Brief Communication

Effects of Atrial Natriuretic Peptide in the Canine Coronary Circulation

Robert J. Bache, Xue-Zheng Dai, Jeffrey S. Schwartz, and Da Guang Chen

Atrial natriuretic peptides have been reported to cause dose-dependent relaxation of isolated preconstricted vascular and intestinal smooth muscle strips and to inhibit vasoconstriction of vascular strips in response to norepinephrine or angiotensin II. However, Wangler et al., using Langendorff-perfused guinea pig hearts, reported that atriopeptin II caused intense and sustained dose-related coronary vasoconstriction that reduced coronary perfusion sufficiently to cause ischemic depression of myocardial function. In addition, these investigations included preliminary data from four blood perfused canine hearts in which atriopeptin II also produced coronary vasoconstriction. Because of potentially important physiologic and therapeutic implications of such an intense endogenous coronary vasoconstrictor, this study was undertaken to characterize the coronary vasomotor response to human atrial natriuretic peptide in the canine coronary circulation. Since we observed only coronary vasodilation in response to this material, additional studies were undertaken to determine whether vasodilation utilized adenosine- or prostaglandin-dependent mechanisms.

Materials and Methods

Studies were performed in 16 adult mongrel dogs weighing 19 to 29 kg. The animals were premedicated with morphine sulphate (1 mg/kg s.c.), anesthetized with α-chloralose (100 mg/kg i.v.), intubated, and ventilated with a respirator. Supplemental oxygen was administered to maintain the arterial Po₂ within the normal range. A left thoracotomy was performed in the fifth intercostal space and the heart suspended in a pericardial cradle. A PVC catheter, 3.0 mm o.d., was introduced into the left carotid artery and advanced into the ascending aorta. A similar catheter was positioned in the left ventricular cavity through a stab wound in the apical dimple and secured in place with a purse-string suture. After administration of heparin sodium, 700 U/kg, the proximal left circumflex coronary artery was cannulated with a thin-wall stainless steel cannula, 3.5 mm o.d., and perfused with blood from the right femoral artery with a peristaltic pump. Pressure at the cannula tip was measured with a small internal tube incorporated into the wall of the cannula. The frequency response of the coronary cannula pressure tube system was determined during the procedure using the free oscillation method. The damped natural frequency of the system for measurement of coronary pressure was 25 Hz with a damping factor of 0.32. Pump flow was adjusted so that pressure at the tip of the perfusion cannula was equal to mean arterial pressure, and thereafter, flow was maintained constant for the duration of the study. All dogs were hemodynamic-stable throughout the study so that coronary pressure remained stable without need for further adjustments of the coronary flow rate. Coronary flow rates ranged from 38 to 80 ml/min (mean 57 ± 6). Since cannula flow remained constant throughout the study, changes in perfusion pressure reflected changes in coronary vascular resistance. Aortic, coronary cannula, and left ventricular pressures were measured with Statham P23ID pres-
sure transducers. Left ventricular pressure was recorded at both normal and expanded scale for measurement of end-diastolic pressure. Left ventricular maximum \(\frac{dP}{dt}\) was obtained by electronic differentiation of the pressure signal. Since left ventricular pressure was measured with a fluid-filled catheter, changes in left ventricular \(\frac{dP}{dt}\) were used only to provide directional information. Hemodynamic data were recorded on a Hewlett-Packard Model 8800 direct writing oscillograph.

After completion of the surgical procedure, data were recorded continuously for 20–30 minutes to ensure that a stable hemodynamic state had been achieved before beginning the study. Dose-response curves were then obtained by administering atrial natriuretic peptide (ANP) 0.0002, 0.002, 0.02, 0.2, and 2.0 \(\mu g/kg\) into the coronary perfusion line. Each drug dosage was administered over a 5-second interval and the peak response observed; subsequent interventions were not performed until measurements had returned to their control values. Multiple dilutions of drug were prepared so that injection volumes did not exceed 0.4 ml. The response to a similar volume of vehicle was observed and subtracted from the response to active drug.

