Effect of estrogen on global myocardial ischemia-reperfusion injury in female rats

PEIYONG ZHAI, THOMAS E. EURELL, ROBERT COTTHAUS, ELIZABETH H. JEFFERY, JANICE M. BAHR, AND DAVID R. GROSS

1Department of Veterinary Biosciences, 2Department of Food Science and Human Nutrition, and 3Department of Animal Sciences, University of Illinois, Urbana-Champaign, Illinois 61802

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Zhai, Peiyong, Thomas E. Eurell, Robert Cotthaus, Elizabeth H. Jeffery, Janice M. Bahr, and David R. Gross. Effect of estrogen on global myocardial ischemia-reperfusion injury in female rats. Am J Physiol Heart Circ Physiol 279: H2766–H2775, 2000.—We investigated the effects of estrogen on global myocardial ischemia-reperfusion injury in rats that were ovariectomized (Ovx), sham-operated, or ovariectomized and then given 17b-estradiol (E2b) supplementation (Ovx+E2b). Hearts were excised, cannulated, perfused with and then immersed in chilled (4°C) calcium-free Krebs-Henselit bicarbonate buffer for 120 min. The coronary flow rate, first derivative of left ventricular pressure, and nitrite production were not consistently different throughout the entire reperfusion period. Ca2+ accumulated more in Ovx rat hearts than in sham-operated or Ovx+E2b hearts, and mitochondrial respiratory function was lower in Ovx hearts than in sham-operated or Ovx+E2b hearts. However, coronary flow rates or nitrate production were not consistently different throughout the entire reperfusion period. The mechanisms by which E2b may exert cardioprotective effects during ischemia-reperfusion are unclear. E2b was reported to improve endothelium-dependent vasodilatation (2), decrease endothelin-1 gene expression and peptide secretion (1), and antagonize Ca2+ influx into vascular smooth muscle cells (22). In experimental models of regional myocardial ischemia-reperfusion, E2b was reported to upregulate the glutathione/glutathione disulfide redox system (26), diminish hydroxyl radical production (33), inhibit tumor necrosis factor-α production, and limit intercellular adhesion molecule-1-mediated binding of leukocytes to injured myocardium (41). E2b was also reported to inhibit polymorphonuclear neutrophil infiltration and subsequent harmful mediator release (8). Although the cardioprotective effects of endogenous and exogenous estrogen on regional myocardial ischemia-reperfusion have received extensive attention, less work has been directed toward elucidating the possible role of estrogen in global, cardioplegia-protected, myocardial ischemia and reperfusion. These experiments were designed to 1) demonstrate whether estrogen plays a protective role in global, cardioplegia-protected myocardial ischemia followed by reperfusion and, if so, 2) gain information about potential mechanisms of the cardioprotective effect.

MATERIALS AND METHODS

Experimental animals. All experiments involving animals were approved by the Institutional Animal Care and Use Committee of the University of Illinois and were conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Female Sprague-Dawley rats (3 mo old) were purchased and fed with standard rat chow for 3 mo. The 6-mo-old rats were divided into three groups and fed with a standard casein-based diet or supplemented with estrogens as described below.

