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Research Article

To study vascular actions of synthetic alpha-human atrial natriuretic polypeptide (alpha hANP) in man, forearm blood flow (FBF) was measured by strain-gauge plethysmograph during the continuous infusion of 100 ng/min alpha hANP dissolved in 5% dextrose into the brachial artery in healthy subjects. alpha hANP increased FBF, with the concomitant increase in ipsilateral limb venous plasma concentrations of alpha hANP. Overall, there was a significant linear correlation between the decrements of ipsilateral forearm vascular resistance (FVR) during infusions of alpha hANP and initial FVR levels ($r = -0.883$, P less than 0.01). Moreover, alpha hANP, at the stepwise increasing doses of 20, 100, and 500 ng/min, increased FBF in a dose-related fashion: alpha hANP elicits a concentration-dependent vasodilation of forearm vascular beds. Concomitantly, infusions of alpha hANP caused a dose-dependent increase in ipsilateral limb venous plasma cyclic guanosine monophosphate (cyclic GMP). Overall, there were direct correlations of FBF either to ipsilateral venous plasma alpha hANP ($r = 0.724$, P less than 0.01) or to cyclic GMP concentrations ($r = 0.637$, P less than 0.01). Subsequently, isoosmolar CaCl₂ solution was infused into the same brachial artery at a rate of 0.09 meq/min, and then, with a 2.5 +/- 0.2-mg/dl increase in ipsilateral venous serum calcium concentrations the incremental responses of both FBF and plasma cyclic GMP to alpha hANP were severely [...]

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Vasodilatory Actions of α -Human Atrial Natriuretic Peptide and High Ca^{2+} Effects in Normal Man

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Abstract

To study vascular actions of synthetic α -human atrial natriuretic polypeptide (α hANP) in man, forearm blood flow (FBF) was measured by strain-gauge plethysmograph during the continuous infusion of 100 ng/min α hANP dissolved in 5% dextrose into the brachial artery in healthy subjects. α hANP increased FBF, with the concomitant increase in ipsilateral limb venous plasma concentrations of α hANP. Overall, there was a significant linear correlation between the decrements of ipsilateral forearm vascular resistance (FVR) during infusions of α hANP and initial FVR levels ($r = -0.883$, $P < 0.01$). Moreover, α hANP, at the stepwise increasing doses of 20, 100, and 500 ng/min, increased FBF in a dose-related fashion: α hANP elicits a concentration-dependent vasodilation of forearm vascular beds. Concomitantly, infusions of α hANP caused a dose-dependent increase in ipsilateral limb venous plasma cyclic guanosine monophosphate (cyclic GMP). Overall, there were direct correlations of FBF either to ipsilateral venous plasma α hANP ($r = 0.724$, $P < 0.01$) or to cyclic GMP concentrations ($r = 0.637$, $P < 0.01$). Subsequently, isoosmolar CaCl_2 solution was infused into the same brachial artery at a rate of 0.09 meq/min, and then, with a 2.5 ± 0.2 -mg/dl increase in ipsilateral venous serum calcium concentrations the incremental responses of both FBF and plasma cyclic GMP to α hANP were severely blunted. There was also a significant positive linear correlation between FBF and venous plasma cyclic GMP during infusions of α hANP with the simultaneous administration of CaCl_2 ($r = 0.807$, $P < 0.01$). Finally, the addition of CaCl_2 infusion did not change the slope of the regression line of the FBF-plasma cyclic GMP relationship during infusions of α hANP.

Evidence presented suggests that α hANP acts directly on the forearm vascular beds in man, eliciting its vascular relaxant effect, possibly by increasing cellular levels of cyclic GMP. Moreover, modest elevations of serum calcium inhibit the α hANP-dependent vasodilation, possibly through the suppression of cyclic GMP activation.

Introduction

Mammalian atrial extracts have been demonstrated to contain peptides with potent natriuretic and diuretic properties (1-5).

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Partial purified atrial extracts were also found to relax isolated vascular smooth muscle (4-6). Several investigators have purified, sequenced, and synthesized atrial peptides (5, 7-10). Recently, the complete sequence of α -human atrial natriuretic polypeptide (α hANP)¹ has been obtained from purified human atrial extracts (11). With the subsequent synthesis of α hANP precise investigation of the renal and vascular actions of this peptide in human beings has been possible. Intravenous administration of α hANP in normal subjects caused marked natriuresis and diuresis (12-20). But, the vasorelaxant action of this peptide has not been defined, despite the occurrence of a mild hypotension (15, 16, 19). In anesthetized rats the infusion of atrial natriuretic peptides (ANP) decreased systemic and regional vascular resistances (21, 22), but in conscious rats and man failed to decrease these vascular resistances (19, 20, 23, 24). Intravenous administration of ANP in conscious animals increased systemic and regional vascular resistances during the fall in blood pressure, possibly through reflex compensatory mechanisms, to maintain blood pressure in the face of the decreased cardiac output (23). In the present study, therefore, we have studied the possible direct effect of α hANP on forearm vascular beds in man, by measurement of forearm blood flow (FBF) followed by the intraarterial administration of synthetic α hANP at such low doses that did not alter heart rate or systemic blood pressure. This method was chosen because it was devoid of circulatory reflexes and so enabled evaluation of direct pharmacological actions on the peripheral vasculature in man (25).

It has been hypothesized that ANP-induced relaxation of vascular smooth muscle may be mediated through the formation of cyclic guanosine monophosphate (cyclic GMP) (26). This hypothesis was based upon the observation that ANP elevated cyclic GMP levels in plasma, vascular smooth muscle, and isolated renal tubules (27-30). Such increases apparently occur through direct activation of particulate guanylate cyclase, rather than inhibition of phosphodiesterase (27, 28). Thus, cyclic GMP may be a second messenger for this hormone (31). It is well established that calcium ion (Ca^{2+}) is an important regulator of hormone-sensitive adenylate cyclase and guanylate cyclase (32). Several investigators have demonstrated that high concentration of extracellular Ca^{2+} blunts the action of antidiuretic hormone, by inhibition of adenylate cyclase activation in the renal tubules (33) and in the toad bladder (34). Moreover, it has been reported that high extracellular Ca^{2+} sharply inhibits particulate guanylate cyclase activity in the heart and lung (35, 36). Therefore, we studied the effect of increasing Ca^{2+} concentration in forearm arterial blood on vasorelaxation and cyclic GMP formation in forearm vascular beds at several concentrations of α hANP.

1. Abbreviations used in this paper: α hANP, α -human atrial natriuretic polypeptide; ANP, atrial natriuretic polypeptide; FBF, forearm blood flow; FVR, forearm vascular resistance.

