Effects of Atrial Natriuretic Peptide on Proximal Epicardial Coronary Arteries and Coronary Blood Flow in Conscious Dogs

Alan Chu and Frederick R. Cobb

The effects of atrial natriuretic peptide (ANP) on proximal epicardial coronary artery dimensions and coronary blood flow were examined in 7 awake dogs chronically instrumented with miniature coronary dimension crystals and Doppler flow probes on the circumflex coronary artery. ANP (10, 50, and 150 μg) was infused as a bolus via the left atrial catheter. Aortic pressure, left ventricular end-diastolic pressure, heart rate, and dP/dt did not change significantly with any dose of ANP. ANP caused transient (1–5 minutes) dose-related increases in coronary blood flow; maximum increases were 28.1 ± 6.9%, 40.2 ± 6.2%, and 73.9 ± 12.5% with the 10-, 50-, and 150-μg doses, respectively. ANP also induced prolonged (average 70.2 ± 28.6 minutes with 150-μg dose) dose-related increases in coronary diameter; maximum increases were 3.1 ± 1.0%, 3.9 ± 1.5%, and 5.7 ± 1.3% with the 10-, 50-, and 150-μg doses, respectively. The increase in diameter was not attenuated when the transient increase in blood flow was prevented by partial occlusion with a pneumatic snare. Combined autonomic blockade with propranolol (1 mg/kg), phentolamine (1 mg/kg), and atropine (0.06 mg/kg) attenuated the relative increase in coronary flow but did not alter the increases in epicardial coronary diameter produced by ANP. These data demonstrate that bolus injection of ANP effects preferential, sustained, dose-dependent, flow-independent increases in epicardial coronary dimensions and relatively brief dose-dependent increases in coronary blood flow. The vasodilator effects of ANP on epicardial vessels are direct and are not mediated via the autonomic nervous system. The effects of ANP on the coronary vasculature are similar to those of nitrates, although the effects of ANP on epicardial vessel dimension are more gradual and more sustained. (Circulation Research 1987;61:485–491)

DeBold et al. demonstrated in 1981 that intravenous infusions of extracts derived from atrial muscle caused a profound natriuretic and diuretic effect and mild systemic hypotension in anesthetized rats. Subsequent studies by Currie et al. showed that atrial extracts caused dose-dependent relaxation of rabbit aortic strips previously contracted with norepinephrine. While the potential role of atrial natriuretic peptide (ANP) in maintaining body fluid and electrolyte homeostasis has since been extensively examined, its role in vasoreactivity, especially in the coronary vasculature, has not been conclusively elucidated.

In vitro studies using vascular strips indicate that the vasoactive effects of ANP vary in different organ systems and species. Garcia et al. found that partially purified ANP produced dose-dependent relaxation of aortic and renal but not mesenteric artery strips from rabbits and rats that were contracted with either norepinephrine or angiotensin. Ishihara et al. measured vascular responses in isolated precontracted canine arterial strips from the heart, brain, kidney, and limbs and concluded that the vasodilator properties of ANP were relatively specific for the renal artery. Rapoport et al. observed that ANP induced relaxation in precontracted denuded proximal coronary artery rings from explanted hearts of patients.

