J. Cell. Mol. Med. Vol 8, No 1, 2004 pp. 85-92

Differential gene expression in infarct scar and viable myocardium from rat heart following coronary ligation

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Received: January 29, 2004; Accepted: February 10, 2004

Abstract

Post-myocardial infarction (MI) remodeling of cardiac myocytes and the myocardial interstitium results in alteration of gross ventricular geometry and ventricular dysfunction. To investigate the mechanisms of the remodeling process of the heart after large MI, the expression of various genes in viable left ventricle and infarct scar tissue were examined at 16 weeks post-MI. Steady-state expression of Na^+-K^+ ATPase α-1 and -2, phospholamban (PLB), α-myosin heavy chain (α-MHC), ryanodine receptor (Rya) and Ca^{2+} ATPase (Serca2) mRNAs were decreased in the infarct scar vs noninfarcted sham-operated controls ($P < 0.05$). On the other hand, Giα2 and β-MHC mRNAs were upregulated ($P < 0.05$, respectively) in the infarct scar whereas Na^+-K^+ ATPase-β, Na^+-Ca^{2+} exchanger and Gs mRNAs were not altered vs control values. In viable left ventricle, the a-1 subunit of Na^+-K^+ ATPase, α-3, β-isoforms, Rya, β-MHC, Giα2, Gs and Na^+-Ca^{2+} exchanger were significantly elevated while expression of the a-2 subunit of Na^+-K^+ ATPase, PLB and Serca2 were significantly decreased compared to controls. Expression of CK2α mRNA was elevated in noninfarcted heart (145 ± 15%) and diminished in the infarct scar (66 ± 13%) vs controls. Expression of β-MHC mRNA was elevated in both viable and infarct scar tissues of experimental hearts (140 ± 31% and 183 ± 30% *vs.* controls, respectively). These results suggest that cardiac genes in the infarcted tissue and viable left ventricle following MI are differentially regulated.

Keywords: myocardial infarction • gene expression • heart failure • infarct scar • casein kinase 2

Introduction

In North America, incidence of myocardial infarction is causal for the majority of patients suffering from gross ventricular remodeling manifest as pathological cardiac hypertrophy, attendant heart failure and sudden death. Cellular and molecular mechanisms of heart failure following myocardial infarction have been the subject of intense scrutiny for the past decade, and a number of candidate genes are implicated in the progression of this disease, including those for the impairment of Ca^{2+} homeostasis [1], a shift from the α- to β-isoform of myosin heavy chain (MHC) [2,3], and an increase in the number of non-cardiomyocytes and overt deposition of extracellular matrix in the heart [4]. These changes are inextricably linked to incidence of cardiac diastolic disorders and reduced cardiac output. On the other hand, a scar is formed in the infarct zone

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through wound healing mediated by cardiac myofibroblasts. Myofibroblasts proliferate and are abundant in the infarct scar within days of the initial insult, are muscular phenotypic variants of quiescent fibroblasts, and are the main cell type populating the "healed" infarct scar [4, 5]. The role of the infarct scar in cardiac function and dysfunction as well as ventricular remodeling is not well understood despite the recent attention this problem has received [6-9].

Finally, casein kinase II (CK2) is a serine-threonine protein kinase composed of catalytic subunits (α) and regulatory units (β). Its activity is elevated in myocardial ischemia/reperfusion [5]. *In vitro* studies have revealed that CK2 phosphorylates Ca^{2+} binding proteins in the sarcoplasmic reticulum (SR) [10], and that CK2 activity is higher in proliferating cells [11]. Thus it is conceivable that CK2 may participate in cardiac hypertrophy and remodeling. We undertook the current comparative study of gene expression in viable and remnant myocardium and that of the infarct scar, and have focused on mRNA expression of genes that participate in cardiac myocyte Ca^{2+} handling, as well as G-proteins, MHCs and CK2 in left ventricle and scar tissues in age-matched sham-operated and coronary artery ligated rat hearts with large myocardial infarction and congestive heart failure.

