Effect of a purified atrial natriuretic factor on rat and rabbit vascular strips and vascular beds

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GARCIA, RAUL, GAETAN THIBAULT, MARC CANTIN, AND JACQUES GENEST: Effect of a purified atrial natriuretic factor on rat and rabbit vascular strips and vascular beds. Am. J. Physiol. 247 (Regulatory Integrative Comp. Physiol. 16): R34–R39, 1984.—Rat atrium cardiocytes contain a powerful natriuretic and diuretic peptide that has been localized in the specific granules. This atrial natriuretic factor (ANF) produced a potent, dose-dependent relaxant effect on rabbit and rat arterial strips previously made to contract by application of either norepinephrine (NE) or angiotensin II. The effect was not seen if KCl was used as contractile agent or under any conditions with rabbit mesenteric strips. After the application of ANF the vascular strips were refractory to subsequent stimulation by either NE or angiotensin II. The infusion of ANF into a high-resistance isolated perfused rat kidney produced a rapid decrease (30 ± 5 mmHg) in perfusion pressure that lasted for 16 ± 3 min. This effect was not seen in the isolated rat mesenteric arterial preparation, even when the perfusion pressure was raised by the infusion of NE. These effects of ANF on vascular smooth muscle are not mediated by prostaglandins, by alpha- and beta-adrenergic and muscarinic receptors, or by an impairment of Ca** influx, but they are mimicked by sodium nitroprusside. A low- and a high-molecular-weight ANF produced the same effects. The existence of specific receptive sites for these peptides is suggested.

A powerful natriuretic and diuretic substance is present in the specific granules of mammalian atrial cardiocytes (4, 11). It has been suggested that this atrial natriuretic factor (ANF) is a peptide that does not inhibit Na* - K* - ATPase (24).

Observations in experiments to modify salt and water balance and atrial granularity (3, 19) and atrial natriuretic activity (24) have suggested that the ANF contained in the specific granules may play a role in regulating natriuresis and diuresis in response to volume changes.

De Bold et al. (5) described a fall in blood pressure during the infusion of a crude atrial extract that was ascribed to a decreased circulating volume. However, in their experiments a direct effect of ANF on vascular smooth muscle could not be ruled out.

We have studied the possible direct effect of a partially purified ANF on rat and rabbit arterial strips and on isolated vascular beds.

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MATERIALS AND METHODS

Vascular Strips

Male New Zealand White rabbits (1.8–2.0 kg) were fed Purina rabbit chow and allowed free access to tap water. Under pentobarbital sodium anesthesia (Nembutal, 30 mg/kg iv) the thoracic aorta and mesenteric and renal arteries were rapidly excised and gently trimmed of excess fat and connective tissue, and the arterial tissue was helically cut as described by Furchgott and Bihadakom (10). Each vascular strip (ca. 2 × 20–25 mm for the mesenteric artery, 1 × 15–20 mm for the renal arteries, and 3 × 30–35 mm for the aorta) was suspended in a 20-ml tissue bath containing a continuously oxygenated Krebs solution (95% O2–5% CO2) at 37°C and pH 7.4. The strips were mounted between a fixed base and a force displacement transducer (Grass, FT-03C). The contractions were registered on a polygraph (Grass, model 7).

A tension of 1,000–1,500 mg was applied to each mesenteric arterial strip, 500–750 mg to each renal strip, and 2,500–3,000 mg to each aortic strip. The tension was adjusted and bathing fluid changed every 15 min. The strips were allowed to equilibrate for 2 h before the experimental procedures began.

Male Sprague-Dawley rats (225–250 g) were fed Purina rat chow and allowed free access to tap water. On the day of each experiment, the rats were killed by decapitation and exsanguinated. The thoracic aorta was rapidly removed and prepared as above. Each vascular strip (2 × 30–35 mm) was subjected to a tension of 250–1,000 mg.

The composition of the solution used in this study was as follows (mmol/l): 119 NaCl, 4.7 KCl, 1.8 KH2PO4, 1.17 MgSO4, 7 H2O, 2.5 CaCl2-6 H2O, 25.0 NaHCO3, and 5.5 dextrose.

For each arterial strip a dose-response curve to norepinephrine (NE; L-norepinephrine bitartrate, Sigma Chemical) was obtained. The interval between doses was at least 15 min. When a maximal response was attained, an ED50 value (concentration of the agonist required to produce 50% of the maximal response) was estimated.

