Integrin-Mediated Collagen Gel Contraction by Cardiac Fibroblasts
Effects of Angiotensin II

Maria Lonnett Burgess, Wayne E. Carver, Louis Terracio, Steven P. Wilson, Marlene A. Wilson, Thomas K. Borg

Abstract Angiotensin II (Ang II), a vasoactive octapeptide, has been implicated in cardiac growth and the development of hypertrophy and fibrosis secondary in hypertensive disease. These consequences of Ang II imply an effect on the function and morphology of cardiac interstitial cells (fibroblasts). The present investigation was designed to (1) determine whether neonatal heart fibroblasts (NHFs) possess functional Ang II receptors on their plasma membrane and (2) examine the effects of Ang II on NHFs in vitro using three- and two-dimensional (3D and 2D, respectively) cultures. Several analytic techniques were used to test the specific questions of the present study. Since cardiac fibroblast phenotype can be influenced by culture conditions, both 2D and 3D cultures were used in the present investigations. Reverse-transcriptase polymerase chain reaction and radioligand binding analysis were used to test for the presence of Ang II receptors on NHFs. Both revealed that NHFs in 2D culture possess Ang II receptor mRNA and Ang II receptors. When isolated NHFs were cultured in 3D collagen gels and treated with Ang II, gel contraction was stimulated by NHFs. This effect was attenuated by the specific Ang II receptor antagonist [Sar\(^1\), Ala\(^8\)]Ang II. Ang II–stimulated gel contraction was completely inhibited by extracellular matrix receptor (β\(_1\)-integrin) antibodies (\(P<.05\)), supporting previous studies indicating that collagen gel contraction is mediated via the integrins. Immunofluorescent staining was used to test the localization of cell-surface integrins. A more intense staining pattern for β\(_1\)-integrin in Ang II–treated versus control cells was observed. Immunoprecipitation of β\(_1\)-integrin revealed more β\(_1\)-integrin protein on the surface of Ang II–treated cells (\(P<.05\)). These results suggest that Ang II mediates collagen gel contraction via Ang II receptors and β\(_1\)-integrin, indicating a functional relation between Ang II and β\(_1\)-integrin on cardiac fibroblasts. We conclude that Ang II influences the ability of cardiac fibroblasts to contract 3D collagen gels and the regulation of membrane-bound integrins in both 2D and 3D cultures of NHFs. (Circ Res. 1994;74:291-298.)

Key Words • angiotensin II • fibroblasts • integrins • collagen gels • heart • AT\(_1\) receptor

Recent evidence has suggested a role for angiotensin II (Ang II), a vasoactive octapeptide, as a growth-regulating factor important both in normal heart development and in hypertrophy from systemic hypertension.\(^1\) Pressure-overload cardiac hypertrophy and neonatal development are characterized by increases in extracellular matrix (ECM) accumulation of collagen and associated glycoproteins as well as by increases in myocyte size and ventricular wall thickness.\(^2\) The association of Ang II with the accumulation of interstitial matrix components (ie, collagen and elastin) in hypertrophy that occurs during development or disease has recently been given considerable attention. For example, reducing endogenous Ang II with angiotensin-converting enzyme inhibitors attenuates the normal accumulation of both collagen and elastin in the hearts of developing young rats,\(^3\) whereas increased Ang II levels stimulate collagen synthesis in cultured vascular smooth muscle cells.\(^4\) In vivo experiments cannot distinguish the variety of serum factors, such as hormones and growth factors, that may be partially responsible for the Ang II–associated effects on cardiac hypertrophy during development and disease. In addition, the most probable site of Ang II action must involve myocardial fibroblasts, since they are responsible for synthesizing the majority of ECM proteins; however, evidence for the presence of Ang II receptors on cardiac fibroblasts has only been recently demonstrated.\(^2,3,5\) Indeed, the direct effects of Ang II on cardiac fibroblasts warrant further research.

