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Contents

Extended Abstracts

Section I: ANP Synthesis, Storage, Secretion, and Radioimmunoassays

Section IV: ANP in Heart Disease and Hypertension

Section V: ANP Effects on the Kidney and Liver

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CHAPTER 16

Atrial Natriuretic Peptide in Normal Humans: Hemodynamic and Renal Effects after Single and Repeated Bolus Injection

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To study cardiovascular and diuretic effects and to establish whether there is an attenuation of response after repeated application, we gave a single or two 30-min-apart bolus injections of 200 μg human atrial natriuretic peptide (α-hANP). Before and after α-hANP bolus injections, echocardiography was performed and plasma atrial natriuretic peptide (ANP) and cyclic guanosine monophosphate (cGMP) as well as urinary cGMP excretion were determined. Plasma cGMP rose from basal 3.4 ± 1.1 to 25.0 ± 6.7 nmol/liter within 10 min after the first injection and from 20.0 ± 6.0 before the second administration to 32.7 ± 11.1 nmol/liter another 10 min later. Urine volume (30-min sampling periods) increased from 2.1 ± 2.5 to 16.1 ± 5.3 after the first and to 22.6 ± 7.0 ml/min after the second bolus. Urine sodium excretion increased from 6.2 ± 2.9 to 43.0 ± 18.5 and 38.7 ± 21.0 mmol/hr. After each α-hANP bolus, there was a significant decrease in mean arterial blood pressure and increase in heart rate. The ejection fraction increased from 66.2 ± 3.7 to 76.4 ± 4.0% after the first bolus and from 66.8 ± 3.8 before the second to 76.9 ± 5.0 after the second α-hANP bolus. All reported changes are significant ($p < 0.01$).

Single and repeated bolus applications of 200 μg of α-hANP in healthy volunteers increases plasma and urinary cGMP, diuresis, and natriuresis as well as echocardiographically determined ejection fraction and decreases arterial blood pressure. Reproducible diuretic effects and improved cardiac performance after repeated bolus application of α-hANP may be beneficial in the treatment of congestive heart failure.

α-hANP is a cardiac hormone with diuretic, natriuretic, and vasodilating properties (1–3). When given either as a bolus or as an infusion intravenously in normal humans, α-hANP causes a rise in urinary volume and sodium excretion (1,2). The hemodynamic actions of α-hANP are yet not well defined. After an α-hANP bolus, Richards et al. observed an increase of heart rate and a decrease of blood pressure (1). During infusion of α-hANP, Cuneo et al. could not detect changes of heart rate or blood pressure (4), but Indolfi et al. reported decreased blood pressure while the heart rate was kept constant by cardiac pacing (5). Vasodilating actions (4) as well as other hemodynamic and diuretic effects of α-hANP depend on the application scheme and the given dose (6).

In this study, our aim was to investigate the effects of single and repeated high dose α-hANP bolus injections in normal humans on (a) cGMP response, (b) hemodynamic parameters detectable by echocardiography, and (c) diuresis and natriuresis.

MATERIALS AND METHODS

The first part of the study (protocol 1) was performed in seven healthy volunteers (six men and one woman; 27.7 ± 1.8 years) without any evidence of heart disease. After an overnight fast and bed rest, 200 μg of α-hANP (Bissendorf Peptide, Wedemark, FRG) were given as a bolus injection intravenously.

Heart rate and blood pressure were monitored noninvasively by an automatic device (Dynamap^{R}). M-mode echocardiography was performed 40 and 20 min before as well as 5, 10, 15, 30, 45, 60, 120, and 180 min after the injection of α-hANP.

Venous blood samples were drawn for the determination of ANP, cGMP, hematocrit, sodium, potassium, and serum osmolality at −20, ±0, +2, +5, +10, +20, +35, +60, +90, and +180 min. Urine was collected at 30-minute intervals for measuring the excretion of sodium, potassium, and cGMP. Urine output was replaced throughout the study by oral water.

Informed consent was given; the study was approved by a local ethics committee.