Material and methods

Experimental model and hemodynamic measurements

Myocardial infarction model was induced in male Sprague-Dawley rats (150-200 g) by occlusion of the left coronary artery as described earlier from this laboratory [12]. Sham operated animals were treated similarly except the coronary artery was not ligated. Experimental animals with an infarct size > 30% of left ventricle were used in this study. For hemodynamic measurement, the rats were anesthetized by an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (10 mg/kg) mixture. The right carotid artery was exposed and a microtip pressure transducer (model SPR-249, Millar Instruments, Houston, TX, USA) was inserted into the left ventricle of the heart. The left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP) and heart rate were recorded using a computer program AcqKnowledge for Windows 3.5.3 (Biopac Systems Inc., Goleta, CA, USA).

RNA isolation and Northern blot analysis

Total RNA from frozen left ventricular tissue was prepared by using guanidinium thiocyanate-phenol-chloroform extraction procedure as described by Chomczynski and Sacchi [13]. Scanning densitometry was performed on autoradiograms to estimate the abundance of each particular mRNA in control and experimental groups. Relative 18S (5'-ACG GTA TCT GAT CGT CTT CGA ACC-3') RNA abundance was used as internal standards to normalize the density values for each target mRNA level [12].

The list of cDNA fragments used in the present study

The cDNA probes of Na^{+}-K^{+} ATPase-α1 (0.332 kb), -α2 (0.381 kb), -α3 (0.278 kb) and -β1 (0.271 kb), CK2-α (1.4 kb) and -β (500 bp), Gsα (1.585 kb), Giα (1.365 kb), α-MHC (5'- CAG GCA TCC TTA GGG TTG GGT AGC ACA AGA - 3') and -β (5'- GGG ATA GCA ACA GCG AGG CTC TTT CTG CTG GAC AGG TTA - 3') were purchased from American Type Culture Collection (Rockville, MD, USA). Ryanodine receptor (RyR, 2.2 kb) and Ca^{2+} ATPase (Serca2a, 0.762 kb) cDNA fragments were gifts from Dr. A.K. Grover (McMaster University, Hamilton, ON, Canada). Phospholamban (PLB, 0.503 kb) was a gift from Dr. D.H. MacLennan (University of Toronto, Toronto, ON, Canada). The cDNA segment coding for exons of Na^{+}-Ca^{2+} exchanger (1.1 kb) was obtained from SWANT (Switzerland).

Statistical analysis

Experimental values were presented as mean ± S.E.M. Comparison of mean values was performed by the Student's "t" test and a P value < 0.05 was considered to reflect a significant difference.

Results

The general characteristics of sham and infarcted rats

As shown in Table 1, the heart weight, the wet lung weight and LVEDP were increased significantly ($P < 0.05$) in infarcted heart, indicating the cardiac hypertrophy and heart failure were developed in this model.

Alteration of Na^+-K^+ ATPase gene expression in scar and viable tissues

As shown in Fig. 1, mRNA level of Na^+-K^+ ATPase were significantly changed in both viable left ventricle and scar tissue (Fig. 1A, B, and C): α1, α3 and β isoforms were upregulated in viable tissue, α1, α2 isoforms were significantly downregulated in scar tissue. The mRNA level of α2 isoform in viable left ventricular tissue is similar to that of the sham group (Fig. 1C). The mRNA of α3 and β isoforms show no significant change in scar tissue (Fig. 1B) in the comparison to the sham group.

Alteration of SR gene expression

In viable tissues, the gene of Rya was overexpressed; PLB and Serca2 had no significant change (Fig. 2A and C). In the scar tissue, the mRNA levels of Rya, PLB and Serca2 were significantly decreased (Fig. 2B)

Alteration of G-protein gene expression

In viable left ventricular tissue, Gs and Giα2 mRNA was significantly higher (Fig. 3A and C). In scar tissue, Gs had no significant change while G_iα2 mRNA was significantly elevated (Fig. 3B).

