Protection of hypoxic myocardium by intracoronary administration of verapamil in open-chest dogs

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ABSTRACT Hypoxic perfusion of the regional myocardium was performed in 34 open-chest dogs. In eight dogs in which the myocardium was perfused with original hypoxic Krebs-Henseleit (K-H) solution for 5 min (group I) there was a marked decrease in adenosine triphosphate content (2.03 ± 0.44 μmol/g) and an increase in lactate content (8.65 ± 2.26 μmol/g). In myocardium of nine dogs (group II) perfused with verapamil-containing (1.3 mg/dl) K-H solution and in that of nine dogs (group III) receiving Ca²⁺-free solution, the degrees of reduction in adenosine triphosphate (2.99 ± 0.73 and 3.25 ± 0.48 μmol/g, respectively) and of lactate accumulation (5.16 ± 0.59 and 4.79 ± 1.02 μmol/g, respectively) at 5 min were significantly less than in group I. Absence of statistically significant differences in hemodynamic parameters among these three groups indicates that the metabolic preservation in group II probably resulted from a direct effect of verapamil on the regional myocardium related to Ca²⁺ metabolism. However, metabolic data in four dogs of group I and four dogs of group III killed at 40 sec of perfusion, in conjunction with results of the analysis of regional contraction, revealed that the time courses of segmental shortening in the three groups did not account for the metabolic differences among them.


THE USEFULNESS of verapamil for myocardial protection from ischemia has been stressed experimentally in preparations in vivo since Wende et al.1 and Smith et al.2 using an epicardial ST mapping method,3 reported a reduction in ischemic area induced by the drug. In an experimental preparation of isolated hearts, inhibition of Ca²⁺ influx across the sarcolemma was indicated as the principal mechanism of the myocardial protection observed.4–13 In contrast, in studies of open-chest dogs receiving the drug systemically, the importance of direct effects on the ischemic myocardium remained unclear.3,4,14 The effects of the drug on the collateral circulation and on peripheral hemodynamics have also been shown to influence the balance of oxygen demand and supply,5,8 obscuring the direct effects on the jeopardized myocardium.

In the present study we attempted to elucidate the effects of local administration of verapamil on the energy metabolism and regional contraction of the hypoxic myocardium in a preparation in vivo in which the peripheral hemodynamic action of the drug was minimized as far as possible. The possibility that the mechanism of myocardial preservation is related to the Ca²⁺-blocking action of verapamil was examined by comparing the effects of verapamil perfusion with those of hypo-Ca²⁺ perfusion.

Materials and methods

Experiments were performed on 34 mongrel dogs weighing 12 to 29 kg that were anesthetized with 25 mg/kg of pentobarbital. Artificial respiration with a mixture of room air and 1 to 2 liters/min of oxygen through a Harvard pump maintained the arterial blood oxygen and carbon dioxide tension at around 100 and 30 mm Hg, respectively, throughout the experiments. After a left thoracotomy and pericardiotomy were performed in each dog, the left anterior descending artery (LAD) was dissected at the distal portion of the junction. The first diagonal branch was used as the connecting site for the tip of a bypass tube from the left subclavian artery (figure 1). The silicone tubing used to complete the bypass had an internal diameter of 3 to 4 mm and a three-way stopcock for hypoxic perfusion of the regional myocardium (initially fed by blood from the subclavian artery) and for measurement of the shunt pressure. An electromagnetic flow probe (Nihon Kohden Co.) was inserted in the bypass tube, and the output of the probe was measured by a flow meter. Aortic blood flow and shunt blood flow were measured by electromagnetic flow meters (Nihon Kohden Co.) and recorded on a pen记录er. Aortic blood flow was recorded using a transducer (Millar Instruments, Inc.) inserted into the ascending aorta. For measurement of systemic arterial pressure, a Statham transducer was inserted into the aortic arch. Systemic arterial pressure and heart rate were recorded on a oscilloscope. Systemic arterial pressure was also recorded simultaneously with the ECG and respiratory volume signals. Parameters of respiration were recorded with a respiratory monitor (Ohmeda, Inc.) and a respiratory pressure transducer (model 1200, Validyne Engineering Co.). The fraction of inspired oxygen was 0.21 to 0.29 during the experiments. As a result of this, the arterial blood oxygen tension was maintained at an average level of 100 ± 5 mm Hg, and the arterial carbon dioxide tension was maintained at an average level of 28 ± 2 mm Hg. Heart rate during the experiments was maintained at an average level of 95 ± 5 beats/min by adjusting the respiration rate and artificial ventilation. Ischemic insult was then produced by interrupting coronary blood flow for 5 min with a prior 2 min period of coronary occlusion. Ischemic insult was produced by interrupting coronary blood flow for 5 min with a prior 2 min period of coronary occlusion. Subsequently, the coronary occlusion was released. Myocardial function was examined by recording changes in ECG, arterial pressure, heart rate, and changes in respiratory volume signals.
heart performance, aortic pressure was monitored with a catheter placed in the ascending aorta, aortic flow was measured with an electromagnetic flowmeter with the probe applied around the ascending aorta, and left ventricular pressure and its first derivative were determined with an elastic catheter inserted from the apex. The tracings were recorded simultaneously with a limb lead electrocardiogram on a polygraph (Nihon Kohden RM 6000).

