Vascular responses to local atrial natriuretic peptide infusion in man

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1 The effect on skin and muscle blood flow of arterial infusion of atrial natriuretic peptide (ANP) directly into the forearm circulation, and on venous tone of direct infusion into a dorsal hand vein, was studied in normal subjects.

2 ANP produced a dose-dependent increase in both skin and muscle blood flow, but at equivalent doses, produced no dilatation of noradrenaline-precontracted dorsal hand veins. These findings indicate that ANP acting locally, is an arterioselective dilator in the upper limb circulation in normal man.

3 Measurements of ANP in venous plasma during arterial infusion suggest marked clearance of ANP across the forearm vascular bed. Such peripheral clearance may, at least in part, account for the short plasma half-life of this peptide.

4 The lowest dose of ANP infused was calculated to produce plasma levels similar to those found in patients with heart failure. The findings with this dose suggest that, in heart failure, circulating levels of ANP may be within a range capable of influencing peripheral vascular resistance directly.

Keywords atrial natriuretic peptide arterioles veins blood flow vasodilation man

Introduction

α -human atrial natriuretic peptide (α -hANP) has been shown clearly to be secreted by the heart, to circulate in blood, and to be removed rapidly during circulation through various tissues (Editorial, 1986; Lang *et al.*, 1987). Specific high-affinity receptors for ANP have now been identified in vascular, renal and adrenal tissue (Cantin & Genest, 1985; Ballerman & Brenner, 1985). The physiological stimuli associated with secretion of ANP suggest that this peptide may play an important role in blood volume homeostasis (Needleman & Greenwald, 1986; Lang *et al.*, 1987). In order to explain this, a physiological vasorelaxant action of ANP has been invoked (Currie *et al.*, 1983; Grammer *et al.*, 1983), additional to its potent natriuretic and diuretic properties (Richards *et al.*, 1985; Anderson *et al.*, 1987).

In vitro studies with vascular preparations have shown that ANP causes relaxation which is mediated by cyclic guanosine monophosphate (cGMP) through the activation of membrane-bound particulate guanylate cyclase by a calcium- and endothelium-independent mechanism (Murad, 1986). Organic nitrates also cause cGMP-dependent, calcium- and endothelium-independent dilatation, though this probably occurs through intracellular activation of soluble guanylate cyclase rather than of the particulate form (Murad, 1986; Holtz *et al.*, 1986). Despite the similarity in effector mechanisms, and evidence that nitrates have a similar profile of effects to ANP on vascular smooth muscle (Garcia *et al.*, 1984a, b; Winkvist *et al.*, 1984), animal studies report conflicting evidence with regard to effects on the venous circulation (Lappe *et al.*,

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1985; Breuhaus *et al.*, 1985; Hintze *et al.*, 1985; Holtz *et al.*, 1986). The relative contributions of arterial and venous dilatation to the actions of ANP in man are also unclear, although there is some evidence (Richards *et al.*, 1985; Bolli *et al.*, 1987) that ANP causes direct arteriolar dilatation, at least within the nanomolar range.

In the present study we have examined the effects of direct brachial artery infusion of α -hANP on blood flow in the forearm and microvasculature of the skin, and of local infusion of ANP on venous tone in the noradrenaline pre-constricted dorsal hand veins, in normal man.

Methods

Forearm blood flow during local ANP infusion

Four healthy normal males, aged 31 to 33 years (MA, NB, JC, DW), were studied while maintaining their usual sodium intake. All of the studies were performed in the Clinical Pharmacology Laboratory at St George's Hospital Medical School, and the subjects gave their written informed consent to take part in the experiments, which had been approved by the local hospital Ethics Committee.

