Protective Effects of Estrogen Against Reperfusion Arrhythmias Following Severe Myocardial Ischemia in Rats

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Background: Female sex hormones may have protective effects against arrhythmias, including reperfusion arrhythmias (RAs), but the mechanisms are not still completely known.

Methods and Results: Serial changes in rat hearts (rhythm, apoptosis and the its influencing factors; cardiac vinculin mRNA expression and connexin43 (Cx43) dephosphorylation) were examined during periods of ischemia–reperfusion with and without estrogen treatment. After reperfusion, although the incidence of arrhythmias became higher in both the vehicle-group and estrogen-group, compared with the ischemia period, estrogen prevented reperfusion-induced upregulation of the incidence of arrhythmias, especially ventricular premature beats (VPB) and ventricular tachycardia (VT). The duration of VT and fibrillation, and the number of VPB and VT, were all significantly decreased in the estrogen-group. The expression of cardiac vinculin mRNA decreased significantly in the vehicle-group but not in the estrogen-group. Cx43 dephosphorylation and myocyte apoptosis increased in both groups, but the values for the estrogen-group were all markedly lower than those for the vehicle-group. A selective estrogen receptor (ER) β agonist prevented reperfusion-induced upregulation of the incidence of both VPB and VT significantly; a selective ERα agonist had no significant influence.

Conclusions: Estrogen can protect the heart against RAs, at least in part, mediated through gap junctions. Upregulation of ERβ but not ERα mediated most of the estrogen-induced cardioprotection against RA. (Circ J 2010; 74: 634–643)

Key Words: Estrogen; Gap junction; Reperfusion arrhythmia

Reperfusion arrhythmia (RA) is a specific phenomenon that can be specifically and reproducibly induced immediately after reperfusion. Clinically, it can result from many therapies, such as coronary artery bypass grafting, thrombolysis, and percutaneous coronary angioplasty, which have already become standard techniques in patients with myocardial ischemic disease. RA can be regarded as an indicator for myocardial reperfusion and successful recanalization of an occluded artery. However, life-threatening ventricular arrhythmias, such as ventricular tachycardia (VT) and ventricular fibrillation (VF), remain the most important causes of sudden cardiac death (SCD) in humans.

Epidemiological studies revealed that women appear to have a lower incidence of arrhythmia-related SCD than men, and that postmenopausal women are much more prone to SCD than premenopausal women. Animal experiments revealed that estrogen could attenuate ischemia- or reperfusion-induced ventricular arrhythmias, which suggests that female sex hormones may have protective effects against arrhythmias, including RA. Although the biological effects of estrogen in males are less well defined than in females, a growing body of clinical evidence demonstrates that endogenous estrogen (17β-estradiol) may protect cardiovascular health not only in female but also in male patients.

The mechanisms by which estrogen may exert cardioprotective effects against RA during ischemia–reperfusion (I/R) are unclear. Because estrogen actions are mediated through its cognate receptors, studying the function of estrogen receptors (ERs) may be helpful to elucidation of the mechanisms of estrogen. Traditionally, estrogen mediates its physiological effects by binding to intracellular ERs (ERα and ERβ) that function as ligand-modulated nuclear transcription factors.

In the context of this study, our goal was to establish for the first time the effects of chronic estrogen and selective...
ER agonist supplementation on gap junctions (GJs) and the influential factors in RAs following severe ischemia in male rats.