Integrins have been recognized as a widely expressed family of cell surface adhesion receptors that are non-covalently linked \(αβ\) heterodimers.\(^6\) Multiple integrins are expressed on the surface of the cellular components of the heart and play an important role as signaling receptors in a variety of cell types.\(^5\) A particularly important feature of integrins is that they undergo activation. For example, in adhesion processes, integrins provide the strong adhesion but only after activation by other stimuli, which can include soluble mediators such as hormones and cytokines.\(^5\) Activation of and signaling by integrins result in a variety of influences on the cell that play roles in development and cellular differentiation,\(^7\) platelet and lymphocyte function, fibroblast cell adhesion, T-cell activation and proliferation, and ECM regulation.\(^8,9\) Integrins are important regulators in early cardiac development, providing positional information for migrating and differentiating cells.\(^9\) They are also important regulators of the intimate actions between myocytes, ECM proteins, and cytoskel-

Received March 22, 1993; accepted October 25, 1993.

From the Department of Developmental Biology and Anatomy (M.L.B., W.E.C., L.T., T.K.B.) and the Department of Pharmacology (S.P.W., M.A.W.), University of South Carolina School of Medicine, Columbia.

Correspondence to Maria Lonnett Burgess, PhD, Department of Developmental Biology and Anatomy, University of South Carolina School of Medicine, Columbia, SC 29208.
etal components in the adult heart. In an in vitro assay using three-dimensional (3D) collagen gels to assess cell function, \( \beta_1 \)-integrin mediates gel contraction by neonatal heart fibroblasts (NHFs) by various growth factors.

Cell phenotype and function can be influenced by culture conditions. Cardiac fibroblasts in situ reside within a collagenous network and are elongate and bipolar in shape. Plated in two-dimensional (2D) culture, these cells will change from elongate to a flat shape with stellate projections and actively synthesize collagens. Fibroblasts plated in 3D collagen gels maintain their elongation and have less projections and lower rates of collagen synthesis (versus cells plated in 2D culture), which are characteristics similar to in vivo conditions. Collagen gel contraction assays are often used as an in vitro model for wound contraction and connective tissue morphogenesis, and the contraction process has been characterized in great detail. The interaction of fibroblasts with a 3D collagen lattice affects cellular activities such as protein synthesis and proliferation, and thus provides a unique model for fibroblast responses in vitro. NHFs in attached collagen gels interact with the collagen via specific integrin receptors, and this interaction results in gel contraction. Cardiac fibroblasts in vivo interact with myocytes, and together, they regulate mechanical properties of the heart. Similarly, 3D collagen gels in vitro reestablish the interaction of fibroblasts with their collagen matrix. Because culture conditions influence cell phenotype and function, the present study examines NHFs in both 2D and 3D cultures.

In the present investigation, a variety of in vitro models were used to (1) determine the presence of functional Ang II receptors on NHFs and (2) determine the specific effects of Ang II on cardiac fibroblast morphology and function. A variety of techniques were used to examine the effects of Ang II on isolated NHFs in vitro in regard to modulation of collagen gel contraction and integrin regulation. The data from each of the techniques used support the presence of Ang II receptors on NHFs as well as the regulation of integrins by Ang II.

Materials and Methods

Animals

All animals were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care with food and water ad libitum. The present investigation was conducted in conformity with the “Guiding Principles for Research Involving Animals and Human Beings,” as approved by the “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health, Bethesda, Md). All experimental protocols involving the animals were approved by the University Institutional Animal Review Board.

Antibodies

A rabbit polyclonal antibody against rat \( \beta_1 \)-integrin was used for immunofluorescent localization and immunoprecipitation of \( \beta_1 \)-integrins and \( \beta_1 \)-integrin-inhibited gel contraction experiments. The purification and characterization of this antibody have been previously described.

