Preconditioning prevents alterations in cardiac SR gene expression due to ischemia-reperfusion

RANA M. TEMSAH, KENICHI KAWABATA, DONALD CHAPMAN, AND NARANJAN S. DHALLA

Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre and Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada R2H 2A6

Received 28 May 2001; accepted in final form 29 November 2001

Temsah, Rana M., Kenichi Kawabata, Donald Chapman, and Naranjan S. Dhalla. Preconditioning prevents alterations in cardiac SR gene expression due to ischemia-reperfusion. Am J Physiol Heart Circ Physiol 282: H1461–H1466, 2002.—We have previously shown that ischemic preconditioning (IP) improves cardiac performance and sarcoplasmic reticulum (SR) function in hearts subjected to ischemia-reperfusion (I/R). In this study, we examined the effect of IP on I/R-induced changes in gene expression for SR proteins such as the Ca$^{2+}$ release channel, Ca$^{2+}$-pump ATPase, phospholamban, and calsequestrin in the isolated rat heart. Normal isolated rat hearts exposed to three brief cycles of IP (5-min ischemia and 5-min reperfusion) exhibited a significant decrease in the transcript levels of SR genes. Nonpreconditioned I/R hearts when subjected to 30-min ischemia and 30-min reperfusion showed a marked decrease in mRNA levels for the SR proteins compared with normal hearts; this decrease was attenuated by preconditioning. Although hearts subjected to Ca$^{2+}$-paradox (CP) have been shown to exhibit intracellular Ca$^{2+}$ overload and SR dysfunction like those in I/R hearts, virtually nothing is known regarding the effect of CP on cardiac SR gene expression. Accordingly, CP (5-min Ca$^{2+}$-free perfusion and 30-min reperfusion with normal medium) was observed to produce dramatic changes in SR gene expression, and the heart failed to contract; these alterations were attenuated by IP. Our results show that 1) both I/R and CP depress SR gene expression in the normal heart, 2) IP attenuates I/R- and CP-induced depression in cardiac function and SR gene expression, and 3) intracellular Ca$^{2+}$ overload may play a role in depressing SR gene expression in both I/R and CP hearts.

METHODS

Heart perfusion and experimental protocol. Male Sprague-Dawley rats weighing 250–300 g were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg). The hearts were rapidly excised, cannulated to the Langendorff apparatus, and perfused with Krebs-Henseleit solution gassed with a mixture of 95% O$_2$ and 5% CO$_2$; pH 7.4. The perfusion medium contained (in mmol/l) 120 NaCl, 25 NaHCO$_3$, 11 glucose, 4.7 KCl, 1.2 KH$_2$PO$_4$, 1.2 MgSO$_4$, and 1.25 CaCl$_2$. The hearts were electrically stimulated at a rate of 300 beats/min (Phipps and Bird; Richmond, VA), and the perfusion rate was maintained at 10 ml/min. A water-filled latex balloon was inserted in the left ventricle and connected to a pressure transducer for the left ventricular systolic and diastolic pressure measurements. The left ventricular developed pressure (LVEDP) and the left ventricular end-diastolic pressure (LVEDP) were measured using the Acknowledge 3.5.3 software for Windows (Biopac Systems; Goleta, CA). LVDP was the difference between left ventricular systolic pressure and LVEDP.

Acknowledgments: This work was supported by the Heart and Stroke Foundation of Canada (to N.S. Dhalla) and the Canadian Medical Research Council (to N.S. Dhalla, R.M. Temsah, K.K. Kawabata, and D. Chapman). We thank Dr. N. Murry for the use of his laboratory for this study, Dr. N. Murry for the use of his laboratory for this study, and Dr. N. Murry for the use of his laboratory for this study. We also thank Dr. N. Murry for the use of his laboratory for this study. We also thank Dr. N. Murry for the use of his laboratory for this study.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: N. S. Dhalla, Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, 351 Taché Ave., Winnipeg, Manitoba, Canada R2H 2A6 (E-mail: cvs0@sbrc.ca).

