Acetylcholine and adenosine diphosphate cause endothelium-dependent relaxation of isolated human pulmonary arteries

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Endothelium-derived relaxing factor(s) (EDRF) are released from endothelial cells in response to a variety of different pharmacological stimuli [1-4]. Since Furchgott and Zawadski [1] first demonstrated the obligatory role of the endothelium in eliciting relaxation of isolated rabbit aorta to acetylcholine, evidence for pulmonary endothelium-dependent relaxation mediated by EDRF has been found in most mammalian species [5-8]. Furthermore, damage or functional disturbance of endothelium is associated with a reduction of EDRF-mediated vasorelaxation in experimentally induced atherosclerosis [9], systemic hypertension [10], coronary artery vasospasm [11], cerebrovascular disease [12] and hypoxic pulmonary vasoconstriction [13]. The latter is the principal cause of secondary pulmonary hypertension [14], which is an important source of morbidity and cause of mortality in man [15]. The mechanism of hypoxic pulmonary vasoconstriction is unknown [16], but there is some evidence from studies in animals that EDRF activity in isolated pulmonary arteries is decreased by hypoxia [13]. To study the role of EDRF in the pathogenesis of human pulmonary vascular disease, it is fundamental to first demonstrate that endothelium-dependent relaxation is indeed present in human pulmonary vessels. Studies in man have been limited by sparsity of tissue [17, 18]. Here we report further evidence of endothelium-dependent relaxation of isolated human pulmonary arteries.

Materials and methods

Tissue preparation

Segments of pulmonary artery were obtained from eight patients (5 men and 3 women), 64.2±6.8 (mean±s.d.) yrs old (range 52-72 yrs), undergoing lobectomy for lung carcinoma. Arterial segments were dissected from areas free of macroscopic malignant disease. Immediately after removal, tissue was placed in cold (4°C), pregassed Krebs-Ringer bicarbonate (KR) solution for transport to the laboratory. The composition of the KR solution was (mmol·l⁻¹): NaCl 120, KCl 4.6, MgCl₂·6H₂O 1.05, CaCl₂·2H₂O 1.2, Na₂HPO₄ 0.7, NaH₂PO₄ 1.5, glucose 10, NaHCO₃ 20.4.

Within half an hour of removal of the lungs, lobar and segmental pulmonary artery branches (first and second order of segmentation from both main pulmonary arteries) were cleaned of excess fat and connective tissue and cut into rings (3-5 mm in length and 2-4 mm outer...
diameter). From half the segments, endothelium was removed by gentle rubbing with a pipe-cleaner inserted into the lumen, whereas it was carefully preserved in the other half of the specimens. Rings with and without endothelium were then mounted over fine rigid wires, in organ chambers filled with 20 ml of KR buffer bubbled with 95% O₂ and 5% CO₂, maintained at 37°C by an outer bath warmed by a recirculating heater (Circulator C-400, Techne Ltd, Cambridge, UK). The lower hook was fixed to the glass chamber, and the upper one was connected to a force transducer (Harvard Bioscience, Ma, USA). Changes in isometric tension were recorded on a two channel chart drive recorder (PM 8252A, Philips). The rings were placed at an initial tension of 1.5 g, which was previously determined as the tension corresponding to the optimal point of the length-tension relationship in a series of preliminary experiments. The rings were allowed to equilibrate in the bath for at least 90 min (range 90–300 min; mean±s 162±52 min) during which time the fluid in the bath was changed every 15 min. Two pairs of rings (one ring with, and one without endothelium) were studied on each occasion.

Dose-response curves

After equilibration, the rings were submaximally precontracted with phenylephrine hydrochloride (PE) (10⁻⁶–10⁻⁵ M) sufficient to obtain a stable plateau of contraction. Acetylcholine chloride (ACh) and adenosine diphosphate (ADP) were then added in a cumulative fashion (10⁻¹–10⁻⁵ M) and the corresponding dose-response curves constructed. A sample of rings was pre-incubated with indomethacin (5·10⁻⁶ M) 15 min before the trial to inhibit the production of prostacyclin. Thereafter indomethacin was present throughout the experiment. Sodium nitroprusside (10⁻⁴ M) was added at the end of all experiments.

The drugs were diluted in distilled water except for indomethacin which was dissolved in 50% ethanol. All the drugs were purchased from Sigma Chemical Co, and solutions were freshly prepared before use.

