ABSTRACT The transmural distribution of myocardial blood flow across the left ventricles of anesthetized dogs was measured with radioactive microspheres during intracoronary infusion of the endothelium-dependent vasodilators acetylcholine (10 μg/min), adenosine triphosphate (ATP; 20 μg/min), and arachidonic acid (600 μg/min) and the endothelium-independent vasodilator nifedipine (5 μg/min). These compounds were administered before and after a 30 min intracoronary infusion of the phospholipase A2 inhibitor quinacrine (300 μg/min). Acetylcholine, ATP, and arachidonic acid produced significant (p < .05) increases in transmural blood flow and in the ratio of subendocardial to subepicardial blood flow (endo/epi) when compared with control. Infusion of quinacrine did not affect this ratio and did not block the increase in transmural blood flow produced by each agent; however, it did block the redistribution of flow to the subendocardium. In contrast, there was no change in endo/epi during intracoronary infusion of nifedipine before and after quinacrine. These results suggest that endothelium-dependent vasodilators produce a preferential increase in subendocardial perfusion via a product of unsaturated fatty acid metabolism.


THE INTIMAL ENDOTHELIUM plays an important role in modulating the vascular response to a large number of compounds. Furchgott and Zawadzki\(^1\) first demonstrated the obligatory role of endothelium in the vasodilatory response to acetylcholine. In addition, it has been shown that adenosine triphosphate (ATP) and arachidonic acid produce endothelium-dependent relaxation of precontracted isolated blood vessels.\(^2\ 3\) In contrast, vasodilation produced by the slow-channel calcium-entry blocker nifedipine is the result of blockade of voltage-sensitive calcium channels\(^4\) and is not dependent on an intact vascular endothelium. In this study, the distribution of myocardial blood flow produced by intracoronary infusion of several endothelium-dependent vasodilators was compared with that produced by nifedipine, an endothelium-independent vasodilator.

Although the precise chemical nature of endothelium-derived relaxing factor (EDRF) is unknown, it has been postulated to be a derivative of arachidonic acid (or some other fatty acid) metabolism.\(^1\ 5\) The phospholipase A\(_2\) inhibitor quinacrine (mepacrine), which interferes with fatty acid metabolism by preventing availability of these compounds for lipoxygenase and cyclooxygenase enzymes, also has been found to inhibit the endothelium-dependent relaxation of isolated arteries produced by acetylcholine in several vascular beds.\(^1\ 6\)

Intracoronary infusion of acetylcholine has been shown previously to produce a greater increase in blood flow to the subendocardium compared with the subepicardium.\(^7\) In contrast, vagal stimulation increased coronary blood flow uniformly across the left ventricular wall.\(^8\) These results suggest that intracoronary infusion of acetylcholine stimulates endothelial cell release of EDRF, which results in a redistribution of blood flow favoring the subendocardium.

Thus the purpose of this study was to characterize the transmural distribution of left ventricular blood flow after vasodilation produced by intracoronary infusion of several endothelium-dependent or independent compounds and to assess the effects of phospholipase A\(_2\) inhibition on the distribution of blood flow produced by these agents.

Methods

General preparation. Adult mongrel dogs of either sex, weighing 15 to 25 kg, were fasted overnight, anesthetized with pentobarbital sodium (30 mg/kg supplemented with 5 mg/kg/hr
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right ventricular free wall, septum, valves, atria, epicardial fat, and blood vessels, the left ventricle was sectioned into normal (unstained) and drug-perfused (stained) regions. Care was taken to avoid any partially stained or border pieces. Each tissue sample was subdivided into subepicardial, midmyocardial, and subendocardial layers of approximately equal weight (0.5 to 1.0 g). The samples were weighed and placed in glass scintillation vials, and the activity of each isotope was determined in duplicate at four energy windows in an Autogamma spectrometer equipped with a dual-channel analyzer (Searle Analytic 1195). Similarly, the activity of each isotope in the reference blood flow samples was also determined. The true activity of each isotope in the tissue sample was calculated by correcting for energy overlap. Myocardial blood flow (Qm, ml/min/g) was calculated from the equation:

\[ Q_m = Q_s \cdot C_m/C_r \]

where \( Q_s \) is the rate of withdrawal (ml/min) of the reference blood sample, \( C_s \) is the true activity (counts/min) of the reference blood sample, and \( C_m \) is the true activity (counts/min/g) of the myocardial tissue sample. Myocardial blood flow values of tissue samples from the stained (drug-perfused) and unstained (normal) areas were pooled for calculation of flow in the subepicardium, midmyocardium, and subendocardium of both regions. Transmural myocardial blood flow was the weighted average of flows in the subepicardium, midmyocardium, and subendocardium in drug-perfused or normal zones.

