Role of intracellular Ca^{2+} overload in inducing changes in cardiac gene expression

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OBJECTIVES: Although intracellular Ca^{2+} overload is believed to cause cardiac abnormalities and subcellular remodelling, its role in inducing alterations in cardiac gene expression has not been investigated.
METHODS: Intracellular Ca^{2+} overload was induced in isolated rat hearts on perfusion with Ca^{2+}-free medium for 5 min followed by reperfusion with medium containing different concentrations of Ca^{2+} for 30 min (Ca^{2+}paradox). Changes in messenger RNA levels for various subcellular proteins were monitored either by Northern blotting or real-time polymerase chain reaction techniques.
RESULTS: Marked depressions in gene expression for sarcolemma Na^+-K^+-ATPase and Na^+-Ca^{2+} exchanger, sarcoplasmic reticulum Ca^{2+}-pump ATPase, Ca^{2+} release channel and phospholamban, as well as myofibrillar α- and β-myosin heavy chain proteins were observed in hearts reperfused with 1.25 mM Ca^{2+} following perfusion with Ca^{2+}-free medium. In contrast, messenger RNA levels for calpain-1 and -2 proteins were elevated in hearts subjected to Ca^{2+}paradox. These changes were dependent on the concentration of Ca^{2+} in the reperfusion medium.
CONCLUSIONS: The results suggest that intracellular Ca^{2+} overload is an important factor in the induction of defects in gene expression, subcellular remodelling and cardiac dysfunction in heart disease.

Key Words: *Cardiac gene expression; Ca^{2+}-pump ATPase; Ca^{2+}-release channel; Intracellular Ca^{2+} overload; Myosin heavy chains; Na^+-K^+-ATPase; Na^+-Ca^{2+} exchanger*

Normal expression of cardiac genes for Ca^{2+}transport and contractile proteins is known to play a critical role in the maintenance of the function of subcellular organelles such as sarcolemma (SL), sarcoplasmic reticulum (SR) and myofibrils (MFs) in cardiomyocytes. Previous studies have revealed varying degrees of depression in gene expression for SL, SR and MF proteins, as well as defects in subcellular organelles during the development of heart failure in patients and experimental animal models (1-6). Accordingly, it has been suggested that cardiac dysfunction in failing hearts is due to subcellular remodelling as a consequence of changes in cardiac gene expression (2,7-9). Although the occurrence of intracellular Ca^{2+} overload is believed to be intimately involved in the genesis of cardiac dysfunction in heart failure (10-15), its role in inducing defects in cardiac gene expression is not fully understood. It was, therefore, the purpose of the present study to investigate whether alterations in gene expression of SL, SR and MF proteins occur during the development of intracellular Ca^{2+} overload in the myocardium.

Isolated heart reperfused with high concentrations of Ca^{2+} following a brief period of perfusion with Ca^{2+}-free medium has been demonstrated to be an excellent model (Ca^{2+}paradox [CP]) for investigating the effects of intracellular Ca^{2+}overload (16-26). We have reported that the inability of CP hearts to recover contractile function was associated with a marked increase in intracellular Ca^{2+}, as well as the development of cardiac contracture, ultrastructural damage and subcellular defects (11,13,15,16,21,23). In the present study, we examined whether hearts perfused with Ca^{2+}-free medium followed by reperfusion with medium containing different concentrations of Ca^{2+} exhibit alterations in gene expression for SL Na^+-K^+ ATPase and Na^+-Ca^{2+} exchanger, SR Ca^{2+}-pump ATPase, Ca^{2+}-release channel and phospholamban (PLB), as well as MF α- and β-myosin heavy chain proteins. Because subcellular remodelling in the failing heart is dependent on the balance between changes in gene expression and activities of proteolytic enzymes, such as calpain (27-33), messenger RNA (mRNA) levels for both calpain-1 and calpain-2 were measured in CP hearts. It may be noted that subcellular remodelling in failing hearts has also been suggested to be the result of activation of different proteolytic enzymes (27,28).