**Methods**

The present study required the use of: DW-2000 animal respirator (Shanghai Jiapeng Technology Co, China); ECG-6511 (Shanghai Photoelectricity Medical Electronic Apparatus Co, China); GALIBOR flow cytometer (American BD Co); 17β-estrogen (Sigma Co); 4,4’,4”-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT; a selective ERα agonist, American Cayman Co); 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN; a selective ERβ agonist, American Cayman Co); ICI 182,780 (ER antagonist, American Enzo Co); One-step RT-PCR kits (Takara Dalian, China); DAB coloring reagent kit (Pierce Biotechnology, Rockford, IL, USA); eNOS kit (Nanjing Jiancheng Biotechnology Institute, China); Orthotopic apoptosis (TUNEL) kit (American BD Co); 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN, a selective ERβ agonist, American Cayman Co); ICI 182,780 (ER antagonist, American Enzo Co); One-step RT-PCR kits (Takara Dalian, China); DAB coloring reagent kit (Pierce Biotechnology, Rockford, IL, USA); eNOS kit (Nanjing Jiancheng Biotechnology Institute, China); Mem-PER membrane protein extraction kit (Pierce Biotechnology, Rockford, IL, USA); HRP-conjugated rat antitransferrin, which was then centrifuged for 15 min at 1,479 g. The acquired ECG was displayed and analyzed as previously reported. The percentage incidence of arrhythmias, numbers of ventricular premature beats (VPB) and VT, the duration of VT and VF, and cases of SCD because of RAs throughout the experiment were recorded and compared between the vehicle and estrogen groups.

**Intracellular Ca²⁺ Measurement by Flow Cytometry**

Myocardium was homogenized and made into 10% homogenate, which was then centrifuged for 15 min at 1,479 g. Finally, the supernatant was used for detecting intracellular calcium [Ca²⁺]-levels with the Ca²⁺-sensitive fluorescent probe Fluo-3-acetoxymethyl ester (Fluo-3/AM) in the GALIBOR flow cytometer. The reaction is followed by colorimetric assay (λexcitation = 488 nm, λemission = 525 nm; n=5).

**Detection of Reactive Oxygen Species (ROS) Generation by Fluorescence Assay**

After being homogenized, the myocardial supernatant was used for detecting ROS. Myocytes (1×10⁶/ml) were preincubated for 20 min with 5 μmol/L of DCFH-DA in a water bath at 37°C with horizontal agitation. DCFH-DA diffuses into cells and is hydrolyzed into nonfluorescent 2’-7’-dichlorofluorescin (DCF). The DCF fluorescence distribution of 20,000 cells was analyzed by flow cytometry (λexcitation = 488 nm, λemission = 525 nm; n=5).

**Measurement of Endothelial Nitric Oxide Synthase (eNOS) Activity**

The eNOS activity was measured by NOS colorimetric assay kit (Nanjing Jiancheng Biotechnology Institute), which determines eNOS activity by enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colo-
Rimetric detection of nitrite as an azo dye product (540 nm) of the Griess reaction. The levels of eNOS were calculated according to the manufacturer’s instructions (n=5).

RNA Isolation and Semiquantitative Reverse Transcriptase (RT)-Polymerase Chain Reaction (PCR) Analysis
Total RNA was extracted from cardiomyocytes (2×10⁶ cells/ml) using the TRIZOL Reagent according to the manufacturer’s directions. For semi-quantitative 1-step RT-PCR analysis for vinculin, 1 μg of total RNA was used as the template in RT-PCR with the forward and reverse primers of vinculin in the kit (TaKaRa Dalian, China) with the following program: RT at 30°C for 10 min, 42°C for 1 h, 99°C for 5 min, and 5°C for 5 min; PCR at 94°C for 2 min, followed by 33 cycles of amplification (94°C for 1 min, 48°C for 45 s, and 72°C for 1 min), and by extension at 72°C for 7 min. The primers for the target and housekeeping genes (GAPDH, performed as the controls) are summarized in Table. After amplification, the samples were separated on 2% gel agarose, visualized, and quantified by Labworks-Analyst (Gene Co) software.