Table 1 The general characteristics of sham and infarcted rat heart.

Parameters	Sham (n=6)	Infarction (n=6)
Body weights (g)	651 ± 20	700 ± 29
Heart weights (g)	1.4 ± 0.04	1.8 ± 0.1*
Lung wet weight (g)	2.34 ± 0.05	2.83 ± 0.1*
LVEDP (mm Hg)	2.6 ± 0.3	13.8 ± 5*
LVSP (mm Hg)	109 ± 5	116 ± 2
Heart rate (beats/min.)	232 ± 22	239 ± 16

*P < 0.05 compared to sham group. The data were obtained 16 weeks after coronary ligation.

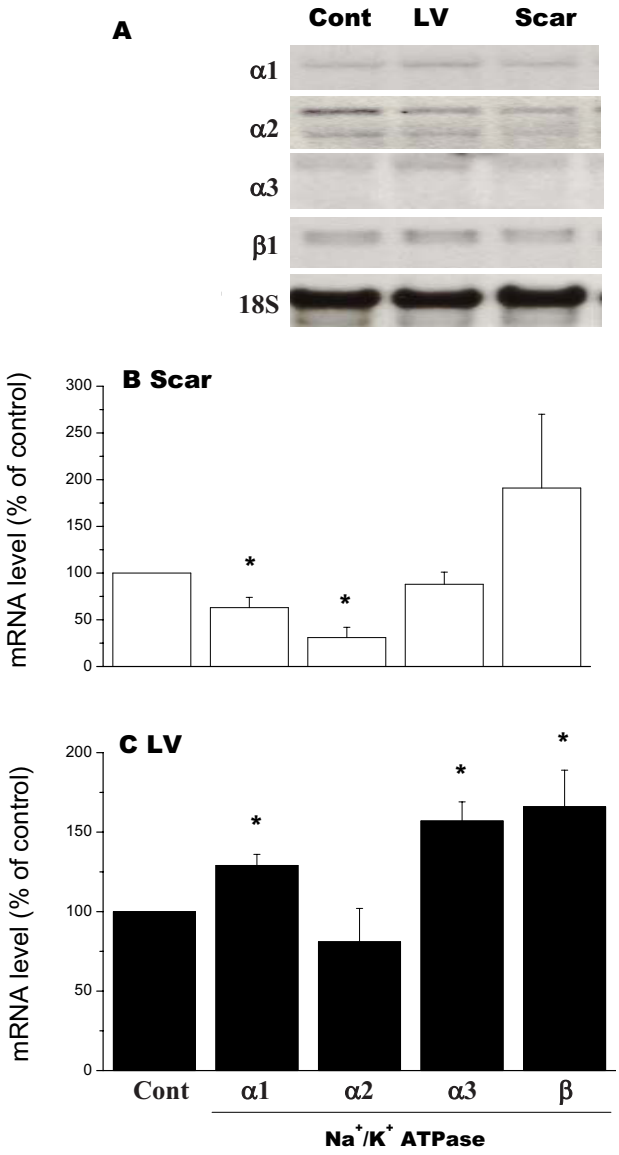

Fig. 1. Gene expression of Na^+-K^+ ATPase in infarcted scar (B) and left ventricular viable tissues (C) by Northern blot analysis. (A) is a representative blot. The relative abundance of each gene was expressed as the ratio of densitometric value of each mRNA band/18S RNA. The results are mean ± S.E. from 4-6 experiments. *p < 0.05 compared to the left ventricular in the sham group (Cont).

Alteration of mRNA level of MHC and Na^+-Ca^{2+} exchanger

In viable left ventricular tissue, the mRNA levels of β-MHC and Na^+-Ca^{2+} exchanger were significantly increased (Fig. 4 A and C). In the scar tissue, the α-MHC mRNA was significantly decreased while β-MHC mRNA level was increased about 180% of control value (Fig. 4A and B).

