Pharmacological Actions of Synthetic Atrial Natriuretic Factor on Coronary Vascular Smooth Muscle

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The pharmacological activities of synthetic atrial natriuretic factor (ANF) on cat coronary artery were studied in vitro. In the preparation of isolated cat coronary artery perfused at a constant flow, ANF (3–300 nM) decreased perfusion pressure in a concentration dependent manner, indicating coronary vasodilating activity. ANF also relaxed feline coronary strips when contracted by U-46,619, CTA₂, angiotensin II, serotonin, leukotriene C₄ and D₄. This relaxant effect was independent in the presence of endothelial cells and occurred in the presence of guanylate cyclase inhibitor, methylene blue. ANF had no direct effect on electrically driven isolated cat papillary muscles signifying a lack of direct inotropic activity. It can be concluded that ANF may potentiate coronary vasodilation and therefore contribute to coronary regulation, without directly altering myocardial performance.

ADMINISTRATION of atrial natriuretic factor (ANF) in animals results in natriuresis and relaxation of vascular smooth muscle.¹⁻³ The mechanism of ANF-induced relaxation of the vascular smooth muscle is understood incompletely but may involve interaction with high affinity receptor cites⁴ resulting in increased levels of cyclic GMP through activation of guanylate cyclase. This is similar to the profile for the nitrate vasodilators such as nitroprusside⁵⁻⁷ On the basis of this information, ANF would be expected to exert a coronary vasodilator effect. However, Wangler et al⁸ reported that synthetic atrial natriuretic peptide (atriopeptin-II) exerted coronary vasoconstrictor activity in Langendorff-perfused heart preparations. To clarify this situation, we studied the possible direct effect of a synthetic ANF³ on isolated coronary artery and papillary muscle preparations.

METHODS

Adult male cats weighing between 2.5 to 3.5 kg were anesthetized with sodium pentobarbital. The hearts were quickly excised and placed in ice-cold Krebs-Henseleit (K-H) solution.

Isolated Perfused Coronary Arteries

According to the method of Olgetree et al⁹ both the left and the right coronary arteries were isolated and perfused with oxygenated (95%O₂ + 5%CO₂) K-H solution warmed at 37°C. Each artery was placed in 10-ml chamber, and the K-H solution was circulated by a Harvard peristaltic pump. Perfusion pressure was continuously monitored. After a 1 h stabilization period, vessel reactivity was tested by adding 30 nM of carboxylic thromboxane A₂ (CTA₂)¹⁰ After washing with fresh perfusate, the coronary artery was used to assess the vascular responses to ANF. Synthetic ANF (26 amino acid fragments, 8–33 COOH-terminal acid) was obtained from Merck

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inhibitor, methylene blue. Data are expressed as percent decrease of developed force to U-46,619 (10 nM) induced coronary tone.

**Papillary Muscle Preparation**
Right ventricular papillary muscle were mounted in 20-ml chambers containing K-H solution. These were electrically stimulated with a frequency of 1.0 Hz according to the method of Lefer. Developed force was recorded on a Grass Model 7 oscillographic recorder using Grass FT-03 force displacement transducers. Each papillary muscle was tested with isoproterenol (100 nM) to demonstrate their responsiveness to positive inotropic agents. Data are expressed as percent change in developed force.

**Statistics**
All values described in the text are means ± S.E.M. The significance of all results was determined with Student's "t"-test. P values less than 0.05 were considered statistically significant.

**RESULTS**

**Isolated Perfused Coronary Arteries**

ANF exerted a significant vasodilator effect in perfused cat coronary arteries (i.e., a decrease in perfusion pressure during constant flow perfusion). Fig. 1 illustrates the concentration-related nature of the coronary vasodilator effect of ANF. ANF produced a decrease in perfusion pressure ranging from 2.7 ± 0.7 mmHg (p < 0.02 from baseline value) at 3 nM to 28.6 ± 3.7 mmHg (p < 0.001) at 300 nM.

**Isolated Coronary Artery Strips**

ANF exerted non-specific vasorelaxation activity in helical strips contracted by U-46,619 (Fig. 2). Acetylcholine (100 nM) relaxed rings with an endothelium and contracted rings with functionally destroyed endothelium. Nevertheless, ANF relaxed strips both with and without an endothelium. This ANF induced endothelium-independent vasorelaxation activity was also observed in preparation which were contracted by angiotensin II (100 nM), serotonin (100 nM), CTA₂ (30 nM), LTC₄ (50 nM) and LTD₄ (50 nM).

To further characterize the ANF-induced coronary relaxation, its interactions with a guanylate cyclase inhibitor methylene blue, were compared with the relaxation effect of acetylcholine and nitroglycerin. ANF stimulates par-

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Institute for Therapeutic Research (West Point, PA, U.S.A.) and prepared fresh daily in K-H solution just prior to use.

