Pharmacologic Preconditioning of Estrogen by Activation of the Myocardial Adenosine Triphosphate-Sensitive Potassium Channel in Patients Undergoing Coronary Angioplasty

Tsung-Ming Lee, MD, FESC,* Sheng-Fang Su, PhD,‡ Tsai-Fwu Chou, MD,§ Chang-Her Tsai, MD, PhD†
Taipei and Tainan, Taiwan

OBJECTIVES
The purpose of this study was to determine whether administration of estrogen produces cardioprotective effects in patients undergoing coronary angioplasty.

BACKGROUND
We have previously demonstrated that estrogen can provide cardioprotection by activating the mitochondrial adenosine triphosphate-sensitive potassium (KATP) channel, a major contributor to ischemic cardioprotection.

METHODS
Fifty patients undergoing angioplasty of a major epicardial coronary artery were randomly allocated to either ischemic preconditioning or intracoronary estrogen administration in the presence or absence of glibenclamide (glyburide).

RESULTS
The coronary collateral circulation, as quantitatively assessed by an intracoronary Doppler flow wire, was similar during balloon inflation among the groups. Patients in the preconditioned and estrogen-treated groups significantly lowered their ischemic burden, as assessed by an ST-segment shift, chest pain score and myocardial lactate extraction ratio, as compared with control subjects. The reduction in the ST-segment shift afforded by estrogen during the first inflation (63% vs. first inflation in the preconditioned group) was similar to that afforded by preconditioning during the second inflation (68% vs. first inflation). In contrast, the patients given glibenclamide developed significantly higher ischemic burden during the first and second inflations, as compared with those in the estrogen-treated group alone.

CONCLUSIONS
It is concluded that intracoronary administration of estrogen before balloon angioplasty rendered the myocardium relatively resistant to subsequent ischemia, and the degree of cardioprotective effect was comparable to that afforded by ischemic preconditioning. The effect of estrogen was abolished by glibenclamide, suggesting that the cardioprotective effect of estrogen may result from activation of myocardial KATP channels. (J Am Coll Cardiol 2002;39:871–7) © 2002 by the American College of Cardiology Foundation

Coronary artery disease is the leading cause of death in women, especially among postmenopausal women (1). Estrogen levels drop markedly after menopause. Epidemiologic studies have shown that estrogen replacement can significantly reduce cardiovascular morbidity by 50% in menopausal women (2), although the mechanism remains unclear. Estrogen has previously been shown to exert cardioprotective effects in the dog (3–5). The cardioprotective effects of estrogen have been attributed to antioxidants (3), increased nitric oxide release (4) and the opening of calcium-activated potassium channels (5). Our previous study showed that 17-beta-estradiol administration provided cardioprotection produced by activation of adenosine triphosphate-sensitive potassium (KATP) channels in canine hearts (6). However, no previous study has addressed the issue of the effects of estrogen in humans on KATP channels during myocardial ischemia.

Ischemic preconditioning is a cardioprotective phenomenon in which short periods of myocardial ischemia result in the myocardium's resistance to a subsequent stress (7). The model of percutaneous transluminal coronary angioplasty (PTCA) as a surrogate of ischemia-reperfusion has gained popularity for assessing the ability of various agents to mimic preconditioning (8). Ischemic preconditioning is thought to be mediated by mitochondrial KATP channels (6). The opening of these channels may be important in ischemic preconditioning, because inhibition of KATP channels with glibenclamide (glyburide) abolishes the cardioprotective effects of ischemic preconditioning in both experimental and clinical studies (9,10). The use of agents to open this channel may mimic a physiologic response that acts to attenuate ischemic injury. Thus, this study investigates whether pretreatment of intracoronary estrogen potentiates ischemic preconditioning before balloon inflations in patients undergoing PTCA. We have also investigated whether the observed cardioprotective effects of estrogen are caused by the activation of KATP channels through the use of glibenclamide, a KATP channel blocker.