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based diet (AIN-76 B-40, ICN Pharmaceuticals) that does not contain phytoestrogen. Two weeks later, one group of rats (n = 10) was ovariectomized (Ovx). The rats were anesthe-
tized with ketamine (20 µg/g ip) and xylazine (0.5 µg/g ip). The skin was prepared, and a lateral abdominal incision was made. The ovaries were isolated and removed along with the oviduct. The abdominal incision was then closed with stain-
less steel wound clips. The animals were monitored in the laboratory until fully recovered from anesthesia and were then put back to the animal care facilities, where they were observed daily. One group (n = 10) was sham operated. The anesthesia and the operative procedure were the same as for the Ovx group except that the ovaries were exposed but not removed. Another group (n = 10) was ovariectomized simi-
larly to the Ovx group, but circulating estrogen concentration was restored by a 17β-estradiol capsule implanted subcuta-
neously (Ovx+E2). Experimental protocol. Rats were anesthetized with ket-
amine (20 µg/g ip) and xylazine (0.5 µg/g ip) and treated with 1,000 units of heparin (intraperitoneally). The heart was quickly removed, weighed, and mounted on a perfusion ap-
paratus. Cardioplegic solution [Plegisol (Abbott Labs) plus 25 mM NaHCO3 and 2 U/ml heparin], pH 7.4 at 4°C, was infused through an aortic catheter into the coronary arteries with the use of a speed-control roller pump at a constant rate of 0.3 ml/min for 5 min at pressures not exceeding 80 mmHg. A balloon-tipped catheter was inserted into the left ventricle (LV) and secured. Infusion of the cardioplegic solution was then stopped, and the heart was immersed in the same cardioplegic solution for a total ischemia time of 30 min. The heart was then mounted in a Langendorff-type isolated heart perfusion system and subjected to 2 h of retrograde coronary artery reperfusion with oxygenated Krebs-Henseleit bicarbo-
nate buffer (Sigma), pH 7.4 at 37°C, at a constant pressure of 120 cmH2O. LV pressure (LVP) was continuously mea-
sured from data continuously recorded during the reperfusion period. Coronary effluent volume was measured at the various time intervals for a total of 120 min. Coronary flow rate (CFR, in ml.min-1.g-1) was de-
fined as the total volume collected during the reperfusion interval divided by the time, normalized by the heart wet weight (g), which was measured at the beginning of the experiment. Measurement of coronary flow rate. Estimation of myocardial Ca2+ accumulation. The Ca2+ concentrations in both coronary perfusate and effluent were measured by inductively coupled plasma atomic emis-
sion spectrometry at the end of each reperfusion period. Myocardial Ca2+ accumulation (µmol/g) was estimated ac-
cording to the Fick principle and was calculated from the difference in Ca2+ concentration (µM) between perfusate and effluent multiplied by the coronary effluent volume (ml) di-
vided by heart wet weight (g). Measurement of nitrite concentration in coronary effluent. Light microscopy. The heart was fixed by immersion in 10% neutral buffered Formalin. Serial sections (6 µm) were made parallel to the atrioventricular groove. Standard hema-
toxylin-eosin (H-E) or hematoxylin-basic fuchsin-picric acid (HBFP) stains (29) were used for histomorphological evalu-
atation. Four digital images of each sample were randomly taken for morphometric analysis using NIH Image software. The contrast, threshold, and magnification of all the images were identical for each stain method. The percentage of myocardium with a positive HBFP stain was calculated. The volume fraction of interstitial space (VFITS) in myocardial tissue was determined from H-E-stained sections by using the equation VFITS = (100% × area of interstitial space/ total tissue area). Ultrastructure study. Small tissue blocks (~1 mm3) were cut from the LV free wall, fixed in Karnovsky’s fixative for 24 h at room temperature, and stored at 4°C until processed. The sample was postfixed in osmium tetroxide, dehydrated in a graded series of alcohols, treated with propylene oxide, and embedded in epoxy. After polymerization, 0.5-µm sections were postfixed in osmium tetroxide, dehydrated in a graded series of alcohols, treated with propylene oxide, and embedded in epoxy. After polymerization, 0.5-µm sections were cut and stained with toluidine blue. The samples were examined using a transmission electron microscope (Hitachi H-600). Estimation of myocardial Ca2+ accumulation. The Ca2+ concentrations in samples of coronary perfusate and effluent were measured by inductively coupled plasma atomic emis-
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were examined under light microscopy, and representative areas of tissue samples were chosen for ultrathin sectioning (0.1 μm). The ultrathin sections were mounted on uncoated copper grids, stained with uranyl acetate and lead citrate, and examined with a Hitachi 600 Transmission Electron Microscope. Four negative films per sample were randomly taken for quantitative analysis. The films were scanned to obtain digital images that were then analyzed using NIH Image software. The mitochondrial cross-sectional area was measured. The number of fragmented mitochondria, the number of mitochondria with amorphous matrix densities or granular densities, and the total number of mitochondria studied in each group were counted.

Statistical analysis. Data were presented as means ± SE and first analyzed with the use of a two-way ANOVA for repeated measures or a single-factor ANOVA as appropriate. If significant differences were observed, Dunnett’s t-test was applied to compare differences between groups and differences between measurements at 15 min and at other time periods within groups. The statistic analyses were done by running appropriate SAS procedures (SAS Institute, Cary, NC). All proportions were compared using a chi-square test. The α level was set at 0.05, and adjustment was made to control experimentwise type I error where appropriate.

RESULTS

Plasma estradiol concentration. The plasma estradiol concentration averaged 60 ± 6 pg/ml in the sham group. The average plasma estradiol concentration of the Ovx group was 27 ± 1 pg/ml, which is statistically significantly lower than that of the sham group (P < 0.0001). The average plasma estradiol concentration from the Ovx+E2β group was 49 ± 2 pg/ml, which is significantly higher than that of the Ovx group (P < 0.0000001) but not significantly different from that of the sham group (P > 0.05).