http://www.ajpheart.org 0363-6135/02 $5.00 Copyright © 2002 the American Physiological Society
pressure and LVEDP. All hearts were stabilized for a period of 30 min before use and were randomly distributed into the different experimental groups (Fig. 1). In one series of experiments, control hearts were perfused for 30 or 60 min after stabilization, and because no significant differences were observed in the level of SR gene expression, the control values were grouped together. The I/R hearts were made globally ischemic by stopping the coronary flow for 30 min, after which the flow was restored for 30 min. For the preconditioning group, one set of hearts was collected after three cycles of 5-min ischemia and 5-min reperfusion (IP), and the second set was collected by subjecting IP hearts to the I/R protocol (IP+I/R). Another series of experiments was conducted to test the effects of IP on Ca2+-induced changes in SR gene expression. For this purpose, control hearts were perfused for 53 min after stabilization, whereas the CP hearts were perfused with normal medium for 18 min followed by 5 min of Ca2+-free perfusion and 30 min of perfusion with normal medium containing 1.25 mM Ca2+. For the preconditioning group, one set of hearts was collected after three cycles of 3-min ischemia and 3-min reperfusion, and the other set was collected after the CP protocol (IP+CP). The perfusion procedures (Fig. 1) were similar to those in our previous reports (9, 16). The experimental protocol in the present investigation was approved by the Animal Care Committee of the University of Manitoba and conforming with the Canadian Council on Animal Care concerning the “Care and Use of Experimental Animals” (Vol. 1, 2nd Ed., 1993).

RNA isolation and Northern blot analysis. Total RNA was extracted from the ventricular tissue by the guanidinium thiocyanate method described previously (25). Samples normalized to 5, 10, 20, 30, and 40 μg total RNA were denatured with formaldehyde and electrophoresed in a 1.2% agarose-formaldehyde gel. The linearity of the procedure used for the quantification of RyR, SERCA2a, PLB, and CQS is shown in Fig. 2, A–D. Twenty micrograms total RNA were used in the rest of the study because it is in the linear range. The Random Primer DNA Labeling System and T4 Polynucleotide Kinase kits (GIBCO-BRL Life Technologies; Gaithersburg, MD) were used to label the cDNAs and oligonucleotides, respectively. Inserts were separated from recombinant plasmids and used as probes (25). 18S mRNA was used as an internal standard.

Statistical analysis. Results are expressed as means ± SE. Statistical analyses were performed using ANOVA followed by Student’s unpaired t-test for multiple comparisons. A linear regression test was used for the linearity study. Values of P < 0.05 were considered to be statistically significant.

Fig. 1. Experimental protocols employed in this study. Hearts perfused by the Langendorff technique were stabilized for 30 min. A: perfusion protocol for investigating the effect of ischemic preconditioning (IP) on ischemia-reperfusion (I/R)-induced changes in the heart. C, control. B: perfusion protocol for studying the effect of IP on Ca2+ paradox (CP)-induced changes in the heart. Arrows indicate the time points at which the experiments were terminated.
RESULTS

Table 1 shows that I/R and CP depressed LVDP by 75% and 94% and increased LVEDP by 13- and 8.6-fold in nonpreconditioned hearts, respectively. IP improved LVDP by 71.5% in the I/R hearts and by 26.8% in the CP hearts, whereas LVEDP was higher than the control level by 4.6-fold in the I/R hearts and 5-fold in the CP hearts. At the level of SR gene expression, three cycles of IP (5-min ischemia and 5-min reperfusion) decreased \((P < 0.05)\) the mRNA levels of RyR (by 33%), SERCA2a (by 21%), and PLB (by 23%) but had no effect on the mRNA levels of CQS (Fig. 3, A–E). Compared with the control group, I/R (30-min ischemia and 30-min reperfusion) decreased the mRNA levels for RyR, SERCA2a, PLB, and CQS by 60%, 34%, 50%, and 46% in the nonpreconditioned hearts, respectively. The alterations in SR gene expression were significantly protected by 70% for RyR, 84% for SERCA2a, 62% for PLB, and 71% for CQS when I/R hearts were preconditioned. Some 30-min ischemic hearts were reperfused for 60 min, but the results with respect to changes in SR gene expression were similar to those observed in ischemic hearts reperfused for 30 min. Results similar to these were obtained when the I/R hearts were preconditioned with three cycles of 3-min ischemia and 3-min reperfusion (data not shown, \(n = 2\)).

