Differential Role of $K_{\text{ATP}}$ Channels Activated by Conjugated Estrogens in the Regulation of Myocardial and Coronary Protective Effects

Tsung-Ming Lee, MD, FESC; Tsai-Fwu Chou, MD, PhD; Chang-Her Tsai, MD, PhD

Background—We have demonstrated that estrogen can reduce myocardial injury in ischemia-reperfusion via activation of ATP-sensitive potassium ($K_{\text{ATP}}$) channels. We sought to determine whether the protective effect of estrogen extends to epicardial coronary artery with attenuated vasoconstriction in patients after angioplasty by activation of such channels.

Methods and Results—The study was designed to prospectively investigate 41 consecutive patients scheduled for elective coronary angioplasty. Pretreatment with estrogen limited myocardial ischemia during coronary occlusion and attenuated postangioplasty coronary vasoconstriction at the dilated and distal segments. An inhibitor of $K_{\text{ATP}}$ channels, glibenclamide, did not affect coronary vasomotor response, although it abolished the beneficial effect of estrogen on myocardial ischemia. Patients to whom estrogen was administered after the second balloon deflation experienced a similar magnitude of myocardial ischemia as controls but showed significantly attenuated vasoconstriction compared with controls ($P<0.0001$). Endothelin-1 levels from the great cardiac vein rose significantly from $1.9\pm0.4$ to $3.1\pm0.6$ pg/mL ($P=0.001$) 15 minutes after angioplasty in the control group; this was attenuated after estrogen was administered. Significant correlation was found between the changes in coronary vasomotion of the dilated segment and endothelin-1 levels ($r=0.65$, $P<0.0001$).

Conclusions—These results demonstrate that estrogen is protective against both myocardial ischemia and coronary vasoconstriction through different mechanisms. The myocardial effect of estrogen was abolished by glibenclamide, which suggests that the cardioprotective effect of estrogen may result from activation of $K_{\text{ATP}}$ channels. In contrast, estrogen-induced attenuated vasoconstriction is associated with an attenuated release of endothelin-1, independent of $K_{\text{ATP}}$ activation. (Circulation. 2003;107:lll-lll.)

Key Words: coronary disease $|$ ion channels $|$ ischemia $|$ reperfusion

Although PTCA provides effective recanalization of coronary atherosclerosis, routine vasoconstriction occurs after angioplasty. Coronary vasoconstriction is associated with a greater residual stenosis and an increased incidence of restenosis after angioplasty. A better understanding of possible mechanisms involved and potential strategies to limit its extent should be sought. We have demonstrated that endothelin-1 (ET-1) plays an important role in coronary vasoconstriction after angioplasty. A specific antagonist of the ET$_A$ receptor (BQ123) has been shown to attenuate coronary vasoconstriction in experimental and clinical studies.

We have demonstrated that estrogen is cardioprotective against infarct of any size and myocardial ischemia by activation of ATP-sensitive potassium ($K_{\text{ATP}}$) channels in animals and in humans, similar to ischemic preconditioning (IP). Cardioprotection via $K_{\text{ATP}}$ channels may be an integral player in IP, in which brief periods of ischemia and reperfusion before a sustained ischemic stress protect the heart. A blocker of $K_{\text{ATP}}$ channels, glibenclamide, abolishes the cardioprotective effects of IP. Cardiomyocytes, vascular smooth muscle cells, and arterial endothelial cells contain $K_{\text{ATP}}$ channels, which can also be inhibited by pretreatment with glibenclamide. The beneficial effect of IP has been shown not to be limited to cardiomyocytes; it can also be observed in the coronary artery, including endothelium and smooth muscle cells, in animals. Despite convincing data from IP studies in animals, it remains unclear in humans whether pharmacological preconditioning with estrogen offers protection against reperfusion-related vascular injury. Furthermore, the effects of estrogen on both myocardium and coronary artery have not been examined simultaneously, which would help determine whether myocardial protection was caused by improvement in coronary function or a direct effect on myocardial function. Thus, the present study sought to evaluate the influence of estrogen on myocardial ischemia...
and coronary vasomotion after angioplasty and to determine whether the effect was related to activation of K<sub>ATP</sub> channels through use of glibenclamide, a K<sub>ATP</sub> channel blocker.