LV function. During reperfusion after 30 min of ischemia, LV function was much better when estrogen was present (Fig. 1). The LV dP/dt of hearts from sham-operated rats that had endogenous estrogen was significantly higher than that of Ovx rats throughout the 120 min of reperfusion, except between 30 and 60 min. The LV dP/dt of hearts from Ovx+E2β rats that have circulating concentration of estrogen restored with a 17β-estradiol subcutaneous implant was significantly higher than that of Ovx hearts throughout the 120 min of reperfusion. Even though the LV dP/dt was significantly decreased in all three groups after 60 min of reperfusion, the LV dP/dt of sham-operated or Ovx+E2β hearts was still higher than that of Ovx hearts.

CPR. CPR was significantly improved in sham-operated or Ovx+E2β hearts during reperfusion (Fig. 2). The CPR of sham-operated hearts was significantly...
higher than that of Ovx hearts within 60 min of reperfusion. The CFR of Ovx+E2β hearts was significantly higher than that of Ovx hearts throughout 2 h of reperfusion. After 45 min of reperfusion, CFR decreased in all three groups, but Ovx+E2β hearts still had a higher CFR than Ovx hearts did.

**Nitrite production.** Nitrite production of sham-operated and Ovx+E2β hearts was significantly higher and decreased later than that of Ovx hearts (Fig. 3). Estimated nitrite production of sham-operated hearts was significantly higher than that of Ovx hearts. Estimated nitrite production of Ovx+E2β hearts was significantly higher than that of Ovx hearts. Nitrite production significantly decreased from 30 min of reperfusion in Ovx hearts, after 60 min of reperfusion in sham-operated hearts, and after 45 min of reperfusion in Ovx+E2β hearts.

**Myocardial Ca2⁺ accumulation.** Estimated myocardial Ca²⁺ accumulation during the first 15 min of reperfusion of Ovx hearts was significantly higher than that of sham-operated hearts but was not significantly different from that of Ovx+E2β hearts (Fig. 4A). Estimated myocardial Ca²⁺ accumulations during the next 15 min and at 45–60 min of reperfusion of Ovx hearts were significantly higher than those of Ovx+E2β hearts (Fig. 4A). In the sham group, Ca²⁺ was taken up during the first 15 min of reperfusion. This Ca²⁺ was then apparently exported, and very little was accumulated during the rest of the measurement periods. In the Ovx group, Ca²⁺ accumulated during most of the reperfusion periods. In the Ovx+E2β group, significant Ca²⁺ accumulation was accumulated during the first 15 min of reperfusion. Ca²⁺ was then apparently washed out, resulting in what appeared to be negative accumulation. The overall Ca²⁺ homeostasis (average of the sum of various time periods within groups) during reperfusion was accumulation in Ovx hearts but was approximately balanced in sham-operated and Ovx+E2β hearts (Fig. 4B).

**Myocardial MTT extraction.** After ischemia-reperfusion, myocardial MTT reduction in Ovx hearts was significantly lower than that in sham-operated hearts, and it was also lower than that in Ovx+E2β hearts (Fig. 5). These results indicate that Ovx hearts had more severe impairment of mitochondrial respiratory function than sham-operated or Ovx+E2β hearts after ischemia-reperfusion.

**Myocardial histology.** After ischemia-reperfusion, marked myocardial damage was found in Ovx hearts. The VFITs in Ovx hearts was significantly higher than that of sham-operated or Ovx+E2β hearts. This suggests prominent interstitial edema in Ovx hearts after ischemia-reperfusion. Myocardial contraction bands were also evident in Ovx heart samples (Fig. 6). Damaged myocytes, detected by a positive HBFP stain, were found in all groups, but the extent of damaged cells (those that did not exclude the stain) were more prominent in Ovx hearts. The percentage of myocardium with a positive HBFP stain in Ovx hearts was
significantly higher than that of sham-operated in Ovx hearts (Fig. 7).

