Inhibition by Acetylcholine of the Norepinephrine Release Evoked by Potassium in Canine Saphenous Veins

PAUL M. VANHOUTTE, M.D., AND TONY J. VERBEEUREN

SUMMARY In the dog's saphenous vein acetylcholine inhibits the norepinephrine release caused by nerve stimulation, but not that caused by tyramine. Experiments were performed to determine whether acetylcholine affects the release of norepinephrine evoked by high K⁺ concentrations. We recorded changes in isometric tension of dog saphenous vein strips. Acetylcholine (5 × 10⁻⁴ to 10⁻⁴ g/ml) caused dose-dependent relaxations during contractions caused by K⁺ > 40 mEq/liter. These relaxations were not depressed by tetrodotoxin (10⁻⁷ g/ml), which abolished the response to nerve stimulation, but were inhibited by atropine (10⁻⁴ g/ml). Strips of saphenous veins were incubated with [³H]norepinephrine and mounted for superfusion (3 ml/min) and isometric tension recording; the total radioactivity and the amount of intact [³H]norepinephrine present in the superfusate were determined. K⁺ at 50 mEq/liter increased tension, total radioactivity of the superfusate, and the [³H]norepinephrine efflux; acetylcholine (10⁻⁴ g/ml) depressed the contractions and diminished the efflux of [³H]norepinephrine. Increasing the K⁺ concentration from 50 to 70 mEq/liter augmented both tension and the evoked release of [³H]norepinephrine. Acetylcholine did not significantly alter the release of [³H]norepinephrine evoked by K⁺ = 120 mEq/liter. These experiments show that acetylcholine inhibits the norepinephrine release evoked by potassium ions, as it does during nerve stimulation. The inhibition of adrenergic neurotransmission is not due to interference with action potential electrogienesis, but probably is caused by hyperpolarization of the adrenergic nerve endings.

IN THE CASE OF the canine saphenous vein, increases in potassium ion concentration (K⁺) to values greater than 30 mEq/liter cause contraction and this is caused in part by release of endogenous norepinephrine.¹ In the same preparation, and in other adrenergically innervated blood vessels of the dog, acetylcholine through activation of muscarinic receptors inhibits the release of norepinephrine evoked by nerve stimulation, but not that caused by tyramine.² To elucidate further the mechanism of action of acetylcholine on the adrenergic nerve terminals of the saphenous vein, the following experiments were performed to determine whether acetylcholine modifies the release of adrenergic transmitter evoked by potassium ions.

Methods

The experiments were performed on isolated saphenous veins taken from dogs (15-25 kg) anesthetized with pentobarbital (30 mg, iv). Helical strips were placed in a chamber filled with Krebs-Ringer solution of the following millimolar composition: NaCl, 118.3; KCl, 4.7; MgSO₄, 1.2; KH₂PO₄, 1.2; CaCl₂, 2.5; NaHCO₃, 25; glucose, 11.1; and calcium disodium ethylenediaminetetraacetate (EDTA), 0.026 (this was added to chelate possible trace amounts of heavy metals which catalyze the auto-oxidation of catecholamines). The solution was maintained at 37°C and continuously aerated with a mixture of 95% O₂ and 5% CO₂. The strips were connected to a strain gauge (Grass FT .03) to record isometric tension.

To stimulate adrenergic nerve endings in the preparation, two rectangular platinum electrodes were placed parallel to the strips, as previously described.¹ ² Four electrical impulses consisted of rectangular waves (9 V, 2 msec) provided by a direct current power supply and switching transistor (Siemens, AD 149) triggered by a Grass stimulator (model S88). The following pharmacological agents were used: acetylcholine chloride, atropine sulfate, phentolamine, and tetrodotoxin. The dose of each drug was contained in 0.1 ml of Krebs-Ringer solution, and this was the volume added to the organ bath. All doses are expressed as final bath concentrations of the salts. To increase the K⁺ concentration the Krebs-Ringer solution in the bath was replaced with solutions containing added KCl in equimolar replacement of NaCl. The drugs, or the high potassium solutions, were removed from the bath by overflowing the preparations with aerated Krebs-Ringer solution at 37°C.

Before the experiments were begun, the preparations were placed at the optimal point on their length-tension relationship when activated by a standard electrical stimulation.³ The strips were allowed to equilibrate at their optimal length for 90 minutes prior to experimentation.

