,30–32] β-arrestins initiate β-arrestin-biased agonism at the AT1-R in the absence of detectable G protein activation. Both G protein- and β-arrestin2-mediated pathways can lead to ERK1/2 activation.[32] β-arrestin2, rather than β-arrestin1, mediates inotropic effects of the AT1-R in isolated cardiomyocytes.[33] Mechanical stretch induces β-arrestin2-biased ERK1/2 signaling of the AT1-R in the absence of AngII or G protein activation.[32] AngII-induced cell proliferation is dually mediated by Src/Yes/Fyn tyrosine kinases-regulated ERK1/2 activation in the cytoplasm and PKCζ-controlled ERK1/2 activity within the nucleus.[34] Src can be activated in a Gα_q protein- or β-arrestin2 dependent manner for EGFR transactivation.[35,36] We recently also confirmed that β-arrestin2-dependent Src activation is a prerequisite for mechanical stretch-induced ERK1/2 phosphorylation.[31]

The AT1-R stimulates tyrosine phosphorylation of the Janus family kinases (Jak1, Jak2, Jak3, and Tyk2).[12] Both AngII and mechanical stretch activate the AT1-R to induce the physical association and activation of Jak2, resulting in the phosphorylation of signal transducers and activators of transcription (STAT) proteins, which translocate to the nucleus and activate gene transcription.[1,12]

The phosphatidylinositol 3-kinase (PI3K)-Akt pathway regulates cardiac hypertrophy through inhibition of glycogen synthase kinase-3β (GSK3β) and activation of the mammalian target of rapamycin (mTOR).[20] The PI3K-Akt pathway can be activated by AngII through G$\beta\gamma$, IGF1R, and β-arrestin2.[20,24,32,37] Overexpression of activated PI3Kα generates a form of physiological cardiac hypertrophy, whereas PI3Kγ contributes to the generation of pathological hypertrophy and cardiac contractile dysfunction during PO.[38] The discrepancy between G protein- and arrestin-induced Akt activation and subsequent effects remains to be elucidated.

Although several effector molecules (RAS components, *c-fos*, β-arrestin2) are differently induced by mechanical stretch vs. AngII manipulation,[15,16,31] it is common that 1 ligand can trigger G protein-dependent and β-arrestin2-dependent pathways to induce the hypertrophic response.[35] It is intriguing that AngII in vitro causes cardiomyocyte hypertrophy with a phenotype indistinguishable from stretch-induced hypertrophy.[10] Moreover, the AT1-R in vivo is activated via AngII or directly by mechanical stretch in the mechanically loaded heart.[1,2] Thus, whether a specific ligand for the AT1-R would improve or worsen cardiac remodeling, through a G protein-dependent or G protein-independent manner, awaits particular assessment before therapeutic use.

Parsing the Roles of the AT1-R in Hearts With Differential Loading Conditions

Mechanical stretch is the primary trigger of the hypertrophic response in the loaded heart.[1] Two types of load, PO and volume overload (VO), can be differentiated.[39] Both PO and VO exert mechanical stretch on the heart to stimulate cardiac hypertrophy. However, PO happens during systole (afterload) and induces concentric cardiac hypertrophy, whereas VO occurs

during diastole (preload) and produces eccentric cardiac hypertrophy.[39,40] The reasons for that discrepancy remain to be elucidated. Given the critical role of the AT1-R in the development of cardiac hypertrophy, increasing evidence shows how the AT1-R is involved in PO- and VO-induced cardiac hypertrophy.

The AT1-R, as well as many other RAS components, such as angiotensinogen, rennin, AngII, ACE, are expressed in cardiomyocytes and upregulated by PO because of aortic coarctation in the heart.[17,41] G protein-dependent and -independent downstream signaling pathways (ERK1/2, ERK5, JNK, p38, PKC, calcineurin, CaMKII, Jak/STAT, Src, PI3Kγ-Akt) of the AT1-R are activated and promote cardiac hypertrophy under PO.[5,20,25,27,28,31,32,39] In basal conditions, the cardiomyocyte-specific over transgene (TG) of the AT1-R induces conspicuous morphologic changes of myocytes and nonmyocytes that mimic the changes observed during the development of cardiac hypertrophy.[42] In a rat model with a lower level of TG expression of the human AT1-R, cardiac changes were minimal under basal conditions. However, when challenged with PO, the heart showed enhanced hypertrophy in TG rats vs. corresponding WT animals.[43] Interestingly, murine hearts with genetic deletion of the AT1A-R neither show significant difference under basal conditions nor demonstrate a significant difference in hypertrophic response to PO, compared with corresponding WT mice.[17] Those findings suggest there are AT1-R-dependent and -independent pathways in the PO heart. Meanwhile, the AT1-R-independent pathway can substitute for the AT1-R-dependent pathway.[17] That notion seems at odds with the great efficacies of ARBs, which effectively downregulate the activation induced by PO in patients and animals.[6,44,45] The paradox between KO mice and pharmacologically manipulated mice may be attributed to ARB blockade not being equivalent to *AT1-R* KO.[46] Genetic deletion of the AT1-R from early embryogenesis may induce a compensatory ability to use AT1-R-independent or/and AT1B-R-dependent pathways for the cardiac hypertrophic response, which is also partly reflected by the fact that, under basal conditions, no difference is found in the cardiac phenotypes of KO and WT mice.

