Effect of Atrial Natriuretic Peptide on Coronary Collateral Blood Flow

Blair Foreman, Xue-Zheng Dai, David C. Homans, David D. Laxson, and Robert J. Bache

This study was carried out to examine the effects of atrial natriuretic peptide on coronary collateral blood flow. Studies were performed in nine adult mongrel dogs 3.4 months after embolic occlusion of the left anterior descending coronary artery had been performed to stimulate collateral vessel growth. At the time of study the anterior descending coronary artery was cannulated to allow estimation of interarterial collateral flow from measurements of retrograde blood flow. Injection of radioactive microspheres during retrograde flow collection allowed simultaneous determination of continuing tissue flow for evaluation of microvascular collateral communications. Atrial natriuretic peptide in doses of 20 and 200 µg administered into the left atrium resulted in 17±3.0% and 34±4.5% increases in retrograde flow, respectively (each p<0.01). Tissue flow in the collateral dependent myocardial region did not change in response to atrial natriuretic peptide. After the larger dose of atrial natriuretic peptide, the administration of nitroglycerin (10 µg/kg into the left atrium) caused no further increase of retrograde blood flow, and no further decrease of collateral vascular resistance. These data indicate that atrial natriuretic peptide causes vasodilation of moderately well-developed interarterial coronary collateral vessels. (Circulation Research 1989;65:1671-1678)

In vitro studies have shown that atrial natriuretic peptide (ANP) causes relaxation of preconstricted blood vessel strips and inhibits contraction of isolated vessels in response to norepinephrine or angiotensin II.1-3 In the intact animal, ANP causes transient vasodilation of coronary resistance vessels with an increase in coronary blood flow.4,5 In contrast to this modest effect on the coronary resistance vessels, Chu and coworkers6-7 reported that ANP caused substantial sustained vasodilation of epicardial coronary arteries.

In response to gradual coronary artery occlusion, collateral vessels may undergo sufficient growth to maintain viability of the dependent myocardium despite development of total arterial occlusion.8 Although these developed collateral vessels provide adequate blood flow to the dependent myocardium during resting conditions, increases in tissue blood flow in response to increased cardiac activity may be subnormal, resulting in stress-induced myocardial ischemia.9 Growth of collateral vessels is associated with development of substantial smooth muscle in the vessel wall, suggesting potential for vasomotor activity.10 Nitroglycerin has been shown to cause vasodilation of developed coronary collateral vessels, thereby enhancing blood flow to the dependent myocardium.11,12 The presence of an endogenous vasodilator of coronary collateral vessels would be of considerable interest. Consequently, this study was carried out to determine whether ANP exerts vasodilator activity on the moderately well-developed canine coronary collateral vasculature. The effect of ANP was compared with nitroglycerin, a known dilator of coronary collateral vessels.

Materials and Methods

Collateral Vessel Development

Studies were performed in 11 adult mongrel dogs weighing 25±0.4 kg (range, 24-28 kg). Growth of coronary collateral vessels was stimulated by placement of a hollow stainless steel plug into the left anterior descending coronary artery. Aspirin (325 mg orally) was administered at least 1 hour before this procedure was begun to retard the rate of platelet aggregation on the coronary artery plug. This was done to delay thrombotic occlusion of the plug, thereby extending the time available for flow to increase across the native collateral vessels before...
total arterial occlusion occurred. In addition, β-
adrenergic blockade was produced with atenolol (50 mg orally) to minimize adrenergic effects on the heart during placement of the coronary artery plug. Morphine sulphate (1.2 mg/kg s.c.) was administered 1 hour prior to induction of anesthesia with sodium thiamylal (20–30 mg i.v.). Animals were intubated and ventilated with a Harvard respirator (Harvard Apparatus, South Natick, Massachusetts) using room air supplemented with oxygen. After administration of heparin sodium (200 units/kg i.v.) and lidocaine (2 mg/kg i.v.), a 7F Judkins JR4 coronary catheter (Bard-USCI, Billerica, Massachusetts) was introduced into the right carotid artery and advanced into the left main coronary ostium for pressure measurement. A similar catheter was introduced into the left femoral artery and positioned in the descending thoracic aorta to allow coronary angiographic images to be obtained. An 8F plastic guidewire was inserted into the coronary catheter and advanced into the anterior descending artery to the cardiac apex. The catheter was then removed, leaving the guidewire in place. A stainless steel hollow plug 3 mm in length (2.3–3.0 mm o.d.; 1.0 mm i.d.) was pushed along the guidewire with a length of flanged PE90 polyethylene tubing until the plug was firmly wedged in the anterior descending coronary artery. The guidewire was then removed, and coronary angiographic images were obtained by injection of 5–10 ml of 60% diatrizoate meglumine to confirm the position of the plug in the proximal or middle portion of the left anterior descending coronary artery. The catheter was then removed, and the animal was allowed to recover.