METHODS
Study group. The study was conducted prospectively. All patients fulfilled the entry criteria: 1) a history of chronic,
stable angina pectoris ≥3 months and a positive standard stress test for myocardial ischemia; 2) no history on the electrocardiogram (ECG) of a previous myocardial infarction, nor pathologic Q-waves or bundle branch block, which could have interfered with the interpretation of ST-segment changes; 3) no angiographically visible collateral blood flow at baseline, so that the collateral circulation of the study patients was homogeneous; 4) a single proximal or mid-epicardial coronary artery lesion with 70% to 90% reduction in the lumen diameter; and 5) successful balloon angioplasty resulting in residual stenosis <30%. No patient had a history of diabetes mellitus. Women included in this study were confirmed to have been menopausal for at least five years by measuring their serum follicle-stimulating hormone and estrogen levels. No patient had received hormone replacement therapy for at least six months before the study began. Medications, including calcium channel blockers and beta-blockers, were held for 24 h before the procedure. Any patient who had taken nitroglycerin within 4 h of catheterization was excluded from the study. A consecutive total of 50 patients were included. Patients were randomly allocated to one of the five groups (Fig. 1). The study was approved by the National Taiwan University Hospital Review Board, and all subjects provided written, informed consent before participation.

**Study protocol. CATHETERIZATION PROCEDURES.** Diagnostic left heart catheterization and angiography were performed from a femoral approach, as previously described (11). After completion of diagnostic catheterization, intravenous heparin was supplemented to maintain an activated clotting time of 300 to 350 s, and a 6F Judkin's guiding catheter was advanced to the ostium of the left or right coronary artery. To assess collateral flow during coronary occlusion, a 0.014-in. (0.035-cm) Doppler wire (FloWire, Cardiometrics, Inc., Mountain View, California) was first introduced through a standard angioplasty-type Y-connector attached to the angiographic catheter. The wire tip was positioned such that a characteristic and stable flow waveform was obtained. Collateral flow during balloon inflations was determined by the sum of systolic and diastolic collateral blood flow velocity integrals, as previously described (12). The distal segment of the guide wire was placed 2 to 3 cm beyond the balloon catheter tip. The external end of the guide wire was connected to the chest lead by a sterile alligator clamp to record the intracoronary ECG. Multiple pairs of perpendicular views (90°) of the left and right coronary arteries were obtained. The precise angle, skew rotation and table height of each projection were recorded to allow the projection to be duplicated. Quantitative measurements of coronary artery dimensions were performed.

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**Figure 1.** Summary of study protocol. Black boxes = period of balloon inflation; arrows = intracoronary electrocardiograms and chest anginal score; arrowheads = lactate measurements from the great cardiac vein and the aorta simultaneously. Est = estrogen; Glib = glibenclamide; PC = preconditioning; S = seconds.
made using a computer-based edge enhancement technique (DCI System, Philips, Inc., Best, The Netherlands), as previously described (11). Nonionic contrast medium was used in all patients to prevent a myocardial depressant effect during coronary angiography. Blood pressure and heart rate were continuously monitored during the procedure. Patients were not sedated.

**ANGIOPLASTY PROCEDURE.** After angiographic collateral assessment and intracoronary ECG monitoring, intracoronary estrogen (Premarin, 5 mg, Wyeth-Ayerst, Radnor, Pennsylvania) was administered through the guiding catheter. The control group was injected with a bolus of the same volume of normal saline. After a 10-min drug-free period, the lesion was crossed with a balloon. To determine the potential role of K\textsubscript{ATP} channels in estrogen-induced preconditioning, glibenclamide (10 mg) was orally administered 60 min before catheterization with a continuous infusion of 10% dextrose at the same time. The optimal balloon size was determined by quantitative evaluation of the coronary artery diameters adjacent to the stenosis. After the balloon was positioned across the lesion, the patients underwent two 2-min balloon inflations separated by 1- or 2-min intervals of reperfusion (Fig. 1), with the Doppler guide wire remaining across the lesion at the same site for each successive recording. A recovery time of 1 min was adequate to re-establish baseline nonischemic conditions, as assessed by intracoronary electrocardiography and chest pain. An interval of 2 min between balloon inflations was used to produce preconditioning (13). During the reperfusion intervals, the angioplasty balloons were withdrawn from the stenotic site, and the guide wire was left at the same position. Because balloon pressure is a determinant of cardiac pain during PTCA (14), identical balloon pressure was maintained during the first and second inflations in each patient, with inflation pressures ranging from 6 to 10 atm.