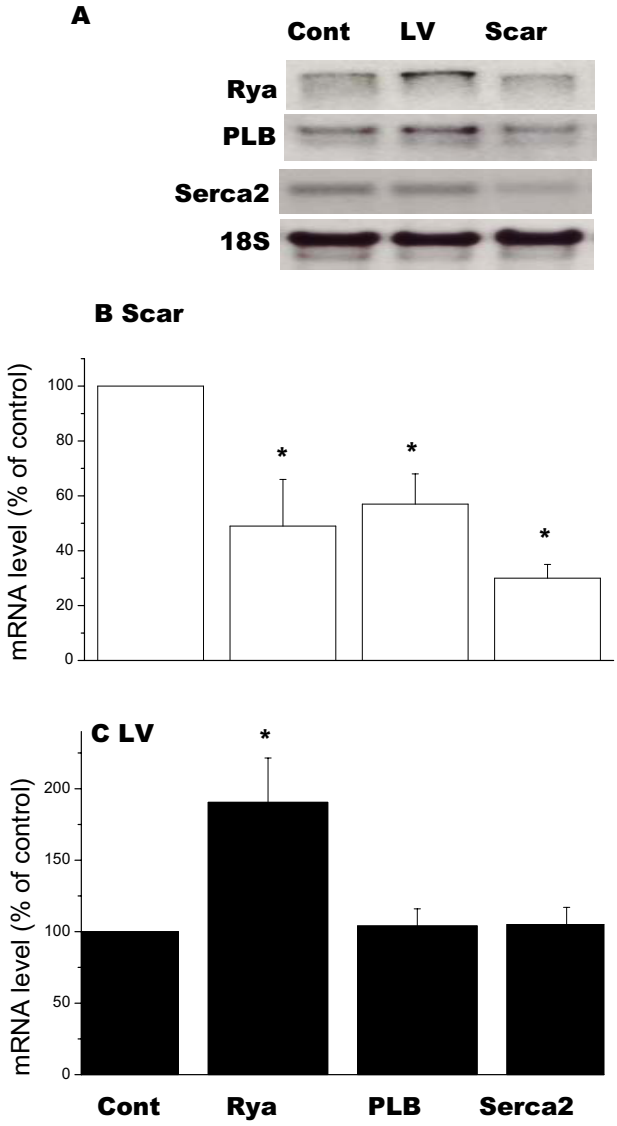

Fig. 2. Gene expression ryanodine receptor (Rya), phospholamban (PLB) and Ca^{2+} ATPase (Serca2) in infarcted scar (B) and left ventricular viable tissues (C) by Northern blot analysis. (A) is a representative blot. The relative abundance of each gene was expressed as the ratio of densitometric value of each mRNA band/18S RNA. The results are mean ± S.E. from 4-6 experiments. *p < 0.05 compared to the left ventricular in the sham group (Cont).