Cell Isolation

Neonatal Sprague-Dawley rats (3 to 5 days of age) were decapitated, and the hearts placed in ice-cold phosphate-buffered saline (PBS). NHFs were isolated by established procedures. Briefly, minced heart tissue was subjected to collagenase digestion, and the dissociated cells were plated in 150-cm\(^2\) culture flasks with Dulbecco’s modified essential media (DMEM) supplemented with 10% neonatal bovine serum, 5% fetal bovine serum, 0.5 U/mL penicillin G, 0.5 \( \mu \)g/mL streptomycin, and 2 \( \mu \)g/mL amphotericin B (10/5-DMEM). After 1 to 2 hours, the medium was aspirated, and the attached cells were washed with Moscona’s saline. Forty milliliters of 10/5-DMEM was added, and cells were grown at 37°C in 5% CO\(_2\)/95% air until confluent. Cells from subsequent passages 3 to 10 were used for all experiments.

Ang II Receptors

Saturation binding analysis was used to test the presence of Ang II receptors on NHFs. Reverse-transcriptase (RT) polymerase chain reaction (PCR) was used to test whether NHFs possessed mRNA for Ang II receptors.

The presence of Ang II receptors on NHFs was determined using a modification of the method of the method of Aceto and Baker. Briefly, NHFs were plated in 35-mm six-well culture plates at a density of 200,000 per well in 10/5-DMEM. After 24 hours, the attached cells were washed with Moscona’s saline and fed with 5 mL of serum-free DMEM. Cells were grown in serum-free DMEM for 4 to 5 days before conducting receptor-binding experiments. Cells were washed three times with 1 mL of an incubation buffer (50 mmol/L Tris, 120 mmol/L NaCl, 4 mmol/L KCl, 1 mmol/L CaCl\(_2\), 1 mmol/L MgCl\(_2\), 2 mg/mL dextrose, 10 \( \mu \)g/mL bacitracin, and 0.25% bovine serum albumin [BSA] at pH 7.5 and 25°C). A saturation radioligand-binding assay was conducted as follows. One milliliter of incubation buffer was then added either alone or in combination with varying concentrations of \( \text{[H]} \)-Ang II. Nonspecific binding was determined in the presence of 10 nmol/L unlabeled Ang II. The cells were incubated for 60 minutes at room temperature, after which the reaction mixture was removed by aspiration. The cells were then washed three times with incubation buffer, after which 200 \( \mu \)L of lysis solution (0.25N NaOH and 0.5% sodium dodecyl sulfate [SDS]) was added to each well. After a 10-minute incubation at room temperature, wells were washed with an additional 500 \( \mu \)L of the NaOH/SDS solution, and the solutions were transferred into vials for gamma counting. Identical wells for each condition were used for determination of protein content. Total protein was determined by the Pierce assay (Pierce Chemical Co, Rockford, Ill). Affinity (K\(_d\)) and receptor number (B\(_{max}\)) were determined using saturation analysis (0 to 8 nmol [125I]-Ang II; specific activity, 2175 Ci/mmol) and calculated according to the method of Lundeen and Gordon. The data were then graphed as a Scatchard plot to demonstrate linearity of transformed data. Ang II receptor-binding experiments were repeated at least three times, and results are expressed as moles per milligram protein.

RT-PCR methodology determined whether NHFs were expressing mRNA for Ang II (type 1) receptors, because this receptor mediates all of the hemodynamic manifestations of Ang II. Total RNA was isolated from confluent NHFs in 2D culture using the one-step guanidium thiocyanate method. RT-PCR was carried out with the GeneAmp RNA PCR kit (Perkin-Elmer, Norwalk, Conn) using 2 \( \mu \)g of total RNA per reaction. Briefly, Moloney murine leukemia virus RT and random primers were used for cDNA synthesis from the RNA. The cDNA was amplified with AmpliTaq DNA polymerase and primers for the rat aortic vascular smooth muscle Ang II (type 1) receptor. Specifically, cDNA (rat) sequences nt 296 to 318 (ACCTGATGAATGCTGCCCCGC) and nt 854 to 875 (TACGCTATGCAGATGGTGATGG) were used. Primer Detective software (Clontech, La Jolla, Calif) was then used to select the Ang II primers for this experiment. These primers were synthesized by the Oligonucleotide Synthesis Facility at the University of South Carolina (Institute for Biological and Research Technology, Columbia, SC). Primers for \( \beta_1 \)-actin
Ang II was
Thermal cycling
A-DNA
Digested
IgG
Neutral
Appropriate
Polymerize
Antibody
1% Triton
Anti-integrin
37°C
Additional
Incubation
Protease inhibitor cocktail
1000 g
1251.21
Student-Newman-Keuls
ANOVA
Immunoprecipitation
Anti-integrin
Immunofluorescence
Anti-integrin
Anti-integrin
Anti-integrin
Anti-integrin
Anti-integrin IgG
Anti-integrin IgG
Anti-integrin IgG
Anti-integrin IgG
Antibody
Anti-integrin IgG
Anti-integrin IgG
Anti-integrin IgG
Anti-integrin IgG
Anti-integrin IgG
Anti-integrin IgG
Significant
Statistics
Data from saturation experiments
Condition x time
ANOVA
Student-Newman-Keuls
Analysis
was used to probe individual differences. Where appropriate, results are presented as mean±SEM.

