Atrial Natriuretic Factor and Cyclic Guanosine 3',5'-Monophosphate in Vascular Smooth Muscle

Makito Sato, Keishi Abe, Kazuhsa Takeuchi, Minoru Yasuima, Ken Omata, Masao Hwataru, Yutaka Kasai, Masaya Tanno, Masahiro Kohzuki, Kei Kudo, Kaoru Yoshinaga, and Tadashi Inagami

SUMMARY To elucidate the molecular mechanism of the vascular action of atrial natriuretic factor (ANF), we investigated the effects of synthetic ANF and sodium nitroprusside on the levels of intracellular cyclic nucleotides and prostacyclin (measured as its stable metabolite 6-keto-prostaglandin F₁α) in cultured vascular smooth muscle cells from rat mesenteric artery and, in some experiments, from rat renal artery. Both ANF and sodium nitroprusside increased intracellular cyclic guanosine 3',5'-monophosphate (cGMP) levels in a dose-dependent manner but did not affect cyclic adenosine 3',5'-monophosphate levels or 6-keto-prostaglandin F₁α synthesis. The stimulatory effects of ANF and sodium nitroprusside on cGMP levels were additive. Neither the deprivation of extracellular Ca²⁺ nor calcium entry blockers affected ANF-stimulated cGMP levels. Preincubation of ANF or sodium nitroprusside with kallikrein attenuated only the effect of ANF on cGMP levels. The effect of kallikrein was abolished by serine protease inhibitors. In contrast, the oxidant methylene blue inhibited the effect of sodium nitroprusside on cGMP levels, but not that of ANF. The stimulatory effect of ANF on cGMP levels was greater in cells from renal artery than in those from mesenteric artery. These results in cultured vascular smooth muscle cells further support the hypothesis that cGMP mediates the vasorelaxant action of ANF. (Hypertension 8: 762-771, 1986)

KEY WORDS • atrial natriuretic factor • sodium nitroprusside • cyclic guanosine 3',5'-monophosphate • vascular smooth muscle cell • methylene blue • kallikrein

A PEPTIDE released from atria, generally termed atrial natriuretic factor (ANF), recently has been suggested to play an important role in renal and cardiovascular homeostasis.¹⁻⁶ This peptide has a potent natriuretic, diuretic, and direct vasorelaxant action, but the molecular mechanisms of its action have not been well elucidated. The action of ANF seem to be independent of prostaglandins (PGs)⁴⁻⁵ and Na⁺, K⁺-ATPase.⁶⁻⁷ Recent studies have focused on the vascular effects of ANF in terms of cyclic nucleotides, especially cyclic guanosine 3',5'-monophosphate (cGMP).⁸⁻¹² Cyclic nucleotides have been shown to act as mediators for vasorelaxant actions of various compounds and hormones,¹³⁻¹⁴ and the vascular action of ANF has been found to be qualitatively similar to that of sodium nitroprusside (SNP),¹⁵⁻¹⁶ which is thought to relax vascular smooth muscle through cGMP accumulation.¹³⁻¹⁴,¹⁷⁻¹⁸ Specific receptors for ANF have been found in vascular tissues¹⁹ and cultured vascular smooth muscle cells from aorta.¹⁰ In addition, ANF has been shown to stimulate guanylate cyclase and to increase cGMP levels in vascular tissues⁸⁻¹² as well as in cultured aortic smooth muscle cells.¹⁰ However, some controversy exists regarding the vascular action of ANF and its relation to cGMP. Methylene blue, an oxidant that inhibits SNP-induced arterial guanylate cyclase activation and relaxation,¹⁷⁻¹⁸ has been reported to inhibit¹² or not to affect the vasorelaxant action of ANF.¹⁶ The role of calcium in the vascular action of ANF is also controversial. Removal of calcium from experimental solutions has been reported to have no effect on the vasorelaxant action of ANF in rabbit and rat aortic
strips or on the stimulatory effect of ANF on cGMP levels in rabbit aortic tissues. In the isolated perfused rat kidney, however, a low calcium solution and the calcium entry blocker verapamil markedly blunted the hemodynamic and natriuretic action of ANF. Although these discrepant results may be explained by the difference in species, experimental conditions, vascular tissues, sources of ANF (crude or partially purified atrial extracts or synthetic ANF), or the doses of ANF, these controversies may weaken the hypothesis that cGMP mediates the vasorelaxant action of ANF.