Brief interruption of the blood supply to the regional myocardium was necessary for cannulation of the tip of the bypass tube into the LAD. The time required for this procedure did not exceed 60 sec. After hemodynamic stabilization, validation of reactive hyperemia, and recording of control data, infusion of hypoxic solution into the LAD was begun with a tubing pump (Cole-Parmer Instrument Co.). Throughout the perfusion period the flow rate was maintained at the rate of LAD blood flow during the control period.

By deoxygenation with a gas mixture of 95% N₂ and 5% CO₂, the oxygen content of the solution was lowered to below 0.1 vol% and the pH was maintained at about 7.4. The temperature of the solution was kept at around 37°C with a thermostat (Komatsu Electronics Co.).

Hypoxic solutions were modified as follows. The dogs in group I (n = 12) received the original Krebs-Henseleit (K-H) solution (in mM, KCl 3.8, KH₂PO₄ 1.2, MgSO₄ 1.2, NaCl 118, NaHCO₃ 25, glucose 10) to which 2.5 mM of Ca²⁺ was added, those in group II (n = 9) received K-H solution with 2.5 mM of Ca²⁺ and 1.3 mg/dl of verapamil, and those in group III (n = 13) received a Ca²⁺-free solution made by omitting CaCl₂.

Eight, nine, and nine dogs from groups I, II, and III, respectively, underwent myocardial perfusion with hypoxic solutions for 5 min. Effects of brief hypoxia (40 sec) on myocardial metabolism were also studied in four dogs from group I and four from group III.

In the group II dogs, the systemic verapamil concentration was measured by sampling of arterial blood after 4 min of perfusion. In each of four dogs from group II the returning coronary venous blood and the hypoxic medium were drained through a catheter inserted into the great cardiac vein from the internal jugular vein to minimize transfer of the drug into the systemic circulation. In these four dogs the systemic verapamil concentration was negligible and even in the remaining five dogs of group II the systemic drug concentration was clearly low (9.3 ± 0.6 ng/ml) compared with the usual therapeutic concentration.¹⁴

In each of 26 dogs from the three groups part of the myocardium, including the site at which microcrystals were implanted, was biopsied transmurally by use of an electric drill⁽¹⁵⁾ (internal diameter 15 mm) for measurements of levels of high-energy phosphates, lactate, and pyruvate after 5 min of perfusion. After 40 sec of perfusion biopsy samples were obtained from eight dogs from groups I and III. In 18 of the 26 dogs undergoing 5 min of perfusion and all eight dogs undergoing 40 sec of perfusion, the nonhypoxic myocardium fed by the left circumflex branch was simultaneously biopsied as a normal control sample. The biopsy samples were quickly compressed with Wollenburger’s forceps precooled in liquid nitrogen. These procedures took about 10 sec. Levels of adenosine triphosphate (ATP), diphosphate (ADP), and monophosphate (AMP) were measured by high-performance liquid chromatography.¹⁶ Assays for creatine phosphate (CP), lactate, and pyruvate were by enzymatic methods.¹⁵

