Impaired Endothelium-Dependent Relaxation After Cardiac Global Ischemia and Reperfusion: Role of Warm Blood Cardioplegia

CHAU-HSIUNG CHANG, MD, PYNG JING LIN, MD, YEN CHU, MS, YING-SHIUNG LEE, MD
Taipei, Taiwan, Republic of China

Objectives. Experiments were designed to determine whether coronary endothelial dysfunction after cardiac global ischemia and reperfusion could be prevented by warm blood cardioplegic solution.

Background. The coronary endothelium produces endothelium-derived relaxing factor (EDRF) to prevent vasospasm and thrombosis. After ischemia and reperfusion, endothelium-dependent relaxation (EDR) is diminished as a result of G-protein dysfunction.

Methods. Dogs were exposed to extracorporeal circulation in 37°C (group 1) or 28°C (groups 2 and 3). The heart was ischemic for 120 min while continuous warm blood cardioplegic solution (group 1) or intermittent cold (4°C) crystalloid cardioplegic solution (group 2) was infused into the aortic root. Cardioplegic solution was not used in group 3 animals. The heart was then allowed to function for 60 min of reperfusion.

Results. Endothelium-derived relaxation in response to acetylcholine, adenosine diphosphate and sodium fluoride of the coronary rings of group 1 was significantly different from that of groups 2 and 3 but was not significantly different from that of group 4. In contrast, EDR in response to the receptor-independent calcium ionophore agonist A23187 was not significantly different between the four groups. Scanning electron microscopic studies showed that platelet adhesion and aggregation, area of microthrombi, disruption of endothelial cells and separation of the intercellular junction could be found in coronary segments of groups 2 and 3 but not in vessels of groups 1 and 4.

Conclusions. These experiments suggest that cardiac global ischemia and reperfusion impair receptor-mediated release of EDRF from the coronary endothelium with G-protein dysfunction. This type of coronary endothelial dysfunction can be prevented by continuous anterograde infusion of warm blood cardioplegic solution during global ischemia.

From the Division of Thoracic and Cardiovascular Surgery and Division of Cardiology, Chang Gung Memorial Hospital, Chang Gung Medical College, Taipei, Taiwan, Republic of China. This study was supported in part by Grant CMRP 421 from the Chang Gung Memorial Hospital, Chang Gung Medical College and by Grant NSC 85-0412-B-182-111 from the National Science Council, Executive Yuan, Taiwan, Republic of China.

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Address for correspondence: Dr. Pyng Jing Lin, Division of Thoracic and Cardiovascular Surgery, Chang Gung Memorial Hospital, 199, Tun-Hwa North Road, Taipei, Taiwan, Republic of China.

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electrocardiogram was continuously monitored by limb leads. Deferoxamine (20 mg/kg) and methylprednisolone (20 mg/kg) were infused intravenously 30 min before the establishment of the cardiopulmonary bypass. The dogs were then placed on total cardiopulmonary bypass. The dogs were then placed on total cardiopulmonary bypass using a bubble oxygenator (Bard, C. R. Bard, Inc.) with a flow rate of 50 ml/kg per min. Myocardial temperature was continuously monitored by epicardial thermistor probes (Shiley, Inc.). After stabilization, the ascending aorta was cross-clamped. A double-lumen aortic root cannula (DPL, Inc.) was inserted for delivery of cardioplegic solution and simultaneous measurement of aortic root pressure. All infusions of cardioplegic solution were administered at 50 mm Hg of pressure.

Animals were randomized into four groups, with 14 dogs in each group. In the dogs of group 1, the heart was protected by continuous warm (37°C) blood cardioplegia infused into the aortic root (anterograde infusion), with the rectal temperature at 37°C. Blood cardioplegia was delivered as a mixture of four parts oxygenated blood to one part of induction or maintenance solution using a Sarns MP-4 cardioplegia delivery system (Sarns 3M Health Care Group). The composition of the induction solution was 51 mEq of NaHCO₃, 25 g of glucose, 100 mEq of KCl, and 10 U of regular insulin in 1,000 ml of lactated Ringer’s solution. The composition of maintenance solution was the same as that of the induction solution except that the potassium concentration was 40 mEq/liter. Initial induction of cardiac arrest was accomplished by using induction solution with a potassium ion concentration of 20 mmol/liter, followed by continuous infusion of maintenance solution with a potassium ion concentration of 8 mmol/liter. After 120 min of aortic cross-clamping, the infusion of blood cardioplegia was stopped and the cross-clamp was removed.