Histology

After the pharmacological test, all the rings were fixed in formalin and stained with haematoxylin and eosin. Sections of paraffin wax-embedded tissue were examined by light microscopy at high power magnification. The intact or disrupted endothelial cell layer in each ring was examined and correlated to the corresponding dose-response curves. The histologist was unaware of the experimental results. In addition, microscopic evidence of carcinomatous spread to vascular rings was sought on histological examination.

Analysis

Results are expressed as the percentage relaxation obtained with either ACh or ADP from submaximal precontraction to PE. Maximal relaxation is the greatest reduction in tone obtained with a given agent. The concentration of drugs causing 50% relaxation (EC₅₀) was determined by interpolation from linear regression and expressed as its negative decimal logarithm (-log). Rings with and without endothelium coming from the same patient were compared by a paired t-test.

Fig. 1. – Endothelium-dependent relaxation responses to cumulative doses of acetylcholine in rings with and without endothelium, precontracted with phenylephrine.

Fig. 2. – Endothelium-dependent relaxation responses to cumulative doses of adenosine diphosphate in rings with and without endothelium, precontracted with phenylephrine.

Histology
ENDOTHELUM-DEPENDENT PULMONARY VASORELAXATION

With endothelium
Acetylcholine (-log M)

Without endothelium

Adenosine diphosphate (-log M)

Fig. 3. – Typical responses of rings of human pulmonary arteries, with and without endothelium, to cumulative doses of acetylcholine and adenosine diphosphate. Each ring was precontracted with phenylephrine (PE; 10⁻⁶ M). Sodium nitroprusside (NP; 10⁻⁴ M) was added at the end of the experiment to assess endothelium-independent vasorelaxation.

A

B

Fig. 4. – Luminal surfaces of two pulmonary artery rings in which endothelium-dependent relaxation was studied. The ring in the left panel, where the endothelium is present, exhibited relaxation to acetylcholine. The ring in the right panel, where the internal elastic lamina is seen deprived of endothelial cell lining, did not.
Further more, our has been found by GREENBERG more potent vasodilator than ATP since nary vasodilation with ADP, allowed not only a clear distinction between arterial rings with and without endothelium, but also definitively excluded the presence of tumour in the specimens studied. There is evidence from the literature [21] that incubation with similar dose, and for the same duration, of indomethacin, a potent cyclo-oxygenase inhibitor [22], is capable of preventing the production of prostacyclin (PGI₂) in isolated organs. Furthermore, in this study, the endothelium-dependent relaxation of the rings was not affected by pretreatment with indomethacin, PGI₂ or any other cyclo-oxygenase product, is therefore unlikely to account for the relaxation evoked by ACh and ADP in those pulmonary artery rings. Although we did not attempt to block the endothelium-dependent vasorelaxation with methylene blue or haemoglobin [23], and although EDRF has not been measured in the medium, we feel reasonably confident, on the basis of these results and those of previous studies [1, 5, 6, 17, 18], that the pulmonary vasorelaxation observed in rings with endothelium was mediated by non-prostanoid product(s) released by endothelial cells, namely the EDRF.

One of the EDRF has recently been identified with the gas nitric oxide (NO) [24] which is synthesized by the endothelial cells from the amino acid L-arginine [25]. Both inhaled NO [26], and infused nitrovasodilators, such as nitroprusside [27], can cause pulmonary vasodilation in man, as a result of increase of the smooth muscle intracellular level of cyclic guanosine monophosphate [19, 20].

### Table 1. - Maximal relaxation and EC₅₀ in pulmonary artery rings with (E) and without (E) endothelium obtained from dose-response curves to acetylcholine (ACh) and adenosine diphosphate (ADP)

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<tr>
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<th>ACh</th>
<th>ADP</th>
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<tr>
<td>Mean±SEM</td>
<td>5.73±0.39</td>
<td>7.10±0.34</td>
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<tr>
<td>Range</td>
<td>5.89-10</td>
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### Results

Both ACh and ADP caused concentration-dependent relaxation in rings with, but not in those without, endothelium (p<0.001, figs 1-3) from human pulmonary arteries precontracted with PE. Rings that failed to respond to ACh and to ADP had gross disruption of endothelium (fig. 4), but they did relax after the addition of sodium nitroprusside (fig. 3). The latter induced some further degree of relaxation in rings with intact endothelium which had already reached maximal relaxation at the highest doses of ACh and ADP (fig. 3).

