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Original Research Communication

Selenium Improves Cardiac Function by Attenuating the Activation of NF-κB Due to Ischemia–Reperfusion Injury

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ABSTRACT

Although selenium, an essential trace element and a component of glutathione peroxidase, is known to protect the heart from ischemia–repefusion (I/R)-induced injury, the mechanisms of this protection are not fully understood. For this purpose, isolated rat hearts were subjected to 30 min of global ischemia followed by 30 min of reperfusion; sodium selenite (25–1,000 n*M*) was added in the perfusion medium 10 min prior to ischemia, as well as during reperfusion. Selenium caused a dose-dependent improvement in cardiac performance and attenuated the decrease in the ratio of reduced glutathione to oxidized glutathione, as well as the increased level of malondialdehyde in I/R heart. Elevated ratios of nuclear factor-κB (NF-κB) in particulate and cytosolic fraction and of phosphorylated NF-κB and total NF-κB in I/R hearts were reduced by selenium. Cardiac dysfunction in hearts perfused with xanthine plus xanthine oxidase mixture, as well as hydrogen peroxide, or subjected to Ca^{2+} paradox was also attenuated by selenium. These data suggest that selenium protects the heart against I/R injury due to its action on the redox state and deactivation of NF-κB in I/R hearts. *Antioxid. Redox Signal.* 7, 1388–1397.

INTRODUCTION

Accumulation of reactive oxygen species (ROS) is considered an important mediator of ischemia–reperfusion (I/R)-induced cardiac dysfunction that occurs in some pathological conditions, as well as following certain surgical procedures (8, 11). It has been shown that hearts exposed to ROS-generating systems exhibit numerous features similar to those of the I/R hearts; these include changes in mechanical and electrical activities, as well as metabolic pathways (21, 33, 34). Striking similarities with respect to cardiac dysfunction and generation of oxyradicals have also been described between I/R-induced injury and the Ca^{2+}-paradox phenomenon (16). In view of the importance of ROS in the activation of nuclear factor-κB (NF-κB), a redox-sensitive transcription factor for the regulation of different cellular processes (39), it has been suggested that ROS may enhance NF-κB activation by modifying the activity of one or more of the kinases responsible for NF-κB activation by changing the cellular redox state (2). Furthermore, the activation of NF-κB has been shown to be induced by prooxidants and several stimuli eliciting oxidative stress (7, 13, 19). In fact, different antioxidants were found to inhibit this activation (5, 7, 20, 22, 30). Thus, it appears that oxidative stress and the activation of NF-κB play a critical role in I/R-mediated cardiac dysfunction.

Selenium is an essential trace element and an important dietary antioxidant (14, 28). Its deficiency has been shown to be associated with different diseases, such as coronary artery disease, osteoarthritis, AIDS, and cancer (23). Cell culture studies have revealed that sodium selenite inhibited the binding of NF-κB to nuclear responsive elements with a substantial increase in the activity of glutathione peroxidase (GSH-Px) and a significant inhibition in the activity of inducible nitric oxide (18, 19). Recently it has been shown that selenium supplementation has some beneficial effects in I/R-induced injury in the intestinal tissue (25), as well as in the heart (27), kidney (37),

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and liver (42). However, the mechanisms of such protection by selenium in I/R-induced injury have not been completely elucidated. Therefore, the present study was undertaken to test if the beneficial effects of selenium in I/R hearts are linked to the level of phosphorylated NF-κB (phospho-NF-κB), as well as the subcellular distribution of NF-κB. The actions of selenium on oxidative stress-induced cardiac dysfunction, as well as in Ca^{2+}-paradox hearts, were also investigated to examine if this agent produces effects similar to those in I/R hearts.

MATERIALS AND METHODS

Experimental model

All experimental protocols were approved by the University of Manitoba Animal Care Committee in accordance with the standards of the Canadian Council on Animal Care. Adult male Sprague–Dawley rats (250–300 g) were anesthetized with a mixture of ketamine (90 mg/kg) and xylazine (9 mg/kg). The hearts were rapidly excised and cannulated to the Langendorff's apparatus. Each heart was perfused at a constant flow rate of 10 ml/min with Krebs–Henseleit (K-H) buffer (at 37°C, pH 7.4), containing 120 m*M* NaCl, 25 m*M* $NaHCO_3$, 11 m*M* glucose, 4.7 m*M* KCl, 1.2 m*M* KH_2PO_4, 1.2 m*M* $MgSO_4$, and 1.25 m*M* $CaCl_2$, and gassed with a mixture of 95% O_2 and 5% CO_2. The hearts were paced at 300 beats/min by an electrical stimulator (Phipps and Bird Inc., Richmond, VA, U.S.A.). A water-filled latex balloon was inserted into the left ventricle, and the left ventricular end diastolic pressure (LVEDP) was adjusted at 9–10 mm Hg at the beginning of the experiment. The left ventricular developed pressure (LVDP), LVEDP, and the rates of pressure development (+dP/dt) and pressure decay (−dP/dt) were measured via a transducer (model 1050 BP, Biopac System Inc., Goleta, CA, U.S.A.). The data were recorded on-line through an analog-to-digital interface (MP-100, Biopac System Inc.) and stored with a computer program (AcqKnowledge 3.5.3) by a Biopac Data-Acquisition System (Biopac System Inc.). The hearts were perfused for 20 min with K-H buffer for stabilization and were subjected to 30 min of global ischemia by stopping the coronary flow followed by reperfusion for 30 min as described previously (34). Sodium selenite (75 n*M*) (Sigma–Aldrich, Oakville, ON, Canada) solution was infused for 10 min before induction of ischemia, as well as throughout the reperfusion period. In some experiments, different concentrations (25, 50, 75, 100, and 1,000 n*M*) of sodium selenite were used. The selection of 75 n*M* concentration of sodium selenite for all the experimental groups was based on our observation showing maximal improvement of cardiac function in I/R hearts. At the end of reperfusion period, the hearts were frozen in liquid nitrogen and stored at −70°C for biochemical analysis.