The relaxant effect of ANF was studied during the contraction induced by the ED50 dose of NE.

Cumulative dose-response curves were built up in some strips, but because of the scarcity of the partially purified ANF most of the experiments were done with one dose of the natriuretic factor—that known to produce maxi-
Vascular Beds

Mesenteric arterial perfusion. Mesenteric arteries of male Sprague-Dawley rats (250–300 g) were perfused as previously described (7). Briefly the rats were decapitated, and the mesenteric artery was quickly cannulated, dissected free from the intestine, and mounted in an organ bath. The arteries were then perfused with oxygenated (95% O2-5% CO2) Krebs solution at 37°C by means of a peristaltic pump (Extracorporeal Medical Specialties, model 925). The flow rate was adjusted to obtain a perfusion pressure of 20–25 mmHg; the rate remained constant during the course of the experiment. The perfusion pressure was monitored with a pressure transducer (Hewlett-Packard, model 7702B) placed around each artery. NE was prepared in 0.01 mmol/l HCl, diluted as needed, and injected in boluses of 0.25, 0.5, 1.0, 5.0, and 10 µg at a rate of one injection every 5 min.

Two approaches have been taken to study the effect of ANF on this vascular territory: 1) continuous infusion of ANF at 100–400 U/min (1 U = 1 µmol Na+ excreted per 20 min in the urine of a bioassayed rat) by means of an infusion pump (Gilson, Minipulse 2) and subsequent challenges with bolus injections of NE and 2) continuous infusion of NE to maintain perfusion pressure between 60 and 80 mmHg and subsequent challenges with ANF in bolus injections between 50 and 200 U.

Renal vascular perfusion. Male Sprague-Dawley rats (300–350 g) were anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg iv). After catheterization of the right ureter with polyethylene tubing (PE-50), the distal end of the superior mesenteric artery was tied and loose ligatures were placed around the superior mesenteric artery and the right renal artery near the aorta. The right kidney was gently freed from the perirenal fat, and the right renal artery was cannulated via the superior mesenteric artery without interruption of flow. As the arterial cannula was passed into the renal artery the kidney was continuously perfused. The cannula was then secured by means of previously placed ligatures. The kidney was then removed and fitted into a kidney-shaped Plexiglas chamber.

The kidney was then perfused by means of a peristaltic pump (Harvard Apparatus, model 1203) with a Krebs solution containing Ficoll 70 (50 g/l, Pharmacia, Sweden) at 37°C and equilibrated with a mixture of 95% O2-5% CO2. Before entering the kidney the fluid passed through a bubble trap and a filter (Whatman grade 10-03) which retained any particles. The perfusion pressure was monitored by a pressure transducer (Hewlett-Packard, model 1280C) and recorded on a polygraph (Hewlett-Packard, model 7702B).

After perfusion was begun, the system was allowed to equilibrate for 20 min, during which time the perfusion flow rate stabilized at 8–12 ml/min per kidney with a perfusion pressure of 90–100 mmHg.

We must emphasize that the purpose of this setup was to study the effect of ANF on renal vasculature, not on diuresis and natriuresis.

ANF was partially purified as previously described (24). Briefly, atria from female Sprague-Dawley rats (200–250 g) were homogenized in acetic acid (1.0 mol/l) and centrifuged at 30,000 g for 20 min. The pellet was reextracted with acetic acid (1.0 mol/l), and the pooled supernatants were lyophilized. The powder was redissolved in acetic acid (0.1 mol/l), and the precipitates were removed by centrifugation at 40,000 g for 10 min. The pellet was washed twice with acetic acid (0.1 mol/l) and discarded.

The supernatant was passed through two sets of three Sep-Pak columns connected in series. The columns were washed with acetic acid (0.1 mol/l) and eluted with 80% acetonitrile in acetic acid (0.1 mol/l), and the active material was dried in a rotary vacuum evaporator. The powder was redissolved in acetic acid (0.1 mol/l), deposited on a Bio-Gel P-10 column (2.5 x 90 cm), and eluted with acetic acid (0.1 mol/l).

After this last chromatographic step, two main peaks with natriuretic activity were found (24), one with a molecular weight of about 5,000 (low-molecular-wt ANF) and the other of about 15,000 (high-molecular-wt ANF).

Because of the scarcity of our active material, most of our experiments were done with a mixture of the low- and high-molecular-weight ANFs. Some experiments, however, were carried out with the two ANFs separately.

