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ARB and Cardioprotection

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Abstract A growing body of evidence has suggested that the use of angiotensin II (Ang II) type 1 (AT_1) receptor blockers (ARBs) leads to a significant decrease in mortality and morbidity in patients with congestive heart failure. The AT_1 receptor is a seven-transmembrane G protein-coupled receptor, and is involved in regulating the physiological and pathological process of the cardiovascular system. Systemically and locally generated Ang II has agonistic action on AT_1 receptor. However, recent in vitro studies have demonstrated that AT_1 receptor is structurally flexible and instable, and has significant and varying levels of spontaneous activity in an Ang II-independent manner. Furthermore, mechanical stress activates AT_1 receptor by inducing conformational switch without the involvement of Ang II. Experimental studies have demonstrated that Ang II-independent activation of AT_1 receptor is profoundly relevant to the pathogenesis of cardiac remodeling in vivo, and that these agonist-independent activities of AT_1 receptor can be inhibited by inverse agonists, but not by neutral antagonists. Therefore, inverse agonist activity emerges as an important pharmacological parameter that contributes to cardioprotective effects of ARBs through inhibiting both Ang II-dependent and -independent activation of AT_1 receptor.

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Introduction

In addition to the systemic effects including elevation of blood pressure, sodium and water retention, and activation of sympathetic nervous system, the renin-angiotensin system (RAS) has direct deleterious effects on the hearts and vessels, especially through a local activation system in tissues [1, 2]. Angiotensin II (Ang II) is the pivotal bioactive molecule of RAS, and most of the pathophysiological actions of Ang II in the cardiovascular system are mainly mediated through Ang II type 1 (AT_1) receptor [3]. The AT_1 receptor is a typical member of the G protein-coupled receptor (GPCR) family, the structure of which is characterized by seven transmembrane-spanning α-helices [4–6]. The AT_1 receptor blockers (ARBs) are non-peptide compounds that selectively bind to the AT_1 receptor and inhibit Ang II-induced receptor activation. At present, several ARBs are clinically available as a highly effective and well-tolerated class of drugs for the management of hypertension. In addition, clinical trials have indicated that the ARBs provide cardiovascular protection [7, 8]. For example, the Valsartan Heart Failure Trial (Val-HeFT) demonstrated that the ARB valsartan significantly reduces mortality and morbidity and improves clinical signs and symptoms in patients with heart failure of New York Heart Association class II, III, or IV [9]. The Candesartan in Heart Failure Assessment of Reduction in Mortality and morbidity (CHARM) program demonstrated that the ARB candesartan significantly reduces cardiovascular death and hospitalization in the broad spectrum of patients with heart failure [9].

In principle, AT_1 receptor is activated upon binding to Ang II, which is produced systemically or locally after sequential proteolytic processing of angiotensinogen. However, recent studies demonstrated that AT_1 receptor inherently shows spontaneous constitutive activity even in the absence of Ang II [10–13]. In addition, AT_1 receptor is activated by mechanical stress independently of Ang II [14–16] through conformational switch of the receptor [10]. These observations have led to identification of ARBs that are able to inhibit agonist-independent receptor activity and/or activation, i.e. inverse agonists [17–19]. This review focuses on the current evidence supporting the use of ARBs in cardiovascular medicine, and molecular basis underlying the cardioprotective effects of ARBs.

Inhibitory effects of ARBs on cardiac remodeling

In a variety of pathological conditions such as hypertension, valvular heart disease, myocardial infarction, and cardiomyopathy, hemodynamic overload induces hypertrophic growth of cardiomyocytes. Pathological enlargement of cardiomyocytes affects the collagen network surrounding the myocardium, and promotes interstitial fibrosis. Although cardiac hypertrophy is initially compensatory and beneficial, prolongation and excess of this process leads to deleterious outcomes such as congestive heart failure, arrhythmia, and sudden death [20]. Ang II infusion in rats induced cardiac hypertrophy in via activation of AT_1 receptor, independently of blood pressure elevation [21], and cardiac-specific overexpression of AT_1 receptor in mice also induced cardiac hypertrophy, interstitial fibrosis and contractile dysfunction [22, 23]. These results suggest that activation of AT_1 receptor is sufficient for inducing cardiac remodeling.

