Nitric Oxide Protects Cardiac Sarcolemmal Membrane
Enzyme Function and Ion Active Transport against
Ischemia-induced Inactivation*

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Nitric oxide (NO) generated from nitric oxide synthase (NOS) isoforms bound to cellular membranes may serve to modulate oxidative stresses in cardiac muscle and thereby regulate the function of key membrane-associated enzymes. Ischemia is known to inhibit the function of sarcolemmal enzymes, including the (Na\(^+\) + K\(^-\))-ATPase, but it is unknown whether concomitant injury to sarcolemma (SL)-associated NOS isoforms may contribute to this process by reducing the availability of locally generated NO. Here we report that nNOS, as well as eNOS (SL NOSs), are tightly associated with cardiac SL membranes in several different species. In isolated perfused rat hearts, global ischemia caused a time-dependent irreversible injury to cardiac SL NOSs and a disruption of SL NO generation. Pretreatment with low concentrations of the NO donor 1-hydroxy-2-oxo-3-(N-3-methyl-aminopropyl)-3-methyl-1-triazene (NOC-7) markedly protected both SL NOSs and (Na\(^+\) + K\(^-\))-ATPase functions against ischemia-induced inactivation. Moreover, ischemia impaired SL Na\(^+\)/K\(^-\) binding, and NOC-7 significantly prevented ischemic injury to the ion binding sites on (Na\(^+\) + K\(^-\))-ATPase. These novel findings indicate that NO can protect cardiac SL NOSs and (Na\(^+\) + K\(^-\))-ATPase against ischemia-induced inactivation and suggest that locally generated NO may serve to regulate SL Na\(^+\)/K\(^-\) ion active transport in the heart.

Myocardial ischemia causes intracellular energy depletion, leading to loss of cellular Na\(^+\)/K\(^-\) homeostasis through inhibition of the sarcolemmal (Na\(^+\) + K\(^-\))-ATPase, resulting ultimately in cellular Ca\(^2+\) overload (1–4). The nature of ischemic injury to the (Na\(^+\) + K\(^-\))-ATPase and its effect on ion pump function has not been fully defined despite its fundamental importance in the maintenance of cell viability. Sarcolemma (SL)\(^3\) contains many bound enzymes that may modify the activity of (Na\(^+\) + K\(^-\))-ATPase, including nitric oxide synthase. NO\(^\cdot\) synthase is a cell type-specific enzyme that catalyzes the synthesis of NO, a short-lived gaseous free radical, which also serves as an important physiological regulator of myocardial function (5). All NOS isoforms, including neuronal NOS (nNOS), inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS\(_{\alpha}\) (nNOS\(_{\alpha}\)) have been identified in cardiac muscle (6–9). However, little is known about specific NOS isoforms on the SL membrane (SL NOSs) and what effect ischemia may have on their function. NO\(^\cdot\) generated at the sarcolemma by SL NOSs could modulate the activity of SL-associated ion pumps, but whether ischemic dysfunction of SL NOSs directly affects SL (Na\(^+\) + K\(^-\))-ATPase activity and SL ion active transport has not been studied. The functional relationships among SL NOSs, (Na\(^+\) + K\(^-\))-ATPase, NO\(^\cdot\), and SL Na\(^+\)/K\(^-\) ion active transport in normal and diseased hearts have remained open questions. In this study, we show that, like eNOS, nNOS is also tightly associated with the SL membrane in several species. Using an assay of NOS activity, we demonstrate that global ischemia causes a time-dependent irreversible injury to the SL NOSs and a disruption of SL NO\(^\cdot\) generation. Pretreatment of isolated rat hearts with an NO\(^\cdot\) donor prior to global ischemia markedly protects SL NOSs and (Na\(^+\) + K\(^-\))-ATPase activities and ion binding to the SL membrane, suggesting that SL NO\(^\cdot\) may play a crucial role in regulating SL membrane ion active transport.