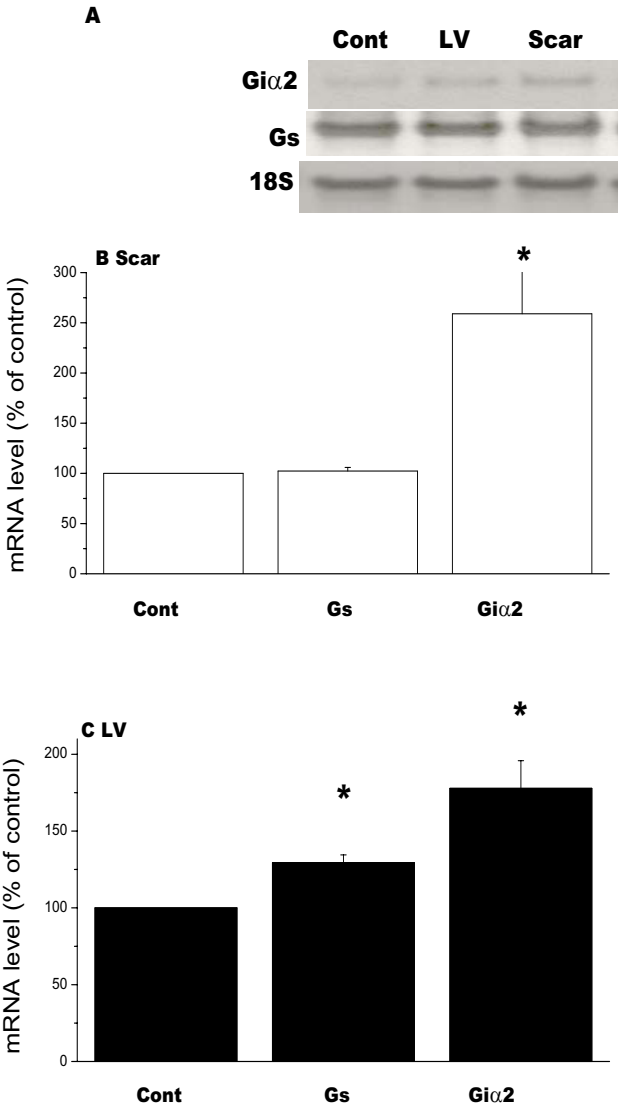

Fig. 3. Gene expression of G-proteins (Gs and Giα2) in infarcted scar (B) and left ventricular viable tissues (C) by Northern blot analysis. (A) is a representative blot. The relative abundance of each gene was expressed as the ratio of densitometric value of each mRNA band/18S RNA. The results are mean ± S.E. from 4-6 experiments. *p < 0.05 compared to the left ventricular in the sham group (Cont).

Alteration of CK2 gene expression in viable left ventricular tissue and scar

As shown in Fig. 5 (A, B, and C), the mRNA level of CK2α is significantly higher in viable left ventricular tissue (145 ± 15%) and significantly lower in scar tissue (66 ± 13%) in comparison to sham group (100%). However, the mRNA levels of β subunits are significantly elevated in both viable left ventricule and scar tissue (140 ± 31% and 183 ± 30%, respectively).

Discussion

Fibrillar collagens types I and III are the major components of cardiac extracellular matrix [4], and