According to a meta-analysis that evaluated the effects of antihypertensive therapy on cardiac hypertrophy, ARB is the most effective drug class for reducing left ventricular mass in patients with essential hypertension [24]. In addition, a randomized controlled trial of the Losartan Intervention for European Reduction in Hypertension (LIFE) study provided evidence that the ARB losartan is superior to the β-blocker atenolol, in reducing left ventricular mass beyond blood pressure lowering [25]. The Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial also demonstrated that candesartan confer more beneficial effects to hypertensive patients with cardiac hypertrophy than the calcium channel blocker amlodipine [26].

A large number of in vitro experiments have demonstrated that activation of AT_1 receptor via $G_{q/11}$ protein coupling stimulates diverse intracellular signaling pathways and enhances production of reactive oxygen species, which consequently evokes hypertrophic responses in cardiomyocytes, and enhances cellular proliferation and production of extracellular matrix proteins such as collagen in cardiac fibroblasts [27, 28]. Especially, AT_1 receptor signals the mitogen-activated protein (MAP) kinase family such as extracellular signal-regulated protein kinases (ERKs) [29, 30], c-Jun NH_2-terminal kinase [31] and p38 mitogen-activated protein (MAP) kinase [32]. Although the signaling pathways linking AT_1 receptor to ERKs vary according to cell-types, protein kinase C and Raf-1 kinase are critically important as the upstream elements of ERKs cascade in cardiomyocytes [30]. Activated ERKs promote protein synthesis by enhancing p70 S6 kinase activity and ribosomal RNA transcription [33]. In addition, ERKs phosphorylate and activate several transcription factors such as GATA-4 and STATs or transcriptional coactivators such as p300 and CBP, and thereby enhance gene expression associated with hypertrophic response [33]. Activation of AT_1 receptor also stimulates G-protein-independent signaling pathways such as activation of Jak/STAT pathway and β-arrestin-mediated activation of ERKs [34].

Constitutive activity of AT_1 receptor and cardiac remodeling

GPCRs are structurally unstable, and show significant levels of spontaneous constitutive activity in an agonist-independent manner [18]. Constitutive activity of wild-type AT_1 receptor under basal conditions is relatively low, but can be detected when AT_1 receptor is overexpressed in cells even in the absence of endogenous expression of angiotensiogen [10, 11, 13, 14, 35]. It has been a challenging problem whether the subtle constitutive activity of AT_1 receptor fulfills a pathophysiological role.

According to recent papers, transgenic overexpression of AT_1 receptor in the hearts induced cardiac hypertrophy and remodeling without alterations in systemic blood pressure [22, 23]. In addition, knockin mice with a constitutively activating mutation (substitution of Asn^{111} to Gly with a C-terminal deletion) showed low-renin hypertension and progressive fibrosis in kidney and heart [36]. These results may raise a possibility that enhancement of constitutive activity, either through up-regulation of receptor expression or activating mutations, is disease-causing. Indeed, AT_1 receptor is up-regulated in stressed hearts of spontaneously hypertensive rats [37], two-kidney one-clip renovascular hypertensive rats [37], Tsukuba hypertensive mice [38], rats with myocardial infarction [39], and pressure-overloaded mice [13]. To corroborate this possibility, we recently generated transgenic mice overexpressing AT_1 receptor under the control of *α-myosin heavy chain* promoter in *angiotensinogen*-knockout background (AT_1Tg-AgtKO mice) [13]. In AT_1Tg-AgtKO hearts, AT_1 receptor is constitutively activated independently of Ang II, because redistribution of

$G\alpha_{q11}$ subunit into cytosol and phosphorylation of ERKs were significantly increased. As a consequence, AT_1Tg-AgtKO mice showed spontaneous systolic dysfunction and chamber dilatation, accompanied by severe interstitial fibrosis. These results suggest that constitutive activity of AT_1 receptor under basal conditions promotes cardiac remodeling even in the absence of Ang II, when AT_1 receptor is up-regulated in the heart [13].

Mechanical stress-induced activation of AT_1 receptor and cardiac remodeling

We recently found a novel mechanism whereby mechanical stress activates AT_1 receptor independently of Ang II [10, 14]. Mechanical stress, along with neurohumoral factors, is the primary stimulus for cardiac hypertrophy. Importantly, mechanical stretching of cultured cardiomyocytes alone induced hypertrophic responses such as activation of many protein kinases including ERKs and reprogramming of gene expression [40, 41].

