Responsiveness to Cardiac Sympathetic Nerve Stimulation During Maximal Coronary Dilation Produced by Adenosine

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SUMMARY. This study examined effects of adrenergic constrictor stimuli on total and transmural myocardial blood flow during maximal coronary dilation produced by adenosine. Our goal was to determine (1) if coronary vessels maximally dilated with adenosine respond to constrictor stimuli and (2) if the constrictor effect is uniform transmurally. In anesthetized dogs, the circumflex coronary artery was perfused at constant pressure. Total circumflex flow was measured with an electromagnetic flowmeter; transmural flow was measured with 9-μm radiolabeled spheres injected into the perfusion tubing. Propranolol (2 mg/kg, iv) was administered to block myocardial and coronary \( \beta \)-receptors. Coronary responses to cardiac sympathetic nerve stimulation (CSNS) at 10 Hz and intracoronary administration of angiotensin (0.125 and 0.50 μg/min), phenylephrine (0.50 and 2.00 μg/min), norepinephrine (0.25 and 1.0 μg/min) and tyramine (200 μg/min) were obtained during intracoronary infusion of adenosine (4.7 μm/min). This dose of adenosine abolished reactive hyperemia; moreover, a higher dose of adenosine did not produce further increases in flow. Control flow during adenosine averaged 382 ± 50 (SE) for endocardium and 524 ± 44 ml/min per 100 g for epicardium. During adenosine, CSNS at 10 Hz decreased epicardial flow to 308 ± 44 ml/min per 100 g; \( P < 0.05 \), but did not decrease endocardial flow (378 ± 58 ml/min per 100 g; NS). In contrast, angiotensin, phenylephrine, norepinephrine, and tyramine produced significant decreases (\( P < 0.05 \)) in both endocardial and epicardial flows. The results indicate: (1) that coronary arteries maximally dilated with adenosine are responsive to constrictor effects of adrenergic and nonadrenergic stimuli, and (2) that during adenosine treatment, the administration of angiotensin, phenylephrine, norepinephrine, and tyramine decreases both endocardial and epicardial flow, but sympathetic nerve stimulation decreases epicardial flow without significant decreases in endocardial flow. This non-uniform influence of nerve stimulation on transmural myocardial flow does not appear to relate to mechanical or metabolic factors or to characteristics of coronary adrenergic receptors. (Circ Res 50: 510-517, 1982)

CARDIAC sympathetic nerves can influence coronary resistance and myocardial blood flow directly through effects on coronary vessels mediated by \( \alpha \)-receptors and indirectly through effects on myocardial metabolism (Ross, 1976). Studies from several laboratories have demonstrated that the direct constrictor effects of cardiac sympathetic nerves can modulate or dominate metabolic vasodilator influences (Mohrm an and Feigl, 1978; Murray and Vatner, 1979; Mudge et al., 1979). Nevertheless, it is often suggested that intense metabolic vasodilation abolishes the constrictor effect of the sympathetic nerves. In this regard, it has been reported that adenosine, a purported mediator of constrictor effects of adrenergic neurotransmission (Verhage et al., 1977).

The goal of this study was to examine the effects of cardiac sympathetic nerve stimulation on coronary blood flow during administration of adenosine. The questions we posed were: (1) can sympathetic nerve stimulation constrict coronary vessels which are maximally dilated with adenosine, and (2) is the vasoconstriction produced by sympathetic nerve stimulation during adenosine uniform transmurally?

Methods

General Preparation

Studies were performed in open-chest dogs anesthetized with \( \alpha \)-chloralose (100 mg/kg, iv) and treated with decamethonium bromide (0.3 mg/kg, iv). Dogs were ventilated with a Harvard respirator, using room air supplemented with oxygen. A water trap was used to maintain an expiratory pressure of about 5 cm of water. Blood gases and acid/base balance were maintained within normal limits.

Arterial pressure was measured with a polyethylene catheter advanced into the aorta through a femoral artery. Heart rate was monitored with a tachometer triggered by the arterial pressure pulse. Left ventricular pressure was measured with a Konigsberg P22 pressure transducer inserted into the left ventricle through an incision in the left atrial appendage. Left ventricular pressure signal with a Gould 13-4615-72 differentiator amplifier. All recordings were made on a Gould Brush 2800 recorder.

