L-Arginine administration recovers sarcoplasmic reticulum function in ischemic reperfused hearts by preventing calpain activation

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Abstract

Objective: Earlier studies have shown that impaired cardiac contractility in ischemia reperfusion (IR) is associated with alterations in sarcoplasmic reticulum (SR) function. Impaired release of nitric oxide (NO) has been reported during IR, while administration of NO donors, such as L-arginine (LA), has been shown to improve cardiac performance in IR hearts. We therefore investigated the mechanisms underlying the recovery of contractile function in IR hearts treated with LA.

Methods: Isolated rat hearts subjected to 30 min of global ischemia were reperfused for 60 min. The effects of LA on cardiac performance, SR function and its regulation were examined.

Results: IR-induced impairment in cardiac performance was associated with a reduction in SR function and its regulation. IR caused an increase in calpain activity and a decrease in the sarcolemmal and SR nitric oxide synthase (NOS) isoform protein content as well as cytosolic NO levels. Administration of LA prevented contractile dysfunction in IR hearts, which was associated with a recovery of SR function and SR regulation by protein phosphorylation. This was consistent with a recovery in protein levels of major SR Ca2+-cycling and Ca2+-regulatory proteins. LA treatment attenuated an increase in calpain activity, possibly by nitrosylation of calpain, and increased cytosolic NO levels and SR NOS protein content in IR hearts.

Discussion: These results suggest that LA administration improved cardiac contractility by preventing alterations in SR Ca2+ handling and calpain activation in IR hearts.

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1. Introduction

Myocardial ischemia reperfusion (IR) has been associated with functional, biochemical, and ultrastructural damage [1–3]. Generation of reactive oxygen species (ROS) and cytosolic Ca2+ overload have emerged as two mutually non-exclusive pathogenic mechanisms underlying IR injury [1–3]. Both mechanisms impair Ca2+ homoeostasis and result in abnormalities in cardiac contractile function. The sarcoplasmic reticulum (SR) is a major regulator of intracellular Ca2+ concentrations and therefore a central player in determining cardiac contractility [4,5]. Our earlier studies have shown that impaired contractility in the IR hearts is intimately associated with alterations in SR function [6–8]. Accordingly any therapeutic interventions to improve SR function would possibly recover cardiac contractility in IR hearts. Previous studies [9,10] reported that release of nitric oxide (NO) was impaired during IR and administration of NO improved cardiac contractility [9–11]. In this regard, abnormalities in contractile function in IR hearts have been associated with endothelial dysfunction [12,13], which in turn has been linked to impairment of NO generating enzyme present on the coronary endothelium, endothelial nitric oxide synthase (eNOS). The presence of NOS isoforms, eNOS and bNOS (brain NOS or neuronal NOS) on the sarclemma (SL) and SR respectively in cardiomyocytes [14,15] have also been reported to play a direct role in modulating myocyte contractility, however nothing is known about their status in IR hearts.

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In view of the protective role of NO in recovering cardiac contractile function in IR hearts we investigated the mechanisms underlying this beneficial effect. The effects of an NO precursor, L-arginine (LA) on SR function, NO content and bNOS protein level were examined in hearts subjected to global IR. In a recent study [8] we showed that activation of a Ca\(^{2+}\) activated protease, calpain, contributed to SR dysfunction during IR by proteolytically modifying key SR Ca\(^{2+}\) handling and regulatory proteins. We therefore examined whether bNOS was a target for calpain action.

2. Materials and methods

The experimental protocol was approved by the Animal Care Committee of the University of Manitoba and conforms to the guidelines of the Canadian Council on Animal Care (CCAC) and the guidelines of the National Institute of Health.

2.1. Perfusion and experimental protocol

Male Sprague–Dawley rats weighing 225–275 g were anaesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg). The hearts were rapidly excised and cannulated to the Langendorff apparatus. Hearts were perfused in a retrograde manner with Krebs–Henseleit (K–H) buffer at 37 °C as described previously [6–8].