**Methods**

**Subjects**

The study was conducted prospectively. All patients fulfilled the following entry criteria: (1) history of chronic stable angina pectoris ≥3 months and a positive standard stress test for myocardial ischemia; (2) neither pathological Q waves or bundle-branch block nor an ECG that could have interfered with the interpretation of ST-segment changes; (3) single proximal or mid-artery lesion of the left anterior descending artery or left circumflex artery; and (4) successful balloon angioplasty resulting in residual stenosis <30% immediately after the procedure. To avoid the effect of the severity of the culprit lesion on coronary vasomotion,16 patients were highly selected, with similar diameter stenosis of between 70% and 90%. To make collateral circulation of these study patients homogenous, patients with angiographically visible collateral blood at baseline were excluded. No patients received hormone replacement therapy for at least 6 months before the study began. Medications, including calcium channel and β-adrenergic blockers and caffeine-containing beverages or substances, except aspirin (100 mg/d), were withheld for 24 hours before the procedure. Any patients who had taken nitroglycerin within 4 hours of catheterization were excluded from this study. A total of 41 consecutive patients were included. Patients were randomized into 5 groups on the basis of the use of intracoronary estrogen and glibenclamide (Figure 1). To determine the potential role of K<sub>ATP</sub> channels in regulation of myocardium and coronary artery, glibenclamide (10 mg) was orally administered 60 minutes before catheterization with a continuous infusion of 10% dextrose at the same time. This dose of glibenclamide will result in an estimated serum concentration of 500 nmol/L,17 which has been shown to block myocardial and vascular K<sub>ATP</sub> channels in humans.18 To further confirm whether IP is mandatory for estrogen-induced attenuated vasoconstriction, a group treated with estrogen immediately after the second balloon deflation was assessed. The clinical characteristics of patients are given in Table 1.

**Study Protocol**

**Catheterization Procedures**

After completion of the diagnostic catheterization, a 6F Judkins guiding catheter was advanced to the ostium of the left or right coronary artery as described previously.9 To assess collateral flow during coronary occlusion, a 0.014-in Doppler wire (FloWire, Cardiometrics, Inc) was first introduced through a standard angioplasty-type Y-connector. Collateral blood flow during balloon inflation was defined as the sum of systolic and diastolic collateral blood flow velocity integral, which has been demonstrated to be a good indicator of the function of collateral vessels.19 The external end of the guidewire was connected to the chest lead by a sterile alligator clamp to record the intracoronary ECG.

**Angioplasty Procedure**

After angiographic collateral assessment and placement of the intracoronary ECG monitor, estrogen (Premarin 5 mg IC, Wyeth-Ayerst) was administered via the guiding catheter. An intracoronary injection of saline was given in the control group. After a 10-minute drug-free period, the lesion was crossed with a balloon. After the balloon was positioned across the lesion, patients underwent two 120-second balloon inflations separated by 60-second intervals of reperfusion, with the Doppler guidewire remaining across the lesion at the same site for each successive recording.

**Angiography Measurements**

For each lesion, the view showing the most severe degree of stenosis was used for analysis. Quantitative measurements of coronary artery dimensions were made by a computer-based edge-enhancement technique (DCL System, Philips, Inc), as described previously.9 Measurements were performed before, immediately after, and 15

