To mark the 60th birthday of *Circulation Research* (1953–2013), the editors have commissioned ***Circulation Research Classics***, a series of commentaries highlighting seminal articles published in the journal over the past six decades that have importantly shaped cardiovascular research. Written by leading experts, ***Circulation Research Classics*** are intended to describe the impact of these articles on the field by putting them in a historical perspective. The concept of "classic" is inextricably linked to time - a classic is something that maintains its value regardless of its age. Thus, an important consideration in selecting the articles to be highlighted is that they have stood the test of time, which is the most reliable indicator of the value of scientific work. By looking back at the illustrious past of *Circulation Research*, we hope to promote a deeper appreciation of the contributions of this journal to the advancement of knowledge.

# Solving the Cardiac Hypertrophy Riddle
## The Angiotensin II–Mechanical Stress Connection

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**Molecular Characterization of Angiotensin II-Induced Hypertrophy of Cardiac Myocytes and Hyperplasia of Cardiac Fibroblasts. Critical Role of the AT1 Receptor Subtype**
Sadoshima and Izumo
*Circ Res*. 1993;73:413–423.

**Signal Transduction Pathways of Angiotensin II-Induced c-fos Gene Expression in Cardiac Myocytes In Vitro. Roles of Phospholipid-Derived Second Messengers**
Sadoshima and Izumo
*Circ Res*. 1993;424–438.

A series of studies conducted 20 years ago, documenting the cardiac hypertrophy phenotype and its underlying signaling mechanism induced by angiotensin II (Ang II) and mechanical stress, showed a remarkable similarity between the effect of the $G_{\alpha}q$ agonist and that of mechanical forces on cardiac hypertrophy. Subsequent studies confirmed the involvement of autocrine/paracrine mechanisms, including stretch-induced release of Ang II in load-induced cardiac hypertrophy. Recent studies showed that the Ang II type 1 ($AT_1$) receptor is also directly activated by mechanical forces, suggesting that $AT_1$ receptors play an important role in mediating load-induced cardiac hypertrophy through both ligand- and mechanical stress–dependent mechanisms.

Hypertrophy, characterized by increased muscle mass as a result of increased cell size, is a compensatory mechanism

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by which the heart adapts to increased mechanical stress. However, it is also considered an independent risk factor for heart disease, because progression to decompensated hypertrophy eventually leads to heart failure. The renin–angiotensin system (RAS) has long been known to be important in maintaining renal and cardiovascular homeostasis, but much of the focus in earlier studies was on its systemic function and its role in the regulation of blood pressure. In 1993, a pair of studies published in *Circulation Research* investigated the effects of Ang II, the primary effector molecule of the RAS, on the cells of heart itself. In cultured neonatal rat cardiac myocytes, incubation with Ang II caused an increase in protein synthesis without a concomitant increase in DNA synthesis, suggesting a hypertrophic effect. Ang II also upregulated transcription of genes known to be induced by hypertrophic stimuli in cardiac myocytes, with rapid induction of the immediate-early genes *c-fos*, *c-jun*, *jun B*, *Egr-1*, and *c-myc* and later induction of the fetal-type genes atrial natriuretic factor and skeletal α-actin.[1] Closer examination of the signal transduction pathways involved in *c-fos* induction revealed that Ang II increased production of several phospholipid-derived second messengers and activated phospholipases C and D, as well as causing sustained activation of protein kinase C. Although intracellular $Ca^{2+}$ seemed to play a permissive role, selective mutation of the *c-fos* promoter showed that protein kinase C–dependent signaling through the serum response element, rather than extracellular $Ca^{2+}$ and $Ca^{2+}$/cAMP response element signaling, is responsible for Ang II responsiveness.[2] These results were remarkably similar to those observed after passive mechanical stretch of cardiac myocytes in an in vitro model of load-induced cardiac hypertrophy,[3,4] suggesting the possibility that Ang II plays an important role in mediating the effects of stretch in the heart.