[³H]NOREPINEPHRINE EFFLUX

In some experiments the strips were incubated for 4 hours in Krebs-Ringer solution containing [³H]norepinephrine in a concentration of 5 × 10⁻⁴ g/ml (specific activity, 8.8 Ci/mmol, Amersham). At the end of incubation period the strips were rinsed in fresh Krebs-Ringer and mounted for superfusion as previously described.¹ ² The preparations were suspended in a moist, tunnel-shaped chamber maintained at 37°C; the strips were superfused at 3 ml/min by a constant flow roller pump with Krebs-Ringer solution or with solutions containing added KCl in equimolar replacement of NaCl. All solutions were maintained at 37°C and aerated with 95% O₂-5% CO₂. The preparations were connected to a strain gauge (Grass FT .03) for continuous tension recording. The initial tension was set at 3 g; after this...
initial stretch, the tension decreased and stabilized within 30 minutes. At that time sampling of the superfusate was begun. The superfusate was collected at 2-minute intervals for direct estimation of the total radioactivity. For selected 5-minute intervals, the superfusate was collected into cooled flasks which contained carrier norepinephrine (20 μg), normetanephrine (20 μg), 3,4-dihydroxymandelic acid (20 μg), 3,4-dihydroxphenylglycol (20 μg), and 3-methoxy-4-hydroxymandelic acid (20 μg) together with 2 n Na hydrochloric acid (0.2 ml), sodium metabisulfite (20 mg), and disodium EDTA (20 mg). These samples were stored at −23°C until chromatographic analysis was performed.

Acetylcholine chloride was infused at a constant rate upstream from the roller pump; the concentration is expressed as the final concentration of the salt in the superfusing fluid.

COLUMN CHROMATOGRAPHY

Norepinephrine was separated from its metabolites in the superfusate as described previously.1, 3 Catechol compounds were separated from non-catechol metabolites and from tritiated water by adsorption on alumina at pH 8.4 and subsequent elution with acid. Norepinephrine was separated from deaminated metabolites in the alumina eluate by adsorption on a Dowex 50-X4 resin.

Activated alumina (500 mg), previously washed by the method of Anton and Sayre,1 was added to 10-ml samples of superfusate. With continuous stirring, the pH was adjusted to 8.4 with 0.5 n sodium carbonate and maintained at 8.4 for 5 minutes. The mixture then was transferred to a glass column (internal diameter, 0.5 cm) that was plugged with glass wool. The alumina was washed with 10 ml of water (collected with the effluent) and catechols were eluted with 7 ml of 0.05 n perchloric acid. Columns (height, 2.5–3 cm; diameter, 0.5 cm) of the Dowex 50 resin (200-400 mesh), previously cycled through the hydrogen and sodium forms, were repeatedly rinsed with Krebs-Ringer solution, minced into small pieces, and homogenized. Intact [3H]norepinephrine accounted for 51 ± 2.6% of the radioactivity contained in the homogenate (mean, 13.73 ± 3.43; 10^6 dpm/mg wet wt).

STATISTICAL ANALYSIS

For each group of preparations the number of strips reported is also the number of dogs used. The data are expressed as means ± SE. For the statistical analysis of the data Student’s t-test for paired or unpaired observations was used.

Earlier work in the saphenous vein has shown that the 1H efflux decays with time in unstimulated preparations and during nerve stimulation or exposure to high K+;1, 2, 9 To correct for this decrease in labeled transmitter output with time the following procedure was used to analyze statistically changes in [3H]norepinephrine efflux. In unstimulated preparations samples for chromatography were collected in control solution, during the infusion of acetylcholine, and after return to control solution; the values obtained in presence of acetylcholine were compared with those obtained by averaging the two control samples. To analyze the effects of acetylcholine on the [3H]norepinephrine release evoked by K+, the strips were superfused for 36 minutes with a solution of high K+ concentration; acetylcholine was infused from the 12th to the 24th minute. Samples for chromatography were collected at the 8th, the 20th, and the 32nd minute of superfusion with high K+. Again, the values obtained in presence of acetylcholine were compared with those obtained by averaging the high K+ samples collected prior to and after the infusion of the agent.

