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Atrial Natriuretic Peptide Decreases Cardiac Output Independent of Coronary Vasoconstriction (42619)

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Abstract. Studies were performed in isolated, Langendorff-perfused rat hearts and anesthetized dogs to determine the effects of synthetic atrial natriuretic peptide (ANP 8-33) on the coronary circulation. *In vitro* studies in the rat examined coronary flow dynamics to ANP 8-33 over a defined range from physiologic to pharmacologic concentrations. No changes in coronary flow or chronotropic and inotropic function of the isolated Langendorff-perfused heart were observed in response to increasing concentrations of ANP 8-33 (10^2 to 10^6 pg/ml). In the dog, a low, nonhypotensive dose of ANP 8-33 (0.05 μg/kg/min) decreased cardiac output with no change in coronary blood flow or coronary vascular resistance. At a high, hypotensive dose (0.3 μg/kg/min) ANP 8-33 decreased cardiac output in association with transient coronary vasodilation. Continued infusion resulted in a decrease in coronary blood flow and arterial pressure with no change in coronary vascular resistance. Thus, *in vitro* physiologic and pharmacologic concentrations of ANP, or *in vivo* low concentrations of ANP, do not result in an alteration in coronary flow. *In vivo* ANP 8-33, at both nonhypotensive and hypotensive concentrations, decreased cardiac output in the absence of coronary vasoconstriction.

Atrial natriuretic peptide (ANP) is a hormone of cardiac origin which, in addition to stimulating natriuresis and diuresis, also possesses significant hemodynamic and vasoactive properties. Administration of ANP *in vivo* has consistently lowered arterial blood pressure (1–4). The mechanism of this reduction in arterial pressure is secondary to a decrease in cardiac output with an increase in systemic vascular resistance. These findings are consistent with a depression of venous return and/or a direct action of ANP on the heart, i.e., negative inotropic and/or chronotropic action (5–9). Recent studies by Wangler *et al.* (10) have reported that ANP is a potent coronary vasoconstrictor which may contribute to the reduction of cardiac output. In isolated, Langendorff-perfused guinea pig heart, atriopeptin II 101–126 resulted in a marked coronary vasoconstriction; this vasoconstrictor response was observed as well in the rat heart and in an anesthetized blood perfused dog heart preparation. These investigators speculated that elevated cardiac interstitial ANP concentrations could occur under physiologic conditions during increased release of ANP and result in a reduction in coronary blood flow and cardiac inotropic function with a subsequent decrease in cardiac output. In contrast, in one conscious dog, a bolus injection of ANP failed to alter coronary blood flow or coronary vascular resistance (11). Bache *et al.* (12) have reported in preliminary studies in the conscious dog that ANP may result in coronary vasodilatation. Thus, the role of ANP in mediating a reduction in cardiac output via coronary vasoconstriction remains unclear. The primary aim of the present study was to define *in vivo* in the anesthetized dog the effect of ANP 8-33 on coronary blood flow at concentrations which were associated with decreases in cardiac output. *In vitro* studies were undertaken in the isolated rat heart to assess the direct action of ANP 8-33 on coronary flow and inotropic function at both physiologic and pharmacologic concentrations.

Materials and Methods. *Anesthetized dogs.* Experiments were performed in 11 mongrel dogs of both sexes weighing 18–25 kg. On the day of the acute experiment, the dogs were anesthesized with pentobarbitol sodium (30 mg/kg, iv). The femoral vein and artery were cannulated and catheters were inserted for measurement of arterial pres-

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sure, arterial sampling, and infusion of ANP 8-33. The trachea was orally intubated using a 9.5-mm endotrachael tube and the animal was mechanically ventilated (Harvard Apparatus). The right external jugular vein was isolated and a thermodilution No. 7 French balloon-tipped pulmonary artery catheter (American Edwards Laboratory) was advanced into the pulmonary artery for measurement of right atrial pressure and pulmonary artery pressure as well as for measurement of cardiac output by thermodilution methods as previously described (13). The heart was exposed through a left thoracotomy incision at the fifth interspace. The pericardium was reflected. The proximal one-third of the left anterior descending (LAD) coronary artery was isolated and a calibrated electromagnetic flow probe was placed on the artery and connected to a flowmeter (Carolina Instruments, King, NC) for the continuous measurement of coronary blood flow.