The reports on the effects of ANP infusion on systemic hemodynamics and regional perfusion in intact animals have been conflicting. Pegram et al. observed that intravenous infusion of partially purified ANP in rats 3 hours after recovery from ether anesthesia caused an immediate decrease in arterial pressure and cardiac output, and blood flow to the heart, brain, kidney, skin, muscle, and mesenteric regions. Lappe et al. also observed that ANP infusion in conscious rats caused dose-dependent decreases in arterial pressure, marked decreases in blood flow to the kidney, mesentery, and limb, and increases in total and regional vascular resistance. It was concluded that the decreases in cardiac output resulted from venodilation with decreased venous filling since central venous and left atrial pressures decreased during the infusion. The increase in resistance was thought to be mediated via reflex mechanisms secondary to decreased cardiac output. Kleinert et al. observed in conscious and anesthetized dogs that ANP caused a decrease in arterial pressure, an initial decrease and then subsequent increase in total peripheral resistance, a sustained decrease in cardiac output and dp/dt, but no change in pulmonary capillary wedge pressure or central venous pressure. Studies by Ishihara et al. in anesthetized dogs...
and Hintze et al\textsuperscript{a} in conscious dogs measured blood flow to multiple organ systems, including the heart, brain, mesentery, limb, and kidneys, and concluded that the vasodilation properties of ANP were relatively specific for the renal arteries. In isolated, Langendorff-perfused guinea pig hearts, intracoronary infusion of ANP caused potent coronary vasoconstriction and severe reductions in left ventricular pressure and dP/dt.\textsuperscript{10} In contrast, Garcia et al,\textsuperscript{10} using radioactive microspheres in conscious rats to evaluate regional blood flow, found that intraventricular injections of ANP caused greater relative increases in blood flow to the heart, lungs, and spleen than to the kidneys with no change in cardiac output. Thus, significant controversy remains concerning the effects of ANP on the coronary vasculature. Studies have not examined the in vivo effects of ANP on the epicardial coronary vessels.

The present study was designed to evaluate the effects of ANP on the epicardial coronary arteries and on phasic coronary blood flow. The studies were performed before and after combined \( \alpha \), \( \beta \), and cholinergic blockade to determine reflex or autonomic influences on the responses. Conscious chronically instrumented dogs were used to eliminate the variables of acute surgery and anesthesia.

Materials and Methods

Nine mongrel dogs (30–35 kg), previously screened for the absence of anemia and infection, underwent left thoracotomy in the fourth intercostal space after intravenous thiopental sodium anesthesia (60–80 mg/kg). The heart was suspended in a pericardial cradle. Heparin-filled polyvinyl catheters were inserted into the left atrium via the left atrial appendage, into the left ventricular cavity via the apex, and into the ascending aorta via the left internal thoracic artery. A 0.5–1-cm segment of the left circumflex coronary artery just distal to the atrial appendage was dissected free. Care was taken to ensure minimal dissection of the vessel. A pair of 7-MHz-piezoelectric crystals (1.5 \( \times \) 2.5 mm, 15–20 mg), insulated with Insl-X (Insl-X Products, Yonkers, N.Y.) and attached to a Dacron backing, were sutured to the adventitia on opposite surfaces of the dissected coronary segment using 6-0 prolene (Ethicon, Inc., Somerville, N.J.) and attached to a Dynacon monitor (Dynacon Instruments, Inc., Belmont, Calif.) and oscilloscope monitoring (Tektronix 2215 A, Beaverton, Oregon). A 10-MHz cuff-type pulse Doppler flow probe was implanted distal to the crystals in 3 dogs. An inflatable balloon snare was also placed distal to the flow probe. One additional dog was not implanted with crystals but had a flow probe and a balloon snare on the circumflex artery. All branches arising from the circumflex artery between the crystals and the snare were carefully ligated. The catheters, wires, and tubings were tunneled to a subcutaneous pouch at the base of the neck.

After a 7–14-day recovery period, the catheters and wires were exteriorized under local lidocaine infiltration anesthesia. The dogs were sedated lightly with intramuscular morphine sulfate (0.3–0.5 mg/kg), allowed to accommodate to the laboratory environment for 60 to 90 minutes, and studied while loosely restrained and lying awake on the right side in a dimly illuminated laboratory. Aortic pressure, left ventricular end-diastolic pressure, dP/dt, external coronary diameter, electrocardiogram, and coronary flow were monitored and recorded continuously throughout the study. The fluid filled catheters and pressure gauges (Statham P23Db) were optimally damped with an attached Corrector device (Norton Health Care Products, Akron, Ohio). Any drift in the pressure and dimension measurements was minimized by frequent repeat calibrations. Nitroglycerin (NTG), 0.4 mg, was infused via the left atrial catheter on a day prior to subjecting the dog to the study protocol in order to ensure a responsive vasculature. Dilations \(<3\%\) were considered inadequate, and those dogs \((n = 2)\) were excluded from the study. None of the dogs implanted with flow probes was excluded. The same dose of NTG was infused at the end of each study day to ensure comparable day-to-day vasoreactivity.