METHODS

Male Sprague Dawley rats, each weighing 250 g to 300 g, were used in the present study. All experiments were conducted according to the protocol approved by the Animal Care Committee of the University of Manitoba (Winnipeg, Manitoba) as per guidelines established by the Canadian Council on Animal Care. Isolated hearts were perfused according to the Langendorff technique with Krebs-Henselite medium (gassed with 95% O_2 and 5% CO_2) containing 1.25 mM Ca^{2+} at 37°C for 20 min. The left ventricular developed pressure and the left ventricular end diastolic pressure (LVEDP) were recorded using a microtip catheter (Millar Instruments Inc, USA) and AcqKnowledge software (Biopac Systems, USA). After a stabilizing period, the hearts were perfused with Ca^{2+}-free medium for 5 min and then reperfused with medium containing different concentrations of Ca^{2+} for 30 min. Control hearts were perfused for 35 min with normal medium containing 1.25 mM Ca^{2+}. Methods for the perfusion of heart, recording of contractile parameters and induction of CP were similar to those used previously (22-26).

At the end of perfusion-reperfusion protocol, total RNA was extracted from the control and experimental left ventricular tissue using the guanidinium thiocyanate method (34). mRNA levels for different subunits (α_1, α_2 and β-isoforms) of SL Na^+-K^+ ATPase and Na^+-Ca^{2+}-exchanger, SR SERCA2a (Ca^{2+}-pump ATPase), ryanodine receptor (Ca^{2+}-release channel) and PLB, as well as MF α-myosin heavy chain and β-myosin heavy chain proteins were measured using previously described Northern blotting techniques (33,35,36). In one set of experiments, gene expression for calpain-1 and calpain-2 proteins was determined using real-time polymerase chain reaction techniques described previously (37). Values are presented as mean ± SE and were

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TABLE 1
Effect of 30 min reperfusion with medium containing different concentrations of Ca^{2+} on the left ventricular end diastolic pressure (LVEDP) in isolated rat hearts following perfusion with Ca^{2+}-free medium for 5 min

Concentration of Ca^{2+} in reperfusion medium	Perfusion in Ca^{2+}-free medium for 5 min	Reperfusion with different Ca^{2+} concentrations for 30 min
	Increase in LVEDP, mmHg	
1.25 mM (CP)	25.2±2.6	78.5±5.1*
0 μM	28.5±2.2	29.3±2.0
30 μM	31.1±3.8	32.2±1.9
100 μM	25.3±2.3	60.7±4.3*
300 μM	29.1±2.1	85.3±6.5*

*LVEDP in hearts before initiating Ca^{2+}-free perfusion varied between 6 mmHg and 8 mmHg. Controls were perfused for 35 min without subjecting to Ca^{2+}-free medium whereas Ca^{2+}-paradox (CP) hearts were subjected to 5 min of Ca^{2+}-free medium followed by 30 min of reperfusion with normal medium containing 1.25 mM Ca^{2+}. Data presented as mean ± SE of 5 to 7 experiments. *Statistically significant (P<0.05)*

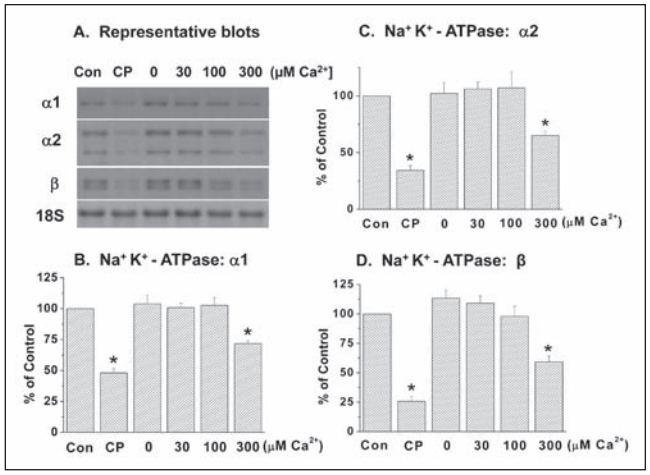

Figure 1) *Effects of 30 min reperfusion with medium containing different concentrations of Ca^{2+} on messenger RNA levels for different Na^+-K^+ ATPase subunits in isolated rat heart following perfusion with Ca^{2+}-free medium for 5 min. The experimental conditions for control (Con) and Ca^{2+}-paradox (CP) hearts are same as described in the legend for Table 1. Data presented as mean ± SE from five to seven experiments. *Significantly different (P<0.05) from control*

