Adenylyl cyclase activity and function are decreased in rat cardiac fibroblasts after myocardial infarction

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Swaney JS, Patel HH, Yokoyama U, Lai NC, Spellman M, Insel PA, Roth DM. Adenylyl cyclase activity and function are decreased in rat cardiac fibroblasts after myocardial infarction. Am J Physiol Heart Circ Physiol 293: H3216–H3220, 2007. First published September 14, 2007; doi:10.1152/ajpheart.00739.2007.—Myocardial infarction (MI) results in left ventricular remodeling (e.g., ventricular hypertrophy, dilatation, and fibrosis). Fibrosis contributes to increased myocardial stiffening, impaired ventricular filling and function, and reduced cardiac output. Adenylyl cyclase (AC) expression and activity are reduced in animal models of heart failure. Stimulation of AC can inhibit extracellular matrix production in isolated cardiac fibroblasts; however, a role for reduced AC expression and activity in fibrosis associated with cardiac remodeling after chronic MI has never been determined. We tested the hypothesis that AC expression and activity are reduced in cardiac fibroblasts after chronic (18 wk) MI. Rats underwent coronary artery ligation or sham surgery (control), and echocardiography was used to assess left ventricular remodeling 1, 3, 5, 7, 10, 12, and 18 wk after surgery. Cardiac fibroblasts were isolated from the noninfarcted myocardium and compared for differences in AC activity and collagen synthesis. End-diastolic dimension was increased [control: 0.76 ± 0.02 cm and MI: 1.0 ± 0.02 cm (means ± SE), P < 0.001] and fractional shortening was decreased [control: 44 ± 2% and MI: 17 ± 2%, P < 0.001] in MI compared with control rats. Basal and forskolin-stimulated cAMP production were decreased by 90% and 93%, respectively, and AC5/6 expression was decreased 39% in fibroblasts isolated from MI rats compared with sham controls. Serum-stimulated collagen production was increased twofold and forskolin-mediated inhibition of collagen synthesis was reduced in fibroblasts from MI rats compared with controls. Our data demonstrate that AC expression and activity are reduced and collagen production is increased in cardiac fibroblasts of rats after MI.

CONGESTIVE HEART FAILURE (CHF) is a leading cause of morbidity and mortality in the United States. CHF was believed to result primarily from systolic dysfunction; however, it is recognized that diastolic dysfunction, or an inability of the heart to fill during diastole, is also an underlying mechanism in many patients with heart failure (29). Myocardial fibrosis, a key contributor to cardiac dysfunction during CHF, reflects hyperplasia and increased deposition of extracellular matrix (ECM) material into the interstitial and perivascular space (5). Exaggerated ECM deposition results in myocardial stiffening and decreased relaxation of the heart, ultimately leading to cardiac dysfunction (19, 20).

The myocardium is composed of cardiac myocytes and nonmyocytes, which include endothelial cells, vascular smooth muscle cells, and fibroblasts (34). Cardiac fibroblasts (CFs), an abundant cell type in the heart (comprising ~2/3 of the total cell population) are responsible for basal ECM homeostasis as well as repair after a cardiac insult (7, 19). Following myocardial infarction (MI), reparative scar formation at the site of injury can initiate maladaptive connective tissue production remote from the infarct zone in the remaining functional regions of the myocardium (1, 21, 31, 33). This increased ECM contributes to the impaired cardiac compliance, reduced filling capacity, and cardiac dysfunction involved in heart failure.

Adenylyl cyclases (ACs), membrane-bound enzymes that catalyze the conversion of ATP to cAMP, are activated upon stimulation of G protein-coupled receptor (GPCR) agonists that signal through Gαs. Of the nine membrane-bound AC isoforms, AC5 and AC6 are the most highly expressed isoforms in the heart and have direct effects on cardiac function (9, 11, 18). Interestingly, AC6 mRNA expression and total AC activity are reduced in animal models of heart failure (26), and overexpression of AC6 attenuates deleterious remodeling and increases function in the heart (17, 27, 28). Our laboratory and others have shown that AC6 activation and expression and increased cAMP production inhibit fibroblast proliferation and collagen synthesis, suggesting an antifibrotic role for AC6 in the heart (4, 6, 12, 24, 32). However, a role for AC expression and activity in fibrosis associated with cardiac remodeling after chronic MI has never been established.

We hypothesized that AC activation negatively regulates collagen production in the heart and that downregulation of AC expression and activity following MI may contribute to cardiac fibrosis. To test this hypothesis, we conducted echocardiographic assessment of left ventricular (LV) function over an 18-wk period after MI or sham surgery and analyzed changes in AC expression and activity and collagen production in adult rat CFs isolated from noninfarcted regions of the LV. We show that total AC activity and AC5/6 expression are reduced concomitantly with increased collagen production in MI rats compared with sham controls. These data suggest that a downregulation of AC function in the heart after MI may exacerbate maladaptive connective tissue production, thereby contributing to cardiac fibrosis and heart failure.

MATERIALS AND METHODS

Animals. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Veterans Affairs Medical Center (125), 3350 La Jolla Village Dr., San Diego CA 92161 (e-mail: droth@ucsd.edu).