Results

ACETYLCHOLINE- AND K+-INDUCED CONTRACTIONS

For five saphenous vein strips single doses of acetylcholine (10^{-10} to 5 × 10^{-4} g/ml) were given, in random sequence, during contractions caused by increasing the K+ concentration of the bath solution from 5.9 to 40 mEq/liter; such contractions are known to be due in part to release of endogenous norepinephrine.1 At 10^{-10} and 5 × 10^{-10} g/ml acetylcholine did not significantly alter the reaction to high K+. Higher concentrations of acetylcholine caused dose-dependent relaxations which were maximal at 10^{-3} g/ml. With a concentration of acetylcholine of 10^{-3} g/ml the relaxations were significantly smaller than those obtained with 10^{-2} g/ml. At 5 × 10^{-3} g/ml acetylcholine caused a further increase in tension in all preparations (Fig. 1).

In six strips, acetylcholine at 10^{-1} g/ml significantly depressed the contractile response to a K+ concentration of 40 mEq/liter from a mean of 1.73 ± 0.18 g to a mean of
1.23 ± 0.15 g. Atropine (10^-7 g/ml) did not significantly alter the response to K^+ (mean, 1.69 ± 0.17 g), but abolished the relaxation caused by acetylcholine (10^-7 g/ml).

In six additional strips a K^+ concentration of 40 mEq/liter caused an increase in tension which was significantly depressed by acetylcholine (10^-7 g/ml). Incubation for 10 minutes with tetrodotoxin (10^-7 g/ml) abolished the response of the preparations to nerve stimulation (15 Hz, 10 seconds), but did not significantly alter the contractile response to K^+ = 40 mEq/liter; acetylcholine still depressed the response to high K^+. The percentage decreases in tension caused by acetylcholine were not significantly different in control solution and in the presence of tetrodotoxin. The same strips then were incubated in solutions containing phentolamine (10^-7 g/ml); the a-adrenergic blocking agent was used to mask the part of the contractile response due to release of endogenous norepinephrine. Phentolamine significantly depressed the contractile response to K^+ = 40 mEq/liter; in this circumstance acetylcholine caused no relaxation but rather a slight increase in tension (Fig. 2; Table 1).

**[3H]Norepinephrine Efflux: Effect of Acetylcholine on K^+-Induced Responses**

K^+ = 40 mEq/liter. In four preliminary experiments the strips were incubated with [3H]norepinephrine and mounted for superfusion. Changing the K^+ concentration from 5.9 to 40 mEq/liter caused contraction and moderate increases in total radioactivity of the superfusate. In each case both the contractile response and the evoked [H] release were reversibly depressed by acetylcholine (Fig. 3; Table 2).

K^+ = 50 mEq/liter. In the experiments in which the efflux of intact [3H]norepinephrine was to be determined, a larger increase in K^+ concentration (from 5.9 to 50 mEq/liter) was used.

For four strips superfused with control solution the superfusate was collected for direct estimation of total radioactivity, and during a selected 5-minute period for chromatographic analysis. The strips then were superfused with solution containing a K^+ concentration of 50 mEq/liter; at 12-minute intervals samples for column chromatography were obtained. Ten minutes after return to control solution, a final control sample was collected. The results are shown in Figure 4 (left). Increasing the K^+ concentration of the superfusing solution from 5.9 to 50 mEq/liter caused an increase in tension (which was maximal at the 6th minute), and an increase in total radioactivity of the superfusate (maximal at the 4th minute). Throughout the further superfusion with high K^+, there was a progressive fall in tension, in total radioactivity of the superfusate, and in efflux of intact [3H]norepinephrine.
A similar experiment was performed, on six additional strips except that from the 12th to the 24th minute of superfusion with K⁺ = 50 mEq/liter, acetylcholine (10⁻⁷ g/ml) was infused (Fig. 4, right). K⁺ at 50 mEq/liter increased the tension, the total radioactivity of the superfusate, and the efflux of [³H]norepinephrine. For each strip acetylcholine caused relaxation, decreased total radioactivity of the superfusate, and reduced [³H]norepinephrine efflux (Table 2). These effects of acetylcholine were reversible.

