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The effect of atrial natriuretic peptide on human isolated resistance arteries

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1 The action of (1-28) α-human atrial natriuretic peptide (ANP) was studied on human isolated resistance arteries.

2 Renal, skeletal muscle, omental and subcutaneous resistance arteries were taken from tissue removed at surgery and isometric tension responses were measured with a myograph.

3 ANP (10^{-9}–10^{-6} M) relaxed precontracted segments of renal and skeletal muscle arteries in a concentration-dependent manner. ANP failed to relax isolated omental or subcutaneous arteries.

4 The effect of ANP on human isolated resistance arteries varies depending on the site of origin of the artery.

Introduction

The discovery of a vasodilator and natriuretic substance, atrial natriuretic peptide (Kangawa & Matsuo, 1984), has excited much interest in the physiological role of this peptide in the control of blood pressure and extracellular fluid volume (Needleman *et al.*, 1985). Alpha human (1–28) atrial natriuretic peptide (ANP) has been shown to circulate in human plasma (Theiss *et al.*, 1987) and to possess natriuretic effects in man at low doses (Anderson *et al.*, 1986a), whilst higher doses have been shown to be hypotensive in man (Richards *et al.*, 1985). Recent work in man, *in vivo*, has shown a local vasodilator effect of ANP infusion into the forearm arterial vasculature (Bolli *et al.*, 1987; Hughes *et al.*, 1988). Studies in several species including man (Winquist, 1985; Rappoport *et al.*, 1986; Hughes *et al.*, 1988) have demonstrated a vasorelaxant effect of ANP on isolated arterial smooth muscle from a variety of sites.

Most of these studies demonstrating a vasorelaxant effect of ANP on isolated vasculature have been conducted on isolated vessels of internal diameter greater than 0.5 mm. Such arteries do not play a significant role in the control of peripheral vascular resistance (Haddy, 1960; Mulvany, 1983) and it is recognised that considerable differences exist between the pharmacological responsiveness of large 'conduit' arteries and smaller resistance arteries (Altura, 1974). Recent studies in the rat (Aalkjaer *et al.*, 1985; Osol *et al.*, 1986) and rabbit (Edwards & Weidley, 1987) in which the effect of ANP on arteries small enough to influence peripheral resistance was examined, have generally failed to show direct vasorelaxant effects of ANP in a variety of sites with the exception of the renal arcuate artery of the rat (Aalkjaer *et al.*, 1985). Consequently we have examined the effect of ANP on a variety of human isolated resistance arteries. Some of these results have been presented previously to the British Pharmacological Society (Sever *et al.*, 1988).

Methods

Tissue was obtained from 14 patients (8 male) undergoing surgery (age range 40–78 years). Omentum and subcutaneous tissue was obtained from abdominal surgery, macroscopically normal renal tissue from nephrectomies for renal carcinoma and skeletal

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Table 1 Characteristics of arteries used in the study

Site	*Normalised diameter* (μm)	*Active tension induced by KDS* ($N\,m^{-1}$)	n
Renal	291 ± 74	2.2 ± 0.5	7
Skeletal	262 ± 44	2.5 ± 0.7	5
Omental	227 ± 35	2.4 ± 0.2	4
Subcutaneous	339 ± 96	3.6 ± 1.5	4

Values are mean ± s.e.mean.

muscle from hip surgery. Tissue was collected in physiological saline solution (PSS) of composition (mM): NaCl 118, KCl 4.7, $MgCl_2$ 1.2, $NaHCO_3$ 21, glucose 20, NaH_2PO_4 1, $CaCl_2$ 2.5, Na_2EDTA 0.03 and used immediately for studies; arteries from all sites were treated similarly. Arteries were dissected from surrounding tissue with a dissecting microscope and mounted on two 40 μm stainless steel wires in a myograph to allow the measurement of isometric tension (Mulvany & Halpern, 1977). The myograph contained 10 ml PSS at 37°C aerated with 95% O_2: 5% CO_2. Following 30 min equilibration, vessels were normalised on the basis of their passive internal circumference to tension relationship to an internal circumference (0.9 L_{100}), that is, 0.9 times the internal circumference which they would possess if distended by 100 mmHg pressure on the basis of the Laplace relationship (pressure = tension/radius) Under these conditions the contractile responses of