Experimental design. After completion of surgery, sotalol (2.0 mg/kg iv) was administered to block myocardial and coronary vascular \( \beta \)-adrenoceptors as previously described.10 After a 30 min equilibration period, the peak reactive hyperemic response after a 20 sec total left anterior descending coronary artery occlusion was obtained. In 25 dogs after \( \beta \)-adrenergic blockade, mean coronary blood flow during reactive hyperemia was increased from 15 ± 2 to 48 ± 5 ml/min. This information allowed selection of a dose of drug to be administered via intracoronary infusion, which produced submaximal vasodilation of the left anterior descending perfusion territory to avoid nonspecific changes in the transmural distribution of coronary flow that occur during maximal vasodilation.11 In addition, intracoronary infusion permitted study of the direct effects of the vasodilators on myocardial blood flow independent of peripheral hemodynamic actions.

The endothelium-dependent vasodilators selected for study consisted of a structurally unrelated group of compounds, including acetylcholine (5, 10, and 20 \( \mu \)g/min), ATP (10, 20, and 50 \( \mu \)g/min), and arachidonic acid (0.3, 0.6, and 1.2 mg/min). The slow-channel calcium-entry blocker nifedipine (2, 5, and 10 \( \mu \)g/min), an endothelium-independent vasodilator, was studied for purposes of comparison. The influence of the phospholipase \( A_2 \) inhibitor quinacrine (300 \( \mu \)g/min iv), which blocks the release of EDRF,14 on the actions of the vasodilator agents was also determined. Separate groups of animals (\( n = 6 \) to 7) were used to study each vasodilator. In each experiment, the first dose of radioactive microspheres was administered during intracoronary infusion of vehicle (control state). Intracoronary infusion of each vasodilator was then performed in increasing doses for 5 min to establish a dose-response relationship for coronary blood flow. Subsequently, the second dose of microspheres was administered at steady-state flow conditions during a 5 min intracoronary infusion of a dose of each vasodilator that produced an increase in coronary blood flow that was 50% to 80% of the peak reactive hyperemic response. The third dose of radioactive microspheres was injected at the end of a 30 min intracoronary infusion of quinacrine (300 \( \mu \)g/min) to assess any direct effects of the phospholipase \( A_2 \) inhibitor on regional

Regional myocardial blood flow. The distribution of coronary blood flow was determined by the radioactive microsphere technique.12 Carbonized plastic microspheres (15 ± 3 \( \mu \)m diameter, New England Nuclear) labeled with \(^{141}\text{Ce}, ^{51}\text{Cr}, ^{103}\text{Ru}, \) or \(^{95}\text{Nb} \) were obtained as 2 mCi nucleol in 10 ml of isotonic saline to which one drop of Tween 80 was added to minimize aggregation. Before the left atrial injection, the mixture was vigorously agitated. A timed collection of reference flow from the femoral artery was started immediately before injection and maintained at a constant rate (7 ml/min) for 3 min. Approximately 2 to 4 \( \times \) 10\(^6\) microspheres were injected into the left atrium in a volume of 0.8 to 1.0 ml and flushed in with 4 to 6 ml of normal saline.

