BENEFICIAL EFFECT OF DILTIAZEM ON ISCHEMIA-REPERFUSION INJURY IN THE DOG

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We determined the effect of the calcium antagonistic agent, diltiazem hydrochloride upon ischemia-reperfusion injury in the dog.

Ischemia was produced by occluding the left anterior descending artery for 40 min. Subsequent reperfusion was accomplished for 120 min by virtue of removal of the occlusion. Sixteen of the dogs studied were randomly assigned to diltiazem (D)-treated group (n = 8) and saline (S)-treated group (n = 8).

D in saline was intravenously infused at a concentration of 3 mcg/kg/min starting 15 min after the occlusion. Myocardial blood flow (MBF) was measured using hydrogen gas clearance method. Infarct size was quantified as percent myocardium at risk by triphenyltetrazolium chloride staining.

D administration caused a slight decline in mean aortic pressure, heart rate, and heart rate x systolic blood pressure throughout the periods of occlusion and reperfusion. However, there was no significant difference observed in both groups of dogs. MBF to ischemic myocardium was not significantly enhanced by D during ischemia. After 5 min of reperfusion subepicardial MBF was increased in group S, indicating a tendency towards reactive hyperemia. After 120 min of reperfusion there was a significant reduction in subendocardial MBF in group S and the transmural blood flow ratio was 1.23 ± 0.59 in group D as compared with 0.53 ± 0.39 in group S (p < 0.05). The infarcted area as a percentage of the risk area was considerably smaller in group D than in group S (27.5 ± 3.0 vs 47.0 ± 6.5%, p < 0.05).

D markedly reduced the elevation of tissue calcium especially in the subendocardium. These findings suggest that D reduces the ultimate infarct size through the beneficial effect on ischemia-reperfusion injury.

DILTIAZEM belongs to a group of drugs known as calcium antagonists which inhibit the movement of calcium ions through calcium specific channels in the sarcolemma. Calcium has frequently been associated with reperfusion. Shen and Jennings reported that a sudden restoration of flow results in a rapid and massive calcium influx into the ischemic reperfused region. In view of the important role of alterations in calcium homeostasis in myocardial ischemia, the protective effect of calcium antagonists has been intensively

Key Words:
- Myocardial ischemia
- Reperfusion injury
- Infarct size
- Myocardial blood flow
- Diltiazem

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The action of diltiazem includes an increase in myocardial blood flow, a decrease in left ventricular afterload and a decrease in myocardial contractility. In addition, diltiazem has been reported to prevent the intramitochondrial accumulation of calcium in the isolated perfused heart after coronary reperfusion, and has been shown to accelerate the restoration of myocardial contractility. Diltiazem has been reported to attenuate ischemia-induced depression of ATP levels and anaerobic glycolysis and mitochondrial dysfunction in acute canine myocardial ischemia. Because of the favorable effects on the determinants of the myocardial oxygen supply-demand ratio and its protective effect after reperfusion, diltiazem therapy has been studied as a mean of diminishing myocardial infarct size. However, the mechanism whereby diltiazem reduces ultimate infarct size after acute coronary occlusion has not been completely determined as yet. Bush has suggested that the reduction of myocardial ischemic injury is due to a lowering of myocardial oxygen demands, indirectly via favorable hemodynamic alterations and directly by limiting transmembrane calcium fluxes during ischemia and reperfusion. In contrast, has reported evidence that nifedipine therapy did not result in any significant reduction in the ultimate infarct size in the baboon. Nifedipine therapy was administered during a 2-hour period of coronary occlusion followed by reperfusion. Thus, evidence of the ability of calcium antagonists to salvage ischemic myocardium is inconclusive. In the present study, the effect of diltiazem on ischemia-reperfusion injury was examined using dog hearts in which myocardial blood flow, percent myocardium at risk and calcium contents were simultaneously measured.