Echocardiography

An IREX III (m-mode) echocardiograph together with a CARDIO 200 computer for the elaboration of the data was used. The left ventricular dimensions were obtained when the ultrasonic beam was directed at the chamber between the mitral valve echos and the papillary muscle echos (7). The end-diastolic diameter (EDD) was taken at the peak of the R-wave and from the trailing edge of the left side of the intraventricular septum to the leading edge of the posterior endocardial echo. The end-systolic diameter (ESD)

was taken at the peak downward motion of the intraventricular septum. Ventricular volumes—end-diastolic volume (EDV) and end-systolic volume (ESV), stroke volume (SV), and cardiac index (CI)—were obtained utilizing the formula of Teicholz et al. (8). The ejection fraction (EF) was calculated according to the method of Quinones et al. (9).

Analytical Methods

ANP (without prior extraction; IBL, Hamburg, FRG) and cGMP (10) were measured by radioimmunoassay.

Calculation and Statistics

Data are given as means ± standard deviation (SD). For statistical analysis, Student's *t*-test for paired data and the analysis of variance for repeated measures over time (11) were used.

In the second part of the study (protocol 2), 200 μg of α-hANP were given twice as intravenous boluses at a 30-minute interval in six healthy volunteers (five men, one woman; 27.5 ± 2.4 years). Again, echocardiography was performed 40 and 20 min before the first administration as well as 5, 10, 15, and 30 min after each bolus and finally at 90, 120, and 180 min. As before, venous blood samples were drawn (−40, −20, ±0, +2, +5, +10, +20, +32, +35, +40, +50, +60, +90, +120, +150, +180 min) and urine was collected at 30-minute intervals.

RESULTS

Within 2 min after each injection, a transient heart rate increase together with a fall of mean arterial pressure occurred (Tables 1 and 2). These

TABLE 1. *Hemodynamic, echocardiographic, and laboratory findings after a single bolus injection of α-hANP*[a]

	Basal	Max/min	Time (min)
Heart rate (min $^{-1}$)	58.6 ± 4.67	79.1 ± 7.4	+4
Mean arterial pressure (mm Hg)	91.3 ± 5.7	81.7 ± 6.4	+2
Stroke volume (ml)	77.5 ± 15.1	90.3 ± 10.2	+10
Cardiac index (liters/min/m^2)	2.44 ± 0.41	2.88 ± 0.41	+10
Urinary cGMP (nmol/min)	0.71 ± 0.25	5.56 ± 1.94	0–30
Hematocrit (%)	41.7 ± 3.3	44.8 ± 3.2	+35

[a]Time: time of maximal deviation from baseline values. The significance of all differences between basal and maximal or minimal values (max/min): $p < 0.01$.

TABLE 2. *Hemodynamic, echocardiographic, and laboratory findings after repeated bolus injections of α-hANP*[a]

	Basal	Max/min	Time (min)
Heart rate (min^{-1})	A 65.5 ± 8.6	79.5 ± 6.4	+3
	B 60.2 ± 7.9	83.3 + 6.7	+3
Mean arterial pressure (mm Hg)	A 90.0 ± 3.9	82.5 ± 6.4	+4
	B 89.8 ± 5.8	81.2 ± 5.3	+2
Stroke volume (ml)	A 83.4 ± 11.0	95.8 ± 13.0	+10
	B 78.6 ± 13.1	90.2 ± 23.7	+15
Cardiac index (liters/min/m^2)	A 2.97 ± 0.51	3.20 ± 0.67	+10
	B 2.63 ± 0.46	3.28 ± 0.93	+15
Urinary cGMP (nmol/min)	0.62 ± 0.42	5.68 ± 1.75	30–60
Hematocrit (%)	42.5 + 2.5	46.7 ± 2.3	+40

[a]A: first α-hANP bolus; B: second α-hANP bolus. Time: time of maximal deviation from baseline values. The significance of all differences between basal and maximal or minimal values (max/min): $p < 0.01$.

changes had disappeared another 5 min later. All subjects experienced mild to moderate facial flushing; no severe side effects were observed.

