Br J Clin Pharmacol 1995; **40**: 585–590

Acute effects of ANP and BNP on hypoxic pulmonary vasoconstriction in humans

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1 Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) have pulmonary vasorelaxant activity with plasma concentrations being elevated in patients with hypoxaemic pulmonary hypertension. However, their effects on acute hypoxic pulmonary vasoconstriction (HPV), the initiating stimulus for pulmonary hypertension have not to date been investigated. We have therefore studied the effects of ANP and BNP on acute HPV in humans.

2 Eight healthy volunteers were studied on three separate occasions. After reaching a resting haemodynamic state (t_0), an infusion of either ANP (10 pmol kg^{-1} min^{-1}), BNP (10 pmol kg^{-1} min^{-1}) or placebo (5% dextrose) was commenced. This was given alone for 30 min (t_{30}) before subjects were rendered hypoxaemic (SaO_2 75–80%) for a further 30 min (t_{60}), with the initial infusion continuing to t_{60}. Pulsed-wave Doppler analysis of pulmonary artery flow was used to measure mean pulmonary arterial pressure (MPAP) and hence total pulmonary vascular resistance (PVR) was calculated.

3 MPAP and PVR both tended to decrease in response to ANP and BNP infusion, although compared with placebo, the difference at t_{30} was only statistically significant for PVR. Hypoxaemia increased MPAP and PVR, although values at t_{60} were significantly lower following both ANP and BNP compared with placebo.

4 In terms of the actual change in PVR (ΔPVR) induced by hypoxaemia (from t_{30} to t_{60}), BNP (146(16) dyn s cm^{-5}), but not ANP (183(21) dyn s cm^{-5}) significantly attenuated ΔPVR compared with placebo (194(26) dyns s cm^{-5}): mean difference BNP versus placebo 48 dyn s cm^{-5}, 95% Cl 3–93. An identical pattern was observed for ΔMPAP where BNP (15.9(1.1) mmHg), but not ANP (18.0(1.2) mmHg) significantly attenuated ΔMPAP compared with placebo (19.0(1.7) mmHg): mean difference BNP versus placebo 3.1 mmHg, 95% Cl 0.7–5.5.

5 Thus, although both ANP and BNP exhibit pulmonary vasorelaxant activity, only BNP significantly attenuated the MPAP and PVR responses to acute hypoxaemia. This suggests that the natriuretic peptides may have a role in attenuating pulmonary hypertension secondary to hypoxaemia.

Keywords natriuretic peptides hypoxaemia pulmonary circulation

Introduction

The vasoconstriction of the mammalian pulmonary vasculature in response to hypoxia has intrigued physiologists since the phenomenon was first described by Von Euler & Liljestrand [1]. The exact mechanism underlying acute hypoxic pulmonary vasoconstriction (HPV) remains unknown but the beneficial effects of lung blood flow redistribution are more clear. Vasoconstriction in areas of alveolar hypoxia leads to diversion of blood flow away from hypoxic regions hence improving overall ventilation/perfusion matching [2]. This response can, however, be detrimental if hypoxia is chronic and affects the whole lung, as is seen in hypoxaemic chronic

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obstructive pulmonary disease. In this setting, the consequences of HPV include development of pulmonary hypertension [3] and eventual right ventricular decompensation [4]. When this situation arises in man, the clinical picture is that of cor pulmonale with attendant high morbidity and mortality [5].

Agents with the ability to attenuate acute HPV may be therapeutically important in preventing or decreasing the pulmonary hypertension secondary to chronic hypoxia. In this respect, the natriuretic peptides of cardiac origin are of interest. Atrial natriuretic peptide (ANP) is released acutely in response to atrial stretching [6, 7] whilst brain natriuretic peptide (BNP) is produced mainly from the cardiac ventricles [8] and appears to be released over a longer time scale in response to a sustained increase in ventricular afterload [7, 9]. The natriuretic and systemic vasorelaxant properties of these peptides are well recognized [10] but their effects in the pulmonary vasculature have been less extensively studied.