After completion of the experiment, intracoronary injection of India ink was performed through the diagonal branch catheter at mean aortic pressure to stain the area of myocardium exposed to intracoronary drug infusions. The heart was fibrillated by electrical stimulation, excised, washed with saline, and fixed in 10% formaldehyde solution for 24 to 48 hr. After removal of the
TABLE 1  
Effects of intracoronary infusion of acetylcholine (10 μg/min) on hemodynamics before and after quinacrine (300 μg/min) 

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acetylcholine</th>
<th>Quinacrine</th>
<th>Quinacrine + acetylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>128 ± 8</td>
<td>128 ± 7</td>
<td>125 ± 8</td>
<td>121 ± 6</td>
</tr>
<tr>
<td>Mean aortic blood pressure (mm Hg)</td>
<td>82 ± 8</td>
<td>78 ± 8</td>
<td>74 ± 7</td>
<td>70 ± 4</td>
</tr>
<tr>
<td>Left ventricular systolic pressure (mm Hg)</td>
<td>110 ± 7</td>
<td>106 ± 6</td>
<td>104 ± 8</td>
<td>101 ± 5</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td>5 ± 2</td>
<td>6 ± 2</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Peak positive dP/dt (mm Hg/sec)</td>
<td>1725 ± 150</td>
<td>1700 ± 150</td>
<td>1375 ± 150</td>
<td>1250 ± 125^A</td>
</tr>
<tr>
<td>Mean coronary blood flow (ml/min)</td>
<td>26 ± 3</td>
<td>48 ± 6^A</td>
<td>31 ± 6</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>Systolic coronary blood flow (ml/min)</td>
<td>10 ± 2</td>
<td>16 ± 3</td>
<td>19 ± 4</td>
<td>22 ± 6^A</td>
</tr>
<tr>
<td>Diastolic coronary blood flow (ml/min)</td>
<td>38 ± 3</td>
<td>72 ± 7^A</td>
<td>39 ± 6</td>
<td>53 ± 10</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 6).  
^Significant difference from control (p < .05).

myocardial perfusion. Each vasodilator was again infused by the intracoronary route in the doses established before administration of quinacrine. The fourth dose of radioactive microspheres was then administered at steady-state flow conditions during infusion of vasodilator in the presence of quinacrine at the same dose as used for the second microsphere, in the absence of quinacrine. In six additional experiments, regional myocardial blood flow was measured during infusion of vehicle (control) and arachidonic acid (600 μg/min ic) before and after indomethacin (1.5 mg/kg iv).

All drugs were prepared freshly on the day of the experiment with the exception of arachidonic acid, which was prepared as a stock solution. Arachidonic acid was dissolved in a solution of 10% ethanol in 100 mM Na₂CO₃ under nitrogen and diluted with isotonic saline to a final concentration of 1 mg/ml^12 and frozen in unit dose vials. Sotalol, acetylcholine, ATP, and quinacrine were dissolved in 0.9% sodium chloride solution. Indomethacin was prepared in a solution of 0.2 mM Na₂CO₃, and the pH was adjusted to 8.0 with 1N HCl. Nifedipine was dissolved in 10% ethanol and polyethylene glycol and diluted with normal saline. Care was taken to avoid contact of the nifedipine solution with light. Syringes and tubing were covered, and these experiments were performed under light emitted from a sodium vapor lamp.

Statistical analysis. All values are reported as mean ± SEM. Analysis of variance within a randomized complete block design with four treatments was used to test of main effects. Multiple comparisons procedure. Probability values of less than .05 were considered statistically significant.

Results

Hemodynamics. Hemodynamic data after intracoronary infusion of acetylcholine (10 μg/min), ATP (20 μg/min), arachidonic acid (600 μg/min), and nifedipine (5 μg/min) are summarized in tables 1 through 4, respectively. None of the compounds produced significant changes in heart rate, left ventricular and aortic pressures, or peak positive dP/dt. In contrast, all four compounds produced significant (p < .05) increases in total coronary blood flow of the drug-perfused region that were equal to 52% to 80% of the peak reactive hyperemic response. Both peak diastolic and systolic coronary blood flows were increased by all four vasodilators, but significantly (p < .05) so only in the presence of arachidonic acid and nifedipine.