**METHODS**

*Experimental procedure*

Thirty dogs weighing between 10 and 18 kg were anesthetized with pentobarbital (25 to 30 mg/kg) and were each placed on an Aika 60 respirator and ventilated under positive pressure with room air supplemented with oxygen to keep arterial oxygen tension and carbon dioxide tension between 95 and 120 mmHg, and 35 and
TABLE I HEMODYNAMIC PARAMETERS

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia</th>
<th>R-5</th>
<th>R-60</th>
<th>R-120</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group S (N = 8)</strong> HR ( /min)</td>
<td>149 ± 5</td>
<td>143 ± 7</td>
<td>141 ± 9</td>
<td>147 ± 11</td>
<td>153 ± 13</td>
</tr>
<tr>
<td>mAoP (mmHg)</td>
<td>116 ± 5</td>
<td>109 ± 4</td>
<td>109 ± 7</td>
<td>115 ± 6</td>
<td>120 ± 7</td>
</tr>
<tr>
<td>HR × SBP × 10³</td>
<td>19.6 ± 1.1</td>
<td>17.2 ± 1.4</td>
<td>17.5 ± 2.3</td>
<td>19.7 ± 2.7</td>
<td>20.9 ± 3.3</td>
</tr>
<tr>
<td>max dp/dt (mmHg/sec)</td>
<td>3740 ± 350</td>
<td>2320 ± 209</td>
<td>2530 ± 477</td>
<td>2860 ± 541</td>
<td>2980 ± 417</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>4.1 ± 0.3</td>
<td>7.3 ± 0.3</td>
<td>7.4 ± 0.4</td>
<td>6.7 ± 0.2</td>
<td>6.2 ± 0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia</th>
<th>R-5</th>
<th>R-60</th>
<th>R-120</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group D (N = 8)</strong> HR ( /min)</td>
<td>139 ± 9</td>
<td>138 ± 8</td>
<td>131 ± 9</td>
<td>135 ± 9</td>
<td>131 ± 10</td>
</tr>
<tr>
<td>mAoP (mmHg)</td>
<td>112 ± 7</td>
<td>105 ± 6</td>
<td>103 ± 6</td>
<td>110 ± 6</td>
<td>111 ± 7</td>
</tr>
<tr>
<td>HR × SBP × 10³</td>
<td>17.9 ± 1.9</td>
<td>16.5 ± 1.3</td>
<td>16.1 ± 1.7</td>
<td>17.5 ± 1.6</td>
<td>16.9 ± 1.8</td>
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<tr>
<td>max dp/dt (mmHg/sec)</td>
<td>3090 ± 156</td>
<td>2700 ± 180</td>
<td>2330 ± 477</td>
<td>3000 ± 378</td>
<td>2640 ± 240</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.3 ± 0.4</td>
<td>5.5 ± 0.3</td>
<td>6.5 ± 0.5</td>
<td>6.8 ± 0.4</td>
<td>6.1 ± 0.6</td>
</tr>
</tbody>
</table>

Mean ± Standard error
R-5, R-60 and R-120 denote the time in minutes after reperfusion, respectively.

TABLE II MYOCARDIAL BLOOD FLOW (ml/min/g) IN THE ISCHEMIC AREA

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia</th>
<th>R-5</th>
<th>R-60</th>
<th>R-120</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group S (N = 8)</strong> END</td>
<td>0.87 ± 0.14</td>
<td>0.10 ± 0.03</td>
<td>0.86 ± 0.11</td>
<td>0.50 ± 0.09</td>
<td>0.36 ± 0.09</td>
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<tr>
<td>EPI</td>
<td>1.14 ± 0.12</td>
<td>0.23 ± 0.04</td>
<td>1.57 ± 0.32</td>
<td>0.91 ± 0.23</td>
<td>0.74 ± 0.16</td>
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<tr>
<td>END/EPI</td>
<td>0.80 ± 0.12</td>
<td>0.70 ± 0.40</td>
<td>0.65 ± 0.11</td>
<td>0.69 ± 0.11</td>
<td>0.52 ± 0.14</td>
</tr>
<tr>
<td><strong>Group D (N = 8)</strong> END</td>
<td>1.03 ± 0.12</td>
<td>0.16 ± 0.03</td>
<td>1.10 ± 0.21</td>
<td>0.97 ± 0.16</td>
<td>0.86 ± 0.17</td>
</tr>
<tr>
<td>EPI</td>
<td>1.16 ± 0.17</td>
<td>0.16 ± 0.03</td>
<td>1.28 ± 0.21</td>
<td>0.75 ± 0.10</td>
<td>0.71 ± 0.10</td>
</tr>
<tr>
<td>END/EPI</td>
<td>0.93 ± 0.09</td>
<td>0.86 ± 0.12</td>
<td>0.96 ± 0.11</td>
<td>1.42 ± 0.24</td>
<td>1.23 ± 0.22</td>
</tr>
</tbody>
</table>

Mean ± standard error.
END = Subendocardium; EPI = subepicardium; END/EPI = The ratio of subendocardium. R-5, R-60 and R-120 denote the time in minutes after reperfusion, respectively. * Significance between groups S and D (p < 0.05).