To determine whether \(\beta\)-adrenergic mechanisms were involved in the response to ANP, in eight dogs \(\beta\)-adrenergic blockade was produced with propranolol, 1.0 mg/kg i.v. The dose-response to intracoronary bolus dosages of ANP 0.0002 to 2.0 \(\mu g/kg\) was repeated beginning 10 minutes after administration of propranolol. The adequacy of beta-blockade was demonstrated by blockade of the response of heart rate and left ventricular \(\frac{dP}{dt}\) to isoproterenol, 1 \(\mu g/kg\) i.v.

To determine whether the coronary vasodilating action of ANP was dependent on an adenosine-mediated mechanism, in 16 dogs adenosine receptor blockade was produced by administration of 8-phenyltheophylline (Calbiochem). The 8-phenyltheophylline was administered in a dosage of 5 mg/kg i.v. dissolved in 3.0 ml of dimethylsulphoxide. The dose-response to intracoronary bolus dosages of ANP 0.0002 to 2.0 \(\mu g/kg\) was repeated beginning 10 minutes after administration of 8-phenyltheophylline. The adequacy of adenosine receptor blockade was demonstrated by examining the coronary vasodilator response to intracoronary bolus injections of 25 \(\mu g\) of adenosine before and after 8-phenyltheophylline.

To determine whether products of arachidonic acid metabolism participated in the vasodilator response to ANP, cyclooxygenase inhibition was produced by administration of indomethacin, 5 mg/kg i.v., in 16 dogs. Indomethacin was not administered until at least 1 hour after 8-phenyltheophylline, when the response to intracoronary adenosine had returned to the control level. The dose-response curve to intracoronary ANP was then repeated beginning 30 minutes after administration of indomethacin. In five dogs, the response to intracoronary arachidonic acid was examined before and after indomethacin. Arachidonic acid was dissolved in a solution of 10% ethanol in 100 mM Na\(_2\)CO\(_3\), under nitrogen. This solution was diluted with isotonic saline to a final concentration of 1 mg/ml and frozen under nitrogen for later use. The response to intracoronary administration of arachidonic acid (0.15-mg bolus) was examined before and after indomethacin.

Heart rate and all pressure measurements were obtained directly from the strip chart recordings. Data are presented as mean \(\pm\) SEM. Comparisons of hemodynamic data before and after each of the interventions were performed using analysis of variance testing. A probability of less than 0.05 was required for statistical significance. While a significant effect was found, individual comparisons were carried out using Duncan's procedure. Half of the dogs given 8-phenyltheophylline and indomethacin had previously received propranolol; consequently, data were first analyzed in two separate groups according to whether or not animals had previously received propranolol. Since there was no significant difference between these groups, the data were combined.

**Results**

Intracoronary administration of ANP in the dosages used in this study did not cause significant alterations of heart rate, aortic or left ventricular pressures, or the maximum left ventricular \(\frac{dP}{dt}\) (Table 1). As shown in Figure 1, ANP caused dose-dependent coronary vasodilation. The smallest dosage that produced a statistically significant coronary vasodilation was 2 ng/kg (9 \(\pm\) 2\% decrease in mean coronary resistance), while a dosage of 2 \(\mu g/kg\) caused a 27 \(\pm\) 4\% decrease in coronary resistance (Figure 2). The onset of coronary vasodilation occurred within 6–8 seconds after administration of ANP, while the peak response was observed 12–15 seconds after drug administration. These time intervals did not vary significantly with the dose of ANP. However, the duration of coronary vasodilation was directly related to the dose of ANP, ranging from 25 \(\pm\) 2.7 seconds at a dose of 0.0002 \(\mu g/kg\) to 142 \(\pm\) 13 seconds at a dose of 2.0 \(\mu g/kg\).

