Coronary Vasoconstriction Induced by Vasopressin
Production of Myocardial Ischemia in Dogs by Constriction of Nondiseased Small Vessels

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Background. We studied the effect of intracoronary administration of arginine-8-vasopressin on blood flow in nondiseased coronary arteries and determined whether this vasoconstriction was severe enough to produce ischemia in 30 dogs.

Methods and Results. In group 1 (n=6), after vasopressin administration coronary blood flow was decreased by 41% (p<0.002) without changes in heart rate or aortic pressure, and left ventricular ejection fraction measured by radionuclide angiocardiography was decreased by 18% (p<0.0005). In group 2 (n=6), ischemia was confirmed by measurement of transmural pH changes. Administration of vasopressin decreased subendocardial pH of the infused zone from 7.40±0.03 to 7.31±0.07 (p<0.01). The subendocardial pH of the zone not infused with vasopressin did not change. To overcome the intrinsic regulation of blood flow, operating primarily in small coronary arteries, we hypothesized that vasopressin must increase resistance primarily in large rather than small coronary arteries. After intracoronary infusion in group 3 (n=6), however, most (94%) of the increase in resistance during vasopressin administration was explained by an increase of resistance in small coronary arteries. In group 4 (n=9), vasopressin decreased coronary blood flow by 50% and decreased local shortening by 90% at a time when systemic hemodynamics were unchanged. Coronary constriction induced by vasopressin, or the recovery from it, also was not altered by cyclooxygenase blockade.

Conclusions. Thus, vasopressin produces myocardial ischemia by constricting small, nondiseased coronary arteries severely enough to overcome the competition from normal coronary regulation, and this ischemic event is not mediated by prostaglandin products. (Circulation 1991;83:2111–2121)

To understand the clinical observation of myocardial ischemia without an increase in myocardial oxygen demand,1 it is necessary to understand two basic issues: first, whether endogenous vasoconstrictors can constrict coronary arteries in experimental animals with no known arterial disease; second, whether the vasoconstriction in nondiseased arteries can be strong enough to compete with and overcome the endogenous regulatory system that controls coronary blood flow to meet myocardial oxygen demand.2–4 For example, vasopressin is known to produce coronary vasoconstriction5–9; and if flow reduction is severe enough, ischemia would be expected to occur. The evidence to suggest that coronary vasoconstriction by vasopressin is severe enough to produce ischemia7,8,10 is questionable.

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Furthermore, if an endogenous peptide such as vasopressin could overcome normal coronary regulation to produce myocardial ischemia, knowing whether the peptide had overcome normal regulation by constriction of primarily large or small coronary arteries is of crucial importance. Because the endogenous coronary regulatory system operates at the level of small coronary arteries,3,4 it would seem difficult for a compound to compete with and overcome endogenous regulation directly at the level of small coronary arteries. On the other hand, it is easier to understand how an agent could cause ischemia by constricting large coronary arteries that are on the epicardial surface (seen on angiography), remote from the metabolic milieu of the myocardium. The large coronary arteries would be less influenced by the vasodilator metabolites, such as adenosine, released from ischemic myocardium.

Thus, the purposes of the present study were, first, to test the hypothesis that intracoronary administration of arginine-8-vasopressin to normal dogs can reduce coronary blood flow enough to produce myocardial ischemia, as indicated by the development of left ventricular dysfunction11 and myocardial acidosis.12 The second purpose of this study was to test the hypothesis that vasopressin would have to constrict primarily large rather than small coronary arteries to produce myocardial ischemia, using the differential pressure and resistance measurements described by Farn and McGregor.13 Last, because cyclooxygenase blockade with ibuprofen prevented 40% of the constrictor effects of another peptide, neuropeptide Y,14 we tested whether ibuprofen altered the response to vasopressin.