It has recently been suggested that serine proteases (trypsin or kallikrein) modulate the natriuretic and diuretic action of ANF or attenuate the stimulatory effect of ANF on particulate guanylate cyclase from kidney or aorta. However, to our knowledge, the effect of kallikrein on ANF-stimulated cGMP levels in vascular smooth muscle cells has not been evaluated. Furthermore, several reports indicate that renal artery is more sensitive than other arteries to the vasorelaxant action of ANF. If cGMP is involved in the vasorelaxant action of ANF, then the cGMP response to ANF may be greater in cells from renal artery than in those from other arteries. However, no data are available on the difference in the cGMP response to ANF among renal and other arteries.

The purpose of the present experiments was to 1) confirm existing findings that ANF increases cGMP levels in vascular tissues, using cultured vascular smooth muscle cells from mesenteric artery, which has been known to play an important role in the regulation of the peripheral vascular resistance; 2) clarify the effects of calcium, kallikrein, and methylene blue on ANF-stimulated intracellular cGMP levels and compare them with those of SNP-stimulated cGMP levels; and 3) evaluate the cGMP response to ANF in cells from mesenteric and renal arteries.

Materials and Methods

Type III collagenase, deoxyribonuclease I, elastase, soybean trypsin inhibitor, calcium ionophore A23187, 3-isobutyl-1-methylxanthine (IBMX), SNP, and 6-keto-PGF1α were purchased from Sigma Chemical (St. Louis, MO, USA). Medium 199, minimal essential medium (MEM) with or without calcium, antibiotic-antimycotic, penicillin-streptomycin, l-glutamine, sodium bicarbonate, basal medium Eagle amino acid solution, and tryptophan ethylenediaminetetraacetic acid (EDTA) were purchased from Gibco Laboratories (Grand Island, NY, USA), and fetal bovine serum was obtained from MA Bioproducts (Walkersville, MD, USA). The ANF was obtained from two sources: a 25 amino acid ANF was synthesized as previously reported, and 24 amino acid atriopeptin III was purchased from Peninsula Laboratories (Belmont, CA, USA). Porcine pancreatic kallikrein (130 U/mg protein) and gabexate mesylate, [ethyl-4-(6-guanidino-hexanoyloxy)benzoate] methane sulfonate (Foy), were gifts from Ono Pharmaceutical (Osaka, Japan). Aprotinin (Trasylo; 6500 KIU/mg) was a gift from Bayer AG (Leverkusen, West Germany), and angiotensin II (Hypertensin) was obtained from CIBA-Geigy (Basel, Switzerland). Methylene blue was purchased from Wako Chemicals (Tokyo, Japan), and arginine vasopressin was obtained from Peptide Institute (Osaka, Japan). Twelve-well sterile culture dishes were purchased from Costar (Cambridge, MA, USA), and six-well dishes were obtained from Nunc (Copenhagen, Denmark). Radioimmunoassay kits for cyclic adenosine 3',5'-monophosphate (cAMP) and cGMP were obtained from Yamasa Shoyu (Choshi, Japan). Antisera for 6-keto-PGF1α were a gift from Dr. Michael J. Dunn, Cleveland, OH, USA.

Isolation and Culture of Cells

Vascular smooth muscle cells were isolated from superior mesenteric arteries and, in some experiments, from renal arteries of Sprague-Dawley rats (weight, 150–200 g) and were cultured according to the methods of Ives et al. and Gunther et al. as modified by Hassid and Williams. With the rats under pentobarbital anesthesia, the superior mesenteric or renal arteries were dissected free of surrounding tissues and placed in a 35-mm culture dish containing MEM (1.8 mM Ca2+). The adventitial adipose tissue around the arteries was gently removed with forceps, and the arterial tissues were incubated for 15 to 20 minutes at 37°C in a modified Krebs-Ringer buffer solution of the following composition (in mM): NaCl, 110; KCl, 5; KH2PO4, 1; N2-hydroxyethylpipеразине-N'-2-этилсульфоnic acid, 25; MgSO4, 1; glucose, 14; CaCl2, 0.2; and NaHCO3, 25. The medium was further supplemented with dissociation enzymes (collagenase, 360 U/ml; deoxyribonuclease 1, 56 U/ml; elastase, 96 U/ml; soybean trypsin inhibitor, 1 mg/ml). Cells that were dissociated during the first 15 to 20 minutes were discarded. The remaining portion was then transferred to a second aliquot of dissociation medium, and incubation was continued for an additional 30 to 45 minutes. Cells dissociated during the second incubation were collected by centrifugation, passed through a sterile 150-μM Nytex gauze filter (Tetko, Elmsford, NY, USA), and seeded into culture wells in Medium 199. After 2 hours, most cells had attached to the bottom of wells and were further cultured in Medium 199 containing 10% fetal bovine serum and penicillin-streptomycin. Cells were subcultured by treatment with 0.08% trypsin EDTA. Morphological examination revealed characteristics of vascular smooth muscle cells with crisscross patterns, hill and valley and nodular structures in confluent cultures with phase-contrast microscopy and myofilaments and dense bodies with electron microscopy. Cells at passage levels 3 to 9 were used for experiments.