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Fig. 1. Cardiac size is increased and cardiac function is reduced 18 wk after myocardial infarction (MI). M-mode echocardiographic images were obtained after MI (A) or sham surgery (control; B). Arrows denote the distance between the anterior left ventricular (LV) free wall and posterior LV (septum) during diastole. Percent fractional shortening (C) and end-diastolic diameter (D) were measured before and 1, 3, 5, 7, 10, 12, 14, and 18 wk after MI or sham surgery to assess LV function and dilatation. Values represent means ± SE of at least 5 experiments compared using two-way ANOVA with a post hoc multiple-comparisons test.

Fig. 2. cAMP production is reduced in adult rat cardiac fibroblasts (CFs) 18 wk after MI. A: homogeneity of the cell preparation was confirmed by discoidin domain receptor 2 (DDR2) and fibroblast-specific protein 1 (FSP1) staining. DAPI, 4′,6-diamidino-2-phenylindole. B: cAMP production by CFs isolated from the noninfarcted LV region of rats 18 wk after MI or sham surgery (control). cAMP production was measured by a radioimmunoassay using CFs grown for 48 h in serum-free media and then stimulated for 10 min with 2.5% FBS alone (basal) or in the presence of forskolin (Fsk; 10 μM). Values represent means ± SE of n = 5–7 rats/group. *P < 0.05 compared with control using two-way ANOVA with a post hoc multiple-comparisons test.
mechanically ventilated with 1.5% isoflurane in 100% O2 to maintain rats/group. *P wk after MI or sham surgery (control). AC5/6-immunoreactive bands were CFs 18 wk after MI. AC5/6 expression was measured by immunoblot analysis (sham control group). All animals were killed 18 wk after surgery.

**Echocardiography:** Echocardiography was performed in anesthetized rats prior to surgery and at 1, 3, 5, 7, 10, 12, 14, and 18 wk postsurgery. Rats were anesthetized using isoflurane (1.5% in O2) supplied via a nose mask. With the use of a pediatric 12-MHz linear probe (Agilent Technologies), a parasternal short-axis view was obtained as a guide for LV M-mode imaging at the papillary muscle level. M-mode images were digitized on the optical disc (HP 5500). With the use of HP 5500 standard software, LV dimensions were obtained as a guide for LV M-mode imaging at the papillary muscle level. Echocardiographic assessment of LV size and function after MI. Compared with control animals, rats with MI exhibited increased LV chamber diameter and thinning of the anterior LV free wall, indicative of myocardial tissue loss in the infarct region (2). Based on these measurements, rats with an echo score ≥4 (18-wk echo) were included in the infarct group.

**Isolation and culture of adult rat CFs.** Eighteen weeks after MI, CFs were isolated from the noninfarcted LV of MI rats or from LV septa of control rats as previously described (32). All CFs were used at early passage (≤2) to minimize loss of the in vivo phenotype as a result of cell division in vitro. Homogeneity of the cell preparation was verified by positive staining for fibroblast-specific markers: discoidin domain receptor 2 (DDR2) and fibroblast-specific protein 1 (FSP1). Immunohistochemical staining and image acquisition were performed as previously described (32).

**Collagen synthesis assay.** Collagen synthesis was measured using collagenase-sensitive [3H]proline incorporation according to previously established methods (25, 32).

**cAMP production.** cAMP production by CFs was measured according to previously described methods (32).

**Immunoblot analysis.** Antibody for AC5/6 was obtained from Santa Cruz Biotechnology. Immunoblot analysis was conducted as previously described (32).

**Data analysis.** Statistical comparisons and graphical representation were performed using Graph Pad Prism 3.0 (GraphPad Software). ANOVA was followed by a post hoc Bonferroni correction for multiple comparisons. Statistical significance was set at P < 0.05.

**RESULTS**

**Echocardiographic assessment of LV size and function after MI.** Compared with control animals, rats with MI exhibited increased LV chamber diameter and thinning of the anterior LV free wall, indicative of myocardial tissue loss in the infarct (Fig. 1, A and B). LV remodeling post-MI was associated with a significant (P < 0.05) decrease in %FS (Fig. 1C) and increased end-diastolic dimension (EDD; Fig. 1D), demonstrating reduced cardiac function and progressive LV chamber dilatation after MI. No significant changes in %FS or EDD were observed in control rats.

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**Fig. 3.** Adenylyl cyclase type 5/6 (AC5/6) expression is reduced in adult rat CFs 18 wk after MI. AC5/6 expression was measured by immunoblot analysis using CFs (passage ≤ 2) isolated from the noninfarcted LV region of rats 18 wk after MI or sham surgery (control). AC5/6-immunoreactive bands were normalized to that of GAPDH, and data are expressed as means ± SE of n = 5–6 rats/group. *P < 0.05 compared with control using an unpaired Student’s t-test.