**Figure 1.** Representative ECGs of ischemia–reperfusion arrhythmias. (A) AVB and bigeminy of VPB. PR interval is approximately 0.16 s (normal value =0.02–0.05 s with 180–520 beats/min), so it is obviously more prolonged than normal, P<0.05. VPB appears to be coupling with QRS; (B) Wenckebach phenomenon of AVB. PR interval approximately (1) 0.084 s, (2) 0.104 s, (3) 0.112 s, (4) 0.128 s, (5) without QRS; (C) Atrioventricular dissociation. Each PR interval differs from the other, but the PP intervals or RR intervals are the same. The shapes of 2 VPB are different; (D) nonsustained VT; (E) VF and QRS waves change from beat to beat in rate and configuration. AVB, atrioventricular block; VF, ventricular fibrillation; VPB, ventricular premature beats; VT, ventricular tachycardia.
Western Blot Analysis of Connexin43 (Cx43) Dephosphorylation

Following the method of Rakotovao et al., membrane proteins of each sample were extracted from cardiomyocytes at a density of 2×10⁶ cells/ml using the Mem-PER membrane protein extraction kit (Pierce Biotechnology) according to the manufacturer’s directions. The quality of membrane protein was assessed by biophotometer (Eppendorf). 50 μg of each sample was separated on 12% polyacrylamide gels and transferred to PVDF membranes (Millipore). The blots were

Figure 2. Ischemia–reperfusion arrhythmias and SCD. (A) Tracing from both groups after 8-min reperfusion. The duration of VT is longer in (a) the vehicle group than in (b) the estrogen group. (B) Incidence of both arrhythmias and mortality in both groups during the whole period. Rats were subjected to 20-min coronary occlusion and 30-min reperfusion. The incidences of AVB, VPB, VT, VF, and SCD are shown. (C) Duration of VT and VF, which occurred in the occlusion and reperfusion periods in both groups. (D) Number of VPB during the occlusion or reperfusion period in surviving rats. (E) Incidence of mortality during the reperfusion period: 16% in the vehicle-group and 4% in the estrogen-group (NS). Compared with the corresponding points in the vehicle-group: †P<0.05, ‡P<0.01; compared with the ischemia point of each group: ☆P<0.05, ★P<0.01 (veh-group, n=21 vs E-group, n=24). AVB, atrioventricular block; SCD, sudden cardiac death; VF, ventricular fibrillation; VPB, ventricular premature beats; VT, ventricular tachycardia.
then blocked with 5% non-fat-milk at 37°C for 1 h. Subsequent-
ly, they were washed three times for 10 min with Tris-buffered
saline (TTBS), and incubated with an anti-dephosphorylated
Cx43 mouse monoclonal antibody (Zymed) diluted to a con-
centration of 1/1,500 in TTBS for 1 h at room temperature.
The blots were then washed three times for 10 min with TTBS,
and incubated with a horse radish peroxidase-conjugated rat
anti-mouse polyclonal antibody (Santa Cruz) diluted to a
concentration of 1/2,000 in TTBS for 1 h at room tempera-
ture. After 3 more 10-min washes with TTBS, the blots were
developed with the ECL Plus kit (Pierce Biotechnology).
Signal analysis was performed by exposure of the blots to
films, which were then scanned and band intensities were
measured with Labworks-Analyst (Gene Co) software.

Measurement of Myocardial Apoptosis (In Situ End-Labeling
TUNEL Assays)
Cardiomyocytes were fixed with 4% paraformaldehyde at
25°C for 30 min and washed 3 times with phosphate-buffered
saline (PBS). Terminal deoxynucleotidyl transferase reaction
solution was added to the cells for 60 min at 37°C. After
being washed with PBS, the cells were incubated with a fluo-
rescein isothiocyanate-tagged anti-biotin monoclonal anti-
body (1:500) at 37°C for 1 h and then observed by fluorescent
microscopy. Peroxidase was transformed with substrate at
37°C for 30 min; finally, slices were stained with DAB and
counterstained lightly with hematoxylin. The samples without
the addition of enzyme were regarded as positive controls.
Apoptosis was identified according to the following criteria:
green fluorescence indicated apoptotic cells by fluorescein
isothiocyanate; the nuclei of cardiomyocytes that were stained
brown-yellow by DAB were regarded as apoptosis positive;
otherwise, they were nonapoptosis cells. The percentage of
apoptotic cells was calculated as the number of DAB-posi-
tive cells/total number of cells counted per slide×100%.

