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Editorial

"Change can happen" by PKA: Proteasomes in in vivo hearts

Rudolf Schoenheimer pioneered the technique to tag amino acids with isotope for tracing their metabolism within living animals [1]. The results of his experiments led to a revolutionary view that the proteins within a body are in a dynamic state of synthesis and degradation. Now, after more than 6 decades, the concept of protein turnover is widely accepted. Especially, to maintain cellular homeostasis, the cells carry out protein quality control through ubiquitin–proteasome system (UPS) and autophagy, and eliminate needless or defective intracellular proteins that are of no use and even hazardous. The UPS is a highly selective degradation process occurring in the cytoplasm, but in contrast, autophagy is a non-selective process that degrades bulk proteins and organelles in the lysosomes to recycle [2]. Insomuch as the UPS participates in proteolysis of thousands of specific proteins, this regulatory system plays an important role in a variety of cellular responses including cell cycle and division, hypoxic response, DNA repair, apoptosis and immune response [3]. Importantly, recent studies have indicated that dysregulation of the UPS is profoundly implicated in human diseases such as inflammatory diseases, neurodegenerative diseases, muscle-wasting disorders, cancer, and cardiovascular diseases [3,4], and the UPS has emerged as a potential therapeutic target for the treatment of these diseases [5].

Postnatal cardiomyocytes scarcely proliferate, and thus are in extraordinary need of removing damaged or misfolded proteins to avoid accumulation of these kinds of garbage within the cells. In addition, since the beating heart is under continuous stress, especially in diseased conditions, myocardial proteins are liable to damaging and misfolding [4]. Furthermore, recent studies have demonstrated that the UPS is dysfunctional in the hearts of rodent models of ischemia/reperfusion (I/R) [6,7] or desmin-related cardiomyopathy [8]. Therefore, elucidation of the regulatory mechanism of the UPS in the heart will be important to understand the pathogenesis of heart diseases. The UPS-mediated proteolysis consists of two sequential steps: covalent attachment of ubiquitin to the protein substrate (ubiquitination) and degradation of the ubiquitinated proteins by 26S proteasome complex [9,10]. Ubiquitination proceed through a series of enzymatic reactions involving ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). Selectivity and specificity of the protein substrate is determined by E3 ligases that have either the RING-finger domain or the HECT domain. Although much knowledge has been accumulated on selective and specific aspects of ubiquitination, the regulatory mechanism of 26S proteasome remains elusive, especially in the heart.

In this issue of Journal of Molecular and Cellular Cardiology, Asai and colleagues have provided unequivocal evidence that protein kinase A (PKA) enhances the assembly and activity of cardiac 26S proteasome both in vitro and in in vivo [11]. The 26S proteasome is a 2.4 MDa multisubunit complex, consisting of a core 670 kDa 20S catalytic subcomplex and two 700 kDa 19S (or PA700) regulatory subcomplexes [9,10,12]. Both ends of the barrel-shaped 20S subunit are capped by 19S regulatory subunits (Fig. 1). The 20S subunit is composed of four axially stacked rings (two identical outer α rings and two identical inner β rings), and each α and β ring contains seven distinct subunits (α1–α7, β1–β7). Three distinct peptidase (chymotrypsin-like, trypsin-like, and caspase-like) activities have been identified, and assigned to three distinct catalytic subunits (β5, β2, and β1, respectively) lining a central lumen. Polyuniquitinated proteins are recognized and unraveled by 19S subunit, which then facilitates the entry and degradation of unfolded polypeptides in the cavity of the 20S subunit.

A couple of studies have shown that PKA can induce serine- or threonine-phosphorylation in 26S proteasome and increase proteolytic activity in vitro [13,14]. The 19S subunit contains six AAA ATPases (Rpt1~6), which contact with outer α rings of the 20S subunit and unfold the polyubiquitinated substrates [12]. According to a recent study, PKA stimulates the proteasome activity by phosphorylating Rpt6 [14]. Ping and colleagues recently delineated a phosphorylation profile of the endogenous 20S subunit of murine hearts and identified phosphorylation in multiple subunits, by using 2-D gel electrophoresis, immunoblotting, and tandem mass spectrometry [13]. In the same study, PKA was identified within the native cardiac 20S complex, and recombinant PKA significantly increased proteasome activity in vitro. The study by Asai et al firstly shows that PKA stimulation enhances the activity of 26S proteasome in in vivo hearts [11]. The proteolytic activities of 26S proteasome in the hearts of anesthetized dogs were significantly increased after PKA stimulation through intracoronary administration of isoproterenol (a β-adrenergic receptor agonist) or forskolin (an activator of adenylate cyclase that increases cyclic AMP and activates PKA) for 30 min. In addition, the 26S proteasome activity was also increased at 30 min after ischemic preconditioning (IP), consisting of 4 cycles of 5 min of ischemia and 5 min of reperfusion. Among a number of signaling pathways involved in IP [15], PKA mediates the enhancement of proteasome activity after IP, because it was attenuated by intracoronary administration of a PKA inhibitor, H-89. As mentioned above, PKA phosphorylates Rpt6 in the 19S subunit and may facilitate diffusion of the polypeptide substrates into the proteolytic cavity of the 20S subunit (Fig. 1), although the precise mechanism remains unclear. The phosphorylations of the 20S subunit may directly or indirectly induce conformational change of the catalytic sites to increase proteolytic activity (Fig. 1).

Alternatively, PKA phosphorylation may be involved in the assembly of proteasome subunits (Fig. 1). Proteasomes with normal function require correct assembly of all subunits, which is orchestrated by multiple proteasome-specific chaperones [16]. Although

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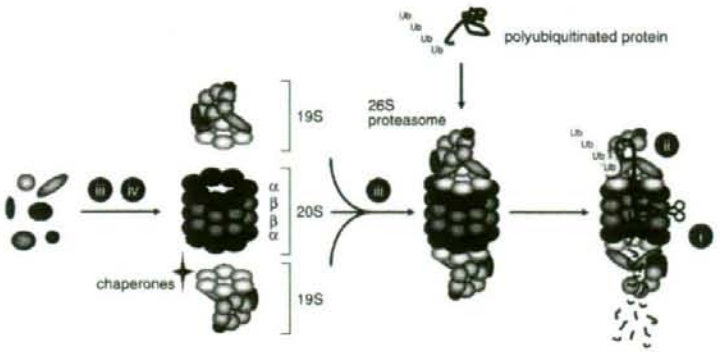

Fig. 1. Potential change in 26S proteasome bought about by PKA. PKA enhances the activity of 26S proteasome potentially i) by increasing proteolytic activity through phosphorylation of 20S subunit, ii) by facilitating translocation of polyubiquitinated substrates through phosphorylation of 19S subunit, iii) by promoting assembly of proteasome subunits through phosphorylation of the subunits or chaperones, or iv) by altering molecular composition of proteasome through an unknown mechanism.

the regulatory kinases are not specified, the phosphorylation of proteasome subunits or chaperones can affect the status of proteasome assembly. For example, the phosphorylation of Rpt6 in the 19S subunit is required for the incorporation of the 19S subunit into 26S proteasome, possibly through the formation of interaction between Rpt6 and α2 subunit [17]. In addition, the phosphorylation of α7 subunit stabilizes the association of the 19S subunit with the 20S subunit to form 26S proteasome [18]. The study by Asai et al shows that PKA stimulation increases the incorporation of Rpt5, α7, and β5 subunits into cardiac 26S proteasome both in vitro and in vivo by immunoblot analysis under non-reducing conditions [11]. Clearly, these results leave many open questions: which subunit phosphorylated by PKA is important in this process? How does the phosphorylation induce an allosteric effect that changes the stability of 26S proteasome? Is the assembly of subunits by PKA critically linked to proteolytic activity of 26S proteasome? Furthermore, recent studies have indicated that certain pathological stress can alter proteasome composition, and that the molecular composition of proteasome is closely related to proteolytic activity [19,20]. It may be possible that PKA alters the proteasome composition, especially in vivo (Fig. 1). Additional studies are necessary to determine the mechanism and consequence of PKA-mediated assembly of 26S proteasome.

Asai et al further investigated the pathophysiological role of proteasome activation by IP in canine hearts subjected to I/R [11]. A significant decrease in the proteasome activity was observed after 90 min of ischemia, which lasted for the following period of reperfusion. It has been reported that free radical-initiated oxidation, such as lipid peroxidation, participates in oxidative modification and inactivation of the 20S proteasome during I/R [6,7]. As a consequence of a decline in the proteasome activity, I/R increased accumulation of ubiquitinated proteins in the hearts. Interestingly, IP counteracted the decline of proteasome activity during I/R, which was associated with a significant reduction in the accumulation of ubiquitinated proteins. Abnormal accumulation of ubiquitinated proteins causes aberrant protein aggregates, and thus is thought to be deleterious to cardiomyocytes [4]. The favorable effect of IP on accumulation of ubiquitinated protein in I/R hearts was abolished by intracoronary administration of a proteasome inhibitor epoxomicin, but surprisingly, the infarct size in I/R hearts was unchanged with or without IP even by epoxomicin at the concentration that reduced proteasome activity by 43%. These results imply that proteasome activation by IP is irrelevant to the alleviative effect of IP on myocardial cell death during I/R. Then, the next question will be whether the beneficial effect of IP on contractile function of viable myocardium is prevented or not by epoxomicin in I/R hearts. Indeed, proteasome inhibitors may lead to deleterious and beneficial outcomes during myocardial ischemia according to the experimental designs [21]. The intracoronary administration of epoxomicin in anesthetized dogs may mitigate the inflammatory response within the hearts, because the NF-κB signaling is regulated by the UPS. Given that the UPS tightly controls turnover of regulatory proteins involved in physiological responses such as intracellular signaling and transcriptional regulation [3], the subtle difference in the concentrations or the pharmacokinetics of the proteasome inhibitors may influence the outcomes in in vivo experiments. In addition, it has been reported that autophagy acts as a compensatory degradation system when the UPS is impaired in a Drosophila model of neurodegenerative disease [22]. Administration of proteasome inhibitors may induce autophagy in I/R hearts, and thereby prevent myocardial cell death by maintaining organelle turnover and energy homeostasis [23]. Further studies are needed to clarify the functional coupling between the UPS and autophagy, especially in I/R hearts.

The proteasome inhibitor bortezomib shows selective cytotoxicity to cancer cells, and is approved for clinical treatment of refractory multiple myeloma [5]. Insomuch as the proteasome activity is hampered in ischemic hearts, pharmacological restoration of the proteasome function has a potential to become a rational strategy for treatment. The study of Asai et al provides an important clue toward this strategy [11]. Manipulation of proteasome function may be applied to treatment of a wide spectrum of heart diseases such as cardiac hypertrophy. Tsukamoto et al revealed that proteasome was dysfunctional in murine hearts of pressure-overloaded hypertrophy [24]. However, Depre et al argued that proteasome function was upregulated during pressure overload in canine hearts and that administration of proteasome inhibitors attenuated cardiac hypertrophy without altering cardiac function [25]. Of course, in-depth assessment of the pathogenic role of the UPS in heart diseases will be a prerequisite for launching a bench-to-bed approach.

Will pharmacological activation of PKA induce "a change we can believe in" in proteasomes of stressed myocardium and produce a clinical benefit in the treatment of heart diseases? Further studies are required to explore the detailed mechanism of proteasome modification and to develop an optimal way in normalization of proteasome function in diseased hearts.

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References

[1] Schoenheimer R. The Dynamic State of Body Constituents. Cambridge, Massachusetts, USA: Harvard University Press; 1942.
[2] Mizushima N. Autophagy: process and function. Genes Dev 2007;21(22):2861–73.
[3] Schwartz AL, Ciechanover A. Targeting proteins for destruction by the ubiquitin system: implications for human pathobiology. Annu Rev Pharmacol Toxicol 2009;49:73–96 [February]. doi:10.1146/annurev.pharmtox.051208.165340.
[4] Wang X, Su H, Ranek MJ. Protein quality control and degradation in cardiomyocytes. J Mol Cell Cardiol 2008;45(1):11–27.
[5] Nalepa G, Rolfe M, Harper JW. Drug discovery in the ubiquitin–proteasome system. Nat Rev Drug Discov 2006;5(7):596–613.
[6] Bulteau AL, Lundberg KC, Humphries KM, Sadek HA, Szweda PA, Friguet B, et al. Oxidative modification and inactivation of the proteasome during coronary occlusion/reperfusion. J Biol Chem 2001;276(32):30057–63.
[7] Gurusamy N, Goswami S, Malik G, Das DK. Oxidative injury induces selective rather than global inhibition of proteasomal activity. J Mol Cell Cardiol 2008;44(2):419–28.
[8] Liu J, Chen Q, Huang W, Horak KM, Zheng H, Mestril R, et al. Impairment of the ubiquitin–proteasome system in desminopathy mouse hearts. FASEB J 2006;20(2):362–4.
[9] Hochstrasser M. Ubiquitin-dependent protein degradation. Annu Rev Genet 1996;30:405–39.
[10] Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem 1998;67:425–79.
[11] Asia M, Tsukamoto O, Minamino T, Asanuma H, Fujita M, Asano Y, et al. PKA rapidly enhances proteasome assembly and activity in in vivo canine hearts. J Mol Cell Cardiol 2009;46:452–62.
[12] Pickart CM, Cohen RE. Proteasomes and their kin: proteases in the machine age. Nat Rev Mol Cell Biol 2004;5(3):177–87.
[13] Zong C, Gomes AV, Drews O, Li X, Young GW, Berhane B, et al. Regulation of murine cardiac 20S proteasomes: role of associating partners. Circ Res 2006;99(4):372–80.
[14] Zhang F, Hu Y, Huang P, Toleman CA, Paterson AJ, Kudlow JE. Proteasome function is regulated by cyclic AMP-dependent protein kinase through phosphorylation of Rpt6. J Biol Chem 2007;282(31):22460–71.
[15] Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiol Rev 2008;88(2):581–609.
[16] Murata S. Multiple chaperone-assisted formation of mammalian 20S proteasomes. IUBMB Life 2006;58(5–6):344–8.
[17] Satoh K, Sasajima H, Nyoumura KI, Yokosawa H, Sawada H. Assembly of the 26S proteasome is regulated by phosphorylation of the p45/Rpt6 ATPase subunit. Biochemistry 2001;40(2):314–9.
[18] Bose S, Stratford FL, Broadfoot KI, Mason GG, Rivett AJ. Phosphorylation of 20S proteasome alpha subunit C8 (alpha7) stabilizes the 26S proteasome and plays a role in the regulation of proteasome complexes by gamma-interferon. Biochem J 2004;378(Pt 1):177–84.
[19] Hanna J, Meides A, Zhang DP, Finley D. A ubiquitin stress response induces altered proteasome composition. Cell 2007;129(4):747–59.
[20] Drews O, Wildgruber R, Zong C, Sukop U, Nissum M, Weber G, et al. Mammalian proteasome subpopulations with distinct molecular compositions and proteolytic activities. Mol Cell Proteomics 2007;6(11):2021–31.
[21] Powell SR. The ubiquitin–proteasome system in cardiac physiology and pathology. Am J Physiol Heart Circ Physiol 2006;291(1):H1–H19.
[22] Pandey UB, Nie Z, Batlevi Y, McCray BA, Ritson GP, Nedelsky NB, et al. HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. Nature 2007;447(7146):859–63.
[23] Gustafsson AB, Gottlieb RA. Recycle or die: the role of autophagy in cardioprotection. J Mol Cell Cardiol 2008;44(4):654–61.
[24] Tsukamoto O, Minamino T, Okada K, Shintani Y, Takashima S, Kato H, et al. Depression of proteasome activities during the progression of cardiac dysfunction in pressure-overloaded heart of mice. Biochem Biophys Res Commun 2006;340(4):1125–33.
[25] Depre C, Wang Q, Yan L, Hedhli N, Peter P, Chen L, et al. Activation of the cardiac proteasome during pressure overload promotes ventricular hypertrophy. Circulation 2006;114(17):1821–8.

