Neutral Endopeptidase 24.11 Inhibition Reduces Pulmonary Vascular Remodeling in Rats Exposed to Chronic Hypoxia[1-3]

ROBERT J. D. WINTER, LAN ZHAO, THOMAS KRAUSZ, and J. M. B. HUGHES

SUMMARY Inhibition of the metabolism of endogenous atrial natriuretic peptide (ANP), by continuous infusion of a specific inhibitor of neutral endopeptidase (membrane metalloendopeptidase E.C. 3.4.24.11), UK 73,967 (candoxatrilat), was undertaken in rats, in which chronic hypoxia was used as a stimulus to induce pulmonary hypertension and right ventricular hypertrophy. Inhibition of neutral endopeptidase 24.11 with low-dose and high-dose UK 73,967 (NEI) increased endogenous plasma ANP by > 155% during the development of pulmonary hypertension. NEI treatment reduced mean pulmonary arterial pressure in hypoxia as follows: vehicle 26.6 ± 4.0 mm Hg; low-dose NEI 22.7 ± 1.9 mm Hg, and high-dose NEI 22.6 ± 2.5 mm Hg (both $p < 0.01$ compared with hypoxic vehicle); however, it was without effect on pulmonary arterial pressure in normoxia (17.6 ± 2.2 mm Hg) or on systemic blood pressure. The development of right ventricular hypertrophy was also reduced in both groups treated with NEI (right ventricular weight/left ventricular weight: 0.43 ± 0.03 vehicle; 0.40 ± 0.02 low-dose NEI and 0.40 ± 0.02 high-dose NEI, both $p < 0.05$ compared with vehicle). Remodeling of the pulmonary vasculature, characterized by extension of the muscle within the small pulmonary arteries toward the periphery of the lung, was reduced by NEI treatment (percentage of thick-walled peripheral vessels; 19.2 ± 3.1% vehicle; 10.4 ± 2.3% low-dose NEI and 8.1 ± 1.8% high-dose NEI, both $p < 0.001$ compared with vehicle). In the isolated blood perfused rat lung pulsed doses of NEI had no effect on pulmonary vascular tone in the absence of ANP. Specific inhibition of the enzyme neutral endopeptidase reduces vascular remodeling, the development of pulmonary hypertension, and right ventricular hypertrophy. Endogenous ANP modulates vascular remodeling *in vivo*. Retarding the metabolism of endogenous ANP through inhibition of neutral endopeptidase 24.11 represents a potential approach toward therapy.

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Introduction

Atrial natriuretic peptide (ANP), a natriuretic, diuretic, and vasorelaxant peptide, is stored within electron-dense granules within both atria and synthesized, additionally, in both ventricles and at certain extracardiac sites. In pulmonary hypertension, primary or secondary to parenchymal lung disease, there is increased synthesis and secretion of ANP and increased plasma ANP despite normal right atrial pressure (1-3). Experimental models of pulmonary hypertension have also shown plasma ANP immunoreactivity to be increased whether the stimulus used to produce this is hypoxia (4, 5) or the alkaloid monocrotaline (6). An important site of increased ANP synthesis may be the hypertrophied right ventricle, as both immunoreactive ANP and ANP messenger RNA (mRNA) in this tissue are increased in pulmonary hypertension (7). Similar increases in plasma ANP have been reported in patients and experimental animals with systemic hypertension (8, 9) as has the increase in ANP mRNA in the hypertrophied ventricle, suggesting that ventricular synthesis of ANP is increased in the hypertrophied state in both right and left ventricles (10).