Methods

All 31 subjects were healthy (5 women, 26 men), young (18–22 yr), Japanese students at University of Tsukuba and were fully informed of the purposes, procedures, and hazards of the experiment; written consent was obtained. These volunteers were studied in the resting, postabsorptive state in an air-conditioned laboratory, with ambient temperature ranging from 24° to 27°C. With the subject comfortable in the supine position and his arms supported at a 45° angle from the long axis of the body, 19-gauge hypodermic needles with attached plastic tubing were inserted in an upstream direction into an antecubital vein of one arm (“ipsilateral” arm) distal to the elbow. Under local xylocaine anesthesia the ipsilateral brachial arteries were also cannulated in an upstream direction with 20-gauge arterial needles. To improve mixing of infusate with brachial arterial blood a jet-injector needle was used for the intraarterial infusions. Infusion rate was 1.0 ml/min (Harvard mini-pump, Harvard Apparatus Co., Natick, MA). This jet-injection system improves mixing of infused substances with brachial arterial blood at infusate kinetic energies too low to cause significant hemolysis. The jet needle was introduced into the brachial artery through the arterial needle, an adaptor on the jet needle hub locking into the hub of the arterial needle. The tip of the jet needle usually protruded up to but not proximal to the intercondylar line at the elbow. To center the jet in the arterial lumen, the position of the jet needle was manipulated to obtain the lowest possible infusion pressure, usually not more than 200 mmHg above pressure during jet infusion into air.

FBF was measured by a plethysmographic technique, as described previously (37, 38). Changes in forearm blood volume were determined by means of a mercury in rubber strain-gauge plethysmograph (39) placed on the midforearm. Strain-gauge was mounted so that its maximal tension was < 10 g, to prevent the gauge from obstructing even the superficial veins beneath it. To eliminate the vessels in the hand from these determinations, a sphygmomanometric cuff 7-cm wide was placed around the wrist and inflated to a level exceeding systolic arterial pressure just before each venous occlusion. A sphygmomanometric cuff 13-cm wide was placed around the upper arm, and forearm venous occlusion was produced by suddenly inflating this cuff to a pressure below the diastolic arterial pressure (40 mmHg) (40), utilizing a tank of compressed air to provide a constant pressure source. FBF was taken as the average of four to eight flow measurements made at 15-s intervals. Calculation of FBF was done independently by two of the authors from the copied records, and the average value was used for statistical analysis. The blood pressure was measured in the other arm with a sphygmomanometer. FBF, expressed as milliliters per 100 ml of forearm volume per minute, was calculated from the change in forearm circumference during venous occlusion. Forearm vascular resistance (FVR) was calculated by dividing mean arterial pressure (diastolic pressure plus one third of the pulse pressure in millimeters of mercury) by forearm blood flow (milliliters per 100 ml of forearm volume per minute); these values are expressed as “units” throughout this report. The study consisted of four interrelated protocols (Fig. 1), each of which is described in detail below.

FBF response to α hANP infusion (protocol I; Fig. 1). 16 normal subjects were studied. After placing catheters and a strain-gauge plethysmograph, at least 15 min were allowed for each subject to become accustomed to the study conditions before beginning the protocol. Control solution was composed of 5% dextrose. The experimental solution containing synthetic α hANP was brought to volume with 5% dextrose. With the subject comfortable in the supine position, control solution was infused for 10 min, followed by infusions of solution containing α hANP delivered 100 ng/min into brachial arterial blood for 10 min. During the last 2 min of each infusion, FBF was measured by strain-gauge plethysmograph. Immediately before the α hANP infusion and at the end of the infusion, we sampled the ipsilateral and contralateral venous blood for the measurement of α hANP into the tube containing EDTA · 2Na (1 mg/ml) and Trasylol (500 KIU/ml).

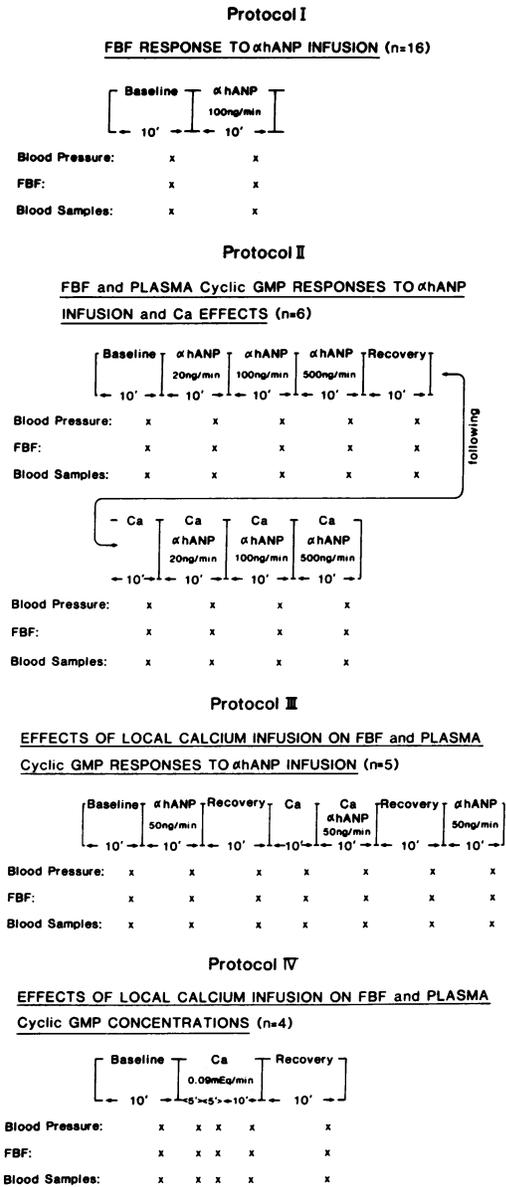


Figure 1. Schematic representation of the protocol design.

FBF and plasma cyclic GMP responses to α hANP infusion and calcium effects (protocol II; Fig. 1). Six normal subjects were admitted to the trial. After the infusion of control solution, α hANP, at stepwise increasing rates of 20, 100, and 500 ng/min, was infused into brachial arterial blood for each 10 min. Subsequently, control solution was again infused for 10 min, and then isoosmolar CaCl_2 solution was infused into brachial arterial blood at a rate of 0.09 meq/min for 10 min, followed by infusions of the mixed solutions containing 20 ng/min α hANP and 0.09 meq/min CaCl_2 delivered into brachial arterial blood, 100 ng/min α hANP and 0.09 meq/min CaCl_2 , and 500 ng/min α hANP and 0.09 meq/min CaCl_2 , respectively. The effect of each infusion of α hANP was compared with forearm hemodynamics measured during a preceding paired infusion of control or CaCl_2 solution. As described in protocol I, at each infusion FBF was measured and venous sampling for measurements of calcium, α hANP and cyclic GMP was performed. Plasma samples for the measurement of cyclic GMP were taken with EDTA 2K (1 mg/ml).