Intracoronary infusion of quinacrine (300 μg/min) had no effect on heart rate, left ventricular and aortic pressures, or coronary blood flow. Peak positive dP/dt was reduced after infusion of quinacrine. The peak

TABLE 2  
Effects of intracoronary infusion of ATP (20 μg/min) on hemodynamics before and after quinacrine (300 μg/min) 

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ATP</th>
<th>Quinacrine</th>
<th>Quinacrine + ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>117 ± 6</td>
<td>117 ± 6</td>
<td>115 ± 6</td>
<td>116 ± 5</td>
</tr>
<tr>
<td>Mean aortic blood pressure (mm Hg)</td>
<td>87 ± 6</td>
<td>88 ± 5</td>
<td>87 ± 8</td>
<td>85 ± 10</td>
</tr>
<tr>
<td>Left ventricular systolic pressure (mm Hg)</td>
<td>107 ± 7</td>
<td>106 ± 7</td>
<td>106 ± 10</td>
<td>105 ± 11</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Peak positive dP/dt (mm Hg/sec)</td>
<td>1950 ± 125</td>
<td>1875 ± 175</td>
<td>1675 ± 150</td>
<td>1625 ± 150</td>
</tr>
<tr>
<td>Mean coronary blood flow (ml/min)</td>
<td>8 ± 1</td>
<td>18 ± 2^A</td>
<td>11 ± 2</td>
<td>16 ± 2^A</td>
</tr>
<tr>
<td>Systolic coronary blood flow (ml/min)</td>
<td>3 ± 2</td>
<td>10 ± 3</td>
<td>7 ± 3</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>Diastolic coronary blood flow (ml/min)</td>
<td>19 ± 2</td>
<td>46 ± 4^A</td>
<td>26 ± 4</td>
<td>37 ± 4^A</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 6).  
^Significant difference from control (p < .05).
reactive hyperemic response to a 20 sec total coronary occlusion was unchanged by quinacrine. In 25 dogs, mean coronary blood flow at the peak of reactive hyperemia increased from 15 ± 2 to 48 ± 5 ml/min (an increase of 320%) before and 14 ± 2 to 41 ± 3 ml/min (an increase of 293%) after quinacrine. However, the reactive hyperemic flow area as determined by planimetry was reduced (p < .05) from 0.91 ± 0.10 square inch before quinacrine to 0.53 ± 0.06 square inch after quinacrine. This suggests a decrease in total flow repayment with no decrease in vasodilator reserve after infusion of quinacrine. An example of the phasic reactive hyperemic response to a 20 sec occlusion before and after infusion of quinacrine is shown in figure 1.

**Regional myocardial perfusion.** Blood flow to the subepicardium, midmyocardium and subendocardium of the normal (left circumflex) and drug-perfused (left anterior descending) regions are summarized in tables 5 to 8. Intracoronary infusion of acetylcholine (10 μg/min), ATP (20 μg/min), arachidonic acid (600 μg/min), or nifedipine (5 μg/min) had no effect on tissue flow in the unperfused region. In contrast, all of the vasodilators increased blood flow to the subepicardium, midmyocardium, and subendocardium and consequently produced significant increases in transmural blood flow in the drug-perfused region (figure 2). The transmural distribution of flow across the left ventricular free wall produced by acetylcholine, ATP, and arachidonic acid preferentially favored the subendocardium, thus increasing the ratio of subendocardial to subepicardial flow (endo/epi) (figure 3). Cyclooxygenase inhibition by indomethacin had no effect on the increase in endo/epi produced by arachidonic acid. In six experiments, arachidonic acid increased endo/epi from 1.01 ± 0.13 to 1.63 ± 0.18 before indomethacin and to 1.51 ± 0.16 after indomethacin. Endo/epi remained unchanged in the presence of nifedipine despite significant increases in transmural flow (figures 2 and 3).