In investigations of the mechanisms of ANF effect, the following pharmacological agents were used: indomethacin (0.14 mmol/l), isoproterenol (0.1 mmol/l, DL-isoproterenol hydrochloride), propranolol (0.01 mmol/l, DL-propranolol hydrochloride), atropine (1 µmol/l), and sodium nitroprusside (0.5 mmol/l). All these materials were purchased from Sigma Chemical (St. Louis, MO); methylene blue (0.01 mmol/l) was purchased from Allied Chemical (New York, NY).

Statistics

When appropriate, results are expressed as means ± SE. Comparisons were made by Student’s unpaired t test.

RESULTS

Partially purified ANF produced a dose-related relaxation in rabbit aortic and renal arterial strips that had been subjected to contraction with an ED50 dose of NE (Figs. 1 and 2). ANF (100 U) induced in almost every assayed strip a 100% relaxation. This relaxation started immediately after ANF was added to the tissue bath and was completed in 8 ± 0.6 min in aortic strips (n = 9) and in a significantly (P < 0.001) shorter period (4 ± 0.5 min) in renal arterial strips (n = 12). After ANF was added to the bath the tissue preparations were refractory to further challenge with NE. This refractoriness was absolute in aortic and partial in renal arterial strips, in which a
small response (10–15%) could still be elicited (Fig. 3, A and B). The initial response to NE was gradually recovered in 123 ± 21 min for the aorta (n = 7) and again in a significantly (P < 0.02) shorter period (57 ± 11 min) for the renal artery (n = 7).

ANF produced neither relaxation of a NE-induced contraction nor refractoriness in the rabbit mesenteric strips (n = 4, Fig. 3C).

As seen with NE, ANF readily relaxes angiotensin II-induced contraction in both aortic (n = 4) and renal arterial (n = 4) strips (Figs. 4B and 5B). The strips were refractory to further stimulation with angiotensin II. When ANF was added to the tissue bath before the vascular strips were stimulated with either NE or angiotensin II, no contractions were elicited. Neither relaxation to ANF nor refractoriness to subsequent doses was observed in the KCl-induced contractions (n = 6) either in aortic or renal arterial strips (Figs. 4C and 5C).

In the rat aortic strips (n = 4) ANF produced the same effects as those observed in the rabbits: relaxation and refractoriness to NE-induced contractions.

Both low- and high-molecular-weight ANF produce relaxation of the NE-induced contractions in the rabbit aortic and renal arterial strips (Fig. 6).

The addition of propranolol, atropine, indomethacin, or methylene blue to the tissue bath 20 min before a contraction was induced by NE did not modify the relaxation produced by ANF nor did the use of a Ca2+-free Krebs solution.

Figure 7 shows that sodium nitroprusside (0.5 mmol/l) induced a relaxation and refractoriness to NE very similar to those seen with ANF. Furthermore, as with ANF, sodium nitroprusside induced neither relaxation nor refractoriness to the contraction produced by depolarization with KCl.

In the rat mesenteric arterial preparation (n = 8), the continuous infusion of ANF from 100 to 400 U/min produced neither changes in the base line nor refractoriness to NE. When ANF was administered in bolus injections (50–200 U) during a continuous NE infusion, no significant changes in the perfusion pressure were observed.

In the rat kidney (n = 6) perfused at 129 ± 7 mmHg, the bolus injection of 100 U of ANF produced an immediate and significant (P < 0.01) fall of 33 ± 5 mmHg in the perfusion pressure; this effect lasted for 18 ± 7 min.
Atrial Natriuretic Factor and Vascular Smooth Muscle

Discussion

The presence in atrial specific granules of a powerful natriuretic factor (ANF) with peptide characteristics has received ample confirmation (4, 6, 11, 24, 24a). It is not yet known, however, whether this peptide actually plays a role in fluid volume regulation when atrial volume receptors (12) are stimulated. Indirect evidence (3, 19, 24) suggests that it could be so. No relationship has been found between ANF and the so-called natriuretic hormone (9, 24).