Methods

**Experimental Preparation**

Thirty foxhounds or mongrel dogs of either sex weighing 20–35 kg were anesthetized with morphine sulfate, 3 mg/kg (i.m. or s.c.) and α-chloralose (100 mg/kg i.v. bolus, then 2–8 mg/kg/hr i.v. infusion). In all animals, a left thoracotomy was performed, and a pericardial cradle was made. A 1–2-cm segment of the left circumflex coronary artery (LCx) was isolated, and a calibrated electromagnetic flowprobe (Carolina Medical Electronics, King, N.C.) was placed proximal to a pneumatic occluder (R.E. Jones Co.) on the artery. The pneumatic occluder was used to stop flow for 3–10 seconds to check the balance of the flow probe. No measurements were made for at least 5 minutes after balancing the flow probe. The flow probes were previously calibrated in blood. A 22-gauge catheter was inserted into the proximal LCx for infusion of vasopressin. Aortic pressure was monitored through a femoral arterial catheter by a Statham P23-ID pressure transducer (Gould-Statham, Oxnard, Calif.). Arterial blood gases were maintained within normal limits by adjusting ventilation or by intravenous infusion of sodium bicarbonate (150 mM).

**Radionuclide Angiography**

To evaluate possible ischemia and to determine whether the changes in coronary blood flow were functionally significant, gated blood pool scintigraphic studies were performed in six dogs before and after infusion of vasopressin (group 1). When conditions were stable, 3 mg stannous pyrophosphate was injected intravenously, which was followed by 20 mCi technetium-99m 30–40 minutes later, as reported previously.11 Electrocardiographic-gated blood pool scintigraphy was performed by imaging the dog with a portable gamma scintillation camera in a modified left anterior oblique projection. The data were collected in list mode on a computer (Hewlett-Packard, Palo Alto, Calif.) using programs developed at the National Institutes of Health (NIH).11 The time-activity curve is proportional to changes in left ventricular volume, and left ventricular ejection fraction was calculated from the following equation: (end-diastolic counts minus end-systolic counts) divided by (end-diastolic counts minus background counts).

After the baseline gated blood pool scan was obtained, measurements of coronary blood flow, aortic pressure, left ventricular pressure, and heart rate were made. Intracoronary saline was injected as a control solution in six dogs, and it caused no change in heart rate, coronary blood flow, aortic pressure, or left ventricular ejection fraction. Vasopressin (a single dose of 0.4 or 0.8 nmol i.c. in 2 ml saline) was infused during 4 minutes, and repeated measurements were made. Most dogs received 0.8 nmol, but no differences were seen in responses to 0.4 or 0.8 nmol, so data were grouped. The same doses and infusion protocols were used in groups 1–3, but in group 4, the lower dose was 0.6 instead of 0.4 nmol. These doses of vasopressin were determined from preliminary studies in other dogs, in which an intracoronary dose was identified that decreased coronary blood flow without changing aortic pressure.

**Intramyocardial pH**

To test for the presence of ischemia by an independent method, intramyocardial pH was measured before and after vasopressin in nine dogs (group 2). Intramyocardial pH was measured continuously during the basal state after intracoronary infusion of vasopressin by the same protocol as above; a single dose of 0.4 or 0.8 nmol in 2 ml saline was infused during 4 minutes. Intramyocardial pH was measured by a miniature fiberoptic pH probe system with four probes developed15–17 and validated12,18,19 at NIH and used by others.20 Detailed description of the probe system is published elsewhere.12,15–19 In brief, pH determination with the fiberoptic probe system is based on the concept of monitoring the change in color of a pH-sensitive dye, phenol red. The dye is contained within a semipermeable membrane housed in a stainless steel hypodermic needle with outer diameter of 0.5 mm (25 gauge).
The probes were calibrated at 37°C with buffers of pH 6.81, 6.98, and 7.46 before every experiment. The probes recorded 90% of their response to a step change in pH within 60 seconds. Two probes were implanted in the zone not infused by vasopressin and two in the infused zone. They were secured by rubber grommets at a depth of 3 mm below the epicardial surface for subepicardial pH and at 6 mm for subendocardial pH measurements. The dogs in which pH was measured received heparin to prevent clotting on the pH probes: 7,000 units initially, followed by 5,000 units every 30 minutes.

