Br. J. Pharmacol. (1989), **99**, 9–10 **SHORT COMMUNICATION**

Impairment of pulmonary endothelium-dependent relaxation in patients with Eisenmenger's syndrome

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A comparison has been made between the endothelium-dependent relaxation of pulmonary arteries (PA) obtained at heart-lung transplantation from 4 patients with Eisenmenger's syndrome and secondary pulmonary hypertension, and PA obtained at lobectomy from 4 patients with lung carcinoma, the controls. All vascular rings were studied immediately after lung excision. PA rings from control patients dose-dependently relaxed to cumulative doses of acetylcholine (ACh, 10^{-10} to 10^{-5} M), achieving a maximal relaxation of 80 ± 5% (mean ± s.e.mean) from precontraction with phenylephrine. By contrast, PA rings from Eisenmenger's syndrome patients achieved a maximal relaxation of only 34 ± 12% ($P < 0.05$, unpaired t test), with even paradoxical contraction at high doses of ACh (10^{-6} to 10^{-5} M). Sodium nitroprusside (10^{-4} M) relaxed all PA rings, with and without endothelium (carefully removed before study), obtained from both control and Eisenmenger's syndrome patients. These results provide the first evidence that endothelium-dependent relaxation of PA mediated by endothelium-derived relaxing factors is impaired in Eisenmenger's syndrome patients with secondary pulmonary hypertension.

Introduction Eisenmenger's syndrome is defined by systemic levels of pulmonary arterial pressure and high pulmonary vascular resistance associated with a variety of congenital cardiac shunts (Wood, 1958a). Infusion of acetylcholine (ACh) into the main pulmonary artery in patients with Eisenmenger's syndrome fails to reduce the elevated pulmonary vascular resistance (Wood, 1958b). This could reflect an impairment of the endothelium-dependent relaxation of pulmonary arteries in these patients, since ACh is known to induce release of endothelium-derived relaxing factors (EDRF) by the endothelial cells (Furchgott & Zawadzki, 1980). However, this impairment has not been confirmed by *in vitro* studies. We have studied isolated pulmonary arteries obtained from the explanted lungs at the time of heart-lung transplantation (HLT), an established treatment for patients dying from Eisenmenger's syndrome (Reitz *et al.*, 1982).

Methods Segments of proximal pulmonary arteries were obtained at HLT from 4 female patients with Eisenmenger's syndrome (aged 16 to 41 years) who had a forced expiratory volume in one second (FEV_1) of 72 ± 14% predicted (mean ± s.d.), and an arterial oxygen tension (Pao_2) of 4.4 ± 0.3 kPa (mean ± s.d.). Control segments of similar sized pulmonary arteries were obtained from lobectomy in 4 patients (2 women and 2 men, aged 56 to 64 years) with bronchial carcinoma who had normal lung function tests (FEV_1 = 101 ± 10% predicted).

Immediately after lung excision, the tissue was placed in pregassed (95% O_2 and 5% CO_2) Krebs-Ringer (KR) bicarbonate solution at 4°C. Pairs of rings (3–5 mm in length and 2–4 mm outer diameter) were cut from the pulmonary arteries. The endothelium was carefully removed from one ring of each pair, with a pipe cleaner. Changes in isometric tension of each ring were recorded in organ baths filled with 20 ml of KR bicarbonate solution and bubbled with 95% O_2 and 5% CO_2 at 37°C, by use of a force transducer (Harvard Bioscience, Ma, USA).

After full relaxation, on an initial tension of 1.5 g, with repeated washes over 90 min, the rings were submaximally precontracted with phenylephrine dichloride (PE) (10^{-6} to 10^{-5} M) to obtain a stable plateau of contraction. Acetylcholine dichloride (ACh) was then added to produce a cumulative dose-relaxation response curve (10^{-10} to 10^{-5} M) of pairs of rings with and without endothelium (Figure 1). Sodium nitroprusside (10^{-4} M) was added at the end of all experiments to assess endothelium-independent vasorelaxation. All rings were pretreated with indomethacin (5×10^{-6} M) to inhibit production of prostacyclin.

The rings were fixed in formalin at the end of each experiment and then reviewed histologically by one of the authors (C.C.) without knowledge of diagnosis.