K⁺ = 120 mEq/liter. For four control strips, the K⁺ concentration was changed from 5.9 to 120 mEq/liter for 42 minutes. Samples for column chromatography were taken from the control solution, during superfusion with K⁺ = 120 mEq/liter (three samples at 12-minute intervals) and after return to control solution (Fig. 5, left). During superfusion with K⁺ at 120 mEq/liter the efflux of tritiated compounds and of [³H]norepinephrine decreased with time.

For five strips, acetylcholine (10⁻⁷ g/ml) was infused during a sustained response to K⁺ at 120 mEq/liter. Acetylcholine caused no significant changes in tension, in total radioactivity of the superfusate, or in efflux of [³H]norepinephrine (Fig. 5, right; Table 2).

**Effect of Increasing K⁺ from 50 to 70 mEq/liter.** For six preparations, the K⁺ concentration was increased from 5.9 to 50 mEq/liter; this caused an increase in tension and in efflux of [³H]norepinephrine. The K⁺ concentration then was raised to 70 mEq/liter. For all strips, this resulted in further significant increases in tension, in total radioactivity of the superfusate, and in efflux of [³H]norepinephrine (Table 2).

**Effect of Acetylcholine on Unstimulated Preparations.** For six strips superfused with control solution (K⁺ = 5.9 mEq/liter) acetylcholine at 10⁻⁷ g/ml caused a small significant increase in tension, but did not affect the [³H]norepinephrine efflux (Fig. 4, middle; Table 2).

**Discussion**

In the case of the saphenous vein of the dog acetylcholine inhibits the release of norepinephrine evoked by nerve impulses. The main purpose of the present experiments was to explore further the mechanism of action of acetylcholine on the adrenergic nerve endings in the blood vessel wall. The experimental procedures used have been discussed in detail elsewhere. In the case of unstimulated preparations acetylcholine at 10⁻⁷ g/ml causes an increase in tension; this confirms that in adrenegically innervated vascular muscle such as the saphenous vein the norepinephrine-releasing action of potassium ions combines with their direct activating effect on the smooth muscle cells to cause contraction; similar conclusions have been reached for other systems. The present experiments stress that, when considering pharmacological effects on K⁺-induced responses, part of these effects can be due to interference with the norepinephrine release evoked by K⁺.

In the case of unstimulated preparations acetylcholine at 10⁻⁷ g/ml causes an increase in tension; this confirms that the direct effect of the drug on the smooth muscle cells of the saphenous vein is to cause contraction. In contrast, during contractions obtained with K⁺ at 40 mEq/liter, a concentration known to release norepinephrine, acetylcholine causes a dose-dependent relaxation that is maximal at 10⁻⁷ g/ml. For strips pretreated with phentolamine, acetylcholine causes only an increase in tension. These experiments suggest that acetylcholine inhibits the release of norepinephrine evoked by K⁺ and that this effect is partly masked by the direct activating effect of the drug on the smooth muscle cells; with high concentrations of acetylcholine, the latter predominates and the relaxations are no longer seen (Fig. 1). This interpretation is supported by the experiments.
in the which the \[^{[H]}\text{norepinephrine efflux}\] was followed. They show that acetylcholine, in a concentration that does not affect the basal efflux of \[^{[H]}\text{norepinephrine}\], reduces the increase in tension and the augmentation in radioactivity level of the superfusate evoked by raising the K\(^+\) concentration from 5.9 to 40 or 50 mEq/liter; the column chromatographic analysis indicates that in the presence of acetylcholine the efflux of \[^{[H]}\text{norepinephrine}\] is markedly depressed by increases in K\(^+\) concentration, as it does during nerve stimulation.\(^{2-4}\)

In the saphenous vein, tetrodotoxin abolishes the response to electrical stimulation, as already reported for this and other vascular preparations.\(^{2-4}\)\(^{13}\) In contrast, tetrodotoxin does not depress the contractile response to K\(^+\) and does not abolish the relaxation caused by acetylcholine, indicating that it does not inhibit the release of norepinephrine evoked by K\(^+\). Tetrodotoxin is believed to act on nerve tissue mainly by selective inhibition of the transient membrane conductance changes for sodium that occur during the action potential, without affecting the steady state conductance for potassium.\(^{14-17}\) Hence, the present experiments suggest that the inhibition of norepinephrine release caused by acetylcholine is not due to interference with spike electrogensis at the adrenergic nerve terminals.