ANP (rat, 28-amino acid, Peninsula Laboratories, Inc., Belmont, Calif.) was dissolved in saline and infused as a bolus via the left atrial catheter. Three different doses (10, 50, and 150 \( \mu g \)) were infused in each of the 7 study dogs. A minimum of 120 minutes was allowed between injections to ensure clearance of the effects of ANP and to allow recovery of baseline coronary dimensions and hemodynamics. Repeat injections were given on different days to ensure reproducible vasoresponsiveness.

To eliminate influences from autonomic reflexes on the vasoreactive response, ANP infusions (150 \( \mu g \)) were repeated in all 7 dogs after \( \alpha \), \( \beta \), and cholinergic blockade with phentolamine (1 mg/kg), propranolol (1 mg/kg), and atropine (0.06 mg/kg), respectively. Adequate blockade was verified by appropriate challenges with \( \alpha \) and \( \beta \) agonists and acetylcholine. In the 4 dogs implanted with flow probes and inflatable balloon snares, ANP infusions (150 \( \mu g \)) were repeated at constant flow controlled by partial occlusion of the balloon snare to determine whether flow contributed to the vasodilation.\textsuperscript{12,13}

Vasoactive changes were expressed as percent change from baseline dimensions. Statistical analysis was performed using Student’s paired \( t \) test.

Results

Figure 1 is a representative recording of the effects of ANP infused as a 150-\( \mu \)g bolus into the left atrium on epicardial coronary dimension, coronary blood flow, and hemodynamics. ANP infusion did not significantly change aortic pressure, left ventricular end-diastolic pressure, heart rate, or dP/dt in the group (Table 1A). In 2 dogs, the maximum dose of ANP decreased aortic pressure by \( \leq 10\% \). Each dose of ANP produced transient significant increases in coronary blood flow in each dog that started 3–10 seconds after infusion and lasted 1–5 minutes (Figure 1). The effects of ANP on coronary blood flow were dose dependent.
ANP-induced coronary dilation in conscious dogs

**Figure 1.** Representative recording of epicardial coronary dimension changes, coronary flow changes, and hemodynamic effects after infusion of 150 μg ANP into left atrium. ANP caused sustained potent epicardial coronary dilation and transient coronary flow increase without significant change in aortic pressure, heart rate, or dP/dt.

Maximum increases in blood flow were 28.1 ± 6.9%, 40.2 ± 6.2%, and 73.9 ± 12.5% with the 10-, 50-, and 150-μg doses, respectively (Figure 2).

ANP caused dose-dependent increases in epicardial coronary diameter; maximum increases were 3.1 ± 1.0%, 3.9 ± 1.5%, and 5.7 ± 1.3% with the 10-, 50-, and 150-μg doses, respectively (Figure 3). In contrast to the immediate but relatively brief increase in blood flow, ANP produced a more delayed and more sustained increase in vessel dimension. The onset of epicardial vasodilation occurred 15–30 seconds after injection and gradually increased to reach peak dilation in 3 to 10 minutes. Epicardial vasodilation persisted for 16.4 ± 4.7 minutes (range 10–22 minutes) after the 10-μg dose and for 70.2 ± 28.6 minutes (range 40–120 minutes) after the 150-μg dose. The peak dilation was maintained usually for greater than two thirds of the duration of the dilation. The effects of ANP on epicardial dimension were not significantly changed by preventing the initial increase in blood flow by partial occlusion with the balloon snare (Figures 4 and 5).