For cardiac sympathetic nerve stimulation, the left ansa subclavia was decentralized and the distal part of the ansa was stimulated at 5 and 10 Hz with 4-msec pulses at supramaximal voltage.

Since the goal of this study was to examine the direct...
constrictor effects of sympathetic nerves on myocardial flow, all experiments (except where specified) were performed after administration or propranolol (2 mg/kg, iv) to block myocardial metabolic effects of nerve stimulation.

**Intravenous Adenosine Study**

In an initial series of experiments (n = 9), we examined the effects of cardiac sympathetic nerve stimulation at 5 and 10 Hz during intravenous adenosine (4.7 µmol/kg per min). Intravenous adenosine produced a stable, albeit low, systemic arterial pressure within 3 minutes. To determine that this dose of adenosine produced maximal coronary vasodilation, we measured the reactive hyperemic response to 20 seconds of coronary occlusion before and during adenosine infusion using a pulsed Doppler velocity probe (Wright et al., 1980). This dose of adenosine consistently abolished the reactive hyperemia produced by coronary occlusion. Microspheres were injected between 15 and 25 seconds after nerve stimulation was begun. Preliminary studies in which a flowmeter was utilized showed that the constrictor response to nerve stimulation after propranolol was maximal by 15–25 seconds.

In all experiments, transmural myocardial blood flow was measured with 9-µm microspheres (3M Company) injected into the left atrium. The spheres were labeled with one of the following isotopes: ⁴¹Sc, ⁴⁶Nb, ⁴⁶Sc, ¹²⁴Ce, ¹⁵²T, or ¹⁵⁵Sn. The techniques for this method have previously been described (Falsetti et al., 1975; Heymann et al., 1977). Briefly, between 1.1 and 10.7 X 10⁶ microspheres were injected into the left atrium through a small catheter for each flow measurement. Before injection, the vial containing the spheres was vigorously agitated mechanically for at least 1 minute. The spheres were injected over 30 seconds with 5 ml of saline warmed to 37°C. For flow determination, reference blood samples were withdrawn simultaneously from a femoral and brachial artery at a constant rate using a Harvard infusion-withdrawal pump.

After the study, the heart was excised and all other chambers, great vessels, valves, and epicardial fat were removed from the left ventricle and interventricular septum. The left ventricle and attached interventricular septum were divided from apex to base into four slices which in turn were subdivided into eight transmural segments each. Each segment then was divided into three layers: endocardium, mid-wall, and epicardium. The myocardial pieces were weighed to the nearest milligram and counted for 3 minutes each in a 3” well-type sodium iodine y counter along with the reference blood samples. Isotope separation was performed utilizing standard techniques (Heymann et al., 1977). Subsequent analysis of flow was performed with a PDP 11/35 computer. Myocardial blood flow was calculated using the formula:

\[ MBF = \frac{CM \times 100}{CR} \times RBF \]

where MBF = myocardial blood flow in ml/min per 100 g; CM = counts/g of myocardium; RBF = reference blood flow (rate of withdrawal from reference artery in ml/min); and CR = total counts from reference blood.

**Constant Perfusion Pressure Studies**

In all subsequent studies, a constant pressure perfusion system was used. A short segment of the circumflex coronary artery near its origin was carefully dissected free of periadventitial tissue. Ligatures were placed around the vessel. The vessel was incised and cannulated. The circumflex coronary artery was perfused at constant pressure. Coronary perfusion pressure was measured within 2–3 mm of the tip of the coronary cannula with a polyethylene catheter within the lumen of the coronary cannula. Adenosine was infused into the perfusion circuit to minimize the systemic effects of adenosine and to study coronary constrictor effects of other agonists. Drugs were infused directly into the coronary perfusion tubing. Heparin (500 units/kg) was administered intravenously. Blood was obtained from a femoral artery and pumped into a reservoir. The blood in the reservoir was mixed with a small magnetic stirring bar. The perfusion circuit from the reservoir to the circumflex coronary artery contained (1) a cannulating electromagnetic flow probe (Carolina Medical Electronics), (2) a piece of thick-walled rubber tubing for injection of drugs and spheres, (3) a 90° bend just distal to the injection tubing to produce turbulent blood flow for adequate mixing of spheres, and (4) two ports, one for measuring perfusion pressure and one for withdrawing reference blood samples for calculation of microsphere blood flows.