Hemodynamic Findings

In general, echocardiographic changes were most pronounced 10 to 15 min after the injection of α-hANP and reached baseline values another 15 to 20 min later.

Single Bolus (Protocol 1)

The results are given in Figs. 1 and 2 and in Table 1. ESD decreased markedly. As EDD (basal, 5.06 ± 0.37 cm) remained constant, this resulted in an increase of the calculated EF. Correspondingly, SV and CI were augmented by about 17%.

Repeated Bolus Injection (Protocol 2)

Figures 3 and 4 and Table 2 give the results. Again, ESD decreased significantly while EDD remained unchanged, leading to an increase of calculated EF, SV, and CI. Two-way analysis of variance did not disclose differences between the effects of a single ANP bolus (protocol 1) and the first of two bolus injections (protocol 2). Repeated injection after 30 min provoked comparable hemodynamic responses; an attenuation or waning effect could not be demonstrated.

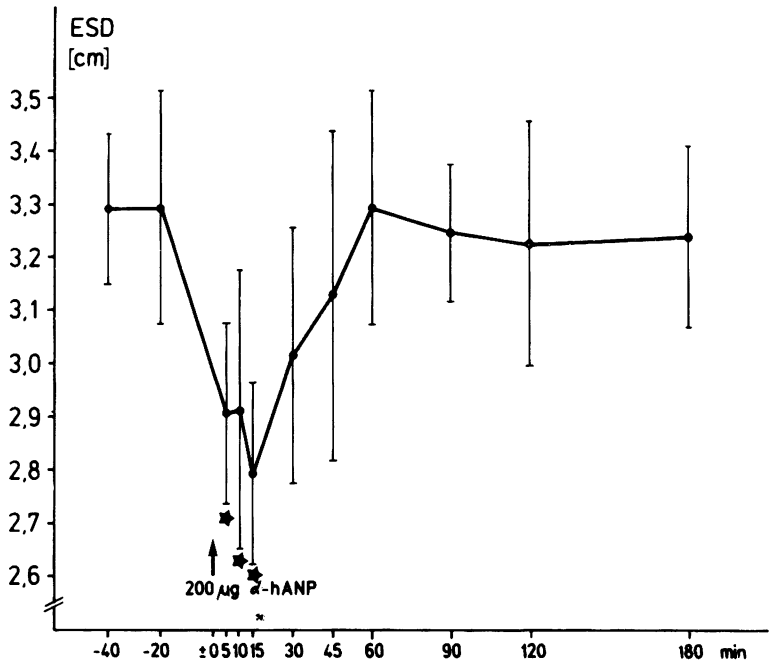

FIG. 1. End-systolic diameter (ESD) after a single bolus injection of 200 µg of α-hANP in volunteers; $^*p < 0.01$.