Results

Ang II Receptor Binding

Receptor binding studies demonstrate the presence of Ang II receptors on NHFs by analysis of saturation experiments (Fig 1, left). Binding demonstrated saturation at \( \approx 7 \) nmol/L (see Fig 1, left, inset). The \( K_d \) for NHFs was 1 nmol/L, and \( B_{max} \) was 24.4 fmol/mg protein as determined by the Lunden method. Competition binding experiments showed appropriate displacement of \( ^{125}\text{I}-\text{Ang II} \) by unlabeled hormone (data not shown). RT-PCR analysis showed positive expression for Ang II (type I) receptor RNA (Fig 1, right). The Ang II (type 1) receptor band was of a size similar to that shown previously.\(^{20}\) Together, these data indicate the presence of Ang II receptors on isolated heart fibroblasts.

Collagen Gel Contraction

Collagen gel contraction experiments showed a significant main effect for area (enhanced contraction evidenced by decreased gel area) of the 3D collagen gels with the Ang II treatment versus control gels (Fig 2, left). Figures express change in gel area as percentage of control gel area. Student-Newman-Keuls post hoc analysis revealed that significant differences in gel contraction occurred at the 24-, 48-, 72-, and 96-hour time points (Fig 2, right; \( P<.05 \)). When collagen gels were incubated with [Sar\(^1\),Ala\(^3\)]Ang II (an Ang II receptor antagonist) or both Ang II and Ang II receptor antagonist, gel contraction was attenuated at all time points examined (Fig 3, \( P<.05 \)). When collagen gels were incubated with \( \beta_1 \)-integrin antibody or both Ang II and 10 \( \mu \)g/mL \( \beta_1 \)-integrin antibody, gel contraction was completely abolished, even after 96 hours (Fig 4, \( P<.05 \)).

Immunofluorescence

Confocal microscopic localization of \( \beta_1 \)-integrin shows more intense staining in the 2D Ang II–treated cells versus control cells (Fig 5). The more concentrated staining appears to be distributed in the bottom focal plane between the cell and the culture dish, with intense staining under the nucleus. The figures represent substrate-level cell-surface sections, where the gain was set for control conditions and held constant for all experimental images. Fig 6 shows more intense staining for \( \beta_1 \)-integrin of NHFs plated in 3D collagen minigels. In Fig 6, the cells in the 3D gels have organized into a ring within the gel. This organization has been previously described.\(^{10}\) The fluorescence shows the ringlike organization of the cells within the gel in addition to the localization of the \( \beta_1 \)-integrins. The micrographs represent a low magnification image of a portion of the cell ring. In both 2D and 3D culture conditions, Ang II–treated cells demonstrated a more intense staining pattern for \( \beta_1 \)-integrin.