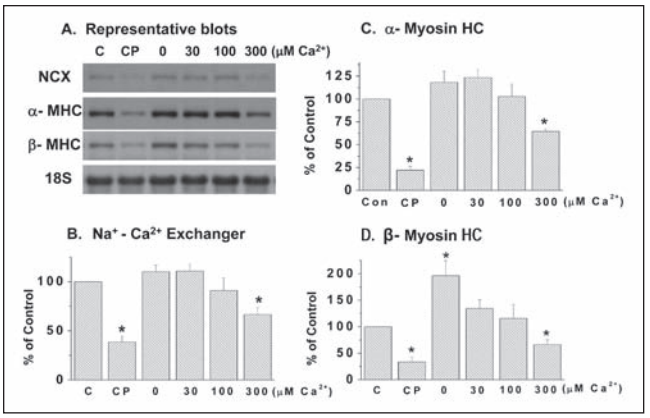

Figure 2) *Effects of 30 min reperfusion with a medium containing different concentrations of Ca^{2+} on messenger RNA levels for Na^+-Ca^{2+} exchanger as well as α-myosin heavy chain (HC) and β-myosin HC proteins in isolated rat heart following perfusion with Ca^{2+}-free medium for 5 min. The experimental conditions for control (C) and Ca^{2+}-paradox (CP) hearts are same as described in the legend for Table 1. Data presented as mean ± SE from five to seven experiments. *Significantly different (P<0.05) from control*

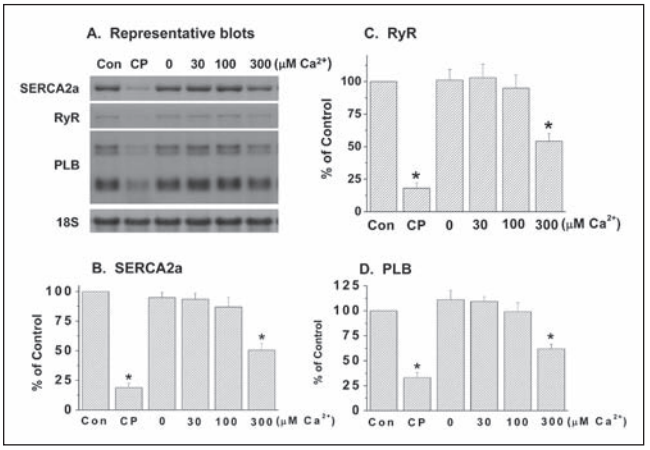

Figure 3) *Effects of 30 min reperfusion with a medium containing different concentrations of Ca^{2+} on messenger RNA levels for sarcoplasmic reticulum (SR) proteins in isolated rat heart following perfusion with Ca^{2+}-free medium for 5 min. The experimental conditions for control (Con) and Ca^{2+}-paradox (CP) hearts are same as described in legends for Table 1. Data presented as mean ± SE from five to seven experiments. *Significantly different (P<0.05) from control. SERCA2a SR Ca^{2+}-pump ATPase; RyR Ryanodine receptor (SR Ca^{2+}-release channel); PLB Phospholamban*

statistically evaluated using one-way ANOVA; differences between the control and experimental groups were considered to be statistically significant at P<0.05.