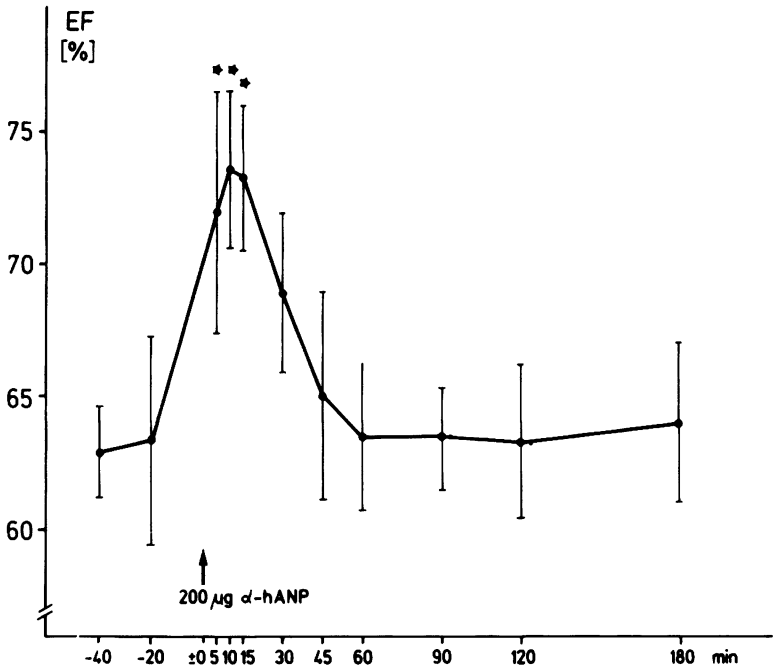

FIG. 2. Ejection fraction (EF) after single bolus injection of 200 µg of α-hANP in volunteers; $^*p < 0.01$.

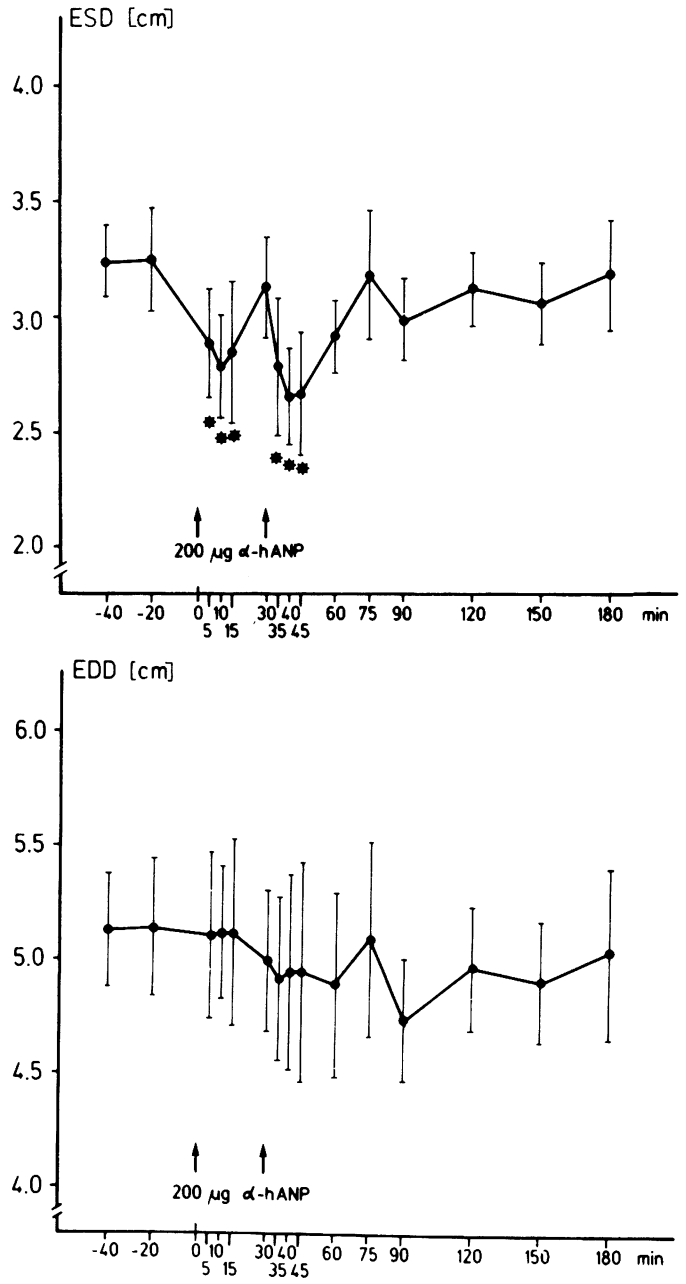

FIG. 3. End-systolic diameter (ESD) and end-diastolic diameter (EDD) after repeated bolus injections of 200 µg of α-hANP at a 30-min interval; $^*p < 0.01$.