It is likely that progressive increases in K\(^+\) concentration cause release of transmitter through progressive depolarization of the adrenergic nerve terminals; this is illustrated best by the experiments showing in the same preparations that increasing the K\(^+\) concentration of the superfusing solution from 50 to 70 mEq/liter augments both the contractile responses and the \[^{[H]}\text{norepinephrine release}\] (Table 2). With solutions containing K\(^+\) = 120 mEq/liter, the depolarization of the nerve endings must be complete; this is suggested by the large amounts of \[^{[H]}\text{norepinephrine}\] appearing in the superfusate. In view of the important changes in membrane potential caused by the depolarizing solution (K\(^+\) = 120 mEq/liter) modulatory influences on the neuronal membrane potential must be less effective; however, pharmacological effects not mediated through changes in membrane potential still should be present. During

### Table 2. Effects of Increased K\(^+\) and Acetylcholine on Tension, Total Radioactivity in Superfusate, and \[^{[H]}\text{Norepinephrine Efflux in Canine Saphenous Veins}\]

<table>
<thead>
<tr>
<th>K(^+) (mEq/liter)</th>
<th>Tension (g)</th>
<th>Total radioactivity (10(^3) dpm/5 min)</th>
<th>[^{[H]}\text{Norepinephrine efflux}] (10(^3) dpm/5 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine (10(^{-7}) g/ml)</td>
<td>1.74 ± 0.12</td>
<td>31.6 ± 1.2</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>Potassium (40 mEq/liter)</td>
<td>1.92 ± 0.13*</td>
<td>28.4 ± 0.8</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K(^+) (40 mEq/liter)</td>
<td>2.10 ± 0.09</td>
<td>7.3 ± 0.3</td>
<td>-</td>
</tr>
<tr>
<td>K(^+) plus acetylcholine (10(^{-7}) g/ml)</td>
<td>3.29 ± 0.13*</td>
<td>13.7 ± 0.9*</td>
<td>-</td>
</tr>
<tr>
<td>Potassium (50 mEq/liter)</td>
<td>2.94 ± 0.12*</td>
<td>10.0 ± 0.7*</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K(^+) (50 mEq/liter)</td>
<td>2.15 ± 0.25</td>
<td>46.5 ± 5.3</td>
<td>2.5 ± 0.9</td>
</tr>
<tr>
<td>K(^+) plus acetylcholine (10(^{-7}) g/ml)</td>
<td>4.58 ± 0.46*</td>
<td>103.5 ± 7.0*</td>
<td>32.9 ± 5.0*</td>
</tr>
<tr>
<td>Potassium (120 mEq/liter)</td>
<td>4.45 ± 0.47*</td>
<td>97.6 ± 9.7</td>
<td>30.3 ± 6.0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K(^+) (50 mEq/liter)</td>
<td>1.79 ± 0.21</td>
<td>22.3 ± 0.7</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>K(^+) plus acetylcholine (10(^{-7}) g/ml)</td>
<td>3.94 ± 0.34*</td>
<td>50.7 ± 3.8*</td>
<td>14.6 ± 2.5*</td>
</tr>
<tr>
<td>Potassium (120 mEq/liter)</td>
<td>2.20 ± 0.33*</td>
<td>30.9 ± 1.3*</td>
<td>4.7 ± 0.9*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K(^+) (50 mEq/liter)</td>
<td>2.63 ± 0.36</td>
<td>52.7 ± 9.3</td>
<td>7.5 ± 1.7</td>
</tr>
<tr>
<td>K(^+) (70 mEq/liter)</td>
<td>4.83 ± 0.62*</td>
<td>71.9 ± 14.3*</td>
<td>17.9 ± 3.9*</td>
</tr>
<tr>
<td>Potassium (120 mEq/liter)</td>
<td>5.85 ± 0.94*</td>
<td>94.2 ± 15.5*</td>
<td>29.7 ± 3.4*</td>
</tr>
</tbody>
</table>

Values shown are means ± 6S. To correct for the spontaneous decrease in labeled transmitter efflux with time, the control values were obtained by averaging the data of the samples collected in control solution prior to and after the administration of acetylcholine or the superfusion with high concentrations of K\(^+\). For the same reason, the first values shown in each K\(^+\) experiment are obtained by averaging the data from the samples collected at the 4th and the 22nd minute of superfusion with high K\(^+\) solutions (see Methods).