Although the pharmacologic effects of ANP on sodium and water handling by the kidney, on vascular smooth muscle, and on the renin-angiotensin-aldosterone cascade have been well described (11), limited information is available about the role of increased endogenous ANP concentration in pulmonary and systemic hypertension. The possibility that endogenous ANP might alter pulmonary vascular tone was investigated in studies in which C-ANP, a ring deleted analogue of ANP that binds to the ANP clearance receptor, was studied in rats with established hypoxic pulmonary hypertension (12). An acute fall in mean pulmonary arterial pressure (Ppa) was produced by C-ANP in rats in which the pulmonary vascular remodeling had been produced by chronic hypoxia but not in normoxic control rats. Lately, we and others have reported that physiologic doses of synthetic ANP attenuates both developing and fully established pulmonary hypertension and pulmonary vascular remodeling (13, 14). The therapeutic potential of ANP is, however, severely limited by its peptidic nature and rapid elimination. A plasma half-life of ANP shorter than 5 min has been reported in all species studied (15), 50 to 80% of ANP being removed from plasma in one passage across a major target organ by the enzyme membrane metalloendopeptidase, E.C. 3.4.24.11 (neutral endopeptidase 24.11) (16), a zinc-dependent peptidase widely distributed in peripheral tissues and at high concentration in the lung and in the kidney (17). Inhibition of this enzyme, also termed atriopeptidase, in rats, normal volunteers, and in patients with heart failure prolongs the half-life of ANP, with corresponding increases in plasma ANP and diuretic response following volume loading (18). We have investigated the effect of potentiating the activity of the endogenous ANP by preventing its *in vivo* degradation. We report the effect of treatment with an atriopeptidase inhibitor UK 73,967 (candox-

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[1] From the Respiratory Division, Department of Medicine, and the Department of Histopathology, Royal Postgraduate Medical School, Hammersmith Hospital, London, United Kingdom.

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[3] Correspondence and requests for reprints should be addressed to Dr. Robert Winter, Department of Medicine (Respiratory Division), Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK.

atrilat; Pfizer, Sandwich, Kent, England) on development of pulmonary hypertension, vascular remodeling, and cardiac hypertrophy.

Methods

Neutral Endopeptidase 24.11 Inhibition

The neutral endopeptidase inhibitor UK 73,967 (NEI) (candoxatrilat: Pfizer), the active enantiomer of UK-69,578 (18) was used. In *in vitro* studies UK-73,967 is a potent inhibitor of EC 3.4.24.11 isolated from both rat K_i 3.6×10^{-8} M) and dog kidney (K_i 5.2×10^{-8} M), the compound displaying classic competitive kinetics. UK-73,967 exhibits negligible inhibition of carboxypeptidase A, leucine aminopeptidase, trypsin, chymotrypsin, and angiotensin-converting enzyme at concentrations up to 10^{-4} M. Thus, UK-73,967 appears to be a specific inhibitor of neutral endopeptidase 24.11, with no significant inhibitory activity demonstrated toward mammalian proteases representative of the zinc- and serine-dependent classes. *In vivo* studies have shown that UK-73,967 is a potent inhibitor of renal and extra renal components of ANP metabolism capable of producing a 2- to 3-fold increment in plasma ANP (19). Two doses of UK-73,967 were used corresponding to a dose of 5.4 mg/kg/day, the concentration required to inhibit 95% of the activity of neutral endopeptidase in the rat (20) and a 3-fold increase (16.2 mg/kg/day).

Drug Delivery and Experimental Model

Specified pathogen-free male Wistar rats (in the weight range 200 to 250 g) were used (Tucks, Battlesbridge, Essex). Water and standard laboratory chow were given without restriction. Intraperitoneally administered pentobarbitone sodium (6 mg/100 g body weight) was used as anesthesia initially for implantation of osmotic minipumps (see below) and subsequently for measurements of Ppa and systemic blood pressure. The left jugular vein was cannulated by a polyethylene catheter (Alzet, Palo Alto, CA) (PE-60), channelled subcutaneously, and connected to osmotic minipumps (Model 2ml1; Alzet), which were also positioned subcutaneously. A washout period of 1 day was allowed at the end of the 7-day period of drug delivery to allow hemodynamic measurements to be made. Six groups (n = 8 in all groups) received vehicle, low-dose NEI, or high-dose NEI, half in normoxia and half in hypoxia for 7 days.

Rats were placed in a hypoxic chamber in which F_{IO_2} was maintained at 10% (21), an established and well-validated method of producing pulmonary hypertension (22, 23). Excess carbon dioxide was removed by self-indicating soda-lime filters, changed daily, and humidity was controlled by refrigeration so that condensation occurred outside the chamber. Gas was sampled periodically for analysis by mass spectrometer: fraction of inspired oxygen (F_{IO_2}) remained within 0.5% of the prescribed level and fraction of inspired carbon dioxide (F_{ICO_2}) was less than 0.04% throughout. No adverse effects were observed due to osmotic minipump implantation, hypoxic exposure, or drug infusion.