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Review

Mechanisms and functions of agonist-independent activation in the angiotensin II type 1 receptor

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ABSTRACT

The angiotensin II (AngII) type 1 (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 AngII has agonistic action on AT_1 receptor, but recent studies have demonstrated that AT_1 receptor inherently shows spontaneous activity even in the absence of AngII. Furthermore, mechanical stress can activate AT_1 receptor by inducing conformational switch without the involvement of AngII, and induce cardiac hypertrophy *in vivo*. These agonist-independent activities of AT_1 receptor can be inhibited by inverse agonists, but not by neutral antagonists. Considerable attention has been directed to molecular mechanisms and clinical implications of agonist-independent AT_1 receptor activation, and inverse agonist activity emerges as an important pharmacological parameter for AT_1 receptor blockers that will improve efficacy and expand therapeutic potentials in cardiovascular medicine.

Contents

1. Introduction

Pharmacological inhibitions of the renin–angiotensin system (RAS) are crowned with one of the greatest success in the current field of cardiovascular medicine. During the past quarter of century, a growing body of evidence has accumulated indicating that RAS blockade can prevent progression of cardiac hypertrophy and reduce the morbidity and mortality in patients with heart failure (Zaman et al., 2002; Jessup and Brozena, 2003). In addition to the systemic effects including elevation of blood pressure, sodium and water retention, and activation of sympathetic nervous system, the RAS has unfavorable direct effects on the hearts, especially through a system of local activation in tissues (Re, 2004; Paul et al., 2006). Angiotensin II (AngII) has been considered to be the pivotal bioactive molecule of RAS, and most of the pathophysiological actions of AngII in the cardiovascular system are mainly mediated through AngII type 1 (AT_1) receptor (Timmermans et al., 1993). According to the results from *in vitro* experiments, activation of AT_1 receptor stimulates diverse intracellular signaling cascade cascades and enhances production of reactive oxygen species, which consequently evokes hypertrophic responses in cardiomyocytes and enhances cellular proliferation and production of extracellular matrix proteins in cardiac fibroblasts (Kim and Iwao, 2000; Hunyady and Catt, 2006).

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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 with an extracellular N-terminus and a cytoplasmic C-terminus (Gether and Kobilka, 1998; Gether, 2000; Miura et al., 2003a). As a matter of course, AT_1 receptor is activated upon binding to AngII, the specific and endogenous agonist. AT_1 receptor can also be activated by autoantibodies against the receptor. These agonistic antibodies bind to epitopes on the second extracellular loop of the receptor, and are involved in the pathogenesis of preeclampsia and renal-allograft rejection (Thway et al., 2004; Dragun et al., 2005). It is now believed that agonist binding facilitates isomerization of a GPCR to an active conformation by disrupting the intramolecular interactions that constrain the receptor in an inactive conformation (Gether and Kobilka, 1998; Gether, 2000; Farrens et al., 1996; Hunyady et al., 2003). However, the classical concept that the receptors switch by a simple 'on-off' mechanism has been challenged since the discovery of spontaneous activity of δ-opioid receptor in the absence of agonist (Costa and Herz, 1989). Inherently, GPCRs are structurally flexible and instable, and have significant and varying levels of spontaneous activity in an agonist-independent manner (Leurs et al., 1998; Milligan, 2003). The constitutive activity has been demonstrated when AT_1 receptor is heterologously expressed in recombinant systems, and becomes manifest as a consequence of specific mutations (Hunyady et al., 2003; Noda et al., 1996; Groblewski et al., 1997; Balmforth et al., 1997; Feng et al., 1998). Furthermore, we have recently obtained compelling evidence that AT_1 receptor is activated by mechanical stress independently of AngII (Zou et al., 2004; Yasuda et al., 2008). These observations have in turn led to identification of the ligands that are able to inhibit agonist-independent receptor activity and/or activation, i.e. inverse agonists (Milligan, 2003; Strange, 2002; Bond and Ijzerman, 2006), and now prompt us to re-evaluate pharmacological actions of AT_1 receptor blockers (ARBs). In this article, we will review the current understanding of the structure–function relationship and the pathophysiological or therapeutical relevance of agonist-independent AT_1 receptor activation.

2. Constitutive activity of AT_1 receptor

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 (Zou et al., 2004; Miura et al., 2006). This phenomenon can be rendered more distinct by introducing specific amino acid substitutions of the receptor (Hunyady et al., 2003; Noda et al., 1996; Groblewski et al., 1997; Balmforth et al., 1997; Feng et al., 1998). The first evidence of constitutively active mutant (CAM) GPCR was obtained in α_{1B}-adrenoreceptor (Cotecchia et al., 1990). Amino acid substitutions of Ala^{293} at the end of the third intracellular loop of the α_{1B}-adrenergic receptor conferred constitutive activity (Kjelsberg et al., 1992). The following studies revealed that the mutational changes in the equivalent residues in this region resulted in constitutive activation of β_2-adrenoreceptor (Samama et al., 1993) and α_2-adrenoreceptor (Ren et al., 1993). These results provided a model that spontaneous signaling is repressed to a low level via the conserved intramolecular constraints, and that agonist binding alters the receptor conformation by relieving these intrinsic constraints (Parnot et al., 2002; Costa and Cotecchia, 2005). CAMs are thought to mimic an active conformation of the wild-type receptor, in which structural constraints are disrupted. In this regard, CAMs have provided plentiful insights into the molecular process of agonist-induced receptor activation (Parnot et al., 2002; Costa and Cotecchia, 2005).

Structure–function analyses have demonstrated that the bindings of AngII to Asn^{111} in transmembrane (TM) 3 and to His^{256} in TM6 of the AT_1 receptor are crucial for receptor activation (Noda et al., 1995a, 1996; Feng et al., 1998; Miura et al., 1999), although two salt bridges between AngII and Lys^{199} in TM 5 or Asp^{281} in the third extracellular loop are important for docking AngII to the receptor (Yamano et al., 1992; Noda et al., 1995b; Feng et al., 1995). Interestingly, substitutions of Asn^{111} to residues of smaller size such as Gly or Ala caused higher constitutive activity in inositol phosphate production, while those to larger residues such as Phe or Tyr resulted in a reduction of the basal activity (Noda et al., 1996; Feng et al., 1998). The mechanism by which the size of the residue at the position of Asn^{111} determines the level of constitutive activity is not clear, but it is likely that the receptor with the activating mutations may emulate the conformational transition that AngII-binding normally induced through altering the van der Waals contact between Asn^{111} and other residues in the AT_1 receptor (Noda et al., 1996; Feng et al., 1998).

The structural transition underlying constitutive activation in AT_1 receptor harboring the Asn^{111} mutations has been explored by studies using the substituted cysteine accessibility mapping (SCAM). The SCAM study is used to probe relative conformational changes of GPCRs by validating the presence of Cys residues within the ligand-binding pocket (Miura and Karnik, 2002; Chen et al., 2002; Boucard et al., 2003; Miura et al., 2003b; Lemaire et al., 2004; Jongejan et al., 2005; Martin et al., 2007) (Fig. 1). The SCAM studies have revealed that the mutations in Asn^{111} confer constitutive activity of the receptor (Groblewski et al., 1997; Feng et al., 1998) by releasing helical constraints involving TM2 (Miura and Karnik, 2002; Miura et al., 2003b), TM6 (Martin et al., 2007) and TM7 (Boucard et al., 2003; Miura et al., 2003b).

3. Pathophysiological relevance of constitutive activity of AT_1 receptor

Activating mutations for several GPCRs are causative of diseases, such as thyrotropin-stimulating hormone receptor in hyperfunctioning thyroid adenoma (Parma et al., 1993) and luteinizing hormone receptor in familial male precocious puberty (Shenker

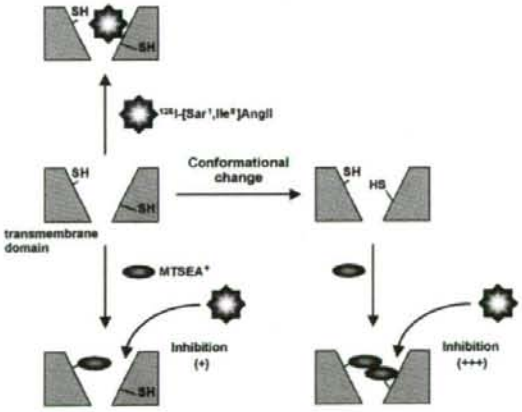

Fig. 1. Substituted cysteine accessibility mappaing (SACM). Sulfhydryl-specific reaction with methanethiosulfonyl ethyl-ammonium (MTSEA$^+$), which reacts a billion times faster with water-exposed and ionized Cys than lipid-exposed and un-ionized Cys. Upon this reaction, a positively charged sulfonylmethylammonium group is added onto water-exposed Cys via a mixed disulfide bond. The chemical modification of Cys in the water-accessible ligand pocket results in interference with the binding of radioisotope-labeled ligand either through steric hindrance or electrostatic repulsion. Thus, changes in the binding of radioisotope-labeled ligand indicate an entry or exit of Cys residues within the ligand pocket, which results from a conformational transition of the GPCR.

et al., 1993). With regard to AT_1 receptor, no germline or somatic mutation has been identified that causes hypertension or primary hyperaldosteronism (Davies et al., 1997; Sachse et al., 1997) through induction of robust constitutive activity.

The question remains to be difficult to solve whether the subtle constitutive activity of native GPCRs fulfills a pathophysiological role. Indeed, constitutive activity of native histamine H_3 receptors is present in rodent brain, and seems to control activities of cerebral histaminergic neurons *in vivo* (Morisset et al., 2000). However, it is still unclear whether this spontaneous activity is crucial to proper function of neurons. Theoretically, higher expression levels of GPCRs are anticipated to increase agonist-independent basal activity in native tissues. The expression of AT_1 receptor is up-regulated in vascular cells by low-density lipoprotein cholesterol (Nickenig et al., 1997), insulin (Nickenig et al., 1998), glucose (Sodhi et al., 2003), progesterone (Nickenig et al., 2000), and inflammatory cytokines such as interleukin-1α or interleukin-6 (Sasamura et al., 1997; Wassmann et al., 2004), providing a potential mechanistic link of enhanced AT_1 receptor expression to atherosclerosis associated with hyperinsulinemia, hypercholesterolemia and estrogen deficiency (Wassmann and Nickenig, 2006; Griendling et al., 1996). However, it is quite difficult to measure the accurate amount of functional AT_1 receptor expression in tissues, and experimental proof is needed that such distinctions of enhanced intrinsic receptor activity contribute to progression of atherosclerosis.

According to recent papers, transgenic overexpression of AT_1 receptor in the hearts induced cardiac hypertrophy and remodeling without alterations in systemic blood pressure (Hein et al., 1997; Paradis et al., 2000). 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 (Billet et al., 2007). These results may raise a possibility that enhancement of constitutive activity, either through up-regulation of receptor expression or activating mutations, is disease-causing. To corroborate this possibility, further studies will be needed to examine whether enhanced intrinsic activity of AT_1 receptor leads to some phenotypic abnormalities even under circumstances where the production of AngII is pharmacologically or genetically inhibited.

4. Mechanical stress-induced activation of AT_1 receptor

We recently found a novel mechanism whereby mechanical stress activates AT_1 receptor independently of AngII (Zou et al., 2004; Yasuda et al., 2008). Mechanical stress, along with neurohumoral factors, is the primary stimulus for cardiac hypertrophy. In isolated hearts perfused as Langendorff preparations, the increase in protein synthesis was most closely related to stretching of ventricular wall as a consequence of increased afterload (Kira et al., 1984). In addition, an increase in protein synthesis was also observed, when cardiomyocytes cultured on deformable silicone rubber dishes underwent passive stretch even in serum-free media (Mann et al., 1989). Furthermore, mechanical stretching of cultured cardiomyocytes induced hypertrophic responses such as activation of many protein kinases including extracellular signal-regulated protein kinases (ERKs) and reprogramming of gene expression (Komuro and Yazaki, 1993; Sadoshima and Izumo, 1997). These results suggest that mechanical stress *per se* induces hypertrophic responses primarily by stretching cardiomyocytes.

Activation of AT_1 receptor is profoundly involved in the development of load-induced cardiac hypertrophy. Many clinical studies have shown that ARBs have superior effects on left ventricular mass reduction in hypertensive patients (Kjeldsen et al., 2002; Klingbeil et al., 2003; Okin et al., 2004). Furthermore, pretreatment of cardiomyocytes with ARBs significantly attenuated hypertrophic responses induced by stretching (Sadoshima et al., 1993; Yamazaki et al., 1995). These results indicate that mechanical stress induces cardiac hypertrophy through the activation of AT_1 receptor. However, it has been a challenging problem to solve how AT_1 receptor is activated by mechanical stress. There is a possibility that AngII is stored in cardiomyocytes, and that mechanical stretch induces the secretion of stored AngII into the culture medium, resulting in the induction of cardiomyocyte hypertrophy by the autocrine mechanism (Sadoshima et al., 1993). However, direct measurement of AngII concentration in the medium conditioned by stretching cardiomyocytes did not reveal a significant increase in AngII concentration (Zou et al., 2004). Furthermore, a neutralizing antibody to AngII did not suppress the stretch-induced ERKs activation in cardiomyocytes, although the antibody abolished AngII-induced ERKs activation (Zou et al., 2004). These results suggest that AngII, even if secreted from cardiomyocytes, plays a marginal role in stretch-induced ERKs activation, and raise quite a different possibility that mechanical stress can directly activate the AT_1 receptor without the involvement of AngII.

