Angiotensin II Receptor–Induced Cardiac Remodeling in Mice Without Angiotensin II

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Cell surface receptors and their ligands cooperatively regulate physiological processes. The receptor activity is regulated positively when agonists bind and negatively when antagonists displace the agonists. Complete absence of a hormone should abrogate physiological and pathogenic functions regulated by the cognate receptor. However, awareness of the constitutive activity, the ability of native receptors to become functionally active in absence of hormone, is changing our view of the robustness of ligand-regulated receptor mechanisms.

Paradigms of constitutive activity of G protein–coupled receptors (GPCRs) and inverse agonist activity of GPCR-targeted drugs are firmly established. The GPCR, angiotensin II (Ang II) type I receptor (AT₁R), can be spontaneously active.¹ Ways such as membrane environment, interacting proteins, receptor autoantibodies, and single nucleotide polymorphisms that increase expression can increase G-protein signaling in the absence of Ang II using the potential energy of the receptor.² Inverse agonists can suppress the constitutive activity of a receptor; however, classic antagonists cannot perform this action¹,² (Figure).

Constitutive activity is an inherent property of a GPCR in all, including human and animal, species.¹–³ Wild-type AT₁R stimulates significant G protein signaling in the absence of Ang II, when 1 to 10 pmol/mg of receptor is expressed in cell lines. The constitutively active pool of wild-type AT₁R is <5%, which is the reason why it is difficult to detect it with the available functional assays in native tissues expressing the receptor in the femtomole per milligram range. In general, effects of constitutive activity of native GPCRs in vivo have been studied in transgenic animals significantly overexpressing the receptors. Constitutive activity of many native GPCRs, including the AT₁R, opioid receptors, D₁ dopamine, and the 5HT2C and 5HT7 serotonin receptors; the H₃ histamine receptor; and the bradykinin B₂ receptor, have been ascertained this way.³ Therefore, the question remains whether constitutive activity observed at such expression levels in the presence of endogenous ligand and GPCR/G protein stoichiometry is of physiological relevance.

In this issue of Hypertension, Yasuda et al⁴ convincingly demonstrate for the first time that cardiac-specific upregulation of wild-type human AT₁R (hAT₁R) expression leads to spontaneous systolic dysfunction and chamber dilatation, accompanied by severe interstitial fibrosis in mice genetically made angiotensinogen (Agt) deficient. The Agt-null mice with the endogenous level of AT₁R expression did not develop the pathology. Conventional Ang II binding to the hAT₁R is thought to initiate signal transduction pathways responsible for the physiological and pathological actions of Ang II. Could enhancement of constitutive activity in vivo, because of overexpression of the native hAT₁R, lead to cardiac abnormalities when the Ang II production is genetically inhibited?

Constitutive activity of the native AT₁R (<5%) in cultured cells is low, but introduced mutations such as N111G and N111S significantly enhance constitutive activity of AT₁R (25% to 40%). Transgenic mice with endothelium-restricted expression of a low level of the AT₁R-N111G mutant produced a hypotensive phenotype.⁵ Transgenic mice with inducible cardiomyocyte-specific expression of wild-type or N111G mutant hAT₁R from the onset of adolescence show enhanced myocyte growth and associated cardiac hypertrophy in the adult.⁶ Gene knock-in mice with the N111S mutant hAT₁R with a C-terminal deletion (to reduce constitutive internalization) showed low-renin hypertension and progressive fibrosis in the kidney and heart.⁷ These studies established that engineered constitutive activating mutations are useful for controlled upregulation of local AT₁R activity and mimic various in vivo disease conditions. However, activating mutations of the AT₁R gene in humans have not been identified, and it remains unclear whether constitutive activity of the native hAT₁R has an in vivo pathogenic role.