Following completion of surgery, the dogs were allowed to stabilize for approximately 1 hr, after which control measurements were obtained during a 15-min period. The control period was followed by intravenous infusion of synthetic rat atrial natriuretic peptide (8-33, Peninsula Laboratories, Belmont, CA) at a rate of 0.05 μg/kg/min in group 1 ($n = 5$) and 0.3 μg/kg/min in group 2 ($n = 6$) for 45 min. We have previously reported that the low dose results in a natriuresis, but without a decrease in arterial pressure, in contrast to natriuresis with decreases in arterial pressure with the higher dose (1, 14). Experimental measurements were assessed beginning 15 min after initiation of ANP infusion and continued for the remaining 30 min. The infusion was then stopped. Sixty minutes after the infusion was stopped, a 15-min recovery period followed during which measurements were repeated.

During each period, the following measurements were obtained: mean systemic arterial pressure, heart rate, right atrial pressure, pulmonary capillary wedge pressure, pulmonary artery pressure, cardiac output, stroke volume, and coronary blood flow. The following hemodynamic parameters were calculated: pulmonary vascular resistance (PVR = mean pulmonary artery pressure minus pulmonary capillary wedge pressure divided by cardiac output), systemic vascular resistance (SVR = mean arterial pressure minus right atrial pressure divided by cardiac output), coronary vascular resistance (CVR = mean arterial pressure minus right atrial pressure divided by coronary blood flow), and stroke volume (SV = cardiac output divided by heart rate). Cardiac output was determined in triplicate with the values averaged.

Retrograde coronary perfusion of isolated rat hearts. Male Wistar-Kyoto rats (250 to 350 g) were anesthetized with sodium pentobarbital (30 mg/kg intraperitoneally). The heart was rapidly removed, placed into ice-cold physiological salt solution, and the aortic stump was cannulated to allow retrograde coronary perfusion (15). The hearts were perfused without recirculation at constant pressure (85 cm H_2O) by Krebs–Ringer bicarbonate solution (control solution; composition in m*M*: NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; calcium disodium EDTA, 0.026; glucose, 11.1; equilibrated with 95% O_2 + 5% CO_2 gas mixture; pH 7.4) via filter (9.45 μ*M* Millipore Type HA), bubble-trap, and heat exchanger (37°C). Coronary flow was continuously measured by an electromagnetic flow probe (Carolina Medical Electronics, Inc., Model EP 300-1-16). The force generated by the spontaneously beating heart was measured by a force transducer (Gould UT2) connected to the apex of the heart. After a 30-min perfusion period with control solution (during which the measured parameters reached steady level), increasing concentrations (10^2 to 10^6 pg/ml, for ten 10-min periods each) of synthetic atrial natriuretic peptide (8-33, Peninsula) were added to the perfusate. After return to perfusion with control solution, the vasodilator effect of adenosine and the vasoconstrictor action of nickel chloride (Sigma Chemical Co., St. Louis, MO) were tested as well. To determine the change of the various measured parameters with time, a group of hearts ($n = 6$) was perfused with control solution only (time control).

Statistical analysis. Data from individual experiments were averaged and expressed as means ± SE. The data were analyzed using

Dunnett's paired *t* test for simultaneous multiple comparisons.

Results. *Effect of ANP on anesthetized dogs.* Table I summarizes the effects of ANP 8-33 infusion on the coronary circulation and hemodynamic parameters in groups 1 and 2.

Hemodynamic effects. In group 1, low-dose ANP did not decrease arterial pressure, but did decrease pulmonary capillary wedge pressure (3.5 ± 0.2 to 2.9 ± 0.3 mm Hg, $P < 0.05$), and cardiac output (4.5 ± 0.4 to 3.9 ± 0.4 liters/min, $P < 0.05$). Mean arterial pressure decreased significantly in group 2 during ANP infusion from 103 ± 4 to 90 ± 4 mm Hg ($P < 0.005$), in association with a decrease in pulmonary capillary wedge pressure from 3.8 ± 0.6 to 3.0 ± 0.4 mm Hg ($P < 0.05$). Cardiac output also decreased significantly in response to ANP from 4.9 ± 0.6 to 3.4 ± 0.5 liters/min ($P < 0.025$).

Effects on coronary circulation. In group 1, low-dose ANP 8-33 failed to alter coronary blood flow or coronary vascular resistance. Initiation of ANP infusion in group 2 resulted in a transient coronary vasodilation during the intial 2.1 ± 0.3 min of infusion, in which coronary blood flow increased from 38.0 ± 4.2 to 42.3 ± 4.0 ml/min, $P < 0.05$, with a decrease in coronary resistance from 2.77 ± 0.32 to 2.38 ± 0.28 mm Hg (ml • min^{-1}), $P < 0.05$. Continued ANP infusion resulted in reduction in coronary flow from 38.0 ± 4.2 to 34.4 ± 4.6 ml/min, $P < .025$, in association with a decrease in arterial pressure. Coronary vascular resistance did not change significantly from 2.77 ± 0.32 to 2.73 ± 0.32 mm Hg (ml • min^{-1}).