Statistical Analysis
The results were analyzed by SPSS 15.0 (Chicago, IL, USA).
Data are expressed as arithmetic means±SEM of the num-
ber (n) of experiments. Samples were analyzed with repeated
measures analysis of variance; differences in the incidences
of arrhythmias were analyzed using Fisher’s exact test (dou-
ble-tail). P<0.05 was considered statistically significant.

Results
Representative ECG Tracings of I/R Arrhythmia
After coronary artery occlusion, all animals exhibited car-

Figure 3. Variations in intracellular calcium [Ca^{2+}], ROS and eNOS of cardiomyocytes treated with and without 17β-estrogen
during 20-min ischemia and 30-min reperfusion. Ratio of (A) [Ca^{2+}], (B) intracellular ROS generation, (C) eNOS activity. Com-
pared with the ischemic point of each group, †P<0.05, ‡P<0.01; compared with the corresponding points of vehicle group,
§P<0.05. Data represent means±SEM, n=5 for all. eNOS, endothelial nitric oxide synthase; ROS, reactive oxygen species.
Diastolic arrhythmias, which occurred as VPBs, VT or VF. Atrioventricular block (AVB) also occurred. To better understand these arrhythmias, some typical ECGs during the I/R periods are shown here (Figure 1).

Protective Effects of Estrogen

SCD The incidence of occlusion-induced AVB, VPB, VT, and VF was 9.5%, 19.0%, 9.5%, and 0.0%, respectively, in the vehicle-group; and 12.5%, 16.7%, 16.7%, and 0.0% in the estrogen-group. There were no significant differences in the AVB, VPB or VF incidence between the vehicle and estrogen groups (vehicle-group, n=21 vs estrogen-group, n=24. Figures 2A, B). The duration of VT in the estrogen-group (74.8±12.2 s) was higher than in the vehicle-group (58.7±13.2 s) but without significance. The numbers of VPB and VT were not significantly different between groups (Figures 2C, D). During the ischemia period, VF or SCD did not occur in any rats.

On subsequent reperfusion, in the vehicle-group the incidences of AVB, VPB, VT, and VF increased to 38.1%,
85.7%, 57.1%, and 9.5%, respectively, and increased to 20.8%, 54.2%, 25.0%, and 4.2%, respectively, in the estrogen-group. Compared with the vehicle-group, the incidences of VPB and VT in the estrogen-group were significantly reduced (P<0.05). The incidences of AVB and VF also decreased but without significance (Figure 2B). The duration and number of arrhythmias all significantly increased compared with their respective occlusion periods. Furthermore, the duration of both VT and VF, and the number of VPB and VT in the estrogen-group (443.0±82.4 s, 178.0±9.9 s, 247.7±60.2 s, and 263.6±39.1 s, respectively) were significantly different from those of the vehicle-group (586.3±123.6 s and 258.7±40.7 s, 433.2±142.8 s and 390.1±91.2 s, respectively) (Figures 2C, D).

In total, 4 of 25 (16%) vehicle rats died from reperfusion-induced irreversible VF, and 1 of 25 (4%) in the estrogen-group. They were excluded from the analysis of arrhythmias and only included in the SCD statistics (Figure 2E).

Variations in [Ca^{2+}], ROS, and eNOS After reperfusion, [Ca^{2+}] and ROS were significantly increased in both groups compared with their ischemia points (P<0.01 and P<0.05 respectively). Figures 3A, B shows that treatment with 17β-estradiol significantly inhibited the elevated intracellular concentration of Ca^{2+} and ROS induced by reperfusion (n=5, P<0.05 for all). During the reperfusion period, the eNOS activity of both groups decreased significantly nearly in parallel (P<0.01 for all), but from ischemia to reperfusion, the values for the estrogen-group were always higher than those of the vehicle-group (n=5, Figure 3C).