The RAS is also activated in cardiac hypertrophy caused by VO.[47–49] Intriguingly, no difference was found in AT1-R mRNA in the LV in a rat model of VO induced by aortocaval shunt.[47] In rats with TG expression of the human AT1-R, the level of the receptor was unchanged in the hearts challenged with aortocaval shunt.[43] Eccentric hypertrophy in experimental VO showed that the G protein-dependent and -independent downstream signaling pathways are different from that in PO-induced concentric hypertrophy. In mouse hearts under the effect of aortocaval shunt or aortic regurgitation, the activation of JNK, p38 and PKC increases, while that of ERK1/2, ERK5, calcineurin, and CaMKII decreases or is unaltered.[39,50,51] However, another study showed increased phosphorylated ERK1/2 in the heart in a rat aortocaval shunt model at 4 weeks and 16 weeks after surgery.[52] The reasons for the discrepancy may be the differences in observation times and animal model (aortocaval shunt vs. aortic regurgitation). Specifically, VO-induced cardiac hypertrophy is characterized by activation of Akt signaling, which is similar to that in physiological hypertrophy.[39,51] Transgenic rats with overexpressed human AT1-R exhibit normal cardiac growth and function under basal conditions, but show more pronounced hypertrophic responses when exposed to VO induced by aortocaval shunt.[43] In contrast, although the hearts from genetic AT1A-R KO mice are phenotypically similar to WT mice, the KO mouse hearts show significantly attenuated LV hypertrophy and dilatation, and less interstitial fibrosis under VO induced by aortic regurgitation.[53] Correspondingly, ARBs have paved a promising path in the treatment of VO cardiac hypertrophy.[46,54]

The widespread experimental findings and clinical applications of ARBs to ameliorate cardiac remodeling under PO and VO are testament to the importance of the AT1-R. Yet, questions remain. It seems that genetic deletion of the AT1-R attenuates VO but not PO cardiac hypertrophy.[17,53] Does that suggest the AT1-R differently perceives and deciphers the initial stretch signals into intracellular signaling networks during systole and diastole? Solving the riddle may also help answer why AT1-R expression is upregulated in PO but negligibly changed in VO.

ARB, Biased Agonist and Cardioprotection: Significance and Perspectives

ARB Development

ARBs are nonpeptide compounds that selectively bind to the AT1-R. Several highly effective ARBs (losartan, valsartan, irbesartan, candesartan, telmixatan, azilsartan, eprosartan and olmesartan) are clinically available for preventing the development of various cardiovascular diseases. Many basic and clinical studies have indicated that ARBs improve cardiovascular outcomes.[45,55]

ARBs effectively inhibit AngII-induced AT1-R activation. They ameliorate the peripheral vascular resistance, cardiovascular hyperplasia and hypertrophy by blocking the deleterious effects of AngII. Meanwhile, blockage of the AT1-R directly causes vasodilation, and reduces secretion of vasopressin and aldosterone, leading to downregulation of blood pressure.

Recent studies have found that most GPCRs, including the AT1-R, are structurally unstable and show spontaneous constitutive activity in an agonist-independent manner.[1,55] Furthermore, the constitutive activity of the AT1-R under basal conditions promotes cardiac remodeling even in the absence of AngII, when the AT1-R is upregulated in the heart.[56] ARBs that suppress the basal activity of the AT1-R are defined as inverse agonists. They are capable of stabilizing the inactive conformation of the AT1-R,[45] thus embracing the property of suppressing both AngII-dependent and -independent activities. That property makes some ARBs mechanically different from traditional ARBs that only inhibit AngII-dependent activities (**Figure 2**). We previously reported that mechanical stretch activated the AT1-R through a conformational switch without the involvement of AngII. That AngII-independent AT1-R activation is relevant to the pathogenesis of cardiac remodeling and can be blocked by inverse agonists.[6,45] We have further indicated that some ARBs, such as candesartan, olmesartan and losartan, function as inverse agonists, because they effectively inhibit PO-induced cardiac hypertrophy even in the absence of endogenous AngII, whereas non-inverse agonists such as telmisartan and valsartan do not exert such inhibitory effects.[45] Therefore, ARBs characterized by inverse agonism have great potential therapeutic benefits for the treatment of cardiovascular diseases, especially those caused by a constitutively active AT1-R.