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**TABLE 1. Clinical Characteristics**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=8)</th>
<th>Estrogen (n=9)</th>
<th>Glibenclamide (n=8)</th>
<th>Estrogen + Glibenclamide (n=9)</th>
<th>Late Estrogen (n=7)</th>
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<tbody>
<tr>
<td>Age, y</td>
<td>55±6</td>
<td>54±6</td>
<td>55±7</td>
<td>53±7</td>
<td>54±10</td>
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<tr>
<td>Male/female</td>
<td>7/1</td>
<td>8/1</td>
<td>6/2</td>
<td>7/2</td>
<td>7/0</td>
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<td>CAD risk factor</td>
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<td></td>
<td></td>
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<td></td>
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<td>Hypertension, %</td>
<td>4 (50)</td>
<td>3 (33)</td>
<td>3 (38)</td>
<td>5 (56)</td>
<td>4 (57)</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>2 (25)</td>
<td>4 (44)</td>
<td>3 (38)</td>
<td>3 (33)</td>
<td>3 (43)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>228±40</td>
<td>237±33</td>
<td>241±34</td>
<td>228±18</td>
<td>221±45</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>247±43</td>
<td>240±86</td>
<td>227±51</td>
<td>242±51</td>
<td>239±58</td>
</tr>
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Vessel disease

<table>
<thead>
<tr>
<th>Vessel disease</th>
<th>LAD</th>
<th>LCx</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; LAD, left anterior descending artery; LCx, left circumflex artery; and Late Estrogen, estrogen administered after second balloon deflation.

Values are mean±SD or n (%).
minutes after angioplasty. The dilated segment was defined as the minimal lumen diameter that was responsible for the clinical symptoms. The distal segment was defined as a vessel segment distal to the dilation site. The proximal segment was defined as a smooth vessel segment proximal to the dilation site. The control segment was defined as a vessel segment that was not reached by the balloon catheter. We have had experience with quantitative coronary arteriography; the intraobserver and interobserver variabilities at the proximal segments were 0.18±0.15 mm (5.7±6.2%) and 0.21±0.23 mm (6.7±6.8%), respectively.

### Assessment of Myocardial Ischemia

The intracoronary ECG was recorded online at a paper speed of 25 mm/s during the 2 balloon inflations and at selected times after deflation. ST-segment elevation was evaluated in a blinded manner by 2 observers (TML, TFC) who viewed the ECGs in random order without knowledge of which patient was being presented. Differences in interpretation were resolved by consensus.

To confirm myocardial ischemia during balloon inflations, selective catheterization of the great cardiac vein was successfully attempted. Simultaneous samples of the aortic root and the great cardiac vein were obtained for measurement of lactate contents. The myocardial lactate extraction ratio (MLR) was calculated by the following formula: \( \frac{[L_{Ao} - L_{CV}] / L_{Ao} \times 100}{} \), where \( L_{Ao} \) and \( L_{CV} \) represent plasma lactate concentrations in the aortic root and great cardiac vein, respectively.

Before coronary angioplasty, patients were informed that they might develop chest pain during balloon inflations. Immediately before termination of balloon inflation, patients were asked to quantify the intensity of cardiac pain using a visual-analog scale\(^2\) on a scale of 0 to 10, from no pain (0) to the most severe pain (10).

### Laboratory Measurements

Because of local release of ET-1 at the dilated sites, blood samples from the aortic root and great cardiac vein were obtained simultaneously for measurements of local ET-1 production at baseline and at the end of the study. ET-1 was measured by immunoassay (R&D System Inc). The detection limit was 1 pg/mL for ET-1. Intra-assay and interassay coefficients of variation were 4.5% and 6.6%, respectively.

Great cardiac vein blood was sampled for measurement of plasma estrogen levels at baseline and at the end of the study. Estrogen concentrations were quantified by enzyme-linked immunosassay (Diagnostic Products Corporation). The detection limit was 10 pg/mL for 17β-estradiol. To determine the confounding roles of glucose in ischemic preconditioning, great cardiac vein blood samples for glucose concentrations were assayed.

### Statistical Analysis

Continuous variables are expressed as mean±SD. Patients were analyzed by ANOVA among the groups. A 2-way repeated-measures ANOVA was used to search for possible effects of estrogen and glibenclamide on the measurements of intracoronary ECG and lactate, and if an F value was found to be significant, a 2-tailed Student’s t test for paired observation with Bonferroni correction was used to test differences. The interaction term of estrogen and glibenclamide effects was incorporated into the model. Visual analog scales were analyzed with the Wilcoxon signed rank test. \( \chi^2 \) Analysis was used for categorical variables, and Fisher’s exact test was used for patient numbers <5. Correlation between ET-1 and other parameters was assessed by Pearson’s correlation coefficient. Probability values were 2-tailed, and a value of \( P<0.05 \) was considered statistically significant.