In animals of group 2, cold (4°C) crystalloid cardioplegia (10 ml/kg) (Plegisol, Abbott Laboratories)—with the following electrolyte composition (in mEq/liter): calcium 2.4, magnesium 32, potassium 16, sodium 120 and chloride 160—was infused into the aortic root with systemic hypothermia (28°C). The cardioplegic solution was reinfused (4 ml/kg) at 20-min intervals during global ischemia. No topical cooling was used. After 120 min of cardiac ischemia, the cross-clamp was removed and the animal was warmed to 37°C.

In group 3 animals, the rectal temperature was kept around 28°C. The cardioplegic solution was not used. After 120 min of aortic cross-clamping, the cross-clamp was removed and the animals were warmed to 37°C.

After declamping, the animals were weaned from cardiopulmonary bypass with a mean arterial pressure of 80 mm Hg. When ventricular fibrillation occurred, direct current countershocks of 10 W-s were applied. Cardiotonic drugs and vasodilators were not used. The heart was maintained in the beating and working state for a total of 60 min, and then it was excised.

An additional 14 dogs served as the control group (group 4). After induction of anesthesia, intubation and median sternotomy, the heart was excised rapidly.

All animals received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health (NIH publication no. 85-23, revised 1985).

Organ chamber experiments (12 animals in each group) for endothelial functional studies and scanning electron microscopic examinations (2 animals in each group) for the ultrastructure of the coronary endothelium were then performed.

**Organ chamber studies.** The heart was quickly removed and immersed in cold oxygenated physiologic solution of the following composition (mmol/liter): NaCl 118.3, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.22, CaCl₂ 2.5, NaHCO₃ 25.0 and glucose 11.1 (control solution). The left anterior descending and/or left circumflex coronary arteries were carefully dissected free and prepared as rings (4 mm in length). During dissection care was taken to remove as much of the surrounding tissues as possible and to avoid stretching and rubbing of the intimal surface against foreign material or the opposite wall of the vessel.

In some rings, the endothelium was removed by gently rubbing the intimal surface with the tip of a pair of watchmaker’s forceps (15–17). The rings were suspended in organ chambers (25 ml) filled with physiologic solution (37°C, aerated with 95% oxygen and 5% carbon dioxide pH 7.4). Each ring was suspended by two stainless-steel clips passed through the lumen. One clip was anchored to the bottom of the organ chamber; the other was connected to a strain gauge (Statham Gould UC2) for the measurement of isometric force. Rings were placed at the optimal point of their length–tension relation by progressively stretching them at each level of distention until the contraction to KCl (20 mmol/liter) was maximal (15–17). In all the experiments, the presence or absence of endothelium was confirmed by the response to acetylcholine (ACh) (10⁻⁶ mol/liter) of rings contracted with potassium ions (20 mmol/liter). After this procedure, the preparations were allowed to equilibrate at their optimal length for 45 min before the experiments.

**Scanning electron microscopic studies.** The scanning electron microscopic procedures were reported previously by our
group (17). After ischemia and reperfusion, the distal part of the ascending aorta of the reperfused hearts (groups 1 to 3) and control hearts (group 4) was cross-clamped during exanguination while the heart was still beating. The coronary arteries were perfused through the aortic root cannula at a controlled pressure of 120 mm Hg for 5 min with buffered physiologic solution of the following composition (mmol/liter): KCl 2.7, NaCl 137.9, Na2HPO4·7H2O 8.1 and KH2PO4 1.1. Glutaraldehyde (1%) in buffered physiologic solution was then infused for 10 min. In this way, the coronary endothelium was fixed in situ at physiologic pressure before being processed for electron microscopic studies. Segments (2 cm in length) of the left anterior descending or left circumflex coronary artery, or both, were carefully harvested and kept in the ice perfusion-fixation solution. The specimens were then fixed with ice 3% glutaraldehyde in 0.1 mol cacodylate buffer (pH 7.2 to 7.4) for 2 h. Subsequently, the specimens were rinsed with cold perfusion-fixation solution several times and postfixed with 1% phosphate buffer osmium tetroxide (pH 7.2 to 7.4) for an additional 2 h. For electroconduction and stabilization of the surface structure, the tissues were immersed in 1% tannic acid in distilled water for 30 min at 4°C and then transferred into 1% osmium tetroxide in distilled water for 30 min at 4°C, followed by a rinse with distilled water. The tissues were then dehydrated in graded concentrations of chilled ethanol. The tissue was subjected to critical point drying. After drying, samples were mounted on specimen stubs and coated with platinum and palladium alloy 4-nm thick. The specimens were examined with a Hitachi S-5000 scanning electron microscope operated at 3 kV by Dr. Lee, who did not know the grouping of the specimens.