**ASSESSMENT OF MYOCARDIAL ISCHEMIA.** The intracoronary ECG was recorded on-line at a paper speed of 25 mm/s during the two balloon inflations and at selected times after deflation. Calibration was performed at the beginning of the procedure (1 mV = 5 mm). At all time points, the ST-segment shift was measured 80 ms after the J point on a minimum of three complexes. ST-segment elevation was evaluated in a blinded manner by two observers (T. M. L., C. H. T.) who viewed the ECGs in random order, without knowledge of which patient was being presented. Differences in interpretation were resolved by consensus. Changes in ST-segment levels at baseline were used as the control, and differences in ST-segment levels recorded between the control value and at the end of the first and second inflations were compared to evaluate the severity of myocardial ischemia.

To confirm myocardial ischemia during balloon inflations, selective catheterization of the great cardiac vein was successfully attempted. Simultaneous samples of aortic root and coronary sinus blood withdrawn at identical sampling rates were obtained for measurements of lactate contents. The myocardial lactate extraction ratio (MLER) was calculated by the following formula:

\[
\text{MLER} = \frac{[\text{L}_{\text{AR}} - \text{L}_{\text{CS}}]/\text{L}_{\text{AR}}] \times 100
\]

where \text{L}_{\text{AR}} and \text{L}_{\text{CS}} represent plasma lactate concentrations in the aortic root and coronary sinus, respectively.

**ASSESSMENT OF CHEST PAIN.** Before PTCA, patients were informed that they might develop chest pain during balloon inflation. Immediately before termination of balloon inflation, patients were asked to quantify the intensity of chest pain by using a visual-analog scale (15) on a scale of 0 (no pain) to 10 (most severe pain).

**LABORATORY MEASUREMENTS.** Coronary sinus blood, reflecting the local concentrations, was sampled for measurement of plasma estrogen levels at baseline and at the end of the study, ~60 min after administration. Estrogen concentrations were quantified by enzyme-linked immunosorbent assay (Diagnostic Products Corp., Los Angeles, California). The detection limit was 10 pg/ml for 17-beta-estradiol. To determine the confounding roles of glucose in ischemic preconditioning, sinus blood samples for glucose concentrations were assayed.

**Statistics.** Continuous variables are expressed as the mean value ± SD. The groups of patients were analyzed using analysis of variance (ANOVA). Two-way repeated-mesures ANOVA was used to search for the possible effects of estrogen and glibenclamide on the measurements of intracoronary electrocardiography and lactate levels and, if an F value was found to be significant, the two-tailed Student t test for paired observations, with the 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 using the Wilcoxon signed rank test. Chi-square analysis was used for categoric variables, and the Fisher exact test was used for patient numbers <5. Probability values are two-tailed, and a p value <0.05 is considered to be statistically significant.

**RESULTS**

The clinical features of the patients are outlined in Table 1. There were no significant differences among the groups in terms of the patients’ age, gender and frequency of cardiovascular risk factors. Coronary stenosis was similarly reduced among the groups (Table 2). Glucose levels remained stable throughout the study. The balloon pressure used was similar. No myocardial injury was reflected in any patients after PTCA, as assessed by electrocardiography.

**Coronary sinus plasma estrogen concentrations.** There were no significant differences in estrogen concentrations among the groups at baseline (Table 1). After estrogen administration, there was a significant increase at the end of the study.

**Hemodynamic data.** No significant changes were seen in the mean blood pressure and heart rate among the five
groups at baseline and after the first and second angioplasties. The rate–pressure product, an index of oxygen consumption, was comparable among the groups. The rate–pressure product, an index of oxygen consumption, was comparable among the groups. The rate–pressure product, an index of oxygen consumption, was comparable among the groups.