EXPERIMENTAL PROCEDURES

Materials—An NOS-detect assay kit, including reduced NADPH, 1-hydroxy-2-oxo-3-(N-3-methyl-aminopropyl)-3-methyl-1-triazene (NOC-7), calmodulin, FAD, FMN, and tetrahydrobiopterin, was purchased from Alexis Biochemicals; l-arginine and EDTA were from Fluka; [\(^3\)H]l-arginine was purchased from Amersham Biosciences; anti-eNOS (catalog no. PA3-031), anti-universal NOS (anti-uNOS, catalog no. MA3-030) and an antibody reacting with iNOS, eNOS, and nNOS isoforms, anti-phospholamban (anti-PLB, catalog no. MA3-922), and anti-ryanodine receptor (anti-RyR, catalog no. MA3-916) were from Affinity BioReagents; Laemmli sample buffer was from Bio-Rad and all other reagents were from Sigma. The anti-nNOS\(_{\alpha}\) antibody (specifically recognizing the major \(\alpha\)-isoform of nNOS and not the \(\beta\)- or \(\gamma\)-isoforms) and the homozygous mutant nNOS (nNOS\(^-\)/- ) knockout mouse model were from the laboratory of Dr. Ted W. Dawson (10). The laboratory of Dr. Arthur L. Burnett provided both eNOS knockout (eNOS\(^-\)/- ) and nNOS\/_eNOS double knockout (nNOS/eNOS\(^-\)/- ) mice for this study.

*This research was supported by American Heart Association Grants 9951350U and 0151308U and National Institute of Health Grants HL52175 (to K. Y. X.), HL33560 (to L. C. B.), NINDS NS37090 (to T. M. D.), and DR02568 (to A. L. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡The abbreviations used are: SL, sarcolemma; NOS, NO synthase; iNOS, inducible NOS; eNOS, endothelial NOS; nNOS, neuronal NOS; uNOS, universal NOS; EPR, electron paramagnetic resonance; NOC-7, 1-hydroxy-2-oxo-3-(N-methyl-3-aminopropyl)-3-methyl-1-triazene; RyR, ryanodine receptor; PLB, phospholamban.
The homozygous mutants nNOS knockout, eNOS knockout, and nNOS/eNOS double knockout mice were backcrossed to the C57BL/6J black mouse. They were originally generated from Dr. Paul L. Huang’s laboratory at the Massachusetts General Hospital, Boston, MA (11, 12).

Isolated Rat Heart Ischemia and Reperfusion—Female Sprague-Dawley retired breeder rats (Harlan Company, Boston, MA) were used for the study, and all experiments were carefully conducted in compliance with the National Institutes of Health guidelines for the use of laboratory animals. The heart was excised after complete anesthesia (65 mg/kg pentobarbital), and the ascending aorta was rapidly cannulated and the heart perfused. Following an equilibrium period of 15 min to allow for functional stabilization, baseline values of coronary flow, left ventricular end diastolic pressure, left ventricular systolic pressure, and left ventricular developed pressure were measured. After 15 min, the hearts were then subjected to global (no-flow) ischemia in different time courses with or without 45 min of reperfusion. The perfusate contained 17 mM glucose, 120 mM NaCl, 25 mM NaHCO3, 5.9 mM KCl, 2.5 mM CaCl2, 1.2 mM MgCl2, and 0.5 mM EDTA. All perfusate solutions were routinely filtered and bubbled with 95% O2/5% CO2 gas mixture at 37 °C (13).

A side arm in the perfusion line located just proximal to the aortic cannula allowed infusion of drug solutions using a Harvard infusion pump (Harvard Apparatus, Holliston, MA). A balloon was connected via a hydraulic line to an ML7844 physiological pressure transducer with pressures amplified to a Gould four-channel strip chart recorder as well as a computer (PowerLab) equipped with a data acquisition software. The balloon volume was adjusted to achieve an initial left ventricular end-diastolic pressure of 8–12 mm Hg, and all subsequent measurements were performed with the same balloon volume.

Studies of ischemia and pretreatment with a membrane-permeable NO doner NOC-7 (14) were performed in four groups as follows: (i) control (perfusion for 20, 40, or 60 min without ischemia or drug treatment); (ii) treatment with NOC-7 infused at concentrations of 10, 30, 60, 90, 120, or 150 ng/kg/min for 10 min; (iii) global zero flow ischemia for 20, 40, or 60 min; and (iv) pretreatment with NOC-7 at concentrations of 10, 30, 60, 90, 120, or 150 ng/kg/min for 10 min prior to ischemia under the same conditions as described previously (3). This NOC-7 compound spontane-ously releases NO at physiological pH with a half-life time of 10 min.