In order to examine if the beneficial effects of selenium on I/R injury are due to its antioxidant activity, the hearts were perfused with either xanthine (X) plus xanthine oxidase (XO), an oxyradical-generating system (11), or hydrogen peroxide (H_2O_2), a potent oxidant (21). These hearts were perfused in the absence (control) and presence of sodium selenite (75 n*M*) with either X (2 m*M*) (Sigma–Aldrich) plus XO (0.03 U/ml) (Sigma–Aldrich) or H_2O_2 (150 μ*M*) (Fisher Scientific, Fair Lawn, NJ, U.S.A.) for 20 min; sodium selenite treatment was started 10 min before either X + XO or H_2O_2 perfusions.

For inducing Ca^{2+} paradox, the hearts were perfused with Ca^{2+}-free medium for 5 min followed by perfusion for 30 min with normal K-H medium containing 1.25 m*M* Ca^{2+} as used previously (43). Sodium selenite (75 n*M*) was infused for 10 min before the start of perfusion with Ca^{2+}-free medium and was carried out during the Ca^{2+}-depletion and Ca^{2+}-repletion periods.

Measurement of biochemical parameters

To gain some information regarding the status of oxidative stress, reduced glutathione (GSH), oxidized glutathione (GSSG), and malondialadehyde (MDA) contents were determined in control, I/R, and selenium-treated hearts. For estimation of GSH and GSSG content, a glutathione assay kit (Cayman Chemical, Ann Arbor, MI, U.S.A.), which contained MES buffer, GSSG standard, cofactor mixture, enzyme mixture, and 5,5'-dithiobis-2-nitrobenzoic acid, was used (36). In brief, myocardial tissue was homogenized in 10 ml of cold buffer (50 m*M* MES) per gram of tissue and centrifuged at 10,000 *g* for 15 min at 4°C. The supernatant was deproteinated by using metaphosphoric acid and triethanolamine reagent before assaying. GSSG was determined by derivatizing an aliquot of the supernatant as described by Griffith (15). Absorbance of standards and samples was determined spectrophotometrically (SPECTRAmax® PLUS^{384}, Molecular Devices, Sunnyvale, CA, U.S.A.) at 450 nm. The lipid peroxidation in whole-heart homogenates was determined by using the thiobarbituric acid reactive substances assay kit (ZeptoMetrix Corp., Buffalo, NY, U.S.A.) for the estimation of MDA content (4). To minimize peroxidation during the procedure, 0.01% butylated hydroxytoluene was added to the thiobarbituric acid reagent mixture. Tubes were covered with glass marble and incubated at 95°C for 60 min. After the tubes were cooled to room temperature in an ice bath for 10 min, the samples were centrifuged at 3,000 *g* for 15 min. The developed color was examined spectrophotometrically at 532 nm.

Measurement of NF-κB protein content

The tissue extract for the measurement of protein content of total NF-κB and phospho-NF-κB was prepared by the method described earlier (40). In brief, the ventricular tissue (50 mg) was homogenized (Polytron PT 3000, Brinkmann Instruments, Mississauga, ON, Canada) on ice (at setting 8 for 2 × 30 s with a 30-s interval in between) in 1 ml of buffer A containing 50 m*M* Tris-HCl, 0.25 *M* sucrose, 10 m*M* EGTA, 4 m*M* EDTA, 20 μg/ml leupeptin, and 200 U/ml aprotinin, pH 7.5. The suspension was sonicated for 2 × 15 s with a 30-s interval in between and centrifuged at 100,000 *g* for 60 min in an ultracentrifuge (Model L70, Beckman Instruments, Fullerton, CA, U.S.A.). The supernatant was collected and labeled the cytosolic fraction. The pellet was suspended in 1 ml of buffer B (buffer A + 1% Triton X-100), incubated on ice for 60 min, and centrifuged at 100,000 *g* for 60 min. This supernatant containing dissolved particulate protein was labeled the particulate fraction. Another piece of 50 mg of ventricular tissue was suspended in buffer B, homogenized, and sonicated as above. The homogenate was incubated on ice for 60 min

and centrifuged at 100,000 *g* for 60 min. The supernatant thus obtained was labeled the homogenate fraction.

The immunoblotting analysis of total NF-κB and phospho-NF-κB was performed by separation of 20 μg of protein on a 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The proteins separated by SDS-PAGE were electroblotted to polyvinylidene difluoride membrane by using a transfer buffer containing 25 m*M* Tris-HCl, 192 m*M* glycine, and 20% methanol (vol/vol) for the determination of relative protein content with immunoblot analysis. The transferred membranes were incubated overnight in the blocking buffer, TBST (10 m*M* Tris-HCl, 150 m*M* NaCl, and 0.1% Tween-20) containing 5% nonfat milk powder at 4°C. The membranes were placed at room temperature for 30 min and incubated for 2 h with primary polyclonal antibody against p65 component or phospho-NF-κB polyclonal antibody at Ser^{536} (1:1,000) (Cell Signaling Technology, New England Biolabs, Mississauga, ON, Canada) in 10 ml of blocking buffer with gentle agitation. The membranes were washed three times for 10 min each with 15 ml of TBST and then incubated with secondary antibody (1:10,000 goat anti-rabbit IgG horseradish peroxidase conjugate, diluted in TBST containing 1% fat-free milk) at room temperature for 1 h. Antigen–antibody complexes in all membranes were detected by the chemiluminescence ECL plus kit (Amersham–Pharmacia Biotech, Baie d'Urfe, QC, Canada). An Imaging Densitometer (GS-800, Bio-Rad, Mississauga, ON, Canada) was used to scan the protein band and quantified using the Quantity One Image Analysis Software Version 4.4 (Bio-Rad, Mississauga, ON, Canada). Protein loading was checked in each experiment by staining the membrane with Ponceau S staining before immunoblotting as described previously (3).

