Br. J. Pharmac. (1987), **91**, 687–691

A comparison of the vasodilator responses to atrial peptides in the pulmonary and renal arteries of the pig *in vitro*

[1] Tim L.Th.A. Jansen, [2]Alyn H. Morice & Morris J. Brown

Clinical Pharmacology Unit, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ

1 Noradrenaline preconstricted pulmonary and renal artery segments from 20 large White pigs were examined *in vitro* for their responses to α-human atrial natriuretic peptide (α-hANP), rat-atriopeptin I (AP I) or rat-atriopeptin III (AP III) added in a cumulative manner. The role of the vascular endothelium in atrial peptide-induced relaxation was examined in the presence of indomethacin and propranolol by removal of the endothelium in one of a pair of arterial segments.

2 Pulmonary artery was significantly ($P < 0.001$) more sensitive than renal artery to α-hANP with a potency ratio of approximately 10. α-hANP appeared to be a more potent relaxant than AP III and AP I in pulmonary artery. Potency ratios were approximately 2 (AP III vs α-hANP; $P < 0.05$) and 30 (AP I vs α-ANP; $P < 0.001$).

3 Removal of the endothelium increased the sensitivity of renal artery to a α-hANP, but made no difference to the pulmonary arterial relaxations.

4 In man the highest circulating concentrations of ANP are found in the pulmonary artery. The demonstration of its potent relaxant effects at this site in the pig indicates a possible role in the modulation of pulmonary arterial tone.

Introduction

Atrial myoctyes release in response to distension a peptide, atrial natriuretic peptide (ANP) (DeBold *et al.*, 1981; Dietz, 1984), which has potent natriuretic and vasodilator properties (Garcia *et al.*, 1985; Needleman & Greenwald, 1986; Waldhausl *et al.*, 1986). In man the highest circulating concentrations of ANP are found in pulmonary arteries (Richards *et al.*, 1986). We hypothesized that ANP may have a role in the regulation of pulmonary artery tone in response to volume load and have therefore investigated the *in vitro* responsiveness of preconstricted arterial rings to ANP and the closely related peptides rat-atriopeptin I (AP I) and rat-atriopeptin III (AP III). We have also investigated the role of the vascular endothelium in these responses, in both the renal and pulmonary artery, in order to determine whether smooth-muscle relaxation induced by atrial peptides is mediated via the recently discovered endothelium-derived relaxing factor (EDRF) (Furchgott *et al.*, 1984).

[1] Present address: University of Nijmegen, The Netherlands.
[2] Author for Correspondence.

Methods

Twenty Large White pigs, 6 months old, were killed by electric shock at the local abattoir. The pulmonary and renal arteries were carefully removed immediately *post mortem* and placed in aerated, room temperature, Krebs solution of the following composition (in mM): KH_2PO_4 1.2, $MgSO_4$ $7H_2O$, 1.2, KCl 4.7, glucose 11.1, $NaHCO_3$ 25.0, NaCl 118, $CaCl_2$ $2H_2O$ 2.6. Those rings which were not used immediately were stored in a refrigerator (+ 4°C) for up to 48 h.

Experimental procedures

Main, hilar and segmental pulmonary arteries were examined, the latter being carefully dissected to the adventitia, clear of surrounding lung parenchyma. Renal vessels were removed en bloc and segments subsequently dissected from the main renal artery. Arterial rings with internal diameter 0.5 to 1.5 cm were mounted in 2 ml baths with cotton thread and immersed in aerated Krebs solution at 36.0 ± 0.5°C. Isometric tension was measured by Grass force-displacement transducers FT03D, connected to Hugo Sachs strain gauge couplers and continuously recor-

ded on a chart recorder mark VII, type WR 3101 (Watanabe Instr Corp). The rings were subjected to a passive tension of 1 g and allowed to equilibrate for 45 min before the addition of any drugs. After equilibration noradrenaline (NA) was added in a cumulative fashion in final bath concentrations from 100 nM to 3 μM, the latter concentration of which produced a submaximal contraction and a similar degree of contractile preload in both renal and pulmonary vessels. ANP, AP I or AP III were then added in a cumulative fashion in bath concentrations from 10 nM to 300 nM.

The role of the vascular endothelium in the relaxation produced by atrial peptides was investigated by removal of the intimal surface in one of a pair of segments. Removal was performed by gentle rubbing of the internal surface of the vessels with a wooden stick. To demonstrate the completeness of this procedure the rings were exposed to acetylcholine (ACh, 10 μM) at the end of each experiment.