45 mmHg, respectively. A left thoracotomy via the fifth intercostal space was performed and the heart was suspended in a pericardial cradle.

NIH Catheters connected to Statham P 23Db pressure transducer were introduced into the left ventricular (LV) cavity and ascending aorta to measure LV enddiastolic pressure (EDP), the peak rate of the rise of LV pressure (max dp/dt), and the aortic pressure. To measure regional myocardial blood flow (RMBF) two pairs of hydrogen electrodes were sutured into the myocardium, one in the normal circumflex area, the other in the central ischemic area. Each pair of electrodes were implanted into the myocardium so that each active part of the electrode, a distance of 3 cm distal to the tip, was in contact with the subendocardium and subepicardium (Fig. 1). Intramyocardial electrocardiogram was taken to confirm the exact placement of each electrode in the subendocardium and subepicardium, and to determine the ST-segment elevation related to myocardial ischemia. The electrode consisted of wire-type platinum, 100 micron in diameter, insulated with epoxy resin. A silver-silver chloride electrode was embedded into the subcutaneous tissue of the thorax as an indifferent electrode.

Hydrogen gas was inhaled into the dogs through the side hole of the tracheal tube at a constant rate of 1 L/min for 60 sec. Almost all desaturation curves obtained from the platinum electrode showed a monoexponential slope, however some fit a biexponential pattern. We calculated RMBF using an initial slope method.

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with one-compartment analysis. A plastic occluder snare was placed around the left anterior descending branch of the left coronary artery (LAD), distal to the first diagonal branch. After establishment of a stable hemodynamic condition, the LAD was totally occluded for 60 min. LAD occlusion caused the anterior wall of the LV to become cyanotic in 16 of 30 dogs and resulted in an ST-segment elevation of ECG. After 60 min of ischemia, the occlusive snare was removed, allowing reperfusion through the stenosed artery. Sixteen out of 30 dogs studied were then randomly assigned to drug-treated or untreated groups and accordingly given diltiazem hydrochloride, 3 μg/kg/min, or saline vehicle for 3 hrs intravenously after 15 min of total occlusion. Measurement of hemodynamic values, ST-segment and regional myocardial blood flow was repeated. After 2 hrs of reperfusion, the chest wound was closed.

Tissue sampling and processing

Approximately 18 hrs after closure of the chest, the dogs were reanesthetized and their chests reopened. Immediately after reocclusion of the LAD, at the same site as the preocclusion methylene blue, 1.5 ml/kg was injected into the left atrium over 30 seconds to define an in vivo epicardial area at risk. The region which was not perfused with methylene blue always coincided geographically with the area of epicardial cyanosis. Immediately after methylene blue staining, the hearts were then excised rapidly and washed with cold distilled water. The left ventricle was sliced into 10 mm transverse sections along the atrioventricular ring. The slices were incubated in 10% triphenyltetrazolium chloride (TTC) for approximately 15 min at 37°C. Infarcted (white in TTC) and ischemic but non-infarcted (red in TTC) areas on both side of the slice were photographed on polaroid film. Non-ischemic, ischemic but non-infarcted, and infarcted total left ventricular areas on both sides of each slice were digitized for subsequent calculation and the following parameters were calculated:

1. the area at risk = the ratio of area unstained by methylene blue to total left ventricular areas of all slices.
2. the area of infarct = the ratio of area unstained by TTC to total areas of all slices.
3. the area of infarct at risk = the ratio of area unstained by TTC to total areas unstained by methylene blue.

Using TTC staining quality as a guide, one slice of left ventricular tissue was dissected radially for calcium analysis. Tissues, approximately, 0.5g, were sampled from the area at risk and the normal area. Calcium levels in the tissue were measured after modification of Sparrow and Johnston. In that method, calcium was extracted into the aqueous phase with glacial acetic acid and 3M trichloroacetic acid. Some of the aliquots of the extract were combusted in the carbon cell and the calcium levels were quantitated by atomic absorption.

Statistical analysis

Differences within and between groups for hemodynamic data and measurements of the area at risk were measured using paired and unpaired t test. A p value of less than 0.05 was considered significant. The results were expressed as mean ± standard error of the mean.