### Results

There were no baseline characteristic differences among the groups shown in Table 1. These groups were comparable in terms of sex, age, lipid profile, heart rate, and blood pressure. Risk factors for coronary artery disease were evenly distributed among the groups. The balloon pressure used was similar (data not shown). During or after the procedure, no patient developed any major complications that might affect vasoconstriction.

### Hemodynamics

There were no significant changes in mean blood pressure and heart rate among the groups at baseline and at the ends of the first and second balloon inflations (data not shown). Rate-pressure product, an index of oxygen consumption, was comparable among the 5 groups (data not shown). The quantitative variables for assessment of the collateral circulation obtained during the first and second balloon inflations indicated the presence of low-grade collaterals and did not differ among the study groups (data not shown). Coronary stenosis was similarly reduced among the groups (data not shown).

### Sinus Blood Measurements

Estrogen levels were similar at baseline in the 5 groups (Table 2). There was a significant increase in the estrogen-treated groups, equivalent to that in human females during the middle...
of their menstrual cycle. Glucose levels remained stable throughout the study (data not shown).

Fifteen minutes after angioplasty, ET-1 concentrations in plasma from the great cardiac vein increased markedly compared with baseline, to $3.1 \pm 0.6 \text{ pg/mL (}}P=0.001\). After administration of estrogen, ET-1 concentration from the great cardiac vein was significantly decreased by 47% 15 minutes after the second inflation compared with the same timing point in the control group, consistent with a blockade of ET-1 release. Glibenclamide administration did not affect the attenuated effect of estrogen on ET-1 concentrations. ET-1 levels from the aorta remained stable throughout the study in the 5 groups.

**Myocardial Ischemia Assessment**

**Chest Pain**

In the control group, severity of chest pain was similar between the first and second inflations (Table 3). The chest pain score in the first and second inflations was significantly less in the estrogen group than in the other groups. Glibenclamide administration significantly increased the severity of chest pain in estrogen-treated patients compared with those treated with estrogen alone. Patients to whom estrogen was given after the second balloon deflation experienced pain similar to that of the control patients.

**Intracoronary ECG**

The ST-segment shift was significantly greater during balloon inflation in the control group than in the estrogen group. In estrogen-treated patients, changes in ST-segment shift were similar between the first and second inflations ($0.40 \pm 0.09$ versus $0.44 \pm 0.09 \text{ mV, } P=\text{NS}$). Conversely, patients to whom glibenclamide was administered developed a significantly higher ST-segment shift during the first and second inflations than those treated with estrogen alone.

**Lactate Measurements**

MLR was more negative in the control group than in the estrogen group during the first and second inflations. The benefits of metabolic features were abolished after administration of glibenclamide.

**Coronary Vasoconstriction**

In the control group, the diameter reduction of the arterial segment at the dilated site averaged $28.1 \pm 11\%$ 15 minutes after the last deflation (Figure 2). The diameter reduction at the distal segments showed a magnitude similar to that at the dilated segments. The control segment and proximal segment did not display any significant diameter changes after angioplasty throughout the study among the 5 groups.

The degree of coronary vasoconstriction at the dilated segment was markedly attenuated in the estrogen-treated groups in the presence or absence of glibenclamide compared with controls ($7 \pm 8\%, P=0.001$ for estrogen alone versus controls; $5 \pm 5\%, P<0.0001$ for the estrogen plus glibenclamide group versus controls). Patients given estrogen after the second balloon