Effects of local calcium infusion on FBF and plasma cyclic GMP responses to α hANP (protocol III; Fig. 1). Five normal subjects were

studied. To examine whether the vascular responses to α hANP can be restored to normal after the discontinuation of CaCl_2 infusion, the following study was performed. The control solution was infused for 10 min, followed by the infusion of the solution which contains α hANP delivered 50 ng/min into brachial arterial blood. After the infusion of this solution, control solution was infused for 10 min, and then isoosmolar CaCl_2 solution was infused into brachial arterial blood at a rate of 0.09 meq/min for 10 min, followed by the infusion of the mixed solution containing CaCl_2 and α hANP delivered 0.09 meq/min and 50 ng/min into brachial arterial blood for each 10 min, respectively. Thereafter, control solution was again infused for 10 min, followed by the infusion of α hANP without CaCl_2 delivered 50 ng/min into brachial arterial blood. The effect of each α hANP infusion was compared with hemodynamics measured during a preceding paired infusion. As described in protocol I, at each infusion FBF was measured and venous sampling for measurements of calcium and cyclic GMP was performed.

Effects of local calcium infusion on FBF and plasma cyclic GMP concentrations (protocol IV; Fig. 1). Four normal subjects were studied. To examine the effect of high Ca^{2+} on FBF and plasma cyclic GMP, isoosmolar CaCl_2 was infused into brachial arterial blood at a rate of 0.09 meq/min for 20 min. FBF was measured immediately before the infusion of CaCl_2 , at 5, 10 and 20 min of CaCl_2 infusion, and at 10 min after switching it to the infusion of 5% dextrose. Venous sampling for measurement for cyclic GMP was performed before, at 10 and 20 min during, and at 10 min after the infusion of CaCl_2 .

Analytical methods. Serum calcium was measured by autoanalyzer. Plasma cyclic GMP was measured by radioimmunoassay (41). Radioimmunoassays of cyclic GMP were done in duplicate after succinylation. Plasma α hANP concentration was determined by radioimmunoassay, as described previously (42). Briefly, radioimmunoassay of α hANP was performed using an antibody generated in NZW rabbits immunized with α hANP conjugated with bovine thyroglobulin. The standard buffer was 0.05 M sodium phosphate buffer, pH 7.4, containing 1% bovine serum albumin (BSA), 0.1% Triton X-100, 0.08 M NaCl, 0.025 M EDTA 2Na, 0.05% NaN_3 , and Trasyloy 500 KIU/ml. ^{125}I - α hANP was used as a tracer (Amersham Japan, Tokyo, Japan) and synthetic α hANP synthesized in our laboratory (11) was used to construct standard curves. The standard α hANP or the unknown sample (100 μ l) was incubated with anti- α hANP antiserum diluent (500 μ l) for 12 h at 4°C. Then, the tracer solution (18,000–20,000 cpm in 200 μ l) was added. After the incubation for 36 h at 4°C, anti-rabbit IgG goat serum diluent (200 μ l) was added. After kept standing for 40 h at 4°C, the tubes were centrifuged at 2,000 \times 30 min at 4°C. Using this proce-

Table I. Systemic and Forearm Hemodynamics, and Plasma α hANP Concentrations before and during α hANP Infusion at a Rate of 100 ng/min in 16 Normal Subjects (Mean \pm SEM)

	Control*	ANP 100
Blood pressure, mmHg		
Systolic	114 \pm 3	114 \pm 4
Diastolic	66 \pm 1	65 \pm 2
Heart rate, bpm [†]	61 \pm 2	62 \pm 3
Forearm blood flow, ml/100 ml/min	2.61 \pm 0.25	4.82 \pm 0.48 [§]
Forearm vascular resistance, U	36.8 \pm 3.6	18.4 \pm 1.6 [§]
Ipsilateral α hANP, pg/ml	106 \pm 14	649 \pm 54 [§]
Contralateral α hANP, pg/ml	101 \pm 13	107 \pm 18

* Control, during the infusion of 5% dextrose; ANP 100, during the infusion of 100 ng/min α hANP.

[†] bpm, beats per min.

[§] $P < 0.01$ vs. control.

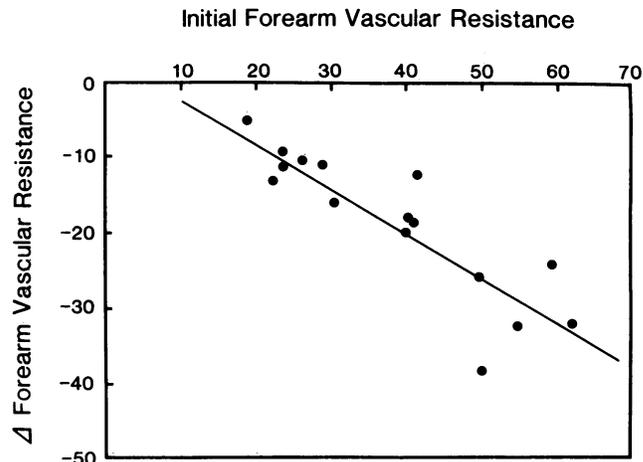


Figure 2. Regression analysis between the decrements of FVR (units) during the infusion of 100 ng/min α hANP and initial levels of FVR (units) in 16 normal volunteers ($r = -0.883$, $P < 0.01$).

dure, the lowest concentration of α hANP yielding a binding significantly different from that in the absence of standard at the 95% confidence interval was 2.5 pg/tube. The 50% intercept was at 59 pg/tube. The interassay variation was 16.8% ($n = 6$), the intraassay variation 7.4% ($n = 10$). The antiserum shows 100% crossreactivity with β hANP and γ hANP.

Statistical analysis. All data are expressed as the mean \pm SEM. Analysis variance for repeated measures, with a post-hoc Dunnett's test, was used to compare observed changes versus the baseline (43). One-way analysis variance, with Bonferroni adjustment of the P value, was used when comparing the changes in FBF and plasma cyclic GMP with α hANP before CaCl_2 infusion, and after CaCl_2 infusion. Paired t test was used to test the significance of single comparisons. Since natural logarithmic transformation rather than absolute values followed a Gaussian distribution, the natural logarithmic transformation of α hANP levels was used for statistical analysis. Regression analysis was performed according to standard procedures. Changes were reported as significant if $P < 0.05$.