After propranolol, intracoronary infusion of adenosine produced a stable increase in flow within 1 minute. We employed two approaches to determine whether adenosine infusion produced maximal coronary dilation. First, during adenosine infusion, release of a 20-second occlusion of the circumflex coronary artery was not followed by any increase in coronary flow. Second, increases in the dose of intracoronary adenosine from 4.7 µmol/min to 9.4 µmol/min did not increase coronary flow.

The adequacy of mixing of spheres with this technique was assessed in vitro as follows. A piece of tygon tubing, terminating in four separate outlets, was attached to the coronary cannula. Blood was then perfused through the system and microspheres injected. The effluent from the four outlets was collected and counted. There was no significant difference between the counts in the four samples over a wide range of pressures and flows. In addition, flows and inner-to-outer ratios were comparable in the perfused and non-perfused hearts, giving further assurance that the spheres were being adequately dispersed in the perfusion tubing.

At the end of the study, Evans blue dye was infused into the perfusion circuit to delineate the area subserved by the cannulated circumflex coronary artery. The central densely stained portion of this area was excised and divided into 11 transmural sections which were then subdivided into endocardial, mid-wall, and epicardial pieces. These tissues and the reference bloods were analyzed as in the first series of experiments. Analysis of the microsphere data showed that each piece of tissue contained at least 600 spheres.

In each of 33 dogs, hemodynamics and total and regional coronary flow were measured during control conditions and during an intracoronary infusion of adenosine. These two measurements were separated by 2–3 minutes. Because the number of measurements of regional coronary flow that could be obtained in each experiment was limited to six, only one of the coronary constrictor stimuli being examined was used with each dog studied.

To determine the specificity and mechanism of the non-uniform effect of sympathetic nerve stimulation, we tested the effects of sympathetic nerve stimulation (10 Hz; n = 11), intracoronary infusion of angiotensin (0.125 and 0.5 µg/min; n = 6), phenylephrine (0.5 and 2.0 µg/min; n = 7), norepinephrine (0.2 µg/min; n = 4), and tyramine (200 µg/min; n = 5). Microspheres were injected intracoronary when the recording of circumflex flow stabilized following sympathetically nervous stimulation or drug infusion. This always occurred within 15–25 seconds. Drug infusion or nerve
stimulation was continued for 2 minutes after microspheres were injected. The interval between infusions of different doses of the agonists was at least 5 minutes. The sequence of drug infusions (high vs. low dose) was alternated.

**Sympathetic Nerve Stimulation in Absence of Adenosine**

In separate experiments, we examined effects of CSNS before \((n = 5)\) and after \((n = 7)\) propranolol in the absence of adenosine. These studies were performed in animals in which the circumflex coronary artery was not cannulated, and microspheres were injected into the left atrium.

CSNS before propranolol was studied to determine effects of stimulation of myocardial \(\beta\)-receptors and increases in myocardial metabolism on transmural distribution of blood flow; this need arose because of the possibility that, even after propranolol, CSNS might have produced a metabolic vasodilator influence and that this might have maintained endocardial but not epicardial flow.

**Dissection and Cannulation of the Circumflex Coronary Artery**

To determine whether dissection and cannulation of the coronary vessel might produce partial denervation of the endocardium, we examined the effects of CSNS on endocardial flow before and after dissection and cannulation of the circumflex artery \((n = 4)\). After propranolol, in the absence of adenosine, we measured myocardial blood flow with intra-atrial injection of microspheres during control and during CSNS at 10 Hz. The vessel then was dissected, cannulated, and perfused at constant pressure. Then, using intracoronary injections of the microspheres, we again measured flow during control state and in response to CSNS at 10 Hz.

**Drugs**

The agents employed in this study were adenosine (Sigma Chemical Company,) angiotensin amide (Ciba), lev-arterenol bitartrate (Sterling Drug), phenylephrine hydrochlide (generously supplied by Ayerst Laboratories), and tyramine hydrochloride (Sigma Chemical Company).

**Statistics**

Statistical analysis was performed using Student's \(t\)-test for paired observations and, when appropriate, a one-way analysis of variance plus a modified \(t\)-test to determine which pairs of means were significantly different. \(P\) values \(< 0.05\) were regarded as statistically significant.