Isolation of Cardiac Sarcolemmal Vesicles—Cardiac SL vesicles were isolated from rat, rabbit, and mouse hearts as reported previously (15). Briefly, animals were anesthetized with sodium pentobarbital. The hearts were removed immediately and rinsed in an ice-cold solution containing 10 mM histidine (free base) and 0.75 mM NaCl. The hearts were cut into 5-mm pieces and homogenized for 5 s at 14,000 rpm. The homogenates were centrifuged at 10,000 rpm for 20 min, and the pellets were resuspended in 10 mM NaHCO3 and 5 mM histidine (free base). The pellets were resuspended, homogenized, and centrifuged three times as described above, and the supernatant was discarded to remove the sarcoplasmic reticulum (SR) of the cardiac tissue for the ultimate isolation of SL vesicles free of significant SR contamination. The pellets obtained from the third centrifugation were resuspended in 10 mM NaHCO3 and 5 mM histidine and homogenized three times for 30 s at 15,000 rpm. The homogenates were centrifuged at 10,000 rpm for 20 min. The supernatant containing partially purified SL vesicles was centrifuged again for 30 min at 20,000 rpm. The pellets were resus-pended and layered on top of the 0.6 M sucrose in each tube. The sample was centrifuged at 60,000 rpm for 54 min in a Beckman Ti70 rotor. After the run, the SL vesicles were observed as distinct snow white protein bands at the interfaces of the 0.25/0.6 M sucrose layers. SL vesicle fractions were then sedimented at 40,000 rpm for 40 min. The final pellets were resuspended with 0.4 M sucrose containing 10 mM MgCl2 at −80 °C. SL vesicles were then transferred to nitrocellulose membranes and incubated with anti-nNOSα, anti-eNOS, anti-iNOS, or anti-uNOS antibodies for 60 min at room temperature. After washing, the membranes were incubated with goat anti-mouse or anti-rabbit IgG (Fc) to which alkaline phosphatase was attached. The distribution of bound antibody was determined by alkaline phosphatase-dependent staining.

Determination of NOS Activity—NOS activity was measured by monitoring the conversion of [3H]arginine to [3H]citrulline. For routine assays (7), 0.1 mg of vesicles and 10 μl of 100 nM [3H]arginine were added to 40 μl of buffer containing 50 mM Tris (pH 7.4), 1 mM reduced NADPH, 0.2 mM CaCl2, 0.1 μM calmodulin, 2 μM FAD, 2 μM FMN, and 3 μM tetrahydrobiopterin. After incubation for 30 min at room temperature, the reaction was initiated by adding ATP (3 mM) and stopped after 30 min by adding 0.75 ml of quench solution and 0.02 ml of developer. The color was allowed to develop for 30 min at room temperature, and the phosphate (reaction product) was determined at 700 nm using a spectrophotometer.

EPR Measurements—Spin-trapping measurements of NO radicals were performed in a flat cell at room temperature (7). The final concentrations of N-methyl-h-glutamate dithiocarbamate, Fe+2, SL vesicles, Ca+2, calmodulin, NADPH, FAD, FMN, tetrahydrobiopterin, and iNOS (14,000 USP units/ml) were then transferred to nitrocellulose membranes and incubated with anti-uNOS antibody, which recognizes all three isoforms of NOS, and anti-eNOS antibodies detected nNOS and eNOS from control mouse (wild-type) SL vesicles (Fig. 1, lanes E and G). No immunodetection of nNOS or eNOS was observed in cardiac SL vesicles isolated from knockout nNOS, eNOS, or nNOS/eNOS double knockout mouse models (Fig. 1, lanes D, F, and H).

Anti-RyR and anti-PLB recognized RyR (Fig. 1, lane I) and PLB (Fig. 1, lane K) in isolated rat cardiac SR vesicles but failed to detect RyR (Fig. 1, lane J) and PLB (Fig. 1, lane L) in SL vesicles, indicating a high purity of our isolated SL vesicles and suggesting a specific molecular expression of nNOS and eNOS in cardiac SL membrane.

Effect of Global Ischemia on SL NOS Function—Next, we examined the effect of ischemia on total SL NOS function. Ca+2-dependent chemical conversion of l-arginine to l-citrul-line was performed in the presence of the NOS coenzyme and cofactors. Total cardiac SL NOS enzymatic activity decreased significantly from 100% (control) to 52 ± 11%, 30 ± 8%, and 5 ± 3% (Fig. 2A) following 30, 60, and 120 min of ischemia, respectively. The loss of enzymatic activity during ischemia was accompanied by a reduction of SL NO generation from 100% (control) to 59 ± 29%, 35 ± 18%, and 2 ± 1.5% (Fig. 2B), respectively, following 30, 60, and 120 min of ischemia.