**Myocardial ultrastructure.** After ischemia-reperfusion, Ovx hearts showed marked ultrastructural damage. Ovx heart samples (Fig. 8A) had a marked loss of characteristic myofibrilar structure, clear areas of sarcoplasmic space resulting from intracellular edema, loss of normal structure, and severely damaged mitochondria with prominent granular densities and amorphous matrix densities compared with sham-operated (Fig. 8B) or Ovx+E2β hearts (Fig. 8C). The mitochondrial densities may represent aggregation of proteins (such as denatured enzymes) and/or deposition of Ca2+ and phosphate (19, 27, 39). In the Ovx group, the mitochondria were markedly swollen, with an average mitochondrial size significantly greater than that in hearts in the sham or Ovx+E2β groups (Fig. 9A). The percentage of mitochondria with granular densities and amorphous matrix densities in Ovx heart samples was significantly greater than that in sham-operated or Ovx+E2β heart samples (Fig. 9B). Many more fragmented mitochondria were found in Ovx heart samples (Fig. 9C).

**DISCUSSION**

In studies of regional ischemia-reperfusion injury, administration of E2β was reported to markedly decrease myocardial necrosis (8), lower the incidence of ventricular arrhythmias, and preserve ventricular function (25). Estrogen replacement in ovariectomized rats was shown to improve LV contractile function in isolated hearts subjected to 15 min of global ischemia followed by 20 min of reperfusion (28). These studies indicated that estrogen plays a protective role in cardiac ischemia-reperfusion. Data presented in this study indicate that LV function was better during 120-min reperfusion after 30-min global hypothermic

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**Fig. 5.** Myocardial reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) after 30 min of ischemia followed by 120 min of reperfusion. The myocardial MTT reduction in sham-operated or Ovx+E2β hearts is significantly higher than that in Ovx hearts. OD, optical density. *Significantly different from Ovx group.

**Fig. 6.** Hematoxylin-eosin stain of myocardium after 30 min of ischemia followed by 120 min of reperfusion. A: sham-operated hearts. B: demonstrations of marked interstitial edema, myofibril destruction, and evidence of contraction bands (arrow) in Ovx hearts. C: demonstrations of occasional, focal loss of myocardial cytosol structure, and very slight interstitial edema in Ovx+E2β hearts. Scale bars, 50 μm. D: volume fraction of interstitial space. Values are means ± SE. *Significantly different from Ovx group.
ischemia and that myocardial damage was less severe after ischemia-reperfusion in hearts from sham-operated rats or Ovx rats with $E_2$ supplementation than in hearts from unsupplemented Ovx rats.

One potential mechanism of the cardioprotective effect of estrogen is through enhanced NO production from endothelial and/or myocardial cells. In our study, Ovx rat hearts had decreased nitrite production (i.e., NO release) compared with hearts from either sham-operated or Ovx + $E_2$ rat hearts. In previous studies, $E_2$ was shown to enhance the activity of NO synthase (NOS-3), and thereby NO production, in human umbilical vein endothelial cells (14). Also, $E_2$ increased NOS-3 gene expression in the rat aorta (12) and increased NOS-3 protein in human aortic endothelial cells (17). Estrogen was also reported to stimulate NO production by activating inducible NOS in isolated coronary artery smooth muscle cells (7). Other mechanisms of improvement of NO production by $E_2$ may include activating second messenger systems and tyrosine kinase or inhibiting the function of NO-degrading systems (24). The basal release of NO from isolated working rat hearts was reduced by ischemia-reperfusion (9). Endothelial NO synthase activity was decreased during ischemia and only partially restored during reperfusion (11). Inhibition of NO synthesis was demonstrated to impair postischemic recovery of function in isolated rat hearts (3, 35). All these studies suggest a protective role of endogenous NO during ischemia-reperfusion. Under these circumstances, NO may improve myocardial perfusion by mediating a rapid recovery of coronary flow (35). Physiological concentrations of estrogen were reported to decrease paracellular permeability of human umbilical endothelial cells via NO-related mechanisms (5). Infusion of $E_2$ into coronary artery was reported to increase NO production, thereby protecting myocardium against regional ischemia-reperfusion injury (34). Chronic administration of $E_2$ to Ovx rats was reported to increase the $Ca^{2+}$-independent NOS activity and improve postischemic LV work in hearts isolated from the same animals (10). In agreement with these findings, our study demonstrated that, in association with the impaired NO production, impaired LV systolic function, decreased CFR, and marked myocardial edema were present in Ovx rat hearts.