Results

FBF response to α hANP infusion. The forearm hemodynamic effects of the intrabrachial arterial infusions of α hANP are presented in Table I. Neither control nor experimental infusions had significant effects on systemic blood pressure or heart rate in normal subjects. Infusion of 100 ng/min α hANP increased FBF from 2.61 \pm 0.25 to 4.82 \pm 0.48 ml/100 ml per min ($P < 0.01$, by paired t test), and decreased FVR from 36.8 \pm 3.6 to 18.4 \pm 1.6 U ($P < 0.01$). Concomitantly, ipsilateral venous plasma α hANP concentrations increased from 106 \pm 14 to 649 \pm 54 pg/ml ($P < 0.01$), without a significant change in contralateral venous plasma α hANP (107 \pm 18 vs. 101 \pm 13 pg/ml, NS). During infusions of α hANP, systemic blood pressure and heart rate remained unchanged. In all 16 subjects, there was a significant linear correlation between the decrements in FVR during infusions of 100 ng/min α hANP and initial FVR levels ($r = -0.883$, $P < 0.01$) (Fig. 2).

FBF and plasma cyclic GMP responses to α hANP infusion and calcium effects. Infusions of 20, 100, and 500 ng/min α hANP increased FBF from 2.39 \pm 0.35 to 3.05 \pm 0.33 (NS), 5.23 \pm 0.60 ($P < 0.01$) and 6.24 \pm 0.64 ($P < 0.01$) ml/100 ml per min, respectively (Table II); FVR decreased from 39.8 \pm 6.2 to

Table II. Effects of CaCl₂ (0.09 meq/min) on Systemic and Forearm Hemodynamics, and Biochemical Parameters during α hANP Infusions at Rates of 20, 100, and 500 ng/min in Six Normal Subjects (Mean \pm SEM)

	Control-1*	ANP 20	ANP 100	ANP 500	Control-2	Ca	Ca + ANP 20	Ca + ANP 100	Ca + ANP 500
Blood pressure, mmHg									
Systolic	111 \pm 3	112 \pm 3	110 \pm 2	110 \pm 2	110 \pm 3	110 \pm 3	110 \pm 2	109 \pm 2	109 \pm 2
Diastolic	66 \pm 3	65 \pm 3	64 \pm 2	64 \pm 2	64 \pm 2	64 \pm 2	64 \pm 2	63 \pm 3	64 \pm 2
Heart rate, bpm	59 \pm 1	58 \pm 1	58 \pm 1	58 \pm 1	58 \pm 1	57 \pm 1	57 \pm 1	58 \pm 1	57 \pm 1
FBF, ml/100 ml/min	2.39 \pm 0.35	3.05 \pm 0.33	5.23 \pm 0.60 [‡]	6.24 \pm 0.64 [‡]	4.06 \pm 0.69	3.06 \pm 0.58	2.92 \pm 0.56	3.21 \pm 0.56	4.15 \pm 0.76
FVR, U	39.8 \pm 6.2	29.8 \pm 4.4	17.8 \pm 2.9 [‡]	14.7 \pm 2.4 [‡]	25.5 \pm 5.3	33.9 \pm 7.7	40.7 \pm 8.8	32.2 \pm 6.4	26.4 \pm 6.5
Ipsilateral α hANP, pg/ml	88 \pm 23	134 \pm 26	608 \pm 48 [‡]	7,160 \pm 1,471 [†]	106 \pm 23	96 \pm 23	161 \pm 30 [§]	743 \pm 66 [§]	8,200 \pm 1,385 [§]
Contralateral α hANP, pg/ml	90 \pm 25	92 \pm 20	94 \pm 22	96 \pm 30	100 \pm 24	96 \pm 24	100 \pm 25	101 \pm 25	103 \pm 32
Ipsilateral cGMP, pmol/ml	3.15 \pm 1.05	4.45 \pm 1.57	5.30 \pm 1.51	8.92 \pm 3.06 [†]	7.05 \pm 1.81	3.57 \pm 0.90	3.65 \pm 0.77	3.90 \pm 1.56	4.41 \pm 0.84
Contralateral cGMP, pmol/ml	3.20 \pm 1.02	3.21 \pm 1.12	3.25 \pm 1.34	3.35 \pm 1.12	3.30 \pm 1.32	3.30 \pm 0.88	3.32 \pm 0.94	3.34 \pm 0.86	3.30 \pm 1.24
Ipsilateral Ca, mg/dl	9.5 \pm 0.2	9.4 \pm 0.2	9.4 \pm 0.2	9.1 \pm 0.2	9.2 \pm 0.2	11.4 \pm 0.3 [†]	11.9 \pm 0.3 [†]	12.0 \pm 0.4 [†]	12.0 \pm 0.3 [†]
Contralateral Ca, mg/dl	9.5 \pm 0.2	9.4 \pm 0.2	9.4 \pm 0.2	9.3 \pm 0.2	9.2 \pm 0.2	9.4 \pm 0.2	9.5 \pm 0.2	9.4 \pm 0.2	9.5 \pm 0.2

* Control-1 and control-2, during the infusions of 5% dextrose; ANP 20, during the infusion of 20 ng/min α hANP; Ca, during the infusion of 0.09 meq/min CaCl₂; Ca + ANP 20, during the infusions of 0.09 meq/min CaCl₂ and 20 ng/min α hANP. [†] $P < 0.01$ vs. the value of control-1. [‡] $P < 0.01$ vs. the value of control-1. [§] $P < 0.01$ vs. the value of Ca. ^{||} Ipsilateral cGMP, ipsilateral venous plasma cyclic GMP concentrations; contralateral cGMP, contralateral venous plasma cyclic GMP concentrations; ipsilateral Ca, ipsilateral serum calcium concentrations; contralateral Ca, contralateral serum calcium concentrations. [†] $P < 0.05$ vs. the value of control-1.