In the CP experiments, three cycles of IP (3-min ischemia and 3-min reperfusion) significantly decreased the expression of RyR (by 29%), SERCA2a (by 27%), and PLB (by 15%) but not that of CQS (Fig. 4, A–E). Hearts subjected to CP (5-min Ca\(^{2+}\)-free perfusion and 30-min reperfusion) showed a marked depression in mRNA levels of RyR (by 62%), SERCA2a (by 89%), PLB (by 59%), and CQS (by 76%) compared with control hearts. IP significantly protected the transcript levels of SR genes in the CP hearts by 61% for RyR, 28% for SERCA2a, 61% for PLB, and 41% for CQS. Results similar to these were obtained when the CP hearts were preconditioning with three cycles of 5-min ischemia and 3-min reperfusion (data not shown, \(n = 2\)).

DISCUSSION

Previous reports have indicated that I/R and CP cause a significant decrease in cardiac performance and SR function (9, 26); moreover, I/R hearts showed a marked decrease in SR and non-SR genes (22, 25, 26). This study not only confirms changes in SR gene ex-
pression in I/R hearts but also provides evidence for the depression of mRNA levels for SR proteins such as RyR, SERCA2a, PLB, and CQS in CP hearts. Several investigators have also reported alterations in SR gene expression in cardiac hypertrophy and different types of heart failure (for a review, see Ref. 3) as well as stunned myocardium (7, 12). Because intracellular Ca\textsuperscript{2+} overload is known to occur in I/R hearts (25, 26) and hypoxia-reoxygenated hearts (24), it is possible that intracellular Ca\textsuperscript{2+} overload may produce alterations at the level of SR gene expression in I/R hearts. This view is supported by our observation that CP, which is known to result in the occurrence of intracellular Ca\textsuperscript{2+} overload and defects in SR function in hearts (1, 2), was found to produce changes in SR gene expression similar to those seen in I/R hearts. In fact, the decrease in the mRNA levels of SERCA2a and CQS in the CP heart was greater than those in the I/R heart. These results suggest that intracellular Ca\textsuperscript{2+} overload may be one of the possible mechanisms that may mod-

Fig. 3. Effect of IP (3 cycles of 5-min ischemia/5-min reperfusion) on sarcoplasmic reticulum (SR) gene expression in the I/R heart. A: representative autoradiogram of mRNA levels of SR genes in control, IP, I/R, and IP+I/R hearts. Total RNA (20 \mu g) was extracted from the heart and assayed for mRNA with respective probes. The 18S mRNA band was used as an internal standard. B–E: Northern blot analyses of RyR (B), SERCA2a (C), PLB (D), and CQS (E) mRNA levels. Each value is the mean ± SE of 6 hearts/group. *P < 0.05 vs. control; †P < 0.05 vs. I/R.

Fig. 4. Effect of IP (3 cycles of 3-min ischemia/3-min reperfusion) on SR gene expression in the CP heart. A: representative autoradiogram of mRNA levels of SR genes in control, IP, CP, and IP+CP hearts. B–E: Northern blot analyses of RyR (B), SERCA2a (C), PLB (D), and CQS (E) mRNA levels. Each value is the mean ± SE of 5 hearts/group. *P < 0.05 vs. control; †P < 0.05 vs. CP.
ulate the SR gene expression in I/R hearts. Oxidative stress has also been shown to explain the I/R-induced changes in heart function, subcellular activities, and SR gene expression (5, 25, 26), and thus it is difficult to indicate whether the observed mRNA changes in I/R and CP hearts are due to oxidative stress and Ca\(^{2+}\) overload.