Drugs. The drugs—ACh chloride, potassium chloride, adenosine diphosphate (ADP), calcium ionophore A23187, (±)-isoproterenol hydrochloride, indomethacin, sodium fluoride, prostaglandin F2-alpha and sodium nitroprusside—were obtained from Sigma Chemical Co. Aluminum chloride was obtained from Aldrich Chemical. All drugs were prepared daily with distilled water, except for indomethacin, which was dissolved in Na2CO3 (10−5 mol/liter), and A23187, which was dissolved in dimethyl sulfoxide (Sigma) and diluted further in distilled water. The concentrations are expressed as final molar concentration in the organ chambers.

Data analysis. The data were expressed as mean value ± SEM. In all experiments, “n” refers to the number of animals from which blood vessels were taken. Although not always mentioned in the protocol, the role of the endothelium in the observed phenomena was determined systematically by comparing blood vessels (rings) with or without endothelium to avoid repetition. The responses gained from rings contracted with prostaglandin F2-alpha were expressed as percent changes from the contracted levels. With regard to relaxations, the negative logarithm of the effective concentration (mol/liter) of agonist that caused 50% inhibition of the contraction (IC50) to prostaglandin F2-alpha was calculated. For contractions, the maximal response (in grams of tension) and concentration of agonist inducing the half-maximal contraction (EC50) were determined. The means of these values were presented. Statistical evaluation of data between groups was performed by analysis of variance (ANOVA), and the Tukey t test was used when ANOVA showed significance. Statistical significance was set at p < 0.05.

Protocol of organ chamber studies. Coronary artery segments with and without endothelium from the same animal were placed in our eight-bath organ chamber system, studied and compared. The following procedures were performed:

Studies of endothelium-dependent relaxation. Coronary artery segments (with and without endothelium) from animals were suspended in organ chambers. They were contracted with prostaglandin F2-alpha (2 × 10−6 mol/liter) and then exposed to increasing concentrations of ACh (receptor-dependent, 10−9 to 10−4 mol/liter), ADP (receptor-dependent, 10−9 to 10−4 mol/liter), A23187 (receptor-independent, 10−9 to 10−6 mol/liter) and sodium fluoride (NaF) (pertussis toxin-sensitive G-protein activator, 1 to 11 mmol/liter).

Studies of endothelium-independent contraction. To test the ability of the smooth muscle to relax, concentration-response curves to sodium nitroprusside (mediated by cyclic guanosine monophosphate, 10−9 to 10−4 mol/liter) and sodium fluoride (NaF) were obtained after they were contracted with prostaglandin F2-alpha (2 × 10−6 mol/liter).

Studies of endothelium-independent contraction. To test the ability of the smooth muscle to contract, concentration-response curves to potassium ions (5 to 50 mmol/liter, voltage-dependent) and prostaglandin F2-alpha (10−9 to 10−4 mol/liter, receptor-dependent) were obtained.

Results

Endothelium-dependent relaxation. Acetylcholine (10−9 to 10−4 mol/liter) induced concentration-dependent relaxation in the coronary artery rings with endothelium of all four groups (n = 12) (Fig. 1). However, relaxation was significantly impaired in the rings of groups 2 and 3 (p < 0.05), and the concentration-response curve was shifted to the right (Fig. 1, Table 1).

Adenosine diphosphate (10−9 to 10−4 mol/liter) caused
EDR in the coronary artery rings of all four groups (n = 12) (Fig. 2). However, as with ACh, relaxation was significantly impaired in the rings of groups 2 and 3 (p < 0.05), and the concentration-response curve was shifted to the right (Fig. 2, Table 1).

A23187 (10^{-9} to 10^{-6} mol/liter) induced comparable endothelium-dependent, concentration-dependent relaxation in the coronary artery rings of all four groups (n = 12, p > 0.05) (Fig. 3, Table 1).