All experiments were peformed in the presence of indomethacin (10 μM) and propranolol (8 μM). All vascular strips were used only once in quantitative measurements. In order to demonstrate adequate vascular responsiveness, NA dose-response curves were obtained in each preparation.

Results are expressed in terms of percentage change of the contractile tension induced by 3 μM NA, a relaxation being a positive percentage. Data, expressed as a mean percentage of relaxation with its standard error (s.e.), consist of 8 groups containing 6 different arterial strips. Comparison of the data was performed by a two-way analysis of variance. Significance was accepted at the 0.05 level of probability.

Drugs

The atrial peptides were obtained from Cambridge Research Biochemicals, Harston, Cambridge, and reconstituted allowing for percentage purity (peptide contents were: α-hANP, 78%; AP III, 75%; AP I, 75%). All other chemicals were of analytic grade. The inorganic salts of the Krebs solution were obtained from Fisons PLC, except calcium chloride which was obtained from BDH. Noradrenaline (Arterenol), acetylcholine (acetylcholine chloride), indomethacin and propranolol were obtained from Sigma.

Results

Vasodilator responses to ANP

ANP potently relaxed the NA-preconstricted renal and pulmonary arteries of the pig. Relaxation was concentration-dependent, slow in onset and sustained (Figure 1). Maximum relaxation was obtained within

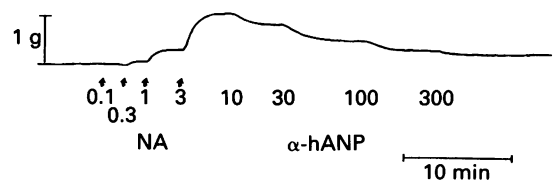

Figure 1 Vasodilator effects of α-human atrial natriuretic peptide (α-hANP) on pig pulmonary artery. The record is an original tracing. Doses of α-hANP (cumulative concentrations in nM) were applied after a dose-response curve had been obtained to noradrenaline (NA), represented in the figure by arrows (cumulative concentrations in μM).

5 min and lasted in excess of 20 min. Control segments preconstricted with 3 μM NA showed a mean loss of constrictor tone over the duration of the experiment of less than 10% ($n = 6$). ANP was significantly more potent at relaxing pulmonary artery when compared with renal artery at all doses ($P < 0.001$) and the potency ratio at 40% of relaxation was approximately 10 ($n = 24$) (Figure 2).

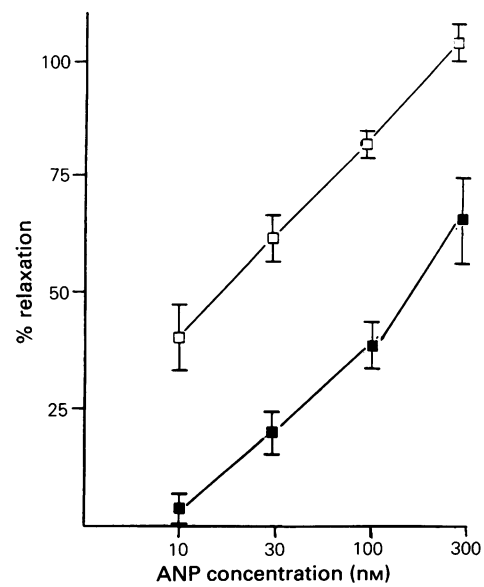

Figure 2 Dose-response curves obtained in pulmonary artery (□, $n = 12$), pooled with and without endothelium and renal artery (■, $n = 6$) with endothelium to α-human atrial natriuretic peptide (α-hANP). Data are presented as mean values; vertical lines show s.e. α-hANP was added in a cumulative fashion to arteries preconstricted with noradrenaline (3 μM) in the presence of indomethacin and propranolol.

Table 1 The effect of endothelial removal on the vasorelaxant responses to atrial peptides of pulmonary and renal arteries in the pig

Endothelium		n	*Concentration* (nM)			
			10	*30*	*100*	*300*
Renal artery						
ANP	+	6	4 (1)	16 (4)	35 (5)	60 (9)
	−	6	18 (4)	33 (5)*	56 (6)*	91 (9)***
Pulmonary artery						
ANP	+	6	44 (10)	62 (7)	81 (4)	99 (3)
	−	6	36 (11)	61 (9)	83 (6)	111 (6)
AP III	+	6	21 (2)	47 (2)	68 (4)	83 (5)
	−	4	27 (5)	53 (4)	78 (3)	94 (3)
AP I	+	6	2 (1)	9 (2)	5 (1)	38 (7)
	−	6	8 (1)	20 (3)*	29 (4)**	52 (8)*

Results are expressed as mean % relaxation or noradrenaline preconstriction with s.e. in parentheses.
* $P<0.05$, ** $P<0.01$, *** $P<0.001$, significantly different from endothelium intact preparation. ANP = α-human atrial natriuretic peptide, AP III = atriopeptin III and API = atriopeptin I.