Quinacrine produced no significant change in sub-

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**TABLE 3**

Effects of intracoronary infusion of sodium arachidonic acid (600 μg/min) on hemodynamics before and after quinacrine (300 μg/min)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Arachidonic acid</th>
<th>Quinacrine + arachidonic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>122 ± 4</td>
<td>125 ± 3</td>
<td>130 ± 5</td>
</tr>
<tr>
<td>Mean aortic blood pressure (mm Hg)</td>
<td>126 ± 5</td>
<td>119 ± 8</td>
<td>127 ± 7</td>
</tr>
<tr>
<td>Left ventricular systolic pressure (mm Hg)</td>
<td>148 ± 4</td>
<td>148 ± 6</td>
<td>150 ± 8</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td>5 ± 1</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Peak positive dp/dt (mm Hg/sec)</td>
<td>2325 ± 250</td>
<td>2300 ± 275</td>
<td>2200 ± 200</td>
</tr>
<tr>
<td>Mean coronary blood flow (ml/min)</td>
<td>11 ± 1</td>
<td>27 ± 3</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Systolic coronary blood flow (ml/min)</td>
<td>6 ± 1</td>
<td>13 ± 3</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>Diastolic coronary blood flow (ml/min)</td>
<td>21 ± 2</td>
<td>52 ± 6</td>
<td>22 ± 3</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 6).

*Significant difference from control (p < .05).

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**TABLE 4**

Effects of intracoronary infusion of nifedipine (5 μg/min) on hemodynamics before and after quinacrine (300 μg/min)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Nifedipine</th>
<th>Quinacrine</th>
<th>Quinacrine + nifedipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>119 ± 6</td>
<td>120 ± 5</td>
<td>117 ± 5</td>
<td>132 ± 10</td>
</tr>
<tr>
<td>Mean aortic blood pressure (mm Hg)</td>
<td>106 ± 5</td>
<td>98 ± 7</td>
<td>95 ± 10</td>
<td>89 ± 8</td>
</tr>
<tr>
<td>Left ventricular systolic pressure (mm Hg)</td>
<td>127 ± 7</td>
<td>119 ± 7</td>
<td>114 ± 10</td>
<td>111 ± 9</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>Peak positive dp/dt (mm Hg/sec)</td>
<td>2025 ± 125</td>
<td>1925 ± 125</td>
<td>1675 ± 150</td>
<td>1625 ± 150</td>
</tr>
<tr>
<td>Mean coronary blood flow (ml/min)</td>
<td>16 ± 2</td>
<td>33 ± 8</td>
<td>17 ± 3</td>
<td>31 ± 7</td>
</tr>
<tr>
<td>Systolic coronary blood flow (ml/min)</td>
<td>6 ± 1</td>
<td>20 ± 6</td>
<td>6 ± 2</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Diastolic coronary blood flow (ml/min)</td>
<td>31 ± 1</td>
<td>57 ± 9</td>
<td>31 ± 2</td>
<td>50 ± 9</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 7).

*Significant difference from control (p < .05).
epicardial, midmyocardial, subendocardial, or transmural blood flow in either normal or drug-perfused regions (tables 5 to 8; figure 2). Likewise, quinacrine produced no change in endo/epi (figure 3). Infusion of quinacrine, however, markedly inhibited the increase in subendocardial flow produced by acetylcholine, ATP, and arachidonic acid, thereby preventing the increase in endo/epi. Thus, whereas the absolute increases in overall tissue perfusion was unaffected (figure 2), the preferential redistribution of blood flow to the subendocardium was prevented (figure 3). The increase in transmural flow produced by nifedipine was unchanged by quinacrine. No redistribution of flow occurred with quinacrine plus nifedipine.

**Discussion**

The purpose of this study was to characterize the regional distribution of coronary blood flow produced by the endothelium-dependent vasodilators acetylcholine, ATP, and arachidonic acid as compared with the endothelium-independent vasodilator nifedipine. The effects of phospholipase A₂ inhibition on the vasodilation produced by these compounds were also investigated. The results demonstrate that endothelium-dependent compounds produce a preferential increase in subendocardial perfusion, thus increasing endo/epi. Endo/epi was unchanged by nifedipine. Blockade of this flow redistribution by quinacrine, a phospholipase A₂ inhibitor, suggests that the increased endo/epi may be mediated by a product of unsaturated fatty acid metabolism.

A preferential increase in subendocardial perfusion produced by intracoronary infusion of acetylcholine has been shown previously to be caused by a specific dilator action on the subendocardial vasculature.