Sodium fluoride (1 to 11 mmol/liter) caused comparable concentration-dependent relaxation in the coronary artery rings with endothelium of groups 1 and 4 (n = 12, p > 0.05) (Fig. 4). However, exposure to NaF induced concentration-dependent relaxation in the coronary artery rings with endothelium of groups 2 and 3 (n = 12) that was significantly less than that of groups 1 and 4 (p < 0.05) (Fig. 4, Table 1).

There was no significant change in tension in response to ACh, ADP, A23187 and NaF between the coronary artery rings without endothelium of all four groups (p > 0.05).

### Table 1. Endothelium-Dependent Relaxation of Segments of Canine Coronary Arteries With Endothelium

<table>
<thead>
<tr>
<th>Group</th>
<th>IC_{50} (-log mol/liter)</th>
<th>Maximal Relaxation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>6.8 ± 0.3</td>
<td>100</td>
</tr>
<tr>
<td>ADP</td>
<td>6.5 ± 0.2</td>
<td>100</td>
</tr>
<tr>
<td>A23187</td>
<td>7.6 ± 0.2</td>
<td>100</td>
</tr>
<tr>
<td>NaF</td>
<td>9.0 ± 0.3</td>
<td>100</td>
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</table>

*Significant difference from control group 4. †50% inhibition of contractions induced by norepinephrine (3 × 10^{-7} mol/liter) could not be obtained with the agonist. Data presented are mean value ± SEM. A23187 = calcium ionophore A23187; ACh = acetylcholine; ADP = adenosine diphosphate; IC_{50} = negative logarithms of the concentration (mol/liter) of agonists causing 50% inhibition of contractions (i.e., median effective dose) induced by prostaglandin F_{2}-alpha (2 × 10^{-6} mol/liter); NaF = sodium fluoride.

**Figure 2.** Concentration-response curves to calcium ionophore A23187 in reperfused (groups 1, 2 and 3) and control (group 4) coronary artery rings with endothelium (n = 12). Gr. = group.

**Figure 3.** Concentration-response curves to sodium fluoride in reperfused (groups 1, 2 and 3) and control (group 4) coronary artery rings with endothelium (n = 12). Gr. = group.

**Endothelium-independent relaxation.** Increasing concentrations (10^{-9} to 10^{-4} mol/liter) of isoproterenol and sodium nitroprusside induced comparable concentration-dependent relaxation in the coronary artery rings with and without endothelium of all four groups. In all groups, the maximal relaxation induced by isoproterenol or sodium nitroprusside was not altered in coronary artery segments without endothelium, nor did this induction change the sensitivity to relaxation of the vascular smooth muscle (Table 2).

**Endothelium-independent contraction.** Increasing concentration of potassium ions (5 to 50 mmol/liter) and prostaglandin F_{2}-alpha (10^{-9} to 10^{-4} mol/liter) induced comparable concentration-dependent contraction of arterial segments with and without endothelium in all four groups. The maximal response or the sensitivity to these agonists did not result in any significant changes in the four groups (Table 3).

**Scanning electron microscopic studies.** In the coronary arterial segments of group 1, scanning electron microscopic observations showed that the endothelium was continuous and was maintained in an integrated form without significant alteration of surface morphology in all specimens examined (Fig. 5). The smooth surface of the endothelium was consistently covered with flat endothelial cells. The interendothelial junctions could not be readily delineated. We rarely observed blood cells that adhered to the endothelial surface. These findings were not significantly different from those of control group 4 vessels. In the scanning electron microscopic studies of the vessels of groups 2 and 3, swelling and disruption of the

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**Figure 4.** Concentration-response curves to sodium fluoride in reperfused (groups 1, 2 and 3) and control (group 4) coronary artery rings with endothelium (n = 12). Gr. = group.
endothelial cells with wide separation of intercellular junctions were frequently demonstrated (Fig. 6A). Platelet adherence and aggregation on the endothelial surface were a constant finding in every specimen investigated (Fig. 6, B and C). In some areas, platelet microthrombi were noted (Fig. 6, B and C). In some instances, extensive disruption of the endothelium was seen (Fig. 6D). Those surface morphologic changes of the endothelium were consistently noted on vessels of groups 2 and 3.

**Discussion**

The major finding of this study was that during cardiac ischemia, intermittent infusion of cold crystalloid cardioplegic solution (group 2) could not, but continuous anterograde infusion of warm blood cardioplegic solution (group 1) could, prevent reperfusion injury to either EDR or ultrastructure of the coronary endothelium.