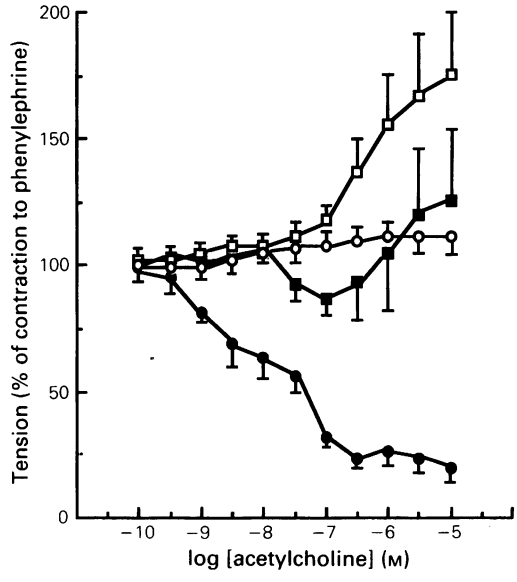

Figure 1 Endothelium-dependent relaxation responses in pulmonary arterial rings without (open symbols) and with (closed symbols) endothelium to cumulative doses of acetylcholine in control (●, ○) ($n = 4$) and Eisenmenger's syndrome (■, □) patients ($n = 4$). Each point represents the mean and vertical lines show s.e.mean.

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Results Pulmonary arterial rings with endothelium obtained from control patients relaxed in a dose-dependent manner with increasing doses of ACh, whilst rings without endothelium showed no response (Figure 1; $P < 0.001$ by paired t test). Pulmonary arterial rings with endothelium obtained from Eisenmenger's syndrome patients also dose-dependently relaxed to low doses of ACh (10^{-8} to 10^{-7} M) (Figure 1). However, endothelium-dependent relaxation with ACh was markedly reduced in pulmonary arteries from Eisenmenger's syndrome patients compared with controls (Figure 1). The maximal relaxation (mean ± s.e.mean) was 34 ± 12% in Eisenmenger's syndrome and 80 ± 5% in control patients ($P < 0.05$, by unpaired t test). Furthermore, higher concentrations of ACh (10^{-6} to 10^{-5} M) resulted in contraction above the baseline precontraction obtained with PE in vascular rings with and without endothelium of Eisenmenger's syndrome pulmonary arteries. The maximal contraction expressed as % from baseline precontraction with PE was 125.5 ± 58% and 175.8 ± 52% (mean ± s.e.mean) in rings with and without endothelium, respectively (Figure 1).

When the highest dose of ACh (10^{-5} M) was reached, addition of sodium nitroprusside (10^{-4} M) further completely relaxed the rings, returning the tension to precontracted levels, i.e. those before the addition of PE. This was seen in rings with and without endothelium obtained from all control patients and from 3 out of 4 patients with Eisenmenger's syndrome. The remaining patient exhibited 73% of maximal relaxation with sodium nitroprusside (10^{-4} M).

On histological examination, unlike the control vessels, all vascular rings of the Eisenmenger's syndrome patients had intimal fibrosis of varying severity. In addition, 2 out of 4 patients's rings showed secondary medial atrophy. The appearances described are consistent with atherosclerotic changes due to pulmonary hypertension (Reid *et al.*, 1986).

Discussion Endothelium-dependent vasorelaxation through release of EDRF occurred in the isolated pulmonary arteries of patients undergoing lobectomy for lung carcinoma who had no evidence of pulmonary vascular disease (Greenberg *et al.*, 1987; Thom *et al.*, 1987). We have recently found that EDRF-mediated pulmonary relaxation is reduced in cystic fibrosis patients with respiratory failure and evidence of cor pulmonale (Dinh Xuan *et al.*, 1989). We now extend these findings to patients with Eisenmenger's syndrome. Furthermore, we provide evidence supporting previous *in vivo* findings of a lack of pulmonary vasodilatation in response to an infusion of ACh into the main pulmonary arteries in 13 of 14 Eisenmenger's syndrome patients (Wood, 1958b). We also found that higher doses of ACh resulted in contraction, even in rings with endothelium. A similar paradoxical vasoconstriction with ACh has been observed in atheromatous coronary artery disease (Ludmer *et al.*, 1986).

Sodium nitroprusside, a nitroso compound which relaxes vascular smooth muscle by releasing nitric oxide (Ignarro, 1989), markedly (in one) or fully (in three others) relaxed the vascular rings that failed to respond to ACh. This makes it unlikely that the structural changes seen in the vascular smooth muscle are responsible for the failure of vascular rings to relax to ACh. The cause is, therefore, more likely to reside within the endothelial cells. Indeed, the results of this study, together with our previous findings in cystic fibrosis pulmonary arteries (Dinh Xuan *et al.*, 1989), support the existence of impaired pulmonary endothelium-dependent relaxation in cases of elevated pulmonary vascular resistance in man. Whether this impairment is a result of failure of endothelial cells to release EDRF or whether it is due to the limitation of diffusion of EDRF through the thickened intima is uncertain.

Pulmonary hypertension is associated with widespread lung destruction in cystic fibrosis whilst in Eisenmenger's syndrome it is associated with cardiac shunts. However, the two disorders share a common sustained hypoxaemic condition. It is therefore tempting to speculate that chronic hypoxaemia may initiate functional and/or structural changes of the pulmonary endothelium. These changes may in turn lead to impairment of endothelium-dependent pulmonary relaxation in chronic hypoxic lung diseases.

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