All hearts were stabilized with K–H buffer for 20 min before being randomly distributed among six experimental groups (Fig. 1) consisting of: control hearts (C) perfused for a period of 120 min with K–H buffer (1), IR was induced by exposing hearts to global no-flow ischemia for 30 min followed by reperfusion for 60 min (3). IR hearts were treated with 1.5 mM LA (Sigma, St. Louis, MO), a biological precursor of NO, for 10 min before inducing ischemia and for 20 min after ischemia beginning at the onset of reperfusion (IR+LA) (4). A dose response was done with 0.75, 1.5, 3, 6 and 12 mM to determine the best cardioprotective dose of LA (Table 1). Another group (C+LA) was added to observe for any possible effects of the drug on control hearts (2). The fifth group (IR+LN) consisted of IR hearts treated with L-NAME, a selective inhibitor of nitric oxide synthase (NOS) (5), while in the sixth group (IR+LA+LN) IR hearts were treated with a combination of LA and L-NAME (6). Two more groups (7 and 8) were added where LA was given either during stabilization (for 10 min) prior to the onset of ischemia (PRE) or immediately after ischemia (for 20 min) at the onset of reperfusion (POST). The protocol for perfusion in different groups is shown in Fig. 1. At the end of the

**Experimental Protocol**

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Fig. 1. Experimental protocol for isolated rat heart perfusion. Panels 1 and 2 show control hearts treated with and without L-arginine (LA). Panels 3 and 4 show IR hearts treated with and without LA, while panels 5 and 6 show IR hearts treated with L-NAME (LN) or a combination of LA and LN. Panels 7 and 8 show IR hearts with pre-ischemic (PRE) treatment and post-ischemic (POST) treatment with LA only. Global ischemia was induced by stopping coronary flow for 30 min and IR by reperfusing the globally ischemic hearts for 60 min. C=Control, IR=Ischemia reperfusion.
experiments the hearts were freeze clamped and stored at −70 °C.

2.2. Isolation of sarcoplasmic reticulum vesicles

SR vesicles were obtained by a method previously described [6–8]. Ventricular tissue was homogenized and centrifuged for 20 min at 10,919 × g. The supernatant was centrifuged for 45 min at 43,666 × g. The resultant supernatant obtained contained the cytosolic fraction and was frozen in liquid nitrogen prior to storage at −80 °C. The pellet was resuspended in a buffer containing 0.6 M KCl and 20 mM Tris–HCl (pH 6.8) and centrifuged for 45 min at 43,666 × g. The final pellet containing SR fraction was suspended in a buffer containing 250 mM sucrose and 10 mM histidine, pH 7.0 and frozen in liquid nitrogen prior to storage at −80 °C. All buffers used for isolation contained a cocktail of protease inhibitors to prevent protein degradation during isolation procedure.

2.3. Measurement of Ca2+-uptake and Ca2+-induced Ca2+-release

SR Ca2+-uptake and release was measured by a procedure described earlier [6–8]. For measuring Ca2+-uptake, Ruthenium red was added as an inhibitor of the Ca2+-release channel. The reaction was initiated by adding SR vesicles (20 μg protein) to the reaction mixture at 37 °C and terminated after 1 min by filtration. The filters were washed, dried and counted in a beta scintillation counter (Beckman, Fullerton, USA). For measuring Ca2+-release, SR fraction was incubated with 10 μM 45CaCl2 (20 mCi/L) and 5 mM ATP for 45 min at room temperature and Ca2+-induced Ca2+-release was carried out by adding 1 mM EGTA plus 1 mM CaCl2 to the reaction mixture. The reaction was terminated at 10 s by filtration. Filters were washed, dried and counted in a beta-scintillation counter. SR Ca2+-induced Ca2+-release was completely prevented (95 to 97%) by the treatment of SR preparations with 20 μM ryanodine.