Activation of AT_1 receptor is profoundly involved in the development of load-induced cardiac hypertrophy. As described above, many clinical studies showed that ARBs have superior effects on left ventricular mass reduction in hypertensive patients [24, 25, 42]. Furthermore, pretreatment of cardiomyocytes with ARBs significantly attenuated hypertrophic responses induced by stretching [29, 43]. These results indicate that mechanical stress induces cardiac hypertrophy through the activation of AT_1 receptor. In human embryonic kidney (HEK) 293 cells or COS7 cells which have no detectable expression of AT_1 receptor and angiotensinogen, neither Ang II nor mechanical stretch activated ERKs, but forced expression of AT_1 receptor conferred the ability to activate ERKs in response to both Ang II and mechanical stretch. Interestingly, candesartan, as an inverse agonist for ARB, inhibited the ERKs activation induced not only by Ang II but also by mechanical stretch in HEK293 cells expressing AT_1 receptor. Stretch stimuli also activated ERKs in HEK293 cells expressing AT_1 mutant which did not bind Ang II [44] and in cardiomyocytes prepared from *angiotensinogen*-deficient mice [45], and these activations were inhibited by candesartan [14]. Furthermore, mechanical stress can induce cardiac hypertrophy in vivo through the AT_1 receptor in the absence of Ang II, because pressure overload induced cardiac hypertrophy in *angiotensinogen*-deficient mice as well as in wild-type mice, which was significantly inhibited by candesartan. These experimental data provided compelling evidence that AT_1 receptor is activated in the absence of Ang II both in vitro and in vivo, and that this Ang II-independent activation of AT_1 receptor is inhibited by candesartan.

Inverse agonist activity of ARBs and cardioprotection

Before the early 1990s, GPCR ligands were simply classified as agonists or antagonists [17–19]. Both agonists and

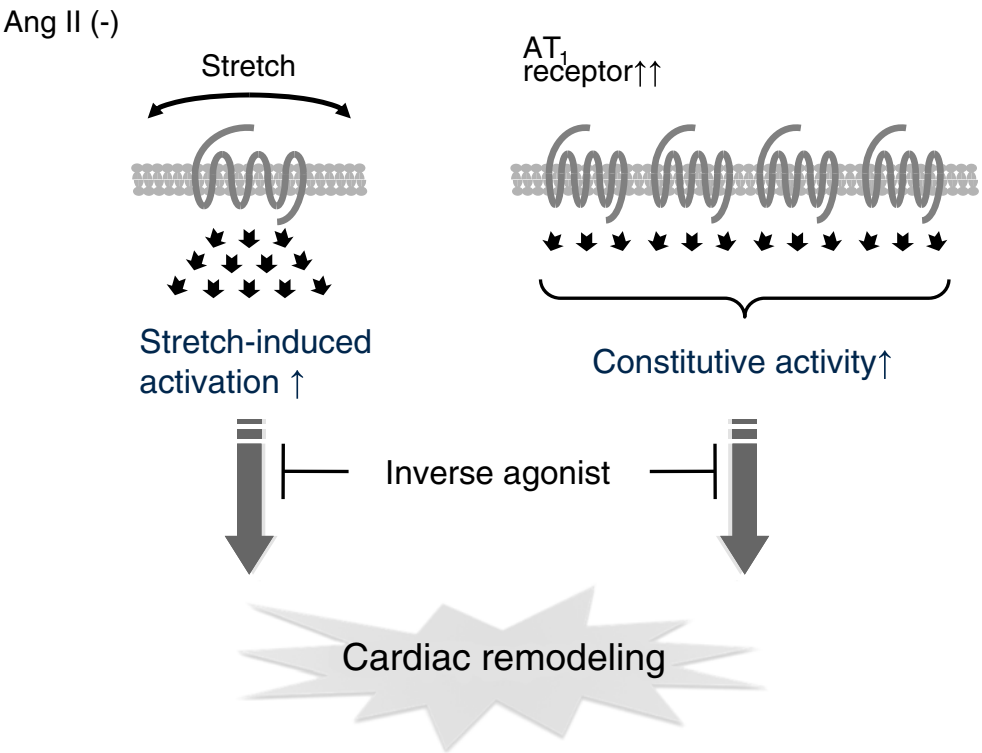

Fig. 1 The inverse agonist activity of ARBs provides clinical advantage of inhibiting both Ang II-dependent and -independent receptor activation, and thus be an important pharmacological parameter defining the beneficial effects on cardioprotection

antagonists bind to the cognate GPCR with high affinity, but only agonists can activate the receptor. Therefore, agonists possess both high affinity and positive efficacy, whereas antagonists posses high affinity without intrinsic efficacy. However, some compounds have been demonstrated to produce effects opposite to those by agonists. Such ligands are classified as "inverse agonists" that have negative efficacy. An inverse agonist stabilizes inactive conformation of the receptor and reduces constitutive activity of the receptor or the agonist-independent receptor activity.