Combined α, β, and cholinergic blockade increased rest heart rate from 68 ± 12/min before to 148 ± 32/min after blockade but did not change aortic or left ventricular end-diastolic pressures or dP/dt (Table 1B). Rest coronary blood flow (expressed in Doppler shift units) increased 45.3 ± 11.9% after blockade (from 2.2 ± 1.1 kHz to 3.2 ± 1.5 kHz). However, epicardial coronary diameter did not change significantly: 3.74 ± 0.33 mm before and 3.75 ± 0.47 mm after blockade. ANP infusion (150 μg) produced comparable changes in epicardial coronary diameter before and after autonomic blockade (Figure 6), but the effect on coronary blood flow was less (maximum increase 73.9 ± 12.5% before vs. 24.4 ± 12.9% after blockade, p < 0.05). The maximal mean flow achieved after infusion of ANP (150 μg), however, was not significantly different before blockade (3.9 ± 2.1 kHz before vs. 3.9 ± 1.4 kHz after). ANP infusion after combined autonomic blockade did not significantly affect heart rate, aortic or left ventricular end-diastolic pressure, or dP/dt (Table 1B).

The effects of ANP on coronary blood flow and coronary dimensions were similar to those produced by bolus injection of NTG, but they differed in several respects. NTG (0.4 mg) caused a transient significant

**Figure 2.** Relation between peak increase in coronary flow (expressed as percent of resting flow) and dose of ANP infused. Increase in flow is significant in each dose when compared with saline control.

**Table 1.** Hemodynamic Data in All 7 Dogs Before and After 150 μg ANP

<table>
<thead>
<tr>
<th></th>
<th>A. No autonomic block</th>
<th>B. After autonomic block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before ANP</td>
<td>After ANP</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>102 ± 12</td>
<td>103 ± 11</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td>10.6 ± 2.0</td>
<td>10.3 ± 1.7</td>
</tr>
<tr>
<td>Left ventricular dP/dt (mm Hg/sec)</td>
<td>2,186 ± 410</td>
<td>2,210 ± 435</td>
</tr>
<tr>
<td>Heart rate (beat/min)</td>
<td>68 ± 12</td>
<td>69 ± 13</td>
</tr>
</tbody>
</table>

*p < 0.05 when compared to no autonomic blockade.
Figure 3. Relation between peak increase in epicardial coronary diameter (expressed as percent of resting diameter) and dose of ANP infused. Increase in diameter is significant in each dose when compared with saline control.

decrease in arterial pressure and increase in heart rate (Figure 7). Both agents caused rapid but relatively brief increases in coronary blood flow, i.e., 1–5 minutes, and sustained increases in epicardial dimension that were not significantly affected by preventing the increase in blood flow. As with ANP, NTG caused less increase in flow after autonomic blockade (maximum increase 162.2 ± 25.9% before vs. 99.5 ± 42.0% after), but the maximum mean flow achieved was not significantly altered (5.2 ± 3.4 kHz before vs. 5.3 ± 2.3 kHz after). The increase in epicardial coronary diameter with the dose of NTG used was slightly greater than with the maximum dose of ANP (150 μg), 7.4 ± 2.6% vs. 5.7 ± 1.3%, respectively. Compared with ANP, NTG effected a more rapid onset of epicardial coronary dilation (<5 seconds vs. 15–30 seconds) and achieved maximal epicardial dilation earlier (1–2 minutes vs. 3–10 minutes). Despite the absence of systemic hemodynamic effects and smaller increases in coronary flow, ANP (150 μg) produced epicardial vasodilation for a significantly longer period than NTG (0.4 mg): 70.2 ± 28.6 minutes, range 40–120 minutes vs. 23.0 ± 4.5 minutes, range 20–30 minutes.

Discussion

The new data provided by this study demonstrate that ANP bolus infusion in the conscious dog produces 1) sustained, dose-dependent vasodilation of the proximal coronary vasculature that is independent of blood flow increases and 2) relatively brief, dose-dependent increases in coronary blood flow that occur in the absence of changes in heart rate, blood pressure, left ventricular end-diastolic pressure, or dP/dt. The preferential dilation of the epicardial vessels was demonstrated not only by the absence of attenuation of the epicardial vasodilation when flow was restricted but also by epicardial vasodilation that was sustained for more than 1 hour after blood flow had returned to baseline with peak vasodilation lasting approximately two thirds of the vasodilation period.