Inhibition of $Ca^{2+}$ accumulation during ischemia-reperfusion may be another mechanism of cardiopro-

![Fig. 7. Hematoxylin-basic fuchsin-picric acid (HBFP) stain of myocardium after 30 min of ischemia followed by 120 min of reperfusion. A: sham-operated hearts. B: positive stain (arrow) was shown in a large number of cells, indicating extensive myocardial damage in Ovx hearts. C: stain was not taken up by a majority of cells, although some uptake (arrow pointing to a positive stain area) indicates myocardial damage in Ovx + $E_2$ hearts. Scale bars are 50 μm. D: percentage of myocardium with a positive stain. Values are means ± SE. *Significantly different from Ovx group.](http://ajpheart.physiology.org/)

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ESTROGEN EFFECTS ON ISCHEMIA-REPERFUSION

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tection by estrogen. Our data demonstrated that rat hearts lacking significant estrogen exposure accumulated significantly more Ca\(^{2+}\) than rat hearts exposed to estrogen. Although it is possible that Ca\(^{2+}\) may also accumulate in vascular endothelial or smooth muscle cells in addition to myocardial cells, we do not have any data to confirm or deny this possibility. Our electron microscopic results indicated that Ca\(^{2+}\) may have accumulated in myocardial cells (Figs. 8B and 9B). It is true that changing Ca\(^{2+}\) concentrations in coronary effluent reflects changes of interstitial Ca\(^{2+}\). It is also true that water-soluble substances, including Ca\(^{2+}\), diffuse very rapidly across intercellular junctions in the capillaries to maintain an equilibrium. It is for this reason that we consider the Ca\(^{2+}\) concentrations in the coronary effluent to reflect the entire myocardial extracellular space. Changes in Ca\(^{2+}\) concentrations in coronary effluent are not totally due to changes in effluent water content or coronary flow.

E\(_2\beta\) has been reported to transiently decrease the inward Ca\(^{2+}\) current and intracellular free Ca\(^{2+}\) in ventricular myocytes (20) and to specifically inhibit L-type Ca\(^{2+}\) channel currents (4). Estrogen was shown, during ischemia-reperfusion, to modify the function of a genetically overexpressed Na\(^+\)/Ca\(^{2+}\) exchanger (6). E\(_2\beta\) (10 \(\mu\)M) has previously been demonstrated to prevent K\(^+\)-induced Ca\(^{2+}\) intracellular loading in isolated guinea pig cardiac myocytes (23). Our study and others suggest that estrogen may play a role in modulating these Ca\(^{2+}\) channels and/or exchangers. This may be important because Ca\(^{2+}\) channels and exchangers are probably involved in Ca\(^{2+}\) overload during ischemia-reperfusion (36). Results from our electron microscopy studies indicate that some of the accumulated Ca\(^{2+}\) may have deposited in myocardial mitochondria. Ca\(^{2+}\) overload in the mitochondria and cytosol is believed to have several harmful effects on myocardial

![Fig. 8. Transmission electron microscopy of myocardium after 30 min of ischemia followed by 120 min of reperfusion. A: sham-operated hearts. B: in Ovx hearts, most of the mitochondria are markedly abnormal in shape and greater in size, with abnormal cristae or areas of loss of matrix. In some mitochondria, the cristae and matrix are cleared out, resulting in what appears to be vacuoles (v). Mitochondrial membranes are occasionally disrupted (r). Dense granular densities (gd) and amorphous matrix densities (amd) are visible within some mitochondria, suggesting mineral deposition. C: myofibrils are intact in Ovx+E\(_2\beta\) hearts. Mitochondria are slightly abnormal in shape, but most have generally distinct cristae and normal matrix. Original magnification, \times10,000; scale bars, 1 \(\mu\)m.

![Fig. 9. Quantitative ultrastructural changes of myocardial mitochondria after 30 min of ischemia followed by 120 min of reperfusion. A: mitochondrial cross-sectional area. B: percentage of mitochondria with granular densities and amorphous matrix densities from all the mitochondria counted in each group. C: percentage of disrupted mitochondria from all the mitochondria counted in each group. *Significantly different from Ovx group.]
cells. It depletes ATP by activating Ca\textsuperscript{2+}-activated ATPases and inhibiting high-energy phosphate production in mitochondria, degrades cellular membrane systems by activating phospholipases and lipases, and accelerates oxygen free radical production via the endothelial xanthine oxidase system (44). In agreement with these findings, our results indicated that hearts with minimal estrogen exposure when subjected to ischemia-reperfusion contained more myocardial contraction bands, more severe myofibrilar destruction, and more prominent mitochondrial damage than hearts with estrogen when subjected to identical ischemia-reperfusion.