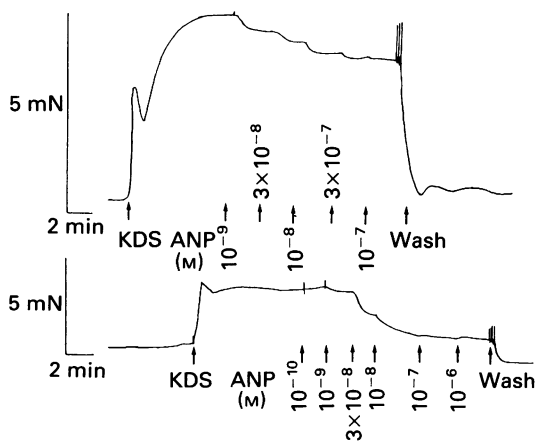

Figure 1 Representative traces showing the effect of cumulative addition of (1–28) α-atrial natriuretic peptide (ANP) (10^{-10}–10^{-6} M) to human isolated resistance arteries taken from kidney (internal diameter = 197 μm) and skeletal muscle (internal diameter = 226 μm). Arteries were contracted with a potassium depolarizing solution (KDS) and ANP added cumulatively as indicated by the arrows.

such small vessels are near to optimal (Mulvany & Halpern, 1977).

Prior to any study the viability of vessels was investigated by exposing them to a sequence of a depolarizing potassium solution (KDS), consisting of PSS containing equimolar KCl substituted for NaCl, followed by washout with PSS and then addition of noradrenaline (NA, 10^{-5} M). Vessels not generating active tension in response to KDS equivalent to or greater than 90 mmHg effective active pressure (calculated by Laplace's relationship) were not used for further studies; 3 out of a total of 23 vessels studied did not satisfy this criterion.

Following washout, arteries were allowed to equilibrate for a further 30 min in PSS before experiments were started. Since arteries rarely possessed much intrinsic tone in PSS, tone was induced with either NA (10^{-5} M) or KDS in order to demonstrate relaxation. ANP (10^{-10}–10^{-6} M) was added cumulatively and responses were calculated as % reduction of induced tone. Concentration-response data from individual arteries was fitted to a logistic function with a computer programme (Barlow, 1984) to derive values for EC_{50}. EC_{50} values are presented as geometric means (95% confidence intervals) and maximum responses (max) as mean ± s.e.mean.

ANP and noradrenaline were obtained from Sigma Chemical Co. Ltd., UK.

Results

The characteristics of the vessels used in these studies are shown in Table 1. Addition of ANP relaxed contracted segments of renal and skeletal muscle arteries in a concentration-dependent manner (Figure 1). The potency and efficacy of ANP was similar in renal and skeletal muscle arteries irrespective of the contractile agonist used, (EC_{50} ($\times10^{-9}$ M) = 11 (3–37) and 8 (3–21); max = 66 ± 5% and 69 ± 16% using KDS and EC_{50} ($\times10^{-9}$ M) = 9 (4–20) and 10 (5–17); max = 73 ± 4% and 69 ± 13% using NA for renal and skeletal muscle arteries respectively). The concentration-response relationships derived from these studies are shown in Figure 2.

In contrast to the results obtained in renal and skeletal muscle vasculature, ANP failed to relax subcutaneous ($n = 4$) or omental arteries ($n = 4$) precontracted by either NA or KDS (Figure 3).