Statistical analysis

Values are given as means ± SEM. The differences between two groups were evaluated by Student's *t* test. The data from more than two groups were evaluated by one-way ANOVA followed by Newman–Keul's test. $p < 0.05$ was considered the threshold for statistical significance between the control and the experimental groups.

RESULTS

Left ventricular dysfunction in I/R hearts

The hearts subjected to global ischemia showed marked a decrease in LVDP, +dP/dt, and −dP/dt with a significant increase in LVEDP (Fig. 1) in accordance with our previous studies (29, 34). Reperfusion of ischemic hearts for 30 min recovered the contractile function with respect to LVDP, +dP/dt, and −dP/dt by 10–15% of the control values, whereas the LVEDP was further increased markedly (Fig. 1). The presence of sodium selenite in the perfusion medium at concentrations from 25 to 1,000 n*M* caused a dose-dependent improvement

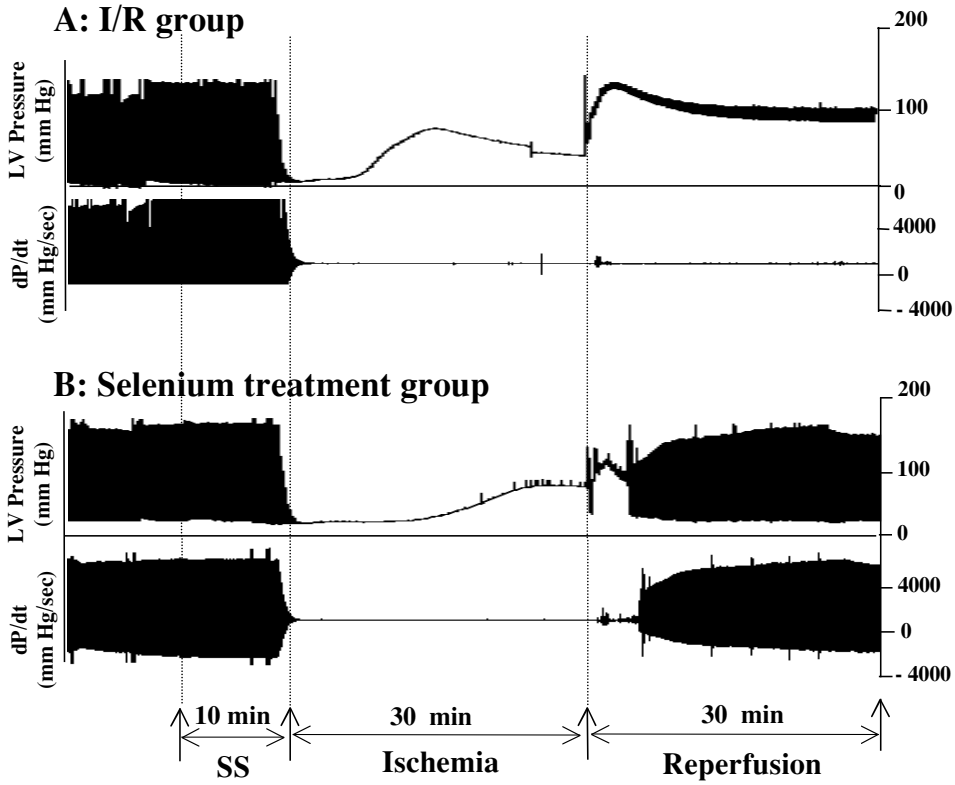

FIG. 1. Representative tracings showing the left ventricular (LV) pressure and change of pressure development (dP/dt) in isolated hearts subjected to I/R without and with sodium selenite (SS) (75 n*M*) treatment. (**A**) Isolated hearts subjected to 30 min of ischemia followed by 30 min of reperfusion. (**B**) Isolated hearts treated with SS 10 min prior to ischemia and during reperfusion period. The exposure times for SS, ischemia, and reperfusion are indicated by the arrows.

of the contractile activity in the I/R hearts (Fig. 2). The sodium selenite treatment elicited a significant recovery in the LVDP, LVEDP, +dP/dt, and −dP/dt at 25 n*M* concentration, whereas maximal beneficial effects were evident at 75 n*M* concentration (Figs. 1 and 2). Although the development of ischemic contracture was delayed in selenium-treated hearts, the maximum contracture developed during ischemia was not significantly different from that in I/R hearts (Fig. 1). Sodium selenite at 1–10 n*M* concentration did not improve the recovery of cardiac function in I/R hearts (data not shown). Therefore, all further experiments were carried out by using 75 n*M* concentration of sodium selenite. It should be mentioned that sodium selenite at 1–1,000 n*M* concentration had no effect on cardiac function of the isolated perfused control heart.