On the morning of each study subjects were admitted to the laboratory where they rested supine, at a constant room temperature of 25–26°C for the duration of the study. Wrist occlusion cuffs, upper arm congesting cuffs and mercury-in-silastic strain gauges, were applied to both arms for measurement of forearm blood flow by venous occlusion plethysmography using our standard method (Webb & Collier, 1987). A fine fibre-line probe (Perimed) was applied to the volar aspect of the left forearm with the probe holder (Perimed) modified so that the probe rested flush with the skin. The probe was connected to a laser Doppler flowmeter (Perimed PF2b) for measurement of skin flux in arbitrary voltage units (V) in a hemisphere beneath the probe with effective penetration depth of 0.6 mm. The Doppler shifted signal within this volume is derived from flow in the cutaneous capillaries, subpapillary plexus and arteriovenous anastomoses (Holloway, 1983). Twelve KHz gain was used throughout, which has been shown to produce a linear increase in V with flow within the range of flows studied in the forearm (Ahn *et al.*, 1987). The voltage signal was stepped down to one tenth, sampled at 2 s intervals using a programme designed for the BBC Master micro-computer and saved on disc for later analysis.

The left brachial artery was then cannulated, under 1% lignocaine local anaesthesia, with a 27

standard wire gauge unmounted needle coupled to an epidural cannula (Portex). This was linked to a constant rate infusion pump (Harvard 994A) for administration of saline and synthetic α -hANP (Bissendorf Peptide GmbH). The purity of the synthetic ANP was assayed by high performance liquid chromatography as described elsewhere (Brown & Corr, 1987) and was found to contain only one chromatographic peak corresponding to the running position of authentic α -hANP. Infusion studies started after the subjects had rested supine for a minimum of 45 min.

Each subject underwent three infusion studies, with at least 4 days separating each study. On two occasions the subjects were infused, via the left brachial artery, with saline as control for 15 min, followed by incremental doses of ANP (in 0.9% saline: 0.45% human serum albumin) of 0.01, 0.05, 0.10 and 0.20 $\mu\text{g min}^{-1}$, each dose for 15 min, apart from the last which was given for 20 min. Skin flux was not measured at the highest dose. The rate of infusion at each dose was 0.5 ml infusate/min. On a further occasion subjects were infused with saline for 15 min followed by incremental doses of ANP of 0.5, 1.0, and 3.0 $\mu\text{g min}^{-1}$, each for 15 min.

The onset of action of ANP was rapid at all doses and clearly present within 60 s. The maximum effect of each dose was produced within 5 min of starting infusion, and no evidence of tachyphylaxis of the vasodilator response to ANP was seen in three preliminary 60 min constant-dose infusion studies (0.4 $\mu\text{g min}^{-1}$). On this basis forearm blood flow was recorded between the 10th and 15th minute of saline infusion, and of ANP at each dose. The collecting cuff was inflated for 10 s in every 15 s, and the average of the final 75 s of each recording run (five measurements) used for analysis. Skin flux was recorded throughout each study, and the 100 s period of measurement preceding each forearm blood flow recording (50 measurements) averaged and used for analysis. Forearm blood flow was additionally recorded for the first 10 min after stopping the highest dose of ANP, and again 5 min later, for a further 5 min. Forearm volume (FAV) was measured by water displacement.

Hand vein tone during local ANP infusion

Six male subjects aged 22 to 58 years (mean 33 years) were studied resting semi-recumbent in the laboratory at a constant room temperature of 26–27°C. The size of a dorsal vein of the right hand, maintained resting and above heart level, with a constant congesting pressure of 30 mmHg from a cuff around the upper arm, was recorded

by a modification of the technique of Collier & Robinson (1974). Changes in size of the congested vein were recorded continuously by means of a lightweight lever, the tip of which was resting on the summit of the vein, and the fulcrum of which was connected to an electronic transducer (Harvard 386 smooth muscle transducer) and recorder (Electromed MX216). Measurements were made with the vein congested and, after cuff deflation, with the vein fully collapsed. The difference between these two measurements was taken as vein size. Superficial limb veins differ from resistance vessels in that they have no background sympathetic tone provided that the subject is warm, supine and relaxed. In this state these vessels show no response to venodilators or sympatholytics (Robinson & Collier, 1979). The hand vein method is well adapted to studying the effects of drugs infused locally into the vein, particularly venodilator substances since the vein can be precontracted easily with noradrenaline (Robinson, 1978).

Saline and ANP were infused into the vein (rate $0.25 \text{ ml infusate min}^{-1}$) via a 23 standard wire gauge needle linked to a constant-rate infusion pump (Harvard 994A). Measurements of vein size were made more than 10 mm downstream of the tip of the needle but before the development of vein branching.