2.4. Measurement of Ca2+-calmodulin protein kinase and cyclic AMP dependent protein kinase activities

Ca2+-calmodulin protein kinase (CaMK) and cyclic AMP dependent protein kinase (PKA) activities of the SR and cytosol were based upon the technique previously established using Upstate Biotechnology (Lake Placid, NY) assay kits [7,8]. For this purpose SR vesicles and cytosol were isolated in the presence of a phosphatase inhibitor (1 mM sodium pyrophosphate) to prevent any dephosphorylation occurring during the isolation procedure.

2.5. Western blot analysis

The protein content of SR Ca2+ cycling proteins; sarcoplasmic reticulum Ca2+-ATPase (SERCA2a), phospholamban (PLB) and its phosphorylated forms—serine-16 PLB and threonine-17 PLB, calsequestrin (CQS), bNOS, ß-CaMK and α-PKA were determined by Western blot analysis as previously described [7,8]. Protein samples (20–25 μg of SR) were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. The membranes were probed with monoclonal anti-SERCA2a (1:1400) and bNOS (1:100) antibodies from Affinity Bioreagents Inc., Golden, CO; monoclonal anti-PLB (1:2000) and polyclonal anti-CQS (1:2000) antibodies from Upstate Biotechnology, Lake Placid, NY; ß-CaMK II (1:500), α-PKA and PLB at Ser-16 (1:500) antibodies were obtained from Santa Cruz Biotechnology Incorporated, Santa Cruz, CA, while PLB Thr-17 antibody was obtained from Badrilla, Leeds, UK. Equal protein loading was checked in every experiment by staining the membrane with Coomassie Brilliant Blue at the end of the experiment. CQS was used as an internal control.

2.6. NO determination

NO content was determined in the cytosol by using the NO colorimetric assay kit (Roche Diagnostics GmbH, Penzberg, Germany). The assay detects NO photometrically
in samples based on nitrite formation from nitrates. Cytosolic sample (2 mg) was added to 2 ml of DDW and incubated at 60 °C for 15 min. One hundred fifty microliters of Carrez-I (potassium hexacyanoferrate) and Carrez-II (zinc sulfate) solutions were added successively after the sample had cooled. The pH was adjusted to 8.0 and final volume adjusted to 5.0 ml. The solution was centrifuged at 5000 × g for 5 min. The supernatant was centrifuged at 9000 × g for 3 min. The supernatant from the last spin was used to perform the assay in a 96-well microtitre plate. Using the standard solution provided (potassium nitrate), calibration curves were made and nitrate content was estimated by measuring the change in absorbance of sample and standard (potassium nitrate).

2.7. Calpain activity determination

Calpain activity in the cytosol (200 μg) was measured using a calpain assay kit from Biovision as described previously [8]. The reaction was carried out in a 96 well plate and the samples were read in a Gemini fluorescence microplate reader (excitation filter 400 nm and emission filter 505 nm) from Molecular Devices. The results are expressed in relative fluorescent units (RFU).

2.8. Detection of calpain nitrosylation

Calpain nitrosylation was investigated by a modified ‘Biotin Switch’ method [16,17]. Cytosolic samples (6 mg)
were blocked with a solution containing methyl methanesulphonate (20 mM) for 30 min at 50°C in an incubator with constant shaking. Samples were acetone (−20°C) precipitated and centrifuged at 5000 rpm for 10 min. The pellet was suspended in Hen buffer containing SDS, sodium ascorbate and Biotin HPDP and incubated at 25°C for 1.5 h in the dark. Biotinylated proteins were acetone precipitated, centrifuged and incubated with 90 μL of streptavidin agarose (resin) at room temperature for 1 h and the beads were extensively washed in neutralization buffer containing 600 mM NaCl (3 times). The proteins were incubated in a neutralization buffer containing 2-mercaptoethanol for 20 min to dissociate proteins from the beads. Protein samples were mixed (1:1) with Laemmli buffer, boiled and separated by 12% SDS-PAGE, transferred to a nitrocellulose membrane and probed with streptavidin conjugated horse-radish peroxidase antibody.