In vitro, both ANP and BNP have pulmonary vasorelaxant effects [11], whilst in humans *in vivo*, both ANP and BNP can lower baseline pulmonary pressure and vascular resistance, and BNP can attenuate the pulmonary pressor effects of angiotensin II [12]. In terms of an interaction with hypoxaemia, animal studies have shown that ANP can attenuate acute HPV in pigs [13] although BNP has not been studied in this respect. Although ANP is released acutely in response to hypoxaemia [14], the nature of any pulmonary vascular interaction with hypoxaemia has not been studied in man. We have therefore investigated for the first time the effects of ANP and BNP on acute HPV in normal humans.

Methods

Subjects

Eight healthy male volunteers, age (mean ± s.e. mean) 26.4 ± 2.3 years (range 20–36 years) were studied. Prior to inclusion, subjects were required to have no abnormality on clinical history or examination and 12 lead electrocardiogram, echocardiogram, biochemical and haematological indices were also normal. No medications were permitted during and for 1 month before the study. Informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics, was obtained.

Study protocol

Subjects were studied on three occasions, separated by at least 3 days with treatments given in random order. On arrival at the laboratory, intravenous cannulae were inserted into veins in both forearms for blood sampling (right) and for infusion (left). Subjects then rested supine for 30 min to obtain baseline resting haemodynamic parameters (t_0). An infusion of either 10 pmol kg^{-1} min^{-1} ANP (Clinalfa AG, Laufelfingen, Switzerland), 10 pmol kg^{-1} min^{-1} BNP (Clinalfa) or 1 ml min^{-1} placebo (5% dextrose) was commenced and continued alone for 30 min (t_{30}). Subjects were then rendered hypoxaemic by breathing a variable mixture of nitrogen and oxygen, sufficient to render arterial oxygen saturation between 75% and 80%, for 30 min (t_{60}). The hypoxic gas mixture was produced from separate cylinders of nitrogen and oxygen fitted with variable flow valves. Gases were mixed in a 25 l Douglas bag, from which subjects breathed through a mouthpiece connected by a series of one-way valves, while wearing an occlusive nose clip. Measurements of pulmonary and systemic haemodynamic variables were made at t_0, t_{30} and at t_{60} whilst venous blood for ANP and BNP assay was taken at t_{60}.

Measurements

Oxygenation Arterial blood oxygen saturation was continuously monitored by transcutaneous pulse oximetry (CSI 503, Criticare Systems Inc, Waukesha, WI, USA).

Haemodynamics Heart rate (HR) was recorded on an electrocardiograph trace and an average rate over 1 min was obtained. Mean arterial blood pressure (MAP) was measured by semi-automatic sphygmomanometer (Vital Signs Monitor, Critikon, Tampa, FL, USA). Pulmonary acceleration time (PAT) in milliseconds was measured as previously described [15] from pulmonary arterial flow by pulsed-wave Doppler echocardiography (Vingmed SD50, Vingmed Sound, Horten, Norway) from the left 3rd/4th intercostal space. The mean of three consistent waveforms at each time point was used for the purpose of analysis. Mean pulmonary artery pressure (MPAP) in mmHg was calculated as MPAP = 73-(0.42 × PAT) [16]. Aortic cross-sectional area (CSA) was measured by M-mode echocardiography (Vingmed SD50). The aortic systolic velocity integral (SVI) was measured by on-line computer assisted determination using pulsed-wave Doppler echocardiography of ascending aortic blood flow from the suprasternal notch. On-line calculations of stroke volume (SV = SVI × CSA) and cardiac output (CO) as the product of SV and HR were also made. Total pulmonary vascular resistance (PVR) was calculated as: PVR = MPAP/CO × 80 dyn $s\,cm^{-5}$, and total systemic vascular resistance (SVR) was calculated as: SVR = MAP/CO × 80 dyn $s\,cm^{-5}$. We have previously shown the short term coefficients of variability for measurement of PAT and SVI in our hands to be 1.7% and 1.2% respectively [15].