In human embryonic kidney (HEK) 293 cells or COS7 cells which have no detectable expression of AT_1 receptor and angiotensinogen, neither AngII nor mechanical stretch activated ERKs, but forced expression of AT_1 receptor conferred the ability to activate ERKs in response to both AngII and mechanical stretch. Interestingly, candesartan, as an inverse agonist for ARB, inhibited the ERKs activation induced not only by AngII 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 AngII (Yamano et al., 1992) and in cardiomyocytes prepared from *angiotensinogen*-deficient mice (Tanimoto et al., 1994), and these activations were inhibited by candesartan (Zou et al., 2004). Furthermore, mechanical stress can induce cardiac hypertrophy *in vivo* through the AT_1 receptor in the absence of AngII, 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 AngII both *in vitro* and *in vivo*, and that this AngII-independent activation of AT_1 receptor is inhibited by candesartan.

Besides AT_1 receptor, several GPCRs, such as the receptors of endothelin 1 (ET-1) and catecholamines, also contribute to induction of cardiomyocyte hypertrophy (Yamazaki et al., 1996; Zou et al., 1999). However, mechanical stretch did not induce significant activation of ERKs in COS7 cells expressing either ET-1 type A receptor or β_2-adrenoceptor in a ligand-independent manner. A recent study using a fluorescence resonance energy transfer approach demonstrated that fluid shear stress induced a conformational change of bradykinin B_2 receptor in endothelial cells (Chachisvilis et al., 2006). These results suggest that the activation of GPCRs by mechanical stretch without the involvement of their agonists is not a general phenomenon but specific to some GPCRs including the AT_1 receptor.

5. Conformational switch of AT_1 receptor during mechanical stress-induced activation

Insomuch as AT_1 receptor is activated by mechanical stress, AT_1 receptor should undergo a conformational switch that couples mechanical stress-induced activation. We recently demonstrated by a SCAM study that mechanical stretch increased the accessibility of Cys^{289} in TM7 to the ligand-binding pocket in a time-dependent manner (Yasuda et al., 2008). According to the results of a series of SCAM experiments using mutant receptors with substitution of the TM7 residue ranging from Thr^{287} to Asn^{295} to Cys one at a time, TM7 undergoes a counterclockwise rotation and a shift into the ligand-

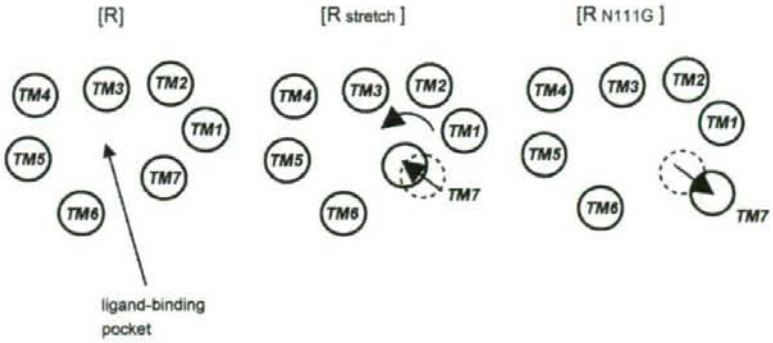

Fig. 2. Helical movements in AT_1 receptor during mechanical stretch-induced activation and in a constitutively active AT_1-N111G receptor. Seven TMs are viewed from the extracellular side. [R] is an unaligned inactive state. [$R_{stretch}$] is an active state stabilized by mechanical stretch. [R_{N111G}] is a state of AT_1-N111G receptor, which mimics a state of AT_1 receptor partially activated by AngII. TM7 rotates counterclockwise and shifts into the ligand-binding pocket in [$R_{stretch}$]. In contrast, TM7 shifts apart from the ligand-binding pocket in [R_{N111G}]. TM, transmembrane domain.

binding pocket in response to mechanical stretch (Yasuda et al., 2008). It is probable that the stabilizing interactions involving TM7 in AT_1 receptor are disrupted by mechanical stress independently of AngII and that counterclockwise rotation of TM7 may cause activation of intracellular signaling pathways. A shift of TM7 to inside the ligand-binding pocket during mechanical stress-induced activation contrasts well with the helical movement observed in a constitutively active AT_1-N111G receptor, because TM7 shifts apart from the ligand-binding pocket in this mutant receptor (Boucard et al., 2003) (Fig. 2). Since AT_1-N111G receptor mimics the state of WT receptor partially activated by AngII (Miura and Karnik, 2002; Le et al., 2003), an active conformation of AT_1 receptor induced by mechanical stress may be substantially different from that by AngII-dependent receptor activation.

Next obvious question is how the AT_1 receptor senses mechanical stress and undergoes a conformational switch. First, membrane tension may directly induce the conformational change of AT_1 receptor. Reconstitution of mechanosensitive channel of large conductance from *Escherichia coli* in synthetic phosphatidylcholines with different chain lengths revealed that thin bilayer favored the open state of channels while thick bilayer stabilized the closed state (Perozo et al., 2002). Likewise, it may be possible that membrane tension causes thinning of the lipid bilayer, which triggers tilting of TM7 of AT_1 receptor to avoid hydrophobic mismatch and to rectify lateral pressure profile (Orr et al., 2006). If so, it follows that AT_1 receptor, a GPCR, functions as a receptor for mechanosensation. It will be intriguing, because GPCRs are involved in mediating senses of vision, olfaction and much of gustation, of Aristotle's five senses (Kung, 2005). Second, mechanical stretch may activate specific mechanosensors, which secondarily activate AT_1 receptor. Potential candidate for mechanosensors, such as muscle LIM protein within the Z-disc (Knoll et al., 2002), integrin-linked kinase (Bendig et al., 2006; White et al., 2006) and melusin (Brancaccio et al., 2003) within the costameres and stretch-sensitive ion channels (Orr et al., 2006; Kung, 2005), might activate the AT_1 receptor, although the underlying mechanism remains to be determined. Recent evidence has shown that mechanical force directly alters conformation or folding of cytoskeletal proteins, which enhances enzymatic activities or susceptibility to enzymatic reactions (Sawada et al., 2006). However, mechanical stretch activated AT_1 receptor even when actin cytoskeleton was disrupted by treatment with cytochalasin D (Yasuda et al., 2008). It will be a great challenge to elucidate the precise mechanism of force sensing by AT_1 receptor.

6. Inverse agonism on agonist-independent activation of AT_1 receptor

Before the early 1990s, GPCR ligands were simply classified as agonists or antagonists (Milligan, 2003; Strange, 2002; Bond and Ijzerman, 2006). Both agonists and 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 (Fig. 3). However, some compounds, originally described as antagonists, have been demonstrated to produce effects opposite to those by agonists. First example was ICI174864, a ligand for δ-opioid receptor, which reduces the basal GTPase activity in membranes of NG108-15 cells (Costa and Herz, 1989). Such ligands are classified as "inverse agonists" that have negative efficacy (Fig. 3). An inverse agonist stabilizes inactive conformation of the receptor and reduces constitutive activity of the receptor or the agonist-independent receptor activity.

Several ARBs are already clinically available for the treatment of hypertension. These drugs share a common action, namely blocking AngII-mediated responses, but show a unique pattern of pharmacological properties (Oparil, 2000). The inverse agonist activity of ARBs could be of clinical advantage to inhibition of both AngII-dependent and -independent receptor activation, and thus be a novel and important pharmacological parameter defining the beneficial effects on organ protection. Candesartan reduces the basal activation of *c-fos* gene promoter by AT_1-WT receptor

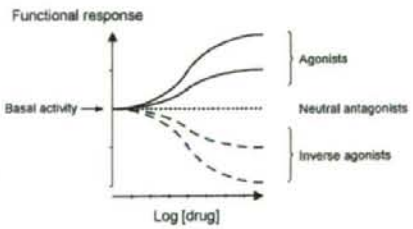

Fig. 3. Classification of GPCR ligands as agonists, neutral antagonists, or inverse agonists. An agonist is a ligand that has a positive efficacy and triggers a functional response. A neutral antagonist is a ligand that has no intrinsic efficacy for a given response, but blocks agonist-induced response. An inverse agonist is a ligand that has a negative efficacy and produces a response opposite to that of the agonist.

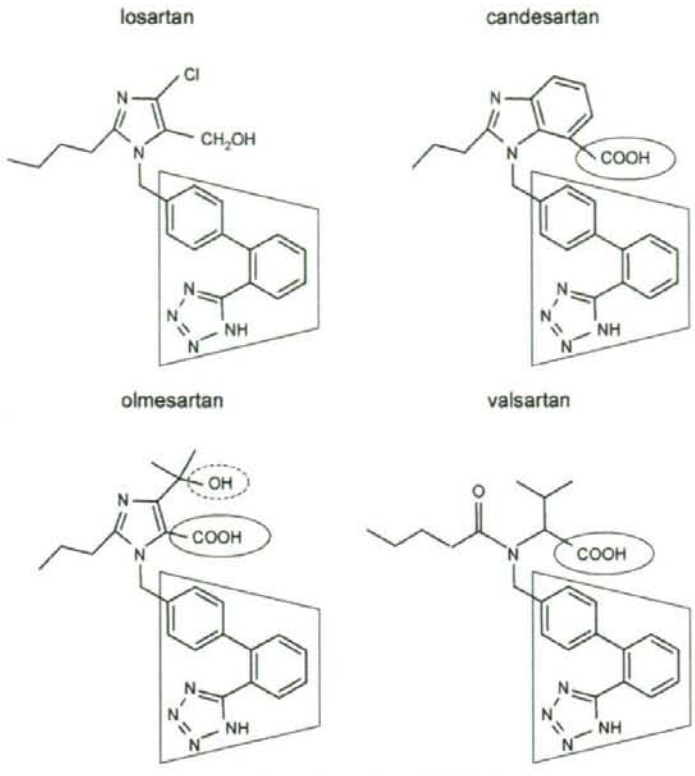

Fig. 4. Chemical structures of losartan, candesartan, olmesartan, and valsartan. A trapezoid indicates biphenyltetrazole ring, a common structure of most ARBs. A circle and a dotted circle indicate carboxyl group and hydroxyl group, respectively. The carboxyl groups and the hydroxyl group in circles are responsible for inverse agonist activity of ARBs.

or a constitutively active AT_1-N111G mutant receptor, suggesting that candesartan is an ARB with potent inverse agonist activity (Yasuda et al., 2008). According to recent papers, olmesartan, valsartan and EXP3174 (active metabolite of losartan) also reduce the constitutive GTPase stimulating activity of AT_1 mutant receptor, while losartan does not reduce it (Miura et al., 2003a, 2006, 2008). Furthermore, candesartan suppressed mechanical stretch-induced helical movement of AT_1 receptor (Yasuda et al., 2008), and thereby inhibited receptor activation (Zou et al., 2004). 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 (Zou et al., 2004).

Although the inverse agonist activity of individual ARBs has not been fully evaluated, we should consider that 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 (Fig. 4), which interacts with Lys^{199} and His^{256} in the AT_1 receptor (Noda et al., 1995b). It was reported that the carboxyl group at the benzimidazole ring of candesartan (Fig. 4) is an important structure for insurmountable inhibition of AngII-induced receptor activation (Noda et al., 1993; Takezako et al., 2004). Insurmountable ARBs depress the maximal agonist responses, in contrast to surmountable ARBs that produce parallel rightward shifts of agonist concentration–response curves in contraction studies using rabbit aortic strip or cell-based functional studies (Vauquelin et al., 2002). It is interpreted that insurmountable inhibition reflects tight drug-receptor complex formation and slow dissociation (Fierens et al., 1999; Vanderheyden et al., 1999). 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 (Yasuda et al., 2008). It is reasonable that the tight binding to AT_1 receptor is prerequisite for an inverse agonist to stabilize the receptor in an inactive conformation, as well as to exert insurmountable inhibition of AngII-induced receptor activation. Besides candesartan, ARBs with potent inverse agonist activity form a complex with AT_1 receptor through tight drug-receptor interactions. For example, olmesartan and valsartan robustly suppresses constitutive production of inositol phosphate by AT_1-N111G receptor (Miura et al., 2006, 2008). 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 requires cooperative interactions between the hydroxyl group and Tyr^{113} in TM3 and between the carboxyl group and His^{256} in TM6 (Miura et al., 2006) (Fig. 4). Interestingly, differential interactions of valsartan to Ser^{105} and Ser^{109} in TM3 and Lys^{199} in TM5 are critical for producing inverse agonism (Miura et al., 2008). Among these docking residues, Ser^{105} binds to the carboxyl group of valsartan (Fig. 4). Thus, the chemical structure of an ARB governs the spatial and kinetic pattern of contacts to the

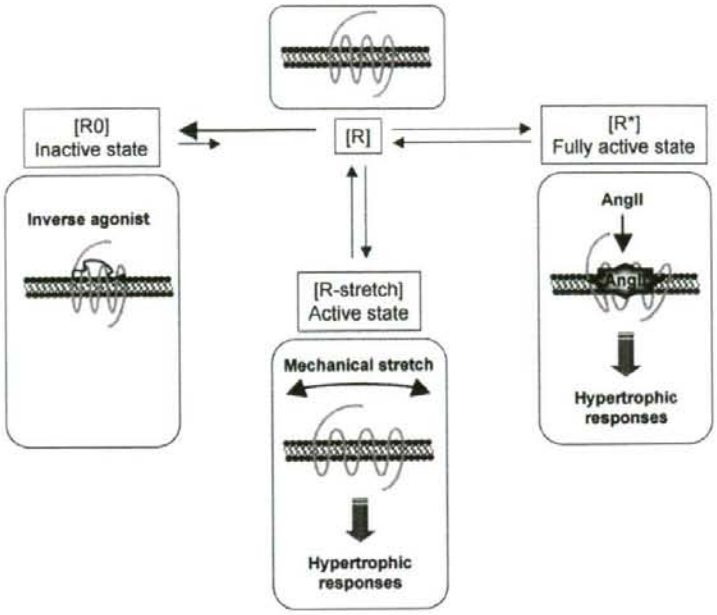

Fig. 5. Distinct conformations of the AT_1 receptor. [R] is an unaligned inactive state, and [R_0] is an inactive state stabilized by an inverse agonist. [R*] is an active state stabilized by the agonist AngII. Mechanical stretch stabilizes AT_1 receptor to another active state [$R_{stretch}$], independently of AngII. An inverse agonist forcibly induces a distinct transition from [R] to [R_0], and prevent a shift of equilibrium to [R*] or [$R_{stretch}$].