Expression of Vinculin mRNA, Cx43 Dephosphorylation and Myocardial Apoptosis Treated With and Without Estrogen During I/R After being ligated for 20 min, compared with the normal points of each group, the expression of cardiac vinculin mRNA markedly declined in the both the vehicle-group and estrogen-group (P<0.05 for all, n=5 for each point, Figure 4A), but Cx43 phosphorylation (n=5) showed no significant variation (Figure 4B). There was no difference in myocardial apoptosis between the groups (Figure 4C). After reperfusion for 30 min, the expression of cardiac vinculin mRNA decreased significantly in the vehicle-group (P<0.01) but not in the estrogen-group.
Dephosphorylation and Apoptosis

Effects on RAs, Vinculin Gene Expression, Cx43 contraction.

Evidence demonstrates that endogenous estrogen may promote the heart in males than in females, a growing body of clinical data indicates that the cardioprotective effects of estrogen are more pronounced in females. Among the female patients, the incidence of VPB and VT, which were 40% and 10%, respectively, in the DPN group (n=10) was significantly lower than in the estrogen-group (P<0.01 and P<0.05, respectively). Furthermore, administration of the estrogen agonist, DPN, prevented the reperfusion-induced upregulation of the incidence of VPB and VT, which were 40% and 10%, respectively, in the DPN group (n=10). There was no significant difference in these parameters between the DPN group and estrogen-group. In order to evaluate whether the cardioprotective effect of DPN was via the ER, a group of rats were treated with DPN and ICI 182,780 (ER antagonist). Administration of ICI 182,780 abolished the DPN-induced attenuation of the incidence of VPB and VT following reperfusion (70% and 30%, respectively, P<0.05). In addition, DPN treatment significantly increased the expression of vinculin in the estrogen-group (vehicle-group, n=21 vs estrogen-group, n=24, P<0.05, Figure 5A). The ERα agonist, PPT, had no significant influence on reperfusion-induced upregulation of the expression of vinculin and VT (n=10). The incidence of VPB and of VT in the PPT group was 80% and 40%, respectively (Figure 5A), both of which were significantly higher than in the estrogen-group (P<0.01 and P<0.05, respectively). Furthermore, administration of the ERβ agonist, DPN, prevented the reperfusion-induced upregulation of the incidence of VPB and VT, which were 40% and 10%, respectively, in the DPN group (n=10). There was no significant difference in these parameters between the DPN group and estrogen-group. In order to evaluate whether the cardioprotective effect of DPN was via the ER, a group of rats were treated with DPN and ICI 182,780 (ER antagonist). Administration of ICI 182,780 abolished the DPN-induced attenuation of the incidence of VPB and VT following reperfusion (70% and 30%, respectively, n=10). In addition, DPN treatment significantly inhibited myocardial apoptosis and Cx43 dephosphorylation (P<0.05 for all), and restored vinculin mRNA expression compared with the vehicle-group, but these effects could be abolished by the administration of ICI 182,780. Meanwhile, compared with the vehicle-group, no significant changes in these parameters were observed in reperfusion rats treated with PPT, but PPT also decreased Cx43 dephosphorylation even without significance (P=0.073). In order to abolish the per se effect of ICI 182,780, we administered it alone. There was no significant difference in the incidence of VPB or VT, vinculin expression, apoptosis or Cx43 dephosphorylation between the vehicle-group, DPN+ICI group and ICI alone group (Figure 5).

Discussion

Although reperfusion is essential for preventing irreversible cellular injury and preserving ventricular function, reperfusion and the attendant recovery from ischemia-induced metabolic, ionic, electrical, and signaling transduction changes cause ventricular arrhythmias, cellular injury, and sudden death. Accumulating experimental and clinical evidence indicates that estrogens are actively involved in protecting the cardiovascular system against ischemic injury and RA. Although the biological effects of estrogen are less well defined in males than in females, a growing body of clinical evidence demonstrates that endogenous estrogen may protect cardiovascular health not only in female but also in male patients. Therefore, understanding the pathophysiological mechanism of RA and how sex hormones protect the heart against RA is important not only for the prevention and control of RA but also for estrogen replacement therapy.