Peptide hormones Venous blood was collected into EDTA tubes containing 4000 kiu aprotinin before centrifugation at 4° C and serum stored at −70° C until assayed in one batch in duplicate at the end of the study. ANP assay was performed after solid-phase plasma extraction with Sep-Pak C18 cartridges (Waters; Millipore Corp, Milford, MA, USA) giving 49% extraction efficiency, using a commercially available radio-

immunoassay kit (Incstar Corporation, Stillwater, MN, USA). BNP assay was performed following extraction with Isolute C8 columns (International Sorbent Technology Ltd, Hengoed, UK) giving 86% extraction efficiency, using a commerically available radioimmunoassay kit (Peninsula Laboratories Inc, Belmont, CA, USA). The intra-assay coefficients of variation for assay of ANP was 8.0% and for BNP was 9.9%. Data for ANP and BNP concentrations were corrected for relative extraction efficiency prior to analysis.

Data analysis

Comparison of values between study days or between serial time points on the same study day were made by multifactorial analysis of variance (MANOVA) and Duncan's multiple-range testing [17]. A probability value of $P<0.05$ (two-tailed) was considered to be statistically significant. Data are presented in the text, tables and figures as means and s.e. mean, and where a difference between means is quoted, the 95% confidence interval for this difference is given.

Results

Natriuretic peptide levels

Final plasma concentration at t_{60} of ANP (corrected for extraction efficiency) during ANP infusion (327 ± 28 pmol^{-1}) was not significantly different from BNP concentration at t_{60} during BNP infusion (290 ± 22 pmol l^{-1}) indicating that at steady-state, molar concentrations of ANP and BNP were similar.

Pulmonary haemodynamics

Absolute values of PAT from which MPAP and PVR were calculated are given in Table 1. Baseline MPAP and PVR at t_0 were similar on each study day (Figure 1).

Table 1 Absolute values (mean ± s.e. mean) of pulmonary acceleration time (PAT) in ms at baseline (t_0), and before (t_{30}) and after hypoxaemia (t_{60}), during infusion of placebo, ANP or BNP

	t_0	t_{30}	t_{60}
Placebo	151 (2.3)	152 (1.9)	107 (4.0)†
ANP	151 (2.6)	156 (2.3)†	113 (3.1)*†
BNP	149 (0.8)	157 (1.3)†	119 (2.9)*†

Asterisk (*) denotes values significantly ($P<0.05$) different from placebo at the same time point, cross (†) denotes values significantly ($P<0.05$) different from t_0 (baseline). PAT was significantly lengthened by infusion of ANP and BNP at t_{30} compared with baseline. Hypoxaemia shortened PAT from baseline on all three study days although in comparison with placebo, levels were significantly higher following both ANP and BNP infusion.