Ppa and Vascular Responses to NEI

Under anesthesia with pentobarbitone (6 mg/100 g body weight) the pulmonary artery was cannulated via the right jugular vein using a precurved catheter, as described by Po and Wenli (24). The left carotid artery was cannulated with a heparinized polyethylene cannula and simultaneous recordings of systemic blood pressure and Ppa in normoxia were made with the three-channel thermal array recorder (Gould RE 550; Gould, Essex, UK).

The effect of a pulsed-dose of NEI on pulmonary vascular tone was studied using the isolated and blood-perfused rat lung (25). In brief, after anesthesia with pentobarbitone (6 mg/100 g body weight, administered intraperitoneally) rats were intubated and ventilated using a small animal ventilator (frequency 34/min, tidal volume 4 to 6 ml, with 4 cm H_2O positive end-expiratory pressure). Lungs were ventilated with either normoxic (21% O_2, 5% CO_2, balance nitrogen) or hypoxic (2% O_2, 5% CO_2, balance nitrogen) gas mixtures. Blood pH was corrected to 7.40 by the addition of sodium bicarbonate. A metal-tipped pulmonary artery cannula was inserted via the right ventricle and secured by ligature. A wide bore preformed left atrial cannula drained blood to the reservoir connected by plastic tubing to the pulmonary artery cannula. Blood flow of 18 ml/min through the lungs was maintained using a roller pump (Watson-Marlow, Falmouth, UK) and measured using an electromagnetic flow meter (Spectramed, Oxnard, CA). Pulmonary arterial and tracheal pressure were measured with transducers and displayed with blood flow on the three-channel recorder. Pulsed doses of UK-73,967 (0.07 and 0.2 mg), ANP 300 ng (3–28, Bachem, CA), or vehicle were injected in volumes of 20 μl into the pulmonary inflow tubing during normoxic ventilation or during the stable phase of pulmonary vasoconstriction during hypoxic ventilation.

Right Ventricular Hypertrophy and Hematocrit

The heart was removed en bloc, the right ventricle (RV) was dissected free from the left ventricle and septum (LV), and the RV and LV and septum were weighed separately using a chemical balance. RV hypertrophy was expressed as RV weight/LV weight (RV/LV) and RV weight/body weight (RV/BW). Data are also shown for initial BW, BW gain and LV weight/BW (LV/BW). Blood, obtained by direct ventricular puncture, was placed in heparinized microtubes that were spun in a microhematocrit centrifuge (Hawskley, Lancing, Sussex, England).

Pulmonary Vascular Remodeling

The trachea was cannulated and the lungs were insufflated and fixed with 10% buffered formol-saline. A block 3-mm in thickness was taken by complete transverse medial to lateral section of the left lung below the hilum. Sections of 3 μm were stained with elastic Van Gieson and coded slides examined systematically using the ×40 magnification objective, following a previously described method with minor modification (22). All vessels with a definite elastic coat adjacent to alveoli and alveolar ducts (25 to 55 μm diameter) were counted, and the proportion of these having a double elastic lamina for between 35 to 100% of the circumference, indicating a muscular media, were counted. The mean number of vessels counted was 152 ± 22 (n = 48) and the proportion of vessels with a double elastic lamina (designated thick-walled peripheral lung vessels [%TWPV]) was expressed as a percentage of the vessels examined.

Plasma ANP

The effect of NEI treatment on plasma ANP during the development of pulmonary hypertension was determined in separate groups of rats treated in identical manner with vehicle, low-dose NEI, or high-dose NEI (n = 8 in all groups) and killed after three days hypoxic exposure. Blood was obtained immediately after decapitation, centrifuged in the presence of EDTA and trasylol, and snap frozen. Plasma samples were later assayed in a single batch without preliminary extraction using a specific and sensitive radioimmunoassay (26).

Statistical Analysis

Results are expressed as means ± standard deviations (SD). Statistical analysis was performed with an analysis of variance and Student's *t* test using Minitab Data Analysis Software, release 6 (Minitab Inc., State College, PA). Significance was assumed when $p < 0.05$.