Maximal relaxation obtained for ACh and for ADP, and EC₅₀ (-log M) for ACh and for ADP in rings with endothelium are listed in table 1. Results obtained in rings pretreated with indomethacin (n=4) were identical to those for untreated rings, EC₅₀ ACh (mean±SEM) 7.67±0.39 versus 7.55±0.41, and EC₅₀ ADP (mean±SEM) 7.71±0.33 versus 7.71±0.31 in pretreated and untreated rings, respectively. No ring had microscopic evidence of carcinoma infiltration.

### Discussion

Our study confirms data previously reported [17, 18] and provides further evidence that endothelium-dependent relaxation occurs in human pulmonary arteries. Unlike Trom et al. [17], who were successful in observing vasorelaxation in arteries taken from only 49% of the patients whom they studied, we were able to demonstrate endothelium-dependent vasorelaxation in all of the pulmonary arteries that we tested. A shorter delay between surgical removal of the tissue and the time that it was actually studied may account for our better results. Because adenosine triphosphate (ATP), which has been used in the study of GREENBERG et al. [18], can also relax the vessels by acting directly on the arterial smooth muscle, we have chosen to assess pulmonary vasodilatation with ADP rather than with ATP. Furthermore, our data provide evidence that ADP is a far more potent vasodilator than ATP since EC₅₀ of the latter has been found by GREENBERG et al. [18] to be four hundred fold higher than that of ACh, the EC₅₀ of which is similar to that of ADP in our study.

Relaxation occurs in response to ACh and ADP only in those arterial rings with intact endothelium. However, rings without endothelium were capable of relaxing with nitroprusside, an endothelium-independent vasodilator [19]. Nitrovasodilators directly initiate relaxation of vascular smooth muscle by elevating intracellular cyclic guanosine monophosphate in a similar fashion to EDRF [20]. This indicates that the procedure for removing the endothelium did not damage the vascular smooth muscle. Histological examination of all the rings that were actually tested, allowed not only a clear distinction between arterial rings with and without endothelium, but also definitively excluded the presence of tumour in the specimens studied.
The importance of demonstrating the presence of an endothelial-dependent mechanism of modulating pulmonary vascular tone other than prostacyclin stems from the observation that the endothelium of the pulmonary vascular bed is often initially involved in disease. For example, hypoxic and hyperoxic injury [28], and primary pulmonary hypertension [29], are associated with morphological changes of the pulmonary endothelial cells. Preliminary reports suggest that hypoxia may reduce EDRF activity in the pulmonary vascular bed of laboratory animals [13]. With the development of heart-lung transplantation in man [30], the way is now open to in vitro studies of the explanted lungs to define the involvement of EDRF-mediated pulmonary vasodilatation in the pathophysiology of human pulmonary vascular disease.

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References

13. Rodman DM, Yamaguchi T, O'Brien RF, McMurry JP. - Decreased pulmonary artery cGMP content during hypoxia is due to decreased endothelium-derived relaxing factor activity. Circulation, 1988, 78, (Suppl. 2), II-320 (abstract).
vasculaires de l’homme. Nous avons ainsi étudié la vasorelaxation endothélio-dépendante de l’artère pulmonaire humaine isolée. Des anneaux vasculaires ont été obtenus à partir de segments d’artère pulmonaire provenant de pièces de lobectomies de 8 patients atteints de cancer bronchique. L’acétylcholine (ACh) et l’adénosine diphosphate (ADP) induisent de façon dose-dépendante, mais seulement en présence de l’endothélium, le relâchement des anneaux vasculaires précontractés par la phényléphrine. Les anneaux dépourvus d’endothélium sont capables de relâcher complètement avec nitroprussiate de sodium qui agit directement sur le muscle lisse vasculaire. L’inhibition de la production de prostacycline par l’indométacine, ne modifie pas la réponse vasculaire à l’ACh et à l’ADP. Ceci suggère qu’un (ou plusieurs) facteur(s) de nature non-prostanoïde dérivé(s) de l’endothélium seraient responsables de la vasorelaxation endothélio-dépendante de l’artère pulmonaire humaine isolée.