RESULTS

Hemodynamic changes

Hemodynamic measurements were made immediately before coronary artery snare (Control), just before coronary reperfusion (Ischemia), after 5 min of reperfusion (R-5), after 60 min of reperfusion (R-60) and after 120 min of reperfusion (R-120). The measurements were made in eight saline (group S) and eight diltiazem-treated (group D) dogs. Changes in heart rate (HR), mean aortic pressure (mAO), the product of HR and systolic blood pressure (SBP), or double product, max dP/dt, and LVEDP are shown in Table I. Control values for all parameters are nearly identical in the two groups of dogs. Diltiazem administration caused slight declines in mAO, HR and HR x SBP, which is an index of myocardial oxygen consumption, throughout the periods of occlusion and reperfusion. The max dP/dt fell in both groups after coronary occlusion and R-5, but tended to return to control levels after R-60 and R-120. However there was no significant difference in both groups of dogs. LVEDP increased in both groups after ischemia and remained elevated throughout the experiment, although again there was no significant difference between both groups.

Regional myocardial blood flow (RMBF)

Changes in RMBF obtained with hydrogen clearance method are shown in Table II. Average blood flow to ischemic LV myocardium before occlusion shows almost similar values in epicardial

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Fig. 2. The ratio of subendocardial (END) to subepicardial (EPI) blood flow in groups S (Saline) and D (Diltiazem) in the ischemic area. R-5, R-60 and R-120 denote the time in minutes after reperfusion, respectively. *Significance between groups S and D.

<table>
<thead>
<tr>
<th>TABLE III ST SEGMENT ELEVATION IN THE ISCHEMIC AREA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td><strong>Group S (N = 8) END</strong></td>
</tr>
<tr>
<td>EPI</td>
</tr>
<tr>
<td><strong>Group D (N = 8) END</strong></td>
</tr>
<tr>
<td>EPI</td>
</tr>
</tbody>
</table>

Mean ± Standard error.
END = Subendocardium; EPI = Subepicardium. R-5, R-60 and R-120 denote the time in minutes after reperfusion, respectively. * = Significance between groups S and D (p < 0.05). † = Significance between ischemia and R-5 (p < 0.05)

and endocardial layers in the two groups of dogs. After 60 min of LAD occlusion, severe reduction in blood flow to myocardium supplied by that artery was observed, and subendocardial and subepicardial blood flow averaged 0.10 ± 0.03, and 0.23 ± 0.04 ml/min/g respectively in group S and 0.16 ± 0.03 and 0.16 ± 0.03 ml/min/g in group D. Blood flow to the ischemic myocardium was not significantly enhanced by diltiazem during LAD occlusion. After 5 min of reperfusion, blood flow returned to control values in both groups and subepicardial blood flow was increased in group S, indicating a tendency towards reactive hyperemia. After 60 min of reperfusion, the blood flow in endocardial and epicardial layers was decreased to the control stage with a greater decrease in endocardial blood flow in group S. After 120 min of reperfusion subendocardial blood flow in group S was further reduced, and there was a statistically significant difference in reduction of subendocardial blood flow between groups S and D. Figure 2 shows changes of transmural flow ratio between the two groups in the ischemic area. Significant differences in the ratio of subendocardial to subepicardial blood flow before occlusion and after 60 min of ischemia and 5 min of reperfusion were not observed. However, after 60 min of reperfu-
The blood flow ratio was significantly greater in group D than in group S. After 120 min of reperfusion the transmural blood flow ratio was $1.23 \pm 0.22$ in group D in comparison to $0.52 \pm 0.14$ in group S ($p < 0.05$).

**Electrocardiographic ST-segment changes**

Table III shows changes of ST-segment in the intramyocardial electrocardiogram in the ischemic area. After 60 min of ischemia, the ST-segment was significantly elevated in both experimental groups above the control values. However 5 min after reperfusion, changes of ST-segment in both endocardial and epicardial layers of group S were significantly greater than those during occlusion, although this elevation of ST-segment was not observed in group D. After 60 and 120 min of reperfusion a marked reduction of ST-segment was observed in both groups.

**Myocardial infarct size**

Figure 3 shows a photograph of a transverse...
Fig. 4. The mean risk area as a percentage of total LV (Risk/LV) is identical in groups S (Saline) and D (Diltiazem). The infarcted area as a percentage of the risk area (Inf/Risk) is significantly smaller in group D than in group S.