Stretched cardiac myocytes were found to secrete soluble factors capable of mimicking the effects of stretch when applied to nonstretched cardiac myocytes in vitro,[4] and these soluble factors were shown to include Ang II released from intracellular granules.[5] Stretch also upregulated expression

of several RAS components in cardiac myocytes in vitro,[5,6] further supporting a role for Ang II produced by the local (cardiac) RAS in mediating the effects of mechanical stress. Understanding of the autocrine/paracrine mechanism stimulated by mechanical stress in cardiac myocytes was further augmented by the discovery that both Ang II and stretch increase synthesis and secretion of the hypertrophic factor endothelin-1, and that Ang II- and stretch-induced hypertrophy is inhibited by inhibition of the endothelin A receptor.[7,8] A variety of secreted growth factors and signaling molecules, including transforming growth factor-β1 and cardiotrophin-1, have been shown to contribute to cardiac hypertrophy through the autocrine/paracrine loop set in motion by Ang II signaling.[9,10]

Ang II can bind to either the $AT_1$ receptor or the Ang II type 2 ($AT_2$) receptor, both G protein–coupled receptors (GPCRs) found in both extracellular and intracellular membranes.[11,12] Inhibition of the $AT_1$ receptor blocked the effects of Ang II and stretch in vitro, but inhibition of the $AT_2$ receptor did not,[1,2,5] suggesting that the hypertrophic effects induced by mechanical stress via Ang II signaling are primarily mediated by the $AT_1$ receptor. In addition, there is both in vitro and in vivo evidence that stretch can also directly activate $AT_1$ receptor signaling even in the absence of Ang II binding.[13] This stretch-induced activation involves a conformational change in the receptor that is distinct from that induced by Ang II binding, with the seventh transmembrane domain undergoing a counterclockwise rotation and shift into the ligand binding pocket.[14] The mechanism by which this occurs is still unknown, but it is possible that physical stretching of the cell membrane directly causes the conformational change that activates the receptor. Alternatively, stretch may activate mechanical sensors that then secondarily activate the $AT_1$ receptor. However, a loss-of-function study found that pressure overload induced hypertrophy, systolic dysfunction, and fibrosis even in the absence of $AT_1$ receptor/Ang II signaling, suggesting the existence of an $AT_1$ receptor–independent pathway.[15] Although the $AT_2$ receptor is generally thought to oppose the actions of the $AT_1$ receptor, there is evidence that it may play a role in cardiac hypertrophy as well. Pressure overload–induced hypertrophy was completely inhibited in knockout mice lacking the $AT_2$ receptor.[16] In addition, overexpression of the $AT_2$ receptor caused constitutive ligand-independent hypertrophy that was additive to the ligand-dependent hypertrophy induced by $AT_1$ receptor overexpression in cardiac myocytes in vitro.[17] Thus, further investigation is needed to clarify the roles of the receptor subtypes in mediating hypertrophy fully.

After activation, the $AT_1$ receptor can trigger downstream signaling through coupled heterotrimeric G proteins. The discovery that Ang II can induce hypertrophy points to the importance of G protein signaling in cardiac remodeling. It was subsequently shown that overexpression of $G_{\alpha}q$ independently induces hypertrophy and, at higher levels, apoptosis in cultured cardiac myocytes and transgenic mouse hearts.[18] Conversely, the absence of $G_{\alpha}q/G_{\alpha}11$ signaling in cardiac myocytes completely prevented pressure overload–induced hypertrophy and fetal gene expression in the heart.[19] Whereas, the $AT_1$ receptor has also been shown to interact physically with other signaling molecules such as Src homology 2 domain-containing phosphatase-2, Janus kinase 2, and phospholipase Cγ,[20,21]

suggesting the presence of alternative downstream signaling pathways. Transgenic mice with cardiac-specific overexpression of an $AT_1$ receptor mutant lacking $G_{\alpha}q/G_{\alpha}i$ coupling exhibited greater hypertrophy and dysfunction, but less apoptosis and fibrosis, compared with mice with overexpression of the wild-type $AT_1$ receptor, and activation of the downstream signaling pathways clearly differed between mice overexpressing the mutant and wild-type receptors.[22] In contrast, disruption of endothelial growth factor receptor transactivation inhibited $AT_1$ receptor–induced hypertrophy, even in the presence of intact G protein signaling.[23] Thus, the $AT_1$ receptor mediates hypertrophy with distinct phenotypes through both G protein–dependent and independent mechanisms.
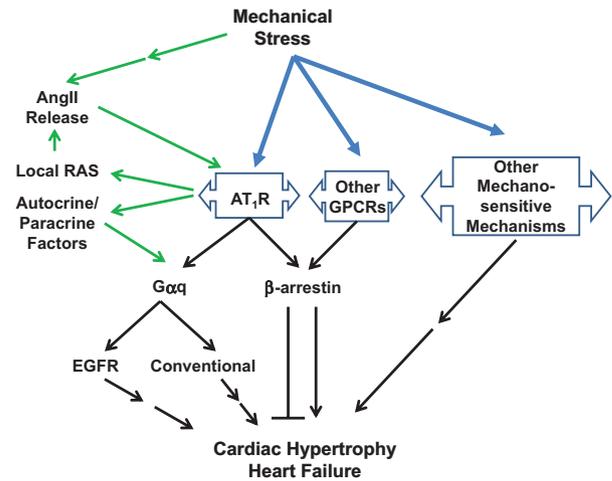