The inverse agonist activity of ARBs could be of clinical advantage to inhibition of both Ang II-dependent and -independent receptor activation, and thus be a novel and important pharmacological parameter defining the beneficial effects on organ protection (Fig. 1). Candesartan reduces the basal activation of *c-fos* gene promoter by AT_1-WT receptor or a constitutively active AT_1-N111G mutant receptor, suggesting that candesartan is an ARB with potent inverse agonist activity [10]. According to recent papers, olmesartan, valsartan, irbesartan, and EXP3174 (active metabolite of losartan) also reduce the constitutive GTPase stimulating activity of AT_1 mutant receptor, while losartan does not reduce it [6, 35, 46, 47]. Furthermore, candesartan suppressed mechanical stretch-induced helical movement of AT_1 receptor [10], and thereby inhibited receptor activation [14]. Inverse agonism of candesartan is especially relevant to its ability to attenuate load-induced cardiac hypertrophy, because pressure overload by constricting the transverse aorta induced cardiac hypertrophy even in *angiotensinogen*-deficient mice as well as in WT mice, which was significantly inhibited by candesartan [14]. In addition, treatment with candesartan effectively prevents cardiac remodeling induced by constitutive activity of overexpressed wild-type AT_1 receptor [13].

Chemical Structure Determining Inverse Agonist Activity of ARBs

The distinctive activity of inverse agonism is primarily determined by chemical structure of the drug. Most of ARBs have a biphenyltetrazole ring structure in common. We recently found that the bindings of the carboxyl group of candesartan to Gln^{257} in TM6 and Thr^{287} in TM7 are responsible for the potent inverse agonism in inhibiting mechanical stretch-induced activation of AT_1 receptor and constitutive activity of AT_1 receptor [10, 13]. Besides candesartan, olmesartan and valsartan robustly suppresses constitutive production of inositol phosphate by AT_1-N111G receptor [35, 46]. Although the interactions of olmesartan with Tyr^{113}, Lys^{199}, His^{256}, and Gln^{257} in the AT_1 receptor are important for the tight drug-receptor binding, its potent inverse agonist activity to suppress constitutive receptor activity requires cooperative interactions between the hydroxyl group and Tyr^{113} in TM3 and between the carboxyl group and His^{256} in TM6 [35]. Furthermore, the inverse agonism of olmesartan against stretch-induced ERKs activation requires an additional drug-receptor interaction involving the tetrazole group of olmesartan and Gln^{257} in the AT_1 receptor [11]. Therefore, multivalent interactions between an inverse agonist and the AT_1 receptor cooperate to stabilize the receptor in an inactive conformation in response to the distinct processes of AngII-independent activation. Interestingly, differential interactions of valsartan to Ser^{105} and Ser^{109} in TM3 and Lys^{199} in TM5 are critical for producing inverse agonism [46]. Among these docking residues, Ser^{105} binds to the carboxyl group of valsartan. Thus, the chemical structure of an ARB governs the spatial and kinetic pattern of contacts to the AT_1 receptor, which will determine the potency of inverse agonist activity.

Conclusions

The use of ARBs has been shown to be beneficial in patients with cardiovascular and metabolic complications. The structure-function analyses of the AT_1 receptor have advanced our understanding of the molecular mechanism underlying receptor activation and inverse agonism. Although inverse agonism is now a well-recognized phenomenon in the field of receptor pharmacology, clinical importance of inverse agonist activity of ARBs is still speculative. At least, in an experimental animal model, inverse agonist activity of ARBs is relevant to its ability to attenuate load-induced cardiac hypertrophy [14] and cardiac remodeling induced by constitutive activity of AT_1 receptor [13]. It is of particular significance to verify whether the inverse agonist activity assayed in recombinant systems contributes to clinical benefits and advantages for cardioprotection in humans.

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