**Results**

**Cardiac Sympathetic Nerve Stimulation during Intravenous Adenosine**

Intravenous adenosine produced a large decrease in mean and diastolic arterial pressure and increased endocardial and epicardial blood flow indicating intense vasodilation (Table 1). The endocardial:epicardial ratio fell during adenosine (Table 1).

Cardiac sympathetic nerve stimulation at 5 and 10 Hz during adenosine resulted in small increases in arterial pressure and significant increases in left ventricular pressure (Table 1). Heart rate did not change (Table 1). Nerve stimulation produced graded decreases in total myocardial blood flow. Epicardial flow decreased markedly, but endocardial flow did not change significantly (Fig. 1; Table 1). Consequently, endocardial:epicardial flow rose with nerve stimulation during adenosine (Table 1).

**Cardiac Sympathetic Nerve Stimulation during Constant Perfusion Pressure**

A constant pressure system was used in these experiments to perfuse the left circumflex coronary artery. Adenosine was infused into the circumflex coronary artery.

Intracoronary administration of adenosine did not significantly alter heart rate, systemic arterial pressure, or left ventricular pressure (Table 2). Coronary perfusion pressure was maintained constant (Table 2). Adenosine greatly increased endocardial and epicardial flow and decreased the endocardial:epicardial ratio (Table 2).

Cardiac sympathetic nerve stimulation at 10 Hz during adenosine increased \((P < 0.05)\) systemic arterial and left ventricular pressures, but coronary perfusion pressure was constant (Table 2). Nerve stimulation under these conditions significantly decreased

### Table 1

**Responses to Cardiac Sympathetic Nerve Stimulation (CSNS) during Intravenous Adenosine (4.7 \(\mu\)g/kg per min) after Propranolol (2 mg/kg, iv)**

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (\text{beats/min})</th>
<th>MAP (\text{mm Hg})</th>
<th>DAP (\text{mm Hg})</th>
<th>LVSP (\text{mm Hg})</th>
<th>LV (\text{dp/dt}} \text{Hg/sec}) per 100 g</th>
<th>Endocardial blood flow (\text{ml/min per 100 g})</th>
<th>Epicardial blood flow (\text{ml/min per 100 g})</th>
<th>Endo:Epi ratio</th>
<th>Total myocardial blood flow (\text{ml/min per 100 g})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>136 ± 5</td>
<td>83 ± 8</td>
<td>71 ± 8</td>
<td>109 ± 10</td>
<td>1984 ± 167</td>
<td>127 ± 19</td>
<td>134 ± 18</td>
<td>124 ± 36</td>
<td>1.32 ± 0.08</td>
</tr>
<tr>
<td>Adenosine</td>
<td>135 ± 4</td>
<td>43 ± 3*</td>
<td>28 ± 4*</td>
<td>75 ± 6*</td>
<td>1766 ± 186</td>
<td>287 ± 38*</td>
<td>208 ± 32*</td>
<td>357 ± 50*</td>
<td>0.64 ± 0.08*</td>
</tr>
<tr>
<td>Adenosine +</td>
<td>135 ± 4</td>
<td>48 ± 4</td>
<td>32 ± 3</td>
<td>94 ± 10†</td>
<td>2111 ± 221</td>
<td>209 ± 19†</td>
<td>194 ± 37</td>
<td>218 ± 34†</td>
<td>1.23 ± 0.13†</td>
</tr>
<tr>
<td>CSNS 5 Hz</td>
<td>128 ± 7</td>
<td>53 ± 5†</td>
<td>37 ± 5</td>
<td>102 ± 10†</td>
<td>2300 ± 284†</td>
<td>162 ± 25†</td>
<td>190 ± 28</td>
<td>146 ± 24†</td>
<td>1.40 ± 0.14†</td>
</tr>
<tr>
<td>Adenosine +</td>
<td>128 ± 7</td>
<td>53 ± 5†</td>
<td>37 ± 5</td>
<td>102 ± 10†</td>
<td>2300 ± 284†</td>
<td>162 ± 25†</td>
<td>190 ± 28</td>
<td>146 ± 24†</td>
<td>1.40 ± 0.16†</td>
</tr>
</tbody>
</table>

Entries are mean ± se; \(n = 9\). MAP = mean arterial pressure; DAP = diastolic arterial pressure; LVSP = left ventricular systolic pressure.