Cardiac dysfunction due to Ca^{2+} paradox-induced injury

A marked depression in LVDP with a subsequent increase in LVEDP was observed during Ca^{2+}-free perfusion for 5 min (depletion phase). As shown in Fig. 3, 30 min of Ca^{2+} perfusion (repletion phase) resulted in a contracture with a further increase in LVEDP with no recovery of LVDP. A significant decrease in +dP/dt and −dP/dt was also observed in hearts subjected to Ca^{2+} paradox (data not shown). Sodium selenite (75 n*M*) pretreatment 10 min before Ca^{2+}-free perfusion and 30 min during the Ca^{2+}-repletion phase of the hearts produced a marked improvement in the recovery of all parameters mentioned above (Fig. 3). The LVEDP increased for 5 min following reperfusion with normal K-H solution (85 ± 12 versus 7 ± 1 mm Hg in the control) and stayed stable around this value until the end of the 30-min reperfusion (95 ± 13 versus 10 ± 2 mm Hg in the control). The LEVDP was significantly decreased by sodium selenite exposure (21 ± 12 and 12 ± 9 mm Hg at 5 min and 30 min of the reperfusion period, respectively) in comparison with the value in the absence of sodium selenite (109 ± 6 and 82 ± 6 mm Hg, respectively). Similarly, sodium selenite exposure had a significant ($p < 0.001$) effect on altered +dP/dt and −dP/dt as these parameters were increased by ~18-fold and ~11-fold, respectively (data not shown).

Cardiac dysfunction due to oxidative stress in control hearts

To test if the protective effects of sodium selenite on I/R-induced heart dysfunction are due to its antioxidant action, the hearts were pretreated with 75 n*M* sodium selenite before exposure to X + XO or H_2O_2. Figure 4A and B show that X +

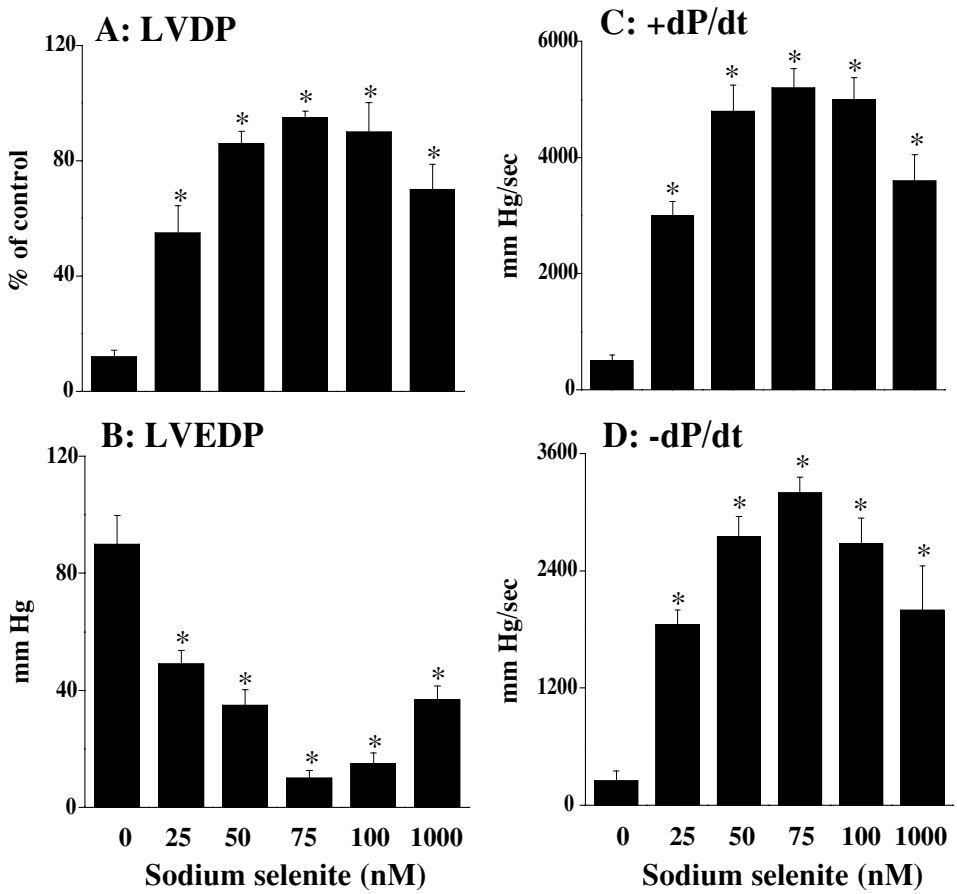

FIG. 2. Dose dependency of sodium selenite (SS) effect on cardiac performance under I/R. LVDP (**A**), LVEDP (**B**), +dP/dt (**C**), and −dP/dt (**D**) are from I/R hearts without and with SS treatment. The hearts were exposed to SS (25–1000 n*M*) 10 min before ischemia and 30 min during reperfusion. Data represent means ± SEM of four to six experiments for each SS concentration. *$p < 0.05$ versus I/R.