* Difference with preceding value is statistically significant (P < 0.05; Student's t-test for paired observations).

† With acetylcholine the decrease in tension during superfusion with K\(^+\) = 50 mEq/liter is significantly greater than the spontaneous decrease in tension noted in control strips (P < 0.001; Student's t-test for unpaired observations).

(*) Inhibition of adrenergic neurotransmission by acetylcholine is not due to interference with spike electrogensis at the adrenergic nerve terminals.

(*) Progressive increases in K\(^+\) concentration cause release of transmitter through progressive depolarization of the adrenergic nerve terminals; this is illustrated best by the experiments showing in the same preparations that increasing the K\(^+\) concentration of the superfusing solution from 50 to 70 mEq/liter augments both the contractile responses and the \[^{[H]}\text{norepinephrine release}\] (Table 2). With solutions containing K\(^+\) = 120 mEq/liter, the depolarization of the nerve endings must be complete; this is suggested by the large amounts of \[^{[H]}\text{norepinephrine}\] appearing in the superfusate. In view of the important changes in membrane potential caused by the depolarizing solution (K\(^+\) = 120 mEq/liter) modulatory influences on the neuronal membrane potential must be less effective; however, pharmacological effects not mediated through changes in membrane potential still should be present. During
supersession with K⁺ at 120 mEq/liter, acetylcholine does not significantly alter the efflux of tritiated transmitter. This suggests that the effect of acetylcholine on the adrenergic nerve endings is mediated through changes in membrane potential. Because acetylcholine decreases the release of labeled transmitter evoked by K⁺ at 50 mEq/liter, and since increases in K⁺ concentration above that level augment it, it is tempting to assume that at the adrenergic nerve ending acetylcholine causes hyperpolarization rather than further depolarization; such a hyperpolarizing effect also would
explain the inhibition of the norepinephrine release evoked by electric impulses. As during nerve stimulation, the inhibitory effect of acetylcholine is blocked by atropine, indicating that muscarinic receptors are involved. Hyper-polarizing muscarinic effects of acetylcholine are well documented in sinoatrial tissue, have been demonstrated in sympathetic ganglion cells, and postulated for the adrenergic nerve endings of the heart.

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Progressive Perfusion Impairment during Prolonged Low Flow Myocardial Ischemia in Dogs

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SUMMARY Recent studies have shown that after total coronary artery occlusion, there is impaired "reflow" of blood accompanied by myocardial and capillary endothelial cell swelling. To investigate the effect of prolonged low flow myocardial ischemia on coronary vascular resistance, regional hyperperfusion of the distal left anterior descending coronary artery was studied in 31 autonomously blocked dogs on right heart bypass. Heart rate, aortic pressure, and, during ischemia, left ventricular end-diastolic pressure were held constant. The distal left anterior descending coronary artery was perfused at a substantially reduced perfusion pressure which resulted in an antegrade coronary blood flow that usually was between 3% and 7% (0.5-1 ml/min) of control. When relative hypothermia (33-34°C) was induced in nine dogs, left anterior descending coronary artery vascular resistance did not change during 2.5-3 hours of low flow ischemia. Under anemic conditions (37-40°C) in 17 dogs there was a consistent progressive increase in distal left anterior descending coronary artery vascular resistance starting at 90 minutes (median) after onset of ischemia. By 110-140 minutes ischemic antegrade flow decreased by 35 ± 4% (SEM) (P < 0.01). Directionally similar flow changes were observed in six anemic experiments using the krypton-85 washout technique. Light microscopy did not reveal hemorrhage as a cause of the increased vascular resistance. The perfusion impairment did not occur in two euthermic, non-ischemic hearts. In five dogs elevation of serum osmolality by 23 ± 11 mOsmol/liter with mannitol attenuated the progressive decrease in flow. Thus, a progressive perfusion defect exists in the ischemic low flow state in the heart which presumably contributes to the extent of eventual necrosis.

THE FACTORS leading to irreversible cell injury during myocardial ischemia are poorly understood. Significantly impaired reperfusion following total arterial occlusion has been demonstrated in the brain1,2 and the kidney.3 In the heart this phenomenon also has been demonstrated during "reflow" of blood following total occlusion of a single coronary artery.4,5 Recent studies from our laboratory6 have demonstrated perfusion defects in the posterior papil-
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