Discussion

This study demonstrates that ANP relaxes human isolated renal and skeletal muscle resistance arteries. The maximum contractile responses of these small human arteries are similar to those reported by others for similar sized arteries obtained from man

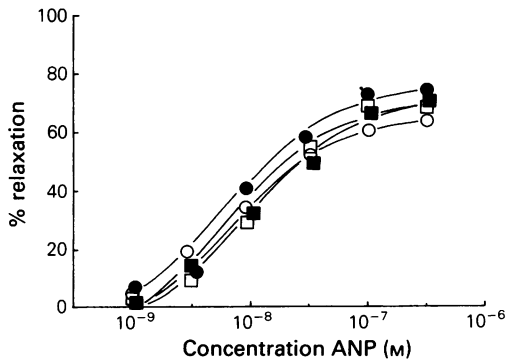

Figure 2 Concentration-response relationships for (1–28) α-atrial natriuretic peptide (ANP)-induced relaxation in renal and skeletal muscle resistance arteries. Arteries were contracted by potassium depolarizing solution (open symbols) or noradrenaline (closed symbols). Points represent means of n observations. Some points have been offset and s.e.means have been omitted for clarity: (○) renal arteries ($n = 7$); (●) renal arteries ($n = 3$); (□) skeletal muscle arteries ($n = 5$); (■) skeletal muscle arteries ($n = 5$).

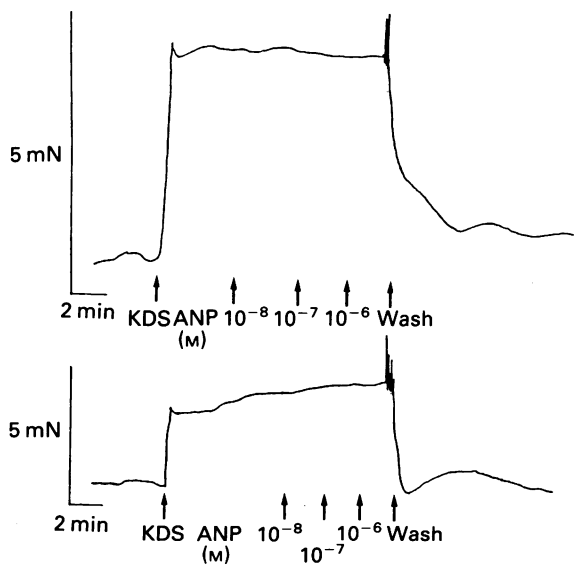

Figure 3 Trace illustrating the failure of (1–28) α-atrial natriuretic peptide (ANP) to induce relaxation in human isolated omental (internal diameter = 264 μm) and a subcutaneous resistance artery (internal diameter = 238 μm). Arteries were contracted with a potassium depolarizing solution (KDS) and ANP added cumulatively as indicated by the arrows. Representative of four separate experiments.

(Aalkjaer & Mulvany, 1983) or other species (Mulvany & Halpern, 1976). The vasorelaxant action of ANP probably involves a direct relaxant effect on vascular smooth muscle cells, as has been suggested by others (Winquist *et al.*, 1984). A notable feature of these results is that the effect of ANP on human isolated resistance arteries varies between arteries taken from different vascular beds. This finding is in contrast to previous studies with large human arteries from a variety of sites *in vitro* (Rappoport *et al.*, 1986; Hughes *et al.*, 1988).

Similar differences with respect to the pharmacological responses of conduit and resistance arteries have been reported previously with respect to neurohypophyseal peptides in the rat (Altura, 1974) and α-adrenoceptor agonists in human vasculature (Nielsen *et al.*, 1987). The findings presented here emphasize the differences between conduit and resistance arteries and indicate caution in extrapolating results obtained in one to the other.

