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

Makito Sato, Keishi Abe, Kazuhisa Takeuchi, Minoru Yasuima, Ken Omata, Masao Hiwatari, 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 F₉α) 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 F₉α 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 seems 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),¹⁻⁵ 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...
materials and methods

Type III collagenase, deoxyribonuclease I, elastase, soybean trypsin inhibitor, calcium ionophore A23187, 3-isobutyl-1-methylxanthine (IBMX), SNP, and 6-keto-PGF$_{1\alpha}$ 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 trypsin 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 (Trasylool; 6500 KIU/mg) was a gift from Bayer AG (Leverkusen, West Germany), and angiotensin II (Hypertensin) was obtained from CIBA-Geigy ( Basel, Switzerland). Methylen 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). Antiserum for 6-keto-PGF$_{1\alpha}$ were a gift from Dr. Michael J. Dunn, Cleveland, OH, USA.
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 ethylene-glycol bis(β-aminoethyl ether)-N,N',N'-,-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 variation was 7.8% for cGMP and 8.1% for cAMP (n = 8). Interassay coefficient of variation 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 × 10⁻³ M ANF. Basal levels of 6-keto-PGF₁α (8.2 ± 0.7 pg/µg protein/30 min) were not affected by ANF (1.1 × 10⁻⁵ to 1.1 × 10⁻³ M). Although angiotensin II (10⁻⁵ M), arginine vasopressin (10⁻⁷ M), and calcium ionophore A23187 (2 × 10⁻⁴ 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 × 10⁻² 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 \((p < 0.001; \text{Figure 3})\) 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.
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 $13.9 \pm 1.6$ to $12.1 \pm 2.0$ or $13.5 \pm 2.1$ fmol/μg protein, respectively). Pretreatment of cells with $10^{-3}$ 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, $0.9 \pm 0.1$; $10^{-3}$ M kallikrein, $1.2 \pm 0.1$; $3.3 \times 10^{-4}$ M SNP, $12.4 \pm 0.5$; SNP and kallikrein, $18.0 \pm 1.3$ 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 $1.2 \pm 0.1$ or $21.4 \pm 2.1$ fmol/μg protein in cells from mesenteric artery and $1.4 \pm 0.1$ or $26.9 \pm 1.5$ fmol/μg protein 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
ANF AND cGMP IN VASCULAR SMOOTH MUSCLE/Sato et al.

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 ± 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\textsuperscript{-11}

We demonstrated that both ANF and SNP selectively increased intracellular cGMP levels without affecting cAMP levels or 6-keto-PGF\textsubscript{1α} 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\textsubscript{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\textsuperscript{-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 $10^{-4}$ 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 ($10^{-2}$-$10^{-5}$ 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 ($3.3 \times 10^{-9}$-$3.3 \times 10^{-8}$ M) or SNP ($10^{-7}$-$10^{-6}$ 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.
tic strips in a calcium-free solution. Winquist et al. found that the ANF-induced increase in cGMP levels was independent of extracellular calcium in rabbit aortic tissues. We also did not observe any change in ANF-stimulated cGMP levels in cultured vascular smooth muscle cells by changing the extracellular calcium concentration or by the addition of the calcium entry blockers verapamil or nifedipine. These results suggest that the vasorelaxant action of ANF may be independent of extracellular calciumlike nitroderivatives. However, we cannot rule out the possibility that the vasorelaxant action of ANF is dependent on the levels of intracellular calcium, since it has been recently suggested that cGMP activates the sarcolemmal calcium extrusion adenosine triphosphatase and relaxes coronary arterial smooth muscle.

Since it has been suggested that the vasorelaxant action of ANF is similar to that of SNP, methylene blue, which inhibits guanylate cyclase activation by SNP, has been used to examine the hypothesis that the guanylate cyclase-cGMP system is involved in the vasorelaxant action of ANF. Ohlstein and Berkowitz found that methylene blue inhibited the ANF-induced vascular relaxation and cGMP elevation in rabbit aortic rings, supporting the role of cGMP in the vasorelaxant action of ANF. Conversely, Garcia et al. failed to observe an inhibitory effect of methylene blue on ANF-induced rabbit mesenteric arterial relaxation, although they speculated that ANF may cause cGMP accumulation by inhibiting cGMP phosphodiesterase. In the present study, we found that methylene blue produced dose-dependent inhibition of SNP-stimulated cGMP but not of ANF-stimulated cGMP. A similar result recently was described by Winquist et al. as an unpublished observation. These results indicate that there may be different receptor sites for ANF and SNP, respectively. This view is furthermore reinforced by the findings that there was an additivity in ANF-stimulated and SNP-stimulated cGMP levels. The present findings that methylene blue did not modify ANF-stimulated cGMP may be explained by the previous reports indicating that SNP predominantly stimulates the soluble form of guanylate cyclase, whereas ANF selectively stimulates the particulate form of guanylate cyclase, which has not been shown to be inhibited by methylene blue. Thus, there may be a potential problem in interpreting experiments in which only methylene blue is employed to evaluate the role of the guanylate cyclase-cGMP system in the vasorelaxant action of ANF. Further studies are required to clarify the effect of methylene blue on particulate guanylate cyclase.

Renal kallikrein, a serine protease, has been known to play an important role in the regulation of sodium-water homeostasis. Recently, Briggs et al. reported that incubation of atrial extracts with pancreatic kallikrein or trypsin, another nonspecific serine protease, reduced the natriuretic activity of ANF, suggesting a possible role for kallikrein in peptide inactivation. Trypsin has also been demonstrated to cleave atrial extracts of the high molecular weight form to that of the low molecular weight form, which is biologically more active, and to abolish the ability of ANF to activate guanylate cyclase from kidney and aorta. In the present study, we also found that incubation of ANF or SNP with pure porcine pancreatic kallikrein abolished the ability of ANF, but not of SNP, to increase intracellular cGMP levels in cultured vascular smooth muscle cells. This effect was concentration-dependent and was inhibited by the serine protease inhibitors aprotinin or Foy, suggesting that the effect of kallikrein was due to enzymatic cleavage rather than to nonspecific effects. Although it is not known wheth-

**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 Shiraishi, and Mayumi Nukiyama and the secretarial assistance of Junko Okazaki and Emiko Sakai.

References

38. Scivoletto R, Carvalho MHC. Cardionatrin 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