Surgical Preparation

Animals were returned to the laboratory 1–4 months (mean, 3.4 months) after placement of the coronary artery plug, premedicated with morphine sulphate (1.2 mg/kg s.c.) and anesthetized with α-chloralose (100 mg/kg i.v.). α-Chloralose was used to obtain prolonged stable anesthesia with minimal myocardial depression and without excessive tachycardia. Animals were intubated and ventilated with room air supplemented with oxygen to maintain the arterial Po2 in the physiological range. A 7F NIH catheter was introduced into the right femoral artery and advanced into the right ventricle for pressure measurement. A similar catheter was introduced into the left femoral artery and positioned in the ascending aorta. A third catheter was introduced into the left carotid artery and advanced into the aorta for reference blood sampling. A left thoracotomy was performed in the fifth intercostal space. A pneumatic cuff occluder was fitted snugly around the descending thoracic aorta to allow control of proximal aortic pressure. The heart was suspended in a pericardial cradle, and a PVC catheter (3.0 mm o.d.) was introduced into the left atrium through the atrial appendage. The location of the plug within the anterior descending artery was determined by palpation, and the artery was dissected free for 2 cm proximal and distal to the plug. After the artery was ligated proximally, a longitudi-

dinal arteriotomy was performed and the plug extracted from the artery. After free retrograde flow displaced any residual thrombus, the anterior descending artery was cannulated with a stainless steel cannula (4.0 mm o.d.) at the site of the previous occlusion. Pressure at the cannula tip was monitored with a 23-gauge tube incorporated into the wall of the cannula. Two dogs were excluded from study because coronary cannula tip pressure did not approach zero when the cannula was opened to allow retrograde flow.

Measurement of Myocardial Blood Flow

Myocardial blood flow was measured with 15 μm diameter microspheres labeled with 125I, 35Co, 51Cr, 85Sr, 86Rb, 113Sn, or 45Sc (3M, St. Paul, Minnesota, and New England Nuclear, Boston, Massachusetts). Microspheres were obtained as 1.0 mCi in 10 ml of 10% low molecular-weight dextran. Microspheres were agitated in an ultrasonic bath for at least 15 minutes before injection. For each intervention, 3×106 microspheres were injected into the left atrium; a reference sample of arterial blood was withdrawn from the aortic catheter at a constant rate of 15 ml/min with a peristaltic pump. Reference sampling was begun at the time of microsphere injection and continued for 90 seconds.

Experimental Protocol

Left ventricular, aortic, and coronary cannula pressures were measured with Statham P23Db pressure transducers (Gould Instruments, Cleveland, Ohio). Left ventricular pressure was displayed at normal and high gain to allow measurement of end-diastolic pressure. Data were recorded on an eight-channel direct writing recorder (model 8800, Hewlett-Packard, Palo Alto, California).

Interarterial coronary collateral blood flow was measured by collecting retrograde flow from the coronary artery cannula into a graduated cylinder for 30 seconds while the cannula tip was maintained at the level of the heart. Initial control measurements of retrograde flow were repeated until consistent measured collections were obtained. Retrograde pressure measurements were recorded during flow collections as well as with the cannula tubing clamped. An initial injection of microspheres was administered with the perfusion cannula clamped. A second injection of microspheres was performed with the perfusion cannula open to air to assess the degree to continuing tissue flow while retrograde flow collection was performed.