AT_1 receptor, which will determine the potency of inverse agonist activity.

According to a sequential binding and conformational model for the molecular mechanism of ligand action on GPCRs (Gether, 2000; Perez and Karnik, 2005), the unaligned receptor in a state [R] can undergo transition to at least two other stabilized states [R_0] and [R*]. [R_0] is an inactive state stabilized by an inverse agonist, and [R*] is an active state stabilized by an agonist. It is consistent with the result of a recent study using a fluorescence resonance energy transfer approach, demonstrating that agonists and inverse agonists for α_{2A}-adrenergic receptor induced distinct conformational changes of the receptor (Vilardaga et al., 2005). With regard to AT_1 receptor, mechanical stretch stabilizes the receptor to another active state [$R_{stretch}$] (Fig. 5). Molecular modeling using the crystal structure of bovine rhodopsin (Palczewski et al., 2000) as a template indicates that, in the inactive state [R_0] in the presence of candesartan, TM6 and 7 move with clockwise rotation, as a consequence of the bindings of the carboxyl group of candesartan to Gln^{257} in TM6 and Thr^{287} in TM7 (Yasuda et al., 2008). The clockwise rotations of TM6 and 7 in this model are consistent with the result of a SCAM experiment demonstrating a decrease in the accessibility of His^{256}, an increase in that of Ile^{290} and a decrease in that of Ala^{291} to the ligand-binding pocket (Yasuda et al., 2008). Therefore, candesartan, as an inverse agonist, forcibly induces a distinct transition from [R] to an inactive conformation [R_0], and prevents a shift of equilibrium to an active conformation [$R_{stretch}$] or [R*] (Fig. 5).

7. Conclusions

The structure–function analyses of the AT_1 receptor have advanced our understanding of the molecular mechanism underlying receptor activation and inverse agonism. Although the structural flexibility of AT_1 receptor, like other GPCRs, may underlie the AngII-independent activation, mechanical stress-induced activation seems to be a phenomenon peculiar to AT_1 receptor. Future investigations with biophysical, biochemical, and pharmacological approaches will elucidate the precise mechanism of force sensing by AT_1 receptor and define the molecular events that link conformational switch of the receptor to the regulation of specific signaling pathways.

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. It is of particular significance to verify whether the drug efficacy assayed in recombinant systems is related to the pharmacological properties *in vivo*. At least, in an experimental animal model, inverse agonist activity of ARBs is relevant to its ability to attenuate load-induced cardiac hypertrophy (Zou et al., 2004). Given that inverse agonist activity is a potential determinant of clinical benefits, molecular dissection of the structure-activity relationship will contribute to the development of a novel and desirable ARB.

We have just taken a first step toward the full understanding of AT_1 receptor activation without the involvement of AngII, and further studies will be required to elucidate the exact molecular mechanisms of receptor activation and to clarify the clinical relevance of inverse agonist activity of ARBs. Recently, crystallizing of native opsin has determined its structure to 2.9 Å resolution, which provides insights into biological process of ligand binding to GPCRs (Park et al., 2008). In addition, crystal structures of squid rhodopsin (Murakami and Kouyama, 2008) and β_1- and β_2-adrenergic receptors (Rasmussen et al., 2007; Cherezov et al., 2007; Rosenbaum et al., 2007) have been obtained, and they reveal several key dif-

ferences with that of bovine rhodopsin. Clearly, crystal structural information of AT_1 receptor will improve our understanding of receptor activation and inactivation at a molecular level.

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References

Balmforth, A.J., Lee, A.J., Warburton, P., Donnelly, D., Ball, S.G., 1997. The conformational change responsible for AT1 receptor activation is dependent upon two juxtaposed asparagine residues on transmembrane helices III and VII. J. Biol. Chem. 272, 4245–4251.

Bendig, G., Grimmler, M., Huttner, I.G., Wessels, G., Dahme, T., Just, S., Trano, N., Katus, H.A., Fishman, M.C., Rottbauer, W., 2006. Integrin-linked kinase, a novel component of the cardiac mechanical stretch sensor, controls contractility in the zebrafish heart. Genes Dev. 20, 2361–2372.

Billet, S., Bardin, S., Verp, S., Baudrie, V., Michaud, A., Conchon, S., Muffat-Joly, M., Escoubet, B., Souil, E., Hamard, G., Bernstein, K.E., Gasc, J.M., Elghozi, J.L., Corvol, P., Clauser, E., 2007. Gain-of-function mutant of angiotensin II receptor, type 1A, causes hypertension and cardiovascular fibrosis in mice. J. Clin. Invest. 117, 1914–1925.

Bond, R.A., Ijzerman, A.P., 2006. Recent developments in constitutive receptor activity and inverse agonism, and their potential for GPCR drug discovery. Trends Pharmacol. Sci. 27, 92–96.

Boucard, A.A., Roy, M., Beaulieu, M.E., Lavigne, P., Escher, E., Guillemette, G., Leduc, R., 2003. Constitutive activation of the angiotensin II type 1 receptor alters the spatial proximity of transmembrane 7 to the ligand-binding pocket. J. Biol. Chem. 278, 36628–36636.

Brancaccio, M., Fratta, L., Notte, A., Hirsch, E., Poulet, R., Guazzone, S., De Acetis, M., Vecchione, C., Marino, G., Altruda, F., Silengo, L., Tarone, G., Lembo, G., 2003. Melusin, a muscle-specific integrin beta1-interacting protein, is required to prevent cardiac failure in response to chronic pressure overload. Nat. Med. 9, 68–75.

Chachisvilis, M., Zhang, Y.L., Frangos, J.A., 2006. G protein-coupled receptors sense fluid shear stress in endothelial cells. Proc. Natl. Acad. Sci. U.S.A. 103, 15463–15468.

Chen, S., Lin, F., Xu, M., Graham, R.M., 2002. Phe(303) in TMVI of the alpha(1B)-adrenergic receptor is a key residue coupling TM helical movements to G-protein activation. Biochemistry 41, 588–596.

Cherezov, V., Rosenbaum, D.M., Hanson, M.A., Rasmussen, S.G., Thian, F.S., Kobilka, T.S., Choi, H.J., Kuhn, P., Weis, W.I., Kobilka, B.K., Stevens, R.C., 2007. High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science 318, 1258–1265.

Costa, T., Cotecchia, S., 2005. Historical review: Negative efficacy and the constitutive activity of G-protein-coupled receptors. Trends Pharmacol. Sci. 26, 618–624.

Costa, T., Herz, A., 1989. Antagonists with negative intrinsic activity at delta opioid receptors coupled to GTP-binding proteins. Proc. Natl. Acad. Sci. U.S.A. 86, 7321–7325.

Cotecchia, S., Exum, S., Caron, M.G., Lefkowitz, R.J., 1990. Regions of the alpha 1-adrenergic receptor involved in coupling to phosphatidylinositol hydrolysis and enhanced sensitivity of biological function. Proc. Natl. Acad. Sci. U.S.A. 87, 2896–2900.

Davies, E., Bonnardeaux, A., Plouin, P.F., Corvol, P., Clauser, E., 1997. Somatic mutations of the angiotensin II (AT1) receptor gene are not present in aldosterone-producing adenoma. J. Clin. Endocrinol. Metab. 82, 611–615.

Dragun, D., Muller, D.N., Brasen, J.H., Fritsche, L., Nieminen-Kelha, M., Dechend, R., Kintscher, U., Rudolph, B., Hoebeke, J., Eckert, D., Mazak, I., Plehm, R., Schonemann, C., Unger, T., Budde, K., Neumayer, H.H., Luft, F.C., Wallukat, G., 2005. Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. N. Engl. J. Med. 352, 558–569.

Farrens, D.L., Altenbach, C., Yang, K., Hubbell, W.L., Khorana, H.G., 1996. Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. Science 274, 768–770.

Feng, Y.H., Noda, K., Saad, Y., Liu, X.P., Husain, A., Karnik, S.S., 1995. The docking of Arg2 of angiotensin II with Asp281 of AT1 receptor is essential for full agonism. J. Biol. Chem. 270, 12846–12850.

Feng, Y.H., Miura, S., Husain, A., Karnik, S.S., 1998. Mechanism of constitutive activation of the AT1 receptor: influence of the size of the agonist switch binding residue Asn(111). Biochemistry 37, 15791–15798.

Fierens, F.L., Vanderheyden, P.M., De Backer, J.P., Vauquelin, G., 1999. Insurmountable angiotensin AT1 receptor antagonists: the role of tight antagonist binding. Eur. J. Pharmacol. 372, 199–206.

Gether, U., 2000. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. Endocr. Rev. 21, 90–113.

Gether, U., Kobilka, B.K., 1998. G protein-coupled receptors. II. Mechanism of agonist activation. J. Biol. Chem. 273, 17979–17982.

Griendling, K.K., Lassegue, B., Alexander, R.W., 1996. Angiotensin receptors and their therapeutic implications. Annu. Rev. Pharmacol. Toxicol. 36, 281–306.

Groblewski, T., Maigret, B., Larguier, R., Lombard, C., Bonnafous, J.C., Marie, J., 1997. Mutation of Asn111 in the third transmembrane domain of the AT1A angiotensin II receptor induces its constitutive activation. J. Biol. Chem. 272, 1822–1826.

Hein, L., Stevens, M.E., Barsh, G.S., Pratt, R.E., Kobilka, B.K., Dzau, V.J., 1997. Overexpression of angiotensin AT1 receptor transgene in the mouse myocardium produces a lethal phenotype associated with myocyte hyperplasia and heart block. Proc. Natl. Acad. Sci. U.S.A. 94, 6391–6396.

Hunyady, L., Catt, K.J., 2006. Pleiotropic AT1 receptor signaling pathways mediating physiological and pathogenic actions of angiotensin II. Mol. Endocrinol. 20, 953–970.

Hunyady, L., Vauquelin, G., Vanderheyden, P., 2003. Agonist induction and conformational selection during activation of a G-protein-coupled receptor. Trends Pharmacol. Sci. 24, 81–86.

Jessup, M., Brozena, S., 2003. Heart failure. N. Engl. J. Med. 348, 2007–2018.

Jongejan, A., Bruysters, M., Ballesteros, J.A., Haaksma, E., Bakker, R.A., Pardo, L., Leurs, R., 2005. Linking agonist binding to histamine H1 receptor activation. Nat. Chem. Biol. 1, 98–103.

Kim, S., Iwao, H., 2000. Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. Pharmacol. Rev. 52, 11–34.

Kira, Y., Kochel, P.J., Gordon, E.E., Morgan, H.E., 1984. Aortic perfusion pressure as a determinant of cardiac protein synthesis. Am. J. Physiol. 246, C247–C258.

Kjeldsen, S.E., Dahlof, B., Devereux, R.B., Julius, S., Aurup, P., Edelman, J., Beevers, G., de Faire, U., Fyhrquist, F., Ibsen, H., Kristianson, K., Lederballe-Pedersen, O., Lindholm, L.H., Nieminen, M.S., Omvik, P., Oparil, S., Snapinn, S., Wedel, H., 2002. Effects of losartan on cardiovascular morbidity and mortality in patients with isolated systolic hypertension and left ventricular hypertrophy: a Losartan intervention for endpoint reduction (LIFE) substudy. JAMA 288, 1491–1498.

Kjelsberg, M.A., Cotecchia, S., Ostrowski, J., Caron, M.G., Lefkowitz, R.J., 1992. Constitutive activation of the alpha 1B-adrenergic receptor by all amino acid substitutions at a single site. Evidence for a region which constrains receptor activation. J. Biol. Chem. 267, 1430–1433.

Klingbeil, A.U., Schneider, M., Martus, P., Messerli, F.H., Schmieder, R.E., 2003. A meta-analysis of the effects of treatment on left ventricular mass in essential hypertension. Am. J. Med. 115, 41–46.

Knoll, R., Hoshijima, M., Hoffman, H.M., Person, V., Lorenzen-Schmidt, I., Bang, M.L., Hayashi, T., Shiga, N., Yasukawa, H., Schaper, W., McKenna, W., Yokoyama, M., Schork, N.J., Omens, J.H., McCulloch, A.D., Kimura, A., Gregorio, C.C., Poller, W., Schaper, J., Schultheiss, H.P., Chien, K.R., 2002. The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. Cell 111, 943–955.

Komuro, I., Yazaki, Y., 1993. Control of cardiac gene expression by mechanical stress. Annu. Rev. Physiol. 55, 55–75.

Kung, C., 2005. A possible unifying principle for mechanosensation. Nature 436, 647–654.

Le, M.T., Vanderheyden, P.M., Szaszak, M., Hunyady, L., Kersemans, V., Vauquelin, G., 2003. Peptide and nonpeptide antagonist interaction with constitutively active human AT1 receptors. Biochem. Pharmacol. 65, 1329–1338.

Lemaire, K., Van de Velde, S., Van Dijck, P., Thevelein, J.M., 2004. Glucose and sucrose act as agonist and mannose as antagonist ligands of the G protein-coupled receptor Gpr1 in the yeast Saccharomyces cerevisiae. Mol. Cell 16, 293–299.

Leurs, R., Smit, M.J., Alewijnse, A.E., Timmerman, H., 1998. Agonist-independent regulation of constitutively active G-protein-coupled receptors. Trends Biochem. Sci. 23, 418–422.

Mann, D.L., Kent, R.L., Cooper, G.t., 1989. Load regulation of the properties of adult feline cardiocytes: growth induction by cellular deformation. Circ. Res. 64, 1079–1090.

Martin, S.S., Holleran, B.J., Escher, E., Guillemette, G., Leduc, R., 2007. Activation of the angiotensin II type 1 receptor leads to movement of the sixth transmembrane domain: analysis by the substituted cysteine accessibility method. Mol. Pharmacol. 72, 182–190.