29.8 \pm 4.4 (NS), 17.8 \pm 2.9 ($P < 0.01$) and 14.7 \pm 2.4 ($P < 0.01$) U, respectively (Table II). Thus, α hANP elicits a concentration-dependent vasodilation of forearm vascular beds. Concomitantly, ipsilateral venous plasma α hANP concentrations increased from 88 \pm 23 to 134 \pm 26 (NS), 608 \pm 48 ($P < 0.01$) and 7,160 \pm 1,471 ($P < 0.01$) pg/ml, respectively (Table II). Overall, there was a significant positive correlation between FBF and ipsilateral venous plasma α hANP concentrations ($r = 0.724$, $P < 0.01$) (Fig. 3). Ipsilateral venous plasma cyclic GMP increased from 3.15 \pm 1.05 to 4.45 \pm 1.57 (NS), 5.30 \pm 1.51 (0.05 $< P < 0.1$), and 8.92 \pm 3.06 ($P < 0.05$) pmol/ml; thus indicating that α hANP caused a dose-dependent cyclic GMP increase. Moreover, the relaxant effect of α hANP was directly correlated to ipsilateral venous plasma cyclic GMP concentrations ($r = 0.637$, $P < 0.01$) (Fig. 4). During the intrabrachial infusion of CaCl₂, FBF was depressed with the accompanying decrease in ipsilateral plasma cyclic GMP (Table II). Ipsilateral venous serum calcium concentrations increased from 9.2 \pm 0.2 to 11.4 \pm 0.3 mg/dl ($P < 0.01$), but contralateral serum calcium did not change during infusions of CaCl₂. Thereafter, infusions of 20, 100, and 500 ng/min α hANP with the simultaneous infusion of CaCl₂ did not significantly increase FBF: from a baseline value of 3.06 \pm 0.58 to 2.92 \pm 0.56 (NS), 3.21 \pm 0.56 (NS), and 4.15 \pm 0.76 (NS) ml/100 ml per min, respectively (Table II). Thus, the increments of FBF with α hANP infusions were significantly less with the simultaneous infusion of CaCl₂ as compared to those without CaCl₂: -4.6 \pm 3.4% vs. 23.6 \pm 10.5% (NS) during the infusion of 20 ng/min α hANP, 8.1 \pm 6.7% vs. 133.3 \pm 29.0% ($P < 0.05$) during 100 ng/min α hANP, and 36.2 \pm 5.7% vs. 181.1 \pm 37.1% ($P < 0.05$) during 500 ng/min α hANP (Fig. 5). Accordingly, with the simultaneous infusion of CaCl₂, infusions of 20, 100, and 500 ng/min α hANP did not significantly increase ipsilateral venous plasma cyclic GMP concentrations: from a baseline value of 3.57 \pm 0.90 to 3.65 \pm 0.77 (NS), 3.90 \pm 1.56 (NS), and 4.41 \pm 0.84 (NS) pmol/ml, respectively (Table II): the increments of ipsilateral venous plasma cyclic GMP with α hANP were significantly less with the simultaneous infusion of CaCl₂ as compared with those without CaCl₂: 0.07 \pm 0.63 vs. 1.30 \pm 0.93 pmol/ml (NS), 0.33 \pm 0.62 vs. 2.16 \pm 0.64 pmol/ml (NS), and 0.84 \pm 0.44 vs. 5.77 \pm 1.89 pmol/ml ($P < 0.05$) during infusions of 20, 100, and 500 ng/min α hANP, respectively (Fig. 6). Thus, the simultaneous infusion of CaCl₂ blunted the responses of both FBF and plasma cyclic GMP to α hANP. The magnitude of the increases in ipsilateral venous plasma α hANP concentrations during the α hANP infusion did not differ between with and without CaCl₂ (Table II). Contralateral venous serum calcium concentrations did not change. Neither did contralateral venous plasma cyclic GMP change. Throughout the study, systemic blood pressure or heart rate did not change (Table II).

Finally, there were significant correlations of FBF either to ipsilateral venous plasma cyclic GMP concentrations during infusions of α hANP without CaCl₂ or to cyclic GMP during infusions of α hANP with CaCl₂ (Fig. 4). Fig. 4 shows no significant difference in the slope between the two regression lines of the FBF-cyclic GMP relationship during infusion of α hANP. Thus, it is suggested that the presence of modest increases in serum calcium results in a blunting of the vasodilator effects of α hANP as well as a blunting of the secondary increase of cyclic GMP, but does not affect the vasodilator responses to the cyclic GMP increase.

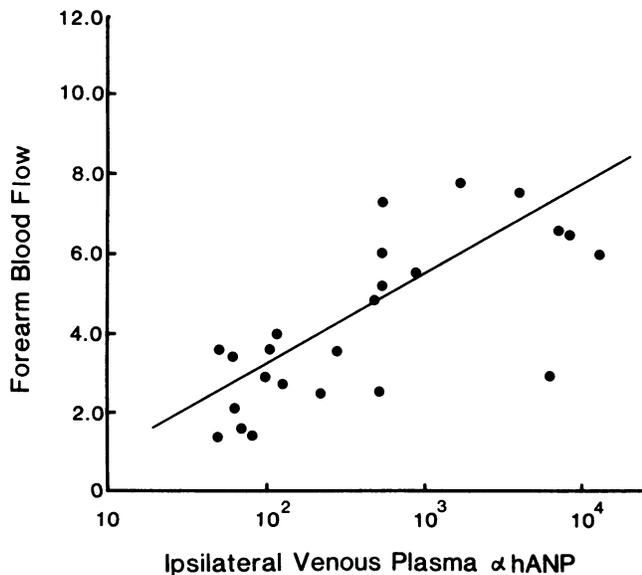


Figure 3. Regression analysis between forearm blood flow (ml/100 ml per min) and ipsilateral venous plasma α hANP concentrations (pg/ml) during the infusions of α hANP at stepwise increasing doses of 20, 100, and 500 ng/min for each 10 min ($r = 0.724$, $P < 0.01$).

Effects of local calcium infusion on FBF and plasma cyclic GMP responses to α hANP. Infusion of 50 ng/min α hANP increased FBF from 2.89 ± 0.75 to 4.21 ± 0.80 ml/100 ml per min ($P < 0.05$) (Table III). The simultaneous infusion of CaCl_2 and α hANP caused an insignificant increase in FBF with α hANP: from 1.62 ± 0.39 to 1.78 ± 0.28 ml/100 ml per min (NS). After the discontinuation of CaCl_2 infusion, however, α hANP pro-