Experiments

All experiments with vascular smooth muscle cells were conducted at least in triplicate using 12-well Costar culture dishes. Before incubation, cells were rinsed...
three times with 0.6 ml of MEM. In most experiments, cells were incubated with effectors in 0.6 ml MEM (1.8 mM Ca²⁺) in the presence or absence of the phosphodiesterase inhibitor IBMX, 10⁻⁴ M, at 37°C in an atmosphere of 5% CO₂, 95% air. A synthetic ANF of 25 amino acids was used only in experiments shown in Figure 1; atriopeptin III was used in all other experiments. In experiments with different extracellular calcium concentrations, Ca²⁺-free medium was made with Ca²⁺-free MEM fortified with 2 mM ethyleneglycol bis(β-aminoethyl ether)-N,N',N³,N⁴-tetraacetic acid and the calcium concentration was changed by adding CaCl₂ solution. Cells were preincubated for 30 minutes in media with different extracellular calcium concentrations or in media containing calcium entry blockers. In experiments involving kallikrein, ANF or SNP was preincubated with kallikrein for 1 hour at 37°C in MEM in the presence or absence of aprotinin or Foy. In experiments in which interactions of methylene blue with ANF or SNP were evaluated, cells were preincubated with methylene blue for 15 minutes. To compare the effect of ANF on cGMP levels in cells from renal and mesenteric arteries, cells were isolated from the same rats and cultured in the same dish, and experiments were performed simultaneously. Experiments were terminated by removal of media, followed immediately by addition of 0.3 ml of 0.1 N HCl. Cells were exposed to the HCl solution for 1 hour, intracellular cyclic nucleotides were extracted into the HCl fraction, and part of the cellular protein was precipitated and left on the bottom of the culture well.

**Assays**

Radioimmunoassays of cGMP and cAMP were done in duplicate after succinylation. We found no cross-reactivity between cGMP or cAMP antisera and ANF, SNP, IBMX, or cell culture incubation media. Samples of 0.1 N HCl solution, diluted 1:5 with 300 mM imidazole buffer, did not interfere with the radioimmunoassay. A 50% displacement of the radioligand was achieved with 30 fmol cGMP or 40 fmol cAMP. Intra-assay coefficient of variance was 7.8% for cGMP and 8.1% for cAMP (n = 8). Interassay coefficient of variance was 11.0% for cGMP (n = 6) and 12.4% for cAMP (n = 6). The 6-keto-PGF₁α was measured by radioimmunoassay of the extracellular media using antisera as previously described. Cellular protein was measured on the precipitate in the culture well and in the 0.1 N HCl solution by the methods of Lowry et al. Since precipitated protein was 56.0 ± 0.3% (n = 25) of total cellular protein and this percentage was constant in each experiment, we measured the precipitated protein in the culture well and corrected the value for total cellular protein by this percentage in some experiments, essentially as described previously.

**Statistics**

The values are expressed as mean ± SE. The statistical significance was evaluated at the 95% confidence level using Student's t test for unpaired observations or one-way analysis of variance.