The role of endothelium in vasodilator responses to artrial peptides

Removal of the vascular endothelium, as demonstrated by failure to relax in response to 10 μM ACh, made no significant difference to the responses of pulmonary artery to ANP and AP III. The data from these studies have therefore been pooled for further analysis.

A slight increase in sensitivity to AP I occurred in rubbed pulmonary artery when compared with the endothelium-intact segments. Renal artery relaxed slightly but significantly more to ANP after removal of the vascular endothelium; the higher the concentration, the more significant the differences were (Table 1). Where endothelial removal caused a significant difference in response, data from endothelium-intact segments have been used to calculate potency ratios.

Vasodilator responses in pulmonary artery to atrial peptides: ANP, AP III and AP I

The closely related atrial peptides AP I and AP III produced potent relaxation of the pulmonary artery segments. AP I was, however, significantly less potent that AP III and APIII was significantly less potent than ANP (Figure 3). Potency ratios at 40% relaxation were approximately 30 ($P<0.001$) for AP I vs ANP and 2 ($P<0.05$) for AP III vs ANP. AP I and AP III produced, in some segments, a brief period of relaxation of less than 3 min, followed by a return to constriction (15 and 30%, respectively $n=5$ for AP I and $n=11$ for AP III). These atypical results have been excluded from analysis but warrant further investigation.

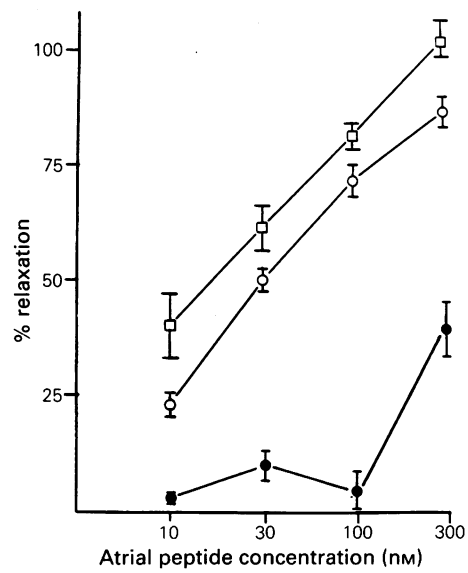

Figure 3 Vasorelaxant potencies of various atrial peptides, α-human atrial natriuretic peptide (α-hANP; □, $n=12$), atriopeptin III (AP III; ○, $n=10$) and atriopeptin I (AP I; ●; $n=6$) in pig pulmonary artery. Each point represents the mean (vertical lines show s.e.) of data pooled regardless of the state of the endothelium, except for the AP I responses which are those of pulmonary artery preparations with endothelium.

Discussion

Following the discovery of peptide-containing granules in the atrial myocytes and the demonstration of the potent natriuretic and vasodilator properties of rat

atrial extracts (De Bold *et al.*, 1981), ANP, a 28 amino acid peptide containing a disulphide bridge, has been hypothesized to be the major circulating hormone of the family of peptides which may be extracted from the atria (Schwartz *et al.*, 1985). Infusions of synthetic ANP in animals and man lead to a natriuresis and diuresis and at higher infusion rates a fall in blood pressure is observed (Kangawa & Matsuo, 1984; Garcia *et al.*, 1985; Waldhausl *et al.*, 1986). Preconstricted, isolated, vascular tissues respond to application of ANP by a relaxation which appears to be mediated by activation of guanylate cyclase (Furchgott & Jothianandan, 1983; Rapoport & Murad, 1983; Furchgott *et al.*, 1984; Hirata *et al.*, 1985). In man circulating levels of ANP are highest in the pulmonary artery; hence, we examined the responses of pulmonary artery and compared these with the responses of a systemic artery of similar size, the renal artery.