**Large and Small Coronary Artery Resistance**

To assess the relative effects of vasopressin on the resistances of large and small coronary arteries in six dogs (group 3), we used the differential pressure gradient technique of Fam and McGregor, as adapted previously in our lab. The 22-gauge catheter for infusion of vasopressin was directed retrograde into the proximal LCx to ensure exposure of the large coronary arteries to the peptide. The tip of this catheter was less than 1 cm from the origin of the circumflex coronary artery.

A 23-gauge blunt needle with a tapered plastic tip was inserted retrograde into the smallest branch of the circumflex coronary artery that could be dissected and cannulated (approximately 0.9 mm in diameter). The branch was ligated distally so that coronary pressure in the branch was measured through the proximally directed catheter connected to a Statham P23-ID pressure transducer. This transducer and a second identical pressure transducer were carefully balanced and calibrated against a mercury manometer in steps from 0 to 150 mm Hg to demonstrate linear responses over this range. These two pressure transducers were connected to a special electronic subtraction circuit, which was calibrated against mechanically calibrated signals in steps and amplified so that the full scale represented 0 to 20 mm Hg. In separate pilot studies, femoral artery pressure was measured through a 23-gauge blunt needle and the usual 8F aortic catheter. The mean pressure difference, when both of these two types of cannulas were inserted into two femoral arteries, was 0 mm Hg. The tubing was flushed with 1,000 units heparin; then the dogs’ blood was heparinized with 7,000 units heparin through the femoral artery initially and 5,000 units/hr.

The mean pressure difference between aorta and the distal coronary branch was between 3 and 13 mm Hg in dogs used for this study. Some dogs were not used for this study because reasonable pressure differences could not be recorded. High values of this difference (more than 14 mm Hg) were attributed to obstruction of the distal coronary branch by the blunt needle cannulation. In such cases, the cannulation was repeated, and if values between 3 and 13 mm Hg could not be obtained, the animal was not used for the study. Similarly, if pressure differences of less than 3 mm Hg were recorded, the animal was not used because we assumed that the coronary branch was too large to reflect a difference between large and small coronary arteries. All decisions of whether to exclude an animal were made before administration of vasopressin to avoid retrospective data selection. Large and small coronary artery resistances were calculated in these dogs as follows: Large coronary artery resistance equals (aortic pressure minus distal coronary pressure) divided by coronary blood flow; small coronary artery resistance equals distal coronary pressure divided by coronary blood flow.

Total coronary vascular resistance, or large plus small coronary artery resistance, was estimated by the formula: mean aortic pressure divided by mean coronary blood flow, acknowledging that the value of coronary back pressure is uncertain but low in the open-chest dog. The system was tested to determine whether it could detect an increase in large coronary artery resistance by a brief inflation of the pneumatic occluder on the proximal coronary artery. Nitroglycerin was not used to test whether the system could detect a decrease in the resistance of large coronary arteries because the effect of nitroglycerin on large vessels is long lasting. We did not want to wait 45 minutes for the effect of nitroglycerin to wear off before administering vasopressin because of the chance that the preparation would deteriorate during the waiting period.

Control measurements included heart rate, aortic pressure, coronary blood flow, and the resistances of large and small coronary arteries. Vasopressin (a single dose of 0.4 or 0.8 nmol i.c.) was infused during 4 minutes (same protocol as above). Repeated measurements of all variables were made during the maximum effect of vasopressin on coronary blood flow. Intracoronary saline was infused at the same volume and rate as control.

**Cyclooxygenase Blockade**

The effects of inhibition of cyclooxygenase blockade were tested in a separate group of nine dogs (group 4). Dogs were anesthetized and surgically prepared as described in “Experimental Preparation.” In addition, a high-fidelity pressure transducer (Millar Instruments, Inc, Houston) was advanced from a femoral artery into the left ventricle to record left ventricular pressure and its first derivative with respect to time (dP/dt). Two pairs of ultrasonic dimension crystals (2-mm diameter) were positioned, one in the posterior myocardium in the ischemic zone supplied by the LCx and the other in the anterior myocardium in the region supplied by the left anterior descending coronary artery for measurement of local segmental function. Care was taken to place the crystals in the midmyocardium, along the circumferential axis.