Effects of ANP in isolated perfused rat hearts (Table II). Coronary flow, spontaneous heart rate, and force generation of isolated rat hearts did not change more than 10% of the initial control value (measured after 30-min perfusion with control solution) between the 30 and the 80 min of perfusion (Table II, time control). Under these perfusion conditions, coronary flow could be increased from 11.5 ± 1.2 to 18.6 ± 1.6 ml/min by adenosine ($10^{-5}M$, $n = 4$) and decreased from 12.4 ± 0.6 to 2.0 ± 0.7 ml/min by infusion of nickel chloride (10^{-5} M, $n = 4$). When compared to time control values, synthetic atrial natriuretic peptide did not cause significant changes in coronary flow, heart rate, and force generation within the entire concentration range (10^2 to 10^6 pg/ml, Table II).

Discussion. The present studies *in vivo* demonstrate that ANP 8-33 decreases cardiac output in anesthetized dogs at both hypotensive and nonhypotensive doses, in the absence of coronary vasoconstriction. Intravenous infusion of atrial natriuretic peptide in the dog caused only a transient coronary vasodilation at pharmacologic concentrations with no active coronary vasoconstriction. *In vitro* studies in the isolated rat heart employing a defined dose range from physiologic to suprapharmacologic doses of ANP demonstrated no effect of ANP on coronary reactivity. Thus, the studies in the isolated rat heart preparation and *in vivo* in the anesthetized dog demonstrate that ANP reduces cardiac output in the absence of coronary vasoconstriction.

Initiation of ANP infusion resulted in a transient but significant coronary vasodilation which is similar to the response described in the renal circulation (1, 3). Of significance was the observation that the decrease in coronary blood flow coincided with the onset of a decrease in systemic arterial pressure in the group of animals receiving high-dose ANP. Such an observation would suggest that the decrease in coronary blood flow in the present *in vivo* studies may be mediated by a decrease in coronary perfusion pressure. In support of this interpretation, the *in vitro* studies demonstrated that coronary flow did not change when perfusion pressure was held constant. The present *in vivo* and *in vitro* studies demonstrate no coronary vasoconstrictor property for ANP and thus are in contrast to the studies of Wangler *et al.* (10) who reported that atriopeptin II evokes a dose-dependent coronary vasoconstriction in isolated perfused guinea pig, rat, and dog hearts, but agree with the studies of Hintze *et al.* (11) who demonstrated no evidence for coronary vasoconstriction in one conscious dog and preliminary studies by Bache and colleagues (12). The lack of effect of ANP 8-33 in isolated perfused rat hearts cannot be attributed to "insensitivity" of the coronary vessels, since similar to previous studies (16), adenosine

TABLE I. CARDIAC AND CORONARY DYNAMIC RESPONSES TO INTRAVENOUS INFUSION OF SYNTHETIC ATRIAL NATRIURETIC PEPTIDE

	Group 1 (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$)			Group 2 (0.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$)		
	Control	ANP	Recovery	Control	ANP	Recovery
MAP, mm Hg	101 ± 3	100 ± 4	102 ± 5	103 ± 4	90 ± 4***	102 ± 7
HR, bpm	144 ± 5	145 ± 5	146 ± 5	140 ± 4	141 ± 6	145 ± 7
RAP, mm Hg	3.0 ± 0.7	2.6 ± 0.6	2.9 ± 0.6	3.2 ± 0.8	2.5 ± 0.8	3.1 ± 0.7
MPAP, mm Hg	11.3 ± 1.8	11.4 ± 2.0	12.0 ± 1.7	12.5 ± 2.0	12.1 ± 1.6	14.1 ± 1.1
PCWP, mm Hg	3.5 ± 0.2	2.9 ± 0.3*	3.4 ± 0.3*	3.8 ± 0.6	3.0 ± 0.4*	4.2 ± 0.6
PVR, mm Hg·(liters·min^{-1})	2.61 ± 0.50	2.95 ± 0.82	2.91 ± 0.71	2.77 ± 0.56	3.72 ± 1.13	3.73 ± 0.47
CVR, mm Hg·(ml·min^{-1})	2.48 ± 0.29	2.47 ± 0.30	2.50 ± 0.27	2.77 ± 0.32	2.73 ± 0.32	2.72 ± 0.29
SVR, mm Hg·(liters·min^{-1})	23.5 ± 3.4	25.9 ± 3.7	24.5 ± 3.6	22.6 ± 3.3	28.0 ± 3.2	27.4 ± 3.5
CO, liters/min	4.5 ± 0.4	3.9 ± 0.4*	4.2 ± 0.6	4.9 ± 0.6	3.4 ± 0.5**	4.0 ± 0.7
SV, ml/min	31.3 ± 4.9	26.4 ± 3.8*	28.5 ± 4.0	35.4 ± 5.4	25.3 ± 4.9*	27.8 ± 5.7
CBF, ml/min	41.2 ± 4.8	40.4 ± 4.5	40.4 ± 4.5	38.0 ± 4.2	34.4 ± 4.6**	38.3 ± 5.2