Milligan, G., 2003. Constitutive activity and inverse agonists of G protein-coupled receptors: a current perspective. Mol. Pharmacol. 64, 1271–1276.

Miura, S., Karnik, S.S., 2002. Constitutive activation of angiotensin II type 1 receptor alters the orientation of transmembrane Helix-2. J. Biol. Chem. 277, 24299–24305.

Miura, S., Feng, Y.H., Husain, A., Karnik, S.S., 1999. Role of aromaticity of agonist switches of angiotensin II in the activation of the AT1 receptor. J. Biol. Chem. 274, 7103–7110.

Miura, S., Saku, K., Karnik, S.S., 2003a. Molecular analysis of the structure and function of the angiotensin II type 1 receptor. Hypertens. Res. 26, 937–943.

Miura, S., Zhang, J., Boros, J., Karnik, S.S., 2003b. TM2-TM7 interaction in coupling movement of transmembrane helices to activation of the angiotensin II type-1 receptor. J. Biol. Chem. 278, 3720–3725.

Miura, S., Fujino, M., Hanzawa, H., Kiya, Y., Imaizumi, S., Matsuo, Y., Tomita, S., Uehara, Y., Karnik, S.S., Yanagisawa, H., Koike, H., Komuro, I., Saku, K., 2006. Molecular

mechanism underlying inverse agonist of angiotensin II type 1 receptor. J. Biol. Chem. 281, 19288–19295.
Miura, S., Kiya, Y., Kanazawa, T., Imaizumi, S., Fujino, M., Matsuo, Y., Karnik, S.S., Saku, K., 2008. Differential bonding interactions of inverse agonists of angiotensin II type 1 receptor in stabilizing the inactive state. Mol. Endocrinol. 22, 139–146.
Morisset, S., Rouleau, A., Ligneau, X., Gbahou, F., Tardivel-Lacombe, J., Stark, H., Schunack, W., Ganellin, C.R., Schwartz, J.C., Arrang, J.M., 2000. High constitutive activity of native H3 receptors regulates histamine neurons in brain. Nature 408, 860–864.
Murakami, M., Kouyama, T., 2008. Crystal structure of squid rhodopsin. Nature 453, 363–367.
Nickenig, G., Jung, O., Strehlow, K., Zolk, O., Linz, W., Scholkens, B.A., Bohm, M., 1997. Hypercholesterolemia is associated with enhanced angiotensin AT1-receptor expression. Am. J. Physiol. 272, H2701–H2707.
Nickenig, G., Roling, J., Strehlow, K., Schnabel, P., Bohm, M., 1998. Insulin induces upregulation of vascular AT1 receptor gene expression by posttranscriptional mechanisms. Circulation 98, 2453–2460.
Nickenig, G., Strehlow, K., Wassmann, S., Baumer, A.T., Albory, K., Sauer, H., Bohm, M., 2000. Differential effects of estrogen and progesterone on AT(1) receptor gene expression in vascular smooth muscle cells. Circulation 102, 1828–1833.
Noda, M., Shibouta, Y., Inada, Y., Ojima, M., Wada, T., Sanada, T., Kubo, K., Kohara, Y., Naka, T., Nishikawa, K., 1993. Inhibition of rabbit aortic angiotensin II (AII) receptor by CV-11974, a new nonpeptide AII antagonist. Biochem. Pharmacol. 46, 311–318.
Noda, K., Saad, Y., Karnik, S.S., 1995a. Interaction of Phe8 of angiotensin II with Lys199 and His256 of AT1 receptor in agonist activation. J. Biol. Chem. 270, 28511–28514.
Noda, K., Saad, Y., Kinoshita, A., Boyle, T.P., Graham, R.M., Husain, A., Karnik, S.S., 1995b. Tetrazole and carboxylate groups of angiotensin receptor antagonists bind to the same subsite by different mechanisms. J. Biol. Chem. 270, 2284–2289.
Noda, K., Feng, Y.H., Liu, X.P., Saad, Y., Husain, A., Karnik, S.S., 1996. The active state of the AT1 angiotensin receptor is generated by angiotensin II induction. Biochemistry 35, 16435–16442.
Okin, P.M., Devereux, R.B., Jern, S., Kjeldsen, S.E., Julius, S., Nieminen, M.S., Snapinn, S., Harris, K.E., Aurup, P., Edelman, J.M., Wedel, H., Lindholm, L.H., Dahlof, B., 2004. Regression of electrocardiographic left ventricular hypertrophy during antihypertensive treatment and the prediction of major cardiovascular events. JAMA 292, 2343–2349.
Oparil, S., 2000. Newly emerging pharmacologic differences in angiotensin II receptor blockers. Am. J. Hypertens. 13, 18S–24S.
Orr, A.W., Helmke, B.P., Blackman, B.R., Schwartz, M.A., 2006. Mechanisms of mechanotransduction. Dev. Cell 10, 11–20.
Palczewski, K., Kumasaka, T., Hori, T., Behnke, C.A., Motoshima, H., Fox, B.A., Le Trong, I., Teller, D.C., Okada, T., Stenkamp, R.E., Yamamoto, M., Miyano, M., 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. Science 289, 739–745.
Paradis, P., Dali-Youcef, N., Paradis, F.W., Thibault, G., Nemer, M., 2000. Overexpression of angiotensin II type I receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. Proc. Natl. Acad. Sci. U.S.A. 97, 931–936.
Park, J.H., Scheerer, P., Hofmann, K.P., Choe, H.W., Ernst, O.P., 2008. Crystal structure of the ligand-free G-protein-coupled receptor opsin. Nature 454, 183–188.
Parma, J., Duprez, L., Van Sande, J., Cochaux, P., Gervy, C., Mockel, J., Dumont, J., Vassart, G., 1993. Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature 365, 649–651.
Parnot, C., Miserey-Lenkei, S., Bardin, S., Corvol, P., Clauser, E., 2002. Lessons from constitutively active mutants of G protein-coupled receptors. Trends Endocrinol. Metab. 13, 336–343.
Paul, M., Poyan Mehr, A., Kreutz, R., 2006. Physiology of local renin–angiotensin systems. Physiol. Rev. 86, 747–803.
Perez, D.M., Karnik, S.S., 2005. Multiple signaling States of G-protein-coupled receptors. Pharmacol. Rev. 57, 147–161.
Perozo, E., Cortes, D.M., Sompornpisut, P., Kloda, A., Martinac, B., 2002. Open channel structure of MscL and the gating mechanism of mechanosensitive channels. Nature 418, 942–948.
Rasmussen, S.G., Choi, H.J., Rosenbaum, D.M., Kobilka, T.S., Thian, F.S., Edwards, P.C., Burghammer, M., Ratnala, V.R., Sanishvili, R., Fischetti, R.F., Schertler, G.F., Weis, W.I., Kobilka, B.K., 2007. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. Nature 450, 383–387.
Re, R.N., 2004. Mechanisms of disease: local renin–angiotensin–aldosterone systems and the pathogenesis and treatment of cardiovascular disease. Nat. Clin. Pract. Cardiovasc. Med. 1, 42–47.
Ren, Q., Kurose, H., Lefkowitz, R.J., Cotecchia, S., 1993. Constitutively active mutants of the alpha 2-adrenergic receptor. J. Biol. Chem. 268, 16483–16487.
Rosenbaum, D.M., Cherezov, V., Hanson, M.A., Rasmussen, S.G., Thian, F.S., Kobilka, T.S., Choi, H.J., Yao, X.J., Weis, W.I., Stevens, R.C., Kobilka, B.K., 2007. GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. Science 318, 1266–1273.
Sachse, R., Shao, X.J., Rico, A., Finckh, U., Rolfs, A., Reincke, M., Hensen, J., 1997. Absence of angiotensin II type 1 receptor gene mutations in human adrenal tumors. Eur. J. Endocrinol. 137, 262–266.
Sadoshima, J., Izumo, S., 1997. The cellular and molecular response of cardiac myocytes to mechanical stress. Annu. Rev. Physiol. 59, 551–571.
Sadoshima, J., Xu, Y., Slayter, H.S., Izumo, S., 1993. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. Cell 75, 977–984.
Samama, P., Cotecchia, S., Costa, T., Lefkowitz, R.J., 1993. A mutation-induced activated state of the beta 2-adrenergic receptor. Extending the ternary complex model. J. Biol. Chem. 268, 4625–4636.
Sasamura, H., Nakazato, Y., Hayashida, T., Kitamura, Y., Hayashi, M., Saruta, T., 1997. Regulation of vascular type 1 angiotensin receptors by cytokines. Hypertension 30, 35–41.
Sawada, Y., Tamada, M., Dubin-Thaler, B.J., Cherniavskaya, O., Sakai, R., Tanaka, S., Sheetz, M.P., 2006. Force sensing by mechanical extension of the Src family kinase substrate p130Cas. Cell 127, 1015–1026.
Shenker, A., Laue, L., Kosugi, S., Merendino Jr., J.J., Minegishi, T., Cutler Jr., G.B., 1993. A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. Nature 365, 652–654.
Sodhi, C.P., Kanwar, Y.S., Sahai, A., 2003. Hypoxia and high glucose upregulate AT1 receptor expression and potentiate ANG II-induced proliferation in VSM cells. Am. J. Physiol. Heart Circ. Physiol. 284, H846–H852.
Strange, P.G., 2002. Mechanisms of inverse agonism at G-protein-coupled receptors. Trends Pharmacol. Sci. 23, 89–95.
Takezako, T., Gogonea, C., Saad, Y., Noda, K., Karnik, S.S., 2004. Network leaning" as a mechanism of insurmountable antagonism of the angiotensin II type 1 receptor by non-peptide antagonists. J. Biol. Chem. 279, 15248–15257.
Tanimoto, K., Sugiyama, F., Goto, Y., Ishida, J., Takimoto, E., Yagami, K., Fukamizu, A., Murakami, K., 1994. Angiotensinogen-deficient mice with hypotension. J. Biol. Chem. 269, 31334–31337.
Thway, T.M., Shlykov, S.G., Day, M.C., Sanborn, B.M., Gilstrap III, L.C., Xia, Y., Kellems, R.E., 2004. Antibodies from preeclamptic patients stimulate increased intracellular Ca2+ mobilization through angiotensin receptor activation. Circulation 110, 1612–1619.
Timmermans, P.B., Wong, P.C., Chiu, A.T., Herblin, W.F., Benfield, P., Carini, D.J., Lee, R.J., Wexler, R.R., Saye, J.A., Smith, R.D., 1993. Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol. Rev. 45, 205–251.
Vanderheyden, P.M., Fierens, F.L., De Backer, J.P., Fraeyman, N., Vauquelin, G., 1999. Distinction between surmountable and insurmountable selective AT1 receptor antagonists by use of CHO-K1 cells expressing human angiotensin II AT1 receptors. Br. J. Pharmacol. 126, 1057–1065.
Vauquelin, G., Van Liefde, I., Vanderheyden, P., 2002. Models and methods for studying insurmountable antagonism. Trends Pharmacol. Sci. 23, 514–518.
Vilardaga, J.P., Steinmeyer, R., Harms, G.S., Lohse, M.J., 2005. Molecular basis of inverse agonism in a G protein-coupled receptor. Nat. Chem. Biol. 1, 25–28.
Wassmann, S., Nickenig, G., 2006. Pathophysiological regulation of the AT1-receptor and implications for vascular disease. J. Hypertens. Suppl. 24, S15–21.
Wassmann, S., Stumpf, M., Strehlow, K., Schmid, A., Schieffer, B., Bohm, M., Nickenig, G., 2004. Interleukin-6 induces oxidative stress and endothelial dysfunction by overexpression of the angiotensin II type 1 receptor. Circ. Res. 94, 534–541.
White, D.E., Coutu, P., Shi, Y.F., Tardif, J.C., Nattel, S., St. Arnaud, R., Dedhar, S., Muller, W.J., 2006. Targeted ablation of ILK from the murine heart results in dilated cardiomyopathy and spontaneous heart failure. Genes Dev. 20, 2355–2360.
Yamano, Y., Ohyama, K., Chaki, S., Guo, D.F., Inagami, T., 1992. Identification of amino acid residues of rat angiotensin II receptor for ligand binding by site directed mutagenesis. Biochem. Biophys. Res. Commun. 187, 1426–1431.
Yamazaki, T., Komuro, I., Kudoh, S., Zou, Y., Shiojima, I., Mizuno, T., Takano, H., Hiroi, Y., Ueki, K., Tobe, K., 1995. Angiotensin II partly mediates mechanical stress-induced cardiac hypertrophy. Circ. Res. 77, 258–265.
Yamazaki, T., Komuro, I., Kudoh, S., Zou, Y., Shiojima, I., Hiroi, Y., Mizuno, T., Maemura, K., Kurihara, H., Aikawa, R., Takano, H., Yazaki, Y., 1996. Endothelin-1 is involved in mechanical stress-induced cardiomyocyte hypertrophy. J. Biol. Chem. 271, 3221–3228.
Yasuda, N., Miura, S., Akazawa, H., Tanaka, T., Qin, Y., Kiya, Y., Imaizumi, S., Fujino, M., Ito, K., Zou, Y., Fukuhara, S., Kunimoto, S., Fukuzaki, K., Sato, T., Ge, J., Mochizuki, N., Nakaya, H., Saku, K., Komuro, I., 2008. Conformational switch of angiotensin II type 1 receptor underlying mechanical stress-induced activation. EMBO Rep. 9, 179–186.
Zaman, M.A., Oparil, S., Calhoun, D.A., 2002. Drugs targeting the renin–angiotensin–aldosterone system. Nat. Rev. Drug Discov. 1, 621–636.
Zou, Y., Komuro, I., Yamazaki, T., Kudoh, S., Uozumi, H., Kadowaki, T., Yazaki, Y., 1999. Both Gs and Gi proteins are critically involved in isoproterenol-induced cardiomyocyte hypertrophy. J. Biol. Chem. 274, 9760–9770.
Zou, Y., Akazawa, H., Qin, Y., Sano, M., Takano, H., Minamino, T., Makita, N., Iwanaga, K., Zhu, W., Kudoh, S., Toko, H., Tamura, K., Kihara, M., Nagai, T., Fukamizu, A., Umemura, S., Iiri, T., Fujita, T., Komuro, I., 2004. Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. Nat. Cell Biol. 6, 499–506.