GJs play a crucial role in cardiac function, because through them major ionic fluxes between neighboring cardiomyocytes are spread, therefore allowing electrical synchronization of contraction. Remodeling of the distribution of GJs has been proposed as a crucial step for the genesis of RAs. But little is known about the effects of estrogen on GJs in the arrhythmogenesis during the I/R process. Recent studies revealed that Cx43 is the principal ventricular coupling protein, and its dephosphorylation has dramatic effects on or can reflect injury to both electrical and chemical coupling in cardiomyocytes. In addition, Zemljic-Harpf et al found that cardiac-myocyte-specific excision of the vinculin gene caused conduction abnormalities (complete AVB, ectopy, and nonsustained polymorphic VT) and high mortality rates in homozygous global vinculin knockout (vCvlKO) mice: gender affected the mortality rate in vCvlKO mice, with male mice showing more rapid mortality than female mice. Because apoptosis also can influence GJs, in the present study we examined for the first time cardiac apoptosis and the expressions of vinculin mRNA and Cx43 on estrogen protection against RA.

We also detected some basic but important factors ([Ca2+], ROS and eNOS) that can influence rhythm and apoptosis. ([Ca2+], homeostasis play a central role in the cardiovascular system, especially rhythm. It has been shown that delayed afterdepolarizations are based on a spontaneous increase in [Ca2+]. In fact, during myocardial I/R injury, Ca2+ overload and ROS were not isolated events but highly relevant. Oxygen free radical generating systems can depress Na+/K+-ATPase, which could indirectly contribute to [Ca2+]i overload by elevating intracellular Na+ levels and secondarily inhibiting Ca2+ efflux by the Na+-Ca2+ exchange. So, attenuation of ROS generation is also an important factor in estrogen protection. In addition, ROS also has a relationship with NO. Cross et al found that higher NO production via eNOS in the perfused heart of female mice was responsible for the ameliorated I/R injury compared with male mice. Figure 3 shows that, after reperfusion in the present study the expressions of [Ca2+]i and ROS in the estrogen-group were higher than those of the vehicle-group, but the variation in eNOS was entirely contrary. These findings, which are all related to apoptosis, strongly imply that apoptosis should have more significance when evaluating the effects of reperfusion on RA, and confirm our hypothesis that estrogen plays a role in the protection of GJs.

Data presented in this study indicate that, during the ischemia period, there were no significant differences between the vehicle-group and estrogen-group in the incidence, duration and number of arrhythmias. At the same time, compared with the normal points of each group, the expression of cardiac vinculin mRNA markedly declined in both the vehicle-group and estrogen-group, but Cx43 dephosphorylation showed no significant variation. Meanwhile, we found no difference in myocardial apoptosis between the two groups either. Most previous works indicated that cardiac ischemia was associated with marked dephosphorylation and intracellular redistribution of ventricular Cx43 during electrical uncoupling. In our study, we did not observe significant variations in Cx43 dephosphorylation in any group during the ischemia period. Michael et al revealed that loss of phosphorylated Cx43 was apparent after 15 min of ischemia when uncoupling had just begun and became more marked after 30 or 40 min of ischemia: in the present study the ischemic period was 20 min. After reperfusion, although the incidence of arrhythmias became higher in both groups, compared with the ischemia period, estrogen treatment prevented the reperfusion-induced upregulation of the incidence of arrhythmias.
especially VPB and VT. The duration of VT and VF, and the number of VPB and VT were all significantly decreased in the estrogen-group than in the vehicle-group. Irreversible VF was only observed in the reperfusion period. SCID was also lower in the estrogen-group. The expression of cardiac vinculin mRNA decreased significantly in the vehicle-group but not in the estrogen-group simultaneously. Cx43 dephosphorylation and myocyte apoptosis increased in both groups, but the expressions in the estrogen-group were all markedly lower than those of the vehicle-group. Vinculin is related to ventricular arrhythmias in dilated cardiomyopathy,²⁵ but its role in RA has not been mentioned before. Although it is still not known precisely how vinculin variation modifies rhythm, from our results it seems likely that variations in vinculin expression, Cx43 dephosphorylation and apoptosis are somehow connected with the changes in rhythm during reperfusion. In our study, variations in RA coincided with changes in the vinculin gene, Cx43 dephosphorylation and myocardial apoptosis, which were prevented by estrogen treatment, suggesting that estrogen provides protection against arrhythmias during reperfusion, mediated, at least in part, through GJs. Further work is needed to test this hypothesis, as well as evaluating the effects of estrogen on signaling molecules in GJ conductance during reperfusion.