Structurally, ARBs have a common biphenyl-tetrazole ring and unique side chains, which contribute to drug-specific differences in their pharmacokinetic and pharmacodynamic properties, and the potency of inverse agonist activity.[57] Each ARB differs in efficacy with regard to its unique chemical structure and molecular interactions with the AT1-R.[58] Structure-function analyses of the AT1-R have advanced our understanding of the molecular mechanism underlying receptor activation and inverse agonism. The AT1-R has 7 TM domains (TM1-7)

(Figure 2). In response to mechanical stress, TM7 undergoes a counterclockwise rotation and a shift towards the ligand-binding pocket. This receptor allosterism activates intracellular signaling molecules.[57] As for inverse agonism, ARBs bind to the AT1-R and induce its rotation in the opposite direction of mechanical activation. With clockwise allosterism of the AT1-R, intracellular pro-hypertrophic signaling pathways are inhibited. The multivalent interactions between an inverse agonist and the AT1-R cooperate to stabilize the receptor in an inactive conformation.[55] In addition to the biphenyl-tetrazole ring, known as the binding site within the AT1-R to antagonize the chemical activation of AngII, unique side chains of inverse agonists play a key role in inhibiting mechanical activation of the AT1-R. For example, the potent inverse agonist activity of olmesartan requires cooperative interaction between the hydroxyl group and Tyr^{113} in TM3 of the AT1-R, between the carboxyl group and Lys^{199} (TM5) and His^{256} (TM6), and between the tetrazole group and Gln^{257} (TM6), which are different from that in non-inverse agonists (Figure 2).

Interestingly, the beneficial effects of the conformational changes of AT1-R induced by inverse agonists are consistent with some findings in clinical trials. The CHARM-Added trial showed a beneficial effect of candesartan in patients already treated with ACE inhibitors (ACEI) to inhibit AngII formation.[44] However, some other clinical studies did not find additional benefits of the combination of ARB and ACEI over ACEI monotherapy.[4] The reasons for the discrepancy remain to be elucidated, but one plausible explanation may be that some ARBs can exert inverse agonism, while others cannot.

It was recently found that olmesartan effectively activates the ACE2-Ang1-7-Mas signaling pathway, which degrades AngII to Ang1-7, resulting in mitigation of vasoconstriction, inflammation, oxidative stress, apoptosis and signaling pathways associated with heart failure.[59] The relation of inverse agonism to this axis is still unclear and elucidation may help develop novel inverse agonists that play promising roles in the homeostasis of the RAS by modulating the interactions of the ACE-AngII-AT1-R axis and the ACE2-Ang1-7-Mas axis.

Biased Agonism at the AT1-R

With the development of bioinformatics, a breakthrough concept in GPCR signaling is β-arrestin-biased agonism, in which specific ligand-activated GPCRs selectively signal through β-arrestins, rather than through G proteins.[32] β-arrestin-biased agonism of the AT1-R in the mechanically stretched heart has recently been demonstrated to initiate cardioprotective signals independent of G protein.[60]

The G protein-independent cardioprotective signals act especially through the ever-expanding cohort of β-arrestin-mediated processes. The most extensively studied β-arrestin signaling pathway is β-arrestin-dependent activation of ERKs. ERK signaling can be activated by a number of GPCRs in a β-arrestin-dependent manner, mostly mediated by transactivation of EGFR.[19,32] Kim et al tested the effect of a β-arrestin2-biased AT1-R ligand, TRV120023, on cardiac performance in response to mechanical stretch, and showed that: (1) TRV120023 increased cardiac contractility in vivo, and this effect was abrogated in β-arrestin2 KO animals; (2) TRV120023 enhanced ERK1/2 and Akt signaling in a β-arrestin2-dependent manner; and (3) TRV120023 promoted cell survival after cardiac injury, compared with treatment with an unbiased ARB losartan, as assessed by TUNEL positivity.[60] Our recent study also indicated that mechanical stretch-induced ERK1/2 activation in a β-arrestin2/Src-dependent and G protein-independent manner.[31] Recent work in animal models of heart failure suggests that a β-arrestin-biased AT1-R agonist [Sar1, Ile4, Ile8]-angiotensin II (SII), enhances MAPK signaling, increases isolated cardiomyocyte inotropy, and attenuates apoptosis and fibrosis.[19,33] Expanded understanding of the mechanism for cardioprotection conferred by β-arrestin2-dependent and G protein-independent signaling would help develop super agonists and super antagonists that target certain arrestin- or G protein-mediated signals.

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Disclosures

Conflict of Interest: None.

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