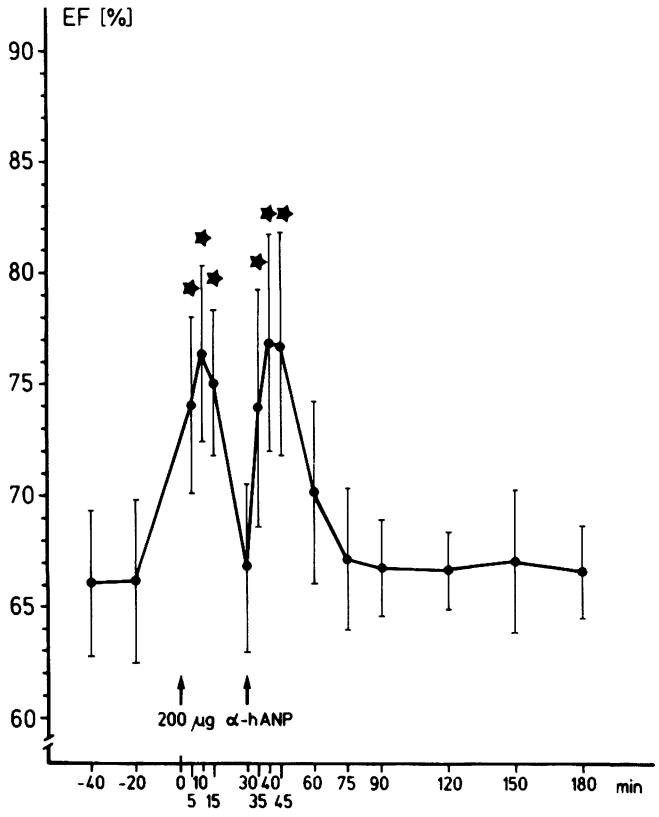

FIG. 4. Ejection fraction (EF) after repeated bolus injections of 200 µg of α-hANP at a 30-minute interval; $^*p < 0.01$.

Renal Findings

Single Bolus

Within the first 30 min after injection, urinary volume increased ninefold and sodium excretion increased fivefold (Fig. 5), whereas potassium excretion only doubled. Enhanced diuresis and natriuresis were still present at least 60 min later.

Repeated Bolus Injections

Again, the first injection of 200 µg of α-hANP produced clear-cut diuresis and natriuresis together with a moderate increase of potassium excretion. These effects were not different from those in protocol 1. Repeated injection

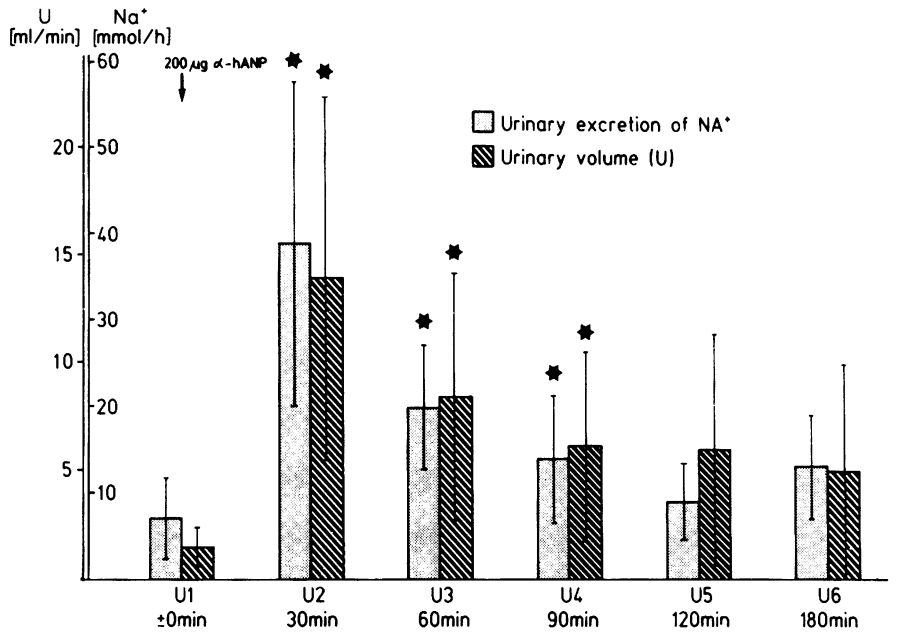

FIG. 5. Urinary volume (U) and urinary sodium excretion (Na^+) after a single bolus injection of 200 µg of α-hANP in volunteers; $^*p < 0.01$.