As shown in Table 2, propranolol, 1 mg/kg i.v., caused significant reductions in heart rate, arterial pressure, left ventricular \(\frac{dP}{dt}\), and coronary pressure. Propranolol reduced the increase in heart rate in response to isoproterenol, 1 \(\mu g/kg\) i.v., from 78 \(\pm\) 16 to 5 \(\pm\) 6 beats/min, and reduced the increase in left ventricular \(\frac{dP}{dt}\) from 2,300 \(\pm\) 480 to 30 \(\pm\) 190 mm Hg/sec (each \(p<0.01\)). As shown in Figure 2, \(\beta\)-adrenergic blockade with propranolol did not alter the vasodilator response to ANP. Administration of 8-phenyltheophylline produced no significant change of heart rate, aortic or left ventricular pressures, or left ventricular \(\frac{dP}{dt}\) (Table 2). However, 8-phenyltheophylline blocked adenosine-induced vasodilation, so that the reduction of coronary pressure in response to intracoronary adenosine, 25 \(\mu g\), decreased from 41 \(\pm\) 4.7 mm Hg during control conditions to 3.8 \(\pm\) 1.8 mm Hg after 8-phenyltheophylline (\(p<0.01\)). Despite effective adenosine receptor blockade, 8-phenyltheophylline did not alter the vasodilator response to ANP (Figure 3).
Hemodynamic Effects of Increasing Doses of Atrial Natriuretic Peptide in the Canine Heart

<table>
<thead>
<tr>
<th></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></td>
<td>Control ANP</td>
<td>Control ANP</td>
<td>Control ANP</td>
<td>Control ANP</td>
</tr>
<tr>
<td>ANP 0.0002 µg/kg</td>
<td>120±4</td>
<td>117±11</td>
<td>90±3</td>
<td>94±9</td>
</tr>
<tr>
<td>ANP 0.002 µg/kg</td>
<td>122±10</td>
<td>123±10</td>
<td>94±8</td>
<td>95±8</td>
</tr>
<tr>
<td>ANP 0.02 µg/kg</td>
<td>123±3</td>
<td>123±10</td>
<td>92±8</td>
<td>93±8</td>
</tr>
<tr>
<td>ANP 0.2 µg/kg</td>
<td>117±6</td>
<td>117±7</td>
<td>90±6</td>
<td>89±7</td>
</tr>
<tr>
<td>ANP 2 µg/kg</td>
<td>120±10</td>
<td>118±9</td>
<td>84±6</td>
<td>84±6</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Measurements were obtained during control conditions prior to each dose of ANP and at the time of maximal response of coronary pressure to ANP.

*p<0.05 in comparison with the respective control measurement.

ANP, atrial natriuretic peptide; LV, left ventricle.

Administration of indomethacin resulted in slight but significant increases of aortic pressure and left ventricular systolic pressure (Table 2). Indomethacin decreased the vasodilator response to intra-arterial arachidonic acid; during control conditions, administration of arachidonic acid caused a 60±6% peak decrease of coronary vascular resistance, but only a 16±6% decrease after indomethacin (p<0.05). However, as shown in Figure 3, indomethacin did not alter coronary vasodilation in response to ANP.

**Discussion**

In the present study, ANP produced dose-related coronary vasodilation in every dog studied. This is in contrast to the study of Wangler and associates, who reported that atriopeptin II caused sustained dose-related coronary vasoconstriction in isolated guinea pig hearts perfused with Krebs-Henseleit buffer. The coronary vasoconstriction observed by these investigators appeared to override normal metabolic mechanisms for control of coronary blood flow and caused depression of left ventricular systolic pressure and dp/dt. The observed decrease in left ventricular systolic function was the result of ischemia and not a direct effect of ANP, since when a constant flow system was used to prevent a decrease in coronary flow during atriopeptin II–induced vasoconstriction, left ventricular dp/dt did not decrease and the reduction of left ventricular developed pressure was much less marked. In preliminary data from four blood-perfused dog hearts, these investigators also reported coronary vasoconstriction in response to atriopeptin II. Several factors could have contributed to the difference in results obtained by Wangler et al and the present study, including a difference in the peptide administered, differences in the dosages used, or differences in responsiveness of...
the coronary vasculature of the experimental preparations. Each of these differences will be discussed below.

Atrial peptides having natriuretic and vasorelaxant properties have been isolated, purified, and amino acid sequences determined. Several investigators have examined the structure-activity relations of ANP-related peptides cleaved either at the N-terminal or at the C-terminal end. The 25-amino acid peptide isolated from human atrium and used in the present study differs from the 23-amino acid rat peptide, atriopeptin II, used by Wangler et al, since atriopeptin II lacks the N-terminal arginine at the 102 position and C-terminal tyrosine at the 126 position. Garcia et al reported that N-terminal truncation to remove arginine from the 102 position only slightly reduced the potency of ANP to relax rabbit aortic strips preconstricted with norepinephrine. However, cleaving the C-terminal tyrosine decreased the vasorelaxant potency by approximately half. These results suggest that vasorelaxant effect of atriopeptin II used by Wangler et al would be less than human atrial natriuretic peptide used in the present study. However, the lesser vasodilator potency of atriopeptin II does not appear adequate to explain the directionally opposite results between Wangler et al and the present study.