Results

NEI treatment produced a 55% increase in plasma ANP during the development of pulmonary hypertension as follows: hypoxic vehicle 16.5 ± 6.4, low-dose NEI 25.5 ± 7.0 ($p < 0.02$ compared with hypoxic vehicle), and high-dose NEI 27.7 ± 5.8 pmol/L ($p < 0.005$ compared with hypoxic vehicle); n = 8 for all groups. There was no difference between low-dose NEI and high-dose NEI on the magnitude of the effect on plasma ANP (figure 1). Chronic hypoxia increased mean pulmonary arterial pressure from 17.6 ± 2.2 to 26.6 ± 4.0 mm Hg (figure 2, upper panel). Ppa in hypoxia was significantly reduced by NEI treatment as follows: vehicle 26.6 ± 4.0 mm Hg; low dose 22.7 ± 1.9 and high dose 22.6 ± 2.5 mm Hg, both $p < 0.01$ compared with vehicle. NEI treatment had no effect on Ppa in normoxia (vehicle 17.6 ± 2.2, low dose 17.9 ± 2.1, and high dose 16.4 ± 3.1 mm Hg) as shown in figure 2. Systemic blood pressure was not influenced by chronic hyp-

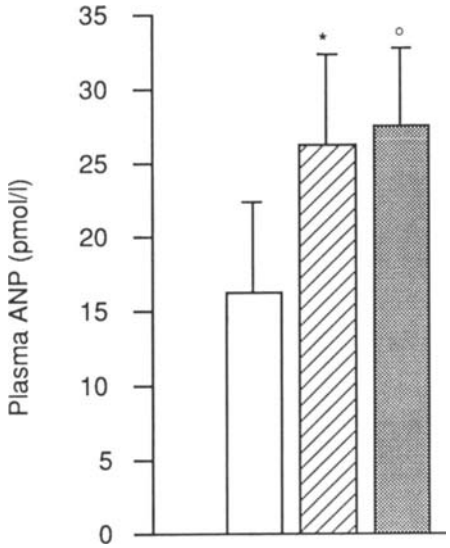

Fig. 1. Plasma ANP (pmol/L, mean ± SD) during continuous infusion of neutral endopeptidase inhibitor, UK 73,967. Asterisk = $p < 0.02$, compared with hypoxic vehicle; circle = $p < 0.005$ compared with hypoxic vehicle. Open bars = vehicle; striped bars = NEI 5.4 mg/kg/day; stippled bars = NEI 16.2 mg/kg/day.

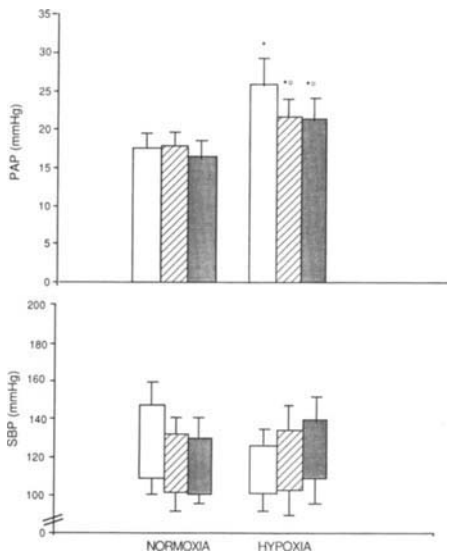

Fig. 2. The effect of low-dose and high-dose NEI in normoxia and hypoxia on Ppa (mm Hg, mean ± SD) (*upper panel*) and systemic blood pressure (mm Hg, mean ± SD) (*lower panel*). Asterisk = $p < 0.001$ compared with normoxic vehicle; circle = $p < 0.01$ compared with hypoxic vehicle. Open bars = vehicle; striped bars = NEI 5.4 mg/kg/day; stippled bars = NEI 16.2 mg/kg/day.

oxia or NEI treatment either separately or together (figure 2, lower panel). In the isolated blood-perfused rat lung, resting Ppa was 16.4 ± 1.3 mm Hg (n = 6) and ΔPpa in hypoxia was 9.9 ± 2.7 mm Hg (n = 6). Pulsed doses of NEI (0.07 mg, 0.2 mg) were without effect on resting tone, and injection of NEI during the stable phase of hypoxic vasoconstriction had no discernable effect (figure 3a). Pulsed doses of synthetic ANP had no effect on baseline Ppa. In the stable phase of hypoxic pulmonary vasoconstriction ANP 300 ng produced a significant fall in Ppa of 2.9 ± 0.8 mm Hg (n = 6); in the presence of 0.07 mg of NEI ANP produced a fall in Ppa of 4.8 ± 0.7 mm Hg, $p < 0.05$ compared with ANP 300 ng alone (figure 3b).