RESULTS

Perfusion of hearts with Ca^{2+}-free medium for 5 min resulted in an immediate loss of their ability to generate left ventricular developed pressure; no recovery of this function was observed on reperfusing these hearts with medium containing different concentrations of Ca^{2+} for a period of 30 min. In contrast, the LVEDP was increased three- to fourfold in hearts perfused with Ca^{2+}-free medium; this elevated LVEDP was markedly augmented (eight- to 10-fold) on reperfusion with medium containing 100 μM to 1.25 mM (CP) Ca^{2+} (Table 1). No significant increase in the LVEDP was apparent when the 5 min Ca^{2+}-free perfused hearts were reperfused for 30 min with either Ca^{2+}-free medium or medium containing 30 μM Ca^{2+} (Table 1). Such augmentation of the LVEDP on reperfusion with medium containing 100 μM to 1.25 mM Ca^{2+} has been demonstrated to be due to the development of intracellular Ca^{2+} overload in hearts perfused with Ca^{2+}-free medium (16,19,21).

Reperfusion of the 5 min Ca^{2+}-free perfused hearts with a medium containing 300 μM and 1.25 mM Ca^{2+} (CP) for 30 min produced varying degrees of depression in mRNA levels for different subunits (α_1, α_2 and β isoforms) of SL Na^+-K^+ ATPase (Figure 1). No changes in mRNA levels for different Na^+-K^+ ATPase isoforms were apparent when the Ca^{2+}-free perfused hearts were reperfused with medium containing no Ca^{2+} or 30 μM and 100 μM Ca^{2+} (Figure 1). It is evident from Figure 2 that mRNA levels for SL Na^+-Ca^{2+} exchanger were also depressed significantly on reperfusing the Ca^{2+}-free perfused hearts with medium containing 300 μM and 1.25 mM Ca^{2+}, unlike that in hearts reperfused with no Ca^{2+} or 30 μM and 100 μM Ca^{2+}.

The data in Figure 2 show that reperfusing the 5 min Ca^{2+}-free perfused hearts with medium containing 300 μM or 1.25 mM (CP) Ca^{2+} depressed mRNA levels for both α- and β-myosin heavy chain in

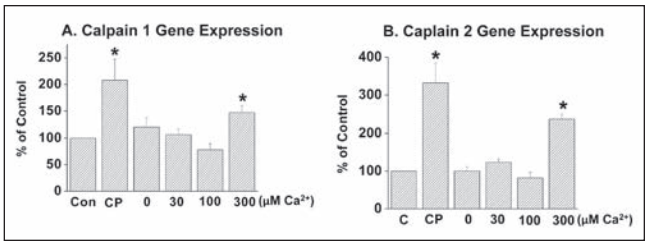

Figure 4) *Effects of 30 min reperfusion with a medium containing different concentrations of Ca^{2+} on messenger RNA levels for calpain-1 and calpain-2 proteins in isolated rat heart following perfusion with Ca^{2+}-free medium for 5 min. The experimental conditions for control (Con) and Ca^{2+}-paradox (CP) hearts are the same as described in the legend for Table 1. Gene expression was determined using real-time polymerase chain reaction techniques. Data presented as mean ± SE from five to seven experiments. *Significantly different (P<0.05) from control*

MF proteins significantly. While mRNA levels for β-myosin heavy chain, unlike that for α-myosin heavy chain, was increased in hearts reperfused with Ca^{2+}-free medium for 30 min, mRNA levels for both α- and β-myosin heavy chains were not altered significantly on reperfusion with medium containing 30 μM or 100 μM Ca^{2+} (Figure 2).