However, these results obtained in human isolated resistance arteries should not be interpreted as definitely excluding the existence of ANP receptors on vascular smooth muscle cells of the omentum and subcutaneous tissue. A recent report by Cauvin and colleagues (1988) suggests that in the rat, at least, although it is not possible to demonstrate vasorelaxant effects of ANP on precontracted mesenteric resistance arteries, ANP does attenuate the phasic component of the response to noradrenaline. This was not studied in our experiments and it is possible that our failure to demonstrate relaxation in response to ANP in subcutaneous and omental arteries similarly reflects the inability of the peptide to relax tonic contraction in these sites. The relaxant effects of ANP in renal and skeletal muscle under the same conditions may therefore reflect a greater efficacy of the peptide in these tissues or some other factor. Further studies will be necessary to establish whether this is the case. The results obtained in these human resistance arteries are nevertheless very similar to those reported by Aalkjaer and colleagues (1985) investigating the vasorelaxant effect of ANP in rat isolated resistance arteries. However, some differences exist, in that skeletal muscle arteries from the rat were found to be unresponsive to ANP, whereas in this study arterioles supplying human skeletal muscle were found to relax in response to ANP. It is possible that this may represent a species difference. In this context, it is interesting that increased central venous pressure, a stimulus to ANP release (Ogihara *et al.*, 1986) is reported to increase skeletal muscle blood flow in man in contrast to some other species (Donald & Shepherd, 1978). However, an involvement of ANP in this response is clearly speculative at present. Indeed the physiological significance of the vasorelaxant effects

of ANP is currently unclear, since the concentrations of ANP necessary, both to produce vasodilatation *in vivo* (Richards *et al.*, 1985; Bussein *et al.*, 1986) or to relax arterial smooth muscle *in vitro* exceed the levels seen circulating under physiological conditions (Anderson *et al.*, 1986b; Ogihara *et al.*, 1986). Further work will be necessary to define the precise role of the receptors for ANP sited on vascular smooth muscle cells and the conditions under which they may be activated.

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References

AALKJAER, C. & MULVANY, M. (1981). Functional and morphological properties of human omental resistance vessels. *Blood Vessels*, **18**, 233–244.

AALKJAER, C., MULVANY, M.J. & NYBORG, N.C.B. (1985). Atrial natriuretic factor causes specific relaxation of rat renal arcuate arteries. *Br. J. Pharmacol.*, **86**, 447–453.

ALTURA, B.M. (1974). Neurohypophyseal hormones and analogues: rat pressor potency versus contractile potency on rat arterioles and arteries. *Proc. Soc. Exp. Biol. Med.*, **146**, 1054–1060.

ANDERSON, J., STRUTHERS, A., CHRISTOFIDES, N. & BLOOM, S. (1986a). Atrial natriuretic peptide: an endogenous factor enhancing sodium excretion in man. *Clin. Sci.*, **70**, 327–331.

ANDERSON, J.V., DONKIER, J., McKENNA, W.J. & BLOOM, S.R. (1986b). The plasma release of atrial natriuretic peptide in man. *Clin. Sci.*, **71**, 151–155.

BARLOW, R.B. (1984). *Biodata Handling with Microcomputers*. Cambridge: Elsevier Science Publishers.

BOLLI, P., MULLER, F.B., LINDER, L., RAINE, A.E.G., RESINK, T.J., ERNE, P., KIOWSKI, W., RITZ, R. & BUHLER, F.R. (1987). The vasodilator potency of atrial natriuretic peptide in man. *Circ.*, **75**, 221–228.

BUSSEIN, J.P., BIOLLAZ, J., WAEBER, B., NUSSBERGER, J., TURINI, G.A., BRUNNER, H.R., BRUNNER-FERBER, F., GOMEZ, H.J. & OFFERBEIN, E.S. (1986). Dose dependent effect of atrial natriuretic peptide on blood pressure, heart rate and skin blood flow of normal volunteers. *J. Cardiovasc. Pharmacol.*, **8**, 216–220.

CAUVIN, C., TERJERINA, M. & VAN BREEMEN, C. (1988). Effects of atriopeptin III on isolated mesenteric resistance vessels from SHR and WKY. *Am. J. Physiol.*, **253**, H1612–H1617.

DONALD, D.E. & SHEPHERD, J.T. (1978). Reflexes from the heart and lungs: physiological curiosities or important regulatory mechanisms. *Cardiovasc. Res.*, **12**, 449–469.