**Isolated Coronary Artery Strips**
The coronary arteries were isolated and cut into helical strips of about 1.5–2.0 mm × 10–15 mm. In some coronary artery strips, the endothelial surface was rubbed by polyethylene tubing which was roughened by a file. The coronary strips were suspended in a 10-ml chambers containing oxygenated K-H solution warmed at 37°C. The strips were suspended under a resting force of 0.5 g and allowed to equilibrate for 2 h prior to the administration of any agent. Isometric contractions were recorded on a Grass Model 7 oscillographic recorder using Grass FT-03 force transducers. Coronary arteries were contracted by 10 nM of 9, 11-methanoepoxy PGH₂ (U-46,619, Upjohn Co., Kalamazoo, MI, U.S.A.), a stable endoperoxide. In some strips, angiotensin II, serotonin, CTA₂, leukotriene (LT) C₄ and D₄ were used to produce initial developed force. Also, ANF vasoactivity was compared with the vascular responsiveness of nitroglycerin (American Critical Care, McGaw Park, IL, U.S.A.) in the presence or absence of guanylate cyclase.

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Fig. 2. Typical responses of isolated helical strips of cat coronary arteries with intact endothelium and without endothelium. Endoperoxide analog U-46,619 (10 nM) was used to produce the initial developed force. Acetylcholine (ACh, 100 nM) relaxed intact coronary strips, but contracted strips with functionally destroyed endothelium. However, synthetic atrial natriuretic factor (ANF) dose-dependently relaxed both coronary strips with intact endothelium and without endothelium.

ticulate guanylate cyclase inducing cyclic GMP accumulation\(^5\) whereas acetylcholine relaxes vascular smooth muscle by EDRF which activates soluble guanylate cyclase resulting in increased cyclic GMP levels.\(^{14}\) On the other hand, nitroglycerin relaxes vascular smooth muscle by non-receptor mediated stimulation of soluble guanylate cyclase which leads to cyclic GMP accumulation.\(^{12}\) Pretreatment with methylene blue (10 μM) reduced the relaxation effect of acetylcholine (100 nM) as well as of nitroglycerin (100 nM). However, the relaxation effect of ANF (3 to 300 nM) was unaffected by methylene blue, indicating a different mechanism of ANF-induced coronary artery relaxation effect from that of acetylcholine and nitroglycerin.

**Isolated Papillary Muscle**

To test the possibility that ANF exerted a direct inotropic effect on cardiac muscle which then alters coronary vascular tone, we measured developed force in isolated cat papillary muscles. Isoproterenol, a positive inotrope agent, produced a significant increase in developed force indicating that the papillary muscles were responsive to an inotropic stimulus. In 5 papillary muscles, ANF (1 to 100 nM) had no direct effect on isolated feline papillary muscles signifying a lack of direct inotropic activity.

**DISCUSSION**

Since ANF has been proposed as a regulator of cardiovascular homeostasis, it is important to characterize its effects on coronary vascular smooth muscle. Our data provide clear evidence that ANF has a very potent coronary relaxation activity in cat coronary vascular smooth muscle. This ANF-induced relaxation was observed in perfused isolated cat coronary arteries and also in isolated helical coronary strips. These findings are consistent with earlier reports that ANF produced a potent, concentration-dependent and endothelium-independent relaxation effect on

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rabbit and rat arterial vascular preparations (renal, mesentric and carotid artery, and aorta)\textsuperscript{7,15,16}. This suggests that the mechanism of ANF-induced relaxation in cat coronary arteries is similar to that of different arterial preparations from other mammalian species.

ANF-induced relaxation of the coronary artery presented in this study is quite different from the findings of Wangler and his coworkers\textsuperscript{3}. They reported that a bolus injection of 100 nanomoles of ANF (atropinepeptin II) raised coronary resistance in blood perfused dog hearts and K-H perfused guinea pig and rat hearts, indicating a coronary constrictor activity of ANF. It is virtually impossible to compare these results because of the different experimental models, species and method of administration of the ANF. However, because of absence of cardiac muscle and coronary veins, responses obtained in isolated perfused coronary arteries and in helical strips of coronary arteries may reflect a more direct vasoactivity of ANF, whereas Langendorff preparations are complicated by mechanical and metabolic response of the cardiac muscle.

Recent reports demonstrate that infusion of ANF into dogs with acute left ventricular failure produced a significant increase in cardiac output and myocardial blood flow and a fall in coronary vascular resistance, without any changes in heart rate, diastolic blood pressure, cardiac contractility\textsuperscript{17}. In such pathologic state, the property of coronary vasoaction of ANF may be beneficial as well as its diuretic effect for the treatment of congestive heart failure. So, synthetic ANF could be expected as a novel diuretic/vasodilating agent for patients with congestive heart failure and coronary artery disease. Further investigations are needed to clarify the actions of ANF on the coronary circulation in a variety of physiological conditions.

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