* \(P < 0.05\), adenosine vs. control.
† \(P < 0.05\), adenosine + CSNS vs. adenosine alone.
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### Table 2

Responses to Constrictor Stimuli during Intracoronary Adenosine (4.7 μg/min) after Propranolol (2 mg/kg, iv)

<table>
<thead>
<tr>
<th>Heart rate (beats/min)</th>
<th>MAP (mm Hg)</th>
<th>LVSP (mm Hg)</th>
<th>LV dp/dt (mm Hg/sec)</th>
<th>Coronary myocardial blood flow (ml/min per 100 g)</th>
<th>Endocardial blood flow (ml/min per 100 g)</th>
<th>Epicardial blood flow (ml/min per 100 g)</th>
<th>Endo:Epi ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>128 ± 8</td>
<td>80 ± 7</td>
<td>91 ± 14</td>
<td>1690 ± 211</td>
<td>104 ± 5</td>
<td>117 ± 10</td>
<td>126 ± 29</td>
</tr>
<tr>
<td>AD</td>
<td>126 ± 6</td>
<td>78 ± 7</td>
<td>91 ± 8</td>
<td>1675 ± 155</td>
<td>106 ± 3</td>
<td>430 ± 40*</td>
<td>382 ± 50*</td>
</tr>
<tr>
<td>AD + CSNS 10 Hz</td>
<td>106 ± 5</td>
<td>117 ± 8†</td>
<td>2157 ± 229†</td>
<td>105 ± 3</td>
<td>280 ± 35†</td>
<td>378 ± 58</td>
<td>308 ± 44†</td>
</tr>
<tr>
<td>AD + ANGIO</td>
<td>100 ± 5</td>
<td>91 ± 6</td>
<td>2037 ± 319</td>
<td>92 ± 8</td>
<td>306 ± 40†</td>
<td>271 ± 33†</td>
<td>375 ± 67†</td>
</tr>
<tr>
<td>AD + ANGIO 0.125 μg/min</td>
<td>105 ± 13</td>
<td>118 ± 8</td>
<td>2262 ± 51†</td>
<td>92 ± 8</td>
<td>276 ± 30†</td>
<td>179 ± 46†</td>
<td>303 ± 54†</td>
</tr>
<tr>
<td>AD + ANGIO 0.50 μg/min (n = 6)</td>
<td>117 ± 11†</td>
<td>112 ± 12†</td>
<td>3150 ± 663</td>
<td>91 ± 3</td>
<td>201 ± 44†</td>
<td>166 ± 38†</td>
<td>226 ± 46†</td>
</tr>
<tr>
<td>Control</td>
<td>129 ± 12</td>
<td>81 ± 9</td>
<td>96 ± 10</td>
<td>2019 ± 196</td>
<td>94 ± 4</td>
<td>126 ± 19</td>
<td>147 ± 27</td>
</tr>
<tr>
<td>AD</td>
<td>115 ± 7</td>
<td>77 ± 12</td>
<td>94 ± 12</td>
<td>2250 ± 295</td>
<td>94 ± 4</td>
<td>566 ± 113*</td>
<td>494 ± 91*</td>
</tr>
<tr>
<td>AD + PE 0.50 μg/min</td>
<td>110 ± 7</td>
<td>96 ± 11</td>
<td>95 ± 14</td>
<td>2276 ± 329</td>
<td>97 ± 3</td>
<td>333 ± 35†</td>
<td>347 ± 30†</td>
</tr>
<tr>
<td>AD + PE 2.00 μg/min</td>
<td>112 ± 11†</td>
<td>99 ± 8†</td>
<td>2036 ± 396</td>
<td>100 ± 4</td>
<td>297 ± 53†</td>
<td>288 ± 46†</td>
<td>325 ± 41†</td>
</tr>
<tr>
<td>Control</td>
<td>137 ± 16</td>
<td>71 ± 18</td>
<td>83 ± 16</td>
<td>1712 ± 208</td>
<td>98 ± 8</td>
<td>133 ± 34</td>
<td>137 ± 28</td>
</tr>
<tr>
<td>AD</td>
<td>126 ± 13</td>
<td>67 ± 17</td>
<td>81 ± 14</td>
<td>1858 ± 300</td>
<td>99 ± 7</td>
<td>488 ± 60*</td>
<td>437 ± 37*</td>
</tr>
<tr>
<td>AD + NE 0.25 μg/min</td>
<td>124 ± 11</td>
<td>78 ± 12</td>
<td>96 ± 11</td>
<td>1836 ± 412</td>
<td>100 ± 4</td>
<td>297 ± 53†</td>
<td>302 ± 87†</td>
</tr>
<tr>
<td>AD + NE 1.00 μg/min</td>
<td>122 ± 14</td>
<td>109 ± 12</td>
<td>2036 ± 396</td>
<td>100 ± 4</td>
<td>245 ± 36†</td>
<td>195 ± 26†</td>
<td>267 ± 56†</td>
</tr>
<tr>
<td>Control</td>
<td>130 ± 4</td>
<td>99 ± 17</td>
<td>114 ± 19</td>
<td>2565 ± 244</td>
<td>100 ± 1</td>
<td>121 ± 14</td>
<td>123 ± 17</td>
</tr>
<tr>
<td>AD + TYR 200 μg/min</td>
<td>128 ± 5</td>
<td>111 ± 19</td>
<td>111 ± 11</td>
<td>2530 ± 234</td>
<td>100 ± 1</td>
<td>525 ± 39*</td>
<td>504 ± 51*</td>
</tr>
<tr>
<td>AD + TYR 1000 μg/min</td>
<td>123 ± 5</td>
<td>94 ± 12</td>
<td>119 ± 10</td>
<td>2732 ± 106</td>
<td>98 ± 1</td>
<td>312 ± 72†</td>
<td>295 ± 87†</td>
</tr>
</tbody>
</table>