Previous in vitro studies have demonstrated that the vasorelaxant effect of ANP is dependent upon preconstriction of the vascular smooth muscle. Similarly, Camargo et al., using the isolated functioning rat kidney, found that the initial level of vascular resistance importantly influenced the subsequent vasomotor response to atrial extract. When the renal vasculature was initially vasoconstricted with endogenously generated angiotensin II, ANP produced a marked decrease of renal vascular resistance. However, when the kidney was perfused in the absence of vasoconstrictor, so that initial renal vascular resistance was low, atrial extract caused vasoconstriction. It is possible that in an analogous manner, ANP causes vasodilation in the normally antiregulating coronary system that has a high level of basal vascular tone, but could produce vasoconstriction in the coronary system that was vasodilated by perfusion with hemoglobin-free oxygen-poor perfusate. This could, at least in part, explain the difference in results between Wangler et al and the present study.

In addition to differences in the specific atrial peptide and experimental preparation used, there were substantial differences in the dosage of ANP employed between the present study and the report of Wangler et al. Thus, Wangler et al used concentrations of atriopeptin II in the coronary perfusate ranging from 1 to 100 nmol, while in the present study dosages ranged from 70 fmol/kg to 3.4 nmol/kg. Using a specific radioimmunoassay, Camargo et al reported a mean value of 156 fmol/ml of ANP in rat aortic blood. It is possible that at the much higher concentrations of ANP used by Wangler et al responses could occur that would not be observed at physiologic concentrations.

Table 1. Continued

<table>
<thead>
<tr>
<th>LV dP/dt (mm Hg/sec)</th>
<th>Coronary pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>ANP</td>
</tr>
<tr>
<td>3,160 ± 170</td>
<td>2,770 ± 370</td>
</tr>
<tr>
<td>3,370 ± 440</td>
<td>3,420 ± 490</td>
</tr>
<tr>
<td>3,350 ± 460</td>
<td>3,420 ± 500</td>
</tr>
<tr>
<td>3,010 ± 330</td>
<td>3,070 ± 310</td>
</tr>
<tr>
<td>2,950 ± 300</td>
<td>2,880 ± 320</td>
</tr>
</tbody>
</table>

Table 2. Responses to Propranolol, 8-Phenyltheophylline, and Indomethacin in Canine Heart

<table>
<thead>
<tr>
<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>
<th>LV dP/dt (mm Hg/sec)</th>
<th>Coronary pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>125 ± 6</td>
<td>92 ± 5</td>
<td>114 ± 5</td>
<td>5.1 ± 1.6</td>
<td>2,580 ± 270</td>
</tr>
<tr>
<td>Propranolol</td>
<td>101 ± 5*</td>
<td>79 ± 5*</td>
<td>100 ± 4*</td>
<td>6.6 ± 1.1</td>
<td>1,870 ± 200*</td>
</tr>
<tr>
<td>Control</td>
<td>106 ± 7</td>
<td>84 ± 5</td>
<td>103 ± 5</td>
<td>5.4 ± 2.1</td>
<td>2,290 ± 140</td>
</tr>
<tr>
<td>8-Phenyltheophylline</td>
<td>101 ± 6</td>
<td>85 ± 3</td>
<td>102 ± 4</td>
<td>5.8 ± 2.4</td>
<td>2,260 ± 80</td>
</tr>
<tr>
<td>Control</td>
<td>91 ± 5</td>
<td>84 ± 6</td>
<td>102 ± 7</td>
<td>6.0 ± 1.8</td>
<td>2,130 ± 170</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>90 ± 5</td>
<td>91 ± 7*</td>
<td>109 ± 8*</td>
<td>6.6 ± 2.1</td>
<td>2,350 ± 150</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *p<0.05 in comparison with the corresponding control value.
pressure, suggesting that indomethacin abolished tonic
cyclooxygenase inhibition did not alter ANP-induced
vasodilation of rabbit aortic strips.2 Of interest was
agreement with previous in vitro studies in which
attenuation of the coronary vasodilation produced by
intra-arterial arachidonic acid. These findings are in
block cyclooxygenase, as demonstrated by marked
attenuation of the coronary vasodilation produced by
ANP, indicating that ANP did not cause coronary
vasodilation through an adenosine-dependent mecha-
nism. Adenosine blockade was produced with 8-
phenylethylphendrine since this agent has been found to
be a highly specific adenosine receptor blocker that is
approximately 100 times more potent than theophyl-
line.14 The adequacy of adenosine receptor blockade
was demonstrated by complete abolition of coronary
vasodilation in response to a dosage of adenosine that
produced a similar degree of vasodilation to the largest
dosage of ANP used.