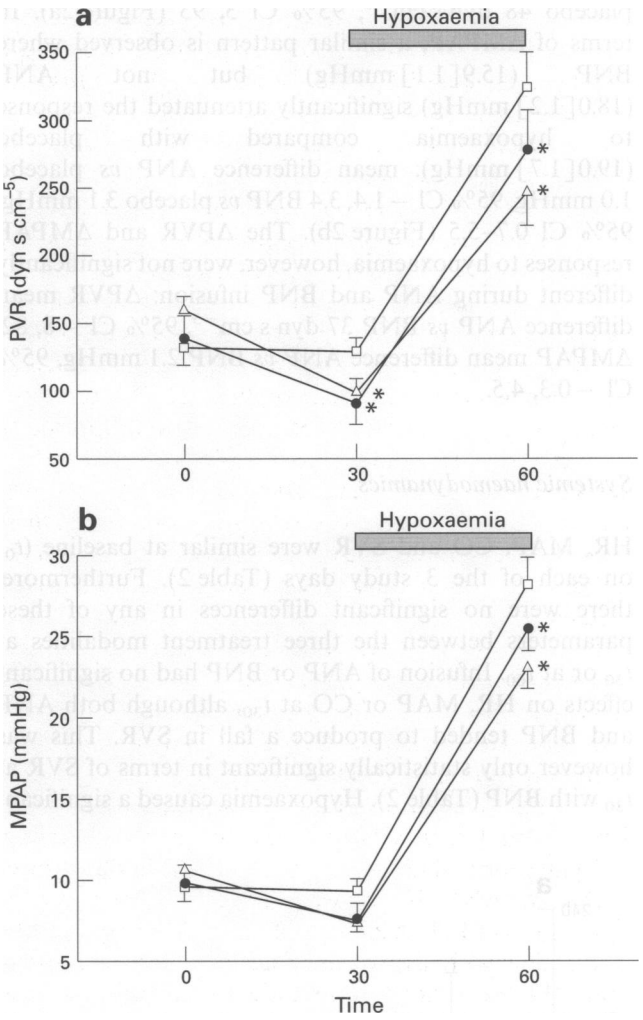

Figure 1 Absolute values (mean and s.e. mean) of a) total pulmonary vascular resistance (PVR) and b) mean pulmonary artery pressure (MPAP) at baseline (t_0), and before (t_{30}) and after hypoxaemia (t_{60}), and during infusion of placebo (□), ANP (●) or BNP (△). Asterisk (*) denotes values significantly ($P<0.05$) different from placebo.

PVR at t_{30} was significantly lower following ANP (91(16) dyn s cm^{-5}) and BNP (98[11] dyn s cm^{-5}) infusion compared with placebo (128[10] dyn s cm^{-5}) (Figure 1a). In terms of MPAP at t_{30}, values after ANP (7.5[0.9] mmHg) and BNP (7.3[0.5] mmHg) were not significantly different from placebo (9.3[0.8] mmHg) (Figure 1b). Hypoxaemia caused a significant increase in PVR on all study days, although values at t_{60} were significantly lower following ANP (276(30) dyn s cm^{-5}) and BNP (246(26) dyn s cm^{-5}) compared with placebo (323(25) dyn s cm^{-5}) (Figure 1a). There was a similar pattern for MPAP with values at t_{60} being significantly lower following ANP (25.5(1.4) mmHg) and BNP (23.1(1.3) mmHg) compared with placebo (28.3(1.6) mmHg) (Figure 1b).

The actual change induced by hypoxaemia (between t_{30} and t_{60}) was also calculated and is expressed as ΔPVR and ΔMPAP. The ΔPVR response to hypoxaemia was significantly lowered by BNP (146[16] dyn s cm^{-5}) but not by ANP (183[21] dyn s cm^{-5}) compared with placebo (194[26] dyn s cm^{-5}): mean difference ANP *vs* placebo 11 dyn s cm^{-5}, 95% CI −34, 56; BNP *vs*

placebo 48 dyn s cm^{-5}, 95% CI 3, 93 (Figure 2a). In terms of ΔMPAP, a similar pattern is observed where BNP (15.9[1.1] mmHg) but not ANP (18.0[1.2] mmHg) significantly attenuated the response to hypoxaemia compared with placebo (19.0[1.7] mmHg): mean difference ANP *vs* placebo 1.0 mmHg, 95% CI −1.4, 3.4 BNP *vs* placebo 3.1 mmHg, 95% CI 0.7–5.5 (Figure 2b). The ΔPVR and ΔMPAP responses to hypoxaemia, however, were not significantly different during ANP and BNP infusion: ΔPVR mean difference ANP *vs* BNP 37 dyn s cm^{-5}, 95% CI −8, 82; ΔMPAP mean difference ANP *vs* BNP 2.1 mmHg, 95% CI −0.3, 4.5.

Systemic haemodynamics

HR, MAP, CO and SVR were similar at baseline (t_0) on each of the 3 study days (Table 2). Furthermore, there were no significant differences in any of these parameters between the three treatment modalities at t_{30} or at t_{60}. Infusion of ANP or BNP had no significant effects on HR, MAP or CO at t_{30}, although both ANP and BNP tended to produce a fall in SVR. This was however only statistically significant in terms of SVR at t_{30} with BNP (Table 2). Hypoxaemia caused a significant increase in MAP at t_{60} during placebo infusion but not during ANP or BNP infusion. HR and CO increased significantly, whilst SVR was significantly reduced at t_{60} in response to hypoxaemia on each of the three study days (Table 2).