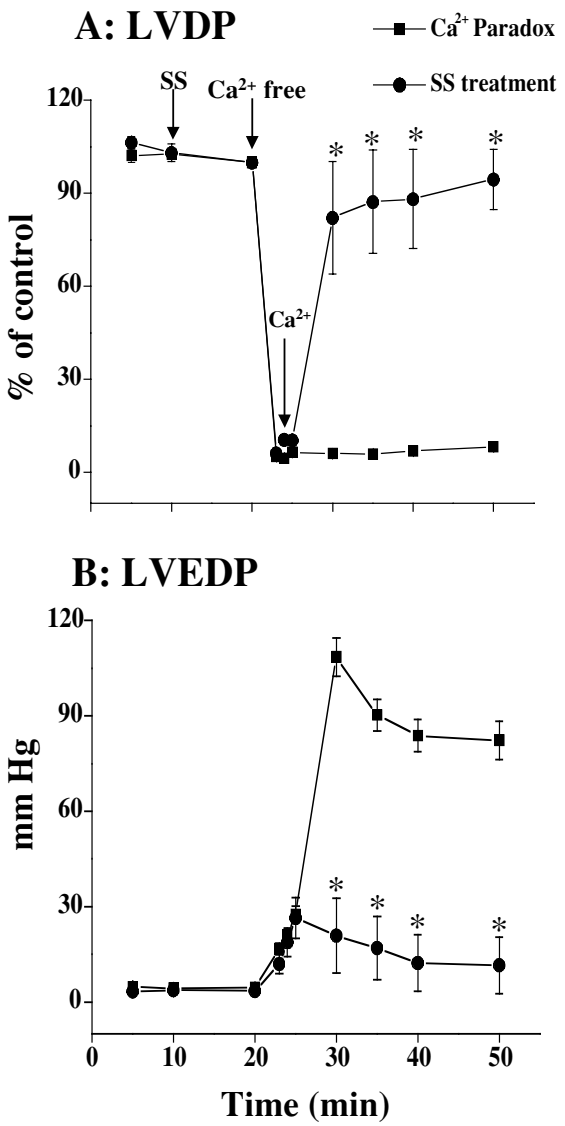

FIG. 3. Effect of sodium selenite (SS) on the mechanical performance of heart in Ca^{2+}-paradox model. Hearts were perfused for 10 min with SS (75 n*M*) before exposure to Ca^{2+}-free solution for 5 min, and then perfusion was continued with Ca^{2+}-containing medium with or without SS for 30 min. The exposure times to SS and Ca^{2+}-free and Ca^{2+}-containing solutions are indicated by the arrows. Each data point represents the mean ± SEM of four to six experiments in each group. (**A**) LVDP (% of the control). (**B**) LVEDP (mm Hg). *$p < 0.05$ versus Ca^{2+}-paradox.

XO perfusion for 20 min induced a seven-fold decrease in LVDP and a 19-fold increase in LVEDP. The sodium selenite treatment was found to exert significant protective effects on alterations in both LVDP and LVEDP. X + XO perfusion decreased +dP/dt by ~50-fold and −dP/dt by ~25-fold at 20 min, whereas in the presence of sodium selenite, these parameters were depressed by ~5-fold and ~4.7-fold, respectively (data not shown). Sodium selenite (75 n*M*) was also found to produce similar beneficial effects in hearts perfused with 150 μ*M* H_2O_2 for 20 min. The LVDP decreased by ~17-fold, whereas the LVEDP increased by ~18-fold by H_2O_2 (Fig. 4C and D); sodium selenite treatment attenuated these alterations. In addition, 20 min of H_2O_2 perfusion decreased +dP/dt by ~29-fold and −dP/dt by ~24-fold, and sodium selenite prevented these changes significantly (data not shown).

Glutathione content and lipid peroxidation in I/R hearts

Cardiac content of MDA, which is indicative of lipid peroxidation (17), increased significantly in the I/R heart with respect to the control perfused group. Sodium selenite (75 n*M*) treatment of the hearts partially, but significantly, attenuated the I/R-induced changes in the MDA levels (Table 1). In addition, I/R significantly decreased the GSH and increased the GSSG contents in cardiac tissue with respect to the control group, leading to a depression in the GSH/GSSG ratio (Table 1), an excellent marker of the redox state, as well as oxidative stress (7). This decrease in GSH/GSSG ratio due to I/R was attenuated significantly by sodium selenite (75 n*M*) treatment (Table 1).

NF-κB protein content in I/R hearts

To gain some information regarding the subcellular distribution of NF-κB, total NF-κB content was measured in cytosolic and particulate fractions of the heart. It can be seen from Fig. 5 that total NF-κB content in the cytosolic fraction was decreased markedly in I/R hearts. Although total NF-κB protein content was also decreased in the particulate fraction of the I/R hearts, this change was less than that seen in the cytosolic fraction. Nonetheless, sodium selenite attenuated these changes in both cytosolic and particulate fractions significantly. The ratio of the protein content of total NF-κB in the particulate to that in the cytosolic fraction was increased in the I/R hearts; sodium selenite treatment decreased this value significantly (Fig. 5). In another set of experiments, the activation of NF-κB protein was studied by measuring the total NF-κB and phospho-NF-κB in the homogenate. The protein content of total NF-κB in homogenate was decreased markedly, whereas the protein content of phospho-NF-κB was increased in the I/R hearts (Fig. 6). The ratio of protein content of phospho-NF-κB to that for the total NF-κB was increased almost 20-fold in the I/R group with respect to the control (Fig. 6). These changes, except for phospho-NF-κB protein content, due to I/R were attenuated by sodium selenite treatment (Fig. 6).