EDWARDS, R.M. & WEIDLEY, E.F. (1987). Lack of effect of atriopeptin II on rabbit glomerular arterioles *in vitro*. *Am. J. Physiol.*, **252**, F317–F321.

HADDY, F.J. (1960). Peripheral vascular resistance. *Am. Heart J.*, **60**, 1–5.

HUGHES, A., THOM, S., MARTIN, G., GOLDBERG, P. & SEVER, P. (1988). Direct effect of alpha human atrial natriuretic peptide on human vasculature *in vivo* and *in vitro*. *Clin. Sci.*, **74**, 207–211.

KANGAWA, K. & MATSUO, H. (1984). Purification and complete amino acid sequence of alpha human atrial natriuretic peptide (alpha ANP). *Biochem. Biophys. Res. Comm.*, **118**, 131–139.

MULVANY, M.J. (1983). Do resistance vessel abnormalities contribute to the elevated blood pressure of spontaneously hypertensive rats? A review of some of the evidence. *Blood Vessels*, **20**, 1–22.

MULVANY, M.J. & HALPERN, W. (1976). Mechanical properties of vascular smooth muscle cells *in situ*. *Nature*, **260**, 617–619.

MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.*, **41**, 19–26.

NEEDLEMAN, P., ADAMS, S.P., COLE, M.G., CURRIE, M.G., GELLER, G.M., MICHENER, M.L., SAPER, C.B., SCHWARTZ, D. & STANDAERT, D.G. (1985). Atriopeptins as cardiac hormones. *Hypertens.*, **7**, 469–482.

NIELSEN, H., THOM, S., HUGHES, A., MARTIN, G., MULVANY, M. & SEVER, P. (1987). Alpha$_2$- adrenoceptors on human resistance arterioles. *Blood Vessels*, **24**, 219.

OGIHARA, T., SHIMA, J., HARA, H., KUMAHARA, Y., KANGAWA, K. & MATSUO, H. (1986). Changes in human plasma atrial natriuretic polypeptide concentration in normal subjects during passive leg raising and whole body tilting. *Clin. Sci.*, **71**, 147–150.

OSOL, G., HALPERN, W., TESFAMARIUM, B., NAKAYAMA, K. & WEINBERG, D. (1986). Synthetic atrial natriuretic factor does not dilate resistance-sized arteries. *Hypertens.*, **8**, 606–610.

RAPPOPORT, R.M., GINSBURG, R., WALDMAN, S.A. & MURAD, F. (1986). Effects of atriopeptins on relaxation and cyclic GMP levels in human coronary artery *in vitro*. *Eur. J. Pharmacol.*, **124**, 193–196.

RICHARDS, A.M., NICHOLLS, M.G., IKRAM, H., WEBSTER, M.W.I., YANDLE, T.G. & ESPINER, E.A. (1985). Renal haemodynamic and hormonal effects of human alpha atrial natriuretic peptide in healthy volunteers. *Lancet*, **i**, 545–549.

SEVER, P.S., HUGHES, A., NIELSEN, H. & THOM, S. (1988). The action of human atrial natriuretic peptide on isolated human resistance vessels. *Br. J. Pharmacol.*, **93**, 239P.

THEISS, G., JOHN, A., MORICH, F., NEUSER, D., SCHRÖDER, W., STASCH, J.-P. & WOHLFEIL, S. (1987). α-h-ANP is the only circulating ANP in humans. *FEBS Letts.*, **218**, 159–162.

WINQUIST, R.J. (1985). The relaxant effects of atrial natriuretic factor on vascular smooth muscle. *Life Sci.*, **37**, 1081–1087.

WINQUIST, R.J., FAISON, E.P., WALDMAN, S.A., SCHWARTZ, K., MURAD, F. & RAPOPORT, R.M. (1984). Atrial natriuretic factor elicits an endothelium independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 7661–7664.

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