Modification of alterations in cardiac function and sarcoplasmic reticulum by vanadate in ischemic-reperfused rat hearts

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Vanadate has been observed to produce beneficial effects on cardiac function in chronic diabetes (12, 20, 22). This action of vanadate on diabetic heart has been mainly attributed to its insulinomimetic property of promoting glucose uptake and oxidation in the cell (10, 27, 33) as a consequence of tyrosine kinase stimulation and/or inhibition of phosphorytensive phosphatase activities (5, 34). Vanadate has also been associated with oxidative stress (7), it is likely that attenuation of cardiac dysfunction in diabetes by vanadate may also be related to its antioxidant activity (17, 25). Although vanadate has been shown to scavenge oxyradicals generated by the xanthine plus xanthine oxidase (X + XO) system (17), the antioxidant effect of vanadate remains to be clearly demonstrated. However, in a rat model of myocardial infarction, in which oxidative stress plays a critical role (19), the cytoprotective effect of vanadate has been shown to be associated with improvement in cardiac function, activation of phosphatidylinositol 3-kinase/protein kinase B (Akt), and inhibition of apoptosis by prevention of caspase-3 activation (30).

Recently, vanadate at low concentrations (1–4 μM) was found to attenuate cardiac dysfunction and changes in sarcoplasmic reticulum (SR) Ca2+ uptake and ryanodine receptor (RyR)-binding activities due to ischemia-reperfusion (I/R) in isolated rat hearts (29). However, the results showing the beneficial actions of vanadate in this study (29) were not conclusive, because the experiments were carried out in hearts perfused at constant pressure, which show a mild I/R injury as a consequence of oxidative stress compared with hearts perfused at constant flow (18, 24, 29, 32). In view of the fact that vanadate pretreatment prevents alterations in the heart and in SR function due to mild I/R injury (29), the present study was undertaken to test the hypothesis that vanadate pretreatment would improve the cardiac performance and SR function, as well as SR protein content, induced by global ischemia and reperfusion in isolated rat hearts perfused at constant flow, which are known to exhibit marked changes due to I/R injury (31, 32). Furthermore, the effects of vanadate on I/R-induced alterations were compared with the effects of superoxide dismutase plus catalase (SOD + CAT), which is known to produce an antioxidant effect due to scavenging of oxyradicals (6, 23, 31). Experiments were also carried out to test the effects of vanadate and SOD + CAT on I/R-induced changes in hearts when these interventions were carried out during reperfusion. To gain further information regarding the mechanisms of vanadate action in I/R hearts, the effects of this agent on changes in cardiac performance and SR function in I/R hearts because of its antioxidant action.

ischemia-reperfusion; oxidative stress

METHODS

Isolated rat heart preparation. All protocols were approved by the University of Manitoba Animal Care Committee in accordance with guidelines of the Canadian Council on Animal Care.

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 quickly excised, mounted on a Langendorff apparatus, and perfused with Krebs-Henseleit buffer gassed with 95% O2-5% CO2, 37°C and pH 7.4, at constant flow of 10 ml/min. Composition of the Krebs-Henseleit buffer was (mM) 120 NaCl, 4.7 KCl, 1.2 KH2PO4, 25 NaHCO3, 1.25 CaCl2, and 11 glucose. The hearts were electrically stimulated using a Phipps and Bird stimulator (Richmond, VA) at 300 beats/min via a square-wave 1.5-ms current throughout the experiment. Left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), rate of change of pressure development (+dP/dt), and rate of change of pressure decay (−dP/dt) were measured under control conditions.