Hypoxia produced right ventricular hypertrophy (table 1). NEI treatment significantly reduced the ratio of RV/LV in hypoxia: 0.43 ± 0.03 vehicle, and 0.40 ± 0.02 low-dose NEI and 0.40 ± 0.02 high-dose NEI, both $p < 0.05$ compared with vehicle without any effect on this ratio in normoxia (vehicle 0.31 ± 0.02, low dose to 0.30 ± 0.01, high dose 0.31 ± 0.01) as shown in table 1. Weight gain was less in all groups in chronic hypoxia (table 1). There was no difference between NEI treatment groups and vehicle-treated control in the weight gained in normoxia or hypoxia. The ratio of heart weight/BW was not influenced by NEI treatment in either environment (table 1). Chronic hypoxia produced significant increases in packed cell volume; however, there were no differences between the vehicle-treated controls and the NEI-treated groups in the hematocrit in either the normoxic or the hypoxic environment (table 1).

Chronic hypoxia produced extension of smooth muscle of the small pulmonary arteries toward the periphery of the lung, reflected by an increase in the percentage of TWPV in the vehicle-treated hypoxic group. NEI treatment did not affect the percentage of TWPV in normoxia (figure 4). Pulmonary vascular remodeling in hypoxia was significantly reduced by NEI treatment (figure 4); %TWPV: 19.2 ± 3.1% vehicle, and 10.4 ± 2.3% low-dose NEI and 8.1 ± 1.8% high-dose NEI, both $p < 0.001$ compared with vehicle, without effect on the percentage of muscularized vessels in the normoxic groups (TWPV vehicle 3.9 ± 1.1%, low-dose NEI 3.5 ± 1.3%, high-dose NEI 3.4 ± 1.8%).

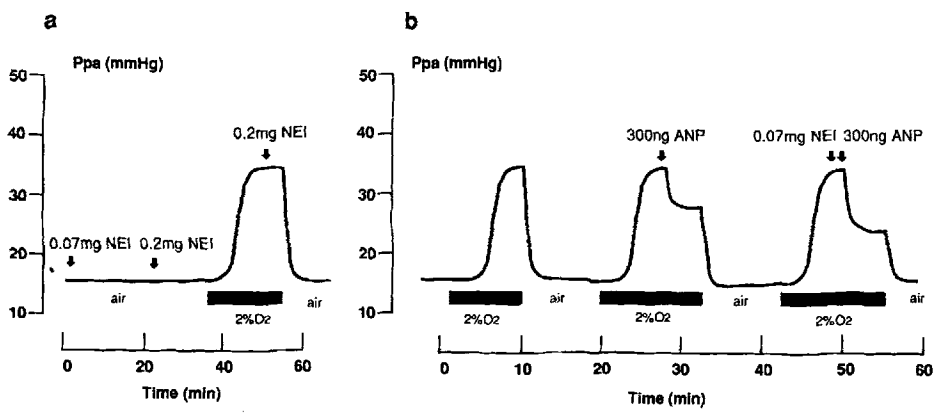

Fig. 3. Tracing showing the effect of pulsed doses of NEI in the absence (a) and in the presence (b) of added ANP on pulmonary artery pressure (Ppa) in normoxia and hypoxia.

Discussion

The use of chronic environmental hypoxia is a well-validated method of producing rapid and profound fibrocellular changes in the pulmonary circulation, termed remodeling (22, 23). The most conspicuous change seen on light microscopy is the development of vessels with a double elastic lamina, reflecting the extension of muscle from the small pulmonary arteries toward the periphery of the lung. The changes in RV hypertrophy, Ppa, and vascular remodeling in the hypoxic vehicle group were identical to those observed in similar studies (13, 14), indicating that the hypoxic stimulus used produced these changes as previously. Based upon the results of Hunter and colleagues (22) we used an exposure period of 1-wk because the changes are well developed at this time. Preliminary study of the delivery characteristics of the osmotic minipumps using C-14 radiolabelled insulin showed that approximately 80% of the loading dose was delivered during the exposure period, and the dose of NEI used was calculated on the basis of this data. Delivery by osmotic minipump, rather than repeated intraperitoneal or subcutaneous administration, enabled drug to be delivered intravenously at a constant rate throughout the exposure period. At the end of NEI administration a 24-h washout period was instituted so that measurements of Ppa would reflect structural changes in the pulmonary circulation rather than acute effects