After completion of control measurements, ANP (20 μg dissolved in 1.0 ml lactated Ringer’s solution) was administered through the left atrial catheter in seven dogs. Duplicate measurements of retrograde flow from the coronary cannula were carried out at 2, 5, and 8 minutes after administration of ANP. Microspheres were injected during retrograde flow collection 5 minutes after administration of ANP in six of these animals.
The response to ANP (200 μg) was observed in nine dogs. In the seven animals that had previously received ANP in a dose of 20 μg, a second dose of 180 μg ANP was administered into the left atrium 10 minutes after the first dose, for a total of 200 μg ANP. Two additional animals received a single dose of ANP (200 μg) into the left atrium. Measurements of retrograde flow from the coronary cannula were obtained 2, 5, and 8 minutes after ANP. Microspheres were administered during retrograde flow collection 5 minutes after the second dose of ANP in eight animals.

In six of the animals that received ANP in a dose of 200 μg, the response of retrograde flow to nitroglycerin, a known dilator of coronary collateral vessels, was assessed. Nitroglycerin was administered into the left atrial catheter in a dose of 10 μg/kg dissolved in 3 ml normal saline. Retrograde flow from the coronary cannula was measured at 4, 5, and 10 minutes after nitroglycerin administration. Microspheres were administered during retrograde flow collection 8 minutes after injection of nitroglycerin.

A final injection of microspheres was performed using the shadow technique of Patterson and Kirk to delineate the area of myocardium perfused by the cannulated left anterior descending coronary artery. During this microsphere injection, the coronary cannula was perfused with nonradioactive arterial blood from a reservoir at a pressure 5 mm Hg greater than arterial pressure while microspheres were injected into the left atrium. In this way, the area of left ventricle perfused by the anterior descending artery was marked with nonradioactive blood to distinguish it from other coronary perfusion beds.

**Tissue Preparation**

At the conclusion of study 10 ml Evans blue dye was injected into the coronary artery cannula to stain the collateral dependent myocardium, and the heart was excised and fixed in 10% buffered formalin. After fixation, the right ventricle, atria, and great vessels were removed, and the left ventricle was sectioned into five transverse rings from base to apex. Each ring was sectioned radially into 12-15 segments that were divided into epicardial and endocardial specimens, weighted on an analytical balance, and placed into vials for counting. Myocardial and blood reference specimens were counted in a gamma counter (model 5912, Packard Instruments, Meriden, Connecticut) with multi-channel analyzer at window settings corresponding to the peak energies of each radionuclide. The activity in each energy window was corrected for background and for overlapping counts between isotopes with a digital computer. Blood flow to each myocardial specimen (Qm) was computed using the formula Qm = Qr × Cm/Cr, where Qr is reference blood flow rate (milliliters per minute), Cm is counts per minute of the myocardial specimen, and Cr is counts per minute of the sample weight and expressed as milliliters per minute per gram of myocardium. To obtain total myocardial tissue flow to the collateral dependent myocardium, absolute blood flows were summed for all specimens identified by the shadow technique to represent myocardium perfused by the anterior descending coronary artery. Duplicate specimens from the posterior wall were used to assess blood flow from normally (noncollateral) perfused myocardium.

**Data Analysis**

Heart rate and all pressures were measured directly from the strip chart recordings. Hemodynamic data were analyzed using analysis of variance for repeated measures. Comparisons of retrograde blood flow between control conditions and during each intervention were analyzed using analysis of variance for repeated measures. A value of p<0.05 was required for statistical significance. Comparisons of tissue flow measurements with microspheres during control conditions and following administration of ANP were performed using Student's t test for paired data. All data are expressed as mean±SEM.

**Results**

**Hemodynamic Data**

Hemodynamic measurements obtained during control conditions, following two doses of ANP, and after nitroglycerin are shown in Table 1. Heart rates tended to decrease after administration of ANP, and heart rate was significantly slower after the second dose of ANP than after the first dose (p<0.01). This difference persisted after administration of nitroglycerin. Mean arterial pressure and left ventricular systolic and end-diastolic pressures did not vary significantly through the study.