**Fig. 4.** Collagen production by CFs is increased following MI. Collagensesensitive [3H]proline incorporation by CFs isolated from the noninfarcted LV region of rats 18 wk after MI or sham surgery (control) is shown. CFs were grown for 48 h in serum-free media and then stimulated for 48 h with 0% FBS (control) or 2.5% FBS in the absence or presence of Fsk (10 μM). Data are normalized for [3H]proline incorporation in cells grown under control conditions. Values represent means ± SE of n = 5–6 rats/group. *P < 0.05 compared with control and #P < 0.05 compared with 2.5% FBS alone using two-way ANOVA with Bonferroni post hoc multiple-comparisons tests.
AC activity of CFs is reduced following MI. Homogeneity of the cell preparation from control and MI rats was confirmed by positive staining with DDR2 and FSP1, specific markers of fibroblasts (Fig. 2A). No observable contamination of other cell types was present. To analyze differences in AC activity by CFs isolated post-MI, cAMP production was measured under basal conditions and in response to the direct AC agonist forskolin (10 μM). Although no differences in basal cAMP production were observed, there was a 93% reduction (P < 0.05) in forskolin-stimulated cAMP production by CFs from MI rats compared with controls (Fig. 2B).

AC expression is reduced following MI. To determine whether decreased cAMP production by CFs results from reduced AC expression, we examined the expression of AC5/6, the predominant AC isoforms expressed in the heart (9, 11, 23), using immunoblot analysis. AC5/6 expression was significantly (P < 0.05) reduced in CFs from MI rats compared with controls (Fig. 3).

Collagen production by CFs is increased following MI. Because AC activation exerts antifibrotic effects on fibroblasts in culture (4, 6, 12, 24, 32), we hypothesized that the decreased AC expression and activity may correlate with increased collagen production by CFs following MI. Consistent with this hypothesis, CFs from MI rats exhibited enhanced (P < 0.05) serum-stimulated collagen production (as indicated by [3H]proline incorporation) compared with control CFs (Fig. 4). In contrast, forskolin reduced (P < 0.05) serum-stimulated collagen production to basal levels in control CFs, whereas CFs from infarcted rats exhibited only moderate inhibition in response to forskolin treatment. Thus, increased collagen production by CFs following MI may result from decreased AC expression and a decreased ability of CFs to produce cAMP.

DISCUSSION

We determined whether the fibrosis and connective tissue production involved in cardiac remodeling after chronic MI results, at least in part, from a downregulation of AC. A previous study (26) has demonstrated that AC6 mRNA expression and total AC activity are reduced following pacing-induced heart failure in pigs. However, no previous studies have examined AC function and collagen production by CFs isolated from the heart 18 wk after MI. Here, we provide the first evidence that AC activity and expression by CFs is reduced in hearts from rats after chronic MI. Furthermore, we show that decreased AC function correlates with increased collagen production by CFs.

Several in vitro studies have demonstrated that AC can have antifibrotic effects, whereby GPCR agonists that activate AC and stimulate cAMP production can inhibit collagen synthesis by fibroblasts from various organs in culture (4, 6, 12, 24). Data from our laboratory have shown that CFs overexpressing AC6 exhibited a greater inhibition of transforming growth factor (TGF)-β-stimulated myofibroblast formation and serum-stimulated collagen production as well as decreased basal and TGF-β-promoted expression of the profibrotic factors PAI-1 and IL-6 (32). Thus, cardiac fibrosis following MI may be potentiated by a downregulation in AC thereby reducing the ability of AC to negatively regulate collagen production by CFs after MI. Antifibrotic effects of AC in isolated cells suggest that AC may have a therapeutic role in post-MI remodeling. It is known that animals post-MI have increased sympathetic drive (10); in this regard, others have shown decreased cardiac AC expression in animal models of heart failure (8, 13, 26). Our results suggest the increased sympathetic tone after MI may be responsible for the decreased expression and activity of AC in CFs and contribute to fibrosis in the noninfarcted myocardium. Our findings have to be considered in the context of other findings that suggest that the decreased expression of AC5 in cardiac myocytes may decrease β-adrenergic receptor-stimulated apoptosis and preserve cardiac function in the cardiac remodeling associated with pressure-overload hypertrophy (14, 22).

A limitation of the present study is the potential loss of the profibrotic phenotype by CFs that are removed from the in vivo cardiac milieu and studied under in vitro culture conditions. For this reason, we conducted all experiments using low-passage cells (passage ≤ 2) to avoid any dedifferentiation of CFs. In a MI model, a previous study (32) has shown that the in vivo CF phenotype is stable and can be studied in culture up to passage 4. The methodologies presented here are consistent with previous reports examining protein expression and signaling by CFs isolated from rats after MI and studied in culture (3, 15, 16, 30). However, unlike these studies, which examined CFs at early time points (≤4 wk) post-MI, we examined the long-term effects of MI on AC function and collagen production to understand the role of AC in CF function in the late phase of remodeling. The progressive fibrosis in the noninfarcted myocardium that leads to diastolic dysfunction in mice occurs much later, reaching a maximum at ~4 mo post-MI (35).

In conclusion, our data provide new information regarding AC expression and activity in CFs after MI. These results suggest a potential role for AC as an antifibrotic mediator of the deleterious connective tissue remodeling that occurs late after MI.

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REFERENCES

FIBROBLAST ADENYLYL CYCLASE AFTER MI