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measured via a transducer (model 1050 BP, Biopac System, Goleta, CA), which was connected with a water-filled latex balloon inserted into the left ventricle. At the beginning of the experiment, LVEDP was adjusted to ~10 mmHg by inflation of the balloon, and left ventricular developed pressure (LVEDP) was taken as the difference between LVSP and LVEDP. Data were recorded online through an analog-to-digital interface (model MP-100, Biopac Systems) and stored in a computer program (AcqKnowledge 3.5.3) by a data acquisition system (Biopac Systems). All hearts were stabilized for 30 min and randomly divided into two experimental groups. In both groups, control hearts were perfused for 60 min after stabilization, and I/R in isolated hearts was induced by interruption of the coronary flow for 30 min followed by 30 min of reperfusion. In the first group, sodium orthovanadate (4 μM; Sigma-Aldrich, St. Louis, MO) or an antioxidant mixture containing SOD (0.31 mM equivalent to 5 × 10⁶ U/l; Sigma-Aldrich, Oakville, ON, Canada) and CAT (0.26 μM equivalent to 7.5 × 10⁶ U/l; Fluka Biochemica, Sigma-Aldrich) was infused into the perfusion stream 10 min before 30 min of ischemia and 30 min of reperfusion. The selection of these doses of vanadate or SOD + CAT is based on our previous studies (23, 29) showing maximal improvement of cardiac performance at these concentrations. In the second group, the same concentrations of vanadate or SOD + CAT were infused only during 30 min of reperfusion without preischemic treatment.

In some of the experiments, to examine whether the beneficial effects of vanadate on I/R injury are due to its antioxidant activity, the hearts were perfused with X + XO, an oxyradical-generating system (9), or H₂O₂, a potent oxidant (14). The hearts were perfused in the absence (control) and presence of vanadate (4 μM) with X (2 mM; Sigma-Aldrich, Oakville, ON, Canada) + XO (1.96 mM equivalent to 0.03 U/ml; Sigma-Aldrich) or H₂O₂ (100 μM; Fisher Scientific, Fair Lawn, NJ) for 30 min; vanadate treatment was started 10 min before X + XO or H₂O₂ perfusion and continued throughout the perfusion period. At the end of all treatments, the hearts were frozen in liquid nitrogen and stored at −70°C for further analysis.

Isolation of SR vesicles. SR vesicles were obtained by a method described previously (1). Briefly, the ventricular tissue was pulverized and homogenized twice for 20 s each at 12,000 rpm via a Polytron homogenizer (model PT3000, Brinkmann Instruments, Mississauga, ON, Canada). The homogenization buffer contained 10 mM NaHCO₃, 5 mM Na₃, 15 mM Tris·HCl (pH 6.8), and protease inhibitors (1 μM leupeptin, 1 μM pepstatin, and 100 μM phenylmethylsulfonyl fluoride). The homogenate was centrifuged for 20 min at 9,500 rpm. The resultant pellet was discarded, and the supernatant was further centrifuged for 45 min at 19,000 rpm (model JA 20, Beckman Coulter, Mississauga, ON, Canada). The pellet was suspended in a buffer containing 600 mM KCl and 20 mM Tris·HCl (pH 6.8) and centrifuged at the speed and duration used in the previous step. The final pellet was suspended in 250 mM sucrose and 10 mM histidine buffer (pH 7.0) and used as the SR fraction. The concentration of the SR proteins was determined by the method of Lowry et al. (15). The activities of glucose-6-phosphate, ouabain-sensitive Na⁺/K⁺-AT-Pase, and rotenone-insensitive NADPH cytochrome c reductase in the SR vesicle were measured as described previously (21) to assess cross-contamination.

Measurement of Ca²⁺ uptake. Ca²⁺ uptake of the SR fraction was determined by the Millipore filtration technique employing ⁴⁴Ca²⁺, as described previously (28). The reaction mixture containing (in mM) 100 KCl, 5 MgCl₂, 5 Tris·ATP, 5 potassium oxalate, 5 sodium azide, 0.1 EGTA, 0.1 ⁴⁴CaCl₂ (20 μCi/l), 20 Tris·HCl (pH 6.8), and 25 μM ruthenium red was preincubated for 3 min at 37°C. The reaction was initiated by addition of SR vesicles (0.04–0.08 mg/ml) to the reaction mixture and terminated after 1, 3, 5, and 10 min by passage through 0.45-μm Millipore filters. The filters were washed, dried, and counted in a liquid scintillation counter (Beckman Coulter, Fullerton, CA). The Ca²⁺-uptake reaction was linear during 2 min of the incubation period. Appropriate blanks without ATP or without membrane fraction were included in all experiments, and values for ATP-dependent Ca²⁺ uptake were calculated after corrections for counts on filters due to simple adsorption of isotope and nonspecific Ca²⁺ binding to the membrane.