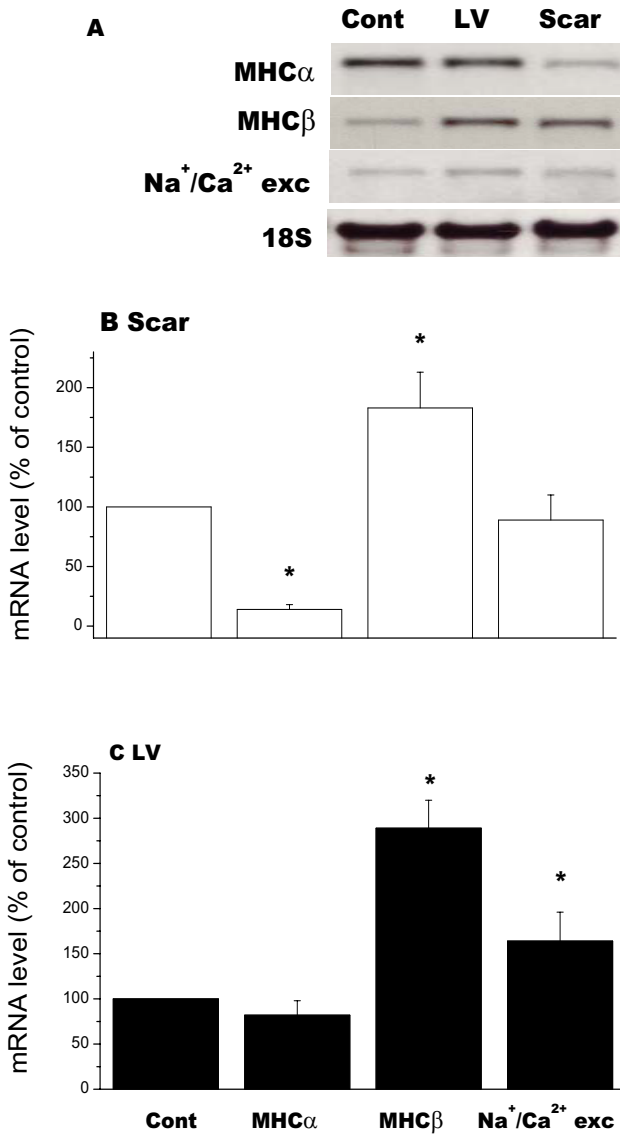

Fig. 4. Gene expression of myosin heavy chain (α-MHC and β-MHC) and Na^+-Ca^{2+} exchanger in infarcted scar (B) and left ventricular viable tissues (C) by Northern blot analysis. (A) is a representative blot. The relative abundance of each gene was expressed as the ratio of densitometric value of each mRNA band/18S RNA. The results are mean ± S.E. from 4-6 experiments. *p < 0.05 compared to the left ventricular in the sham group (Cont).

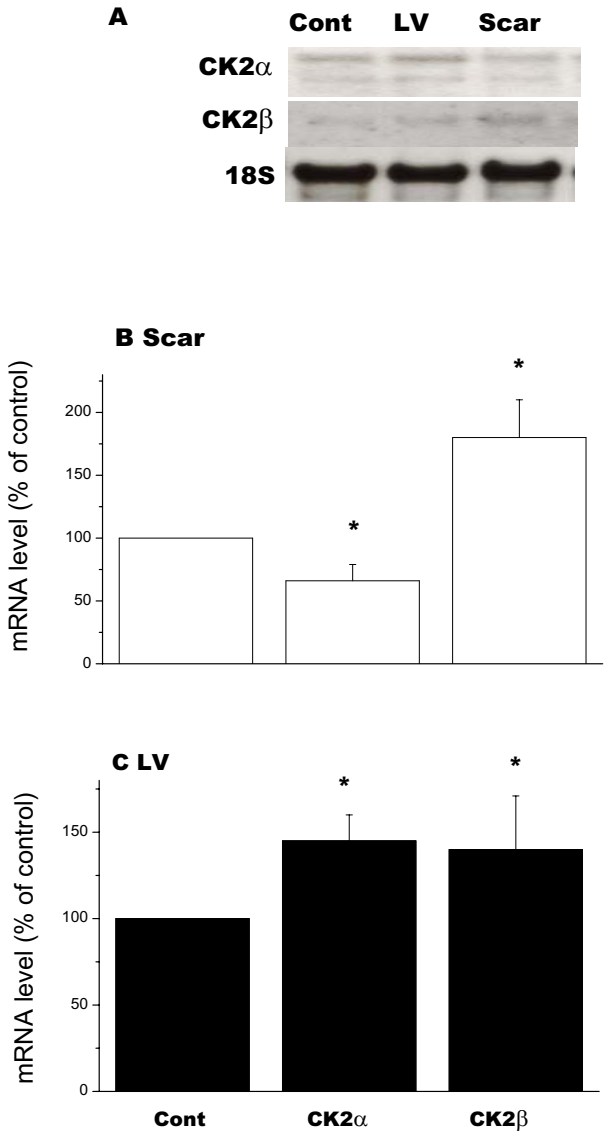

Fig. 5. Gene expression of casein kinase 2 (CK2) isoforms in infarcted scar (B) and left ventricular viable tissues (C) by Northern blot analysis. (A) is a representative blot. The relative abundance of each gene was expressed as the ratio of densitometric value of each mRNA band/18S RNA. The results are mean ± S.E. from 4-6 experiments. *p < 0.05 compared to the left ventricular in the sham group (Cont).