RESULTS
Both nNOS and eNOS Are Associated with Cardiac SL Membrane—We first determined the specific isoforms of NOS that are associated with cardiac SL membrane. Using an anti-uNOS antibody, which recognizes all three isoforms of NOS, i.e., iNOS, eNOS, and nNOS, Western blotting revealed that only nNOS (155 kDa) and eNOS (140 kDa) are present in rat, rabbit, and mouse cardiac SL membrane vesicles (Fig. 1, lanes A, B, and C), whereas iNOS was not detected (data not shown). Anti-nNOSα and anti-eNOS antibodies detected nNOS and eNOS from control mouse (wild-type) SL vesicles (Fig. 1, lanes E and G). No immunodetection of nNOS or eNOS was observed in cardiac SL vesicles isolated from knockout nNOS, eNOS, or nNOS/eNOS double knockout mouse models (Fig. 1, lanes D, F, and H).

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Effect of Global Ischemia on SL NOS Function—Next, we examined the effect of ischemia on total SL NOS function. Ca+2-dependent chemical conversion of l-arginine to l-citrul-
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To determine whether ischemia affects the binding of nNOS and eNOS to the SL membrane and whether the low NOS activity following ischemia is due to loss of enzyme from the SL membrane, Western blotting experiments were performed using SL vesicles isolated from rat cardiac muscle under various ischemic conditions as shown in Fig. 3. The results demonstrated similar concentrations of SL nNOS and eNOS proteins in the SL membrane vesicles isolated from normoxic and ischemic hearts following 30, 60, and 120 min of ischemia. This demonstrates the structural stability of NOS isoforms in SL vesicles.

To investigate the potential reversibility of ischemic dysfunction of cardiac SL NOS enzymes, ischemia/reperfusion experiments were performed to test the recovery of depressed total SL NOS activity. Fig. 4 shows that ischemia-injured cardiac SL NOS activity did not recover following 45 min reperfusion after 30 or 60 min of ischemia. Global ischemia appeared to cause a time-dependent irreversible injury to cardiac SL NOS function.

Role of NO in SL Membrane Enzyme Function—To investigate whether ischemic dysfunction of SL NOSs could affect SL membrane ion active transport, we tested whether NO selectively protects SL (Na⁺ + K⁺)-ATPase activity under various ischemic conditions. We first examined the effect of NOC-7 on cardiac SL (Na⁺ + K⁺)-ATPase function without ischemia. Isolated rat hearts were perfused with the NO donor NOC-7 at different concentrations as shown in Fig. 5A. No inhibitory effect of NOC-7 (from 10 ng/kg/min to 150 ng/kg/min) on SL (Na⁺ + K⁺)-ATPase function was observed as shown in Fig. 5A. We then tested whether perfusion with 10 ng/kg/min NOC-7 would affect enzyme functions under different ischemic conditions. Experimental data show that pretreatment of 10 ng/kg/min NOC-7 provided modest protection to both SL
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FIG. 4. Lack of recovery of cardiac SL NOS activity in isolated rat hearts subjected to ischemia and reperfusion. Perfused rat hearts were subjected to 30 and 60 min of global ischemia followed by 45 min of reperfusion. The SL vesicles were then isolated, and total SL NOS activities were measured. Cardiac SL NOS activities did not recover following 45 min of reperfusion, suggesting that this process may be a time-dependent irreversible ischemic injury (n = 8). Data are plotted as mean ± S.E.

(\(\text{Na}^+ + \text{K}^+\))-ATPase and SL NOS activities; SL (\(\text{Na}^+ + \text{K}^+\))-ATPase activity was 105 ± 43%, 85 ± 28%, and 71 ± 24% (Fig. 5B) compared with control hearts and 88 ± 37%, 73 ± 48%, and 56 ± 19% for SL NOS (Fig. 5C) after 20, 40, and 60 min of no-flow ischemia, respectively. However, pretreatment with 30 ng/kg/min NOC-7 markedly protected SL (\(\text{Na}^+ + \text{K}^+\))-ATPase activity against ischemia-induced inactivation; enzyme activities remained at 103 ± 36%, 102 ± 22%, and 91 ± 21% following 20, 40, and 60 min of ischemia, respectively. In contrast, (\(\text{Na}^+ + \text{K}^+\))-ATPase activity decreased to 45 ± 15%, 32 ± 10%, and 15 ± 6% in the absence of the NO donor under the same ischemic conditions. Furthermore, Fig. 6 shows that 40 min of global ischemia disrupted the ion binding sites on the (\(\text{Na}^+ + \text{K}^+\))-ATPase by reducing the optimal binding of \(\text{Na}^+\) and \(\text{K}^+\) ions to the enzyme. The \(\text{Na}^+\) and \(\text{K}^+\) binding sites were completely protected by pretreatment of NO-donor NOC-7, further suggesting that NO generation in cardiac muscle may be crucial to the SL membrane ion active transport. In addition, pretreatment of NOC-7 markedly maintained SL NOS activity at 100 ± 20%, 99 ± 18%, and 94 ± 13% after 20, 40, and 60 min ischemia, respectively, presumably by scavenging toxic oxygen free radicals that were generated during ischemia.