Measurement of SR Ca²⁺ release. Ca²⁺ release from the ⁴⁴Ca²⁺-loaded SR vesicles was studied according to the procedure described earlier (1, 21, 28). SR protein (0.5 mg/ml) was suspended in a buffer containing (in mM) 100 KCl, 5 MgCl₂, 5 potassium oxalate, 5 Na₃, and 20 Tris·HCl (pH 6.8) and incubated with 10 μM ⁴⁴CaCl₂ (20 μCi/l) and 5 mM ATP for 45 min at room temperature. Ca²⁺-induced Ca²⁺ release was initiated by addition of 1 mM EGTA and 1 mM CaCl₂ to the reaction mixture. The reaction was terminated at 15 s and 1 min by filtration through the 0.45-μm Millipore filter. Filters were washed, dried, and counted in a liquid scintillation counter (Beckman Coulter). SR Ca²⁺ release was completely prevented (95–97%) by treatment of SR preparations with 20 μM ryanodine in all experimental groups.

[^H]ryanodine-binding assay. The status of Ca²⁺ release-channel was determined by [^H]ryanodine binding with SR preparations in the presence of 1, 2, 5, 10, and 40 nM [^H]ryanodine by a procedure described elsewhere (21, 32). Briefly, SR membranes (50 mg) were incubated at 37°C for 60 min in a total volume of 1 ml containing 25 mM imidazole (pH 7.4), 1 M KCl, 1–40 nM [^H]ryanodine, 0.95 mM EGTA, and 1.013 mM CaCl₂. The reaction was terminated by filtration of 0.3 ml of reaction mixture through a 0.45-μm Millipore filter and then washed twice with 5 ml of the washing buffer, dried, and counted in the liquid scintillation counter (Beckman Coulter); the nonspecific binding was determined in the presence of 10 μM ryanodine. The dissociation constant (Kd) and maximum receptor density (Bmax) were calculated by Scatchard plot analysis with use of Prism 4 for Windows (version 4.02, GraphPad Software, San Diego, CA).

Western blot analysis. Western blot analysis was used to determine the protein contents of RyR and sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA2a), as described previously (31). The protein content of each sample was measured by the method of Lowry et al. (15). Protein samples (20 μg for SERCA2a and 25 μg for RyR) were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes for SERCA2a and nitrocellulose membranes for RyR. Membranes were probed with monoclonal anti-RyR and monoclonal anti-SERCA2a antibodies (Affinity Bioreagents). Appropriate secondary antibodies (Affinity Bioreagents) were used, and antigen-antibody complexes were detected by chemiluminescence (ECL plus kit, Amersham-Pharmacia Biotech, Baie d’Urfe, PQ, Canada). An imaging densitometer (model GS-800, Bio-Rad, Mississauga, ON, Canada) was used to scan the protein band; quantification was carried out using Quantity One Image Analysis Software version 4.4. Equal protein loading was checked in each experiment by staining the membrane with Coomassie brilliant blue, as described previously (28).

Statistical analysis. Values are means ± SE. Statistical analysis was performed using Origin version 6 (Microcal Software, Northampton, MA). Differences between the 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-Keuls test. P < 0.05 was considered statistically significant.

RESULTS

IR-mediated alterations of cardiac function. Ischemia for 30 min followed by 30 min of reperfusion caused a marked depression in cardiac function indicated by a significant decrease in LVDP, +dP/dt, and −dP/dt and a marked elevation in LVEDP (Fig. 1). This depression in cardiac function was significantly higher in the constant-flow than in the constant-pressure model, as observed previously (18, 24, 29). The presence of vanadate (4 μM) in the perfusion medium 10 min
before ischemia and during reperfusion elicited a significant recovery in cardiac function, with an increase in LVDP, +dP/dt, and −dP/dt and a decrease in LVEDP (Fig. 1). Similar improvement in these parameters was also observed in hearts treated with the antioxidant mixture containing SOD + CAT. In addition, posts ischemic treatment with vanadate and SOD + CAT has also been shown to prevent I/R-induced alterations in cardiac function (Table 1) similar to those observed before and after treatment.