Two baseline measurements of vein size were made, separated by at least 2 min, after which noradrenaline ($16\text{--}64 \text{ ng min}^{-1}$) was infused at a dose sufficient to cause approximately 50% constriction. This dose was then continued throughout the experiment (rate $0.25 \text{ ml infusate min}^{-1}$) together with saline initially, then incremental doses of ANP of 0.01, 0.1 and $0.25 \text{ } \mu\text{g min}^{-1}$, each for 10 min. In three subjects, only the highest dose of $0.25 \text{ } \mu\text{g min}^{-1}$ was given for 10 min. This was followed by glyceryl trinitrate at $0.4 \text{ } \mu\text{g min}^{-1}$ (a dose previously shown to cause full dilatation), again for 10 min. Two measurements of vein size were made at 6 and 8 min after starting each infusion, and the average value used for analysis.

Blood sampling for ANP assay

Samples for ANP assay were collected into chilled tubes containing potassium-EDTA as an anticoagulant and sufficient aprotinin (Trasylol, Bayer) to give a final concentration of 400 kallikrein inhibitor units ml^{-1} . The samples were immediately centrifuged at 1000 g for 10 min at 4°C . The plasma was stored at -20°C until assayed. Plasma α -hANP was measured after extraction on a Bond-Elut C-8 cartridge (Analytichem International) and elution with acetonitrile:

water: trifluoroacetic acid (60%: 40%: 0.1%). ANP in the eluate was assayed using a commercial radio-immunoassay (Amersham, UK).

Blood samples (10 ml) were taken from the left antecubital fossae of two subjects at the end of a period of at least 10 min infusion via the brachial artery of saline, and ANP at doses of 0.5 and $1.0 \text{ } \mu\text{g min}^{-1}$ in two subjects and at $0.4 \text{ } \mu\text{g min}^{-1}$ in four subjects. Four samples of ANP infusate, made up to $0.8 \text{ } \mu\text{g ml}^{-1}$, but not used in experiments, were also taken and stored at -20°C , for assay as for the blood samples, in order to estimate ANP losses during preparation of solutions for infusion.

Results

Forearm blood flow during local ANP infusion

In the lower dose infusion study with ANP (Figure 1a), blood flow in the infused forearm increased in a dose-dependent manner from $5.3 \pm 1.4 \text{ ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$ during saline infusion to $6.6 \pm 1.5 \text{ ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$ during infusion of ANP at $0.1 \text{ } \mu\text{g min}^{-1}$. Results are shown as mean $\pm$ s.e. mean. Forearm blood flows in the non-cannulated arm were 5.8 ± 2.0 and $5.6 \pm 1.7 \text{ ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$ during saline and $0.1 \text{ } \mu\text{g min}^{-1}$ ANP respectively. The ratio of flows (CNC)

Figure 1 Percentage increase ($\pm$ s.e. mean) in (a) forearm blood flow (FBF) and (b) skin flux during brachial artery infusion of α -hANP in four subjects, each studied on three occasions.

increased from 0.95 ± 0.08 to 1.30 ± 0.15 across the period of ANP infusion. After stopping ANP blood flow returned to basal levels by 10 min.

In the higher infusion study with 0.5 to $3.0 \mu\text{g min}^{-1}$ ANP (Figure 1a), blood flow in the infused forearm increased in a dose-dependent manner from $3.9 \pm 0.5 \text{ ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$ during saline infusion to $10.2 \pm 2.1 \text{ ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$ during infusion of ANP at the highest dose. Forearm flows in the non-cannulated arm were 4.1 ± 0.6 and $3.8 \pm 0.5 \text{ ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$ during infusion of saline and $3.0 \mu\text{g min}^{-1}$ ANP respectively. The ratios of flows (C/N) increased from 0.98 ± 0.04 to 2.70 ± 0.45 across the period of ANP infusion. Offset of the vasodilation produced by ANP was prolonged after the highest dose, with 60% of the maximum effect still present 15 min after returning to saline infusion. Forearm volumes of the infused arms of the individual subjects were 850, 900, 950 and 1050 ml.