Many endothelium-dependent compounds, including those used in this study, have additional mechanisms for producing direct smooth muscle relaxation. A summary is included in table 9, which illustrates the effect of various vasodilators on endo/epi. In contrast, intracoronary infusion of nifedipine, as well as several other vasodilators, has been shown to produce no change in endo/epi while significantly increasing transmural myocardial blood flow. Although there were small differences in control hemodynamics between groups,
these were not reflected as differences in myocardial blood flow distribution. Also, the size of the drug-perfused region was a constant fraction of the size of the left ventricle to ensure that changes produced by the vasodilators would have similar effects on myocardial and systemic hemodynamics. No readily identifiable hemodynamic factors capable of altering endo/epi such as myocardial contractility, diastolic perfusion time, or coronary perfusion pressure were changed by any of the vasodilators investigated. Thus, global hemodynamic changes do not appear to account for the preferential increases in subendocardial blood flow produced by acetylcholine, ATP, or arachidonic acid.

Previous results from this laboratory have shown that intracoronary infusion of acetylcholine at the doses used in this study has no effect on regional contractility as assessed by isometric strain gauge arches or by sonomicrometry.7,16 Similarly, Abrahamsson et al.15 have demonstrated no changes in regional segment shortening until very high doses (40 μg/min) of nifedipine were infused by the intracoronary route. In this study, peak positive dP/dt, a measure of global contractility, was unchanged by any of the vasodilators. Although dP/dt does not directly measure regional wall function, it may reflect regional changes in muscle function in these experiments, since the drug-perfused territory was large enough that any changes in this region might be observed as a change in global contractility.

Quinacrine had no significant effect on systemic hemodynamics but produced decreases in peak positive dP/dt in the acetylcholine-, ATP-, and nifedipine-treated groups. Despite these negative inotropic effects of quinacrine, there was no change in the distribution of myocardial blood flow (endo/epi). Infusion of quinacrine in the presence of acetylcholine, ATP, or nifedipine produced significant (p < .05) decreases in global contractility, indicating a possible additive negative inotropic effect of these compounds. However, Gross et al.17 have demonstrated contractility to have only minimal effects on endo/epi provided left ventricular pressures were unchanged, as in this study. The regional distribution of increased blood flow produced by nifedipine was unchanged after quinacrine. Thus the selective blockade of subendocardial perfusion in the animals treated with endothelium-dependent vasodilators is most likely unrelated to any negative inotropic effects of quinacrine.

Activation of phospholipases with corresponding release of arachidonic or other unsaturated fatty acids from cellular storage sites and subsequent formation of metabolites may be the basis for endothelium-dependent relaxations.18 Many stimuli, including chemical, hormonal, immunologic, and mechanical, are thought to activate phospholipases. The effects of quinacrine, a phospholipase A2 inhibitor,19 on the vasodilation produced by acetylcholine, ATP, and arachidonic acid were investigated in this study and the results compared with those of nifedipine. Quinacrine has been shown previously to block endothelium-dependent vasodilation produced by acetylcholine and ATP20 in rabbit aorta but not those produced by ATP in the canine femoral artery.21 The basis for these results may be related to either species differences or altered reactivities of various vascular beds. In this study, infusion of quinacrine alone had no effect on regional myocardial
Nevertheless, quinacrine accumulation unrelated secretion before fatty rated may which cells stimulates then be blood of lipid redoxes have all been shown to activate guanylate cyclase directly, causing vasodilation. Thus these compounds have been suggested as possible candidates for EDRF. In this study, quinacrine blocked the redistribution of blood flow to the subendocardium produced by arachidonic acid. This would not be expected if EDRF were a metabolite of arachidonic acid itself, since phospholipase A2 inhibition would not affect the activity of exogenously administered arachidonic acid. Indeed, other saturated and unsaturated fatty acids produce endothelium-dependent relaxations that are not inhibited by cyclooxygenase inhibitors. These results agree with the previous suggestion that the unsaturated fatty acid precursor of EDRF is not arachidonic acid but another unsaturated fatty acid.