Note. Values are means ± SE. MAP, mean arterial pressure; HR, heart rate; RAP, right atrial pressure; MPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance; PCWP, pulmonary capillary wedge pressure; CBF, coronary blood flow; CVR, coronary vascular resistance; CO, cardiac output; SV, stroke volume; SVR, systemic vascular resistance.

* $P < 0.05$; ** $P < 0.025$; *** $P < 0.005$.

TABLE II. EFFECTS OF SYNTHETIC ATRIAL NATRIURETIC PEPTIDE ON ISOLATED PERFUSED RAT HEARTS

	Time control ($n = 6$)				Atrial natriuretic peptide ($n = 4$)		
Time of perfusion (min)	Coronary flow % (ml/min)	Heart rate % (bpm)	Force % (g)	Concentration (pg/ml)	Coronary flow % (ml/min)	Heart rate % (bpm)	Force % (g)
30-min control	100 (11.4 ± 0.7)	100 (186 ± 14)	100 (6.32 ± 0.21)	30-min control	100 (10.6 ± 1.4)	100 (200 ± 29)	100 (6.81 ± 0.16)
40	97.6 ± 3.3	97.8 ± 3.1	102.9 ± 5.6	10^2	94.3 ± 9.2	98.2 ± 0.5	98.8 ± 4.1
50	97.6 ± 4.1	95.5 ± 6.6	100.6 ± 4.6	10^3	101.9 ± 9.7	104.9 ± 12.5	92.8 ± 9.1
60	100.7 ± 2.4	92.1 ± 5.3	99.1 ± 7.6	10^4	100.1 ± 9.5	100.8 ± 11.8	93.5 ± 5.6
70	103.1 ± 3.3	97.2 ± 4.8	98.2 ± 8.4	10^5	93.4 ± 9.6	103.6 ± 10.4	89.2 ± 4.2
80	98.2 ± 5.4	96.3 ± 5.0	98.4 ± 9.9	10^6	89.6 ± 9.1	101.2 ± 13.1	86.7 ± 8.1

Note. Data were measured at the end of the 10-min periods, expressed as percentages of the control values obtained in the 30th minute, and shown as means ± SEM.

was able to increase and nickel chloride to decrease coronary flow under the present experimental conditions. However, the use of a different peptide fragment in the present studies which more appropriately reflects the active circulating peptide (17) and differences in the experimental procedures (i.e., different perfusion pressure, EDTA in the perfusate, infusion of ANP instead of boluses) may, at least in part, be responsible for the present findings which conflict with the report of Wangler *et al.* (10). Nevertheless, caution should be advised in regard to interpreting the action of atrial peptides on the coronary circulation, as differing peptide sequences may have different effects on the coronary circulation. Further, the use of general anesthesia in the dog may have attenuated the ability of the dog to reflexively increase systemic vascular resistance in response to ANP-mediated hypotension. Previous studies have reported increases in systemic vascular resistance in conscious and anesthetized animals in response to decreases in arterial pressure during ANP infusion (7–9).

Arterial pressure decreased significantly during intravenous infusion of ANP at pharmacologic concentrations, which was associated with a reduction in cardiac output with an increase, although not significant, in systemic vascular resistance. The present studies are consistent with previous investigations which demonstrate that ANP decreases arterial pressure and cardiac output without a decrease in systemic vascular resistance (5–8). Also, the observed decrease in stroke volume may be related to the decrease in cardiac filling pressure and suggest that the primary cause of ANP-induced decrease in cardiac output is a reduction in venous return (7). Such an observation was also noted but to a lesser magnitude at nonhypotensive concentrations of ANP. This decrease in venous return may be caused by an increase in venous capacitance or transudation of fluid into the interstitial space.

In summary, our studies demonstrate that intravenous administration of synthetic atrial natriuretic peptide 8-33 *in vivo* results in a decrease in cardiac output at hypotensive and nonhypotensive concentrations in the absence of an increase in coronary vascular resistance. Further, in the isolated rat Langendorff preparation employing both physiologic and pharmacologic doses of ANP 8-33, no direct coronary vasoconstrictor action of atrial natriuretic peptide has been observed.

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