DISCUSSION

The studies reported here show immunohistochemically that nNOS, as well as eNOS, is tightly associated with cardiac SL membrane vesicles (Figs. 1 and 3). The fact that anti-nNOS and anti-eNOS antibodies recognized nNOS and eNOS in wild-type mice (Fig. 1, lanes E and G) but failed to recognize nNOS and eNOS (Fig. 1, lanes F and H) in SL vesicles from knockout nNOS or eNOS mice and that no immunodetection of nNOS or eNOS was observed in cardiac SL vesicles isolated from nNOS/eNOS double knockout mouse model (Fig. 1, lane D) further confirm our observation that these two NOS isoforms are localized on the cardiac SL membrane. RyR and PLB are SR membrane protein markers. Anti-RyR and anti-PLB antibodies recognized RyR (Fig. 1, lane I) and PLB (Fig. 1, lane K) in isolated rat SR vesicles but failed to detect RyR (Fig. 1, lane J) and PLB (Fig. 1, lane L) in isolated SL vesicles, suggesting a specific molecular expression of nNOS and eNOS in cardiac SL membrane. It is generally accepted that eNOS is closely associated with SL on endothelial cells and cardiac myocytes through binding with caveolin-1 and caveolin-3, respectively (19, 20). Miethke et al. detected nNOS in rat atrial cardiomyocyte SL but not in ventricular myocytes (21). Hare and coworkers (22), using immunoprecipitation and Western blotting techniques of mouse heart homogenates, reported that eNOS precipitated with caveolin-3, whereas nNOS precipitated with the ryanodine receptor. Subcellular localization was not reported (22). It has been demonstrated that rat skeletal muscle sarcolemma contains an nNOS and synthesizes NO (8, 23). Our experimental results extend these reports by identifying subcellular localization of nNOS in cardiac SL membrane from several animal species. In addition, we also detected eNOS resides in SL membrane, which is consistent with previous observations (24).

Although numerous studies have examined the effect of ischemia on myocardial function, none have focused on whether ischemia may injure cardiac SL NOS function. Our results show that there is a time-dependent loss of SL NOS activity and SL NO generation (Fig. 2, A and B) during global ischemia. Thirty minutes of ischemia resulted in an −50% loss of SL NOS activity and NO generation. Similar protein concentrations of SL NOSs from control and ischemic rat hearts were observed.
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Cardiac SL vesicles were isolated from the hearts of control (no ischemia, black circles) and 40 min of ischemia with (open circles) or without NO-donor NOC-7 (black triangles). Enzyme activity was tested in the presence of 3 mM MgATP under different Na\(^+/\)K\(^-\) concentrations as indicated in the figure. The data show that pretreatment by NOC-7 (30 ng/kg/ml) significantly protected SL Na\(^+/\)K\(^-\) ion binding and (Na\(^+\) + K\(^+)\)-ATPase function against ischemia-induced inactivation.