Marked depressions in mRNA levels for SR Ca^{2+}-pump ATPase (SERCA2a), Ca^{2+}-release channel (ryanodine receptor) and PLB proteins were observed in the 5 min Ca^{2+}-free perfused hearts on reperfusion with medium containing 300 μM and 1.25 mM (CP) Ca^{2+} (Figure 3). In contrast, no significant alterations were observed in mRNA levels for the Ca^{2+}-pump ATPase, Ca^{2+}-release channel and PLB proteins on reperfusing the 5 min Ca^{2+}-free perfused hearts with medium containing no Ca^{2+} or 30 μM and 100 μM Ca^{2+} (Figure 3). In another set of experiments, mRNA levels for calpain-1 and -2 proteins were determined in CP hearts. From the results in Figure 4, it can be observed that mRNA level for both calpain-1 and calpain-2 proteins were markedly elevated in the 5 min Ca^{2+}-perfused hearts on reperfusion with medium containing 300 μM or 1.25 mM (CP) Ca^{2+} for 30 min. In contrast, no significant changes in mRNA levels for both calpain-1 and -2 proteins were observed on reperfusion with medium containing no Ca^{2+}, or 30 μM and 100 μM Ca^{2+} (Figure 4).

DISCUSSION

We have shown a marked increase in LVEDP and dramatic depressions in mRNA levels for SL Na^+-K^+ ATPase (different isoforms) and Na^+-Ca^{2+} exchanger, MF α- and β-isoforms of myosin heavy chain, SR Ca^{2+}-pump ATPase, Ca^{2+}-release channel and PLB, as well as calpain-1 and -2 proteins in hearts on reperfusion with 1.25 mM Ca^{2+} for 30 min following perfusion with Ca^{2+}-free medium for 5 min. Because the magnitude of changes in cardiac LVEDP and gene expression were found to be dependent on the concentration of Ca^{2+} in the reperfusion medium, it is likely that the observed alterations in cardiac gene expression in hearts subjected to CP are due to the development of intracellular Ca^{2+} overload. This view is supported by the fact that a marked cardiac contracture was demonstrated to be intimately associated with a marked increase in the intracellular Ca^{2+} content in the CP heart (16). Varying degrees of alterations in functional and biochemical activities of SL, SR and MF activities have also been shown to occur in CP hearts as a consequence of intracellular Ca^{2+} overload (13-15,17,21-23). Although elevated levels of intracellular Ca^{2+} have been observed to activate proteases, such as calpains, directly the observed increase in mRNA levels for both calpain-1 and -2 proteins may also contribute to increased calpain activity in hearts under different pathological conditions associated with the occurrence of intracellular Ca^{2+} overload (27-31). Thus, it appears that the observed depressions in SL, SR and MF gene expression, as well as the increased gene expression for calpains due to intracellular Ca^{2+} overload, may account for remodelling of subcellular organelles observed in the hearts subjected to CP.

Although perfusing the hearts for 35 min with no Ca^{2+} was observed to significantly increase LVEDP, no depression in cardiac gene expression for different subcellular proteins was observed under this experimental condition. In fact, mRNA levels for β-myosin heavy chain were increased on perfusing the heart with no Ca^{2+} for 35 min. It appears that a critical level of LVEDP needs to be achieved for the occurrence of intracellular Ca^{2+} overload for inducing depression in cardiac gene expression in hearts subjected to CP. It should be emphasized that there are varying degrees of depression in SL, SR and MF gene expression, protein content and functional activities in hearts subjected to ischemia-reperfusion and these alterations have been attributed to the occurrence of intracellular Ca^{2+} overload (7-9,31-36,38). Furthermore, intracellular Ca^{2+} overload has been suggested to play a critical role in the development of alterations in SL, SR and MF gene expression, protein content and functional activities in hearts failing due to myocardial infarction (1-6). Although the exact mechanisms by which intracellular Ca^{2+} overload induces changes in cardiac gene expression and subsequent subcellular remodelling are not clear at present, a marked increase in the production of different cytokines including tumour necrosis factor and the activation of nuclear factor-kB have been observed in the CP heart (24,39). Furthermore, the role of various microRNAs and other transcription factors, which are known to regulate cardiac gene expression (40), needs to be investigated in hearts on inducing intracellular Ca^{2+} overload. Nonetheless, the experiments performed in the present study provide compelling evidence that intracellular Ca^{2+} overload is a potential mediator of inducing defects in gene expression and subsequent subcellular remodelling and cardiac dysfunction.

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