**Results**

A synthetic ANF of 25 amino acids increased intracellular cGMP levels in a dose-dependent manner (p < 0.001) but did not affect cAMP levels (Figure 1). Threshold stimulation of cGMP levels was observed at 1.1 × 10⁻⁹ M, while half-maximal stimulation was seen around 1.1 × 10⁻⁷ M, and maximal stimulation at 1.1 × 10⁻⁵ to 1.1 × 10⁻⁴ M ANF. Basal levels of 6-keto-PGF₁α (8.2 ± 0.7 pg/µg protein/30 min) were not affected by ANF (1.1 × 10⁻⁹ to 1.1 × 10⁻³ M). Although angiotensin II (10⁻⁷ M), arginine vasopressin (10⁻⁷ M), and calcium ionophore A23187 (2 × 10⁻⁶ M) stimulated 6-keto-PGF₁α synthesis about 1.5-fold, 2.0-fold, and 3.1-fold, respectively, preincubation with ANF, 3.3 × 10⁻⁴ to 3.3 × 10⁻³ M, for 30 minutes did not affect stimulated 6-keto-PGF₁α synthesis (data not shown). As shown in Figure 2, incubation of cells with 3.3 × 10⁻⁴ M ANF resulted in a significant increase in cGMP levels within 1 minute. The response was maximal at 2 minutes and then gradually declined over the next 30 minutes in the absence of the phosphodiesterase inhibitor IBMX. The presence of 10⁻⁴ M IBMX...
potentiated both the peak and the duration of ANF-stimulated cGMP levels, while IBMX, 10^{-4} or 5 \times 10^{-4} M, potentiated basal cGMP levels (from control values of 0.2 \pm 0.1 to 0.3 \pm 0.1 or 0.8 \pm 0.2 fmol/\mu g protein) and ANF (3.3 \times 10^{-4} M)-stimulated cGMP levels (from control values of 1.0 \pm 0.2 to 12.5 \pm 3.9 or 42.0 \pm 6.0 fmol/\mu g protein, respectively). The presence of SNP, 10^{-7} M or above, also increased intracellular cGMP levels ($p<0.001$; Figure 3) but did not affect cAMP levels or 6-keto-PGF\(_{1\alpha}\) synthesis (data not shown). Although the amounts of ANF and SNP did not necessarily achieve maximal stimulation of cGMP.

**Figure 2.** Time course for intracellular cGMP levels in the presence or absence of 3.3 \times 10^{-8} M atrial natriuretic factor (ANF) or 10^{-4} M 3-isobutyl-1-methylxanthine (IBMX), or both. Results shown are means \pm SE of six to eight cultures pooled from two experiments.

**Figure 3.** Effect of sodium nitroprusside (SNP) on intracellular cGMP levels. Incubations were performed for 5 minutes with SNP (10^{-7}–10^{-3} M) in the presence of 10^{-4} M 3-isobutyl-1-methylxanthine. At concentrations of 10^{-4} M or above, SNP increased cGMP levels ($p<0.001$). Results shown are means \pm SE of eight cultures pooled from two experiments.
levels, the stimulatory effects of ANF, $3.3 \times 10^{-7}$ to $3.3 \times 10^{-6}$ M, and SNP, $3.3 \times 10^{-6}$ to $10^{-3}$ M, on cGMP levels were additive (Figure 4).

To assess the role of extracellular calcium on ANF-stimulated cGMP levels, experiments were performed with Ca$^{2+}$-free solution or calcium entry blockers. Decreasing extracellular calcium concentration from 1.8 to 0.18 or 0 mM slightly modified the basal cGMP levels from 0.5 ± 0.1 to 0.5 ± 0.2 or 0.3 ± 0.2 fmol/μg protein (nonsignificant change) and did not affect ANF ($3.3 \times 10^{-7}$ M)-stimulated cGMP levels (from 13.9 ± 1.6 to 12.1 ± 2.0 or 13.5 ± 2.1 fmol/μg protein, respectively). Pretreatment of cells with $10^{-5}$ M verapamil or $10^{-5}$ M nifedipine for 30 minutes also failed to affect ANF-stimulated cGMP levels (data not shown).

Figure 5 shows the effect of porcine pancreatic kallikrein on ANF-stimulated cGMP levels in the presence or absence of the serine protease inhibitors aprotinin and Foy. Although kallikrein did not change basal cGMP levels, preincubation of ANF with kallikrein for 1 hour at 37°C significantly attenuated ANF-stimulated cGMP levels ($p < 0.01$). The effect of kallikrein was concentration-dependent and was abolished by concomitant treatment with aprotinin or Foy in a dose-dependent manner. However, kallikrein, in a concentration as high as $10^{-3}$ M, did not inhibit SNP-stimulated cGMP levels (basal, 0.9 ± 0.1; $10^{-3}$ M kallikrein, 1.2 ± 0.1; $3.3 \times 10^{-4}$ M SNP, 12.4 ± 0.5; SNP and kallikrein, 18.0 ± 1.3 fmol/μg protein; $n = 8$). Figure 6 shows the effect of methylene blue on ANF-stimulated or SNP-stimulated cGMP levels. Pretreatment of cells with methylene blue, $10^{-7}$ and $10^{-6}$ M, for 15 minutes inhibited the cGMP response to SNP but not to ANF. The inhibitory effect of methylene blue was concentration-dependent.