### TABLE 3. Myocardial Ischemia Assessed by Subjective Cardiac Pain, Values of ST-Segment Shift, and MLR Throughout the Study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=8)</th>
<th>Estrogen (n=9)</th>
<th>Glibenclamide (n=8)</th>
<th>Estrogen + Glibenclamide (n=9)</th>
<th>Late Estrogen (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac pain score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflation 1</td>
<td>$5.6 \pm 1.1$</td>
<td>$3.3 \pm 1.2^*$</td>
<td>$5.8 \pm 1.0$</td>
<td>$5.8 \pm 1.0$</td>
<td>$5.4 \pm 1.0$</td>
</tr>
<tr>
<td>Inflation 2</td>
<td>$6.0 \pm 1.1$</td>
<td>$3.2 \pm 0.7^*$</td>
<td>$5.9 \pm 1.5$</td>
<td>$5.9 \pm 1.4$</td>
<td>$6.0 \pm 1.2$</td>
</tr>
<tr>
<td>ST-segment shift, mV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflation 1</td>
<td>$1.14 \pm 0.16$</td>
<td>$0.40 \pm 0.09^*$</td>
<td>$1.36 \pm 0.21$</td>
<td>$1.40 \pm 0.22$</td>
<td>$1.13 \pm 0.17$</td>
</tr>
<tr>
<td>Inflation 2</td>
<td>$1.29 \pm 0.27$</td>
<td>$0.44 \pm 0.09^*$</td>
<td>$1.33 \pm 0.18$</td>
<td>$1.36 \pm 0.19$</td>
<td>$1.29 \pm 0.29$</td>
</tr>
<tr>
<td>MLR, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>$18 \pm 11$</td>
<td>$15 \pm 25$</td>
<td>$14 \pm 18$</td>
<td>$13 \pm 17$</td>
<td>$16 \pm 9$</td>
</tr>
<tr>
<td>Inflation 1</td>
<td>$-120 \pm 52$</td>
<td>$-47 \pm 29^*$</td>
<td>$-132 \pm 29$</td>
<td>$-122 \pm 28$</td>
<td>$-127 \pm 53$</td>
</tr>
<tr>
<td>Inflation 2</td>
<td>$-124 \pm 30$</td>
<td>$-39 \pm 27^*$</td>
<td>$-137 \pm 34$</td>
<td>$-127 \pm 36$</td>
<td>$-125 \pm 32$</td>
</tr>
</tbody>
</table>

Values are mean ± SD. An ST-segment shift was defined as difference in ST-segment levels between baseline and ends of first and second inflation.

* $P<0.05$ vs control, Glibenclamide, Estrogen + Glibenclamide, and Late Estrogen sampling at same time.

Figure 2. Percent change in coronary diameter from basal values (immediately after angioplasty). There is significant vasoconstriction at dilated and distal segments in control and glibenclamide groups, whereas vasoconstriction was prevented in estrogen-treated groups in presence or absence of glibenclamide and in late estrogen group. * $P<0.05$ vs control and glibenclamide groups. Est indicates estrogen; Glib, glibenclamide.
Patients with estrogen administration had significantly attenuated vasoconstriction after angioplasty. Significant correlation was found between changes in coronary vasomotion of the dilated segment and ET-1 levels ($r=0.65$, $P<0.0001$, 95% CI 0.12 to 0.26 mm; Figure 3). There was no significant correlation between changes in MLR or intracoronary ECG and coronary vasoconstriction at the dilated segments ($r=0.07$, $P=0.66$ for MLR; and $r=0.27$, $P=0.09$ for intracoronary ECG), which suggests that there was no relationship between vasoconstriction and the degree of myocardial ischemia.

Discussion

The present results clearly show for the first time that estrogen at physiological doses exerts its beneficial effects on both myocardial protection and attenuated vasoconstriction in patients undergoing coronary angioplasty through different mechanisms. The myocardial protection of estrogen was abolished by glibenclamide, which suggests that the cardioprotective effect of estrogen may result from activation of myocardial KATP channels. However, activation of KATP channels does not appear to be involved in attenuated postangioplasty vasoconstriction, which was not affected by administration of glibenclamide at a dose that blocks vascular KATP channels. A close relationship was observed between changes in coronary vasomotion of the dilated segment and ET-1 levels, which suggests that estrogen administration may blunt routine coronary vasoconstriction by reducing the production of ET-1 in the great cardiac vein. Our findings imply that estrogen has an important mechanistic difference in regulating myocardium and epicardial coronary artery.