**Blood Flow to Collateral-Dependent Tissue**

Blood flow measured with microspheres in a cross-sectional ring of left ventricle from a typical study during control conditions (with the coronary cannula closed) and during the shadow technique is shown in Figure 1. During control conditions mean blood flow for all animals in the collateral dependent anterior descending coronary artery perfusion bed (0.82±0.06 ml/min/g) was not significantly different from the normally perfused area (0.76±0.06). Measurements during the shadow technique demonstrated a sharp boundary between tissue perfused with nonradioactive blood through the coronary cannula and normally perfused myocardium; an example from a typical dog is shown in Figure 1. The total mass of collateral-dependent myocardium ranged from 4.6 to 31.0 g (mean, 14.6±3.9 g). Total left ventricular weight was 113.1±6.0 g; collateral dependent tissue represented 12.8±3.2% of the left ventricle.
The response of tissue blood flow in the collateral-dependent myocardial area during each experimental intervention is shown in Table 2. Since these measurements were made with the coronary cannula open, they indicate continuing tissue flow not diverted during the retrograde flow collection. Mean tissue flow decreased from 0.82±0.06 ml/min/g during control conditions to 0.42±0.08 ml/min/g with the coronary cannula open (p<0.01). Neither ANP nor nitroglycerin caused significant change in tissue flow in the collateral-dependent area.

**Retrograde Blood Flow**

The response of retrograde blood flow to ANP (20 μg) over time is shown in Figure 2. Retrograde blood flow was 52±9 ml/min during control conditions and increased to 64±10 ml/min 2 minutes after administration of ANP (p<0.05). This increase in retrograde blood flow persisted throughout the observation period. Mean retrograde flow measurements are shown in Table 3. ANP in a dose of 20 μg caused a 17±3.0% increase (p<0.01), while ANP in a total dose of 200 μg produced a 34±4.5% increase in retrograde flow (p<0.01 in comparison with ANP, 20 μg). There was no further increase in retrograde flow with the addition of nitroglycerin.

**Total Collateral Flow**

Total collateral flow (retrograde flow plus tissue flow to the collateral-dependent myocardium) increased 16±2.8% in response to ANP, 20 μg (p<0.01 vs. control), with an additional increase to 27±5.4% above control in response to ANP in a total dose of 200 μg (p<0.01) (Table 3 and Figure 3). Since arterial pressure did not vary significantly throughout the study, while total collateral blood flow increased, collateral vessel resistance decreased 15±2.4% after 20 μg ANP and 21±2.7% after 200 μg ANP (each p<0.01) (Table 3). The subsequent addition of nitroglycerin caused no further increase in total collateral flow or decrease in collateral resistance.

**Normal Zone Blood Flow**

Blood flow to normally perfused myocardium is shown in Table 4. Neither ANP nor nitroglycerin caused significant change in normal zone blood flow.

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**Table 1. Hemodynamic Data During Control Conditions, After Two Doses of Atrial Natriuretic Peptide, and With Addition of Nitroglycerin**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Heart rate (beats/min)</th>
<th>Mean aortic pressure (mm Hg)</th>
<th>LV systolic pressure (mm Hg)</th>
<th>LV end-diastolic pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>124±14</td>
<td>86±5</td>
<td>100±5</td>
<td>7±1</td>
</tr>
<tr>
<td>ANP (20 μg) (n=7)</td>
<td>117±14</td>
<td>84±5</td>
<td>98±5</td>
<td>6±1</td>
</tr>
<tr>
<td>Control</td>
<td>120±11</td>
<td>89±5</td>
<td>111±8</td>
<td>7±1</td>
</tr>
<tr>
<td>ANP (200 μg) (n=9)</td>
<td>109±12†</td>
<td>88±4</td>
<td>108±7</td>
<td>8±1</td>
</tr>
<tr>
<td>Control</td>
<td>132±13</td>
<td>89±5</td>
<td>104±5</td>
<td>7±1</td>
</tr>
<tr>
<td>ANP+TNG (10 μg/kg) (n=6)</td>
<td>109±13†</td>
<td>87±6</td>
<td>96±1</td>
<td>8±1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. ANP, atrial natriuretic peptide; TNG, nitroglycerin.