Skin flux during local ANP infusion

Skin flux measured on the infused forearm increased in a dose-dependent manner (Figure 1b) from $2.6 \pm 0.4 \text{ V}$ during saline infusion to $3.2 \pm 0.6 \text{ V}$ at $0.1 \mu\text{g min}^{-1}$. Skin flux also increased from $2.6 \pm 0.3 \text{ V}$ during saline infusion to a higher level of $4.3 \pm 0.5 \text{ V}$ during infusion of ANP at $3.0 \mu\text{g min}^{-1}$.

Venous tone during ANP infusion

Congested vein size (Figure 2) was 30 ± 3 arbitrary units (a.u.) before, and 13 ± 2 a.u. after constriction with noradrenaline (Levophed, Winthrop). Infusion of ANP, even at the highest dose of $0.25 \mu\text{g min}^{-1}$, produced no appreciable increase in vein size (14 ± 4 a.u.). However infusion of glyceryl trinitrate (Tridil, American Hospital Supply) produced a clear increase in vein size to

Figure 2 Percentage change in size of dorsal hand vein of six subjects during local infusion of noradrenaline, alone and with simultaneous infusion of ANP ($0.25 \mu\text{g min}^{-1}$) and glyceryl trinitrate (GTN, $0.4 \mu\text{g min}^{-1}$).

30 ± 4 a.u. ($P < 0.05$; Wilcoxon signed rank test), completely reversing the effect of noradrenaline.

Plasma ANP measurements

In the four samples of infusate made up to provide $0.8 \mu\text{g ml}^{-1}$ ANP the recovered concentration was $0.70 \pm 0.02 \mu\text{g ml}^{-1}$, indicating a peptide loss of 12.5% during preparation of solutions.

Venous plasma ANP concentrations during the various arterial infusion rates are shown in Table 1. In two subjects (JC, MA) basal recumbent plasma ANP concentrations were 12 and 15 fmol ml^{-1} respectively. Assuming that haematocrit is 50% and that changes in haematocrit with ANP infusion are small (Almeida *et al.*, 1986), that ANP is distributed into plasma rather

Table 1 Extraction of ANP across the forearm circulation calculated from measured venous and derived arterial plasma concentrations of α -hANP in the forearms of subjects during arterial infusion at doses of 0.4, 0.5 and $1.0 \mu\text{g min}^{-1}$. Arterial ANP was derived from the known dose of ANP infused and the measured forearm blood flow during infusion (for assumptions see text)

Subject	Dose ANP prepared ($\mu\text{g min}^{-1}$)	Dose ANP infused ($\mu\text{g min}^{-1}$)	Forearm blood flow (ml min^{-1})	Calculated arterial ANP (fmol ml^{-1})	Measured venous ANP (fmol ml^{-1})	% extraction
DW	0.4	0.37	109	2196	617	72
JC	0.4	0.33	75	2862	2030	29
MA	0.4	0.35	79	2870	2286	20
NB	0.4	0.33	38	5642	1458	74
JC	0.5	0.44	128	2230	2204	1
JC	1.0	0.88	100	5727	2429	58
MA	0.5	0.44	107	2676	2436	9
MA	1.0	0.88	124	4602	2486	46

than whole blood, and that mixing of plasma allows representative sampling from the antecubital fossa, then extraction across the forearm vascular bed would be $39 \pm 10\%$.

Discussion

The present study shows that α -hANP is a dilator of arterial vessels supplying both forearm muscle and superficial layers of the dermis in healthy normotensive man (Figure 1), confirming the findings of earlier systemic infusion studies (Richards *et al.*, 1985; Bussien *et al.*, 1986). The lack of an effect in the control arm provides evidence that dose-dependent vasodilatation occurs in response to a local action of ANP on the blood vessels, rather than through a centrally-mediated mechanism. In order to account for the large increases in forearm blood flow with ANP, dilatation of resistance vessels in skeletal muscle must be invoked, although dilatation of larger arteries may also occur. Arteriovenous anastomoses are not found in the forearm (Greenfield, 1962). Dilatation of dermal arteriolar vessels must therefore occur to account for the changes in skin flux.

