Regulation of Cardiac Collagen
Angiotensin and Cross-Talk With Local Growth Factors

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In the myocardium, collagen fibers provide a supporting framework for myocytes and blood vessels and act as lateral connections between muscle bundles. These functional properties of collagen serve to maintain tissue architecture and to coordinate the delivery of force generated by myocytes on the ventricular chamber. The accumulation of excess collagen is believed to be an important pathophysiologic process that contributes to diastolic heart failure. Diastolic heart failure accounts for 30% to 50% of heart failure in clinical practice, and hypertensive disease is the major cause of this type of heart failure.1 The precise mechanisms responsible for excess fibrillar collagen accumulation in the pathological heart are poorly understood. Fibrosis of both the injured and noninjured myocardium2 indicates that humoral mechanisms are responsible for this process.

Cross-Talk and Regulation of Collagen Synthesis

In the failing heart, several humoral, autocrine, and paracrine systems are activated,3 suggesting that cross-talk between synergistic and opposing signaling pathways constitutes the predominant form of regulation under these conditions. Several factors have been identified as potentially important mediators of cardiac collagen production. In vitro studies of neonatal and adult rat cardiac fibroblasts have shown that angiotensin II (Ang II) directly stimulates cardiac fibroblast proliferation and collagen synthesis via Ang II type 1 (AT1) receptors.4–6 In this issue of Hypertension, Pathak et al7 provided evidence that a myocyte cofactor was an important mediator of Ang II–induced collagen type I and type III mRNA synthesis in a rat cell coculture model. This work, together with other studies, provides strong evidence that Ang II indirectly regulates cardiac fibroblast function via specific growth factors.8–21 Although the primary autocrine and paracrine mediators of Ang II effects on fibrillar collagen synthesis remain to be elucidated, principal candidates include transforming growth factor-β1 (TGF-β1), osteopontin (OPN), and endothelin-1 (ET-1).

A primary mediator of Ang II effects is thought to be TGF-β, which has been shown to stimulate collagen production in vitro10 and activates a wide array of processes that collectively increase extracellular matrix production.11 Increased expression of TGF-β1 precedes the increase in fibronectin and collagen type I and type III in cardiac hypertrophy.12 In vivo studies further reveal that Ang II is correlated with TGF-β1 expression in the repair of tissues, including infarcted heart, suggesting Ang II stimulates fibrous tissue formation by promoting TGF-β1 synthesis via AT1 receptor binding.13 Ang II has been shown to stimulate TGF-β1 production in neonatal and adult cardiac fibroblasts4,14; however, a definitive a link between Ang II and TGF-β1 remains to be established in the myocardium. There also is evidence that TGF-β1 has differential effects in the intact heart. In a recent study,15 the selective expression of TGF-β1 by cardiac myocytes resulted in overt fibrosis in atria, but not ventricles, of transgenic mice. This suggests that TGF-β1 is not sufficient to promote fibrosis in ventricular myocardium without expression of requisite ancillary factors, such as receptors or activating proteins.

In addition to TGF-β1, OPN has been proposed to mediate Ang II effects on extracellular matrix production in the human heart.8 It was initially identified in bone but is now known to be synthesized in many tissues.16 OPN is a secreted phosphoprotein factor with extracellular matrix and cytokine-like properties that is upregulated in ventricular myocardium of rats with heart failure17 and humans with cardiac hypertrophy.18 In vitro experiments have demonstrated that Ang II is a potent stimulator of OPN mRNA levels in cultures of neonatal and rat cardiac fibroblasts,9 as well as cultured human cardiac fibroblasts.8 Monoclonal antibody directed toward OPN completely blocks the mitogenic effect of Ang II on cultured rat cardiac fibroblasts and attenuates Ang II induction of cardiac fibroblast collagen gel contraction, a model of fibroblast scar contraction behavior.9 These findings suggest that OPN may be an important mediator of Ang II cardiac remodeling. However, it remains to be determined whether fibroblasts contribute to OPN synthesis during heart failure, because cardiac myocytes appear to be the primary source of OPN in myocardium of rats with pressure-overload–induced heart failure and humans with cardiac hypertrophy.17,18 ET-1 also appears to mediate cardiac effects of Ang II. ET-1 is synthesized by cardiac myocytes and fibroblasts19 and has been shown to stimulate collagen I and III
synthesis in isolated coronary artery vascular smooth muscle cells. In rats with chronic heart failure, blockade of endothelin receptors has been shown to decrease left ventricular collagen accumulation. A link between Ang II and ET-1 has been established under in vitro conditions, in which autocrine release of ET-1 was shown to mediate Ang II–induced cardiac myocyte hypertrophy. In a transgenic, Ang II–dependent rat model, ET-1 receptor blockade also reduced collagen III gene expression in the kidney, suggesting that endothelin participates in Ang II–induced end-organ damage. However, it remains to be determined whether a similar mechanism is operational in the failing human heart.

