Review

Angiotensin II, adhesion, and cardiac fibrosis

Janet M. Schnee, Willa A. Hsueh

*a University of California–Los Angeles, School of Medicine, Division of Endocrinology, Diabetes, and Hypertension, Warren Hall, 2nd Floor, Rm 24-130, 900 Veteran Avenue, Mail Code 178622, Los Angeles, CA 90024, USA
b Molecular Biology Institute, Los Angeles, CA, USA

Received 26 November 1999; accepted 17 February 2000

Abstract

Angiotensin II (AII) plays a critical role in cardiac remodeling. This peptide promotes cardiac myocyte hypertrophy and cardiac fibroblast interstitial fibrotic changes associated with left ventricular hypertrophy, post myocardial infarction remodeling and congestive heart failure. AII mediates cardiac myocyte hypertrophy directly via induction of immediate early genes through a MAP kinase dependent pathway. In addition, it mediates cardiac hypertrophy indirectly by stimulating release of norepinephrine from cardiac nerve endings and endothelin from endothelial cells. AII also has multiple effects on cardiac fibroblasts: it induces cardiac fibroblast proliferation, synthesis and secretion of adhesion molecules and extracellular matrix proteins, and expression of integrin adhesion receptors. In addition it stimulates cardiac fibroblasts to adhere more vigorously to defined matrices. This review will discuss the molecular pathways that have been implicated in these AII induced effects in the cardiac fibroblast.

Keywords: Angiotensin; Extracellular matrix; Fibrosis; Hypertrophy

1. AII and cardiac fibrosis

The improvement in outcomes for patients with myocardial infarction and heart failure treated with angiotensin converting enzyme inhibitors (ACEI) compared to placebo have been well demonstrated [1–4]. However, the specific mechanisms by which ACEI exert their protective actions are not known. Although it was initially hypothesized that they are beneficial due to their hemodynamic effects to reduce afterload, it is now known that their efficacy is not completely accounted for on this basis. In the V-HeFT II study, enalapril was shown to be more effective in preventing progression of heart failure than treatment with hydralazine plus isosorbide dinitrate despite similar blood pressure responses [5]. Thus, direct tissue effects have been implicated. ACE is responsible for catalyzing the degradation of kinins as well as the conversion of angiotensin I (AI) to angiotensin II (AII) [6]. Both actions have been implicated in the cardiac protective effects of ACEI. However, the ELITE trial compared outcomes of patients over age 65 with heart failure (HF) treated with either an ACEI or with an AII subtype I (AT1) receptor blocker; mortality was decreased for the AT1 receptor blocker [7]. In a subsequent trial, ELITE II, AT1 receptor blockade and ACEI were associated with similar mortality. Thus, the majority of the benefit imparted by ACE inhibitors appears to result from blocking untoward effects of angiotensin II mediated by the AT1 receptor.

Based on these observations, ongoing studies of the effects of AII on the cardiovascular system are being vigorously pursued. The heart expresses several components of the renin–angiotensin system, and AII has multiple effects on both the cardiomyocytes and cardiac fibroblasts [8,9]. AII promotes cardiomyocyte hypertrophy, cardiac fibroblast hyperplasia, and interstitial cardiac fibrosis [8–14]. These latter two actions, which increase the volume of the non-myocyte compartment of the heart, are predicted to have a particularly negative impact to promote the progression of LVH and the globular heart formation associated with post MI remodeling and HF (for review, see Ref. [8]). Firstly, interstitial fibrosis contributes to ventricular wall stiffness and consequently impairs cardiac compliance, contributing to impaired diastolic function.
[15,16]. Secondly, since neither the ECM nor the fibroblasts contribute to systolic contraction, increased ECM and fibroblast volume means that systolic work is being performed by a smaller proportion of the cardiac mass, contributing to systolic dysfunction. Thirdly, interstitial fibrosis leads to increased distance that oxygen must diffuse and therefore potentially lowers PaO₂ for the working myocytes [17]. Finally, electrical coupling of the cardiomyocytes may be impaired by the accumulation of ECM proteins and fibroblasts since such accumulation causes morphologic separation of myocytes [9]. Thus, changes in the interstitium profoundly affect myocyte metabolism and performance, and ultimately, ventricular function. Therefore, factors that contribute to cardiac interstitial fibrosis should be recognized, and investigations should address whether approaches that attenuate fibrosis improve ventricular function and remodeling.

2. Integrins: mediators of adhesion

While the exact mechanisms by which AII induces cardiac fibrosis is not known, recent work supports a critical role for adhesion, a complex process involving interactions between different families of factors, including integrins, ECM proteins, and adhesion molecules. An important relevant issue is the regulation of integrin expression in cardiac fibroblasts, and the functional impact of modulation of integrin expression both in vitro and in vivo.