Our finding that a given concentration of ANP relaxes pulmonary artery significantly more than it does renal artery suggests a possible role of ANP in the modulation of pulmonary vascular tone at concentrations which are unlikely to affect systemic blood pressure. ANP is released in response to a fluid load and the stimulus for release is thought to be right atrial distension. The modulation of pulmonary vascular resistance in response to fluid load would be a useful mechanism in the control of left atrial pressure.

Other atrial peptides have been shown to be released into the circulation in animals and we have therefore examined the effect on the pulmonary artery of the two atrial peptides most closely related to ANP. AP III differs from ANP by the loss of 4 amino acids on the N-terminal (Geller *et al.*, 1984; Kangawa & Matsuo, 1984). Rat AP III was used in these experiments and this differs from human AP III in respect of an isoleucine substitution for methionine at position 12 (Kangawa *et al.*, 1984). AP I differs from AP III by the loss of a further 3 amino acids on the C-terminal (Geller *et al.*, 1984). Both peptides still contain the cystine disulphide bridge, which is essential for its biological activity (Misono *et al.*, 1984; Hirata *et al.* 1985) and the Phe-Arg sequence in the carboxyl terminus which appears to be required for the vasorelaxant property (Currie *et al.*, 1984; Geller *et al.*, 1984; Hirata *et al.*, 1985). In the pulmonary artery the decreased potency of these peptides when compared to ANP was correlated with the loss of amino acids from the parent ANP molecule. The finding that AP I produced some degree of vasorelaxation is in contrast to the results of other workers (Currie *et al.*, 1984; Oshimi *et al.*, 1984), but corresponds to the data of Sugiyama *et al.* (1984). Because circulating levels of these peptides are much lower than those of ANP, they are unlikely to have a role in the physiological maintenance of pulmonary arterial tone.

Removal of the vascular endothelium did not alter the responses of pulmonary artery to ANP or AP III, demonstrating that relaxation is independent of release of EDRF. Winquist *et al.* (1984) have previously demonstrated vasorelaxation independent of EDRF in the rabbit aorta. This contrasts with the findings in the renal artery, which was more readily relaxed by ANP after rubbing of the internal surface. The mechanism of this phenomenon is unknown, but may be related to increased access of the peptide to the vascular smooth muscle via the disrupted endothelium or to a loss of vasoconstrictor factors. The magnitude of this effect was small, however, when compared with the difference in potency of ANP between the pulmonary and renal arteries.

Whilst others have demonstrated the endothelial independence of atrial peptide-induced vasorelaxation in the pulmonary artery (Ignarro *et al.*, 1986), this is the first demonstration of the increased sensitivity to atrial natriuretic peptide of the pulmonary vasculature in comparison with other systemic vessels. Coupled with the knowledge that circulating ANP levels are highest in the pulmonary artery, this indicates a possible role for the atrial peptides in the modulation of pulmonary vascular tone.

Our grateful thanks to Dr R.G. Hill and the staff of the Parke Davis Research Unit for their helpful advice and for allowing us to use their facilities. A.H.M. is in receipt of a Grant from the British Lung Foundation.

References

CURRIE, M.G., GELLER, D.M., COLE, B.R., SIEGEL, N.R., FOK, K.F., ADAMS, S.P., EUBANKS, S.R., GALLUPPI, G.R. & NEEDLEMAN, P. (1984). Purification and sequence analysis of bioactive atrial peptides (atriopeptins). *Science*, **223**, 67–69.

DE BOLD, A.J., BORENSTEIN, H.B., VERESS, A.T. & SONNENBERG, H. (1981). A rapid and potent natriuretic response to intravenous injection of atrial myocardial extracts in rats. *Life Sci.*, **28**, 89–94.

DIETZ, J.R. (1984). Release of natriuretic factor from rat heart-lung preparation by atrial distension. *Am. J. Physiol.*, **247**, R1093–R1096.

FURCHGOTT, R.F., CHERRY, P.D., ZAWADZKI, J.V. & JOTHIANANDAN, D. (1984). Endothelial cells as mediators of vasodilation of arteries. *J. cardiovasc. Pharmac.*, **6**, (suppl. 2), S336–343.

FURCHGOTT, R.F. & JOTHIANANDAN, D. (1983). Relation of cyclic GMP levels to endothelium-dependent relaxa-

tion by acetylcholine in rabbit aorta. *Fedn. Proc.*, (abstract) **42,** 619.

GARCIA, R., THIBAULT, G., GUTKOWSKA, J., HAMET, P., CANTIN, M.M. & GENEST, J. (1985). Effect of chronic infusion of synthetic atrial natriuretic factor (ANF 8–33) in conscious two-kidney, one-clip hypertensive rats. *Proc. Soc. exp. Biol. Med.*, **178,** 155–159.