2.9. Statistical analysis

Results are expressed as mean±S.E and statistically evaluated by one-way analysis of variance (ANOVA) test for multiple comparisons and the student t-test. Linear regression test was used for the linearity study. A level of \( P<0.05 \) was considered the threshold for statistical significance between the control and various experimental groups and the groups themselves.

3. Results

3.1. Cardiac function

Reperfusion of IR hearts caused recovery in contractile function as represented by a 30%–35% improvement in

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<td>(+dP/dt)</td>
<td>1683±67</td>
<td>47.2±2.7*</td>
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<td>(-dP/dt)</td>
<td>1658±65</td>
<td>37.8±3.8*</td>
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Left ventricular developed pressure (LVDP), left ventricular end diastolic pressure (LVEDP), left ventricular pressure development (+dP/dt) and left ventricular pressure decay (−dP/dt). C=control, IR=Ischemia reperfusion. \( n=5 \) for each group. Data expressed as Mean±SE. *\( P<0.05 \) in comparison to the control and **\( P<0.05 \) in comparison with IR.

Fig. 3. Immunoreactive band and Western blot analysis for protein content of SR from control and IR hearts treated with and without LA (LA). Panel A: SERCA2a, Panel B: PLB. Panel C: PLB to SERCA2a ratio and Panel D: CQS. (\( n=5–6 \) for each group). Data expressed as Mean±SE. *\( P<0.05 \) in comparison to control, # \( P<0.05 \) in comparison to the IR group.
LVDP, +dP/dt and −dP/dt (Fig. 2A, C and D) of the respective pre-ischemic values but there was a marked increase in the LVEDP (Fig. 2B). Contractile activity in IR hearts was significantly improved by LA (1.5 mM) treatment as observed by an 80%–85% recovery of LVDP and about 65% recovery in +dP/dt and −dP/dt, in comparison with pre-ischemic values (Fig. 2A, C and D). A marked reduction in LVEDP was also observed with LA treatment in comparison to the IR group (Fig. 2B). The 1.5 mM dose of LA was selected based upon a dose response study carried out with different concentrations of LA (Table 1).

3.2. SR function

SR function is integral to cardiac Ca\(^{2+}\) homeostasis and therefore any alterations can adversely affect cardiac contractility. SR Ca\(^{2+}\)-uptake and release in IR hearts were assessed upon treatment with 1.5 mM LA. A marked depression in both SR Ca\(^{2+}\)-uptake and Ca\(^{2+}\)-release activities was observed (35%–40% of control values) in IR hearts. LA treatment significantly improved SR Ca\(^{2+}\)-release and Ca\(^{2+}\)-uptake in IR hearts (Fig. 2E,F). The 1.5 mM dose of LA was selected based upon a dose response studying SR Ca\(^{2+}\)-uptake in IR hearts treated with different concentrations of LA (Table 1).

3.3. SR protein content

The content of SERCA2a, a key SR Ca\(^{2+}\) cycling protein and its regulator PLB would directly and indirectly, respectively, influence SR function and therefore their protein levels were examined. Reperfusion of the ischemic hearts decreased the protein content of SERCA2a and PLB (60%–75% of control values) (Fig. 3A and B) and increased the ratio of PLB:SERCA2a (Fig. 3C). LA treatment attenuated the decrease in protein content of SERCA2a...
and PLB as well as normalized the PLB: SERCA2a (Fig. 3C) in IR hearts. To examine whether the reduction of SR Ca\(^{2+}\)-cycling proteins was a general phenomenon in IR hearts we examined the expression of calsequestrin (CQS) an SR protein, which is known to be unaffected in most cardiac pathologies. There was no significant change in the protein content of CQS in the IR group in comparison to the control and LA treated IR hearts (Fig. 3D).