To collect coronary venous blood for the measurement of regional blood gases, a catheter (model P4.0-25-70-M-35-0, Cook Incorporated) was inserted into the jugular vein and advanced into the
coronary sinus and then into one of the marginal veins draining the LCx region. The sideports of the catheter were at least 7–10 mm into the regional vein, thereby ensuring that blood collected from the catheter represented LCx venous blood. The catheter was flushed periodically with heparin until instrumentation was complete, at which time the animal’s blood was heparinized (20,000 units initial bolus and supplemented 1 hour later with 7,000 units). After heparin was given, the catheter was allowed to drain freely. Blood was returned to the animal, except during vasopressin infusion.

Arterial blood pressure, left ventricular pressure and dP/dt, coronary blood flow, and posterior and anterior wall segmental function were recorded continuously. Heart rate was calculated from the dP/dt signal. Fractional shortening of each region was calculated as an estimate of segmental function: [(end-diastolic length minus end-systolic length)/end-diastolic length] multiplied by 100%. End-diastolic length was measured at the point of the initial upsweep of the left ventricular pressure or dP/dt signal. End-systolic length was measured 20 msec before peak negative dP/dt. Respiration was stopped at end expiration during the recording of segmental function.

After instrumentation, the animal was allowed to stabilize for 30 minutes. Then, blood was sampled simultaneously from the systemic artery and coronary vein for measurement of blood gases. At this time (0 minutes), an intravenous infusion of saline (control, n=4) or 5 mg/kg ibuprofen (n=5) (Upjohn Co., Kalamazoo, Mich.) was given during 10 minutes. This dose of ibuprofen was shown previously to attenuate by 40% the coronary constrictor effects of neuropeptide Y, a potent vasoconstrictor. At 60 minutes, blood was sampled, and an intracoronary infusion of vasopressin (a single dose of 0.6 or 0.8 nmol during 4 minutes) was given (same protocol as above except that the lower dose was 0.6 instead of 0.4 nmol). Blood was sampled at 63, 65, and 70 minutes or 3, 5, and 10 minutes after beginning the 4-minute vasopressin infusion, respectively.

Analysis of Data

Data are presented as mean±SD. We tested the significance of differences in each variable from control to the maximum change in coronary blood flow after administration of vasopressin by Student’s t test for paired data. A p value less than 0.05 was considered statistically significant. For left ventricular ejection fraction, only one average value was available, and for intramyocardial pH, we used the maximum change (averaged over a 10–20-second period) in this variable that occurs a few minutes after the maximum change in flow. We tested the significance of differences in parameters between control and ibuprofen-treated subgroups of group 4 at specific time points by Student’s t test for unpaired data. A p value of 0.05 or less was considered statistically significant; a p value between 0.05 and 0.10 was noted as marginally significant. We also tested the differences in parameters measured in group 4 after beginning the 4-minute vasopressin infusion (at 63, 65, and 70 minutes) with respect to 60 minutes (before vasopressin) by Student’s t test modified with the Bonferroni technique for multiple comparisons.

Results

Hemodynamic Effects of Vasopressin

The four groups of dogs studied were similar with respect to baseline hemodynamic parameters. Coronary blood flow was 56±24 in group 1 (radionuclide angiocardiography), 42±8 in group 2 (pH), 37±7 in group 3 (resistance of arteries), and 38±6 ml/min in group 4 (ibuprofen). Mean aortic pressure was 106±12 in group 1, 95±18 in group 2, 109±15 in group 3, and 99±10 mm Hg in group 4. Heart rates were also similar among the first three groups: 131±24, 128±21, and 118±28 beats/min but lower in group 4, 85±15 (p<0.05). Coronary vascular resistance was 2.16±0.78 in group 1, 2.38±0.77 in group 2, 3.02±0.66 in group 3, and 2.30±0.45 mm Hg/ml/min in group 4.

The intracoronary infusion of vasopressin reduced coronary blood flow by 41% in group 1, by 45% in group 2, by 40% in group 3, and 50% in group 4 (Figures 1 and 2, Table 1). During the time of vasopressin infusion, aortic pressure and heart rate were unchanged (Figure 2, Table 1). Thus, the calculated coronary resistance increased during vasopressin by 72%, 77%, 72%, and 125% in groups 1 through 4, respectively.