Because estrogen can protect heart against RA, we were interested in determining the role of the ER during this process. Estrogen responses are often categorized as either rapid/non-genomic,⁵³,⁶⁴ occurring within minutes of cell stimulation, or genomic, characterized by changes in gene transcription occurring in the time frame of hours.⁵⁵ The N-terminal truncated form of the ER (ERα6) is described as a receptor for non-genomic effects in endothelial cells.⁵⁶,⁵⁷ The present report suggests a genomic response of estrogen, as we just detected the effects of the classical ERs (ERα and ERβ), which are all expressed in cardiomyocytes.⁵⁸ DPN acts as an agonist on both ER subtypes but has a 70-fold higher relative binding affinity and 170-fold higher relative estrogenic potency in transcription assays with ERβ, than with ERα.¹³ PPT on the other hand is a selective agonist for the ERα and is the best agonist for ERα out of a series of tetrassubstitutes pyrazole analogs.⁵⁹ PPT binds to ERα with high affinity, displaying 410-fold binding selectivity over ERβ.⁵⁹ Many observations have led to the concept that selective ER modulators (SERMs) selectively activate estrogen-dependent protection in an organ such as the heart without stimulating breast or ovarian proliferation.¹¹

Our results provide evidence that following reperfusion after severe ischemia, estrogen-induced cardioprotection against ventricular RA is mediated by upregulation of ERα activation. DPN prevented the reperfusion-induced upregulation of the incidence of both VPB and VT significantly compared with the vehicle-group; the ERα agonist, PPT, had no significant influence. The ER antagonist, ICI 182,780, could abolish DPN-induced protection against ventricular arrhythmias following reperfusion, suggesting that these effects are mediated via the ER. We administered ICI 182,780 alone in our study to exclude the per se effect and the results showed that ICI 182,780 alone did not influence the aforementioned parameters significantly compared with the vehicle or DPN+ICI group. Although the precise mechanism by which DPN mediates its salutary effects remains unknown, our finding confirms that DPN (or ERα) mediated the estrogen-induced upregulation of vinculin gene expression, inhibition of Cx43 dephosphorylation and myocardial apoptosis. Compared with the vehicle-group, PPT also decreased the Cx43 dephosphorylation even though it was without significance (P=0.073), but the expression of vinculin mRNA was essentially unchanged. We do not know the explanation for these differences, but it might well be that DPN (or ERβ) play a major role in the protective effect of estrogen on GJs, and the effect of PPT can not be ignored entirely. Further studies will be required to define in detail the precise pathophysiological effects of different ERs on GJ communication. Ultrastructural analysis has revealed disarray of individual cardiomyocytes and structural abnormalities within cell-to-cell contacts involving disrupted intercalated discs in ERα−/− mice but not in wild-type (WT) mice.⁶⁰ Yu et al found that ERβ could upregulate heat shock proteins (Hsp90) to protect the heart of male rats.¹¹ However, Zhai et al revealed that ERα knockout mice had more severe damage following I/R than wild-type mice.⁶¹ So there remains a great deal of controversy over the role of female hormones and the ERs.¹¹,¹³,¹⁹,²¹,⁴³,⁴⁴ We think that the ERs are associated with adverse effects on cardioprotection that require further investigation.

We consider that selecting male rats as the experimental subjects in the present study has significance in the development of pharmaceutical agents for patients of either sex.²⁹ The present study has for the first time reported the in vivo effects of estrogen and a selective ER agonist on GJs and the influential factors in RAs following severe ischemia. Protection of GJs, which can affect cellular coupling, may be responsible for the ameliorated I/R arrhythmias, and we hope that our results and reported phenomena provide fresh insight into the role of estrogen and ER subtypes in its protective effect against RA and a new direction for other researchers.

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