increased deposition of these matrix components in the heart eventually leads to increased myocardial stiffness and diastolic dysfunction, which is a major mechanism of heart failure after myocardial infarction. As fibrillar collagens are synthesized and secreted by cardiac fibroblasts and myofibroblasts, stimulation of myofibroblast proliferation in the heart is a phenomenon which is implicit in cardiac remodeling and failure. The current study provides information to support differential expression of key genes in the remnant myocardium and infarct scar in post-MI rat heart. To our knowledge, our observation that CK2 gene is overexpressed in the viable left ventricle (remnant left ventricular tissue) and infarct scar is the first of its kind. CK2, and especially the CK2α subunit, plays an important role in cell growth and proliferation [14] possibly *via* phosphorylation of histone deacetylase 2 (HDAC2) [15]

and subsequent alteration of HDAC2 activity. Despite the controversy that surrounds the issue of CK2's effects on cell proliferation [16], the current results support the suggestion that CK2 may participate in the pathogenesis of cardiac hypertrophy. Trophic ligands that may, in turn, activate CK2 are involved in the pathogenesis of cardiac hypertrophy and failure after infarction. For example, Yamagishi *et al.* [17] observed that angiotensin II (angiotensin) concentration was increased in the scar tissue and Sun and Weber [18] demonstrated that angiotensin receptor expression is elevated in both viable left ventricle and scar tissue. Furthermore, Fraccarollo *et al.* [19] reported that endothelin-1 stimulates collagen accumulation at the site of infarction and ET-1 type A receptor antagonists have been shown to improve ventricular remodeling and survival in rats after infarction [20]. It will be interesting to test the effect of ET-1 on CK2 activity in normal heart as well as failing heart since CK2 proteins and activity in rat heart were reduced during development [21]. Thus CK2 may play an important role in the growth of cardiomyocytes and fibroblasts.

Previously, Peterson *et al.* [4] reported that Gs- and Giα-protein expression were highly expressed (relative to noninfarcted controls) in both viable left ventricle and scar tissue in rat heart failure model. Our results regarding G-protein subtype mRNA expression are in agreement with these observations, and provide further support for their involvement in cardiac remodeling after infarction. Reports of fluctuations in SR gene expression in the failing heart in the literature are controversial. Lai *et al* [22] demonstrated that Rya and SERCA2a mRNAs were downregulated while PLB was not significantly altered in the failing dog heart. Furthermore in failing rabbit heart, the expression of these genes was downregulated [23]. However, Heerdt *et al* [24] found that these three genes were overexpressed in human heart failure. In the current work, we first assayed remnant myocardium from animals in heart failure and found that Rya was upregulated while there was no significant change in the expression of SERCA2 and PLB gene in viable left ventricular tissue in the failing post-MI rat heart. The inconsistency of these observations may be due to the difference of species and the stage of heart failure or may be due to the influences of concomitant application of pharmacologic therapeutic regimens in patient populations. In the infarct scar samples, we found that all SR genes studied are decreased vs normal myocardium in comparison to normal heart tissue. To the best of our knowledge, this is the first report showing that the gene expression of Rya, SERC2A and PLB is sourced from myofibroblasts of the infarct scar. Although cardiac myofibroblasts are contractile in nature, it would appear that the mechanism of Ca^{2+} delivery to these cells differs significantly from that of cardiac myocytes. This view is based on the current survey and the significant differences in classical SR gene expression patterns between what is myocyte- and myofibroblast-dominated tissue types, respectively. This is not surprising given the unique modes of Ca^{2+} entry present in various phenotypic variants of smooth muscle cell types.