**Ischemia and reperfusion injury.** Endothelium-dependent relaxation of the coronary artery in response to aggregating platelets was impaired immediately or long after local ischemia and reperfusion (18,19). After cardiac global ischemia and reperfusion, EDR in response to aggregating platelets and to the receptor-dependent agonists ACh and ADP was also impaired (3). Our studies confirmed these findings in that EDR was impaired after cardiac global ischemia and reperfusion (group 3).

G-protein dysfunction was found recently after cardiac global ischemia and reperfusion with impairment of EDR (4). In our study, EDR of the coronary endothelium in response to the receptor-dependent agonists ACh and ADP and to the pertussis toxin–sensitive G-protein activator NaF was impaired. However, EDR in response to receptor-independent A23187 was not impaired. This indicated that global myocardial ischemia and reperfusion selectively impaired receptor-mediated release of EDRF (nitric oxide) due to G-protein dysfunction. However, the ability to produce EDRF by the coronary endothelium was not impaired.

In the epicardial coronary artery, reperfusion injury appears to be an endothelium-dependent phenomenon (18,19). In our study, smooth muscle contraction in response to potassium

### Table 2. Endothelium-Independent Relaxation of Segments of Canine Coronary Arteries

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
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<tbody>
<tr>
<td><strong>IC50 (−log mol/liter)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With endothelium</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Isoproterenol</td>
<td>7.5 ± 0.1</td>
<td>7.4 ± 0.1</td>
<td>7.3 ± 0.2</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>7.7 ± 0.1</td>
<td>7.7 ± 0.2</td>
<td>7.6 ± 0.1</td>
<td>7.8 ± 0.2</td>
</tr>
<tr>
<td>Without endothelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>7.7 ± 0.1</td>
<td>7.6 ± 0.1</td>
<td>7.5 ± 0.2</td>
<td>7.6 ± 0.1</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>7.9 ± 0.1</td>
<td>7.9 ± 0.1</td>
<td>7.9 ± 0.1</td>
<td>8.0 ± 0.2</td>
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**Maximal Relaxation (%)**

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>With endothelium</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>100</td>
<td>100</td>
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</table>

Data presented are mean value ± SEM. **IC50** = negative logarithms of the concentration (mol/liter) of agonists causing 50% inhibition of contractions (i.e., the median effective dose) induced by prostaglandin F2-alpha (2 × 10−6 mol/liter).
ions (voltage-dependent) or prostaglandin F$_2$-alpha (receptor dependent) was well preserved after ischemia and reperfusion. The smooth muscle relaxation in response to isoproterenol (cyclic adenosine monophosphate mediated) or sodium nitroprusside (cyclic guanosine monophosphate mediated) was also preserved in all groups. These indicated that the vascular smooth muscle function was not injured by ischemia and reperfusion.

**Endothelium function and cardioplegia.** In modern cardiac surgery, crystalloid or blood cardioplegic solutions have been used routinely for myocardial protection. Endothelium-derived relaxation of the coronary arteries after crystalloid cardioplegia and reperfusion was impaired (10–12). Warm blood cardioplegic solution has become more popular in myocardial protection in recent years and can offer a benefit in operations on hearts that are metabolically depleted or acutely ischemic (13). Previous studies suggested that warm blood cardioplegic solution could preserve EDR (11,12). Our studies further demonstrated that warm blood cardioplegic solution can preserve G-protein function and prevent impairment of EDR after ischemia and reperfusion injury. Our experiments also confirmed that crystalloid cardioplegia cannot prevent ischemia and reperfusion injury to the coronary endothelium.

**Ultrastructure.** The ultrastructural change of the endothelium after reperfusion injury is obvious. VanBenthuysen et al. (20) and Lin et al. (17) previously demonstrated significant coronary endothelial ultrastructural injury after ischemia and reperfusion by scanning and transmission electron microscopy. In the present study, significant surface morphologic changes of the endothelium could be found in the coronary arteries of group 2 and 3 animals (Fig. 6). The surface morphology of the coronary endothelium of group 1 (Fig. 5) was well preserved, indicating that warm blood cardioplegic solution could effectively protect the morphology of the coronary endothelium from ischemia and reperfusion injury.

**Conclusions.** Global cardiac ischemia and reperfusion with or without intermittent cold crystalloid cardioplegia impair receptor-mediated release of EDRF from the coronary endothelium with G-protein dysfunction and damage the ultrastructure of the coronary endothelium. This kind of impairment can be prevented by continuous anterograde infusion of warm blood cardioplegic solution.
References