of α-hANP evoked prolonged renal actions when compared to single bolus application (Fig. 6; Table 2).

cGMP

There was a marked rise of plasma cGMP in protocol 1, maximal values being observed between 10 and 20 minutes after injection (Fig. 7). Correspondingly, urinary cGMP excretion increased nearly eightfold during the second collection period (0 to 30 min) (Table 1).

The second α-hANP bolus resulted in a further increase of plasma cGMP (Fig. 8) together with a more pronounced and persisting cGMP excretion up to the third collection period (30 to 60 minutes) (Table 2). During protocol 2, urinary excretion rose from a basal value of 0.62 ± 0.42 to 4.70 ± 1.99 mmol/min after the first bolus and to 5.68 ± 1.75 mmol/min after the second bolus.

Other Findings

ANP (basal value, 195 ± 94 pg/ml) rose nearly 15-fold after each injection and had nearly normalized 30 min later. After a single bolus injection, the

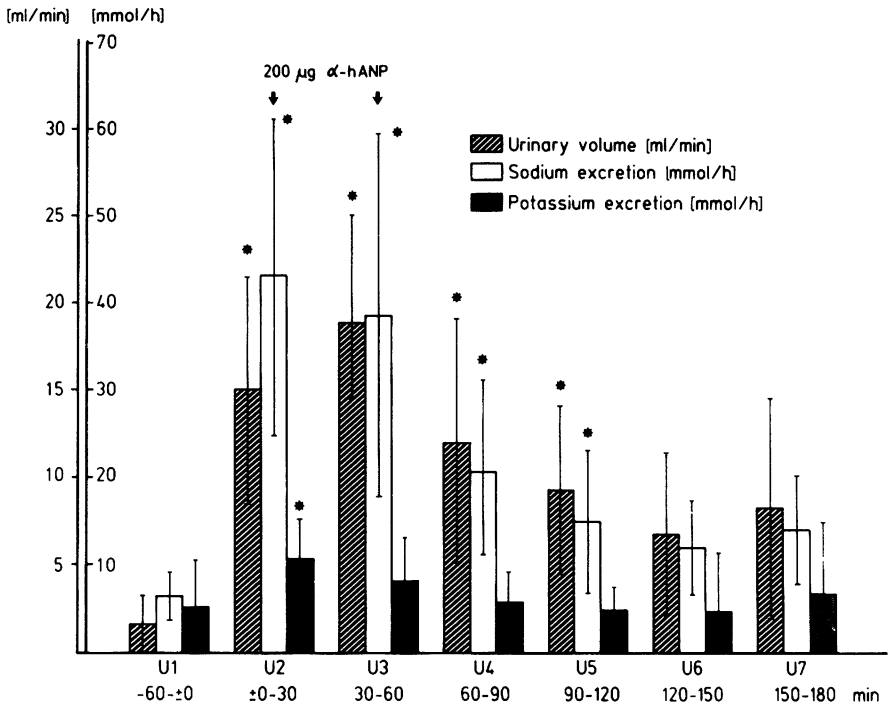

FIG. 6. Urinary volume and urinary sodium and potassium excretion after repeated bolus injections of 200 μg of α-hANP at a 30-min interval; $^*p < 0.01$.

venous hematocrit had risen after half an hour by 7% and not yet returned to a basal value by the end of the study. Repeated injection produced a slightly greater rise of 10% (Tables 1 and 2). Serum concentrations of sodium and potassium remained unchanged during both protocols. Apart from a moderate, symptomatic flush, no side effects were observed in the healthy volunteers.

DISCUSSION

The data demonstrate reproducible transient increases of heart rate and a reduction of blood pressure after single and repeated bolus injections of 200 μg of α-hANP. These results correspond well with previous observations after a single bolus of 100 μg of α-hANP (1).