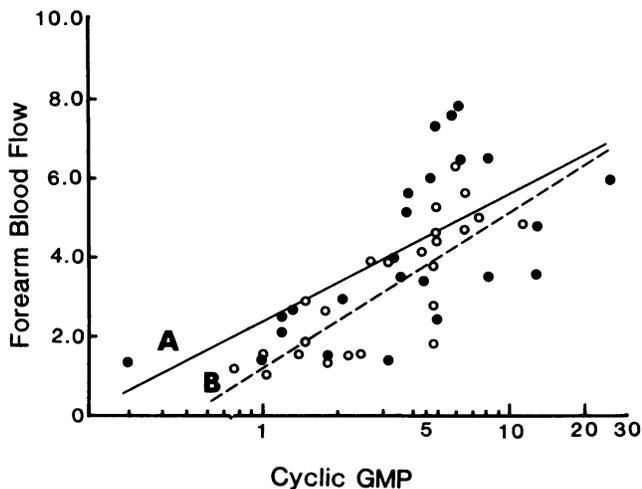


Figure 4. (A) Regression analysis of FBF (ml/100 ml per min) to ipsilateral venous plasma cyclic GMP concentrations (pmol/ml) during the infusions of α hANP at stepwise increasing doses of 20, 100, and 500 ng/min without CaCl_2 (closed circles) ($r = 0.637$, $P < 0.01$), and with the addition of isoosmolar CaCl_2 at a rate of 0.09 meq/min (open circles) ($r = 0.807$, $P < 0.01$). A straight line (A) denotes the regression line of the FBF-plasma cyclic GMP relationship during infusions of α hANP without CaCl_2 ; a dotted line (B), the regression line during the simultaneous infusions of α hANP and CaCl_2 . Note that there was no significant difference in the slope between these two regression lines of the FBF-plasma cyclic GMP relationship.

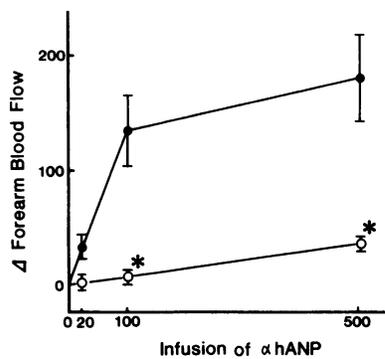


Figure 5. Changes (mean \pm SEM) in FBF (%) during the infusions of the stepwise increasing doses of α hANP with (open circles) and without (closed circles) the simultaneous infusion of CaCl_2 . * $P < 0.05$ vs. the respective value without CaCl_2 .

duced the significant increase in FBF again: from 2.33 ± 0.50 to 3.53 ± 0.71 ml/100 ml per min ($P < 0.05$). With the simultaneous infusion of CaCl_2 , percent increments of FBF during infusions of α hANP were significantly smaller ($20.9 \pm 15.9\%$) as compared with those before ($58.1 \pm 13.5\%$, $P < 0.05$) and after CaCl_2 infusion ($55.5 \pm 17.4\%$, $P < 0.05$) (Fig. 7). Infusion of 50 ng/min α hANP increased ipsilateral venous plasma cyclic GMP from 3.20 ± 0.44 to 5.10 ± 0.82 pmol/ml ($P < 0.05$), from 3.90 ± 0.94 to 4.70 ± 0.85 pmol/ml (NS), and from 4.20 ± 0.78 to 5.84 ± 0.67 pmol/ml ($P < 0.05$), before, during and after the simultaneous infusion of CaCl_2 , respectively (Table III); the increments of cyclic GMP with α hANP were significantly less during the simultaneous infusion of CaCl_2 (0.80 ± 0.52 pmol/ml) as compared to both those before CaCl_2 infusion (1.90 ± 0.92 pmol/ml, $P < 0.05$, by paired t test) and those after CaCl_2 infusion (1.64 ± 0.64 pmol/ml, $P < 0.05$). Thus, the administration of CaCl_2 inhibited the vascular actions of α hANP, and after the discontinuation of CaCl_2 infusion the blunted responses of FBF and cyclic GMP to α hANP were restored to normal.

Throughout the study, systemic blood pressure and heart rate did not change. Contralateral venous plasma cyclic GMP did not change during infusions of α hANP (Table III).

Effects of local calcium infusion on FBF and plasma cyclic GMP concentration. During the infusion of CaCl_2 , FBF was significantly decreased from 3.11 ± 0.59 to 2.27 ± 0.52 ml/100 ml per min (NS) at 5 min of CaCl_2 infusion, to 2.10 ± 0.36 ml/100 ml/min ($P < 0.05$) at 10 min of the infusion, and to 2.09 ± 0.46 ml/100 ml/min ($P < 0.05$) at 20 min of CaCl_2

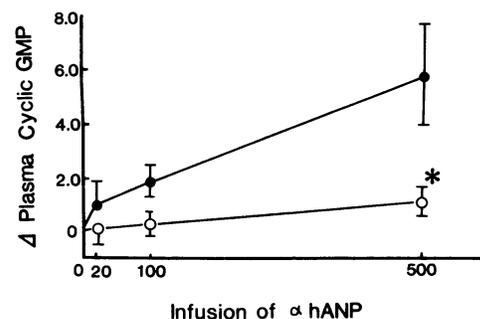


Figure 6. Changes (mean \pm SEM) in ipsilateral venous plasma cyclic GMP (pmol/ml) during the infusions of α hANP at the stepwise increasing doses of 20, 100, and 500 ng/min with (open circles) and without (closed circles) the simultaneous infusion of CaCl_2 . * $P < 0.05$ vs. the value during the infusion of 500 ng/min α hANP without CaCl_2 .

Table III. Systemic and Forearm Hemodynamics, and Biochemical Parameters during α hANP Infusion at a Rate of 50 ng/min before, during, and after the Simultaneous Infusion of CaCl_2 at a Rate of 0.09 meq/min in Five Normal Subjects (Mean \pm SEM)

	Control-1*	ANP 50	Control-2	Ca	Ca + ANP 50	Control-3	ANP 50
Blood pressure, mmHg							
Systolic	124 \pm 5	122 \pm 5	122 \pm 4	122 \pm 3	122 \pm 4	122 \pm 4	121 \pm 4
Diastolic	70 \pm 3	69 \pm 3	70 \pm 3	70 \pm 3	69 \pm 3	69 \pm 3	69 \pm 3
Heart rate, bpm	66 \pm 2	66 \pm 2	65 \pm 3	64 \pm 2	64 \pm 2	65 \pm 2	64 \pm 2
FBF, ml/100 ml/min	2.89 \pm 0.75	4.21 \pm 0.80 [‡]	2.60 \pm 0.53	1.62 \pm 0.39	1.78 \pm 0.28	2.33 \pm 0.50	3.53 \pm 0.71 [§]
FVR, U	42.2 \pm 8.1	26.3 \pm 7.4 [‡]	42.0 \pm 10.1	66.4 \pm 13.0	57.2 \pm 12.2	50.3 \pm 14.3	34.5 \pm 11.8 [§]
Ipsilateral cGMP	3.20 \pm 0.44	5.10 \pm 0.82 [‡]	4.31 \pm 0.84	3.90 \pm 0.94	4.70 \pm 0.85	4.20 \pm 0.78	5.84 \pm 0.67 [§]
Contralateral cGMP	3.10 \pm 0.32	3.23 \pm 0.54	3.21 \pm 0.64	3.10 \pm 0.61	3.23 \pm 0.43	3.21 \pm 0.41	3.35 \pm 0.46
Ipsilateral Ca, mg/dl	9.4 \pm 0.2	9.3 \pm 0.2	9.1 \pm 0.2	11.3 \pm 0.3	11.9 \pm 0.3	9.4 \pm 0.2	9.4 \pm 0.1
Contralateral Ca, mg/dl	9.3 \pm 0.1	9.2 \pm 0.3	9.2 \pm 0.2	9.2 \pm 0.1	9.4 \pm 0.1	9.4 \pm 0.1	9.3 \pm 0.1