Figure 4. Representative recording of coronary diameter changes and hemodynamic effects after infusion of ANP (150 μg) into left atrium of the same dog in Figure 1 in the presence of controlled constant flow. Abolition of flow increase did not alter peak epicardial vasodilation effect.
NTG. Sustained increases in epicardial dimensions that coronary vasculature was similar to that observed with (expressed as percent resting diameter) before and after FIGURE 6. ANP (150 μg) induced epicardial coronary dilation of the resistant coronary arteries. Combined blockade had a similar effect on the response to NTG; epicardial coronary response to ANP. Combined autonomic blockade with propranolol (1 mg/kg), phentolamine (1 mg/kg), and atropine (0.06 mg/kg). Autonomic blockade did not significantly alter ANP-induced vasodilation.

Combined α, β, and cholinergic blockade did not change the epicardial vasodilation produced by ANP, indicating that vasodilation was not mediated or influenced by autonomic mechanisms. Combined autonomic blockade significantly increased heart rate and resting coronary blood flow and reduced the relative increase but not the maximum blood flow achieved in response to ANP. Combined blockade had a similar effect on the response to NTG; epicardial coronary dilation and maximum blood flow were not changed, but the relative increase in blood flow was reduced. The attenuation of the relative increase in blood flow may have been secondary to increased resting flow as a result of increased heart rate and reduced resting tone of the resistant coronary arteries.

The preferential effect of ANP on the proximal coronary vasculature was similar to that observed with NTG. Sustained increases in epicardial dimensions that were independent of blood flow changes and relatively brief increases in coronary blood flow were produced by both agents. The dosage of NTG used caused a variable decrease in blood pressure and significant increases in heart rate that would be expected to increase myocardial metabolic needs and blood flow secondarily. Although studies have demonstrated that NTG also effects direct vasodilation of the distal vasculature, it is believed that NTG is a more selective dilator of the large rather than small coronary arteries. ANP produced increases in coronary blood flow without changing heart rate, blood pressure, left ventricular end-diastolic pressure, or dP/dt, indicating that the flow increases were not due to increases in major factors that increase myocardial metabolism and secondarily increase myocardial blood flow. These observations support the view that ANP has direct vasodilation effects on the distal coronaries. It has been suggested that ANP and nitrates may act via certain common mechanisms. Winquist et al observed that ANP and nitroprusside demonstrate similar vasodilator profiles. In addition, cyclic guanosine monophosphate (cGMP) formation has been correlated in vitro with smooth muscle relaxation following both ANP and nitrates. However, Rapoport et al have observed that although both ANP and nitroprusside increase cGMP in rat aortic strips, a variety of agents, including methylene blue, reducing agents, and cyanide, inhibit the elevated cGMP levels in response to nitroprusside but not ANP. Their findings are consistent with the belief that nitrates act via soluble guanylate cyclase, whereas ANP acts via the particulate enzyme. Although this study was not designed to provide quantitative comparisons of the effects of NTG and ANP on epicardial dimensions and coronary blood flow, several important differences in the epicardial vasodilation responses to NTG and ANP were apparent. Compared with NTG, ANP effected a more delayed onset of epicardial coronary dilation (13-30 seconds vs. <5 seconds) and a more delayed maximum epicardial dilation (3-10 minutes vs. 1-2 minutes). Since NTG, but not ANP, infusion resulted in tachycardia and reflex increases in dP/dt, the enhanced early metabolic drive and the accompanying further increase in coronary flow may contribute to the earlier onset of epicardial coronary dilation; however, peak increase in coronary flow occurred prior to reflex changes. Despite the smaller increase in coronary flow and epicardial coronary diameter, a much longer duration of vasodilation was observed after ANP infusion (70.2 ± 28.6 minutes vs. 23.0 ± 4.5 minutes). These differences suggest that although NTG and ANP may act through some common pathway, there may be certain differences in the basic mechanism of vasodilation in the intact awake animal. The sustained epicardial coronary dilation seen after ANP in the absence of other hemodynamic changes also suggests that ANP may be a more selective epicardial coronary vasodilator than NTG.