DISCUSSION

One of the important findings of this study is that sodium selenite improved the depressed cardiac performance of the isolated rat heart subjected to I/R injury. This beneficial effect of selenium on I/R-induced changes in LVDP, +dP/dt, −dP/dt, and LVEDP was evident at nanomolar concentrations in a dose-dependent manner. Previous studies have also shown that treatment with selenium prior to the induction of I/R improved the recovery of ventricular contraction during the

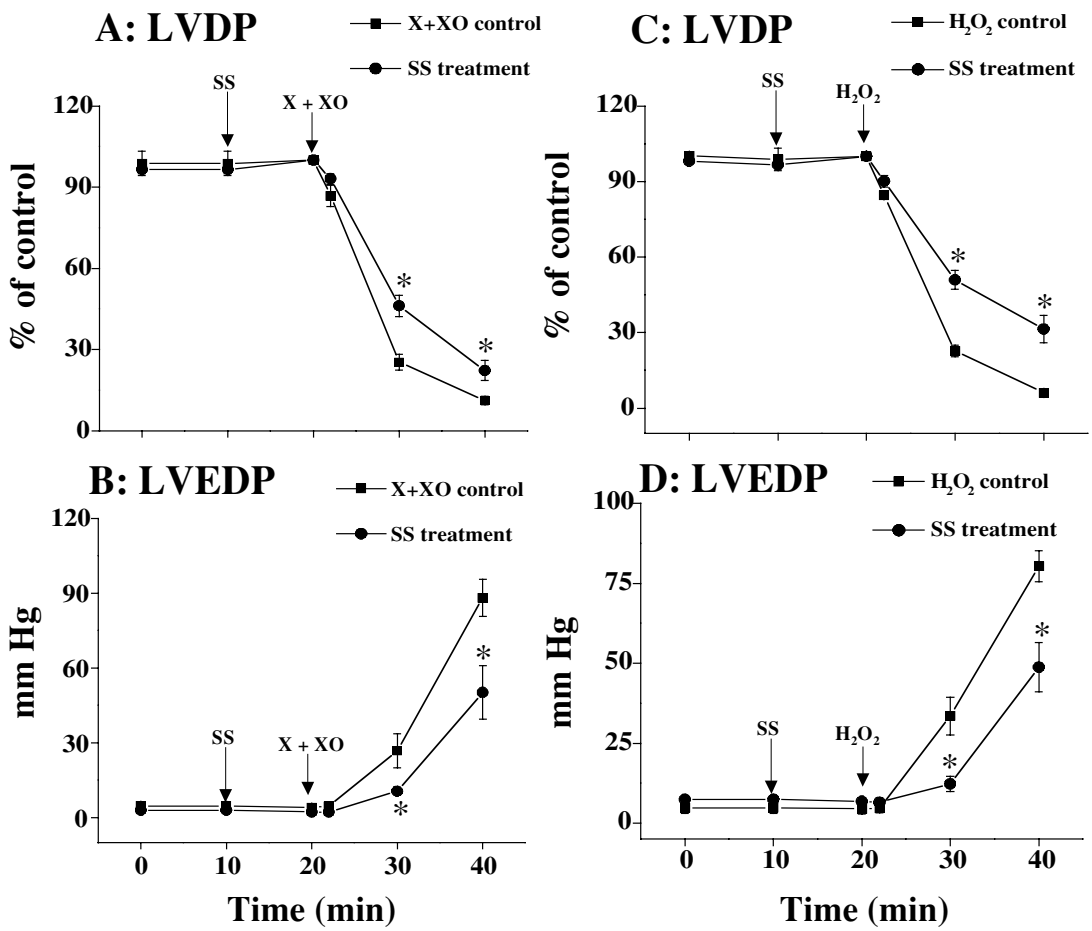

FIG. 4. Effect of sodium selenite (SS) on mechanical performance in hearts perfused with X + XO or H_2O_2. Hearts were perfused for 10 min with SS (75 n*M*) before X + XO or H_2O_2 perfusion, and then continued to be perfused with SS during 20 min of X + XO or H_2O_2 perfusion. The exposure times to SS and X + XO or H_2O_2 are indicated by arrows. Each data point represents the mean ± SEM of four to six experiments in each group. (**A** and **C**) LVDP (% of the control) in hearts treated with X + XO and H_2O_2, respectively. (**B** and **D**) LVEDP (mm Hg) in hearts treated with X + XO and H_2O_2, respectively. *$p < 0.05$ versus X + XO or H_2O_2.

reperfusion period (27) and reduced the myocardial infarct size (31). It should be mentioned that we have not measured changes in coronary flow and heart rate due to selenium in the present study because our experiments were conducted under constant flow rate, as well as continuous pacing of the hearts; however, earlier studies have shown no difference in coronary flow and heart rate in isolated perfused hearts from rats fed with or without a selenium-containing diet (24). The beneficial effects of selenium supplementation have been observed in other organs undergoing I/R injury (25). As I/R-mediated alterations in cardiac function have been reported to occur mainly due to the development of oxidative stress and intracellular

TABLE 1. EFFECT OF SODIUM SELENITE (SS) ON MDA CONTENT AND THE GSH TO GSSG RATIO IN THE I/R HEARTS

Groups	*Control*	*I/R*	*I/R + SS*
MDA (nmol/g wt)	48.7 ± 2.7	68.1 ± 3.6*	60.7 ± 2.9†
GSH content (μmol/g of tissue)	67.4 ± 1.6	45.2 ± 1.3*	55.9 ± 1.7†
GSSG content (μmol/g of tissue)	11.0 ± 0.9	16.3 ± 0.3*	13.2 ± 0.7†
GSH/GSSG	6.1 ± 0.8	2.7 ± 1.2*	4.2 ± 1.9†

Values are means ± SEM of six hearts in each group. Control, I/R, and I/R + SS groups represent 60-min perfused hearts, 30-min global ischemia (I) plus 30-min reperfused (R) hearts, and 10-min SS perfusion prior to I plus 30-min global I plus 30-min R (with SS) hearts, respectively.

*$p < 0.05$ versus control.