3.4. Cytosolic NO content and SR bNOS level

Because NO regulates cardiac contractility we examined whether alterations in cardiac contractile function in IR hearts were associated with any changes in NO content. The NO content in cytosol of IR hearts was reduced by 50% as compared to controls (Fig. 4A). Treatment with 1.5 mM LA restored NO content to control levels. Isoforms of NOS reside on the SR and sarcolemma (SL) and play roles in regulating SR and SL function and cardiac contractility. In view of the reduction in NO levels and alterations in SR function observed in IR hearts we examined whether these changes were associated with any abnormalities in the SR bNOS and SL eNOS protein contents. Our results show significant decrease (85% of control values) in the bNOS protein content (Fig. 4B) in IR. The bNOS protein level was recovered upon treatment with LA (Fig. 4B). The SL eNOS protein content was also significantly reduced by 55% in IR (Control 100%; IR 45%).

3.5. SR protein phosphorylation

SR Ca\(^{2+}\)-uptake is regulated by SR associated CaMK and PKA phosphorylation of PLB. Any changes in SR function can therefore be partly attributed to abnormalities in PLB phosphorylation. PLB phosphorylation at both Ser-16 and Thr-17 was reduced (50%–75% of control values) in IR hearts (Fig. 5A and B). Treatment of IR hearts with LA significantly improved PLB phosphorylation at both sites. To examine whether attenuation in PLB phosphorylation was due to alterations in SR associated PKA and CaMK activities, their enzymatic activities were studied. Our results show a significant reduction in SR-associated PKA and CaMK activities in the IR group to about 35% of the control values (Fig. 6A and B). Treatment of IR hearts with LA markedly improved the SR associated PKA and CaMK activities. In order to determine if the changes in CaMK and PKA activities in IR hearts were compartmentalized to the
SR alone, we studied the activities of these enzymes in the cytosolic compartment also. Both cytosolic CaMK and PKA activities were unaffected by IR in comparison to the control hearts (data not shown). A decrease in the enzymatic activities of CaMK and PKA could be due to alterations in the levels of their protein content. We therefore examined the protein levels of SR associated PKA and CaMK. Hearts exposed to IR showed decreased protein content of PKA (α isoform by 40%) and CaMK II (δ isoform by 44%) in comparison with the controls (Fig. 6C and D). Treatment of IR hearts with LA significantly improved α-PKA and δ-CaMK II protein levels.

3.6. Effect of pre- or post-ischemic LA treatment on cardiac contractile function in IR hearts

To differentiate the beneficial effects observed with LA at the pre-ischemic or post-ischemic stages, IR hearts were treated with LA for 10 min before ischemia or for 20 min after ischemia respectively. Our results show significant increase (40%–50%) in LVDP, +dP/dt and −dP/dt of IR hearts upon pre-ischemic treatment (Fig. 7A, C, D). Post-ischemic treatment increased LVDP, +dP/dt and −dP/dt by 50%–60% in IR hearts (Fig. 7A, C, D). Post-ischemic and not pre-ischemic treatment reduced LVEDP in IR hearts (Fig. 7B).

3.7. Effect of L-NAME in the absence and presence of LA in IR hearts

In order to examine whether the mechanisms underlying the beneficial effects of LA on IR hearts were mediated through NO, IR hearts were treated with L-NAME, an inhibitor of NOS and therefore NO production, in the absence and presence of LA. Treatment of IR hearts with L-NAME had no significant effects on the IR induced depression in LVDP, +dP/dt and −dP/dt (Fig. 8A, C and D). However, the increase in LVEDP was further accentuated (Fig. 8B). On the other hand, the beneficial effects of LA on contractile function were attenuated by L-NAME (Fig. 8A–D). Similarly, L-NAME attenuated improvement in SR Ca^{2+} uptake of IR hearts by LA treatment (Fig. 8E). There was no significant improvement in SR Ca^{2+} uptake of IR hearts with L-NAME alone (Fig. 8E).