**Modification of SR function.** Because the SR plays an important role in maintaining Ca$^{2+}$ homeostasis and cardiac contractility (2), its function was determined in control and I/R hearts, as well as in I/R hearts treated with vanadate or SOD + CAT. SR Ca$^{2+}$ uptake and ryanodine-sensitive Ca$^{2+}$ release were significantly reduced in hearts subjected to I/R (Fig. 2, Table 2). Ca$^{2+}$ uptake was depressed in I/R hearts after 3 min of incubation and saturated at 5 min, whereas Ca$^{2+}$ release was attenuated even after 15 s of incubation, with no further decrease after 1 min (Fig. 2, Table 2). Treatment with vanadate (4 μM) before ischemia and during reperfusion caused a significant improvement in SR Ca$^{2+}$ uptake and release in hearts subjected to I/R (Fig. 2, Table 2). Similar improvement in SR Ca$^{2+}$ uptake and release was also observed in hearts treated with vanadate only during reperfusion (Table 1). In addition, treatment of I/R hearts with SOD + CAT (before and after ischemia) as well as after ischemia only, prevented the attenuation in SR Ca$^{2+}$ uptake and release (Fig. 2, Table 2).

**Alterations in ryanodine binding.** Because the release of activator Ca$^{2+}$ from SR occurs by Ca$^{2+}$-induced Ca$^{2+}$ release through RyR (11), specific binding of $[^{3}H]$ryanodine was determined at various concentrations (1–40 nM) of ryanodine. Figure 3 shows a depression in the maximal binding of $[^{3}H]$ryanodine in SR preparations from I/R hearts ($B_{\text{max}} = 0.91 \pm 0.04$ vs. $2.51 \pm 0.13$ pmol/mg for the control group) without

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**Table 1. Effect of posts ischemic treatment of I/R hearts with vanadate or SOD + CAT on cardiac performance and SR Ca$^{2+}$ transport**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>I/R</th>
<th>I/R + Vanadate</th>
<th>I/R + SOD + CAT</th>
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<tbody>
<tr>
<td><strong>Cardiac performance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LVDP, mmHg</td>
<td>120 ± 5.6</td>
<td>36 ± 2.8*</td>
<td>82 ± 5.9†</td>
<td>78 ± 4.3†</td>
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<tr>
<td>LVEDP, mmHg</td>
<td>9.3 ± 0.7</td>
<td>71 ± 4.9*</td>
<td>39 ± 2.5†</td>
<td>32 ± 2.8†</td>
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<tr>
<td><strong>Ca$^{2+}$ transport</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>Ca$^{2+}$ uptake, nmol Ca$^{2+}$·mg$^{-1}$·min$^{-1}$</td>
<td>21.8 ± 0.64</td>
<td>8.5 ± 0.71*</td>
<td>14.2 ± 1.7†</td>
<td>14.0 ± 0.8†</td>
</tr>
<tr>
<td>Ca$^{2+}$ release, nmol Ca$^{2+}$·mg$^{-1}$·15 s$^{-1}$</td>
<td>7.1 ± 0.32</td>
<td>1.8 ± 0.14*</td>
<td>4.0 ± 0.26†</td>
<td>5.2 ± 0.43†</td>
</tr>
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</table>

Values are means ± SE of 4 hearts in each group. Vanadate (4 μM) or superoxide dismutase (SOD, 0.31 mM) + catalase (CAT, 0.26 μM) was present in reperfusion medium for 30 min after ischemia. I/R, ischemia-reperfusion; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; SOD + CAT, superoxide dismutase + catalase; SR, sarcoplasmic reticulum. *P < 0.05 vs. control. †P < 0.05 vs. I/R.
any change in $K_d$ (3.20 ± 1.71 vs. 3.55 ± 2.12 nM for the control group). Treatment with vanadate during, before, and after ischemia attenuated the I/R-induced depression in SR $[^3H]$ryanodine binding ($B_{\text{max}} = 1.44 \pm 0.09 \text{ pmol/mg}$, $P < 0.05$; Fig. 3). Similarly, SOD + CAT treatment also prevented the I/R-mediated attenuation of $[^3H]$ryanodine binding ($B_{\text{max}} = 1.83 \pm 0.17 \text{ pmol/mg}$, $P < 0.05$; Fig. 3).