Fig. 5. The relation between infarct and risk area in groups D (Diltiazem) and S (Saline). The infarct areas relative to risk areas are smaller in group D than in group S.

Fig. 6. Tissue calcium content. Diltiazem markedly reduced the elevation of tissue calcium content in the subendocardium in the infarcted area (Ischemia). There is no difference between groups S (Saline) and D (Diltiazem) in normal area (Normal). END = subendocardium; EPI = subepicardium; **Significance between groups S and D (p < 0.01).
while infarcted tissue appeared pale yellow. The risk area was usually larger than the infarcted area. The mean risk area as a percentage of total LV was almost identical in groups S and D in Fig. 4. The infarcted area as a percentage of entire LV was considerably smaller in group D than in group S (14.0 ± 1.5 vs. 28.0 ± 5.0%) (p < 0.05). The infarcted area as a percentage of the risk area was considerably smaller in group D than in group S (27.5 ± 3.0 vs. 47.0 ± 6.5%). Figure 5 shows the relation between infarct area and risk area for both groups. The area of infarcted tissue correlated closely with the area at risk in the two groups. Two regression lines for both groups were different from each other and infarct areas relative to risk areas were smaller in group D than in group S. Thus it is suggested that diltiazem decreases myocardial infarct size relative to risk area.

**Tissue calcium**

As shown in Fig. 6, the calcium content of infarcted tissue within the risk area was significantly higher than that of the non-ischemic area. Diltiazem markedly lessened the elevation of tissue calcium in the infarcted area and in particular the calcium content was significantly reduced in subendocardial tissue. The tissue calcium content of non-ischemic myocardium was also less in group D, compared to group S. However, this difference was not significant.

**DISCUSSION**

**Validation of methods**

The hydrogen gas clearance method was first applied to the measurement of RMBF by Aukland. This method has been validated by comparison with the radioactive microsphere technique which is the most accurate method currently available for measuring RMBF. There was a high correlation (r = 0.96) between the RMBF obtained by hydrogen gas clearance and that derived from the radioactive microsphere method. Winbury also used the hydrogen clearance technique to measure RMBF.

We compared infarct size, expressed as a percent of an anatomic perfusion bed of the left ventricle, in saline- and diltiazem-treated dogs. This in vivo method for assessing myocardium at risk was originally described by Darsee who demonstrated Flurbiprofen salvaged lateral and epicardial border zone. There is a wide variation in infarct size in the dog, even with coronary occlusion at the same distance from the coronary orifice. To combat this variability in the infarct size several investigators have utilized their own techniques to accurately delineate the anatomic perfusion bed of the occluded artery. This permitted the infarct size to be expressed as a percent of the myocardium at risk and hence provided for a more accurate assessment of the effects of diltiazem treatment in infarct size.

**Mechanism of the protective effect of diltiazem**

Our results show that diltiazem reduces the ultimate size of myocardial infarct resulting from 60 min of LAD coronary artery occlusion. This beneficial effect does not appear to be due to increased myocardial blood flow to the ischemic region of the myocardium, since hydrogen washout measurements during coronary occlusion had no significant effect on collateral flow into the ischemic area. Whether or not the slow channel blocker can increase the collateral blood flow after coronary occlusion is controversial. Millard and Nakamura showed that diltiazem improved myocardial blood flow at ischemic margins without concomitant myocardial depression although it did not increase the blood flow in the ischemic center. However, the ischemic margin described by Nakamura may include peninsulas of normally perfused and ischemic myocardium, which may result in an apparent increase in myocardial blood flow at ischemic margins. Mitsunami in our laboratory demonstrated that the ischemic region contained peninsulas of normal and intermediated injured cells mainly in its subepicardium and partly at its lateral edge. Therefore even in the central area there should be a salvageable border zone depending on the amount of collateral circulation. Bush could not demonstrate the improvement of collateral blood flow during acute coronary occlusion. However, diltiazem did blunt the vasodilation that occurred in response to a brief total coronary occlusion, and partially corrected the subendocardial underperfusion. Nifedipine did not affect myocardial blood flow in the ischemic zone. In the present experiment the increase in the regional myocardial blood flow after 60 and 120 min following reperfusion may be secondary to the reduced injury in ischemic myocardium. Several investigators have shown that a brief coronary artery occlusion caused a microvascular injury in addition to myocardial damage, resulting in a progressive increase in coronary vascular resist-