The time course of an intracoronary infusion of vasopressin is shown in Figure 1. By 2 minutes after the onset of the infusion, coronary blood flow had decreased from 42 to 20 ml/min. Aortic pressure, left ventricular pressure, and heart rate were unchanged. Left ventricular dP/dt was decreased by 10±4%. The hemodynamic consequences of vasopressin had abated by 12 minutes after the onset of the infusion.

Effect of Vasopressin on Left Ventricular Function

In group 1, infusion of vasopressin reduced left ventricular ejection fraction by 0.10 units (18%) from 0.57±0.05 (p<0.0005). In contrast, infusion of vehicle control (saline) in this group did not alter left ventricular ejection fraction (absolute difference, 0.01±0.01). These ejection fractions were measured a few minutes apart in the same anesthetized dog; the dog and camera were not moved, and there were no changes in heart rate or aortic pressure. The value of 0.57 is normal by this method in dogs and people.

In group 4, irrespective of the drug treatment (ibuprofen or saline), infusion of vasopressin reduced global function as measured by dP/dt (Table 1) and local segmental shortening in the posterior, infused, LCx region (Figures 1 and 2). Fractional shortening in the infused region decreased between 40% and 150% during 5 minutes after beginning vasopressin administration. In some animals, sys-
Effect of Vasopressin on Intramyocardial pH

To test for ischemia by an independent method, intramyocardial pH was measured in group 2. As described earlier, coronary blood flow was decreased by 45% in this group. The decrease in intramyocardial pH of the infused zone followed closely the decrease in coronary blood flow, whereas the pH of the noninfused, normal zone did not change (Figure 3). Intracoronary administration of vasopressin reduced the subendocardial pH of the infused zone from 7.40±0.03 to 7.31±0.07 (p<0.01). Administration of vasopressin also decreased the subepicardial pH of the infused zone from 7.38±0.05 to 7.27±0.12 (p<0.01). There was no transmural gradient of pH from the subendocardium to the subepicardium as we found previously with mechanical constriction of a large coronary artery.12

In control studies, coronary blood flow and intramyocardial pH did not change after intracoronary infusion of saline.19 The 95% confidence limits were defined by an increase in pH of 0.007 or a decrease in pH of 0.013 units in the subendocardium in these studies. Thus, a decrease of more than 0.014 units after vasopressin would be significant at a p value of less than 0.05. The mixing of vasopressin in blood in vitro (at concentrations predicted by the rate of infusion and coronary blood flow in vivo) did not change pH. At concentrations 1,000 times the calculated maximum in vivo concentration, pH was increased by 0.006 units.
Effect of Vasopressin on Large and Small Coronary Artery Resistance

In group 3, after vasopressin administration, total coronary resistance was increased by 72%, from 3.02±0.66 mm Hg/ml·min (p<0.0005, Figure 4). The resistance in large vessels was increased by 68% from 0.22±0.13 mm Hg/ml·min, but this change was not significant because of variability of the measurement. The resistance in small vessels was increased by 72% (p<0.01); resistance in small vessels accounted for 93% of the total coronary resistance before and after vasopressin administration (Figure 4). Of the increase in total coronary resistance, 94% was due to an increase in small vessels, and only 6% was due to the increase in large vessels.

Effect of Cyclooxygenase Blockade

As previously reported, the administration of ibuprofen slightly increased baseline coronary resistance in group 4 (p<0.05, Table 2). The increase in resistance was brought about by a reduction in coronary blood flow; no changes were observed in aortic pressure, dP/dt, heart rate, or fractional shortening in either region. The increase in baseline resistance was accompanied by an increase in oxygen extraction so that calculated myocardial oxygen consumption did not change during the hour.
Prior administration of ibuprofen, a cyclooxygenase blocker, did not attenuate or magnify the coronary constrictor effect of vasopressin (Figure 2, Table 1). Coronary blood flow, segmental shortening of the infused zone, and dP/dt were reduced to similar degrees as in saline-treated animals. We also did not observe any effect of cyclooxygenase blockade on the duration or pattern of recovery from the effects of vasopressin.