Alterations in SR protein content. To investigate the underlying mechanisms of the cardioprotective effect of vanadate, the protein content of the SR was determined by Western blot analysis. A significant reduction in protein contents of SERCA2a as well as RyR was observed in I/R hearts (Fig. 4), which is in agreement with our previous observations (32). Treatment with vanadate in I/R hearts significantly prevented the attenuation of SERCA2a and RyR protein content (Fig. 4). Additionally, SOD + CAT prevented the decrease in protein content of SERCA2a and RyR in hearts subjected to I/R (Fig. 4).

Cardiac dysfunction and SR defects due to oxidative stress in control hearts. To test further whether the protective effects of vanadate on I/R-induced heart dysfunction are due to its antioxidant action, the hearts were treated with vanadate (4 μM) for 10 min before exposure to $XO$ or $H_2O_2$ and during 30 min of perfusion with these agents. Data in Table 3 indicate that $XO$ or $H_2O_2$ perfusion for 30 min induced a significant decrease in LVDP, $+dP/dt$, and an increase in LVEDP. Vanadate (4 μM) treatment exerted significant protective effects on cardiac performance and attenuated the depressed SR Ca$^{2+}$ uptake and release due to $XO$ or $H_2O_2$ perfusion (Table 3).

DISCUSSION

The present study employing the isolated hearts perfused at constant flow exhibited a marked depression in LVDP, $+dP/dt$, $dP/dt$, and $dP/dt$, which were attenuated by treatment with vanadate or SOD + CAT during, before, and after ischemia. These results suggest that vanadate has a cardioprotective effect on I/R-induced heart dysfunction, possibly through its antioxidant action.

Table 2. Effects of vanadate and SOD + CAT on I/R-induced Ca$^{2+}$ release from the SR in rat heart

<table>
<thead>
<tr>
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<th>Ryanodine-Sensitive Ca$^{2+}$ Release, nmol Ca$^{2+}$/mg protein</th>
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<tbody>
<tr>
<td></td>
<td>15 s 1 min</td>
</tr>
<tr>
<td>Control</td>
<td>7.0±0.45 10.6±0.64</td>
</tr>
<tr>
<td>I/R</td>
<td>2.6±0.17* 3.9±0.25*</td>
</tr>
<tr>
<td>I/R + vanadate</td>
<td>5.7±0.38* 7.1±0.36†</td>
</tr>
<tr>
<td>I/R + SOD + CAT</td>
<td>5.9±0.41† 7.8±0.47†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 experiments in each group. Vanadate (4 μM) or SOD (0.31 mM) + CAT (0.26 μM) was present in perfusion medium 10 min before ischemia and 30 min during reperfusion. Pre + Post, pre- and postischemic treatment. *$P < 0.05$ vs. control. †$P < 0.05$ vs. I/R.
Effect of vanadate on H2O2-induced or XO-induced changes in cardiac performance and SR Ca2+ transport

Table 3. Effect of vanadate on H2O2-induced or X + XO-induced changes in cardiac performance and SR Ca2+ transport