* Control-1 and control-2, during the infusions of 5% dextrose; ANP 50, during the infusion of isosmolar CaCl_2 (0.09 meq/min); Ca + ANP 50, during the infusion of 0.09 meq/min CaCl_2 and 20 ng/min α hANP. [‡] $P < 0.05$ vs. the value of control-1. [§] $P < 0.05$ vs. the value of control-3. ^{||} $P < 0.01$ vs. the value of control-1.

infusion (Table IV). At 10 min after switching the CaCl_2 infusion to 5% dextrose infusion, FBF restored toward normal: 2.86 \pm 0.61 ml/100 ml per min. In contrast to the CaCl_2 -induced decrease in FBF, plasma cyclic GMP concentrations did not change throughout the infusion of CaCl_2 .

Discussion

The results of the present studies indicate that in man intraarterial infusions of synthetic α hANP decrease FVR and increase FBF in a dose-related fashion. Thus, α hANP elicits a concentration-dependent vasodilation of forearm vascular beds. Previous studies showed that pronounced natriuresis and diuresis and a mild hypotension occurred when α hANP was intravenously injected in animals (1–6) and man (12–20). The depressor actions of synthetic α hANP were thought to be mediated through vasodilation, since several investigators have demonstrated that ANP are potent relaxants of norepinephrine-constricted aortic-strips or are dilators of renal blood vessels in isolated perfused rat kidneys that are constricted by norepinephrine (6, 44). However, the continuous intravenous infusion of synthetic ANP in conscious rats failed to decrease systemic and regional vascular resistances, rather accompanied by the increase in vascular resistances and the decrease in cardiac output (23, 24), although in anesthetized rats the administration of ANP decreased systemic and regional vascular resistances (21, 22). Thus, in conscious rats these vascular resistances were not decreased, possibly through reflex compensatory mechanisms, in order to maintain arterial pressure in

the face of decreased cardiac output (23). Similar hemodynamic changes with infusions of α hANP have been recently demonstrated in man; the intravenous infusion of α hANP decreased blood pressure, associated with a moderate decrease in renal blood flow (19, 20). Because it is devoid of circulatory reflexes, in the present study α hANP was infused into the brachial artery at doses so low that they did not alter heart rate or systemic arterial pressure, and so we could evaluate direct pharmacological actions of α hANP on the forearm vascular beds in man. Thus, it suggests that α hANP elicits a pronounced vascular relaxant effect in man, which is consistent with the result of the recent study using a similar intraarterial model (45).

A significant positive linear correlation between level of initial limb vascular resistance and magnitude of limb vascular response to vasodilator agents has been reported (25). In the present study there was a similar significant correlation between initial resistance and magnitude of dilator response to α hANP. The fact that the assumption of linearity was correct for the case of α hANP is consistent with the hypothesis that α hANP is a true vasodilator agent.

The second important observation is that intrabrachial infusion of α hANP at the stepwise increasing rates induced the dose-dependent increase in ipsilateral venous plasma cyclic GMP, indicating α hANP-dependent cellular cyclic GMP formation. In the present study, moreover, there appeared to be an intimate relationship between the increase in cyclic GMP production and the vascular relaxation induced by α hANP, since there was a significant positive correlation between FBF and ipsilateral venous plasma levels of cyclic GMP during infusions of α hANP. It is consistent with the result of in vitro studies that α hANP caused a time-dependent and concentration-dependent increase in tissue levels of cyclic GMP that corresponded with vascular relaxation in the same vascular smooth muscle of the rabbit thoracic aorta (29). Thus, cyclic GMP is thought to mediate vascular relaxation induced by ANP (26–31). Furthermore, the increase in cellular cyclic GMP formation is apparently due to an ANP-induced activation of particulate guanylate cyclase rather than to an inhibition of phosphodiesterases, since ANP-induced vascular relaxation and elevation of cyclic GMP levels could be inhibited by

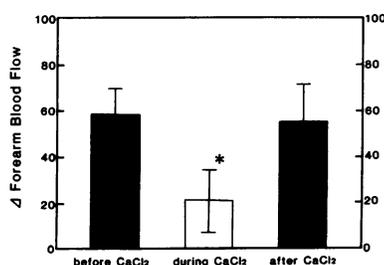


Figure 7. Changes in FBF (%) with infusions of 50 ng/min α hANP, before, during, and after the simultaneous infusion of CaCl_2 (mean \pm SEM). * $P < 0.05$ vs. both before CaCl_2 infusion and after the discontinuation of CaCl_2 infusion.

Table IV. Effects of CaCl₂ Infusions (0.09 meq/min) on FBF and Plasma Cyclic GMP (Mean±SEM)

	Control*	Ca-5 min	Ca-10 min	Ca-20 min	Recovery
FBF, ml/100 ml/min	3.11±0.59	2.27±0.52	2.10±0.36 [‡]	2.09±0.46 [‡]	2.86±0.61
Ipsilateral cGMP, pmol/ml	4.83±1.04	ND	4.26±0.68	4.59±0.92	4.80±0.96
Contralateral cGMP	4.94±1.27	ND	4.56±0.88	4.63±0.99	4.62±0.99

* Control, during the infusion of 5% dextrose; Ca-5 min, at 5 min of CaCl₂ infusion; Ca-10 min, at 10 min of CaCl₂ infusion; Ca-20 min, at 20 min of CaCl₂ infusion; recovery, 10 min after the discontinuation of CaCl₂ infusion. [‡] *P* < 0.05 vs. control.