Intravenous ANP in humans produces an increase of plasma cGMP and of urinary cGMP excretion (10). Probably because of the higher α-hANP bolus dose of 200 μg in the present study, the plasma and urinary response was more pronounced than previously reported after a bolus of 50 μg (10).

Cyclic GMP seems to be not only a marker but also a mediator of ANP

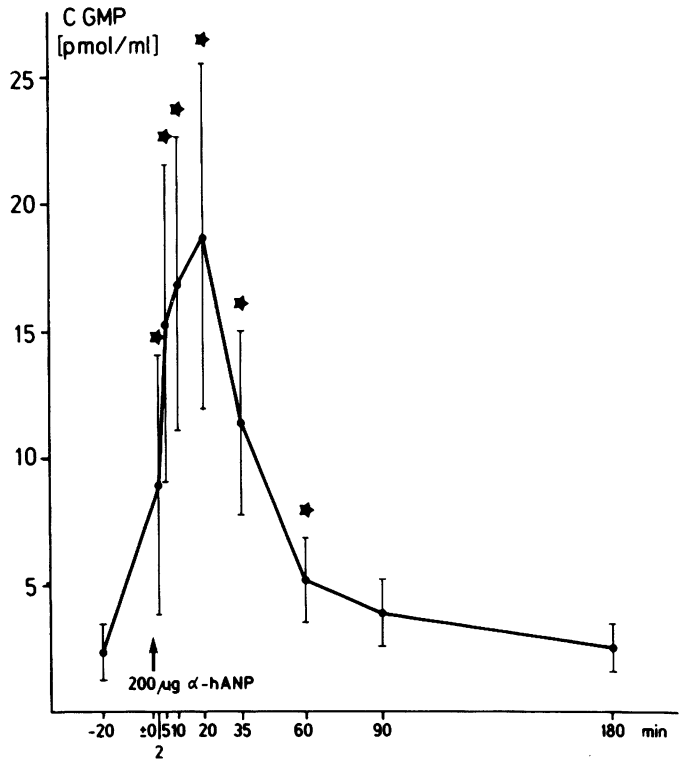

FIG. 7. Serum cyclic guanosine monophosphate (C GMP) after a single bolus injection of 200 µg of α-hANP in volunteers; $^*p < 0.01$.

activity in vascular smooth muscle cells and endothelial cells (12). *In vitro* the stimulation of cGMP production by ANP seems to be relatively irreversible and the responsiveness to ANP is downregulated by prior exposure to ANP (12). Interestingly, we could not demonstrate any attenuation of plasma cGMP or urinary cGMP excretion after the second of two consecutive α-hANP bolus injections. After the second bolus, plasma cGMP rose from a still elevated level to an even higher peak concentration (Fig. 8). Correspondingly, urinary cGMP excretion was also higher after the second than after the first α-hANP bolus. Thus, there seems to be no evidence of a diminished cGMP response after high dose α-hANP bolus applications at a 30-min interval.

Echocardiography after α-hANP application revealed significant decreases of ESD which, with EDD remaining unchanged, led to an increase of computed EF after each α-hANP injection. Also, SV and CI increased after α-hANP. Similar to the cGMP response, there was no diminution of the echocardiographic effects of the second of two consecutive bolus injections in protocol 2.