**Figure. A current hypothesis regarding how mechanical forces induce cardiac hypertrophy and how angiotensin II (Ang II) and Ang II type 1 ($AT_1$) receptors are involved in this process.** Load-induced hypertrophy is mediated by direct activation of mechanosensitive mechanisms (indicated by blue arrows) as well as autocrine/paracrine factors (indicated by green arrows). As a result, $AT_1$ receptors are activated by ligand-dependent and mechanical stress–dependent mechanisms. Ang II released by stretch is generated through the local (cardiac) renin–angiotensin system (RAS). Although both Ang II and the $AT_1$ receptor significantly contribute to the development of load-induced cardiac hypertrophy, other autocrine/paracrine factors (endothelin-1, cardiotrophin-1, transforming growth factor-β, etc), stretch-induced activation of other G protein–coupled receptors (GPCRs; apelin [APJ] receptor, histamine H1 receptor, muscarinic M5 receptor, etc), and mechanosensitive molecules (integrins, Src family tyrosine kinases, phospholipase A2, mechanosensitive ion channels, transient receptor potential channel, tandem of P domains in weak inward rectifier $K^+$ channels (TWIK)-related potassium channel, epithelial sodium channel, etc[27]) may also contribute to it. Despite advancement in the understanding of the signaling mechanisms of cardiac hypertrophy, the mechanism by which cardiomyocytes initially sense mechanical forces and transmit the signal to downstream signaling mechanisms remains to be fully understood. Although stretch allows the $AT_1$ receptor and others to interact with β-arrestin, how stretch affects the structure and function of other GPCRs requires further investigation. GPCRs, including $AT_1$ receptors, couple to diverse signaling mechanisms, including conventional phospholipid-derived second messengers, transactivation of the epidermal growth factor receptor (EGFR), and β-arrestin–dependent signaling pathways, which presumably mediate both pathological and adaptive aspects of cardiac hypertrophy. Biased agonism of $AT_1$ receptors that preserves signaling mediating adaptive hypertrophy may be a promising way to treat cardiac hypertrophy induced by hemodynamic overload.

Hypertrophy can also be triggered directly by mechanical stimulation, as well as by autocrine/paracrine ligand-dependent signaling (Figure). As mentioned earlier, the $AT_1$ receptor can be directly activated by stretch, even in the absence of Ang II.[13] Stretch-induced activation leads to downstream phosphorylation of extracellular signal-regulated kinase that is independent of G protein signaling and increases β-arrestin recruitment and $AT_1$ receptor internalization.[24] Furthermore, Ang II binding and stretch lead to distinct β-arrestin conformations, indicating that the stretch-induced recruitment of β-arrestin does not just enable $AT_1$ receptor desensitization but rather is part of an alternative cell signaling pathway.[24] In fact, β-arrestin–biased agonism of the $AT_1$ receptor had a cardioprotective effect that was distinct from and potentially superior to that of the $AT_1$ receptor blocker losartan, increasing contractility and decreasing apoptosis while increasing phosphorylation of extracellular signal-regulated kinase and AKT after mechanical stress and ischemia and reperfusion.[24,25] The apelin (APJ) receptor, a GPCR that is structurally similar to the AT1 receptor, was also recently shown to be activated by mechanical stress as well as by ligand binding, with the 2 modes of activation leading to distinct downstream responses. Although apelin binding triggers $G_\alpha i$ signaling and inhibits cardiac hypertrophy, activation of apelin (APJ) receptor by stretch inhibits $G_\alpha i$ signaling and promotes hypertrophy via a β-arrestin–dependent mechanism.[26] Whether this mechanosensitivity is a common characteristic of all GPCRs or limited to specific receptors remains controversial, but there is some evidence that other GPCRs may act as mechanosensors as well.[27]