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>H2O2</th>
<th>H2O2 + Vanadate</th>
<th>X + XO</th>
<th>X + XO + Vanadate</th>
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<tr>
<td><strong>Cardiac performance</strong></td>
<td></td>
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<tr>
<td>LVDP, mmHg</td>
<td>121±7.1</td>
<td>15.9±1.4*</td>
<td>162±4.4†</td>
<td>13.4±1.3*</td>
<td>56±3.7†</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>8.3±0.4</td>
<td>74±3.8*</td>
<td>56±2.3†</td>
<td>81±2.8*</td>
<td>42±1.8†</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>2.850±148</td>
<td>102±7.5*</td>
<td>1.571±78†</td>
<td>94±4.4*</td>
<td>1,482±67†</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>2.284±156</td>
<td>84±6.2*</td>
<td>1,490±65†</td>
<td>78±3.8*</td>
<td>1,406±55†</td>
</tr>
<tr>
<td><strong>Ca2+ transport</strong></td>
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<tr>
<td>Ca2+ uptake, nmol·mg⁻¹·min⁻¹</td>
<td>20.1±0.74</td>
<td>10.2±1.23*</td>
<td>16.7±0.92†</td>
<td>9.4±0.66*</td>
<td>15.4±0.43†</td>
</tr>
<tr>
<td>Ca2+ release, nmol·mg⁻¹·15 s⁻¹</td>
<td>7.2±0.56</td>
<td>2.3±0.26*</td>
<td>5.4±0.12†</td>
<td>1.8±0.14*</td>
<td>4.9±0.14†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 hearts in each group. X + XO, xanthine + xanthine oxidase; +dP/dt, rate of pressure development; −dP/dt, rate of pressure decay. Concentrations of X, XO, H2O2, and vanadate were 2 mM, 1.96 mM, 100 μM, and 4 μM, respectively. Vanadate treatment was started 10 min before X + XO or H2O2 perfusion and continued throughout 30-min perfusion. *P < 0.05 vs. control. †P < 0.05 vs. respective H2O2 or X + XO.

Fig. 4. Effect of pre- and postischemic treatment with vanadate or SOD + CAT on Ca2⁺-ATPase (A) and ryanodine receptor (RyR, B) protein content in SR. Western blot bands are shown. Both bands for RyRs were taken into account in density reports. Values are means ± SE of 4 preparations in each group. SERCA2a, sarco/endoplasmic reticulum Ca2⁺-ATPase. *P < 0.05 vs. control. #P < 0.05 vs. I/R.

and −dP/dt with a significant increase in LVEDP due to I/R. These findings are in agreement with our previous observations using the same experimental model (23, 28, 31, 32). The observed functional changes in I/R hearts perfused at constant flow were markedly greater than those in hearts perfused at constant pressure (18, 24, 29). Because Ca2⁺ overload and oxidative stress are the two mechanisms proposed to explain I/R-induced cardiac injury (3), it is possible that such differences in these experimental models may be due to disparity in the extent of injury caused by these two pathways. However, the exact mechanisms explaining the differences in these two models remain to be examined. In contrast to the hearts perfused at constant pressure (29), the results of the present study show marked improvement in cardiac function with respect to I/R-induced changes in LVEDP, LVDP, +dP/dt, and −dP/dt by vanadate treatment before ischemia and during reperfusion. Because postischemic treatment of hearts with vanadate also produced effects similar to those of treatment before and after ischemia, it is possible that a major portion of the action of vanadate may be elucidated during the reperfusion period. Because vanadate at the concentration used in the present study has also been shown to have a positive inotropic effect in isolated control rat hearts (26), this mechanism may contribute to the beneficial effect of vanadate in I/R hearts. Although vanadate treatment reduced the depression in cardiac function in hearts perfused with X + XO, an oxyradical-generating system (9), or H2O2, a potent oxidant (14), it is likely that vanadate may protect the heart by decreasing the magnitude of oxidative stress during I/R injury. Although vanadate has been shown to promote glucose uptake and oxidation (10, 27, 33), the beneficial effect of vanadate in I/R hearts may not be related to increased glycolysis. This view is supported by our previous observations indicating prevention of X + XO-induced loss of contractile force and increase in resting tension by vanadate treatment in glucose-free medium (17). Vanadate treatment in diabetic animals reduced the decreases in the activities of SOD, CAT, and glutathione peroxidase, as well as glutathione content (25), which are known to serve as antioxidant defense mechanisms (6). Moreover, X + XO-induced depression in sarcolemmal Ca2⁺-pump and Na⁺/Ca2⁺ exchange activities was prevented by vanadate (17). Additionally, X + XO-mediated production of superoxide radicals, as detected by electron paramagnetic resonance spectroscopy, was also attenuated by vanadate treatment (17). Nonetheless, the participation of other mechanisms, such as activation of Akt, prevention of fodrin breakdown, and inhibition of apoptosis by prevention of caspase-3 activation during I/R (30), cannot be ruled out on the basis of the present study.
Furthermore, the protective effect of vanadate in I/R may be associated with preservation of myocardial energy metabolism in terms of increase in myocardial contents of ATP, creatine phosphate, and cytosolic phosphorylation, as observed in diabetic hearts (20).