Immunoprecipitation

Results from immunoprecipitation experiments show a relative increase in \( \beta_1 \)-integrin on the surface of Ang II–treated cells versus control cells (Fig 7) by gel electrophoresis. The arrows on Fig 7 mark the \( \beta_1 \)-integrin bands. Identical incorporated counts of \( ^{125}\text{I} \) were used for immunoprecipitation (\( \approx 1.4 \times 10^6 \) counts per condition). Although not quantitative, the immunoprecipitation procedure is used for relative comparisons in the present study. The bands directly above the \( \beta_1 \)-integrin bands have migrated to molecular weights similar to the \( \alpha_5 \) or \( \alpha_6 \)-integrin chains; however, monospecific antibody immunoprecipitation to those \( \alpha \) chains was not performed. Analysis by laser densitometry revealed a relative 56% increase in the cell surface accumulation of \( \beta_1 \)-integrin in Ang II–treated versus control cell extracts (\( P<.05 \)).
Discussion

The ECM in the heart is important for contraction of the extracellular collagen network by tension.5,9 Directly involved in the regulation of ECM components and contraction are the cardiac fibroblasts, which are at least in part responsible for the normal ECM adaptation of the myocardium, which may in turn set the stage for pathological hypertrophy and ventricular dysfunction in hypertension and hypertrophy.2,8 Although Ang II has been implicated in regulating cardiac ECM morphology and function, the present study provides evidence for direct localization and effects of Ang II on cardiac fibroblasts, which are responsible for the synthesis of many ECM components in the myocardium.21,22

The present study used both 2D and 3D culture systems to examine the effects of Ang II on neonatal heart fibroblasts to control for the phenotypic changes often seen when isolating cells into culture. The results

![Image](http://circres.ahajournals.org/)

**Figure 2.** Left, Bar graph shows angiotensin II (Ang II)–stimulated collagen gel contraction (expressed as percentage of control value) by neonatal heart fibroblasts at 0, 24, 48, 72, and 96 hours. At time 0, the diameter of the collagen gels completely filled the culture well and therefore is expressed at 100% of the control value at this time point. Ang II (10 μg/mL) significantly stimulated gel contraction at all time points (*P<.05). Right, Photomicrographs show actual collagen gels treated with 10 μg/mL Ang II (right) or sterile water (control gels, left) for 24 hours (1), 48 hours (2), 72 hours (3), and 96 hours (4). Increased contraction by neonatal heart fibroblasts in Ang II–treated gels can be noted at all time points.

**Figure 3.** Bar graph shows that angiotensin II (All)–stimulated collagen gel contraction (expressed as percentage of control) was significantly attenuated similar to that of control gels when neonatal heart fibroblasts were treated with Ang receptor antagonist [Sar¹, Ala⁸]All (All antag) vs All-stimulated gels (*P<.05 vs All). When both All and [Sar¹, Ala⁸]All were administered simultaneously in a dose of 10 μg/mL (both), gel contraction was still significantly attenuated (*P<.05 vs All).

**Figure 4.** Bar graph shows that, in collagen gel cultures treated with 10 μg/mL anti-β₁-integrin antibody (B1), gel contraction by neonatal heart fibroblasts was completely abolished at all time points (*P<.05 vs angiotensin II [All]-treated cultures). Treatment with both 10 μg/mL All and 10 μg/mL anti-β₁-integrin antibody (both) also resulted in complete inhibition of gel contraction as well (*P<.05 vs All).
of most experimental procedures in the present study exhibited similar responses to Ang II in both 2D and 3D culture conditions. Specifically, both culture conditions resulted in a more intense staining pattern in NHFs for \( \beta_1 \)-integrin, and immunoprecipitation for \( \beta_1 \)-integrin in 2D culture revealed a greater number of integrins on the cell surface. These results may suggest an important role for Ang II in the remodeling of the ECM during pathological hypertrophy, where elevated levels of Ang II are present.