AD = adenosine; CSNS = cardiac sympathetic nerve stimulation; ANGIO = angiotensin; PE = phenylephrine; NE = norepinephrine; TYR = tyramine.

* P < 0.05, adenosine vs. control.
† P < 0.02, adenosine plus constrictor stimulus vs. adenosine alone.

FIGURE 1. Effects of cardiac sympathetic nerve stimulation at 5 and 10 Hz during intravenous adenosine after β-blockade. Entries are mean ± SEM, n = 9. AD = adenosine, *P < 0.05 compared to adenosine. Nerve stimulation decreased epicardial (EPI) but not endocardial (ENDO) flow.
epicardial flow ($P < 0.05$) but did not significantly decrease endocardial flow so that endocardial:epicardial ratio increased (Table 2; Fig. 2).

Like sympathetic nerve stimulation, intracoronary angiotensin, phenylephrine, norepinephrine, and tyramine increased systemic arterial and left ventricular pressure (Table 2), while coronary perfusion pressure remained constant. However, in contrast to nerve stimulation, all these vasoconstrictor stimuli produced significant decreases in both endocardial and epicardial flow (Table 2; Figs. 3 and 4). These agents did not increase the endocardial:epicardial ratio (Table 2).

Cardiac Sympathetic Nerve Stimulation in the Absence of Adenosine Infusion

Before propranolol, CSNS at 10 Hz produced large increases in heart rate and left ventricular systolic pressure and dP/dt (Table 3). These responses were associated with significant increases in epicardial, but not endocardial flow (Table 3). The endocardial:epicardial ratio fell with nerve stimulation before propranolol and adenosine (Table 3).

After propranolol, CSNS at 10 Hz produced smaller increases in left ventricular pressure and dP/dt and significantly decreased both endocardial and epicardial flow (Table 3). The endocardial:epicardial ratio did not change (Table 3).

Effects of Dissection and Cannulation

After propranolol but before dissection and cannulation of the circumflex coronary artery, nerve stimulation at 10 Hz decreased endocardial flow from 140 ± 14 in control state to 98 ± 19 ml/min per 100 g, a 30% decrease ($P < 0.05$). After dissection and cannulation, and in the absence of adenosine, nerve stimulation at 10 Hz decreased endocardial flow from 128 ± 16 to 79 ± 30 ml/min per 100 g, a 38% decrease ($P < 0.05$). The responses before and after dissection and cannulation of the vessels were not different.