Much has been learned during the past 2 decades about the role of Ang II and the RAS in the development of cardiac hypertrophy and cardiovascular disease. The widespread clinical use of Ang-converting enzyme inhibitors and $AT_1$ receptor blockers to treat patients with hypertension and myocardial infarction is testament to the importance of that role, yet questions remain: (1) The RAS was once thought to be a fairly simple systemic pathway, but is now known to act locally as well as systemically and to be much more complex than originally thought. How does the local RAS in the heart differ characteristically and functionally from the systemic RAS? What impact does the emerging intracrine function of Ang II have on cardiac myocyte cell growth and function? There is substantial and growing evidence that signaling by Ang(1–7), a smaller Ang peptide produced by the action of Ang-converting enzyme 2 on Ang II, acts in opposition to Ang II signaling, thereby inhibiting cardiac remodeling. However, several other biologically active Ang peptides, including Ang(1–12), an alternative precursor of Ang II, are now known to exist.[28] What role do these other peptides play? Furthermore, the fact that these previously unknown Ang peptides exist suggests that the question of how Ang II is produced by the local RAS in cardiomyocytes should be revisited. (2) Despite the obvious importance of cardiac myocytes, nonmyocytes, and fibroblasts in particular, represent a large percentage of the cells in the heart. Unlike in cardiac myocytes, Ang II treatment caused hyperplasia, rather than hypertrophy, in cardiac fibroblasts.[1] Although static stretch did not increase secretion of Ang II from nonmyocyte cultures,[5] secretion of Ang II from neighboring myocytes would presumably affect growth and

function of fibroblasts and vascular endothelial cells in vivo. By the same token, fibroblasts and vascular endothelial cells secrete a variety of growth factors and signaling molecules into the local cellular environment in response to Ang II and different forms of mechanical stress, such as cyclic stretch and shear stress. Conditioned medium from Ang II-treated cardiac fibroblasts was shown to induce hypertrophy in cultured cardiac myocytes.[10] How does the cross-talk between myocytes and nonmyocytes contribute to cardiac remodeling? (3) Little is known about how cells sense stretch and how that signal is transmitted to downstream signaling pathways. A Z-disc protein complex that includes muscle LIM protein and telethonin has been identified as playing an important role in sensing mechanical stretch in cardiac myocytes,[29] but how the signal is mediated from sensor to effector remains to be discovered. It has been suggested that the $Na^+/H^+$ exchanger may play a role in sensing mechanical stress and inducing hypertrophy,[30] but its activation seems to be primarily the result of endothelial growth factor receptor transactivation by the $AT_1$ receptor,[31] rather than a direct effect of mechanical stretch. Integrins and their associated proteins have also been implicated as being important in sensing mechanical stress and inducing physiological hypertrophy,[32] but how they might trigger a growth response remains a mystery. Furthermore, the $AT_1$ receptor can be activated in a ligand-independent manner,[13] suggesting that it may itself undergo a conformational change as a direct response to stretch. Thus, although understanding of the downstream signaling pathways responsible for the development of hypertrophy in response to mechanical stress has progressed tremendously, the initial stretch-sensing mechanism is still largely unknown. What are the mechanosensors responsible for sensing stretch in cardiac myocytes, and how do they interact with downstream signaling pathways? How do these differ under conditions of pressure and volume overload?

Although currently used therapies have proven to be highly effective, cardiovascular disease and heart failure continue to be a significant problem. A more complete understanding of how the heart senses and responds to mechanical stress should allow for the development of still more effective therapeutic strategies, with the ultimate goal of eradicating the problem entirely through prevention as well as treatment. Most current approaches aim for complete blockade of RAS function.[28] However, the discovery that GPCRs, and the $AT_1$ receptor in particular, have multiple modes of activation and can signal through multiple downstream signaling pathways, some of which are cardioprotective rather than harmful, suggests a new paradigm in which better outcomes might be attained through the use of biased agonism to block only those downstream pathways that are detrimental to cardiac function while preserving those that are beneficial. Cardiac cellular signaling and remodeling continue to be the focus of much investigation, and there is reason to be optimistic that the answer to the cardiac hypertrophy riddle may be found before another 2 decades go by.

## Disclosures

None.

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