**Discussion**

Several studies by others have not answered the question of whether vasopressin-induced coronary constriction produces myocardial ischemia. There is evidence to suggest that vasopressin can produce ischemia, such as ST abnormalities in the electrocardiogram of patients receiving vasopressin, but possible coronary atherosclerosis and multiple other medical conditions of these critically ill patients prevent clear interpretation. There is also experimental evidence that vasopressin decreases overall left ventricular function. These studies, however, did not determine whether the decreased left ventricular function was due to an increase in aortic pressure, increased afterload, a direct or reflex-mediated negative inotropic effect, or ischemia. An increase in aortic pressure would decrease left ventricular function because of increased afterload or because of baroreceptor reflex-mediated withdrawal of sympathetic inotropic stimulation. In vitro data have produced conflicting evidence as to whether vasopressin exerts a direct positive or negative inotropic effect. A direct negative inotropic effect, for example, could explain reports of vasopressin-induced reductions in regional contraction. Injection of adenosine into the coronary cannula after vasopressin administration caused transient increases in both coronary blood flow and local force development measured by Walton-Brodie strain gauge arches, suggesting that the reduction in local contraction induced by vasopressin was mediated by ischemia.

**Table 1. Hemodynamic and Metabolic Effects of Intracoronary Administration of Vasopressin in Saline- or Ibuprofen-Treated Dogs**

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<tr>
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<th>60 min</th>
<th>% 63 min</th>
<th>% 65 min</th>
<th>% 70 min</th>
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Values are mean±SD. n=4 for saline-treated dogs; n=5 for ibuprofen-treated dogs. Values at 60 minutes were obtained just before starting a 4-minute infusion of vasopressin (60–64 minutes). Values reported at 63, 65, and 70 minutes are taken as percent changes from 60 minutes. The oxygen extraction reflects that of the infused, posterior region. Fractional shortening was calculated for both infused, posterior (POS) and normal, anterior (ANT) regions.

*p<0.05 vs. saline-treated dogs.
found increased lactate concentration in the myocardium perfused by blood containing vasopressin. Increased lactate was detected in only one of three studies\(^1\) and only by comparing the normal zone with the zone infused with vasopressin through a coronary perfusion cannula (paired t test). No control lactate measurements were reported before vasopressin in the zone dependent on the cannula. This study did not assess the adequacy of perfusion via the cannula after the period of coronary occlusion required for cannula insertion. Thus, there is some question whether the only previous study\(^1\) to report abnormal lactate metabolism has demonstrated conclusively that vasopressin can cause coronary constriction severe enough to overcome coronary regulation and produce ischemia.

The results of the present study indicate that intracoronary administration of vasopressin to dogs with no known coronary artery disease caused a 43% reduction in coronary blood flow with no change in heart rate, aortic pressure, or oxygen consumption. One major new finding of the present study is that this decrease in flow produced myocardial ischemia as shown by three lines of evidence: left ventricular dysfunction measured by radionuclide angiocardiography, myocardial acidosis measured by miniature fiberoptic pH probes, and the reduction in regional shortening at a constant, calculated myocardial oxygen consumption. Thus, vasopressin-induced reductions in coronary blood flow can, indeed, produce ischemia in a dog with no evidence of coronary arterial disease.

Because vasopressin can overcome the endogenous coronary regulation, it is of special interest to know whether the peptide produces ischemia by constricting large\(^1\) or small\(^34\) coronary arteries. It is easier to understand how the endogenous coronary regulatory system might be overcome by constricting the large coronary arteries that lie on the epicardial surface, remote from the metabolic milieu of ischemic cells and small coronary arteries. Thus, metabolic mediators of vasodilation that are released from ischemic myocardial cells would have less access to large coronary arteries than to small coronary arteries. Furthermore, recent evidence indicates that sympathetic, \(\alpha\)-mediated activation of small vessels (<100 \( \mu \)m diameter) leads to vasodilation of a metabolic or myogenic nature,\(^35\) indicating that it may be difficult for a small-vessel constrictor to overcome normal regulation to produce myocardial ischemia. Last, direct visualization of coronary arteries by contrast arteriography indicated that vasopressin exerted an important constrictor effect on large coronary arteries,\(^36\) although neither the large- nor small-vessel effects were quantified. In the present study, vasopressin caused a uniform transmural reduction in intramyocardial pH in the subendocardial (0.09 units) and subepicardial (0.11 units) layers of the zone infused by vasopressin, which, according to Khayyal et al.,\(^10\) would be consistent with predominant small-vessel constriction. In contrast, during mechanical occlusion of a large coronary artery that decreased blood flow by 50%, we found that pH decreased much more in the subendocardium than in the subepicardium.\(^12\)