The present study demonstrates the presence of Ang II receptors on neonatal rat heart fibroblasts by saturation-binding analysis. Binding of Ang II to cardiac fibroblasts could be displaced by competitive agonists. RT-PCR also indicates that these cells are expressing mRNA for the Ang II (type I) receptor. Until very recently, support for the presence of Ang II receptors on cardiac fibroblasts has been only indirect. Ang II receptors in cultured fibroblasts prepared from neonatal skin 1 day after birth were reduced by 80% compared with a 19-day fetus.\(^{23}\) Unfortunately, Millan et al\(^{23}\) did not examine the presence of Ang II receptors on the fetal or neonatal heart cells. Other investigations have implied the presence of Ang II receptors on cardiac fibroblasts as evidenced by increased collagen accumulation,\(^4\) the presence of Ang II hormone in cultured rat cardiac fibroblasts,\(^{24}\) and fibroblast proliferation.\(^{25}\) Only very recently have binding studies on cardiac fibroblasts demonstrated the presence of Ang II receptors.\(^{26}\) As evidenced by the different receptor densities in the present study and that in the study of Schorb et al,\(^{26}\) culture conditions play an important role in the expression of AT\(_1\) receptors on cardiac fibroblasts. The present study demonstrated enhanced collagen gel contraction by cardiac fibroblasts treated with Ang II, which was attenuated in the presence of Ang II receptor antagonist. This result indicates that Ang II stimulation of gel contraction is Ang II receptor–mediated. The
function of cardiac fibroblasts in the 3D culture system (ie, the ability of the fibroblasts to contract the collagen gels) can be altered by inhibition of Ang II receptors, implying that Ang II is important in mediating this aspect of cardiac fibroblast function. Previous investigations have shown that Ang II mediates cardiac fibroblast metabolism. For example, angiotensin-converting enzyme inhibitors will suppress normal accumulation of ECM proteins in the heart, whereas treatment with Ang II will promote collagen synthesis in vascular smooth muscle cells and murine mesangial cells. Enhanced remodeling of the ECM by Ang II treatment of neonatal heart fibroblasts in the collagen gel system implies that Ang II may in part be responsible for the dramatic reorganization of interstitial collagen observed in hypertrophy from growth and hypertension.

For example, an increased Ang II concentration seen by the cardiac fibroblast in some forms of renovascular hypertension might stimulate collagen synthesis because of the enhanced metabolic action of this cell observed during normal cardiac growth and in hypertrophy resulting from pressure overload (hypertension). Although the present study did not examine specific mechanisms of action, it does imply that Ang II may impose an increased resistance to the contracting myocytes in the heart by causing fibroblasts to constrict the ECM around the contracting myocytes. Support for this speculation is that altered ECM tension, metabolism, and synthesis have been seen in disease states with elevated Ang II levels. For example, collagen accumulation in hypertensive disease has been held responsible for abnormal myocardial stiffness and impaired pumping capacity of the heart. In addition, administration of the angiotensin-converting enzyme inhibitor enalapril to rats after abdominal aortic constriction prevented the left ventricular hypertrophy usually seen with this model. Enalapril treatment of young growing rats suppressed accumulation of elastin and collagen in large and small arteries as well as total collagen in both left and right ventricles. Ang II has also been implicated in normal cardiac development. For example, Beinlich et al showed decreased left ventricular weight, RNA content, total RNA, and RNA/DNA ratio in left ventricle of newborn pig hearts treated with an Ang II antagonist.

Since fibroblasts produce ECM proteins (ie, collagen) that bind to their membrane-bound integrin receptor, the present study tested whether Ang II effects might be associated with the integrins. By culturing cardiac fibroblasts in substrata (such as hydrated collagen gels), these cells would bind with the substrate via the integrin and then contract by pulling on the collagen, releasing water and thus shrinking the gel. Such an event occurs in wounding and tumor metastasis, as evidenced by the 3D collagen gel system. The observed increase in β-integrin under Ang II treatment may explain the behavior of the fibroblasts in the collagen gel cultures. Specifically, increased β-integrin in the Ang II–treated collagen gels would provide more adhesion sites for the cells, thus enhancing gel contraction. These results support a relation in the mechanism(s) of action of both Ang II and β-integrin in influencing cardiac fibroblast function in the 3D culture system. Specifically, Ang II stimulation of collagen gel contraction may be due to the upregulation of β-integrins by this vasoactive peptide.