†$p < 0.05$ versus I/R group.

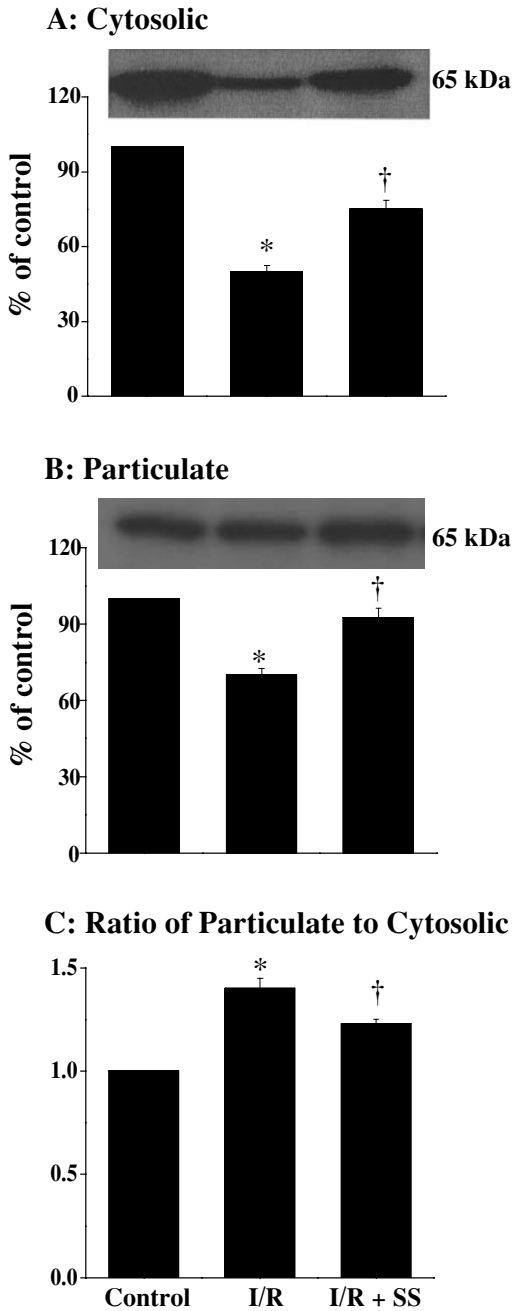

FIG. 5. Effect of sodium selenite (SS) treatment on the protein content of total NF-κB. (**A**) Western blot bands showing the total NF-κB protein content in the cytosolic fraction of the hearts from control, I/R, and SS-treated I/R (I/R + SS) groups. The histographic representation of quantified data (as % of the control) of the total NF-κB content are given below each band. (**B**) The data for total NF-κB content in the particulate fraction of the hearts from the same groups as given in A. (**C**) The ratio of particulate to cytosolic fraction from the hearts representing the mean ± SEM values of four hearts in each group. The concentration of SS was 75 n*M*. **p* < 0.05 versus control; †*p* < 0.05 versus I/R.

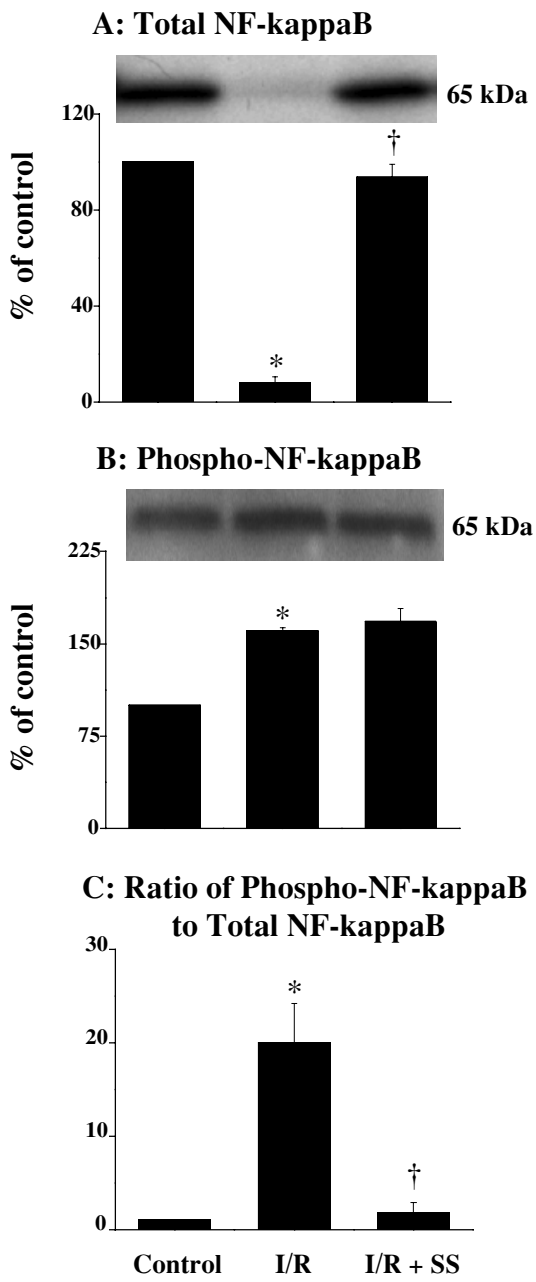

FIG. 6. Effect of sodium selenite (SS) treatment on the protein content for total NF-κB and phospho-NF-κB in homogenate. (**A**) Western blot bands showing the protein contents of total NFκB in the homogenate of hearts from the control, I/R, and SS-treated I/R (I/R + SS) groups. The histographic representations of quantified data (as % of the control) of the total NF-κB content in the homogenates are given below each band. (**B**) The protein contents of phospho-NF-κB in homogenate of hearts from the same groups as shown in A. (**C**) The ratio of phospho-NF-κB to total NF-κB in homogenate from hearts representing the mean ± SEM values of four hearts in each group. The concentration of SS was 75 n*M*. **p* < 0.05 versus control; †*p* < 0.05 versus I/R.