3.8. Calpain activity

In view of the activation of calpain in IR hearts and the possible association between NO and calpain, we examined the effects of NO donor LA on activation of calpain during IR. Our results show an almost 2-fold increase in the cytosolic calpain activity during IR (Fig. 9A). Treatment with NO donor, LA, significantly attenuated the increase in

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Fig. 7. Cardiac function of isolated perfused rat hearts subjected to IR treated with LA (1.5 mM) for 10 min only before ischemia (PRE) and 20 min only after ischemia at the onset of reperfusion (POST). Panel A: Left ventricular developed pressure (LVDP), Panel B: Left ventricular end diastolic pressure (LVEDP), Panel C: Rate of pressure development (+dP/dt) and Panel D: Rate of pressure decay (−dP/dt). n=5–6 for each group. Data expressed as Mean±SE. *P<0.05 in comparison to IR, # P<0.05 in comparison to the IR+LA group.
calpain activity during IR (Fig. 9A). Treatment with L-NAME alone did not affect the increase in calpain activity during IR but L-NAME administration in combination with LA inhibited the beneficial effects observed with LA alone (Fig. 9A).

3.9. Calpain nitrosylation

The decrease in cytosolic calpain activity observed by LA treatment in IR hearts could be due to nitrosylation of the active cysteine thiol groups on calpain—we therefore examined calpain nitrosylation. Our results show nitrosylation of a ~80 kD protein band which was identified as calpain. An almost 10-fold increase in calpain nitrosylation was observed in LA treated hearts in comparison to IR hearts (Fig. 9B).

4. Discussion

Our results show that induction of myocardial IR in rat hearts caused a depression in cardiac function as evident from a decrease in LVDP, +dP/dt and −dP/dt and an increase in LVEDP. Depressed cardiac contractility could be associated with a decrease in SR function. The reduction in SR Ca\textsuperscript{2+} uptake may in part be due to a direct reduction in

![Graphs showing Cardiac function and SR Ca\textsuperscript{2+} uptake](image-url)

Fig. 8. Cardiac function and SR Ca\textsuperscript{2+} uptake of the isolated perfused rat hearts subjected to IR treated with and without LA (1.5 mM) or L-NAME (100 mM) or a combination of the two. Panel A: Left ventricular developed pressure (LVDP), B: Left ventricular end diastolic pressure (LVEDP), Panel C=Rate of pressure development (+dP/dt) and Panel D=Rate of pressure decay (−dP/dt). Panel E: SR Ca\textsuperscript{2+} uptake. n=5–6 for each group. Data expressed as Mean±SE. *P<0.05 in comparison with IR, # P<0.05 in comparison with the IR+LA group.
the protein content of the major SR Ca\(^{2+}\) cycling protein, SERCA2a. Although the protein level of PLB, the regulator of SERCA2a, was also decreased the ratio of SERCA2a to PLB was increased suggesting increased inhibition of SERCA2a by PLB in IR hearts. The reduction in levels of SR proteins was not a generalized phenomenon because the CQS protein content was unaltered. Regulation of SR Ca\(^{2+}\) uptake was also affected in IR hearts as evident from reduced PLB phosphorylation at Thr-17 and Ser-16, mediated by CaMK and PKA, respectively. Decreased PLB phosphorylation was consistent with a decrease in the CaMK II and PKA activities, which in turn was associated with a reduction in their protein levels.

The above results showing contractile abnormalities in IR hearts being associated with impairment in SR function related to alterations in SR Ca\(^{2+}\)-cycling proteins and SR regulatory proteins are consistent with our previous studies [6–8]. Recovery of cardiac contractile function by LA treatment was associated with improved SR Ca\(^{2+}\)-uptake in IR hearts. SR function was enhanced in IR hearts by improving the SERCA2a to PLB protein ratio and by recovering PLB phosphorylation by CaMK and PKA in IR hearts. Both the above effects relieve PLB inhibition of SERCA2a and stimulate SR Ca\(^{2+}\)-uptake. Although nitrosylation in skeletal muscle SR has been considered to affect Ca\(^{2+}\)-uptake activity [18], it remains to be seen whether cardiac SR function is directly altered by nitrosylation.