TABLE 1

EFFECTS OF CHRONIC INFUSION OF LOW DOSE AND HIGH DOSE UK 73,967 ON BODY WEIGHT GAIN, RATIO OF RV WEIGHT TO LV WEIGHT, RV WEIGHT TO FINAL BW, HEART WEIGHT TO FINAL BW, AND HEMATOCRIT (N = 8, ALL GROUPS)

	Normoxia			Hypoxia		
	Vehicle	NEI (low)	NEI (high)	Vehicle	NEI (low)	NEI (high)
IW, g	215 ± 11	215 ± 12	211 ± 6	218 ± 11	213 ± 10	210 ± 11
BWG, g	44 ± 9	43 ± 13	39 ± 5	11 ± 10*	13 ± 14*	12 ± 8*
RV/LV	0.31 ± 0.02	0.30 ± 0.01	0.31 ± 0.01	0.43 ± 0.03*	0.40 ± 0.02*†	0.40 ± 0.02*†
RV/BW, × 10^{-4}	6.7 ± 0.3	6.4 ± 0.3	6.9 ± 0.5	8.8 ± 1.1*	7.9 ± 1.0*†	7.8 ± 0.7*†
LV + RV/BW, × 10^{-3}	2.88 ± 0.12	2.76 ± 0.08	2.93 ± 0.17	2.91 ± 0.17	2.75 ± 0.23	2.74 ± 0.27
Hct, %	48.1 ± 2.1	48.9 ± 1.4	48.9 ± 2.5	56.5 ± 1.8*	57.4 ± 3.1*	58.3 ± 2.8*

Definition of abbreviations: NEI = UK 73,967; IW = initial weight; BW = body weight; BWG = body weight gain; RV = right ventricle; LV = left ventricle; RV/LV = ratio of RV to LV; Hct = hematocrit.

* $p < 0.001$ compared with normoxic vehicle group.

† $p < 0.05$ compared with hypoxic vehicle group.

of raised plasma ANP levels. The adequacy of the washout period, incorporated to overcome acute effects of the drug, was confirmed by the measurements of plasma ANP at the end of the study. No differences in plasma ANP were seen between the treated and vehicle groups at the time of Ppa measurement, consistent with the elimination half-life of the UK-73,967 of 0.15 h in the rat (20) although plasma ANP levels were significantly higher in hypoxic than normoxic groups, in agreement with earlier findings (5).

During the development of pulmonary hypertension and RV hypertrophy plasma ANP was raised to > 155% control by NEI treatment, showing that when the synthesis and secretion of ANP is increased in pulmonary hypertension (4–7) inhibition of neutral endopeptidase 24.11 retards enzymatic degradation of endogenous ANP and is associated with increased plasma ANP levels. The absence of effect of pulsed doses of NEI on normoxic and hypoxic tone, and the augmentation by NEI of the effect of ANP in attenuating hypoxic pulmonary vasoconstriction further suggests that the effects of NEI were mediated through endogenous ANP.

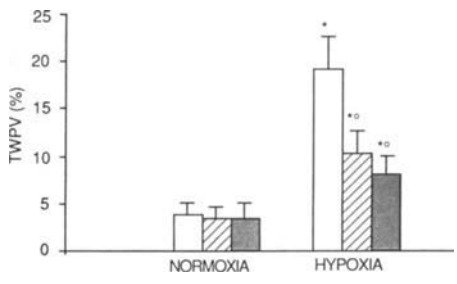

Fig. 4. The effect of low-dose and high-dose NEI in normoxia and hypoxia on pulmonary vascular remodeling. Asterisk = $p < 0.001$ compared with normoxic vehicle; circle = $p < 0.001$ compared with hypoxic vehicle. Open bars = vehicle; striped bars = NEI 5.4 mg/kg/day; stippled bars = NEI 16.2 mg/kg/day.