* p<0.05 vs. control.
† p<0.05 vs. 20 μg ANP.

**Figure 1. Plot of myocardial blood flow measured with microspheres in a ring of left ventricular myocardium of a typical animal measured during control conditions (A), and with the shadow technique, when the cannulated left anterior descending coronary artery was perfused from a reservoir with nonradioactive blood during left atrial injection of microspheres (C). The left ventricular ring was divided into 12 radial sections beginning with the posterior wall (PO), proceeding to the lateral (LAT) and anterior (AN) walls, then to the interventricular septum (SEP), and finally back to the posterior wall. During the shadow technique, blood flow to the anterior free wall perfused by the left anterior descending coronary artery fell to a low level with a sharp boundary from the adjacent normally perfused area. During control conditions with the cannula closed, blood flow in the collateral-dependent anterior region was similar to the normally perfused myocardial areas.**

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TABLE 2. Tissue Blood Flow in the Collateral-Dependent Area Measured During Control Conditions, After Two Doses of Atrial Natriuretic Peptide, and After Addition of Nitroglycerin

<table>
<thead>
<tr>
<th>Condition</th>
<th>Collateral tissue weight (g)</th>
<th>Flow per gram (ml/min/g)</th>
<th>Total tissue flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.6±4.1</td>
<td>0.39±0.10</td>
<td>6.3±2.0</td>
</tr>
<tr>
<td>ANP (20 μg)</td>
<td>13.8±3.5</td>
<td>0.49±0.11</td>
<td>5.8±1.5</td>
</tr>
<tr>
<td>Control</td>
<td>18.2±4.5</td>
<td>0.44±0.10</td>
<td>7.4±2.0</td>
</tr>
<tr>
<td>ANP+TNG (10 μg/kg)</td>
<td>18.2±4.5</td>
<td>0.52±0.10</td>
<td>8.0±1.5</td>
</tr>
</tbody>
</table>

Values are mean±SEM. ANP, atrial natriuretic peptide; TNG, nitroglycerin.

*\( p<0.05 \) vs. control.
**\( p<0.05 \) vs. control.

Discussion

In the present study, collateral flow was measured as the sum of flow diverted in a retrograde direction when the coronary cannula was opened plus continuing tissue flow in the collateral-dependent myocardium. This was done because Downey et al. reported that these two components of collateral flow reflect development of collateral communications at two distinct levels in the coronary vasculature. These investigators measured myocardial blood flow with microspheres in dogs in which ameroid constrictors had been placed on the proximal left anterior descending coronary artery 6–9 weeks before study. When the occluded artery was cannulated and opened to atmospheric pressure to allow retrograde flow, microsphere administration demonstrated that flow continued in the collateral-dependent myocardium at a rate equal to 67% of the control rate before cannulation of the artery. Similarly, in the present study tissue flow measured with microspheres continued at 51% of the control rate when the cannula was opened to permit retrograde flow. These findings suggest that two separable components of collateral vasculature develop in response to chronic coronary occlusion. One component, which is accounted for by the

![Figure 2](http://circres.ahajournals.org/)
retrograde flow measured with microspheres) in response to atrial natriuretic peptide, 20 μg (ANP dose 1), 200 μg (ANP dose 2), and after addition of nitroglycerin, 10 μg/kg i.v. (ANP+TNG). *p<0.05 in comparison with the pretreatment control value.

FIGURE 3. Bar graph of percent change in total collateral blood flow (retrograde flow plus continuing tissue flow measured with microspheres) in response to atrial natriuretic peptide, 20 μg (ANP dose 1), 200 μg (ANP dose 2), and after addition of nitroglycerin, 10 μg/kg i.v. (ANP+TNG). *p<0.05 in comparison with the pretreatment control value.