Table 2 Absolute values (mean ± s.e. mean) at baseline (t_0), and before (t_{30}) and after hypoxaemia (t_{60}), of heart rate (HR), mean arterial pressure (MAP), cardiac output (CO) and cystemic vascular resistance (SVR) during infusion of placebo, ANP or BNP

	Systemic haemodynamics		
	Placebo	*ANP*	*BNP*
HR(beats min^{-1})			
t_0	65.3 (2.1)	67.3 (2.7)	62.3 (2.9)
t_{30}	62.1 (2.7)	67.8 (3.9)	63.9 (2.2)
t_{60}	74.1 (3.1)*†	82.1 (4.7)*†	80.0 (3.2)*†
MAP(mmHg)			
t_0	84.4 (2.7)	87.8 (2.2)	86.4 (2.9)
t_{30}	83.0 (2.5)	86.6 (2.9)	85.4 (3.5)
t_{60}	88.3 (1.2)†	87.0 (2.3)	85.7 (2.8)
CO(l min^{-1})			
t_0	5.80 (0.23)	5.77 (0.35)	5.40 (0.27)
t_{30}	5.77 (0.23)	6.33 (0.47)	6.14 (0.41)
t_{60}	7.10 (0.25)*†	7.45 (0.62)*†	7.25 (0.67)*†
SVR(dyn s cm^{-5})			
t_0	1174 (56)	1268 (124)	1300 (72)
t_{30}	1159 (42)	1144 (109)	1147 (87)*
t_{60}	1006 (46)*†	990 (108)*†	1001 (107)*†

Asterisk (*) denotes values significantly ($P<0.05$) different from t_0 (baseline), cross (†) denotes values significantly ($P<0.05$) different from t_{30}.

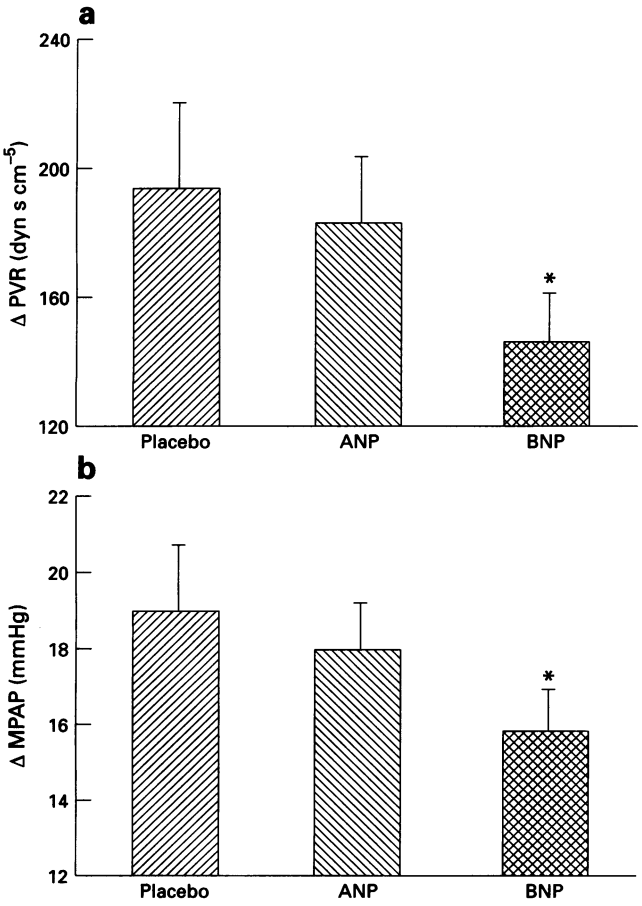

Figure 2 Change in a) total pulmonary vascular resistance (ΔPVR) and b) mean pulmonary artery pressure (ΔMPAP) in response to hypoxaemia (between t_{30} and t_{60}). Values are depicted as means and s.e. mean. Asterisk (*) denotes values significantly ($P<0.05$) different from placebo.