It seems that the natriuresis induced by ANF is secondary to an inhibition of NaCl transport in the distal nephron (1, 23). However, it has been also shown (1) that at higher doses ANF may also enhance glomerular filtration rate. Whether this late change is due to circulatory changes inducing an increase in renal blood flow is not known. There are indications that atrial extracts may indeed induce circulatory changes when injected intravenously (1, 4, 23), but whether this effect was secondary to a loss of circulatory volume or to a direct effect on vascular smooth muscle was not clear. Our results showing that a partially purified ANF has a potent relaxant and vasodilator activity on several vas-
cular preparations suggest the truth of the second hypothesis. The mechanism by which ANF exerts these effects is not clear. The fact that ANF opposes contractions induced by NE as well as angiotensin II rules out the possibility of competition for \( \alpha \)-adrenergic receptors. Since propranolol, a \( \beta \)-adrenergic blocker, had no effect on the relaxation effect of ANF, the possibility of ANF acting on \( \beta \)-adrenergic receptors may be excluded. Furthermore, because the relaxant effect of ANF was not blocked by the use of atropine, we may deduce that muscarinic receptors are not involved.

The contraction induced in vascular smooth muscle by K\(^+\)-induced depolarization depends largely on a net extracellular Ca\(^{2+}\) uptake (20, 26), which is also partially true for the tonic phase of the NE-induced contraction (22). ANF neither relaxed nor inhibited the KCl-induced contraction; furthermore, lack of Ca\(^{2+}\) in the extracellular medium did not prevent the relaxing effect of ANF on a NE-induced contraction. These findings suggest that the effect of ANF is not mediated by an impairment of Ca\(^{2+}\) influx from the extracellular medium.

It has been shown (25) that another peptide, bradykinin, shorter than ANF, also produces relaxation of contracted vascular strips. This effect of bradykinin was inhibited by aspirin and indomethacin, which suggested that its effect is mediated by prostaglandin synthesis. In our experiments, indomethacin has no effect on the relaxation induced by ANF. This finding agrees with that of Keeler (18) who found no inhibition of the natriuretic effect of atrial homogenates by indomethacin.

Sodium nitroprusside mimics both observed effects of ANF (Fig. 7). The action of sodium nitroprusside as well as other nitrous compounds may be due to an intracellular increase in the concentration of guanosine 3′,5′-cyclic monophosphate (cGMP) (21). The degree of relaxation induced by these compounds correlated well with the intracellular concentration of cGMP (14, 15, 17). This increase in cGMP concentration is probably due to the activation of guanylate cyclase (13).

The incubation of ANF with renal tissue induces an increase in the tissue levels of cGMP, with a simultaneous decrease in the tissue levels of cAMP (14, 15). ANF neither relaxed nor inhibited the KCl-induced contraction; furthermore, lack of Ca\(^{2+}\) in the extracellular medium did not prevent the relaxing effect of ANF on a NE-induced contraction. These findings suggest that the effect of ANF is not mediated by an impairment of Ca\(^{2+}\) influx from the extracellular medium.

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The incubation of ANF with renal tissue induces an increase in the tissue levels of cGMP, with a simultaneous decrease of cGMP phosphodiesterase (16). Whether the same mechanism is responsible for the effect of ANF on vascular smooth muscle relaxation remains to be seen.

Methylene blue, an oxidant, has been shown to inhibit both arterial guanylate cyclase activation and relaxation elicited by sodium nitroprusside and other compounds (13, 17). In the present experiments, methylene blue did not inhibit the relaxation produced by ANF, which suggests that if cGMP increase is involved in this relaxation, it may be due to an inhibition or a decrease in cGMP phosphodiesterase, as in the kidney, and not through an activation of guanylate cyclase as produced by sodium nitroprusside.

The fact that both low- and high-molecular-weight ANF produced similar effects suggests that the two are molecularly closely related; this has been confirmed by amino acid analysis and sequencing (24a, 24b).

Using preparations cruder than our own, several authors have demonstrated that atrial extracts contain a vasoactive substance (2, 8); however, they could not clearly establish its relationship to ANF. Our results show that both a low- and a high-molecular-weight ANF have a natriuretic and a relaxant and vasodilator effect on vascular smooth muscle.

The heterogeneity in the response of different vascular preparations to ANF may be due to differences in quantity or sensitivity of specific receptive sites for this peptide.

In conclusion, ANF is a peptide or pair of peptides extracted from rat atria that shows, besides its natriuretic activity, a potent relaxant and vasodilatatory effect on vascular smooth muscle. These effects are apparently not mediated by prostaglandins or by \( \alpha \)- and \( \beta \)-adrenergic or muscarinic receptors. ANF shows a certain tissue selectivity. Its actual role in general homeostasis as a natriuretic and vasodilator peptide remains to be elucidated.

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