The responses of cyclic nucleotides to ANF, SNP, or isoproterenol were compared in cells from renal and mesenteric arteries. Cells at passage level 3 were used for this series of experiments. Basal levels of cGMP or cAMP were 1.2 ± 0.1 or 21.4 ± 2.1 fmol/μg protein in cells from mesenteric artery and 1.4 ± 0.1 or 26.9 ± 1.5 fmol/μg in cells from renal artery (no significant difference between the two cell types). However, cGMP responses to SNP and especially to ANF were much greater in cells from renal artery than in those from mesenteric artery (Figure 7), whereas the cAMP response to isoproterenol did not show any significant difference between the two cell types (Figure 8).

Discussion

The present results in cultured vascular smooth muscle cells from rat mesenteric artery are consistent with previous findings that ANF increases cGMP levels in vascular tissues. 8-12 Although accumulating evidence indicates that ANF increases cGMP levels in several tissues, including kidney, liver, and lung, 8,9 the mechanism of this increase has not been well elucidated. Hamet et al. 8 reported that ANF inhibited cGMP phosphodiesterase but did not activate soluble or particulate guanylate cyclase in arterial tissues and cultured aortic smooth muscle cells. In the present
FIGURE 5. Effect of kallikrein on atrial natriuretic factor (ANF)-stimulated intracellular cGMP levels in the presence or absence of aprotinin or gabexate mesylate (Foy). Incubations were performed for 5 minutes in the presence of 10^{-4} M 3-isobutyl-1-methylxanthine with 3.3 \times 10^{-7} M ANF that had been preincubated with kallikrein (3-300 \mu g/ml) for 1 hour at 37°C in the presence of 10^{-4} M aprotinin or Foy (10^{-4}-10^{-3} M). Kallikrein inhibited the stimulatory effect of ANF on cGMP levels (p<0.001), but this effect was blunted by the pretreatment of kallikrein with aprotinin or Foy. Results shown are means \pm SE of eight cultures pooled from two experiments. Single (p<0.05) and double (p<0.001) asterisks indicate significant difference compared with control values. Dagger (p<0.05), and section mark (p<0.001) indicate significant difference compared with respective values of ANF alone.

...study, the time course of ANF-stimulated cGMP levels showed a rapid elevation followed by a gradual decline after the peak concentration at 2 minutes in the absence of the phosphodiesterase inhibitor IBMX. In addition, IBMX markedly potentiated the ANF-stimulated cGMP levels. These results support the previous findings that ANF-induced intracellular accumulation of cGMP is due mainly to increased synthesis rather than inhibition of cGMP degradation by phosphodiesterase.9,11

We demonstrated that both ANF and SNP selectively increased intracellular cGMP levels without affecting cAMP levels or 6-keto-PGF_{1\alpha} synthesis, suggesting a common molecular mechanism for the vascular action of ANF and SNP. Although these results provide no direct evidence that cGMP mediates the vasorelaxant action of ANF, it is of note that the threshold concentration of the 25 amino acid ANF for cGMP stimulation in the present study was identical to that shown to relax rabbit aortic strips that were precontracted by norepinephrine.38 Several investigators have demonstrated that the vasorelaxant action of ANF, like that of SNP,14 is not dependent on the presence of vascular endothelial cells,12,15,38 and the present results in cultured vascular smooth muscle cells clearly support these findings. Also, the finding that ANF did not affect prostacyclin synthesis in vascular smooth muscle cells may be consistent with the finding that PG synthesis inhibition with indomethacin and aspirin had no inhibitory effect on renal function and the blood pressure fall induced by ANF.5 In addition, it has been reported that ANF did not change urinary excretion of PGs in the perfused, hydronephrotic rabbit kidney.4 We also have recently found that chronic infusion of ANF does not induce any changes in urinary PGE_{2} excretion in conscious rats.39 Recently, ANF has been reported to inhibit adenylate cyclase activity in several tissues, including cultured vascular smooth muscle cells.40 However, we did not observe any effect of ANF on intracellular cAMP levels in the present study, and our experience is consistent with more recent studies in aortic tissues.9,12 Further studies may be necessary to clarify these discrepancies.