The resistances of large and small coronary arteries were estimated directly in the present study, using the technique of Fam and McGregor\(^12\) as modified for use in our laboratory.\(^19,21,22\) The results indicate that our initial hypothesis was wrong because 94% of the absolute increase in total coronary resistance (large plus small vessel) was due to small coronary arteries and only 6% to large coronary arteries.

These results can only localize the effect of vasopressin to vessels greater than or less than the diameter of the smallest artery that could be cannulated (about 0.9 mm). This resolution is sufficient to distinguish whether the primary effect of vasopressin was on the large coronary arteries, as it is in focal narrowing of large arteries seen when the balloon occluder was inflated or on coronary arteriograms during ergonovine testing in susceptible patients.\(^1\) The present results clearly eliminate the possibility that vasopressin causes decreased flow because of focal spasm of large coronary arteries and, therefore, indicate that vasopressin can overcome endogenous coronary regulation by competing at the level of small coronary arteries. In addition, these results are consistent with clinical studies suggesting that constriction of small rather than large coronary arteries can
TABLE 2. Effects of Saline or Cyclooxygenase Blockade on Baseline Hemodynamic and Metabolic Parameters in Dogs

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>% 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aortic pressure (mm Hg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>121±9</td>
<td>-9±7</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>77±5</td>
<td>0.5±2</td>
</tr>
<tr>
<td><strong>dP/dt (mm Hg/sec)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>2,083±260</td>
<td>4±2</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>2,013±321</td>
<td>-0.6±5.0</td>
</tr>
<tr>
<td><strong>Heart rate (beats/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>99±19</td>
<td>-14±6</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>69±4</td>
<td>-15±2</td>
</tr>
<tr>
<td><strong>Coronary blood flow (ml/min·100 g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>45±3</td>
<td>7±7</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>41±6</td>
<td>-13±8*</td>
</tr>
<tr>
<td><strong>Coronary vascular resistance (mm Hg/ml·min/100 g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>2.67±0.24</td>
<td>-13±12</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>2.02±0.23</td>
<td>18±11†</td>
</tr>
<tr>
<td><strong>Oxygen extraction (ml O₂/ml blood)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>9.8±1.8</td>
<td>-3±8</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>9.4±1.4</td>
<td>16±15†</td>
</tr>
<tr>
<td><strong>Fractional shortening (POS) (mm%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>13.8±1.8</td>
<td>-3±10</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>14.0±0.8</td>
<td>11±6</td>
</tr>
<tr>
<td><strong>Fractional shortening (ANT) (mm%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>14.6±1.6</td>
<td>-0.2±5</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>20.3±1.8</td>
<td>2.0±2.6</td>
</tr>
</tbody>
</table>

Data are mean±SD. n=4 for saline-treated dogs; n=5 for ibuprofen-treated (cyclooxygenase blockade) dogs. Values at 0 minutes and 60 minutes were obtained just before starting a 4-minute infusion of vasopressin. Values at 60 minutes are taken as percent changes from 0 minutes. % 60 min, percent change at 60 minutes from 0 minutes. POS, infused, posterior region; ANT, normal, anterior region.