There have been conflicting reports on the effects of ANP infusion on systemic hemodynamics and coro-
Aortic mmHg

Mean Aortic Pressure mmHg

Coronary Diameter mm

Mean Coronary Diameter mm

Coronary Flow KHz

Coronary Flow KHz
dP/dt mmHg/sec

Left Ventricular Pressure mmHg

Nitroglycerin 0.4 mg

FIGURE 7. Representative recording of coronary flow changes, epicardial coronary diameter changes, and hemodynamic effects after infusion of nitroglycerin (0.4 mg). Nitroglycerin caused an immediate increase in flow and epicardial coronary dilation.

In contrast to the absence of hemodynamic effects of bolus injections of ANP (10–150 µg, approximately 0.3–5.0 µg/kg) in the present study, studies by Lappe et al in conscious rats (ANP dose 0.25–4.0 µg/kg i.v.), Pegram et al in rats 3 hours after recovery from anesthesia (partially purified atrial extracts), and Kleinert et al in conscious dogs (ANP dose 3.0 µg/kg followed by 0.3 µg/min/kg i.v.) observed that ANP decreased arterial pressure and cardiac output. Pegram et al also observed decreases in coronary blood flow. Ishihara et al, using anesthetized dogs, observed that ANP (0.1–1.0 µg/kg i.v.) caused dose-dependent decreases in arterial pressure and relatively specific increases in renal blood flow but no significant change in coronary blood flow. However, Hiitze et al, using conscious dogs, observed that ANP (5 µg/kg i.v.) did not significantly affect heart rate, arterial pressure, or coronary blood flow but did significantly increase renal blood flow (27%); they also concluded that ANP was a selective renal vasodilator.

In isolated, Langendorff-perfused guinea pig hearts, Wangler et al observed severe reductions in coronary flow, left ventricular pressure, and dP/dt when ANP was infused into the coronary arteries. In contrast, Garcia et al, using conscious rats and radioactive microspheres, observed that ANP caused greater increases in blood flow to the heart than to the kidney. A variety of factors may have contributed to differences between previous reports and the present study, including species differences; different routes of administration, i.e., intravenous versus injection into the left heart; different study conditions such as isolated, perfused organ system versus intact animal system; acute surgery and anesthesia versus nonanesthesia; and different types and potencies of ANP. Although the dose range in the present study was comparable to that in most studies, the 28-amino acid ANP used has been reported to be of higher potency. In the present study, the maximum dose of ANP caused a slight reduction in aortic pressure in 2 dogs; it is possible that larger doses of ANP would have affected systemic hemodynamics. It is also possible that intravenous infusions and passage through the pulmonary circulation may decrease the coronary effects of ANP since coronary vasodilation was observed in this investigation and in the study by Garcia et al who infused ANP into the left ventricle. In preliminary studies in anesthetized dogs, Bache et al have observed decreases in coronary vascular resistance after intracoronary injection of ANP. Bauman et al also demonstrated increases in coronary blood flow following intracoronary injection of ANP in open-chest anesthetized dogs.

In summary, this study demonstrates that ANP causes direct, sustained, dose-dependent vasodilation.
of the proximal coronary vasculature that is independent of blood flow changes and autonomic reflexes. A relatively brief, dose-dependent increase in coronary blood flow which occurs in the absence of other hemodynamic changes has also been demonstrated. The preferential epicardial vasodilation of this endogenous vasodilator is similar to that observed with NTG but is more delayed and more sustained.

Acknowledgments

We wish to thank Joseph C. Greenfield, Jr., M.D., for his continuing support; Paul Klotman, M.D., for supplying the atrial natriuretic peptide; Adrienne Stakeley and Michael Miller for their technical assistance; Kirby Cooper for surgical assistance; the Durham VA Medical Media Production Service for their help with illustrations; and Cathie Collins for her expert secretarial assistance.

References


KEY WORDS • atrial natriuretic peptide • epicardial coronary dilation • coronary flow • autonomic nervous system • nitroglycerin
Effects of atrial natriuretic peptide on proximal epicardial coronary arteries and coronary blood flow in conscious dogs.

A Chu and F R Cobb

doi: 10.1161/01.RES.61.4.485

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/61/4/485

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/