Ca^{2+} overload (1), it is likely that selenium may protect the heart against I/R injury by attenuating the magnitude of both oxidative stress and intracellular Ca^{2+} overload. In this regard, it is pointed out that treatment with selenium was found to reduce the depression in LVDP, as well as the elevation in LVEDP, induced upon perfusing the heart with X + XO, an oxyradical-generating system (11), or H_2O_2, a well-known oxidant (21). Furthermore, selenium was observed to improve the recovery of markedly depressed LVDP and greatly elevated LVEDP in Ca^{2+}-paradox heart, a well-known model of the intracellular Ca^{2+} overload (43). Improvement in ryanodine receptor function, which is known to be depressed during Ca^{2+} paradox (35), may be the mechanism of protection of selenium in hearts subjected to Ca^{2+} paradox. This fact is supported by the previous studies as selenium treatment has been shown to modulate the Ca^{2+} release from isolated sarcoplasmic reticulum (SR) vesicles by stimulating ryanodine receptor (41) and improves the Ca^{2+}-handling ability of ventricular myocardium (38). Nonetheless, the effect of selenium on changes in other Ca^{2+}-handling mechanisms, including sarcolemmal Na^+,K^+-ATPase, Na^+/Ca^{2+} exchanger, Ca^{2+} pump, and Ca^{2+} channels, as well as SR Ca^{2+}-uptake and SR Ca^{2+}-release channels, which are known to be altered during I/R (10, 12, 32, 34, 35), cannot be ruled out on the basis of the present study. It should be pointed out that the effects of selenium in preventing the I/R-induced alterations in cardiac performance appear to be similar to those of ischemic preconditioning, which is known to reduce both oxidative stress and intracellular Ca^{2+} overload (9, 35).

The observed effects of selenium on changes in cardiac function in I/R hearts seem to be due to its antioxidant property. In this regard, it should be noted that the actions of selenium on cardiac performance are simulated by treatment of I/R hearts with superoxide dismutase and catalase, an oxyradical-scavenging mixture (29). As the elevated MDA level, an index of lipid peroxidation (17), and reduced GSH/GSSG ratio, an index of redox state (7), in the I/R hearts were significantly modified by treatment with selenium, these data further support the view that selenium may provide cardioprotection against I/R injury by attenuating the degree of oxidative stress. In fact, selenium supplementation has been shown to preserve the function of GSH-Px and GSH/GSSG ratio in I/R hearts, as well as blood samples collected from the animals undergoing I/R (27, 31). Endogenous activity and mRNA expression of antioxidant enzymes, including thioredoxin reductase and GSH-Px, have also been increased in I/R hearts pretreated with selenium (26). Furthermore, selenium supplementation has been shown to reduce tissue MDA levels in an experimental intestine I/R model (25).

As oxidative stress has been reported to produce subcellular redistribution and activation of NF-κB and antioxidants such as *N*-acetylcysteine have been shown to prevent this alteration (7), it is possible that selenium-induced NF-κB translocation as observed in the present study may be due to its antioxidant action. In fact, the present study has indicated that the elevated ratio of particulate to cytoplasmic levels of NF-κB and the increase in the ratio of phospho- to total NF-κB content, two well-known parameters of NF-κB activation (2), in the I/R hearts were reduced by treatment with selenium. Although total NF-κB contents in homogenate, particulate, and cytosolic fractions were decreased (most probably by leakage from the cell) in I/R hearts, these changes were prevented by selenium. Thus, it appears that selenium not only prevents the activation of NF-κB due to I/R, but may also attenuate the leakage of this transcriptional factor from the I/R hearts. It is also pointed out that NF-κB activation and leakage of NF-κB have also been reported to occur in the Ca^{2+}-paradox model of intracellular Ca^{2+} overload (43). It should be noted that selenium treatment has been shown to reduce the tumor necrosis factor-α (TNF-α)-mediated activation of NF-κB in Jurkat and Esb-LT lymphocytes (22), as well as inhibit the binding of NF-κB to nuclear responsive elements in human T cells and lung adenocarcinoma cells (18). As high amounts of TNF-α has been reported to be synthesized upon activation of NF-κB in I/R and Ca^{2+}-paradox hearts (6, 43), it appears that improvement of cardiac function by selenium treatment in I/R hearts may be due to the reduction in the formation of TNF-α. Thus, the results presented here suggest that selenium may improve the recovery of cardiac function by attenuating the activation of NF-κB. The protective effect of selenium may be due to a decrease in the oxidative stress and as a result of reduction in intracellular Ca^{2+} overload.

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ABBREVIATIONS

+dP/dt, rate of pressure development; −dP/dt, rate of pressure decay; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; I/R, ischemia–reperfusion; K-H, Krebs–Hanseleit; LVDP, left ventricular developed pressure; LVEDP, left ventricular end diastolic pressure; MDA, malondialdehyde; NF-κB, nuclear factor-κB; phospho-NF-κB, phosphorylated NF-κB; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SR, sarcoplasmic reticulum; TBST, Tris-buffered saline with Tween-20; TNF-α, tumor necrosis factor-α; X, xanthine; XO, xanthine oxidase.

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