*0.05<p<0.10 vs. saline-treated group; †p<0.05 vs. saline-treated group.

cause myocardial ischemic syndromes in some patients with variable threshold angina.37

**Critique of Methods**

One must determine whether the observed decrease in left ventricular ejection fraction from a normal value of 0.57±0.05 to 0.47±0.06 in group 1 can be interpreted as evidence of ischemia. First, aortic pressure did not increase in group 1, so the decreased ejection fraction could not be attributed to increased afterload31 or to baroreceptor reflex-mediated inhibition of contractile function.9 Second, the possibility that intracoronary vasopressin has a direct negative inotropic effect must be considered unlikely because the hormone has been reported to exert a slight positive inotropic effect on heart muscle in vitro, although there are conflicting results.7 Most important, a negative inotropic effect would not cause the decrease in intramyocardial pH seen in group 2; and calculated oxygen consump-

tions remained constant despite decreased function in group 4.

One must determine also whether the observed decrease in intramyocardial pH in the subendocardium and subepicardium of the infused zone represents ischemia. Decreasing pH correlated with the severity of decreased coronary blood flow produced by a mechanical occlusion over a wide range (r=0.80).12 The decrease in pH was directly proportional to the depletion of ATP (r=0.78) and inversely proportional to lactate accumulation in tissue (r=−0.88) 15 minutes after coronary blood flow reduction in our laboratory (unpublished observations). Although one would not always expect close correlation between intramyocardial pH and intracellular metabolites, these correlations 15 minutes after a decrease in coronary blood flow support the concept that intramyocardial pH reflects the severity of ischemic injury. Furthermore, the vasopressin effect was also associated with increased myocardial oxygen extraction in group 4.

Resistance of small and large coronary arteries was determined from pressure differences between the aorta and a small coronary artery. The limiting step in estimating the site of vasoconstriction by this method is the size of the coronary branch that can be cannulated for pressure measurements (about 0.9 mm). Available techniques including those used here cannot distinguish constriction of arterioles from that of small-to-medium size arteries, but this pressure gradient method has permitted important distinctions about the primary site of action of several vasoactive compounds.13,19,21,23,24,28 During inflation of the pneumatic occluder on the LCx, there was a large decrease in pressure between the aorta and the distal coronary catheter to prove that the system could detect physiologically important spasm of large coronary arteries19,21 such as is observed during angiography in humans.1

The maximum level of vasopressin in coronary artery blood achieved in this study was calculated from the infused rate of vasopressin and the coronary blood flow to be only three to 30 times the level of vasopressin in patients undergoing the stress of cardiopulmonary bypass, 0.16 nmol/L.39 The concentration of vasopressin in the coronary circulation of humans during angina at rest is not known, but the purpose of this study was not to determine whether release of endogenous vasopressin causes vasoconstriction in humans.

These studies cannot identify the mechanism by which vasopressin causes vasoconstriction, although we have found that it constricts primarily small coronary arteries. Work in other tissues has suggested that the mechanism involves prostaglandins30; however, we were unable to show any involvement of cyclooxygenase products. We also showed that ischemia elicited by vasopressin is not dependent on prostaglandin products of cyclooxygenase. In contrast, the effect of neuropeptide Y, which also leads to ischemia due to constriction of small coronary
vessels,19 is mediated in part by cyclooxygenase products.14 Also, this study demonstrates that the mechanism of left ventricular dysfunction caused by vasoconstrictor agents involves ischemia due to vasoconstriction. The present finding clarifies previous studies of this issue.8,10,32,35

In conclusion, this study shows that vasoconstrictor agents, an endogenous peptide that we found to increase resistance primarily in small vessels, can overcome the powerful system of coronary blood flow regulation in nondiseased coronary arteries to produce ischemia. The present studies cannot implicate vasoconstrictor agents as the cause of coronary artery vasoconstriction in humans, but they do indicate constriction of small arteries as a plausible mechanism for production of myocardial ischemia. Thus, standard coronary angiographic techniques alone may not be sufficient to identify or rule out coronary artery vasoconstriction as the cause of myocardial ischemia in patients with unexplained chest pain. Also, the fact that nondiseased coronary arteries can be constricted with sufficient intensity to produce myocardial ischemia indicates that research into mechanisms should consider excessive concentrations of vasoconstrictors as well as abnormal biology of the arterial wall.

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**KEY WORDS** • vasoconstriction • vasopressin • coronary arteries • intramyocardial pH • myocardial ischemia • left ventricular ejection fraction • radionuclide angiography • ibuprofen • prostaglandins • cyclooxygenase blockade
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