Table 4. Tissue Blood Flow in Normally Perfused Myocardium During Control Conditions, After Two Doses of Atrial Natriuretic Peptide, and After Addition of Nitroglycerin

<table>
<thead>
<tr>
<th>Drug</th>
<th>Myocardial blood flow (ml/min/g)</th>
<th>ENDO/EPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Drug</td>
</tr>
<tr>
<td>ANP (20 μg)</td>
<td>EPI</td>
<td>0.78±0.19</td>
</tr>
<tr>
<td>(n=6)</td>
<td>ENDO</td>
<td>0.94±0.21</td>
</tr>
<tr>
<td>ANP (200 μg)</td>
<td>EPI</td>
<td>0.78±0.14</td>
</tr>
<tr>
<td>(n=8)</td>
<td>ENDO</td>
<td>0.94±0.15</td>
</tr>
<tr>
<td>ANP+TNG (10 μg/kg)</td>
<td>EPI</td>
<td>0.88±0.20</td>
</tr>
<tr>
<td>(n=5)</td>
<td>ENDO</td>
<td>1.01±0.24</td>
</tr>
</tbody>
</table>

Values are mean±SEM. ENDO/EPI, flow ratio of endomyocardium to epicardium; ANP, atrial natriuretic peptide; TNG, nitroglycerin.
so it is not surprising that myocardial blood flow was not increased. Chu and coworkers\textsuperscript{19} have recently reported that ANP facilitates subendocardial blood flow when a coronary stenosis prevents flow from increasing in response to ischemic dilation of the coronary resistance vessels. Nitroglycerin also produces a similar effect on subendocardial perfusion.\textsuperscript{20} These investigators suggested that both nitroglycerin and ANP dilate the intramural vessels, which deliver blood to the subendocardium and which are not dilated by ischemia. However, these effects on the intramural collateral vasculature would not be expected to alter retrograde blood flow.

In contrast to the increased collateral flow observed in the present study, Chu et al\textsuperscript{19} reported that ANP did not increase collateral flow during acute coronary artery occlusion in dogs. This difference is likely related to differing characteristics of the poorly developed native collateral vessels in that study as compared with the moderately developed collateral vessels in the present study. The native collateral vessels, which are present at the time of acute coronary occlusion, are small and thin walled with little smooth muscle coat and likely with limited ability for vasomotor activity.\textsuperscript{10} In contrast, epicardial collateral vessels, which develop in response to chronic coronary occlusion, take on the appearance of small arteries, with a well-developed muscular media and with well-documented vasomotor ability.\textsuperscript{10,12} It is likely that the failure of Chu et al\textsuperscript{19} to observe an increase in collateral flow in response to ANP was related to the immature state of the collateral vessels in their preparation.

In the present study, the larger dose of ANP resulted in a slight decrease of heart rate. In studies performed in anesthetized rats, Ackermann et al\textsuperscript{21} found that ANP caused direct stimulation of chemosensitive cardiac receptors, which increased vagal tone and produced reflex slowing of the heart rate. Most studies of ANP in the dog have not found a significant effect of ANP on heart rate, and the effect observed in the present study was quite small, although statistically significant.

Chu et al\textsuperscript{19} pointed out that ANP produces effects that are similar to those of nitroglycerin, causing sustained vasodilation of epicardial and intramural arteries, but only modest transient dilation of the coronary resistance vessels. The present study extends this concept by demonstrating that, like nitroglycerin, ANP also causes vasodilation of moderately well-developed interarterial coronary collateral vessels. Clearly, the importance of these vascular effects depends on demonstration that they can occur at blood levels of ANP that are observed during physiological or pathological states. Using a dose of ANP midway between the two doses used in the present study (2.5 μg/kg i.v.), Kuehl et al\textsuperscript{22} reported that plasma levels 1 minute after drug administration were 6.9-fold greater than control measurements; plasma levels of ANP fell rapidly to return to baseline within 25 minutes. In four animals used in the present study, measurements of retrograde flow were continued for 12-18 minutes after administration of the second dose of ANP. During this interval, there was no tendency for the effect on retrograde flow to subside. During exercise in normal individuals ANP has been found to undergo a threefold increase,\textsuperscript{23} while the increased heart rate of supraventricular tachycardia has been reported to result in a 2.4- to 15-fold increase in plasma levels of ANP.\textsuperscript{24} It is likely that these values would equal the plasma levels of ANP present during the later observation period in the present study in which a persistent effect on retrograde flow was observed.

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References


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