To elucidate the pathogenic role of Ang II–independent AT₁R activation in the heart, Yasuda et al⁸ crossed transgenic mice overexpressing hAT₁R under the control of α-myosin heavy chain (MHC) promoter with the Agt-knockout mice to create AT₁Tg-AgtKO hybrid mice, in which the production of Ang II is genetically deficient. Overexpression of hAT₁R in the AT₁Tg parental mice was shown previously to induce cardiac remodeling in the presence of endogenous levels of Ang II that are prevented by treatment with the AT₁R blocker losartan.⁸ The AT₁Tg-AgtKO hybrid mice allowed the authors to unequivocally evaluate the
Ang II–independent constitutive activity in the hearts of mice in vivo, which, until now, was shown only in cultured cells.

The density of AT\(_1\)R was increased by >200-fold in AT\(_1\)Tg-AgtKO hearts compared with AgtKO hearts. Constitutive activation of the hAT\(_1\)R in the AT\(_1\)Tg-AgtKO hearts was showed by significantly increased distribution of Gaq/11 in the cytosol and phosphorylation of extracellular signal–regulated kinases in AT\(_1\)Tg-AgtKO hearts compared with AgtKO controls. These molecular changes in AT\(_1\)Tg-AgtKO mice hearts were associated with progressive chamber dilatation, contractile dysfunction, and interstitial fibrosis compared with normal cardiac structure and function in AgtKO mice. Progressive cardiac remodeling in AT\(_1\)Tg-AgtKO mice was prevented by treatment with the AT\(_1\)R inverse agonist, candesartan. Cardiac remodeling in offspring of Agt\(^{+/−}\) females or Agt\(^{−/−}\) females was similar, suggesting that maternal or placental Agt did not predispose postnatal development of cardiac remodeling in AT\(_1\)Tg-AgtKO mice. The most logical explanation for the observed G-protein and extracellular signal–regulated kinase activation, cardiac remodeling, and the AT\(_1\)R inverse agonist effect in AT\(_1\)Tg-AgtKO mice is the constitutive receptor activity.

Cells, including cardiomyocytes, harbor mechanisms to downregulate activated receptors. Ligand-activated and constitutively activated mutant AT\(_1\)R is phosphorylated by GPCR kinases and recruits β-arrestin, leading to internalization. However, the distributions of GPCR kinase 2 and β-arrestins in the particulate fraction relative to the cytosolic fraction were comparable between AT\(_1\)Tg-AgtKO and AgtKO hearts, implying a lack of receptor downregulation. Yasuda et al\(^4\) suggest that stochastic transient activated conformation in wild-type hAT\(_1\)R may be subtle and not induce detectable receptor internalization. Thorough experiments are needed for consolidating this mechanism and, if proven, would be novel.

In classic models of endocrine regulation, abnormal change in the efficacy or level of the hormone is thought to cause pathology. Consequently, with regard to pathologies of the renin-angiotensin system, the focus of therapeutic strategies has been on controlling circulating and local Ang II levels.\(^8\,10\)

Upregulation of AT\(_1\)R in stressed hearts and vessels in response to various hormones, cytokines, inflammation, or metabolic stress would proportionally enhance constitutive activity of the AT\(_1\)R and accelerate the progression of disease in these tissues, which cannot be effectively prevented by strategies targeting Ang II supply (eg, angiotensin-converting enzyme inhibitors) or clearance (eg, angiotensin-converting enzyme 2), but would require blockade of constitutive activity of the receptor directly through inverse agonists of AT\(_1\)R. Indeed, AT\(_1\)R blockers have been superior to angiotensin-converting enzyme inhibitors in newly treated patients.\(^9\,10\) The inverse agonists are even better therapeutics than neutral antagonists in treating diseases caused by genetic variations and constitutively activating mutations of GPCRs.

Although a hormone-negative condition in vivo may never arise, the proof-of-principle study by Yasuda et al\(^4\) details the importance of constitutive activity of a native GPCR in disease pathogenesis.\(^3\) Activating GPCR mutations underlying diverse diseases have been isolated, and transgenic mice expressing these mutant GPCRs have been developed as animal models of human diseases.\(^1\) The models created will be useful research tools for discovering and evaluating comparative potencies of inverse agonists. The regulatory principle that Yasuda et al\(^4\) have firmly confirmed will have wider relevance across the entire GPCR family.

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References


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