The dilator response to ANP was apparent at the lowest dose infused ($10 \pm 4\%$; $0.01 \mu\text{g min}^{-1}$). Estimation of the local concentrations of ANP in the forearm circulation at doses of 0.01 and $0.05 \mu\text{g min}^{-1}$ gives concentrations (50 and 250 fmol ml^{-1}) similar to those seen in heart failure. Our observations would support the hypothesis (Bolli *et al.*, 1987) that circulating levels of ANP in heart failure (Tikkanen *et al.*, 1985; Richards *et al.*, 1986) may be in a range capable of affecting peripheral vascular resistance, and may be of functional importance in this circumstance. The effect of ANP was rapid in onset and suggests that sudden increases in atrial pressure and consequent secretion of ANP may allow short-term modulation of peripheral vascular resistance.

Measurements of venous plasma ANP from the antecubital fossa draining the infused forearm suggest that marked clearance of ANP occurs across the forearm circulation, even with allowances for the assumptions made in calculation. This degree of clearance occurring during circulation through the peripheral vasculature may, at least in part, account for the short plasma half-life of the peptide, and is very similar to the 50% clearance of ANP across the femoral bed in man reported by Crozier *et al.* (1986). Whether this occurs through uptake of ANP by vascular receptors or by enzymatic degradation remains unclear but plasma proteases are unlikely to make a

substantial contribution to clearance (Richards *et al.*, 1987).

In the present study noradrenaline-preconstricted hand veins were not affected by local infusion of ANP, but constriction was completely reversed by glyceryl trinitrate. In general the dose of a drug required to produce an effect in a single vein is about 10-fold lower than that for brachial artery infusion. From theoretical considerations single hand vein flow is unlikely to exceed 4 ml min^{-1} and more likely to approximate 1 ml min^{-1} (Robinson, 1978). Thus ANP produced no venodilatation at the highest dose infused locally, which was clearly in relative excess of that producing dilatation in the arteriolar vessels.

A number of studies have demonstrated reduction in right atrial pressure and consequent fall in cardiac output during systemic infusion of ANP (Breuhaus *et al.*, 1985; Pegram *et al.*, 1986; Allen & Gellai, 1987; Marks *et al.*, 1987). The assumption has been made from these studies that ANP increases venous capacitance. However, there are other explanations for the findings in these various studies. These include fluid shifts between the vascular compartments, as suggested by the development of haemoconcentration and fall in plasma volume which occur independent of any renal mechanism (Almeida *et al.*, 1986) and, alternatively, reduction in plasma volume as a direct response to the potent natriuretic and diuretic effects of ANP, though changes in atrial pressure tend to occur earlier than the renal response (Breuhaus *et al.*, 1985). Our studies demonstrate no direct effect of ANP on venous tone in dorsal hand veins in man, suggesting that ANP does not increase venous capacitance, although we have no information related to larger capacitance vessels. Our results, however, are not inconsistent with alternative explanations for reduction in right atrial pressure through changes in plasma volume.

ANP antagonises the renin-angiotensin system at a number of levels (Anderson *et al.*, 1986; Harris & Morgan, 1987; Bolli *et al.*, 1987; Hirata *et al.*, 1987). The direct effect of angiotensin II (ANG II) on veins occurs only at high doses and rapidly undergoes marked tachyphylaxis (Collier, 1974). However ANG II may cause constriction of veins indirectly via enhancement of sympathetic activity (Benjamin *et al.*, 1988; Zimmerman, 1981), which may be blocked by converting enzyme inhibitors (Clough *et al.*, 1982; Xiang *et al.*, 1985). This may provide the basis for the efficacy of converting enzyme inhibitors in heart failure. Similarly, by opposing the actions of the renin-angiotensin system, ANP may produce venodilatation in an indirect manner.

In conclusion, the present study shows that ANP is a locally acting arteriolar dilator of vessels in both human forearm muscle and skin, with no effect on tone in noradrenaline-precontracted dorsal hand veins. The lowest dose of ANP associated with dilatation suggests that circulating levels in heart failure are within a range which may directly affect arteriolar tone, though plasma levels circulating in normal subjects might be expected to produce little arteriolar dilatation.

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