Infusion of quinacrine did not alter the peak flow measured during the reactive hyperemic response after a 20 sec total coronary artery occlusion. However, quinacrine did reduce the total flow repayment as assessed by area under the reactive hyperemia flow curve. These findings corroborate those of Kimura and

**TABLE 5**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acetylcholine</th>
<th>Quinacrine</th>
<th>Quinacrine + acetylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.63 ± 0.05</td>
<td>0.64 ± 0.06</td>
<td>0.61 ± 0.05</td>
<td>0.74 ± 0.09</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.73 ± 0.08</td>
<td>0.74 ± 0.06</td>
<td>0.67 ± 0.11</td>
<td>0.82 ± 0.15</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.79 ± 0.07</td>
<td>0.87 ± 0.08</td>
<td>0.74 ± 0.10</td>
<td>0.83 ± 0.08</td>
</tr>
<tr>
<td>Drug-perfused region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.60 ± 0.06</td>
<td>1.26 ± 0.25A</td>
<td>0.82 ± 0.18</td>
<td>1.25 ± 0.33A</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.57 ± 0.05</td>
<td>1.50 ± 0.31A</td>
<td>0.62 ± 0.14</td>
<td>1.30 ± 0.39A</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.62 ± 0.07</td>
<td>1.84 ± 0.40A</td>
<td>0.80 ± 0.21</td>
<td>1.31 ± 0.41</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 6).

**TABLE 6**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ATP</th>
<th>Quinacrine</th>
<th>Quinacrine + ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.72 ± 0.13</td>
<td>0.81 ± 0.20</td>
<td>0.91 ± 0.23</td>
<td>0.85 ± 0.16</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.78 ± 0.12</td>
<td>0.89 ± 0.18</td>
<td>0.95 ± 0.19</td>
<td>1.04 ± 0.24</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.84 ± 0.12</td>
<td>0.91 ± 0.15</td>
<td>0.93 ± 0.15</td>
<td>0.97 ± 0.20</td>
</tr>
<tr>
<td>Drug-perfused region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.77 ± 0.17</td>
<td>1.32 ± 0.31</td>
<td>1.32 ± 0.26</td>
<td>1.43 ± 0.32</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.84 ± 0.14</td>
<td>1.82 ± 0.40A</td>
<td>1.31 ± 0.28</td>
<td>1.68 ± 0.35</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.88 ± 0.17</td>
<td>2.18 ± 0.44A</td>
<td>1.31 ± 0.30</td>
<td>1.79 ± 0.33A</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 6).

*Significant difference from control (p < .05).
Satoh,25 who showed that quinacrine reduced the hyperemic response to coronary occlusion or infusion of adenosine. These investigators postulated that myocardial ischemia activates coronary and/or myocardial phospholipases with subsequent release of arachidonic acid or other fatty acids that are converted to a metabolic product responsible for the hyperemic response. This explanation is consistent with involvement of vascular endothelium in the reactive hyperemic response. In addition, flow-mediated release of EDRF may be important in reactive hyperemia. If quinacrine blocks formation and/or release of EDRF,1 it could reduce the total flow repayment during reactive hyperemia. Infusion of quinacrine had no effect on the increase in coronary blood flow produced by acetylcholine in the study of Kimura and Satoh25 or in our investigation. The redistribution of blood flow to the subendocardium consistently blocked by quinacrine in this study is postulated to be caused by an endothelium-derived factor. The blockade of endothelial-dependent flow changes by quinacrine were limited to alterations of flow redistribution that were not measured during the reactive hyperemic response. Increases in total coronary blood flow produced by the various vasodilators were unchanged by phospholipase A2 inhibition.

Increases in flow have been shown to mediate endothelium-dependent dilation26,27 and stimulate release of EDRF.28 The flow-induced effects of EDRF have been demonstrated in isolated canine coronary arteries18 or isolated rat mesenteric resistance vessels29 as well as in canine femoral30 and epicardial coronary arteries31 in vivo. Quinacrine was shown to attenuate this response in canine femoral arteries in vivo.30 We have postulated that redistribution of blood flow to the subendocardium is dependent on EDRF. The present data do not support a flow-mediated release of EDRF with effects on the microvasculature, since nifedipine did not preferentially increase subendocardial flow although it produced similar increases in total transmural perfusion when compared with acetylcholine, ATP,