Discussion

The present study has demonstrated that in addition to possessing direct pulmonary vasorelaxant activity, both ANP and BNP can decrease PVR and MPAP during acute hypoxaemia. However, in terms of the actual change in PVR and MPAP, despite the molar concentrations of both natriuretic peptides at steady-state being equivalent, only BNP was found to significantly attenuate HPV although the responses to hypoxaemia during ANP and BNP infusions were not significantly different. A larger sample size might therefore have been able to detect the much smaller effect of ANP on acute HPV in humans. This is the first demonstration in man however that any of the cardiac natriuretic peptides can modulate the pulmonary vascular response to hypoxaemia.

The physiological role of the natriuretic peptides in the pulmonary circulation remains unclear. Whilst the systemic haemodynamic, renal and endocrine effects of ANP and BNP are well characterized [10], relatively less research has been conducted to clarify their actions in the pulmonary vascular bed. The demonstration of an interaction with hypoxaemia in the human pulmon-

ary circulation is therefore of considerable interest and suggests a possible modulatory role in the pathophysiology of hypoxaemic pulmonary hypertension.

Acute HPV is the initiating stimulus which, if sustained, eventually leads to development of pulmonary hypertension. In an animal model, as well as blunting acute HPV, ANP has also been shown to attenuate the development of pulmonary hypertension and pulmonary vascular remodelling [18]. BNP has not been studied with respect to attenuation of acute or chronic pulmonary vascular responses to hypoxaemia. In the present study, we have achieved plasma concentrations of ANP and BNP similar to those observed at the upper end of the pathological range seen in patients with pulmonary hypertension secondary to chronic hypoxaemic lung disease [19]. ANP and especially BNP, may therefore have a counter-regulatory role in such patients which may attenuate the adverse pulmonary haemodynamic consequences of chronic hypoxaemia.

The observed interaction of HPV with the natriuretic peptides may be due to a specific interaction between the effects of the natriuretic peptides and of hypoxaemia in the pulmonary vasculature. Such an interaction is possible at a number of sites in their respective signal transduction pathways. In terms of HPV, the role of potassium channels in sensing, and subsequently closing in response to hypoxia is of interest [20]. The consequent calcium channel opening and increase in free intracellular calcium [21] leads to vascular smooth muscle contraction. This effect may be diminished by the natriuretic peptides in two ways. Activation of the type A natriuretic peptide receptor (NPR-A) leads to an increase in intracellular cGMP [22] which in turn appears to reduce intracellular calcium by a number of mechanisms [23]. Alternatively, a direct effect on ion channels has been proposed for ANP [24] where either intracellular cation concentration changes may affect other voltage sensitive channels or prevent closure of the potassium channel directly.

ANP and BNP are equipotent agonists of NPR-A [22] and thus it is difficult to explain the differential effects observed in this study. Furthermore, a pharmacokinetic explanation is not tenable to account for differences between the two peptides since steady-state molar concentrations were approximately equal. We decided to evaluate the effects of a single high dose infusion of both peptides in order to be sure of achieving steady-state within the 60 min infusion period. Constructing a dose-response curve would have required shorter infusion times, with the attendant risk that steady-state concentrations would not have been reached at each dose level. The dose of 10 pmol kg^{-1} min^{-1} was chosen to be identical to that used in a previous study in which the pulmonary pressor response to angiotensin II was significantly attenuated by BNP but not by ANP [12]. Whether there exists another receptor more specific for BNP, or BNP has further non-receptor mediated effects in the pulmonary vasculature, remains speculative.

We have shown the use of pulsed-wave Doppler to be highly reproducible [15], whilst others have validated its use in comparison with catheter measurement of MPAP [16, 25, 26]. However, one possible limitation of this non-invasive methodology is that our calculation of total pulmonary vascular resistance excludes measurement of pulmonary capillary wedge pressure (PCWP) as we would consider it unethical to insert Swan-Ganz catheters into healthy volunteers solely for research purposes. As previous studies have shown that acute hypoxaemia has no effect on invasive measurement of PCWP [27], we would not therefore expect changes in PCWP and left atrial pressure to occur and thus we believe PVR to be a valid measure of changes in pulmonary vascular tone. Furthermore, the cross-over design of the study means that comparison of derived variables between study days are still valid.