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ance and a decrease in the blood flow to formerly ischemic tissue.\textsuperscript{28–31}

While the mechanism responsible for the no-reflow phenomenon is unclear, intracellular and interstitial edema that accompany ischemia and reperfusion have been implicated.\textsuperscript{32} Interventions that attenuate the no-reflow phenomenon may also reduce the ultimate infarct size. Hyperosmotic mannitol has been demonstrated to augment blood flow and improve cardiac function during acute myocardial ischemia,\textsuperscript{33} but Parker\textsuperscript{34} could not obtain any data consistent with that of Powell.\textsuperscript{30} It is likely that the ability of diltiazem to reduce the myocardial infarct size is related to the preventive action from the progressive increase in the coronary vascular resistance.\textsuperscript{5} The mechanism by which diltiazem reduces the ultimate size of myocardial infarct is suggested by Bush\textsuperscript{7,35} who has demonstrated that diltiazem reduces myocardial energy demands by reducing both arterial blood pressure and heart rate. It has also been shown that diltiazem possesses noncompetitive sympathetic blocking activity\textsuperscript{35} which may antagonize the adrenergically mediated increases in heart rate during acute coronary occlusion. In fact propranolol has been reported to have a favorable effect on microvascular injury in acute myocardial ischemia.\textsuperscript{37} However, in the present experiment, heart rate and systolic blood pressure tended to be lower in the treated dog than in saline control dogs, but the difference was not significant. There was no decrease in left ventricular enddiastolic pressure. Therefore, no definite evidence for decreased oxygen consumption was available. Therefore it appeared that the beneficial effects of diltiazem were in its direct action in reducing myocardial injury by preventing the rapid and massive mitochondrial calcium overloading which normally accompanies reperfusion of severely ischemic myocardium.\textsuperscript{8} Ashraf\textsuperscript{11} has suggested that diltiazem exerts its protective effect through an ability to prevent the cellular separation and alterations in the gaw junctions during calcium entry into the cells after reperfusion with calcium-containing medium. The decrease in adenosine triphosphate in the ischemic region was halved with inhibition of anaerobic glycolysis\textsuperscript{5} and the extent of ischemia-induced mitochondrial dysfunction was reduced.\textsuperscript{12,38} The left ventricular function was preserved in the ischemia-reperfusion injury by calcium channel blocking agents.\textsuperscript{4,8} Sasayama\textsuperscript{39} demonstrated that the post-stimulation deterioration of shortening was significantly improved in conscious dogs by diltiazem. The ability of diltiazem to markedly reduce excessive myocardial calcium content of injured myocardium within the area at risk also suggests its direct protective action through the inhibition of transmembrane calcium influx. Bush\textsuperscript{7} has shown marked elevations of both myocardial tissue and mitochondrial calcium after 60 or 90 min of ischemia and reperfusion. It has been suggested that the protective effects of the other calcium channel blockers, nifedipine,\textsuperscript{4} verapamil,\textsuperscript{12,13} nisoldipine\textsuperscript{40} may be explained by direct action.

The attenuation of calcium overload in ischemic myocardium is related to the decrease in reperfused injury with diltiazem. The present study was consistent with the decrease in reperfusion injury since ST-segment elevation after reperfusion was significantly suppressed in diltiazem-treated dogs. Mitochondria in ischemic and reperfused myocardium have been protected from increases of intracellular free calcium which spares ATP.\textsuperscript{2} This, in turn, allows the maintenance of ionic gradients, which may be related to electrocardiographic ST segment deviation. In contrast verapamil could not prevent loss of creatine kinase and accumulation of calcium in the isolated heart.\textsuperscript{39–41} Geary\textsuperscript{21} also could not obtain any favorable effect of nifedipine upon the ultimate size of myocardial infarct after coronary occlusion in the baboon. In their experiment an increase in contractility was noted before coronary occlusion with pretreatment of nifedipine. This increase in contractility may deteriorate the myocardial oxygen supply-demand balance, which results in no significant decrease in the ultimate size of myocardial infarct. Diltiazem may be a more favorable agent in decreasing the ischemia-reperfusion injury than nifedipine due to the absence of increasing contractility.

\textbf{Acknowledgement}

\begin{quote}
We thank Miss Sachiko Matsuyama for secretarial assistance and Mrs Mikio Nakagawa and Hiroshi Okuno for technical aid and collaborative efforts.
\end{quote}

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