Previous studies have shown a dilator action of ANP on vascular smooth muscle, where its magnitude was greater on pulmonary than on similar sized renal vessels (27). Infusion of ANP attenuated the pulmonary pressor response to acute alveolar hypoxia in the pig (28), and radioligand binding studies in conjunction with acute hemodynamic studies in chronically instrumented conscious rats have suggested that ANP may protect against RV overload (29, 30). An acute vasodilator action of ANP on both the pulmonary and systemic circulation was found in rats in which the pulmonary vasculature has been remodeled by monocrotaline, although in this study systemic effects were greater than those on the pulmonary circulation (31). However, the use of the chronically hypoxic rat to assess the effect of pharmacologic interventions during development of pulmonary hypertension has shown many differences between acute and chronic experiments so that only a portion of the several compounds that have an acute vasodilator action on pulmonary arteries are able to attenuate the development of pulmonary hypertension (32, 33). It is therefore not possible to infer from acute studies with ANP an action on the development of pulmonary hypertension. Infusions of ANP varying in duration between 30 min and 3 days provide additional evidence for important differences between acute and chronic effects with respect to natriuresis and diuresis (34).

Infusions of synthetic ANP can modify vascular remodeling in the pulmonary circulation. For example, infusion of exogenous ANP reduced developing and established pulmonary vascular remodeling produced in response to chronic hypoxia (13, 14). Jin and colleagues (13) studied a longer period of exposure and of ANP delivery (4 wk) by changing the osmotic minipumps after 2 wk exposure. Nevertheless, in a 1-wk exposure period to hypoxia, synthetic ANP infusion (14) modulated pulmonary hypertension and vascular remodeling in a similar manner. Examination of the acute effect of the ring analogue C-ANP, which potentiates the half-life of ANP by binding to the ANP clearance receptor, showed that this agent reduced Ppa acutely only in the remodeled pulmonary circulation and was without acute effects on the normal pulmonary circulation in the rat (12). Because the increment in plasma ANP produced by C-ANP was similar in hypoxic and control groups, this finding implied differential effects of ANP in the remodeled pulmonary circulation. Although neutral endopetidase 24.11 was reported to cleave a large number of peptides *in vitro*, raising the possibility of additional other effects of NEI treatment, the number of active peptides proved to be cleaved *in vivo* was found to be far fewer (35). The demonstration of raised plasma ANP, the similarity between the effect of NEI treatment and the effects with continuous infusion of synthetic ANP (13, 14) and the effect of ANP and NEI in the isolated perfused lung in the present study all suggest that the effect of NEI was mediated through its effect on ANP.

In vitro experiments have provided information to suggest that the effect of ANP could have been mediated by an action independent of its actions on vascular tone. ANP inhibited proliferation of cultured vascular smooth muscle cells by platelet-derived growth factor (PDGF) in a dose-dependent manner (36). Later studies have also shown that ANP can act as both an antihypertrophic and antiproliferative factor, exerting potent inhibitory effects not only on DNA replication and cell division in smooth mus-

cle cells, but also on RNA and protein synthesis that may mediate the increase in vascular cell mass (37). The actions of ANP are mediated through the membrane form of guanylate cyclase/receptor (38), a complex family of proteins through which either a direct effect or transmodulation of other growth factors could have occurred (39, 40). In studies of vascular smooth muscle hypertrophy, 8-bromo cyclic guanylate monophosphate (GMP) mimicked the antihypertrophic action of ANP (37). An alternative, less direct basis for the effects on vascular remodeling is provided by the effect of ANP on PDGF-stimulated vascular smooth muscle proliferation (36): increased expression of receptors for PDGF in various vascular proliferative lesions has been proposed as a common mechanism (41), and oxygen tension is known to regulate the expression of PDGF B-chain gene in human endothelial cells (42).

The present experiments have shown an inhibitory effect on vascular remodeling and cardiac hypertrophy when increments in endogenous ANP are produced by inhibiting its major metabolic pathway. The results also suggest that endogenous ANP acts in a negative feedback manner during pulmonary vascular remodeling and the development of associated RV hypertrophy. Further study of the effect of endogenous ANP on vascular remodeling may provide additional insights into normal mechanisms operating to regulate vascular growth and remodeling. Inhibition of neutral endopeptidase 24.11 activity, by use of specific inhibitors, provides a potential approach toward therapy in pulmonary hypertension; the present enquiry may also encourage evaluation of these novel agents in other disorders in which vascular remodeling and ventricular hypertrophy are clinical features.

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