Integrins are a ubiquitous family of transmembrane receptors, which interact with and induce responses of the cell to its external environment (Fig. 1). They mediate a number of cellular functions including adhesion, migration, growth and apoptosis (for reviews, see Refs. [18–21]). Integrins are heterodimers composed of α and β subunits, of which there are many isoforms, accounting for the first of several levels of diversity imparted by these proteins. The large extracellular portion of integrins binds directly to ECM proteins. Specific integrin heterodimers bind to specific yet overlapping subsets of integrin ligands. β1 containing integrins are known to bind collagen, the αvβ5 integrin to bind vitronectin, and the αvβ3 integrin to bind a variety of ECM proteins containing the peptide sequence arginine–glycine–aspartate (RGD sequence) including collagen, vitronectin, fibronectin, thrombin, fibrinogen and thrombospondin. The relatively short integrin cytoplasmic tail binds to various adapter proteins. The adapter proteins in turn interact with transmembrane growth factor receptors, cytoplasmic kinases, and cytoskeletal proteins. Integrins, thereby, mediate changes in cell shape and signal transduction, which impact on nearly all critical cell functions.

When integrins bind to ECM ligands, they cluster within

---

**Fig. 1. Integrins and signal transduction.** Integrins are heterodimeric transmembrane proteins composed of an α and a β chain. Their extracellular portion binds ECM proteins particularly via the tripeptide motif arginine–glycine–aspartate (RGD), while their intracellular domain can interact with proteins such as talin, vinculin, and paxillin, both directly and via complexes, shown in this diagram simply as "adapter proteins". Through these interactions integrins exert effects on growth factor receptors, cytoskeletal components such as actin and α-actinin, as well as various cytoplasmic kinases, such as focal adhesion kinase (FAK) and extracellular response kinase (ERK). These interactions ultimately affect transcriptional regulation of a large number of genes including those encoding several extracellular matrix proteins, cell cycle control proteins, and integrins themselves.
the cell membrane, and promote assembly of actin filaments into large stress fibers [19]. The stress fibers promote further integrin clustering. The result is the formation of focal adhesions that affect multiple signaling pathways. Focal adhesions formed by most integrins activate focal adhesion kinase (FAK), a tyrosine kinase which can bind and activate Src. These interactions can lead to cytoskeletal changes, as well as activation of the MAP kinase pathway. In addition, some αv and β1 integrins have been shown to activate Fyn, a tyrosine kinase which recruits and activates the adaptor protein Shc. Integrins which activate Fyn and thereby Shc have the potential to be strong activators of ERK and of cell proliferation. Integrins can also impact cellular proliferation via associations with growth factor receptors, which also accumulate in focal adhesions; some growth factor receptors may require such interaction for full activation [22–27]. Additionally, integrin interactions can impact expression of multiple components of cell cycle control, e.g. cyclin D1 and the cyclin dependent kinase (cdk) inhibitors p21 and p27 [28,29]. Finally, ECM–integrin interactions may result in signal transduction events which ultimately effect transcriitional regulation of ECM proteins, as well as integrins, themselves [21,30,31].

2.1. Angiotensin II and integrin mediated adhesion

To determine if RGD binding integrins might play a role in AII induced cardiac interstitial fibrosis, we investigated the expression pattern of αv, β1, β3, and β5 integrins that we detected in cardiac fibroblasts [32]. These integrins were found via fluorescence activated cell sorting (FACS) analysis to be present on the surface of both rat and human cardiac fibroblasts. All up-regulated αv, β1, β3, and β5 integrin message and protein, which was blocked by the AT1 receptor blocker irbesartan, but not by the AT2 receptor blocker PD 123319. We tested the functional significance of the induction of expression of these genes by determining the ability of cardiac fibroblasts to adhere to several ECM integrin ligands. All promoted attachment of rat cardiac fibroblasts to the ECM proteins collagen I, fibronectin, vitronectin, and laminin, and AII treatment was associated with increased activation of FAK when fibroblasts were allowed to adhere to these ECM proteins. Pre-treatment with AT1 receptor blockers, irbesartan or losartan, inhibited AII enhancement of cardiac fibroblast adhesion to these substrates. Significantly, pretreatment with anti-β3 integrin antibody also attenuated the induction of adhesion to all four of these substrates by AII, while antibody directed at αvβ5 attenuated the increased adhesion only to vitronectin, its putative ligand. These results indicate that β3 integrin plays a significant role in AII induced adhesion to all four of these substrates, while the αvβ5 role is limited to vitronectin. Thus, blocking the increased integrin expression or integrin ligand binding by AII attenuated cell adhesion to its extracellular matrix milieu (for summary of above results, see Table 1).