The results described in this study show that IP not only attenuated changes in SR gene expression in I/R hearts but also produced a similar effect on alterations in SR gene expression in the CP hearts. The I/R-induced alterations in cardiac performance, SR function, and mRNA levels for SR genes were also prevented by different treatments such as antioxidants (26) and \(\beta\)-adrenergic receptor blockers (25). Likewise, IP has been shown to be an effective intervention for attenuating I/R-induced changes in infarct size, arrhythmias, cardiac performance, and SR function (4, 8, 9, 15, 16, 19). Furthermore, IP has been documented to reduce the rise in intracellular acidosis as well as intracellular Na\(^+\) and Ca\(^{2+}\) concentrations due to I/R (21) in addition to inhibiting glycolysis and maintaining glucose oxidation (6). The results described in this study show that IP itself depressed the transcript levels for SR proteins such as RyR, SERCA2a, and PLB but not those of CQs. On the other hand, both I/R and CP depressed the gene expression of the SR proteins to varying extents; the changes observed due to IP were less severe than those observed due to I/R and CP. Moreover, CP induced a more drastic decrease in mRNA levels for SERCA2a and CQs compared with the changes induced by I/R. Although these transcripts belong to the SR Ca\(^{2+}\)-cycling proteins, it is clear that IP, I/R, and CP affect these genes disproportionately. These results indicate that, for yet unknown reasons, SR genes are differentially regulated under different conditions. More studies are needed to investigate the transcription factors regulating the expression of each gene under different diseased conditions. Studies conducted by Schaper and co-workers (7, 12) showed an increase in the transcript levels for SERCA2a, PLB, and CQs due to one cycle of 10-min coronary occlusion and 90-min reperfusion (7), whereas two cycles of 10-min coronary occlusion followed by 60-min reperfusion under in vivo conditions showed a tendency toward a decrease in the mRNA levels. In fact, transcriptional modulation has been implicated as a major player in alterations of SR gene expression in cardiac pathology (12) as well as during cardiac differentiation and myogenesis (13, 17). On the other hand, differential changes in the mRNA stability cannot be ruled out.

Although IP caused a significant decrease in mRNA levels of the SR genes, these short episodes of IP were able to precondion SR genes against the deleterious effect of I/R- or CP-induced injury. The protection provided by three cycles of IP (3- or 5-min duration) was not surprising because IP cycles longer than 1 min were shown as essential to induce protection of SR protein perfusion (29). Under the experimental conditions employed in this study, we (10, 16) previously reported that the recovery in cardiac performance and SR function observed in I/R and CP hearts due to IP were related to the preserved protein content of SR Ca\(^{2+}\)-cycling proteins. However, it is important to mention that the functional recovery of SR proteins may not be linked to the status of their gene expression (18) because the short duration of our experiment (30-min reperfusion) may not be sufficient to impart the expression of the corresponding proteins, and therefore the improvement in gene expression will not result in de novo protein synthesis in the heart. On the other hand, it is likely that IP may rescue the SR protein levels from degradation by Ca\(^{2+}\)-dependent proteases that are activated during I/R (27). In this regard, it should be noted that one cycle of IP was found sufficient to reduce the elevation in the intracellular Ca\(^{2+}\) concentration in subsequent ischemic episodes (20). Thus it is noteworthy that, although Ca\(^{2+}\) overload, a common feature between I/R and CP, would lead to cell death, our study reports a novel finding regarding the potential of preconditioning in preserving the molecular structure of Ca\(^{2+}\)-regulating organelles.

In conclusion, the major findings of this study were that 1) cardiac SR gene expression is depressed by IP, 2) intracellular Ca\(^{2+}\) overload may be a possible mechanism for inducing alterations in SR gene expression in the heart, and 3) IP was observed to attenuate changes in SR gene expression of the Ca\(^{2+}\)-cycling proteins in both I/R and CP hearts.

This study was supported by a grant from the Canadian Institutes of Health Research (CIHR Group in Experimental Cardiology).

N. S. Dhalla holds the CIHR/Pharmaceutical Research and Development Chair in Cardiovascular Research supported by Merck Frosst Canada Incorporated. R. M. Temsah was a predoctoral fellow of the Heart and Stroke Foundation of Canada.

**REFERENCES**

10. Kawabata KI, Netticadan T, Osaka M, Tamura K, and Dhalla NS. Mechanisms of ischemic preconditioning effects on...