Treatment of IR hearts with LA at either the pre-ischemic or the post-ischemic stages significantly recovered cardiac contractile function; however pre-ischemic treatment did not reduce LVEDP in IR hearts. These results suggest that post-ischemic treatment of IR hearts was more beneficial than pre-ischemic treatment. Although pre- or post-ischemic treatments by themselves were beneficial, maximal recovery was observed with a combination of pre and post-ischemic treatment.

Recently a significant role has been advocated to NO in regulating cardiac contractility [19–21]. Our results show that the IR induced contractile dysfunction and impaired SR function was associated with alterations in NO production as evident from depressed cytosolic NO levels in IR hearts. Reduced levels of NO during IR are consistent with some of the earlier studies [9,12]. Cytosolic NO could be generated by NOS enzymes within the cardiomyocyte. Recent evidence suggests that bNOS, an isoform of NOS localized to the SR and eNOS resident to the SL play important roles in regulating cardiac contractility [15,22–25]. Furthermore, the bNOS isoform influences the cardiac force frequency response and also facilitates the beta-adrenergic response [24,25]. The decrease in protein content of bNOS and eNOS, during IR could therefore in part explain the decreased NO levels in cytosol as well as contribute to impairment of SR function observed in this study. This view is supported by a recent report indicating impairment of SR Ca\(^{2+}\)-uptake in bNOS knockout mice [15]. LA treatment improved cytosolic NO content by recovering SR bNOS levels in IR hearts.

L-NAME given in combination with LA attenuated the improvement in cardiac contractility and SR function observed by LA treatment alone. L-NAME is a specific inhibitor of NOS and therefore an inhibitor of NO production. These results suggest a critical role for NO in maintaining SR function and cardiac contractility. Furthermore, attenuation of the beneficial effects of LA treatment (of IR hearts) by L-NAME may in part be due to inhibition of bNOS.

A recent study from our laboratory showed a reduction in SR Ca\(^{2+}\) cycling and regulatory protein levels in IR hearts that was associated with an increase in the activity of a Ca\(^{2+}\) activated neutral protease, calpain [8]. The present study shows an increase in calpain activity during IR and a decrease in protein content of bNOS. Thus it seems probable that bNOS may be a target for proteolytic modification by calpain and account for the depressed production of NO during IR. This view is supported by in
vitro studies [26,27], which showed that NOS isoforms are substrates for calpain. In our study, treatment with LA attenuated IR induced increase in calpain activity and improved protein content of bNOS. A combination of LA and L-NAME did not completely abolish the beneficial effects observed with LA alone in IR hearts. These results suggest that NO may contribute to the inhibition of calpain—a possible mechanism being nitrosylation. In the present study we demonstrate NO mediated nitrosylation of calpain in IR hearts treated with LA; LA induced nitrosylation of calpain could therefore be responsible for attenuating calpain activity in IR hearts. This view is supported by earlier studies [28,29], which have shown NO (generated by exogenous donors) to reversibly inactivate calpain [28] and inhibit calpain-mediated proteolysis in skeletal muscle [29]. Our results in no way exclude the cardioprotective effects of LA through its action as an antioxidant suggested by some other studies [30–32].

This study provides a novel mechanism for attenuating cardiac contractile abnormalities by improving SR function in IR hearts using a NO donor. Thus prevention of SR dysfunction and attenuation of calpain activity may be part of the spectrum of beneficial effects observed with LA, in addition to its known effects on coronary vasculature in improving cardiac performance in clinical situations of IR [33,34].

Acknowledgements

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