SR Ca\(^{2+}\) uptake, Ca\(^{2+}\) release, and protein contents of SR RyR and SERCA2a were reduced in I/R hearts. Similar alterations in Ca\(^{2+}\) handling, as well as protein contents, in SR have also been shown previously (32). The protective effects of vanadate in I/R-induced myocardial injury seem to be associated with improvement in Ca\(^{2+}\) handling at the SR level, because the I/R-mediated alterations in SR function were attenuated by vanadate treatment. Alterations in SR function that are similar but of less magnitude were seen in our previous study as a result of vanadate treatment in I/R hearts perfused at constant pressure (29). Because changes in SR function have been shown to explain I/R-induced cardiac dysfunction (31, 32), it is likely that the protective effect of vanadate on SR during I/R may be related to the beneficial effects of vanadate on cardiac performance. Vanadate treatment also prevented the attenuation of protein contents of SERCA2a and RyR due to I/R. Moreover, vanadate has been found to inhibit ATP-dependent Ca\(^{2+}\) uptake in isolated cardiac SR vesicles (26). In addition, the I/R-induced decrease in \(^{[3H]}\)ryanodine-binding sites (B\(_{\text{max}}\)) was reduced by vanadate treatment. Because changes in SR function are mainly determined by SR Ca\(^{2+}\) uptake, Ca\(^{2+}\) release, and protein content of Ca\(^{2+}\)-cycling proteins (31, 32), it appears that the effect of vanadate on SR function may be related to the attenuation of I/R-induced changes in SR protein content. In addition, this action of vanadate may be associated with its free radical-scavenging property, inasmuch as treatment with vanadate prevented the attenuation of SR function in hearts perfused with X + XO or H\(_2\)O\(_2\). Moreover, the effect of vanadate on SR Ca\(^{2+}\) function and protein content was similar to that of an antioxidant mixture containing SOD + CAT. Although in the study of Temsah et al. (32) SOD + CAT did not prevent the decrease in SERCA2a and RyR protein content in I/R hearts, such conflicting results seem to be due to the duration of SOD + CAT treatment during reperfusion. In this regard, SOD + CAT was present throughout the reperfusion period, in contrast to the previous study (32), where SOD + CAT was present only for half of the reperfusion period.

In conclusion, the present study reveals that the beneficial effects of vanadate in improvement of cardiac performance and SR function in I/R hearts may be related to its antioxidant property. However, some caution must be exercised in interpretation of these results with regard to the effects of vanadate on I/R hearts, inasmuch as vanadate has been shown to increase lactate dehydrogenase release, an index of cell death (4), in ischemic neonatal rat cardiomyocytes and increase their susceptibility to cell death by blocking tyrosine phosphatases and preventing inactivation of p38 mitogen-activated protein kinases (16). Moreover, the antioxidant activity of vanadate is questioned by the observation that vanadate has been shown to cause production of free radicals (13), with subsequent activation of transcriptional activation protein-1 in mouse epithelial JB6P+ cells, and these alterations were prevented by antioxidants such as N-acetylcysteine, as well as SOD + CAT (8). In addition, vanadate enhanced H\(_2\)O\(_2\)-triggered apoptosis in rat mesangial cells (35). On the other hand, in mouse C127 cells, vanadate-induced expression of actin and c-Ha-ras mRNA was unaffected by oxidants, redoxants, and antioxidant enzymes (36). Differences in the availability of vanadate species, concentration of vanadate, animal species, and type of the preparations, as well as experimental conditions, may be the reason for these discrepancies. Nonetheless, the present study seems to support the view that vanadate may exert beneficial effects in I/R hearts in a manner similar to that seen in hearts subjected to myocardial infarction (30) or diabetes (20).

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