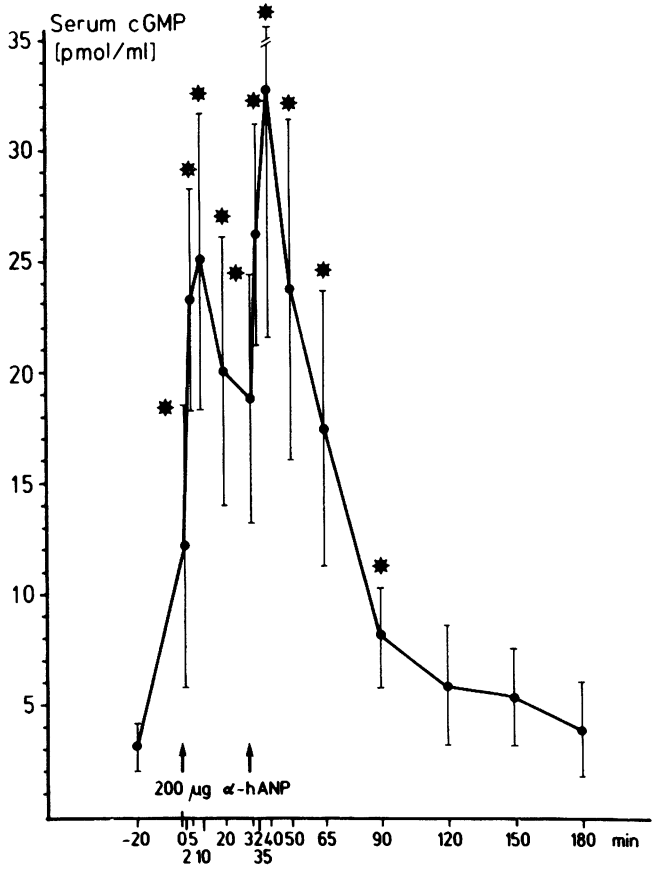

FIG. 8. Serum cyclic guanosine monophosphate (cGMP) after repeated bolus injections of 200 µg of α-hANP at a 30-min interval; $^*p < 0.01$.

In healthy volunteers, there seems to be no evidence for a cardiodepressive effect of the drug, which had been reported earlier in animals (13). Instead, the data point toward improved cardiac performance which may, because of reported vasodilating effects of α-hANP (3), be due to reductions of preload and afterload. Apart from possible direct myocardial effects, temporarily elevated catecholamine levels as a consequence of vasodilatation (14) may contribute to improvement of cardiac performance.

Like others (1,2,4,10), we observed increased diuresis and natriuresis after α-hANP application in non-salt restricted healthy volunteers. Increases of diuresis and natriuresis in this study were larger than those previously reported after bolus injections, probably because of the higher dose of α-hANP (1,2,4,10). The recorded renal effects of α-hANP persisted up to 60 min after a bolus injection, which is remarkably longer than the duration of the cardiovascular effects.

Because of the prolonged renal action of a single dose, the second bolus in protocol 2 was given with an overlap of the renal effect of the first bolus. Nevertheless, there was no attenuation of diuretic and natriuretic effects after the second bolus.

Endogenous plasma α-hANP is elevated in patients with congestive heart failure and may have regulatory functions (15,16). Studies in small numbers of patients with congestive heart failure showed beneficial effects of α-hANP due to its vasodilating and diuretic properties. Responses to α-hANP in those patients has been varying. Although Riegger et al. (17) and Saito et al. (18) observed vasodilatory as well as diuretic effects after bolus injections, Firth (19) found only renal but no cardiovascular effects. After continuous infusion of α-hANP in patients with heart failure, Mollina et al. (20) observed beneficial effects in cardiovascular and renal parameters, but several others (21–23) could not confirm diuretic effects after α-hANP infusion.

In the present study, reproducible improvement of cardiac performance and prolonged diuretic effects could be demonstrated in healthy volunteers after single and two consecutive bolus injections of 200 μg of α-hANP. Although conclusions from data obtained in healthy volunteers must be drawn with care, one can speculate that improved cardiac performance together with sustained diuresis and natriuresis after repeated high dose bolus application of α-hANP might be beneficial in the acute treatment of congestive heart failure.

CONCLUSIONS

Single and repeated bolus applications of 200 μg of α-hANP in healthy volunteers produces increases of plasma cGMP concentrations and urinary cGMP excretion, diuresis and natriuresis, heart rate and echocardiographically determined ejection fraction, and decreases in arterial blood pressure. Comparing the effects of two consecutive bolus injections of α-hANP at a 30-minute interval, there was no evidence for attenuation of cGMP response, diuresis, natriuresis, or cardiovascular effects.

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