**Collateral circulation.** The quantitative variables used to assess the collateral circulation and obtained during the first and second balloon inflations indicated the presence of low-grade collateral channels and did not differ between the study groups (Table 3).

**Myocardial ischemia. CHEST PAIN.** Before each inflation, all patients were asymptomatic. In the control group, the severity of chest pain was similar between the first and second inflations. Chest pain in the preconditioned group during the second inflation was significantly less than during the first inflation, indicating effective ischemic preconditioning. In contrast, the chest pain score during the first inflation was significantly less in the estrogen-treated group than in the other groups. In patients treated with estrogen, the pain was the same during the first and second inflations. Although patients in the preconditioned and estrogen-treated groups experienced less anginal severity during the second inflation, chest pain was significantly increased in patients pretreated with glibenclamide.

**INTRACORONARY ELECTROCARDIOGRAPHY.** The ST-segment shift values at the end of the two inflations are reported in Table 3. Before each inflation, there was no ST-segment shift on the intracoronary ECG. In the control group, the mean ST-segment shift during the second inflation was significantly less than during the first inflation. Although patients in the preconditioned and estrogen-treated groups experienced less anginal severity during the second inflation, chest pain was significantly increased in patients pretreated with glibenclamide.

### Table 1. Clinical Characteristics and Estrogen Concentrations

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n = 10)</th>
<th>PC (n = 10)</th>
<th>Estrogen (n = 10)</th>
<th>PC + Glibenclamide (n = 10)</th>
<th>Estrogen + Glibenclamide (n = 10)</th>
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<tr>
<td>Age (yrs)</td>
<td>53 ± 6</td>
<td>51 ± 4</td>
<td>53 ± 8</td>
<td>55 ± 6</td>
<td>53 ± 7</td>
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<td>Male/female</td>
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<td>8/2</td>
<td>9/1</td>
<td>10/0</td>
<td>9/1</td>
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<td>CAD risk factor</td>
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<td></td>
<td></td>
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<tr>
<td>Hypertension (%)</td>
<td>5 (0)</td>
<td>4 (0)</td>
<td>4 (0)</td>
<td>5 (0)</td>
<td>5 (0)</td>
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<tr>
<td>Smoking (%)</td>
<td>3 (30)</td>
<td>4 (0)</td>
<td>4 (0)</td>
<td>4 (40)</td>
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<td>Total cholesterol (mg/dl)</td>
<td>221 ± 34</td>
<td>201 ± 43</td>
<td>220 ± 38</td>
<td>221 ± 26</td>
<td>230 ± 18</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>233 ± 76</td>
<td>257 ± 55</td>
<td>252 ± 57</td>
<td>249 ± 62</td>
<td>245 ± 49</td>
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<td>Vessel disease</td>
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<tr>
<td>LAD</td>
<td>8</td>
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<td>LCx</td>
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<tr>
<td>RCA</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
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<tr>
<td>17-beta-estradiol (pg/ml)</td>
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<tr>
<td>Baseline</td>
<td>26 ± 3</td>
<td>26 ± 2</td>
<td>25 ± 2</td>
<td>24 ± 3</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>At end of study</td>
<td>24 ± 3</td>
<td>22 ± 4</td>
<td>94 ± 8*</td>
<td>23 ± 4</td>
<td>118 ± 6*</td>
</tr>
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*p < 0.0001 compared with respective baseline data and the groups without estrogen administration at the end of the study. Data are presented as the mean value ± SD or number (%) of patients or control subjects. CAD = coronary artery disease; LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; PC = preconditioning; RCA = right coronary artery.