the guanylate cyclase inhibitor methylene blue, but not inhibited by the phosphodiesterase inhibitor M&B 22,948 (27, 28, 30). Supporting this hypothesis, Garcia et al. have demonstrated that lack of Ca²⁺ in the extracellular medium did not prevent the relaxing effect of ANP on rat renal arterial strips previously made to contract by application of norepinephrine or angiotensin II (46). Moreover, the vasorelaxant effect of ANP in isolated kidneys perfused in the presence of vasoconstrictors is at least partially independent of extracellular Ca²⁺ (47). These findings suggest that the effect of ANP is not mediated by an impairment of Ca²⁺ influx from the extracellular medium. In addition, Winquist et al. have suggested that ANP increase cyclic GMP independently of extracellular Ca²⁺, since the ANP-induced increases in cyclic GMP were observed in isolated rabbit aortic segments pretreated with calcium-free buffer (28). Thus, similarly to other vasorelaxants such as nitroprusside, it is possible that cyclic GMP is the second messenger of the ANP-induced vasorelaxant effect (48). This hypothesis is supported by the results of the present study that the CaCl₂-induced inhibition of the ANP-dependent vascular relaxation was accompanied by the attenuation of increases in plasma cyclic GMP, which might reflect cellular cyclic GMP formation in vascular smooth muscle.

Regarding high extracellular Ca²⁺ effects on the vascular actions of αhANP, in the present study one should consider the down regulatory mechanisms (49); the blunted vascular response to the infusion of αhANP with CaCl₂ might be influenced by the preceding infusion of αhANP. However, ipsilateral venous plasma αhANP concentrations immediately before the second infusion of αhANP with CaCl₂ were almost same as those before the first infusion of αhANP without CaCl₂. Finally, after the discontinuation of the αhANP and CaCl₂ infusion these blunted responses of vasodilation and cyclic GMP formation to αhANP infusion were restored to normal. Therefore, high Ca²⁺ effects on the vascular actions of αhANP should not be explained by "down regulation" of vascular αhANP receptors but be attributed to the action of Ca²⁺ per se. Since Ca²⁺ has a direct effect upon insulin receptor binding and affinity (50), there are some possibilities that high Ca²⁺ may change the affinity of αhANP receptors, which have a dissociation constant consistent with the concentration of αhANP that causes cyclic GMP formation and vasorelaxation (51), and thus attenuate the αhANP-induced increase in cellular cyclic GMP production and the vascular relaxation. However, there is no evidence about the modulating effect of extracellular Ca²⁺ on vascular ANP-receptor.

It is established that Ca²⁺ is an important regulator of hormone-sensitive adenylate cyclase and guanylate cyclase (32). Several investigators have described that high concentration of extracellular Ca²⁺ sharply inhibited particulate guanylate cy-

clase activity in the heart and lung (35, 36). Moreover, high Ca²⁺ blunted the action of antidiuretic hormone, by inhibition of adenylate cyclase activation in the renal tubules (33, 34). Therefore, it is suggested that high Ca²⁺ might suppress αhANP-dependent cyclic GMP production in the smooth muscle cell, through the inhibition of particulate guanylate cyclase activation, although high Ca²⁺ did not change basal plasma cyclic GMP levels. Other possibilities may include the stimulation of the cyclic GMP hydrolysis, through the Ca²⁺-activated cyclic nucleotide phosphodiesterase (52).

Regarding the αhANP-induced vasorelaxation, alternative explanations are not ruled out, including the possibility that the apparently close association between αhANP-elicited cyclic GMP production and relaxation is merely fortuitous. Haass et al. have recently suggested that in the pithed, vagotomized rat ANP resemble the calcium channel blocker nifedipine rather than the general vasodilator sodium nitroprusside, and attenuate the slow, calcium flux dependent component of alpha adrenoreceptor-mediated pressor responses (53). Thus, it is possible that αhANP relax vascular smooth muscle, by inhibiting an influx of extracellular calcium rather than by the increased cyclic GMP production.

In the present study, infusion of CaCl₂ alone caused vasoconstriction without changes in plasma cyclic GMP, suggesting that the mechanism for Ca²⁺-induced vasoconstriction might not involve the vascular cyclic GMP systems. Although increased Ca²⁺ in medium could relax arterial strips (54), intraarterial infusion of calcium produced vasoconstriction in man (55). Regarding Ca²⁺-induced vasoconstriction, in the presence of circulating vasoconstrictors such as norepinephrine and angiotensin the increase in extracellular calcium concentration may augment calcium entry into cell through receptor-operated calcium channels, and thus, constricting the vascular beds by the increased intracellular calcium concentrations. Moreover, it has been demonstrated that a relatively high extracellular Ca²⁺ inhibits specific calcium channels in vascular smooth muscle cells, possibly by the augmented inactivation of calcium channels, and then antagonizes the inhibitory effect of the calcium antagonists, cobalt (56) and magnesium ions (57). In this study, therefore, it is suggested that the increased Ca²⁺ might inhibit the ANP-dependent calcium channels, and thus attenuate the decrease in intracellular Ca²⁺, leading to the blunted ANP-dependent vasodilation. However, there is no evidence about Ca²⁺ effects on the inactivation of ANP-dependent calcium channels.

An intricate relationship between cyclic nucleotide-mediated events and changes in the activity of different Ca²⁺ translocating mechanisms is likely to be important in most excitatory tissues. Ca²⁺ regulates cyclic nucleotides metabolism in several ways, conversely cyclic nucleotides can regulate

calcium metabolism in either a positive or negative way (32). Therefore, there are some possibilities that pretreatment with calcium blunted the vascular relaxant effect of α hANP, through the interaction of cyclic nucleotides and intracellular Ca^{2+} . It should be noted, however, that in the present study modest elevations of serum calcium could attenuate the vascular responses to α hANP, whereas most of the above-mentioned in vitro studies concerned the effects of calcium at clearly superphysiological level. Further studies are needed to clarify these issues.

In summary, synthetic α hANP was infused into the brachial arterial blood in healthy volunteers, because it was devoid of circulatory reflexes. The intrabrachial infusion of α hANP caused a dose-dependent vasodilation of forearm vascular beds, with the concomitant increase in ipsilateral venous plasma cyclic GMP concentrations. There appeared to be a significant positive linear relationship between the cyclic GMP increase and the vascular relaxation induced by α hANP. Moreover, the simultaneous infusion of CaCl_2 not only inhibited the α hANP-induced vascular relaxation but also blunted the accompanying increase in cyclic GMP levels. Therefore, it is suggested that α hANP acts directly on the forearm vascular beds in man, eliciting its vascular relaxant effect, possibly by increasing cellular cyclic GMP formation. Moreover, high extracellular calcium inhibits the α hANP-induced vasodilation, possibly through the suppression of cyclic GMP increase.

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