The precise mechanism(s) by which Ang II regulates ECM morphology and function is yet to be determined. The present study demonstrated increased collagen gel contraction and β-integrin localization and content in Ang II–treated cells. It is possible to speculate that Ang II, acting through its receptor in the plasma membrane, may induce these changes in integrins via a similar mechanism as that proposed by Katz. Specifically, binding of Ang II to its receptor increases phospholipase C activity through a mechanism that probably involves a G protein. Phospholipase C then hydrolyzes phosphatidylinositol 4,5-bisphosphate and forms diacylglycerol and inositol 1,4,5-triphosphate (InsP3). InsP3 increases cytosolic calcium, which may explain the ability of Ang II to promote contraction in smooth and cardiac muscle. Protein kinase C (activated by diacylglycerol) and calcium appear to induce the c-fos proto-oncogene within the nucleus, and c-fos is believed to accelerate transcription and thus increase protein synthesis, possibly accounting for the growth-promoting effects of Ang II.

Integrins are intimately associated with the cytoskeleton and transmit extracellular signals across the sarcolemma to the internal milieu of the cell. This is especially true for mechanical signals. Ang II may provide a chemical signal to the cytoplasm, similar to platelet-derived growth factor (PDGF) and other growth factors, with the subsequent surfacing of cytosolic β-integrins and/or enhanced gene expression of β-integrin. The mechanism proposed by Katz is supported by concomitant increases in integrin expression, protein synthesis, and cardiac hypertrophy in vivo. Previous studies have demonstrated a role for the α-integrin chains in recognition sites for ECM substrates, such as collagen. In addition, it is thought that the α-chains dictate integrin substrate specificity. Regulation of the α-chains by Ang II may provide more evidence of the relation and mechanisms of action between Ang II–mediated and integrin-mediated effects in cardiac fibroblasts. Characterization of the α-integrin chains in fibroblasts in response to Ang II treatment is currently in progress. Other factors have been shown to influence collagen gel contraction by cardiac fibroblasts.
PDGF stimulates collagen gel contraction via the integrins,10 whereas transforming growth factor-β exhibits no such effect (authors’ unpublished data). Studies examining the effects of Ang II and other growth factors may serve to elucidate the precise mechanism(s) by which Ang II and cell surface integrins are related to the control of cardiac fibroblast function.

The present study documents the expression of Ang II receptors on the surface of cardiac fibroblasts by pharmacologic, biochemical, and molecular techniques. There appears to be an interaction between Ang II receptors and integrins, as revealed by the correlation of the data from immunofluorescent staining, immunolabeling, and collagen contraction assays. Specifically, the neonatal rat heart fibroblast up-regulates its expression of surface β1-integrins by Ang II treatment. These results suggest a role for Ang II in mediating heart fibroblast function and may provide insight into the precise role of Ang II on heart fibroblasts in both health (physical training) and disease (hypertension).

Acknowledgments

This study was supported in part by National Institutes of Health grants HL-24935 and HL-40424. Maria Lonnett Burgess, PhD, was the recipient of National Research Service Award HL-08713-01 from the National Institutes of Health. The authors acknowledge Marge Terracio and Margaret Currie for their excellent technical assistance with the cell culture, Titus A. Reeves for assistance with electrophoresis, Will Sharp for assistance with the immunofluorescence, L. Annette Smith for Ang II ionization, and William A. Burgess, Dr William A. Clark, Chris Coakley, and Dr David G. Simpson for their critical review of the manuscript.

References

9. Borg TK, Burgess ML. Holding it all together: organization and function(s) of the extracellular matrix in the heart. Heart Failure. 1993;8:230-238.
Integrin-mediated collagen gel contraction by cardiac fibroblasts. Effects of angiotensin II.
M L Burgess, W E Carver, L Terracio, S P Wilson, M A Wilson and T K Borg

doi: 10.1161/01.RES.74.2.291

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/74/2/291

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/