### Table 2. Hemodynamic Data

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n = 10)</th>
<th>PC (n = 10)</th>
<th>Estrogen (n = 10)</th>
<th>PC + Glibenclamide (n = 10)</th>
<th>Estrogen + Glibenclamide (n = 10)</th>
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</thead>
<tbody>
<tr>
<td>Mean blood pressure (mm Hg)</td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>92 ± 9</td>
<td>98 ± 12</td>
<td>96 ± 15</td>
<td>97 ± 15</td>
<td>98 ± 16</td>
</tr>
<tr>
<td>Inflation 1</td>
<td>95 ± 11</td>
<td>94 ± 15</td>
<td>92 ± 12</td>
<td>97 ± 14</td>
<td>94 ± 15</td>
</tr>
<tr>
<td>Inflation 2</td>
<td>95 ± 12</td>
<td>94 ± 10</td>
<td>99 ± 10</td>
<td>95 ± 13</td>
<td>93 ± 13</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>65 ± 6</td>
<td>67 ± 9</td>
<td>67 ± 7</td>
<td>67 ± 7</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>Inflation 1</td>
<td>66 ± 7</td>
<td>71 ± 11</td>
<td>68 ± 8</td>
<td>68 ± 10</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>Inflation 2</td>
<td>70 ± 10</td>
<td>68 ± 8</td>
<td>68 ± 6</td>
<td>68 ± 8</td>
<td>68 ± 11</td>
</tr>
<tr>
<td>RPP (×10¹³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8.92 ± 1.98</td>
<td>9.24 ± 1.33</td>
<td>9.22 ± 1.71</td>
<td>9.18 ± 2.17</td>
<td>9.17 ± 1.46</td>
</tr>
<tr>
<td>Inflation 1</td>
<td>9.13 ± 1.88</td>
<td>9.31 ± 1.43</td>
<td>9.26 ± 1.12</td>
<td>9.24 ± 1.23</td>
<td>9.15 ± 1.16</td>
</tr>
</tbody>
</table>

Severity of stenosis (%)        |                 |             |                  |                            |                                  |
| Before angioplasty             | 80 ± 4          | 80 ± 5      | 79 ± 4           | 79 ± 5                     | 79 ± 4                           |
| After angioplasty              | 19 ± 6          | 19 ± 4      | 18 ± 6           | 20 ± 4                     | 17 ± 5                           |

Inflation pressure (atm)        |                 |             |                  |                            |                                  |
| Inflation 1                    | 7.3 ± 1.3       | 7.0 ± 0.9   | 7.0 ± 1.1        | 7.1 ± 1.0                  | 7.1 ± 1.0                        |
| Inflation 2                    | 7.3 ± 1.3       | 7.0 ± 0.9   | 7.0 ± 1.1        | 7.1 ± 1.0                  | 7.1 ± 1.0                        |

Data are presented as the mean value ± SD. RPP = rate–pressure product; other abbreviations as in Table 1.
balloon inflation was similar to that observed during the first inflation (1.46 ± 0.26 mV vs. 1.48 ± 0.21 mV, p = NS). In the preconditioned patients, the ST-segment shift was significantly greater during the first inflation than during the second inflation, consistent with ischemic preconditioning. In the estrogen-treated patients, the changes in ST-segment shift were similar between the first and second inflations (0.50 ± 0.16 mV vs. 0.47 ± 0.09 mV, p = NS). The reduction in the ST-segment shift afforded by estrogen during the first inflation (−63% vs. first inflation in the preconditioned group) was similar to that afforded by preconditioning during the second inflation (−68% vs. first inflation). In contrast, the patients who received glibenclamide developed a higher ST-segment shift during the first and second inflations, as compared with those in the estrogen-treated group alone.

**Lactate Measurements.** The respective baseline values were positive and similar, indicating the absence of significant lactate production in the pre-angioplasty state. The MLER was more negative in the control group than in the preconditioned and estrogen groups during the second inflation, indicating less lactate production from the ischemic myocardium in the latter two groups. The benefits of metabolic features were abolished after glibenclamide administration.