Results

The mean perfusion rates in dogs given 5 min perfusions were 34.9 ± 17.2, 29.9 ± 9.9, and 26.9 ± 10.5 ml/min in groups I, II, and III, respectively. Mean values and SDs for the hemodynamic parameters in these groups are summarized in table 1. Values before and after the 5 min perfusion in each group of dogs were analyzed by use of the paired t test and statistically significant differences are indicated in the table.

Regional hypoxic perfusion did not result in significant alterations in heart rate or aortic pressure in any of the three groups of dogs. However, a slight increase in aortic flow and a somewhat greater increase in left ventricular end-diastolic pressure were noted. These may be explained, at least in part, by the transfer of K-H solution into the systemic circulation throughout the 5 min perfusion.

The differences in these four indexes among the three groups were analyzed with the nonpaired t test or Cochran-Cox’s approximation⁽¹⁸⁾ after the F test. No
TABLE 1
Values for hemodynamic indexes at control and at 5 min of perfusion in the three groups of dogs (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>HR (beats/min)</th>
<th>Mean Ao P (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>Ao F (ml/beat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>5 min</td>
<td>0 min</td>
<td>5 min</td>
</tr>
<tr>
<td>Group I</td>
<td>161.4</td>
<td>161.6</td>
<td>103.0</td>
<td>109.0</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>± 22.4</td>
<td>± 23.2</td>
<td>± 18.6</td>
<td>± 19.9</td>
</tr>
<tr>
<td>Group II</td>
<td>154.9</td>
<td>154.1</td>
<td>106.0</td>
<td>99.3</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>± 14.6</td>
<td>± 13.6</td>
<td>± 25.8</td>
<td>± 20.5</td>
</tr>
<tr>
<td>Group III</td>
<td>155.7</td>
<td>153.3</td>
<td>88.4</td>
<td>89.0</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>± 30.3</td>
<td>± 29.0</td>
<td>± 24.5</td>
<td>± 19.9</td>
</tr>
</tbody>
</table>

Statistical analysis with the paired t test revealed that 5 min of perfusion did not result in significant increases in aortic pressure and heart rate, but did so in aortic flow and left ventricular end-diastolic pressure in all of the three groups. The differences in values for these four indexes among the groups were analyzed with the nonpaired t test or Cochran-Cox’s approximation after F test and no significant differences were found for either of the periods.

HR = heart rate; Ao P = aortic pressure; LVEDP = left ventricular end-diastolic pressure; Ao F = aortic flow.

statistically significant difference was noted in any of these indexes either during the control or the perfusion period.

The sequential changes in end-diastolic length normalized to the control value and the percent segmental shortening in 20 dogs that underwent 5 min of perfusion are summarized in table 2. The process of regional contractile deterioration clearly began earlier in group III than in groups I and II, as indicated by the values at both 20 and 50 sec. Except for a significantly smaller end-diastolic length in group II at 50 sec, there was no statistically significant difference between groups I and II.

The mean and SD levels of ATP, ADP, AMP, lactate, and pyruvate in each group of dogs given 5 min of perfusion are summarized in figure 2. The nonhypoxic myocardium of 18 dogs was characterized by high levels of ATP (4.37 ± 0.74 μmol/g), high ATP/ADP (4.52 ± 0.91), low levels of lactate (3.79 ± 1.27 μmol/g), and low lactate/pyruvate (10.63 ± 4.71). The ATP content and ATP/ADP were significantly reduced, whereas the lactate and lactate/pyruvate levels were significantly increased, in dogs from all of the three groups that underwent 5 min of perfusion as compared with the levels in the controls. The degree of reduction in ATP level in groups II (2.99 ± 0.73 μmol/g) and III (3.25 ± 0.48 μmol/g) was significantly less (p < .01 and p < .001, respectively) than that in group I (2.03 ± 0.44 μmol/g), and the ATP/ADP value was also smaller. The degree of lactate accumulation in groups II (5.16 ± 0.59 μmol/g) and III (4.79 ± 1.02 μmol/g) was also significantly less (p < .005 and p < .001, respectively) than that in group I (8.65 ± 2.26 μmol/g) and the lactate/pyruvate value was smaller.