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Peroxisome Proliferator-Activated Receptor γ and Cardiovascular Diseases

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Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily and form heterodimers with retinoid X receptor. Three PPAR isoforms have been isolated and termed α, β (or δ) and γ. Although PPARγ is expressed predominantly in adipose tissue and associated with adipocyte differentiation and glucose homeostasis, PPARγ is also present in a variety of cell types. Synthetic antidiabetic thiazolidinediones (TZDs) are well known as ligands and activators for PPARγ. After it was reported that activation of PPARγ suppressed production of pro-inflammatory cytokines in activated macrophages, medical interest in PPARγ has grown and there has been a huge research effort. PPARγ is currently known to be implicated in various human chronic diseases such as diabetes mellitus, atherosclerosis, rheumatoid arthritis, inflammatory bowel disease, and Alzheimer's disease. Many studies suggest that TZDs not only ameliorate insulin sensitivity, but also have pleiotropic effects on many tissues and cell types. Although activation of PPARγ seems to have beneficial effects on cardiovascular diseases, the mechanisms by which PPARγ ligands prevent their development are not fully understood. Recent data about the actions and its mechanisms of PPARγ-dependent pathway in cardiovascular diseases are discussed here. (*Circ J* 2009; **73:** 214–220)

Key Words: Atherosclerosis; Cardiac hypertrophy; Heart failure; PPARγ; Thiazolidinedione

Peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor superfamily that heterodimerize with the retinoid X receptor (RXR) and bind to specific response elements termed PPAR responsive elements (PPREs) in target gene promoters. The PPREs are direct repeats of the hexameric consensus sequence AGGTCA, separated by 1 nucleotide. These nuclear receptors are ligand-dependent transcription factors, and activation of target gene transcription depends on the binding of the ligand to the receptor. PPARs have 3 isoforms, α, β (or δ) and γ. PPAR α regulates genes involved in fatty acid oxidation, whereas PPARγ promotes adipocyte differentiation and glucose homeostasis. The main function of PPARβ/δ has yet to be ascertained, but involvement in the regulation of fatty acid oxidation seems likely. PPARα is present mainly in the liver, kidney, and muscle, whereas PPARγ is expressed predominantly in adipose tissue. PPARβ/δ is almost ubiquitously expressed. It was recently demonstrated that PPARγ is also expressed in a variety of cell types. After it was reported that activation of PPARγ suppresses production of inflammatory cytokines in activated macrophages, medical interest in PPARγ has grown, along with a huge research effort.

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PPARγ

Peroxisome is a subcellular organelle that plays a crucial role in cellular metabolism. Peroxisome enzymes are implicated in a broad range of catabolic and anabolic enzymatic pathways, such as fatty acid oxidation, biosynthesis of both glycerolipids and cholesterol, and metabolism of reactive oxygen species. Peroxisome proliferation induced in rodents is associated with cellular responses to a range of chemical compounds. In 1990, Issemann and Green reported that peroxisome proliferators activate a member of the steroid hormone receptor superfamily in mouse liver.[1] This nuclear receptor was named PPAR. Soon after, 3 major types of PPAR (α, β/δ, and γ) were recognized. PPARγ is associated with adipocyte differentiation and glucose homeostasis. PPARγ is expressed in a variety of cell types, including adipocytes, macrophages, vascular smooth muscle cells (VSMCs), endothelial cells (ECs), and cardiomyocytes[2–7] Several lines of evidence have demonstrated the functional significance of PPARγ in atherosclerotic lesions[8,9]

Activity of PPARγ is depressed by phosphorylation of a serine residue (Ser^{112}) in the N-terminal domain, mediated by a member of the mitogen-activated protein (MAP) kinase family, extracellular signal-regulated protein kinase (ERK). In addition, another member of MAP kinase family, c-Jun N-terminal kinase (JNK) also phosphorylates PPARγ at Ser^{82} and reduces the transcriptional activity of PPARγ. The association of PPARγ polymorphism with metabolic syndrome has also been examined.[10,11] In the presence of ligand, PPARγ binds to coactivator complexes, resulting in the activation of target genes. In the absence of ligand, PPARγ binds to the promoters of several target genes and associates with a corepressor complex, leading to active repression of target genes. This process is referred to as active repression (**Fig 1**). The corepressor complex constitutes corepressor proteins, such as nuclear receptor corepressor

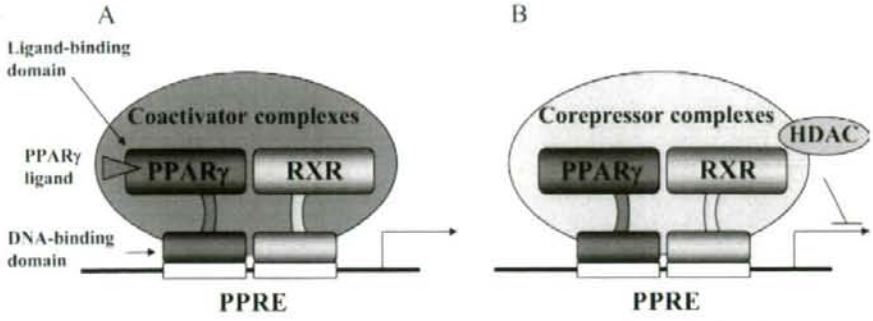

Fig 1. Transactivation and active repression. PPARγ functions as a heterodimer with RXR. (**A**) In the presence of ligand, PPARγ binds to coactivator complexes, resulting in the activation of target genes. (**B**) In the absence of ligand, PPARγ binds to the promoters of several target genes and associates with corepressor complexes, leading to active repression of target genes. HDAC, histone deacetylase; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR responsive element; RXR, retinoid X receptor.

(NCoR) and silencing mediator of retinoid and thyroid hormone receptors, histone deacetylases (HDACs) and transducin β-like protein 1 (TBL1). HDACs are essential in maintaining repressed chromatin structure and TBL1 exchanges a corepressor complex for a coactivator complex in the presence of ligand.[12]

Many nuclear receptors are proposed to sequester inflammatory transcription factors, such as nuclear factor-κB (NF-κB) and AP-1, by inhibiting their DNA-binding activities, resulting in inhibition of inflammatory target genes. In the presence of ligand, PPARγ also interacts with inflammatory transcription factors and inhibits their DNA-binding activities. PPARγ blocks clearance of the corepressor complex in a ligand-dependent manner, and PPARγ stabilizes the corepressor complex bound to the promoter of inflammatory genes.[13] It was demonstrated that PPARγ associates with the protein inhibitor of activated STAT1 (PIAS1), which is a small ubiquitin-like modifier (SUMO)-E3 ligase, in a ligand-dependent manner. PIAS1-induced SUMOylation of the ligand-binding domain of PPARγ enables the receptor to maintain NCoR on the promoter of inflammatory genes.[14] These are the suggested mechanisms of PPARγ transrepression.

PPARγ Ligands

Natural and synthetic ligands bind to PPARγ, resulting in conformational change and activation of PPARγ. The PGD_2 metabolite, 15d-PGJ_2, was the first endogenous ligand for PPARγ to be discovered. Although 15d-PGJ_2 is the most potent natural ligand of PPARγ, the extent to which its effects are mediated through PPARγ in vivo remains to be determined. Two components of oxidized low density lipoprotein (ox-LDL), the 9-hydroxy and 13-hydroxy octadecadienoic acids (HODE), are also potent endogenous activators of PPARγ.[15,16] Activation of 12/15-lipoxygenase induced by interleukin (IL)-4 also produced endogenous ligands for PPARγ;[17] however, whether these natural ligands act as physiological PPARγ ligands in vivo remains unknown. The antidiabetic thiazolidinediones (TZDs), such as troglitazone, pioglitazone, ciglitazone and rosiglitazone, which are used to control glucose concentration in patients with diabetes mellitus (DM), are pharmacological ligands of PPARγ. They bind PPARγ with various affinities and it is conceivable that their insulin-sensitizing and hypoglycemic effects are exerted by activating PPARγ. However, the molecular mechanisms by which TZDs affect insulin resistance and glucose homeostasis are not fully understood. They seem to mediate their effects primarily through adipose tissue, because TZDs alter the expression level of genes that are involved in lipid uptake, lipid metabolism and insulin action in adipocytes. TZDs enhance adipocyte insulin signaling and reduce the release of free fatty acids. TZDs also decrease the inflammation of adipose tissue that is induced by obesity and contributes to increased insulin resistance. There is a possibility that TZDs improve insulin sensitivity in skeletal muscle and liver, the main insulin-sensitive organs, through these multiple adipocentric actions. PPARγ has been demonstrated to have an antiinflammatory effect, leading to initiation of treatment trials for patients with inflammatory diseases. RXR, which interacts with the PPARs, is activated by 9-cis retinoic acid. When combined as a PPAR:RXR heterodimer, the PPAR ligands and 9-cis retinoic acid act synergistically on PPAR responses.

PPARγ and Atherosclerosis

Atherosclerosis is a complex process to which many different factors contribute. Injury of the endothelium, proliferation of VSMCs, migration of monocytes/macrophages, and the regulatory network of growth factors and cytokines are important in the development of atherosclerosis. In addition, chronic inflammation of the vascular wall is also involved. As mentioned earlier, PPARγ has antiinflammatory effect. PPARγ ligands have been shown to reduce production of inflammatory cytokines, such as IL-1β, IL-6, inducible nitric oxide synthase and tumor necrosis factor-α (TNF-α), by inhibiting the activity of transcription factors such as activator protein-1 (AP-1), signal transducers and activators of transcription (STAT), and NF-κB in monocytes/macrophages.[2,3] Those findings suggest that PPARγ activation may have beneficial effects in modulating inflammatory responses in atherosclerosis. Interestingly, expression of PPARγ has been demonstrated in atherosclerotic plaques.[8] Macrophages affect the vulnerability of plaque to rupture and they are implicated in the secretion of matrix metalloproteinases (MMPs), enzymes that are important in the degradation of extracellular matrix. In macrophages and VSMCs, PPARγ

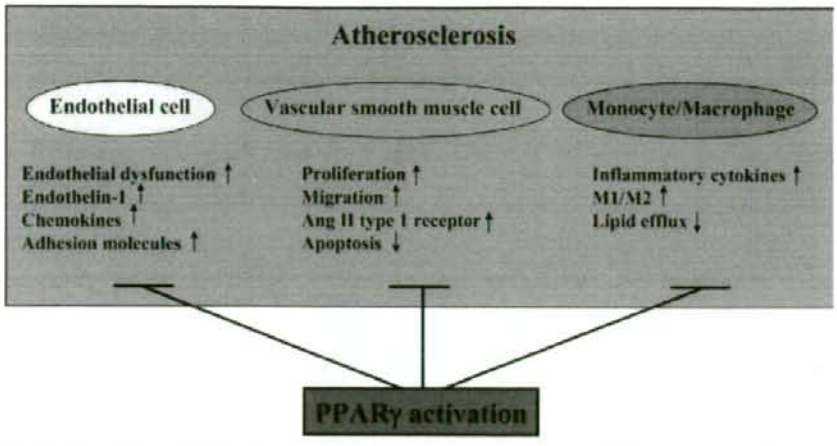

Fig 2. In atherosclerosis, PPARγ inhibits progression of the atherosclerotic lesion. PPAR, peroxisome proliferator-activated receptor.

ligands have been shown to reduce the expression of MMP-9, resulting in the inhibition of migration of VSMCs, and plaque destabilization[3,4] Although activation of T lymphocytes represents a critical step in atherosclerosis, PPARγ ligands also reduce the activation T lymphocytes.[18] Recently, it was reported that PPARγ is a key regulator of M1/M2 polarization.[19] Classically activated macrophages (M1) express a high level of pro-inflammatory cytokines and reactive oxygen species, whereas alternatively activated macrophages (M2) play an antiinflammatory role in atherosclerosis. PPARγ agonists prime monocytes into M2 and PPARγ expression is enhanced by M2 differentiation.[20]

VSMC proliferation and migration are also critical events in atherosclerosis and vascular-intervention-induced restenosis. TZDs inhibit both these changes in the VSMCs and neointimal thickening after vascular injury.[21–24] Furthermore, TZDs induce apoptosis of VSMCs via p53 and Gadd45.[25,26] Angiotensin II (AngII) plays an important role in vascular remodeling via the AngII type 1 receptor (AT1R) and accelerates atherosclerosis. Although AngII induces transcriptional suppression of PPARγ, activation of PPARγ inhibits AT1R gene expression at a transcriptional level in VSMCs.[27–29] Expression of adhesion molecule by ECs, leading to adhesion of leukocytes, is a critical early step in atherosclerosis. PPARγ ligands inhibit the expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 and decreased production of chemokines, such as IL-8 and monocyte chemotactic protein-1 (MCP-1) via suppressions of AP-1 and NF-κB activities in ECs.[30–32] PPARγ ligands also inhibit MCP-1-induced monocytes migration.[33] Endothelin-1 (ET-1) is involved in the regulation of vascular tone and endothelial functions, and induces proliferation of VSMCs. In bovine aortic ECs, PPARγ ligands suppressed transcription of the ET-1 promoter by interfering with AP-1.[34]

PPARγ activation by major oxidized lipid components of ox-LDL, 9-HODE and 13-HODE has an important role in the development of lipid-accumulating macrophages through transcriptional induction of CD36, a scavenger receptor.[35] These findings suggest that atherogenic ox-LDL particles could induce their own uptake through activation of PPARγ and expression of CD36, leading to atherosclerosis. However, several studies have demonstrated that activation of PPARγ does not promote lipid accumulation in either mouse or human macrophages.[36–38] Liver X receptor α (LXRα) is an oxysterol receptor that promotes cholesterol excretion and efflux by modulating expression of ATP-binding cassette transporter 1 (ABCA1).[37,38] LXRα was recently identified as a direct target of PPARγ in mouse and human macrophages.[39,40] Although the PPARγ-induced increase in CD36 expression might accelerate lipid uptake in macrophages, subsequent activation of LXRα and upregulation of ABCA1 appear to induce lipid efflux.

Diep et al have demonstrated that rosiglitazone and pioglitazone attenuate the development of hypertension and structural abnormalities, and improve endothelial dysfunction in AngII-infused rats.[41] These TZDs also prevented upregulation of AT1R, cell cycle proteins, and inflammatory mediators. Rosiglitazone, but not the PPARα ligand fenofibrate, prevented hypertension and endothelial dysfunction in DOCA-salt hypertensive rats.[42] It has been reported that serum levels of the soluble CD40 ligand are elevated in acute coronary syndrome and associated with increased cardiovascular risk. Treatment with rosiglitazone decreased the serum levels of soluble CD40 and MMP-9 in type 2 diabetic patients with coronary artery disease.[43] Taking all the evidence together, PPARγ ligands may prevent the progression of atherosclerotic lesions, particularly in patients with DM (**Fig 2**).