**DISCUSSION**

This study showed that pretreatment with estrogen provided myocardial adaptation to ischemia during coronary PTCA that was as effective as preconditioning and was independent of collateral channel recruitment. The cardioprotective effect of estrogen was abolished by glibenclamide, suggesting that the cardioprotective effect of estrogen may result from activation of myocardial $k_{\text{ATP}}$ channels.

Patients in the preconditioned and estrogen groups showed that myocardial ischemia was significantly ameliorated during the second balloon inflation, as assessed by a subjective anginal score, ST-segment shift and MLER. The ST-segment shift and chest pain score noted during the first inflation in the estrogen-treated patients were indistinguishable from those observed during the second inflation in the preconditioned patients, suggesting that the degree of protection afforded by estrogen was comparable to that afforded by previous exposure to ischemia in the preconditioned group. This finding was further corroborated by the objective observation that in the preconditioned group, the magnitude of MLER decreased after the second inflation, whereas in the estrogen-treated group, no significant changes occurred in this variable during the second inflation as compared with the first inflation—a feature of preconditioning. Tanonaka et al. (16) showed that preconditioning induced by estrogen enhanced the reperfusion-induced restoration of myocardial high-energy phosphate and attenuated the ischemia-induced increase in tissue lactate content, consistent with our results showing a reduction of sinus lactate accumulation. This finding was not consistent with that of Sbarouni et al. (17), who showed that intracoronary estrogen administration did not affect the subsequent myocardial tolerance to ischemia. However, the latter study was not designed to assess the effect of estrogen on ischemic preconditioning. The investigators did not control the balloon inflation duration, nor the interval between the two inflations, both of which are important determinants of ischemic preconditioning. Furthermore, surface electrocardiography was used to measure the ST-segment changes in that study. Several
reports have shown that during PTCA, transient ischemia was more readily detected, and with greater sensitivity, on the intracoronary ECG than on the surface ECG (18).

**Estrogen mechanisms and K\textsubscript{ATP} channels.** The mechanisms by which estrogen affects ischemic preconditioning remain undefined. Clearly, estrogen did not exert any hemodynamic effects, nor was it associated with an increase in myocardial collateral blood flow at the dose used here.

The results from this study have established similarities between estrogen and ischemic preconditioning. ischemic preconditioning and estrogen appear to share a common mediator—the K\textsubscript{ATP} channels, although the signal transduction pathway in estrogen treatment, which results in increased activation of K\textsubscript{ATP} channels, is unknown. One possible mechanism is that the channel is opened by adenosine, which is increased by estrogen (19). Adenosine is one of the agonists binding to membrane receptors to trigger K\textsubscript{ATP} channels (20). Second, Noda et al. (5) demonstrated that estrogen increases the production of nitric oxide, and previous studies have shown that nitric oxide facilitates the opening of K\textsubscript{ATP} in cardiac cells (21). Furthermore, estrogen has been shown to induce rapid pharmacodynamic changes of the G-protein-coupled mechanism, regulating the potency of opening K\textsubscript{ATP} channels (22).

**Clinical implications.** This study shows that preconditioning was blocked by glibenclamide, a commonly used sulfonylurea in patients with diabetes. Previous studies have shown that diabetic patients have a substantially higher mortality rate after acute myocardial infarction, as compared with nondiabetic patients, a circumstance that has been partly attributed to tablet treatment (23,24). A recent retrospective study, however, did not find that survival was influenced by sulfonylurea (25). Because the analysis was retrospective, it has several shortcomings. Randomized, prospective studies can better answer the question. Our study shows that glibenclamide prevents ischemic preconditioning, which could contribute to excessive mortality from cardiovascular causes in diabetic patients receiving sulfonylurea. The notion was compatible with the suggestion that high-risk diabetic patients should switch from sulfonylurea to insulin when undergoing a coronary intervention (24).