To examine whether these metabolic differences

TABLE 2
Sequential changes in end-diastolic length normalized to control (EDLc) and percent segmental shortening (%SS) in the three groups of dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>20 sec</th>
<th>50 sec</th>
<th>2 min</th>
<th>5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDLc (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>10.00</td>
<td>10.81 ± 0.42a</td>
<td>11.33 ± 0.53</td>
<td>11.67 ± 0.70</td>
<td>11.79 ± 0.68</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>10.00</td>
<td>10.38 ± 0.43c</td>
<td>10.62 ± 0.50c,d</td>
<td>11.10 ± 0.59</td>
<td>11.15 ± 0.60</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>10.00</td>
<td>11.37 ± 0.41</td>
<td>11.69 ± 0.53</td>
<td>11.77 ± 0.48</td>
<td>12.00 ± 0.76</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
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</table>

%SS

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17.7 ± 6.8</td>
<td>16.6 ± 7.2</td>
<td>22.8 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>5.6 ± 6.1b</td>
<td>8.9 ± 9.0b</td>
<td>2.6 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>0.6 ± 4.5b</td>
<td>-1.7 ± 5.0</td>
<td>-4.9 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>-2.9 ± 6.8</td>
<td>-5.7 ± 4.2</td>
<td>-6.5 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>-3.3 ± 7.6</td>
<td>-6.3 ± 2.9</td>
<td>-5.5 ± 4.5</td>
</tr>
</tbody>
</table>

Statistical analysis with the nonpaired t test or Cochran-Cox’s approximation after F test: a p < .05; ab p < .01; c p < .005, respectively, compared with group III. d p < .05 compared with group I.
among the three groups could be related to the difference in sequence of bulge formation, the early metabolic changes were compared in the dogs from groups I and III killed after 40 sec of perfusion. No significant difference was observed in either ATP or CP content (table 3).

Discussion

New observations on the present study and their clinical implications. Fleckenstein et al.\(^{19}\) initially reported the inhibition of excitation-contraction coupling and reduction in oxygen consumption induced by verapamil in a preparation of isolated papillary muscle. They also reported\(^{20}\) that subcutaneous administration of the drug prevented the development of isoproterenol-induced cardiac necrosis and that this could be related to the preservation of myocardial contents of ATP and CP. In preparations of isolated hearts, Nayler et al.\(^{12}\) and Watts et al.\(^{13}\) found that verapamil protected myocardial structures and function against the deleterious effects of ischemia and reperfusion by the sparing of ATP in tissue. In these preparations, the effects of the drug on the peripheral circulation can be excluded as a mechanism of action, so the results can be considered evidence for the direct protective effects of the drug. There was also some evidence to suggest that this protective effect was not achieved by a decrease in heart work.\(^{21}\)

However, these results in isolated hearts cannot necessarily be extrapolated to pharmacologic action in preparations in vivo for the reasons pointed out by Ham and Opie.\(^{21}\) In fact, the actions of Ca\(^{2+}\) antagonists observed in preparations in vivo\(^{22}\) are considerably different from those in preparations in vitro and

![FIGURE 2. Metabolic changes in dogs from each group that underwent 5 min of perfusion. Mean and SDs of the levels of the metabolites in the regional myocardium are shown. The scale on the left is for the ATP, ADP, AMP, and pyruvate content, while that on the right is for the lactate content. On the left are the normal control levels of the metabolites obtained from nonhypoxic myocardium of 18 of the study dogs. The risk of error in statistical analysis of differences between the control group and groups I, II, or III is indicated by circles, and that in analysis of differences between group I and II or III is indicated by triangles.](http://circ.ahajournals.org/doi/fig/10.1161/01.01.84.745)
factors such as the timing of drug administration produce further variations in drug action.14, 6, 7, 23

The present results confirm those presented in previous studies of the isolated heart and, furthermore, provide clear evidence of the importance of the direct action of verapamil on the jeopardized myocardium in a preparation in vivo. Between groups I and II there were no significant differences in hemodynamic indexes throughout the experimental period. Furthermore, the systemic verapamil concentration at 4 min was either negligible, or, at most, lower than the therapeutic plasma concentration. These findings indicate that the peripheral hemodynamic effects of verapamil did not account for the differences in metabolic effects observed in groups I and II.