It is interesting to briefly speculate as to the possible clinical relevance of these findings and as to the role of the natriuretic peptides in the pulmonary vasculature. Studies in man are limited, but in addition to the observation that ANP and BNP levels are elevated in patients with pulmonary hypertension [19], ANP given by infusion has beneficial effects on pulmonary haemodynamics in such patients [28]. Inhibition of neutral endopeptidase leads to an increase in plasma concentrations of both ANP and BNP [29], and is the most practical method of increasing these peptides in man. In rats, this treatment has been shown to attenuate pulmonary vascular remodelling in response to hypoxaemia [30] and may therefore produce similar benefits in man. This effect might conceivably be seen if neutral endopeptidase inhibitors were given at an early stage in hypoxaemic lung disease, by attenuating the development of pulmonary hypertension, or as adjunctive therapy in established disease where haemodynamic benefit might be additive to the effects of oxygen therapy.

This research programme has been supported by a grant from the Scottish Hospital Endowments Research Trust (RIC is a Cruden Medical Research Scholar). The authors wish to thank Mrs Wendy Coutie for measurement of plasma ANP and BNP.

References

1 Von Euler US, Liljestrand G. Observations on the pulmonary arterial blood pressure in the cat. *Acta Physiol Scand* 1946; **12**: 301–320.

2 Cutaia M, Rounds S. Hypoxic pulmonary vasoconstriction: Physiologic significance, mechanism and clinical relevance. *Chest* 1990; **97**: 706–718.

3 Weitzenblum E, Loiseau A, Hirth C, Mirhom R, Rasaholinjanahary J. Course of pulmonary haemodynamics in patients with chronic obstructive pulmonary disease. *Chest* 1979; **75**: 656–662.

4 Klinger JR, Hill NS. Right ventricular dysfunction in chronic obstructive pulmonary disease: evaluation and management. *Chest* 1991; **99**: 715–723.

5 Report of the Medical Research Council Working Party. Long-term domiciliary oxygen therapy in chronic hypoxic cor pulmonale complicating chronic bronchitis and emphysema. *Lancet* 1981; **i**: 681–685.

6 Anderson JV, Donckier J, McKenna WJ, Bloom SR. The plasma release of atrial natriuretic peptide in man. *Clin Sci* 1986; **71**: 151–155.

7 Cargill RI, Coutie WJ, Lipworth BJ. The effects of

angiotensin II on circulating levels of natriuretic peptides. *Br J Clin Pharmacol* 1994; **38**: 139–142.

8 Mukoyama M, Nakao K, Hosoda K, Suga S, Saito Y, Ogawa Y, *et al.* Brain natriuretic peptide (BNP) as a novel cardiac hormone in humans. *J Clin Invest* 1991; **87**: 1402–1412.

9 Lang CC, Choy AMJ, Turner K, Tobin R, Coutie W, Struthers AD. The effect of intravenous saline loading on plasma levels of brain natriuretic peptide in man. *J Hypertension* 1993; **11**: 737–741.

10 Lang CC, Choy AMJ, Struthers AD. Atrial and brain natriuretic peptides: a dual natriuretic system potentially involved in circulatory homeostasis. *Clin Sci* 1992; **83**: 519–527.

11 Lippton H, Hao Q, Hyman A. Pulmonary vasodilator properties of natriuretic peptides. *Circulation* 1992; **86** (Suppl 1): 604 (abstract).

12 Cargill RI, Lipworth BJ. Pulmonary vasorelaxant activity of atrial natriuretic peptide and brain natriuretic peptide in humans. *Thorax* 1995; **50**: 183–185.

13 Adnot S, Chabrier PE, Brun-Buisson C, Viossat I, Braquet P. Atrial natriuretic factor attenuates the pulmonary pressor response to hypoxia. *J Appl Physiol* 1988; **65**: 1975–1983.