**Negative Coupling of the AT1 Receptor to Collagen Degradation**

In addition to collagen synthesis, Ang II has been shown to regulate collagen degradation by attenuating interstitial matrix metalloproteinase-1 activity in adult human cardiac fibroblasts and by enhancing tissue inhibitor of metalloproteinase-1 (TIMP-1) production in endothelial cells. The negative coupling of the AT1 receptor to these collagen degradation pathways is poorly understood. Hepatocyte growth factor (HGF) has been shown to reverse fibrosis in liver through induction matrix-degrading pathways and inhibition of TGF-β1 synthesis. A possible role for HGF in the regulation of collagen accumulation has been described in the cardiomyopathic hamster heart. In this study, Ang II blockade prevented myocardial fibrosis, which was accompanied by increased myocardial HGF production, suggesting that this factor may have a role in the prevention of myocardial injury as a result of Ang II blockade. In a genetic rat model of hypertension, the administration of an AT1 receptor antagonist has been shown to normalize TIMP-1 expression and collagenase activity, suggesting that Ang II may facilitate myocardial fibrosis by upregulating TIMP-1 and decreasing collagenolytic activity. It remains to be determined whether Ang II regulates TIMP-1 expression directly or indirectly through a cytokine such as TGF-β1.

**The AT2 Receptor Is a Negative Regulator of Collagen Synthesis**

The reversal of cardiac collagen deposition after AT2 blockade may be due in part to activation of the Ang II type 2 receptor (AT2). AT2 receptor antagonists can direct cardiac effects via a combination of blockade of the AT1 receptor and an unhindered stimulation of the AT2 receptor. Blockade of the AT1 receptor results in increased plasma renin and circulating Ang II levels, and the increase in Ang II will activate AT2 receptors, which are already upregulated in the failing human heart. In the cardiomyopathic hamster, Ohkubo et al provided evidence that suggest chronic activation of the AT2 receptor mediates the beneficial effects of AT1 receptor blockade. However, little is known regarding how the AT2 receptor may counteract actions of the AT1 receptor. One possibility is that the AT2 receptor inhibits collagen synthesis by stimulating kinin production. In support of this concept, bradykinin has been shown to decrease the expression of collagen type I and type III mRNA in rabbit cardiac fibroblasts by stimulating the release of 6-keto prostaglandin F1α. A similar mechanism is likely to be operational after treatment with ACE inhibitors, which increase cardiac bradykinin levels through the inhibition of kinin destruction.

**Contribution of the Local Renin-Angiotensin System**

Upregulation of the renin-angiotensin system (RAS) is a primary feature associated with heart failure in humans and experimental animal models. The complete RAS cascade has been described in cultures of neonatal and adult rat cardiac fibroblasts and myocytes. In addition, phenotypically transformed fibroblasts, termed myofibroblasts, have been shown to actively express components responsible for Ang II production and to contain receptors for Ang II and TGF-β2. Interstitial fibroblasts are responsible for collagen synthesis in the normal myocardium, whereas myofibroblasts are primarily responsible for fibrogenesis at sites of rebuilding and remodeling after myocardial injury. The development of cardiac remodeling induced by hemodynamic overload and myocardial infarction is very likely triggered by mechanical stress. Evidence from experimental studies suggests that Ang II and other factors released on mechanical stretch may be important mediators of cardiac fibrosis. In rat cardiac myocytes, exposure to mechanical stretch results in the autocrine release of Ang II, increased expression of RAS components, and AT1 receptor–mediated growth responses. There is recent in vivo evidence to suggest that cardiac Ang II can mediate myocardial fibrosis independent of mechanical load. Increased expression of human ACE in rat myocardium has been demonstrated to stimulate myocyte hypertrophy and to increase collagen content when examined 2 weeks after transfection. The lack of systemic effects (ie, increased blood pressure, heart rate, or serum ACE activity) indicated that locally synthesized Ang II promotes cardiac growth and fibrosis in the absence of hemodynamic changes. In a rat model of pure pressure overload, treatment with an AT1 receptor antagonist has been demonstrated to prevent interstitial fibrosis in the left ventricle. Because the aortic band was placed close to the heart, this ruled out that a potential lowering of blood pressure could have an indirect effect on cardiac remodeling. There is evidence to suggest that the heart is equipped with a functional aldosterone system that may participate in cardiac fibrosis during cardiac remodeling and is regulated by Ang II. This raises the possibility that local variations in Ang II modify cardiac levels of aldosterone, thereby effecting changes in collagen accumulation. The cardioprotective effects of spironolactone may explain the prognostic value of antialdosterone therapy in patients with severe chronic heart failure evaluated in the Randomized Aldactone Evaluation Study (RALES) mortality trial. Further evidence is required to prove that a local aldosterone system is functional in the human myocardium.

**Conclusion and Future Directions**

The importance of local humoral factors in the regulation of cardiac tissue remodeling has become increasing evi-
dent. Pharmacological interventions with ACE inhibitors and AT₁ receptor antagonists have underscored the importance of Ang II in the mediation of cardiac fibrosis in humans and animal models with heart failure. These studies suggest that circulating or locally produced Ang II stimulates fibrillar collagen accumulation in the heart via AT₁ receptors, whereas AT₂ receptors negatively couple to collagen synthesis. Additional exploration is necessary to determine the various autocrine/paracrine mechanisms involved in Ang II actions on collagen deposition in the failing heart. Unraveling these pathways represents a major challenge that will require the use of gene transfer techniques to control transgenes within selected cardiac cell types. In addition, it will be essential to use new tools such as cardiac microdialysis, which affords the unique opportunity to peer into the cardiac interstitium and to analyze the complex humoral environment of the cardiac fibroblast.

References


**Key Words**

- angiotensin II
- cardiac myocytes
- collagen
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