Discussion

The major findings in this study were:

1. Coronary arteries intensely dilated with adenosine remain responsive to constrictor effects of both adrenergic and non-adrenergic constrictor stimuli.
2. During intense vasodilation produced by adenosine, the direct constrictor effects of sympathetic

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**FIGURE 2.** Effects of cardiac sympathetic nerve stimulation during intracoronary adenosine after β-blockade, n = 11. Entries are mean ± SEM. *P < 0.05. AD = adenosine. In these studies, the circumflex coronary artery was perfused at constant perfusion pressure. Under these conditions, nerve stimulation again decreased epicardial (EPI), but not endocardial (ENDO) flow.

**FIGURE 3.** Effects of intracoronary angiotensin infusion during intracoronary adenosine after β-blockade, n = 6. Entries are mean ± SEM. AD = adenosine and ANG1 and ANG2 = angiotensin 0.125 and 0.50 μg/min, respectively. *P < 0.05 compared to adenosine. Angiotensin decreased both endocardial (ENDO) and epicardial (EPI) flows.
nerve stimulation produce greater decreases in epicardial than endocardial flow.

3. In contrast to the non-uniform transmural effects of sympathetic nerve stimulation during adenosine, intracoronary administration of other coronary vasoconstrictors (angiotensin, phenylephrine, norepinephrine, and tyramine) causes significant decreases in both endocardial and epicardial flow.

The discussion of these findings has two major parts. The first will examine mechanical and metabolic factors which might cause a nonuniform effect of nerve stimulation during adenosine independent of a direct vasomotor effect. The second will discuss adrenergic mechanisms which might produce a nonuniform vasomotor effect of sympathetic nerve stimulation.

Mechanical and Metabolic Factors

The following are several factors that must be considered in interpreting the non-uniform effect of sympathetic nerve stimulation during adenosine.

Coronary Pressure

In the first group of experiments which employed intravenous adenosine, coronary perfusion pressure was not controlled, and increased slightly during sympathetic nerve stimulation. In the presence of intense dilation, this small increase could have preserved endocardial flow in the face of a direct vasomotor effect of sympathetic nerve stimulation (Rouleau et al., 1979). However, in the second series of experiments, coronary perfusion pressure was held constant, and sympathetic nerve stimulation still produced greater decreases in epicardial than endocardial flow. Thus, the results cannot be explained by increases in coronary perfusion during sympathetic stimulation.

Systolic Squeeze

During sympathetic nerve stimulation, there were increases in left ventricular systolic pressure, even after propranolol. Increases in systolic squeeze pro-
duced by increases in left ventricular pressure would be expected to produce greater decreases in endocardial than epicardial flow (Downey and Kirk, 1974). The reverse occurred with nerve stimulation during adenosine. We conclude that the non-uniform effect of nerve stimulation occurred despite, and not because of, increases in left ventricular systolic squeeze.

**Heart Rate**

There was no change in heart rate during nerve stimulation.

**Metabolic Vasodilation**

As noted previously, there were increases in left ventricular systolic pressure and dP/dt with nerve stimulation, even *after* propranolol. One might contend that this would produce a metabolic vasodilator stimulus that would offset sympathetic vasoconstriction to the endocardium. Several observation make this improbable. First, the coronary vessels were already subject to an intense metabolic vasodilator stimulus, adenosine, before sympathetic stimulation. The dose of adenosine employed abolished reactive hyperemia, and doubling the dose of adenosine did not produce further increases in flow. Second, somewhat similar increases in left ventricular pressure and dP/dt occurred with the other constrictor stimuli, and these interventions decreased endocardial as well as epicardial flow. Third, large increases in left ventricular pressure, dP/dt, and presumably myocardial metabolism with nerve stimulation in the absence of propranolol produced greater increases in epicardial than endocardial flow in our study (Table 3) and in another study (Giudicelli et al., 1980). Taken together, these three observations make it improbable that failure of sympathetic nerve stimulation to decrease endocardial flow resulted from a residual metabolic vasodilator effect of nerve stimulation after propranolol.