Our conclusions are supported by 3 lines of evidence. (1) Myocardial KATP channels were activated by administration of estrogen, and the effect of estrogen on preconditioning was abolished by pretreatment with glibenclamide, which confirms the predominant role of KATP channels in this phenomenon. (2) The lack of effect of glibenclamide on estrogen-treated vessels suggests that KATP channels are not involved in the beneficial effect of estrogen. (3) The group with estrogen administration after the second balloon deflation had attenuated vasoconstriction, although they experienced myocardial ischemia similar to the control group. This finding further supports the different mechanisms involved in myocardial and coronary protection.

Estrogen Mechanism

Myocardium Protection

The ischemia-limiting effect of estrogen observed in the present study confirms our previous studies performed in animals and patients. Estrogen has an important mechanistic difference in regulating myocardium and coronary protection. Several vasoconstrictors released during angioplasty have been proposed to be responsible for distal vasoconstriction, such as ET-1. Postangioplasty vasoconstriction was observed selectively at distal vessels in ischemic myocardium. Once ET-1 is released into the bloodstream during coronary angioplasty, it rapidly attaches itself to tissue and is quickly broken down by

Vascular Protection

Because estrogen-induced attenuation of coronary vasoconstriction after angioplasty was not paralleled in the proximal and control segments, it would appear that this effect is not a nonspecific steroid-mediated action. The mechanisms by which acute administration of physiological levels of estrogen influences arterial tone are unclear. The observation of a lack of effect of glibenclamide in estrogen-induced vascular protection suggests that KATP channels are not critical to vascular response and that other mechanisms are involved. The observed luminal changes are the net result of the opposing effect of vasoconstrictors released during angioplasty and estrogen-induced vascular protection. Several vasoconstrictors released during angioplasty have been proposed to be responsible for distal vasoconstriction, such as ET-1. Postangioplasty vasoconstriction was observed selectively at distal vessels in ischemic myocardium. Once ET-1 is released into the bloodstream during coronary angioplasty, it rapidly attaches itself to tissue and is quickly broken down by...
a plasma enzyme; therefore, it is unlikely that ET-1 induces vasoconstriction at the proximal segments. The increased levels of ET-1 released into the coronary sinus during coronary angioplasty can be blunted by administration of estrogen. The present results were compatible with previous findings that showed that ET-1 production was inhibited by a physiological concentration of estrogen via an estrogen receptor–independent mechanism, an effect detectable as early as 20 minutes after intracoronary infusion.

Study Limitations

There appears to be bias with regard to the inclusion of males in this study (35 males and 6 females). Patients were included for cardiac catheterization to assess the severity of coronary arteriosclerosis, which is more prevalent in males than in females. Thus, more clinical studies are needed to confirm the beneficial effects of acute administration of estrogen in females, although we have demonstrated in canine hearts that estrogen limits myocardial infarction size resulting from coronary artery occlusion and reperfusion regardless of sex. Blumenthal et al demonstrated an improvement in coronary vasodilation in males with intravenous conjugated estrogen. However, Collins et al have shown a lack of effect in male coronary arteries in response to acute loading of estrogen for low availability or activity of estrogen receptors in male tissues. The latter suggested that a period of estrogen “priming” to induce receptor-mediated nitric oxide synthesis may be required to yield an improvement in vascular function in males, which is in contrast to the present results. The discrepancy can be explained, at least in part, by the different model and experimental protocol used. In the present study, myocardial ischemia during balloon inflation stimulated the production of ET-1. Estrogen can interact with ET-1 without participation of estrogen receptors. Thus, it is not surprising that estrogen can attenuate vasoconstriction not only in females but also in males without a period of estrogen priming.

Conclusions

The present study showed that estrogen can provide protection at the level of myocardium and epicardial coronary artery by different mechanisms. The myocardial effects of estrogen were abolished by glibenclamide, which suggests that the cardioprotective effect of estrogen may result from activation of myocardial KATP channels. However, estrogen reversed the vasoconstriction changes by an attenuated release of ET-1, independent of KATP channel activation.

References

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