GELLER, D.M., CURRIE, M.G., WAKITANI, K., COLE, B.R., ADAMS, S.P., FOK, K.F., SIEGEL, N.R., EUBANKS, S.R., GALLUPPI, G.R. & NEEDLEMAN, P. (1984). Atriopeptins: a family of potent biologically active peptides derived from mammalian atria. *Biochem. biophys. Res. Commun.*, **120,** 333–338.

HIRATA, Y., TOMITA, M., TADAKA, S. & YOSHIMI, H. (1985). Vascular receptor binding activities and cyclic GMP responses by synthetic human and rat ANP and receptor down-regulation by ANP. *Biochem. biophys. Res. Commun.*, **128,** 538–546.

IGNARRO, L.J., WOOD, K.S., HARBISON, R.G. & KADOWITZ, P.J. (1986). Atriopeptin II relaxes and elevates cGMP in bovine pulmonary artery but not vein. *J. appl. Physiol.*, **60,** 1128–1133.

KANGAWA, K. & MATSUO, H. (1984). Purification and complete amino acid sequence of α-human atrial natriuretic polypeptide. *Biochem. biophys. Res. Commun.*, **118,** 131–139.

KANGAWA, K., FUKUDA, A., KUBOTA, I, HAYASHI, Y. & MATSUO H. (1984). Identification in rat atrial tissue of multiple forms of natriuretic polypeptides of about 3,000 daltons. *Biochem. biophys. Res. Commun.*, **121,** 585–591.

KATSUBE, N., WAKITANI, K., FOK, K.F., TSJOENG, F.S., ZUPEC, M.E., EUBANKS, S.R., ADAMS, S.P. & NEEDLEMAN, P. (1985). Differential structure-activity relationships of atrial peptides as natriuretics and renal vasodilators in the dog. *Biochem. biophys. Res. Commun.*, **128,** 325–330.

MISONO, K.S., FUKUMI, H., GRAMMER, R.T. & INAGAMI, T. (1984). Rat atrial natriuretic factor: complete amino acid sequence and disulfide linkage essential for biological activity. *Biochem. biophys. Res. Commun.*, **119,** 524–529.

NEEDLEMAN, P. & GREENWALD, J.E. (1986). Atriopeptin: a cardiac hormone intimately involved in fluid, electrolyte, and blood-pressure homeostasis. *New. Eng. J. Med.*, **314,** 828–834.

OSHIMA, T., CURRIE, M.G., GELLER, D.M. & NEEDLEMAN, P. (1984). An atrial peptide is a potent renal vasodilator substance. *Circulation Res.*, **54,** 612–616.

RAPOPORT, R.M. & MURAD, F. (1983). Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circulation Res.*, **52,** 352–357.

RICHARDS, A.M., CLELAND, J.G.F., TONOLO, G., McINTYRE, G.D., LECKIE, B.J., DARGIE, H.J., BALL, S.G. & ROBERTSON, J.I.S. (1986). Plasma α natriuretic peptide in cardiac impairment. *Br. med. J.*, **293,** 409–412.

SCHWARTZ, D., GELLER, D.M., MANNING, P.T., SIEGEL, N.R., FOK, K.F., SMITH, C.E. & NEDDLEMAN, P. (1985). Ser-Leu-Arg-Arg-Atriopeptin III: The major circulating form of atrial peptide. *Science*, **229,** 397–400.

SUGIYAMA, M., FUKUMI, H., GRAMMER, R.T., MISONO, K.S., YABE, Y., MORISAWA, Y. & INAGAMI, T. (1984). Synthesis of atrial natriuretic peptides and studies on structural factors in tissue specificity. *Biochem. biophys. Res. Commun.*, **123,** 338–344.

WALDHAUSL, W., VIERHAPPER, H. & NOWOTNY, P. (1986). Prolonged administration of human atrial natriuretic peptide in healthy men: evanescent effects on diuresis and natriuresis. *J. clin. Endocrinol. Metab.*, **62,** 956–959.

WINQUIST, R.J., FAISON, E.P. & NUTT, R.F. (1984). Vasodilator profile of synthetic atrial natriuretic factor. *Eur. J. Pharmac.*, **102,** 169–173.

(Received February 4, 1987.
Revised March 30, 1987.
Accepted April 1, 1987.)