Acknowledgments

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provided by Dr. Rodney W. Lappe of Wyeth Labora-
tories, Inc. The authors wish to acknowledge the expert
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Maak T: Ca-dependent hemodynamic and natriuretic effect of
vasodilator prostaglandin release. This finding has
previously been reported in open-chest dogs, but not in
chronically instrumented awake dogs, and may be
related to manipulation of the coronary artery during
surgical preparation.12

Agents such as dipyridamole and lidoflazine, which
interfere with cellular reuptake of adenosine released
into interstitial fluid, cause coronary vasodilation. These
adenosine-dependent mechanisms for coronary
vasodilation are blocked by adenosine receptor antag-
onsists such as theophylline.13 In the present study, the
adenosine receptor antagonist 8-phenylethylphendrine
did not inhibit the coronary vasodilation produced by
ANP, indicating that ANP did not cause coronary
vasodilation through an adenosine-dependent mecha-
nism. Adenosine blockade was produced with 8-
phenylethylphendrine since this agent has been found to
be a highly specific adenosine receptor blocker that is
approximately 100 times more potent than theophyl-
line.14 The adequacy of adenosine receptor blockade
was demonstrated by complete abolition of coronary
vasodilation in response to a dosage of adenosine that
produced a similar degree of vasodilation to the largest
dosage of ANP used.

few previous studies are available in which the
response of coronary flow to ANP was measured.
Sweet et al10 administered the 26-amino acid ANP to
closed-chest dogs in which acute left ventricular failure
had been produced by coronary artery embolization
with 50-μm plastic microspheres. ANP infused intra-
venously at a rate of 100 or 200 pmol/kg/min caused
an increase of cardiac output with no change in arterial
pressure but a significant reduction of coronary vas-
cular resistance with an increase in myocardial blood
flow. In contrast, Breuhaus et al, using chronically
instrumented awake sheep with normal left ventricular
function, found that atriopeptin II in a dosage of 0.1
mmol/kg/min caused a significant reduction of cardiac
output. These investigators suggested that the decrease
of cardiac output produced by atriopeptin II was in part
related to depression of myocardial function secondary
to coronary vasoconstriction, although direct measure-
ments of coronary blood flow were not obtained.

In the present study the response to ANP was not
altered by β-adrenergic blockade with propranolol,
indicating that ANP-induced coronary vasodilation did
not occur as the result of either release of endogenous
catecholamines or stimulation of coronary or myocar-
dial β-adrenoceptors. This is in agreement with the
finding that ANP did not exert either a chronotropic or
inotropic effect on the heart. In addition, indomethacin
did not inhibit coronary vasodilation caused by ANP.
The dosage of indomethacin used was sufficient to
block cyclooxygenase, as demonstrated by marked
attenuation of the coronary vasodilation produced by
intra-arterial arachidonic acid. These findings are in
agreement with previous in vitro studies in which
cyclooxygenase inhibition did not alter ANP-induced
vasorelaxation of rabbit aortic strips.2 Of interest was
the finding that indomethacin caused a significant
increase of arterial pressure and coronary perfusion
pressure, suggesting that indomethacin abolished tonic

FIGURE 3. Effects of adenosine receptor blockade with 8-
phenylethylphendrine and cyclooxygenase inhibition with indo-
methacin on the vasodilator response to intracoronary adminis-
tration of ANP.


Key Words • atrial natriuretic factor • coronary vasodilation • methylxanthines • indomethacin
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