AII has similar but somewhat different actions on human cardiac fibroblasts, although the overall profibrotic impact of AII appears to be conserved across species. In fibroblasts obtained from explanted human cardiomyopathic hearts, we found an inverse relationship of AII appears to be conserved across species. In diomyopathic hearts, we found an inverse relationship between the mRNA levels of atrial natriuretic peptide (ANP) vs. AT1 receptor, suggesting progressive down regulation of the AT1 receptor with increasing heart failure [33]. However, northern and FACS analysis demonstrated no acute down regulation of AT1 receptor by AII treatment of the human cardiac fibroblasts in culture, in contrast to the downregulation seen with rat cardiac fibroblasts. Similar to findings in rodent cardiac fibroblasts [10], AII induced c-fos and EGR-1 mRNA, as well as MAPK activity and a marked increase (5×) in DNA synthesis. Treatment with the MAPK inhibitor PD98059 blocked this increase in DNA synthesis. AII also stimulated an increase (2×) in the expression of the pro-fibrotic factor TGF-β, the ECM proteins laminin and fibronectin, and the inhibitor of protease activation, plasminogen activator inhibitor-1 (PAI-1). AII also increased attachment of cells to collagen I and III, the dominant ECM proteins of the human heart; this attachment was associated with an increase in focal adhesion kinase (FAK) activity, supporting a role for integrins in this effect. Each of these effects were blocked by irbesartan but not by an AT2 receptor blocker. However, unlike our findings in rodent cardiac fibroblasts, AII did not increase attachment to fibronectin, vitronectin or laminin or enhance integrin expression in human cardiac fibroblasts.

3. Osteopontin: a novel adhesion protein in the heart

Osteopontin (OPN) is an adhesion protein implicated as one important mediator of the profibrotic effects of AII in the heart. It was initially identified in bone, but is now known to be synthesized in many tissues [34]. It has been
proposed to bind calcium as well as fibronectin and collagen. Additionally, it contains an RGD sequence recognized by several integrins. Recent studies have demonstrated that it binds to αvβ1, αvβ3, and αvβ5 integrins and in vascular smooth muscle cells is acutely upregulated by cytokines and growth factors such as transforming growth factor β (TGFβ), epidermal growth factor (EGF), platelet derived growth factor (PDGF), and interleukin 2 (IL-2), supporting a role for it as a mediator of cellular responses [35–38]. In fact, OPN is recognized to contribute to vascular smooth muscle cell (VSMC) migration, adhesion, and spreading, and has been cloned as a gene differentially expressed in VSMC following arterial injury [39,40]. OPN has also been shown to play a critical role in the kidney in the generation of interstitial fibrosis due to obstructive nephropathy [41]. In this model the renin–angiotensin system is markedly activated and ACEI has protective anti-fibrotic effects. OPN knock-out mice were shown to be resistant to development of renal interstitial fibrosis as compared to wild type mice. Thus, in at least two organ systems OPN substantially contributes to interstitial changes.

We demonstrated that OPN is expressed in the myocardium, in both myocytes and fibroblasts ([42,43]; for review, see Ref. [44]). Its ventricular expression positively correlates with ventricular hypertrophy, and AII (10⁻¹¹ M) upregulates expression of OPN message several-fold within 6–24 h in cardiac fibroblasts. Monoclonal antibody directed against OPN completely blocks the mitogenic effect of AII on cultured rat cardiac fibroblasts, and partially blocks AII induction of cardiac fibroblast collagen gel contraction, a model of fibroblast scar contraction behavior. These findings suggest OPN may be an important mediator of AII induced cardiac remodeling. In addition, anti-integrin (β3) antibody also blocked these effects, suggesting that OPN acts via an integrin-dependent pathway.

4. Summary

The multiple pro-fibrotic effects of AII in the heart are summarized in Fig. 2 and include promotion of fibroblast proliferation, as well as synthesis and secretion of ECM and of multiple pro-fibrotic factors, such as TGF-β and PAI-1. TGF-β is known to stimulate ECM production and enhance fibrosis in a variety of tissues [45]. PAI-1 prevents degradation of the ECM, allowing for its accumulation [46]. Recent studies further support a critical role of AII in adhesion. We have shown that integrins are upregulated by AII, and that this upregulation is associated with an increase in FAK activity and adhesion. The increase in adhesion is blocked by treatment with AT1 receptor blockers irbesartan or losartan, as well as by integrin specific antibodies. Additionally, AII exerts pro-fibrotic effects via induction of the adhesion molecule osteopontin.

Fig. 2. Summary of AII effects on cardiac fibroblasts. Angiotensin II (AII) acting via the AT1 receptor, has direct effects on the cardiac fibroblast cytoskeleton as well as on cardiac fibroblast adhesive and fibrotic properties. AII stimulates expression of the cytoskeletal protein α-actinin. AII also increases expression of integrins and the adhesion protein osteopontin, thus promoting cardiac fibroblast adhesion. AII induces synthesis of extracellular matrix proteins, both directly as well as indirectly by stimulating expression of the profibrotic factor TGFβ. Finally, it stimulates the expression of plasminogen activator inhibitor 1 (PAI-1), thus decreasing the activation of metalloproteases and thereby the degradation of extracellular matrix.

Antibodies against OPN or β3 integrin block the proliferative effect of AII on cardiac fibroblasts in vitro, demonstrating that this effect is mediated via an OPN and integrin β3 dependent pathway. Studies are presently underway to determine the exact role of OPN in the development of cardiac fibrosis.

References