Although cardioprotection has been demonstrated by short-term loading of estrogen in this study, it does not necessarily imply the similar benefit in long-term administration of estrogen. Epidemiologic studies have repeatedly demonstrated favorable associations between hormone replacement therapy and cardiovascular morbidity and mortality. However, the Heart and Estrogen/progestin Replacement Study (HERS), the first randomized trial of hormone replacement therapy, showed no overall benefit in the secondary prevention of heart disease in menopausal women (26). Clearly, the use of hormone replacement therapy for secondary prevention of heart disease is more complex than was initially believed. A more detailed analysis of the HERS results showed that the risk of heart disease was reduced at three to five years, but this reduction was offset by a 50% higher cardiac event rate during the first year. This time trend pattern of an early increase and late reduction in risk may be reconciled with epidemiologic studies. The early mortality might be related to an increase in thrombosis risk, whereas the late benefit might be related to a decreased risk of atherosclerosis (26). Besides, the drug used in HERS was a combination of estrogen and progestin, and progestin has been shown to attenuate the cardioprotective effect of estrogen. It is likely that progestin may have contributed to the increase in infarction rates. However, in the Estrogen Replacement and Atherosclerosis trial, there were no benefits in the group treated with estrogen alone (27). Thus, one should be cautious to apply our results to patients using estrogen over a long term.

**Study limitations.** There were several limitations of this study. First, there appears to be a bias toward the inclusion of more males in this study (45 men and 5 women). Patients underwent cardiac catheterization to assess the severity of coronary atherosclerosis, which is more prevalent in males than in females (28). Thus, the effects of short-term administration of estrogen in women need more clinical studies to confirm the benefits. Second, blood samples for measuring lactate production were obtained 10 to 15 s after balloon deflation. Because coronary sinus flow was not measured, our results were not expressed in terms of lactate efflux. The less elevated concentrations in the coronary sinus underestimated a reduction in lactate production in the preconditioned and estrogen-treated groups, because the relative hyperemia was significantly lower than that in the control group (29). Third, the interval between the two balloon inflations was 1 min in the non-preconditioned groups. No previous studies in humans have shown whether the interval was adequate to re-establish a nonischemic baseline value. However, the baseline re-establishment was confirmed in the study assessed by intracoronary electrocardiography and chest pain, although no lactate measurement was performed at the time. The observation was in agreement with the finding of Dunn et al. (30), showing that after a brief coronary occlusion, lactate from the coronary sinus returned to control levels within 30 s after ligation release in dogs.

Fourth, previous studies have demonstrated that intracoronary infusion of a large dose of glibenclamide (50 μg/kg/min) reduced coronary blood flow and elevated the ST segment (31). Kondo et al. (32) demonstrated no ST-segment changes at the dose of 1 mg/kg. The low dose of glibenclamide used in this study should not have created any ST-segment changes. Another possibility is that the opening of K\textsubscript{ATP} channels leads to elevation of the ST segment because of a shortening of the action potential duration and accumulation of extracellular potassium concentrations during myocardial ischemia (33), and those were blocked by glibenclamide. Thus, it is possible that there was an underestimation of the blockade of estrogen-induced preconditioning by glibenclamide in our patients. Finally, a potential problem with the present study is the use of glibenclamide as an antagonist of K\textsubscript{ATP} channels when there are many
potential nonspecific targets of glibenclamide, including the inhibition of Na⁺ channels and the opening of Ca²⁺ channels (34). These alternative effects could confound the interpretation of the present study. Use of a more selective antagonist of K<sub>ATP</sub> channels, 5-hydroxydecanoate, would have strengthened the hypothesis. However, 5-hydroxydecanoate is not available for use in humans.

Conclusions. The present study demonstrates that intracoronary administration of estrogen before PTCA enhances the tolerance of the heart to subsequent ischemia, as assessed by clinical, ECG and metabolic evidence, in a manner analogous to that observed during ischemic preconditioning. Responses are inhibited by the K<sub>ATP</sub> antagonist glibenclamide, suggesting that they act on the same sites.

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Reprint requests and correspondence: Dr. Chang-Her Tsai, Department of Surgery, Cardiology Section, National Taiwan University Hospital, 7, Chung-Shan S. Road, Taipei, Taiwan 10002. E-mail: tsaicher@ha.mc.ntu.edu.tw.

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