Little is known about the role of calcium in ANF-induced vasorelaxation. It has been reported that the hemodynamic and natriuretic effects of atrial extracts were blunted markedly by a low calcium perfusate or verapamil in the isolated, perfused rat kidney.20 whereas no change was observed in the vasorelaxant action of ANF on norepinephrine-contracted rabbit or rat aor-
Figure 6. Effect of methylene blue on atrial natriuretic factor (ANF)-stimulated or sodium nitroprusside (SNP)-stimulated intracellular cGMP levels. Incubations were performed for 5 minutes in the presence of 10^{-6} M 3-isobutyl-1-methylxanthine with 3.3 \times 10^{-7} M ANF or 3.3 \times 10^{-6} M SNP after a 15-minute preincubation of cells in the presence or absence of methylene blue (10^{-8} - 10^{-6} M). Methylene blue inhibited SNP-stimulated cGMP but not basal or ANF-stimulated cGMP. Results shown are means ± SE of eight cultures pooled from two experiments. Single (p<0.05) and double (p<0.01) asterisks indicate significant difference compared with control values.

Figure 7. The cGMP response to atrial natriuretic factor (ANF) and sodium nitroprusside (SNP) in cultured vascular smooth muscle cells from renal and mesenteric arteries. Incubations were performed for 5 minutes in the presence of 10^{-4} M 3-isobutyl-1-methylxanthine with ANF (3.3 \times 10^{-8} - 3.3 \times 10^{-4} M) or SNP (10^{-7} - 10^{-4} M). Cells from renal artery showed greater response to both ANF and SNP than did cells from mesenteric artery. Single (p<0.05) and double (p<0.001) asterisks indicate significant differences compared with the respective values of cells from mesenteric artery. Results shown are means ± SE of eight cultures pooled from two experiments.
ANF AND cGMP IN VASCULAR SMOOTH MUSCLE/Sato et al.

FIGURE 8. The cAMP response to isoproterenol in cultured vascular smooth muscle cells from renal and mesenteric arteries. Incubations were performed for 5 minutes with isoproterenol (10⁻⁴-10⁻⁵ M) in the presence of 10⁻⁴ M 3-isobutyl-1-methylxanthine. There was no significant difference between the two cell types. Results shown are means ± SE of eight cultures pooled from two experiments.
er kallikrein is physiologically important in the vascular action of ANF, it is of note that the kallikreinlike enzyme is present in the rat mesenteric artery. Inactivation of ANF by vascular kallikreinlike enzyme may be a local mechanism for in vivo regulation of vasorelaxant activity.

Several reports indicate that renal artery is more sensitive than other arteries to the vasorelaxant action of ANF. In the present study, the cGMP response to ANF was much greater in cultured cells from renal artery than in those from mesenteric artery. The difference between two cell types may be explained by the differences in synthesis, degradation, or cGMP efflux from the cells; however, this selective cGMP response to ANF in renal arterial cells should be due mainly to the increased synthesis rather than to a difference in phosphodiesterase activity, since the differences in the cGMP response to ANF between two cell types was more prominent than that to SNP. Also, the cAMP response to isoproterenol was similar in two cell types. Although we did not elucidate the specific mechanism of this selectivity, the present results may explain the selective vasodilation of renal artery induced by ANF if cGMP mediates the vasorelaxant action of ANF.

In conclusion, ANF and SNP share a number of similarities in their effects on cultured vascular smooth muscle cells, but their responses to kallikrein and methylene blue differ. Both ANF and SNP increased the levels of intracellular cGMP. Kallikrein abolished the action of ANF but not that of SNP, whereas methylene blue inhibited the action of SNP but not that of ANF. Our results are consistent with the hypothesis that cGMP mediates the vasorelaxant action of ANF.

Acknowledgments

We are grateful for the excellent technical assistance of Kaori Matsuura, Keiko Shiraiishi, and Mayumi Nukayama and the secretarial assistance of Junko Uzakazi and Emiko Sakai.

References

38. Scivoletto R, Carvalho MHC, Cardionatin causes vasodilation in vitro which is not dependent on the presence of endothelial cells. Eur J Pharmacol 1984;101:143-145
43. Rapoport RM, Draznin MB, Murad F. Nitrovasodilator and endothelium-dependent vasodilator-induced relaxation may be mediated through cyclic GMP formation and cyclic GMP-dependent protein phosphorylation. Trans Assoc Am Physicians 1983;96:19-30
Atrial natriuretic factor and cyclic guanosine 3′,5′-monophosphate in vascular smooth muscle.
M Sato, K Abe, K Takeuchi, M Yasujima, K Omata, M Hiwatari, Y Kasai, M Tanno, M Kohzuki and K Kudo

Hypertension. 1986;8:762-771
doi: 10.1161/01.HYP.8.9.762

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/8/9/762

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension 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 Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/