**Denervation**

The possibility that dissection and cannulation of the circumflex coronary artery could have influenced the results was considered. This could have pertained if the dissection and cannulation interrupted sympathetic innervation of endocardial but not epicardial vessels. However, in separate experiments we found that dissection and cannulation of the vessel did not alter the decrease in endocardial flow during nerve stimulation. These observations indicate that the dissection and cannulation cannot account for the failure of nerve stimulation to decrease endocardial flow during adenosine.

**Adrenergic Mechanisms Involved in Non-Uniform Effects of Sympathetic Nerve Stimulation**

We considered three mechanisms: non-uniform distribution of α-adrenergic receptors, non-uniform sympathetic innervation, and nonuniform prejunctional effect of adenosine on adrenergic neurotransmission.

The non-uniform influence of sympathetic nerve stimulation on transmural flow cannot be explained by non-uniformity in density or properties of coronary α-receptors, since stimulation of α-receptors with phenylephrine, norepinephrine, or tyramine decreased both endocardial and epicardial flow. In addition, our results cannot be explained by activation of coronary vascular β2 receptors during nerve stimulation. Previous studies have demonstrated that sympathetic nerve stimulation does not produce significant effects on coronary beta2 receptors (Mark et al., 1972; Hamilton and Feigl, 1976). Moreover, our experiments were performed in the presence of β-receptor blockade produced by propranolol.

Our observations might be explained by a difference in the sympathetic innervation of coronary vessels regulating flow to epicardium vs. endocardium. There are regional variations in sympathetic innervation of myocardium and the conduction system, and it is possible that there might be transmural differences in innervation of coronary vessels. Pace (1977) has reviewed work on the anatomical relationship between sympathetic nerves and coronary vessels. He concluded from electron microscopic and fluorescence histochemical studies that the major anatomic substrate for effective neuromuscular control lies at the level of small arterioles as well as large arteries. To our knowledge, there have been no systematic studies of transmural variation in sympathetic innervation of coronary arteries. There is, however, one line of reasoning which suggests that the non-uniform influence of nerve stimulation on transmural flow during adenosine might not relate to non-uniform innervation. If there were non-uniform innervation, then one might expect nerve stimulation to produce non-uniform effects under various experimental conditions. In our experience, this does not occur. We found that sympathetic nerve stimulation after propranolol (but in the absence of adenosine) decreased both endocardial and epicardial flow (Table 3). Thus, the available evidence does not clearly support the view that our present findings are explained by nonuniform transmural innervation of coronary vessels.

Verhaeghe et al. (1977) have demonstrated that adenosine acts prejunctionally to inhibit adrenergic neurotransmission. If this effect were more pronounced in endocardium than epicardium, this might explain our observation that nerve stimulation decreases epicardial but not endocardial flow during adenosine. A recent study suggests that the vasodilator effect of adenosine is more pronounced in the endocardium (Rembert et al., 1980), and it is tempting to speculate that this may also be true of prejunctional effects. This is a difficult problem to address definitively. An indirect approach is to evaluate the effects of tyramine, since the release of norepinephrine from adrenergic nerve endings produced by this agent is not influenced by prejunctional receptors (Thoa et al., 1975). In contrast to nerve stimulation, we found that—during adenosine—tyramine decreases both en-
docardial and epicardial blood flow (Table 2). This observation is consistent with the view that the innervation may be uniform and that the differential effect of nerve stimulation may relate to prejunctional effects of adenosine. However, there is one observation which is somewhat inconsistent with this explanation. Prejunctional receptor modulation of adrenergic neurotransmission is reportedly most pronounced at low levels of nerve stimulation and is masked at high levels of sympathetic stimulation (Yamaguchi et al., 1977). In our studies, the non-uniform effect of sympathetic nerve stimulation was seen during a high level (10 Hz) of stimulation.

In summary, this study demonstrates that coronary arteries maximally dilated with adenosine remain responsive to constrictor effects of adrenergic and non-adrenergic stimuli. Administration of angiotensin, phenylephrine, norepinephrine, and tyramine decreased both endocardial and epicardial flow, but sympathetic nerve stimulation decreased epicardial flow without significant decreases in endocardial flow. This non-uniform influence cannot easily be attributed to metabolic or mechanical factors regulating myocardial flow or to characteristics of coronary adrenergic receptors. The precise mechanism is not clear; possible roles of transmural non-uniformity in innervation of coronary arteries and in prejunctional effects of adenosine are discussed.

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