<p>| TABLE 7 |
| Effects of sodium arachidonic acid (600 μg/min) on regional myocardial blood flow (ml/min/g) before and after quinacrine (300 μg/min) |</p>
<table>
<thead>
<tr>
<th>Normal region</th>
<th>Control</th>
<th>Arachidonic acid</th>
<th>Quinacrine</th>
<th>Quinacrine + arachidonic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subepicardium</td>
<td>0.79±0.07</td>
<td>0.78±0.12</td>
<td>0.85±0.06</td>
<td>0.72±0.11</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.88±0.07</td>
<td>0.92±0.14</td>
<td>0.91±0.10</td>
<td>0.90±0.14</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>1.05±0.09</td>
<td>1.06±0.15</td>
<td>1.10±0.10</td>
<td>0.96±0.16</td>
</tr>
<tr>
<td>Drug-perfused region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.85±0.10</td>
<td>1.31±0.20</td>
<td>0.78±0.14</td>
<td>1.42±0.14</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.99±0.10</td>
<td>1.69±0.24</td>
<td>0.99±0.10</td>
<td>1.67±0.22</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.94±0.06</td>
<td>2.10±0.33</td>
<td>0.94±0.10</td>
<td>1.76±0.30</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 6).

^Significant difference from control (p<.05).

<p>| TABLE 8 |
| Effects of nifedipine (5 μg/min) on regional myocardial blood flow (ml/min/g) before and after quinacrine (300 μg/min) |</p>
<table>
<thead>
<tr>
<th>Normal region</th>
<th>Control</th>
<th>Nifedipine</th>
<th>Quinacrine</th>
<th>Quinacrine + nifedipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subepicardium</td>
<td>0.65±0.11</td>
<td>0.76±0.15</td>
<td>0.64±0.09</td>
<td>0.77±0.18</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.81±0.10</td>
<td>0.84±0.14</td>
<td>0.70±0.10</td>
<td>0.81±0.18</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.97±0.14</td>
<td>0.97±0.14</td>
<td>0.80±0.11</td>
<td>0.93±0.18</td>
</tr>
<tr>
<td>Drug-perfused region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.77±0.09</td>
<td>1.83±0.40</td>
<td>0.91±0.14</td>
<td>1.63±0.31</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.78±0.11</td>
<td>1.81±0.43</td>
<td>0.86±0.14</td>
<td>1.59±0.39</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.81±0.12</td>
<td>1.86±0.41</td>
<td>0.78±0.11</td>
<td>1.56±0.32</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 7).

^Significant difference from control (p < .05).
and arachidonic acid. Flow-mediated release of EDRF in epicardial vessels would be difficult to distinguish among the vasodilators tested. There were significant increases in subepicardial flow after administration of all compounds tested, thus there may have been an endothelium-dependent component due to this increased flow. However, it is possible that nifedipine, while stimulating flow-mediated EDRF release, offsets this effect by blockade of calcium entry. Rubanyi et al.\textsuperscript{32} have demonstrated that nitrendipine inhibits endothelium-dependent vasodilation induced by the calcium ionophore A23187 in canine femoral artery. This the role of flow-mediated release of EDRF is unresolved for these compounds.

The physiologic significance and pathophysiologic implications of endothelium-dependent vasodilation are subjects of current interest. Since few vasodilators have been shown to produce a selective increase in endo/epi, it may be of interest to study release of EDRF by other stimuli. For example, hypoxic or flow-mediated release of EDRF may produce a similar pattern of vasodilation, providing no confounding hemodynamic alterations are also present (i.e., an increase in heart rate or reduction in diastolic pressure).

Our results indicate that the endothelium-dependent vasodilators acetylcholine, ATP, and arachidonic acid produce an increase in endo/epi presumably via release of an endothelium-derived relaxing factor(s) that is the product of fatty acid metabolism. The compounds studied stimulate the release of EDRF via receptor stimulation (acetylcholine and ATP) or possibly by nonspecific changes in endothelial cell membrane fluidity (arachidonic acid).\textsuperscript{33} The mechanism by which this release of EDRF alters transmural perfusion gradients has not been shown but is possibly related to a higher endothelial cell volume compared with smooth muscle cell volume within the microcirculation of the subendocardium rather than to factors involving the means of endothelial cell stimulation.

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