Estrogen may also protect the myocardium against ischemia-reperfusion injury by preserving mitochondrial structure and function. A reduction in energy production by mitochondria in vivo is reflected by a decrease in tissue high-energy phosphate content. The latter has been shown to correlate with the recovery of cardiac function at reperfusion (38). In isolated rat hearts, decreased mitochondrial function and reduced ATP and creatine phosphate content were demonstrated to correlate with myocardial ischemic contracture during normothermic ischemia (40). In another isolated rat heart model, hypothermia and high-K\textsuperscript{+}, high-Mg\textsuperscript{2+} cardioplegia together were shown to maintain a higher level of ATP and creatine phosphate in heart tissue than hypothermia alone (16). In addition, functional recovery was better in the hypothermia plus cardioplegia group, demonstrating an additive protective role of hypothermia and cardioplegia during myocardial ischemia. The whole purpose of hypothermic, cardioplegic arrest is to provide myocardial protection during global ischemia. This model is quite different from normothermic ischemia in the quantity and quality of protection afforded the ischemic tissue. Possible beneficial effects of estrogen in a normothermic, constantly perfused, beating-heart ligation model with blood reperfusion are not addressed by our experimental paradigm.

In our study myocardial MTT reduction, an indirect indicator of mitochondrial respiratory function, was significantly lower in hearts without estrogen than in hearts with estrogen after ischemia-reperfusion. MTT is a tetrazolium salt that can be reduced by active mitochondrial enzymes. Two sites on the mitochondrial electron transport chain, coenzyme Q and cytochrome c, are thought to catalyze the reduction of MTT to formazan, which accumulates in the endosomes and lysosomes or is exported by exocytosis (31). MTT formazan can be extracted by permeabilizing the cell with agents such as DMSO and isopropanol. In our study, the significant dysfunction observed in myocardial mitochondria from the Ovx group with increased granular densities, which are thought to be due to Ca\textsuperscript{2+} deposition (39), and the amorphous matrix densities, which presumably are an aggregation of denatured proteins (such as enzymes) (19) or Ca\textsuperscript{2+} deposits containing lipids (27). These dense inclusions could substantially impair cellular respiratory function because mitochondrial Ca\textsuperscript{2+} overload has been reported to decrease ATP synthesis (37). In addition to denaturation of enzymes, the substantial loss of mitochondrial enzymes because of the loss of cristae, which provide most of the capacity for oxidation and phosphorylation, may also contribute to mitochondrial dysfunction in Ovx rat hearts subjected to ischemia-reperfusion. The impaired mitochondrial function, Ca\textsuperscript{2+} accumulation, and other changes probably form a vicious circle that leads to progressive myocardial damage.

We acknowledge the limitations of this study. Although the isolated heart perfusion system is widely used in studies of global myocardial ischemia-reperfusion, denervation of the heart and the lack of blood perfusion make the model distinct from in situ conditions. Neutrophils especially have been shown to have harmful effects on ischemia-reperfused myocardium (8). Eliminating the influence of neutrophils confines the interpretation of the experimental results but also helps dissect out the function of other factors, for example, NO and Ca\textsuperscript{2+} during ischemia-reperfusion. Hypothermia and high-K\textsuperscript{+}, high-Mg\textsuperscript{2+} cardioplegia solution have been widely used in open-heart surgery, and this was the focus of our original hypothesis. High-K\textsuperscript{+} cardioplegia previously demonstrated an increase in Ca\textsuperscript{2+} uptake in isolated cardiac cells (22). Estrogen was reported to inhibit this adverse effect of the high-K\textsuperscript{+} cardioplegia (23). Since all three groups in this study were subjected to identical protocols, we believe that the influence of other factors were well controlled and that we were able to focus on estrogen effects on a limited number of variables. An obvious next experimental protocol would be to repeat these experiments by reperfusing with autologous blood.

In conclusion, estrogen may play a protective role in global myocardial ischemia-reperfusion in females. Our experimental results suggest that the hearts of Ovx rats are associated with more severe myocardial damage and cardiac dysfunction following ischemia-reperfusion injury than hearts of either intact female rats exposed to endogenous estrogen or Ovx female rats administered exogenous estrogen. The actions of estrogen in myocardial ischemia and reperfusion appear to be 1) improving NO release, 2) attenuating myocardial Ca\textsuperscript{2+} accumulation, and 3) preserving mitochondrial structure and function.

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