The isoform shift from α-MHC to β-MHC expression in cardiac myocytes is now considered to be a classical molecular marker of the onset of heart failure [3]. Huang *et al*. [25] reported that the level of α-MHC mRNA was not different between sham-operated and hypertrophied rat heart and significantly decreased in the failing heart, but that β-MHC expression was significantly increased in both hypertrophied and failing heart. Lowes *et al.* [3] observed that α-MHC was downregulated while β-MHC was upregulated in human heart failure. The present study revealed that α-MHC was slightly decreased while β-MHC mRNA was significantly elevated in viable left ventricular myocardium of failing hearts. In the infarct scar, α-MHC mRNA expression was dramatically reduced to ~20% of control while β-MHC expression was doubled. In this regard, Sjuve *et al.* [26] reported that β-MHC was not detectable in non-muscle cells of normal bladders but is overexpressed in non-muscle cells from hypertrophied tissue, supporting the concept that expression of β-MHC is inducible in myofibroblasts under pathological conditions. Further, a recent review of the literature by Gabbiani [7] underscores the variable expression of smooth muscle (SM) markers by myofibroblasts, including desmin and SM myosin heavy chains. In this regard, SMemb (the embryonic form of SM myosin) has been demonstrated to be highly expressed in cardiac myofibroblasts in the infarct scar [8]. The suggestion that β-MHC is expressed by cardiac myofibroblasts is interesting, however, other possibilities must be considered to account for the relative expression of this gene in the infarct scar, including i) the β-MHC probe may cross-react with SMemb mRNAs, and ii) the putative

de novo appearance of newly differentiated myocytes from stem cells in adjacent hypertrophied myocardium [27]. It is also pointed out that we did not assess β-MHC protein expression in the infarct scar, and that this gene may be subject to asynchronous mRNA/protein expression patterns.

Na^+-K^+ ATPase plays an important role in the maintenance of homeostasis of intracellular Na^+ concentration. The depression of the activity of Na^+-K^+ ATPase will cause the increase of intracellular Na^+ concentration, cell edema and Ca^{2+} overload through Na^+-Ca^{2+} exchanger. Semb *et al.* [28] reported the activity of Na^+-K^+ pump was significantly attenuated in the failing heart. In addition, α-2 isoform was replaced by α-3 isoform in the failing heart. Trouve *et al.* [29] suggested that the changes in the levels of α-1 and α-3 isoforms appear physiologically irrelevant in compensated left ventricular hypertrophy (CLVH) and decompensated left ventricular hypertrophy (DLVH). However, it is known that the mRNA level of α-2 isoform is upregulated in CLVH and downregulated in DLVH. In our hands, α-2 is slightly decreased while α-3 is significantly upregulated in viable tissues; these results are consistent with previous reports. In the infarct scar tissue, both α-1 and α-2 isoforms were reduced compared to normal ventricular tissues. These results may suggest that the Na^+-K^+ pump expression and possibly its activity may be relatively low in cardiac myofibroblasts vs cardiomyocytes. Na^+-Ca^{2+} exchanger is important to maintain Ca^{2+} homeostasis in the heart. It has been reported that the expression of this gene is elevated in the end stage of heart failure [30, 31]. Our data from 16 weeks heart failure model are consistent with these previous observations.

Based on our data and the previous observations of others [4], manipulation of CK2 gene expression may be a useful and novel approach for treatment of post-MI heart failure. We have also described the expression of several genes in tissues dominated by either hypertrophied myocytes or cardiac myofibroblasts, and have observed markedly different patterns of gene expression among these tissues in post-MI hearts. Taken together, the results in this study regarding the differences in profiles of cardiac gene in the infarct scar and viable myocardium in heart failure due to MI support the view that these genes are regulated differentially. Thus extensive studies are needed to examine the cardiac gene profiles at different stages of heart failure.

Acknowledgements

This work was supported by the Canadian Institutes for Health Research (CIHR) Group grant in Experimental Cardiology and a grant from the CIHR Institute of Circulatory and Respiratory Health on Gene Environment Interaction in Heart Failure. NSD holds the CIHR/Pharmaceutical Development and Research Chair in Cardiovascular Research support by Merck Frosst Canada. IMCD is a scholar of the Myles Robinson Memorial Heart Fund.

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