Cardiac SL (Na\(^+\) + K\(^+)\)-ATPase (25) is an integral membrane protein that couples the hydrolysis of ATP directly to the vectorial transport of Na\(^+\) and K\(^-\) ions across the SL membrane. This enzyme controls the contractility of myocardium by regulating intracellular sodium concentration and membrane potential. Dysfunction of (Na\(^+\) + K\(^+)\)-ATPase in ischemic myocardial injury may contribute to intracellular calcium overload and persistent depression of heart function. We hypothesized that endogenous NO generated from cardiac SL NOSs may be a critical protector of SL (Na\(^+\) + K\(^+)\)-ATPase function and SL Na\(^+\) and K\(^-\) ion transport. If the hypothesis is correct, the (Na\(^+\) + K\(^+)\)-ATPase activity should be resistant to myocardial ischemia when pretreated with a NO donor. To test our hypothesis, we utilized the NO donor NOC-7 (185 nM), and significant protection was found from an OH \((\bullet OH)\) radical generation and leading ultimately to dysfunction of the myocardium (26). Bolli and co-workers have demonstrated that hydroxyl radicals are generated in vivo after myocardial ischemia (27). Exposure of isolated rabbit interventricular septa, isolated rat or rabbit papillary muscles, and isolated rat or rabbit hearts to free radical generating solutions or H\(_2\)O\(_2\) has uniformly resulted in decreased mechanical function and ATP levels (28). These important observations suggest that toxic oxygen free radicals may be responsible for injury to the SL NOSs and (Na\(^+\) + K\(^+)\)-ATPase during myocardial ischemia. Kukreja et al. found that O\(_2\)\(^{=}\) causes relatively little damage to the cardiac SR (29) and SL (30), but hydroxyl (OH) radicals disrupt cardiac SL function by inhibiting the (Na\(^+\) + K\(^+)\)-ATPase activity and ouabain binding sites (31) following ischemia-reperfusion. Our previous studies also show that OH radicals inhibit nNOS activity (32) and damage SR Ca\(^{2+}\)-ATPase function by direct attack on the ATP binding sites (15). These important findings suggest a latent inactivation mechanism for both SL NOSs and (Na\(^+\) + K\(^+)\)-ATPase in which OH radicals, generated during the global ischemia, may attack ATP and substrate or cofactor and ion binding sites of the enzymes, resulting in loss of SL NOSs and (Na\(^+\) + K\(^+)\)-ATPase function.

It has been reported that NO reacts with both OH and O\(_2\)^{=} radicals at rate constants of \(K_{OH} = 1 \times 10^{10} \, M^{-1} \, s^{-1}\) and \(K_{O_2} = 6.7 \times 10^6 \, M^{-1} \, s^{-1}\), respectively (34, 35). In our previous studies, we tested the effect of NO in scavenging OH radicals. EPR spin trapping measurements revealed that when purified nNOS was added to an OH generating system, endogenous NO from nNOS markedly quenched OH radicals (32). These results clearly demonstrated that NO scavenges toxic OH radicals. The scavenger effect of NO was further tested using the NO donor NOC-7 (32). Exogenous NO, generated from NOC-7, completely eliminated OH from an OH radical generation system (H\(_2\)O\(_2\) plus the ferric iron chelate Fe\(^{3+}\)-NTA) (32), and prevented OH radical-induced inactivation of both purified nNOS and (Na\(^+\) + K\(^+)\)-ATPase. These in vitro studies further confirm the interaction between NO and OH radicals and provide a potential mechanism for NO-mediated protection of SL NOS and SL membrane ion active transport function during ischemia. Because both SL NOSs and (Na\(^+\) + K\(^+)\)-ATPase are bound to the SL membrane, endogenously produced NO may be particularly effective in scavenging toxic free radicals within the microenvironment of the (Na\(^+\) + K\(^+)\)-ATPase. Whether pretreatment of NO-donor affects NO-mediated signaling pathways is not known. We do not exclude the involvement of this possible process that may also contribute to the NO protective effect (33). More detailed investigations of functional links between the pretreatment of NO donor and signaling pathways, including activation of guanylate cyclase and cGMP-dependent protein kinase, should increase our understanding of the mechanisms of bioprotective role of NO.

The studies reported here reveal a significant inactivation of cardiac SL NOSs function following global ischemia. Dysfunction of cardiac SL NOSs may contribute to the mechanisms of ischemic injury by removing a source of NO, generated at the SL membrane, resulting in reduced scavenging of toxic oxygen radicals. Our findings that NO protects cardiac SL NOSs and (Na\(^+\) + K\(^+)\)-ATPase against ischemia-induced inactivation suggest that locally generated NO may serve to regulate SL membrane Na\(^+\)/K\(^-\) ion active transport in the heart.
Acknowledgments—We thank Daniel Guastella and Jennifer Kiluk for maintaining the nNOS knockout colony and Robyn Becker for the eNOS knockout and nNOS/eNOS double knockout colonies.

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J. Biol. Chem. 2003, 278:41798-41803.
doi: 10.1074/jbc.M306865200 originally published online August 6, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M306865200

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