14 Kawashima A, Kubo K, Hira K, Yoshikawa S, Matsuzawa Y, Kobayashi T. Plasma levels of atrial natriuretic peptide under hypoxia in normal subjects. *Respir Physiol* 1989; **76**: 79–92.

15 Lipworth BJ, Dagg KD. Comparative effects of angiotensin II on Doppler parameters of left and right heart systolic and diastolic blood flow. *Br J Clin Pharmacol* 1994; 37: 273–278.

16 Dabestani A, Mahan G, Gardin JM, Takenaka K, Burn C, Allfie A, Henry WL. Evaluation of pulmonary artery pressure and resistance by pulsed Doppler echocardiography. *Am J Cardiol* 1987; **59**: 662–668.

17 Brown RA, Beck JS. Comparison of several groups: The analysis of variance. In *Medical Statistics on Microcomputers*; London; British Medical Journal Publishing; 1990: 34–42.

18 Zhao L, Winter RJD, Krausz T, Hughes JMB. Effects of continuous infusion of atrial natriuretic peptide on the pulmonary hypertension induced by chronic hypoxia in rats. *Clin Sci* 1991; **81**: 379–385.

19 Lang CC, Coutie WJ, Struthers AD, Dhillon DP, Winter JH, Lipworth BJ. Elevated levels of brain natriuretic peptide in acute hypoxaemic chronic obstructive pulmonary disease. *Clin Sci* 1992; **83**: 529–533.

20 Post JM, Hume JR, Archer SL, Weir EK. Direct role for potassium channel inhibition in hypoxic pulmonary vasoconstriction *Am J Physiol* 1992; **262**: C882–C890.

21 Cohen CJ, McCarthy RT, Barrett PQ, Rasmussen H. Ca channels in adrenal glomerulosa cells: K^+ and angiotensin II increase T-type Ca channel current. *Proc Nat Acad Sci* 1988; **85**: 2412–2416.

22 Suga S, Nakao K, Hosoda K, Mukoyama M, Ogawa Y, Shirakami G, *et al.* Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide and c-type natriuretic peptide. *Endocrinol* 1992; 130: 229–239.

23 Northridge DB, McMurray J. The cardiovascular effects of atrial natriuretic factor. In *Atrial natriuretic factor.* ed Struthers, AD, Oxford; Blackwell Scientific Publications; 1990: 77–80.

24 Sato K, Murakami K, Nishimura K, Ito K, Kangawa K, Matsuo H. Possible involvement of sodium pump in the relaxation of rat aorta induced by α-human atrial natriuretic polypeptide. *Jap J Physiol* 1988; 38: 187–198.

25 Graettinger WF, Greene ER, Voyles WF. Doppler predictions of pulmonary artery pressure, flow and resistance in adults. *Am Heart J* 1987; 113: 1426–1436.

26 Kitabatake A, Inoue M, Asao M, Masuyama T, Tanouchi J, Morita T, *et al.* Noninvasive evaluation of pulmonary hypertension by a pulsed Doppler technique. *Circulation* 1983; **68**: 302–309.

27 Beard JT, Newman JH, Loyd JE, Byrd BF. Doppler estimation of changes in pulmonary artery pressure during hypoxic breathing. *J Am Soc Echo* 1991; **4**: 121–130.

28 Adnot S, Andrivet P, Chabrier PE, Piquet J, Plas P, Braquet P, *et al.* Atrial natriuretic factor in chronic obstructive lung disease with pulmonary hypertension. *J Clin Invest* 1989; **83**: 986–993.

29 Lang CC, Motwani J, Coutie WJ, Struthers AD. Influence of candoxatril on plasma brain natriuretic peptide in heart failure. *Lancet* 1991; **338**: 255.

30 Stewart AG, Sheedy W, Thompson JS, Morice AH. Effects of SCH 34826, a neutral endopeptidase inhibitor on hypoxic pulmonary vascular remodelling. *Pulm Pharmacol* 1992; **5**: 111–114.

(Received 17 March 1995, accepted 9 August 1995)