Critical alterations in the peripheral hemodynamics or the pump function of the heart were absent even in the dogs treated with verapamil in which the coronary venous mixture was not drained. This indicates that intracoronary administration of verapamil may be clinically useful as a supplementary method for protecting the jeopardized myocardium from irreversible ischemic damage before restoration of blood supply to the myocardium by aortocoronary bypass or coronary angioplasty. The antispastic effect on the coronary artery and/or selective suppression of the regional contractility of the ischemic myocardium by the drug may offer additional benefits of clinical application. However, there are still many problems to be solved before clinical use can be established; it is not known, for example, whether similar effects can be produced in ischemic preparations. However, the present study does at least indicate the usefulness and safety of intracoronary administration of large doses of verapamil for metabolic preservation of the jeopardized myocardium in a preparation in vivo.

The similar metabolic preservation noted in dogs receiving the Ca²⁺-free solution supports the view that the primary mechanism of protection of hypoxic myocardium by verapamil is related to Ca²⁺ metabolism. In isolated muscles perfused with oxygenated solution, the developed tension, which is a major determinant of oxygen consumption,24 was directly affected by the Ca²⁺ concentration of the perfusate,19, 25, 26 as well as by the administration of verapamil.19 In the present study, however, the sequence of segmental shortening did not provide any evidence to suggest that lowering the extracellular Ca²⁺ concentration or local administration of verapamil further suppressed the regional contractility of the hypoxic myocardium.

Two explanations have been offered for this dissociation between the mechanical and metabolic changes. Gibbs and Vaughn27 and Chapman et al.28 demonstrated a decrease in the tension-independent heat production per contraction by Ca²⁺ depletion in rabbit papillary muscle bathed in oxygenated K-H solution. Thus one possibility is that metabolic preservation by verapamil or perfusion with Ca²⁺-free solution arises from a decrease in tension-independent consumption of energy in the hypoxic myocardium. The other possibility is that metabolic preservation results from a decrease in development of tension in the hypoxic myocardium, but the degree of segmental shortening does not reflect this decrease quantitatively. It seems reasonable to speculate that the degree of systolic elongation in the hypoxic segment is affected by many factors.29 Besides the developed tension of the involved myocardium, these factors could include the site of microcrystal implantation, the extent of the hypoxic area, the left ventricular systolic pressure, etc.

Consideration of the experimental preparation. In the isolated heart, the degree of reduction in ATP level induced by hypoxic perfusion is generally less than that induced by ischemia, probably as a result of glycolytic inhibition by accumulated metabolites in the latter preparation.30–33 In this context, however, the rapid fall in ATP content in the present preparation of hypoxic perfusion should be noted.

In a recent study34 we examined differences between ischemia and hypoxia using the same experimental preparation as in the present study, and reduction in ATP level after 5 min of regional ischemia was not significantly different from that after 5 min of hypoxia. In this study the rapid fall in ATP level during the initial 5 min of regional hypoxia is in spite of the lack of severe metabolite accumulation may be explained, in part, by the relatively high distal coronary pressure that was the result of hypoxic perfusion itself, which probably reduces the contribution of collateral flow.

The energy charge of the normal tissue from the dogs undergoing 5 min of perfusion in the present study, 0.83, is somewhat low compared with that observed in other studies. This may be, at least in part, related to the method we used to obtain high-energy phosphate values, in which the pH of the buffer solution was set to obtain accurate ATP and ADP levels on the chromatogram at the cost of some accuracy in AMP measurement.

References
LABORATORY INVESTIGATION–MYOCARDIAL PROTECTION

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