PPARγ and Ischemic Heart Disease

As the effects of PPARγ on the heart are not fully understood, we and others have examined whether PPARγ is involved in various heart diseases. Although the expression of PPARγ in cardiac myocytes is low compared with adipocytes, PPARγ ligands seem to act on cardiac myocytes.[7,44] We demonstrated that PPARγ ligands inhibited the cardiac expression of TNF-α at the transcriptional level, in part by antagonizing NF-κB activity.[7] Because TNF-α expression is elevated in the failing heart and has a negative inotropic effect on cardiac myocytes, treatment with PPARγ ligands may prevent the development of congestive heart failure. Diabetic cardiomyopathy, which is characterized by

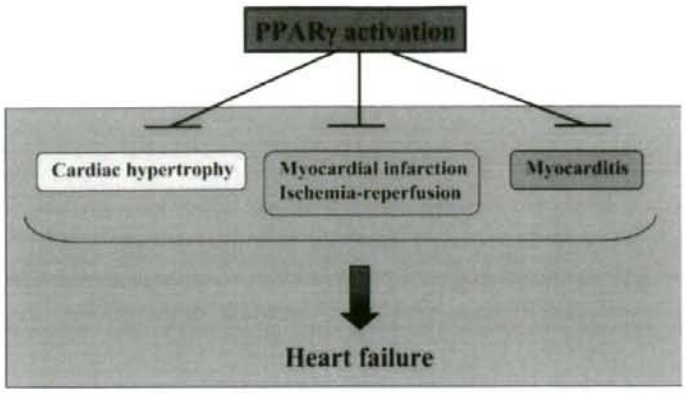

Fig 3. Actions of PPARγ in heart diseases. PPARγ inhibits the progression of heart failure following cardiac hypertrophy, myocardial infarction, ischemia–reperfusion injury, and myocarditis. PPAR, peroxisome proliferator-activated receptor.

systolic and diastolic dysfunction, is a major complication of DM, and therefore TZDs seem to be beneficial for the impaired cardiac function in patients with DM. Following our study, the role of PPARγ in myocardial ischemia–reperfusion (IR) injury has been elucidated[45–48] In animal models, PPARγ ligands reduced the size of the myocardial infarct and improved contractile dysfunction after IR through inhibition of the inflammatory response. IR injury activates JNK, and subsequently JNK induces increases in both AP-1 DNA-binding activity and apoptotic cells. It has been shown in rats that rosiglitazone inhibits the activation of JNK and AP-1 after myocardial IR.[46] Furthermore, pioglitazone has been reported to attenuate left ventricular remodeling and heart failure after myocardial infarction (MI) in mice.[49] Both of these effects of TZDs ligands were associated with decreases in inflammatory cytokines and chemokines.[49,50]

PPARγ and Cardiac Hypertrophy

The PPARγ ligands, troglitazone, pioglitazone and rosiglitazone, inhibited AngII-induced hypertrophy of neonatal rat cardiac myocytes.[51–53] Because generalized PPARγ gene deletion causes embryonic lethality, we examined the role of PPARγ in the development of cardiac hypertrophy in vivo using heterozygous PPARγ-deficient ($PPAR\gamma^{+/-}$) mice.[53] Pressure overload-induced cardiac hypertrophy was more prominent in heterozygous $PPAR\gamma^{+/-}$ mice than in wild-type (WT) mice. Treatment with pioglitazone strongly inhibited the pressure overload-induced cardiac hypertrophy in WT mice and moderately in $PPAR\gamma^{+/-}$ mice.[53] Thereafter, 2 other groups examined the role of PPARγ in the heart by using cardiomyocyte-specific PPARγ knockout mice.[54,55] Duan et al reported that these mice develop cardiac hypertrophy through elevated NF-κB activity;[54] and unexpectedly, rosiglitazone induced cardiac hypertrophy in both the WT mice and cardiomyocyte-specific PPARγ knockout mice through activation of p38 MAP kinase independent of PPARγ. Ding et al reported that cardiomyocyte-specific PPARγ knockout mice displayed cardiac hypertrophy from approximately 3 months of age and then progress to dilated cardiomyopathy;[55] most mice died from heart failure within 1 year after birth. Mitochondrial oxidative damage and reduced expression of manganese superoxide dismutase were recognized in the cardiomyocyte-specific PPARγ knockout mice.[55] These mice models demonstrate that PPARγ is essential for protecting cardiomyocytes from stress and oxidative damage, although the expression level of PPARγ in cardiomyocytes is low. On the other hand, Son et al demonstrated that cardiomyocyte-specific PPARγ transgenic mice develop dilated cardiomyopathy associated with increased uptake of both fatty acid and glucose.[56] Rosiglitazone increased this glucolipotoxicity in cardiomyocyte-specific PPARγ transgenic mice. If PPARγ in the heart is expressed at a high level, rosiglitazone may cause cardiotoxic effects; however, as noted earlier the expression level of PPARγ in the heart is quite low.

PPARγ and Myocarditis

Experimental autoimmune myocarditis (EAM) is a T-cell-mediated disease characterized by infiltration of T cells and macrophages, leading to massive myocarditis necrosis, which develops into heart failure in the chronic phase.[57] The onset of EAM in rats occurs approximately 2 weeks after the first immunization with porcine cardiac myosin. At this time, small numbers of $CD4^+$ T cells and macrophages start to infiltrate into the myocardium and various cytokines are expressed. Macrophage inflammatory protein-1α (MIP-1α) is a C-C chemokine that induces leukocyte accumulation in tissue sites of inflammation. We previously demonstrated that MIP-1α mRNA and protein are highly expressed in the hearts of rats with EAM from day 11 after first immunization.[57] Th1 cells produce interferon-γ (IFN-γ), which is mainly involved in cell-mediated immune responses, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13, which participate in humoral responses. Immune dysfunction associated with autoimmune disease is known to involve an imbalance between Th1 and Th2 cells.

It has been reported that pioglitazone treatment markedly reduces the severity of myocarditis in a rat model of EAM.[58,59] Pioglitazone suppressed expression of inflammatory cytokines and activation of myocardiogenic T cells in the myocardium of EAM rats.[58] The mRNA levels of MIP-1γ were upregulated in the hearts of EAM rats, but not in the hearts of those in the pioglitazone group. Furthermore, treatment with pioglitazone decreased the expression levels of pro-inflamatory cytokine (TNF-α and IL-1β) genes and Th1 cytokine (IFN-γ) genes, and increased the expression levels of Th2 cytokine (IL-4) gene.[59] These results suggest that PPARγ ligands may have beneficial effects on myocarditis by inhibiting MIP-1α expression and modulating the Th1/Th2 balance (**Fig 3**).

Efficacy and Safety of TZD Treatment in the Clinical Setting

Despite the beneficial effects of TZDs in the basic experiments, their propensity to cause fluid retention is a serious side-effect. Clinical studies report TZD-induced peripheral fluid retention, and an increase in plasma volume in 2–5% of patients on monotherapy[60] Fluid retention was more likely to occur with concomitant insulin use, and in patients with underlying cardiac dysfunction or renal insufficiency. The exact mechanisms for TZD-induced fluid retention are not well understood, and it remains unclear whether TZDs directly cause the development of de novo congestive heart failure. It is known that the level of vascular endothelial growth factor is increased in the patients who develop fluid retention with TZD therapy[61] and this may lead to peripheral edema through increased vascular permeability. The insulin-sensitizing action of TZDs also induces water and salt retention. PPARγ is highly expressed in the kidney and collecting-duct-specific PPARγ knockout mice demonstrated no effects of TZD on fluid retention or the expression level of sodium channel ENaC-γ[62,63] These findings suggest that activation of the sodium channel in the collecting duct cells expressing PPARγ may be a mechanism of fluid retention. In patients without evidence of heart failure, careful examination did not reveal any worsening of left ventricular function by TZDs[64] There are very few studies investigating the safety of TZDs in patients with preexisting heart failure. Although a recent study demonstrated that there is not a direct association between the risk of fluid retention and the baseline degree of severity of heart failure in diabetic patients treated with TZDs, the prescription of TZDs for patients with established heart failure should be avoided at present[60,65]

The PROactive (Prospective Pioglitazone Clinical Trial in Macrovascular Events) study has shown that pioglitazone significantly decreases the occurrence of all-cause mortality, nonfatal MI, and nonfatal stroke in patients with type 2 DM and macrovascular diseases[66] Pioglitazone significantly reduced the occurrence of fatal and nonfatal MI by 28% in the PROactive study[66] Although there was a 1.6% absolute increase in heart failure hospitalizations in the pioglitazone group compared with the placebo group, the number of heart-failure-related deaths was almost identical. In contrast to the PROactive study, it has been recently reported that rosiglitazone treatment is associated with increased incidence of MI by meta-analysis[67,68] Although meta-analysis has a number of limitations and the increased risk in MI is still controversial, those results attracted the attention of many clinicians. There are some differences in the actions of pioglitazone and rosiglitazone. Pioglitazone has more beneficial effects on the lipid profile than rosiglitazone[69] As mentioned earlier, rosiglitazone, but not pioglitazone, induced cardiac hypertrophy by a non-PPARγ-mediated pathway[54] Pioglitazone represses NF-κB activation and VCAM-1 expression in a PPARα-dependent manner[70] Pioglitazone was recently reported to increase the number and function of endothelial progenitor cells (EPCs) in patients with stable coronary artery disease and normal glucose tolerance[71] Pioglitazone may induce angiogenesis by modulating EPC mobilization and function. In the future, more mechanistic studies are required to investigate the differences in action between pioglitazone and rosiglitazone.

Conclusions

The American Heart Association (AHA) and American Diabetes Association (ADA) have released a consensus statement that advises caution regarding the use of TZDs in patients with known or suspected heart failure[72] Because there is a possibility that TZDs may unmask asymptomatic cardiac dysfunction by increasing plasma volume, they should be avoided in patients with congestive heart failure of New York Heart Association (NYHA) class III or IV. The data from in vitro studies suggest that TZDs exert direct actions on vascular cells and cardiomyocytes, independent of their glucose-mediated mechanisms. Further studies using tissue-specific gene targeting mice are necessary to address in vivo the pleiotropic effects of PPARγ on the cardiovascular system. If the beneficial roles of PPARγ can be solved, modulation of PPARγ may become a promising therapeutic strategy for cardiovascular diseases. Because cardiac hypertrophy can be seen even in normotensive diabetic patients, and diabetic cardiomyopathy is a major complication of DM, antidiabetic agents such as the TZDs would be expected to have beneficial effects on cardiac hypertrophy and dysfunction in patients with DM. It has been already clarified that 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, statins, have pleiotropic effects in cardiovascular diseases. The effects of PPARγ ligands are similar to those of statins in many respects. A recent study demonstrated that statins activate PPARγ through ERK and p38 MAP-kinase-dependent cyclooxygenase-2 expression in macrophages[73] Further studies are needed to elucidate the molecular mechanisms of the pleiotropic effects of PPARγ ligands in cardiovascular disease.

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References

1. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 1990; **347:** 645–650.
2. Jiang C, Ting AT, Seed B. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998; **391:** 82–86.
3. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature* 1998; **391:** 79–82.
4. Marx N, Schonbeck U, Lazar MA, Libby P, Plutzky J. Peroxisome proliferator-activated receptor γ activators inhibit gene expression and migration in human vascular smooth muscle cells. *Circ Res* 1998; **83:** 1097–1103.
5. Iijima K, Yoshizumi M, Ako J, Eto M, Kim S, Hashimoto M, et al. Expression of peroxisome proliferator-activated receptor γ (PPARγ) in rat aortic smooth muscle cells. *Biochem Biophys Res Commun* 1998; **247:** 353–356.
6. Benson S, Wu J, Padmanabhan S, Kurtz TW, Pershadsingh HA. Peroxisome proliferator-activated receptor (PPAR)-γ expression in human vascular smooth muscle cells: Inhibition of growth, migration, and c-fos expression by the peroxisome proliferator-activated receptor (PPAR)-γ activator troglitazone. *Am J Hypertens* 2000; **13:** 74–82.
7. Takano H, Nagai T, Asakawa M, Toyozaki T, Oka T, Komuro I, et al. Peroxisome proliferator-activated receptor activators inhibit lipopolysaccharide-induced tumor necrosis factor-α expression in neonatal rat cardiac myocytes. *Circ Res* 2000; **87:** 596–602.
8. Marx N, Sukhova G, Murphy C, Libby P, Plutzky J. Macrophage in human atheroma contain PPARγ: Differentiation-dependent peroxisomal proliferator-activated receptor γ (PPARγ) expression and reduction of MMP-9 activity through PPARγ activation in mononuclear phagocytes in vitro. *Am J Pathol* 1998; **153:** 17–23.

9. Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, et al. Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. *Proc Natl Acad Sci USA* 1998; **95:** 7614–7619.
10. Rhee EJ, Kwon CH, Lee WY, Kim SY, Jung CH, Kim BJ, et al. No association of Pro12Ala polymorphism of PPAR-gamma gene with coronary artery disease in Korean subjects. *Circ J* 2007; **71:** 338–342.
11. Dongxia L, Qi H, Lisong L, Jincheng G. Association of peroxisome proliferator-activated receptor γ gene Pro12Ala and C161T polymorphisms with metabolic syndrome. *Circ J* 2007; **72:** 551–557.
12. Perissi V, Aggarwal A, Glass CK, Rose DW, Rosenfeld MG. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. *Cell* 2004; **116:** 511–526.
13. Ogawa S, Lozach J, Jepsen K, Sawka-Verhelle D, Perissi V, Sasik R, et al. A nuclear receptor corepressor transcriptional checkpoint controlling activator protein 1-dependent gene networks required for macrophage activation. *Proc Natl Acad Sci USA* 2004; **101:** 14461–14466.
14. Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, et al. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature* 2005; **437:** 759–763.
15. Nagy L, Tontonoz P, Alvarez JGA, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. *Cell* 1998; **93:** 229–240.
16. Tontonoz P, Nagy L, Alvarez JGA, Thomazy VA, Evans RM. PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell* 1998; **93:** 241–252.
17. Huang JT, Welch JS, Ricote M, Binder CJ, Willson TM, Kelly C, et al. Interleukin-4-dependent production of PPAR-γ ligands in macrophages by 12/15-lipoxygenase. *Nature* 1999; **400:** 378–382.
18. Marx N, Kehrle B, Kohlhammer K, Grub M, Koenig W, Hombach V, et al. PPAR activators as antiinflammatory mediators in human T lymphocytes: Implications for atherosclerosis and transplantation-associated arteriosclerosis. *Circ Res* 2002; **90:** 703–710.
19. Bouhlel MA, Derudas B, Rigamonti E, Dièvart R, Brozek J, Haulon S, et al. PPARγ activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. *Cell Metab* 2007; **6:** 137–143.
20. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, et al. Macrophage-specific PPARγ controls alternative activation and improves insulin resistance. *Nature* 2007; **447:** 1116–1120.
21. Law RE, Meehan WP, Xi XP, Graf K, Wuthrich DA, Coats W, et al. Troglitazone inhibits vascular smooth muscle cell growth and intimal hyperplasia. *J Clin Invest* 1996; **98:** 1897–1905.
22. Goetze S, Xi X P, Kawano H, Gotlibowski T, Fleck E, Hsueh WA, et al. PPARγ-ligands inhibit migration mediated by multiple chemoattractants in vascular smooth muscle cells. *J Cardiovasc Pharmacol* 1999; **33:** 798–806.
23. Hsueh WA, Jackson S, Law RE. Control of vascular cell proliferation and migration by PPAR-γ: A new approach to the macrovascular complications of diabetes. *Diabetes Care* 2001; **24:** 392–397.
24. Takata Y, Kitami Y, Okura T, Hiwada K. Peroxisome proliferator-activated receptor-γ activation inhibits interleukin-1β-mediated platelet-derived growth factor-α receptor gene expression via CCAAT/enhancer-binding protein-δ in vascular smooth muscle cells. *J Biol Chem* 2001; **276:** 12893–12897.
25. Okura T, Nakamura M, Takata Y, Watanabe S, Kitami Y, Hiwada K. Troglitazone induces apoptosis via the p53 and Gadd45 pathway in vascular smooth muscle cells. *Eur J Pharmacol* 2000; **407:** 227–235.
26. Aizawa Y, Kawabe J, Hasebe N, Takehara N, Kikuchi K. Pioglitazone enhances cytokine-induced apoptosis in vascular smooth muscle cells and reduces intimal hyperplasia. *Circulation* 2001; **104:** 455–460.
27. Sugawara A, Takeuchi K, Uruno A, Ikeda Y, Arima S, Kudo M, et al. Transcriptional suppression of type 1 angiotensin II receptor gene expression by peroxisome proliferator-activated receptor-γ in vascular smooth muscle cells. *Endocrinology* 2001; **142:** 3125–3134.
28. Takeda K, Ichiki T, Tokunou T, Funakoshi Y, Iino N, Hirano K, et al. Peroxisome proliferator-activated receptor γ activators downregulate angiotensin II type 1 receptor in vascular smooth muscle cells. *Circulation* 2000; **102:** 1834–1839.
29. Tham DM, Martin-McNulty B, Wang YX, Wilson DW, Vergona R, Sullivan ME, et al. Angiotensin II is associated with activation of NF-κB-mediated genes and downregulation of PPARs. *Physiol Genomics* 2002; **11:** 21–30.
30. Jackson SM, Parhami F, Xi XP, Berliner JA, Hsueh WA, Law RE, et al. Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte-endothelial cell interaction. *Arterioscler Thromb Vasc Biol* 1999; **19:** 2094–2104.
31. Pasceri V, Wu HD, Willerson JT, Yeh ETH. Modulation of vascular inflammation in vitro and in vivo by peroxisome proliferator-activated receptor-γ activators. *Circulation* 2000; **101:** 235–238.
32. Lee H, Shi W, Tontonoz P, Wang S, Subbanagounder G, Hedrick CC, et al. Role of peroxisome proliferator-activated receptor α in oxidized phospholipid-induced synthesis of monocyte chemotactic protein-1 and interleukin-8 by endothelial cells. *Circ Res* 2000; **87:** 516–521.
33. Kintscher U, Goetze S, Wakino S, Kim S, Nagpal S, Chandraratna RA, et al. Peroxisome proliferator-activated receptor and retinoid X receptor ligands inhibit monocyte chemotactic protein-1-directed migration of monocytes. *Eur J Pharmacol* 2000; **401:** 259–270.
34. Delerive P, Martin-Nizard F, Chinetti G, Trottein F, Fruchart JC, Najib J, et al. Peroxisome proliferator-activated receptor activators inhibit thrombin-induced endothelin-1 production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway. *Circ Res* 1999; **85:** 394–402.
35. Han J, Hajjar DP, Tauras JM, Feng J, Gotto AM Jr, Nicholson AC. Transforming growth factor-β1 (TGF-β1) and TGF-β2 decrease expression of CD36, the type B scavenger receptor, through mitogen-activated protein kinase phosphorylation of peroxisome proliferator-activated receptor-γ. *J Biol Chem* 2000; **275:** 1241–1246.
36. Repa JJ, Turley SD, Lobaccaro JA, Medina J, Li L, Lustig K, et al. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science* 2000; **289:** 1524–1529.
37. Venkateswaran A, Laffitte BA, Joseph SB, Mak PA, Wilpitz DC, Edwards PA, et al. Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR alpha. *Proc Natl Acad Sci USA* 2000; **97:** 12097–12102.
38. Costet P, Luo Y, Wang N, Tall AR. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J Biol Chem* 2000; **275:** 28240–28245.
39. Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, et al. A PPARγ-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. *Mol Cell* 2001; **7:** 161–171.
40. Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP, et al. PPAR-α and PPAR-γ activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat Med* 2001; **7:** 53–58.
41. Diep QN, El Mabrouk M, Cohn JS, Endemann D, Amiri F, Virdis A, et al. Structure, endothelial function, cell growth, and inflammation in blood vessels of angiotensin II-infused rats: Role of peroxisome proliferator-activated receptor-γ. *Circulation* 2002; **105:** 2296–2302.
42. Iglarz M, Touyz RM, Amiri F, Lavoie MF, Diep QN, Schiffrin EL. Effect of peroxisome proliferator-activated receptor-α and -γ activators on vascular remodeling in endothelin-dependent hypertension. *Arterioscler Thromb Vasc Biol* 2003; **23:** 45–51.
43. Marx N, Imhof A, Froehlich J, Siam L, Ittner J, Wierse G, et al. Effect of rosiglitazone treatment on soluble CD40L in patients with type 2 diabetes and coronary artery disease. *Circulation* 2003; **107:** 1954–1957.
44. Mehrabi MR, Thalhammer T, Haslmayer P, Glogar HD, Wieselthaler G, Humpeler S, et al. The peroxisome proliferator-activated receptor γ (PPARγ) is highly expressed in human heart ventricles. *Biomed Pharmacother* 2002; **56:** 407–410.
45. Yue T, Chen J, Bao W, Narayanan PK, Bril A, Jiang W, et al. In vivo myocardial protection from IR injury by the peroxisome proliferator-activated receptor-γ agonist rosiglitazone. *Circulation* 2001; **104:** 2588–2594.
46. Khandoudi N, Delerive P, Berrebi-Bertrand I, Buckingham RE, Staels B, Bril A. Rosiglitazone, a peroxisome proliferator-activated receptor-γ, inhibits the Jun NH(2)-terminal kinase/activating protein 1 pathway and protects the heart from IR injury. *Diabetes* 2002; **51:** 1507–1514.
47. Wayman NS, Hattori Y, McDonald MC, Mota-Filipe H, Cuzzocrea S, Pisano B, et al. Ligands of the peroxisome proliferator-activated receptors (PPAR-γ and PPAR-α) reduce myocardial infarct size. *FASEB J* 2002; **16:** 1027–1040.
48. Zhu P, Lu L, Xu Y, Schwartz GG. Troglitazone improves recovery of left ventricular function after regional ischemia in pigs. *Circulation* 2000; **101:** 1165–1171.
49. Shiomi T, Tsutsui H, Hayashidani S, Suematsu N, Ikeuchi M, Wen J, et al. Pioglitazone, a Peroxisome proliferator-activated receptor-γ agonist, attenuates left ventricular remodeling and failure after experimental myocardial infarction. *Circulation* 2002; **106:** 3126–3132.

50. Ikejima H, Imanishi T, Tsujioka H, Kuroi A, Muragaki Y, Mochizuki S, et al. Effect of pioglitazone on nitroglycerin-induced impairment of nitric oxide bioavailability by a catheter-type nitric oxide sensor. *Circ J* 2008; **72:** 998–1002.
51. Takano H, Zou Y, Akazawa H, Toko H, Mizukami M, Hasegawa H, et al. Inhibitory molecules in signal transduction pathways of cardiac hypertrophy. *Hypertens Res* 2002; **25:** 491–498.
52. Yamamoto K, Ohki R, Lee RT, Ikeda U, Shimada K. Peroxisome proliferator-activated receptor γ activators inhibit cardiac hypertrophy in cardiac myocytes. *Circulation* 2001; **104:** 1670–1675.
53. Asakawa M, Takano H, Nagai T, Uozumi H, Hasegawa H, Kubota N, et al. Peroxisome proliferator-activated receptor γ plays a critical role in inhibition of cardiac hypertrophy in vitro and in vivo. *Circulation* 2002; **105:** 1240–1246.
54. Duan SZ, Ivashchenko CY, Russell MW, Milstone DS, Mortensen RM. Cardiomyocyte-specific knockout and agonist of peroxisome proliferator-activated receptor-γ both induce cardiac hypertrophy in mice. *Circ Res* 2005; **97:** 372–379.
55. Ding G, Fu M, Qin Q, Lewis W, Kim HW, Fukai T, et al. Cardiac peroxisome proliferator-activated receptor γ is essential in protecting cardiomyocytes from oxidative damage. *Cardiovasc Res* 2007; **76:** 269–279.
56. Son NH, Park TS, Yamashita H, Yokoyama M, Huggins LA, Okajima K, et al. Cardiomyocyte expression of PPARγ leads to cardiac dysfunction in mice. *J Clin Invest* 2007; **117:** 2791–2801.
57. Toyozaki T, Saito T, Shiraishi H, Tsukamoto Y, Takano H, Nagai T, et al. Macrophage inflammatory protein-1α relates to the recruitment of inflammatory cells in myosin-induced autoimmune myocarditis in rats. *Lab Invest* 2001; **81:** 929–936.
58. Yuan Z, Liu Y, Liu Y, Zhang J, Kishimoto C, Wang Y, et al. Peroxisome proliferation-activated receptor-γ ligands ameliorate experimental autoimmune myocarditis. *Cardiovasc Res* 2003; **59:** 685–694.
59. Hasegawa H, Takano H, Zou Y, Qin Y, Hizukuri K, Odaka K, et al. Pioglitazone, a peroxisome proliferator-activated receptor γ activator, ameliorates experimental autoimmune myocarditis by modulating Th1/Th2 balance. *J Mol Cell Cardiol* 2005; **38:** 257–265.
60. Wang CH, Weisel RD, Liu PP, Fedak PW, Verma S. Glitazones and heart failure: Critical appraisal for the clinician. *Circulation* 2003; **107:** 1350–1354.
61. Sotiropoulos KB, Clermont A, Yasuda Y, Rask-Madsen C, Mastumoto M, Takahashi J, et al. Adipose-specific effect of rosiglitazone on vascular permeability and protein kinase C activation: Novel mechanism for PPAR γ agonist's effects on edema and weight gain. *FASEB J* 2006; **20:** 1203–1205.
62. Zhang H, Zhang A, Kohan DE, Nelson RD, Gonzalez FJ, Yang T. Collecting duct-specific deletion of peroxisome proliferator-activated receptor γ blocks thiazolidinedione-induced fluid retention. *Proc Natl Acad Sci USA* 2005; **102:** 9406–9411.
63. Guan Y, Hao C, Cha DR, Rao R, Lu W, Kohan DE, et al. Thiazolidinediones expand body fluid volume through PPARγ stimulation of ENaC-mediated renal salt absorption. *Nat Med* 2005; **11:** 861–866.
64. St John Sutton M, Rendell M, Dandona P, Dole JF, Murphy K, Patwardhan R, et al. A comparison of the effects of rosiglitazone and glyburide on cardiovascular function and glycemic control in patients with type 2 diabetes. *Diabetes Care* 2002; **25:** 2058–2064.
65. Tang WH, Francis GS, Hoogwerf BJ, Young JB. Fluid retention after initiation of thiazolidinedione therapy in diabetic patients with established chronic heart failure. *J Am Coll Cardiol* 2003; **41:** 1394–1398.
66. Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): A randomised controlled trial. *Lancet* 2005; **366:** 1279–1289.
67. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007; **356:** 2457–2471.
68. Singh S, Loke YK, Furberg CD. Long-term risk of cardiovascular events with rosiglitazone: A meta-analysis. *JAMA* 2007; **298:** 1189–1195.
69. Goldberg RB, Kendall DM, Deeg MA, Buse JB, Zagar AJ, Pinaire JA, et al. A comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia. *Diabetes Care* 2005; **28:** 1547–1554.
70. Orasanu G, Ziouzenkova O, Devchand PR, Nehra V, Hamdy O, Horton ES, et al. The peroxisome proliferator-activated receptor-γ agonist pioglitazone represses inflammation in a peroxisome proliferator-activated receptor-α-dependent manner in vitro and in vivo in mice. *J Am Coll Cardiol* 2008; **52:** 869–881.
71. Werner C, Kamani CH, Gensch C, Böhm M, Laufs U. The peroxisome proliferator-activated receptor-γ agonist pioglitazone increases number and function of endothelial progenitor cells in patients with coronary artery disease and normal glucose tolerance. *Diabetes* 2007; **56:** 2609–2615.
72. Nesto RW, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES, et al. Thiazolidinedione use, fluid retention, and congestive heart failure: A consensus statement from the American Heart Association and American Diabetes Association. *Circulation* 2003; **108:** 2941–2948.
73. Yano M, Matsumura T, Senokuchi T, Ishii N, Murata Y, Taketa K, et al. Statins activate peroxisome proliferator-activated receptor γ through extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase-dependent cyclooxygenase-2 expression in macrophages. *Circ Res* 2007; **100:** 1442–1451.