British Journal of Pharmacology (1996) 119, 1337-1340 © 1996 Stockton Press All rights reserved 0007-1188/96 $12.00

Increased responsiveness of human pulmonary arteries in patients with positive bronchodilator response

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1 The effects of noradrenaline, endothelin-1, acetylcholine and sodium nitroprusside were studied in isolated pulmonary arteries obtained from 14 patients undergoing lobectomy for lung carcinoma. Seven patients had shown increased response to a bronchodilator test prior to operation. In the remaining patients (control) the bronchodilator test was negative.

2 Artery rings from patients with a positive bronchodilator response showed greater contraction to noradrenaline ($pD_2 = 6.44 \pm 0.1$; $E_{max} = 93 \pm 9\%$ of response to 100 mM KCl) and endothelin-1 ($pD_2 = 8.92 \pm 0.1$; $E_{max} = 130 \pm 16\%$) than the rings from control patients ($pD_2 = 6.04 \pm 0.08$; $E_{max} = 56 \pm 8\%$ for noradrenaline; $pD_2 = 8.29 \pm 0.1$; $E_{max} = 78 \pm 10\%$ for endothelin-1). There was no significant difference in the contractile responses to 100 mM KCl between arteries from either group of patients.

3 Arterial rings from patients with a positive bronchodilator test achieved $96 \pm 3\%$ of maximal relaxation in response to acetylcholine, whereas rings from control patients achieved a maximal relaxation of $72 \pm 5\%$. Rings from both the controls and the patients with a positive bronchodilator test achieved complete relaxation in response to sodium nitroprusside but pD_2 values were significantly higher in patients with a positive bronchodilator test.

4 Removal of endothelium or treatment with N^G-nitro-L-arginine methyl ester of artery rings from both the control and the patients with a positive bronchodilator test reduced the relaxation to acetylcholine ($P < 0.05$) but did not modify relaxation to sodium nitroprusside.

5 It is concluded that responsiveness of pulmonary arterial smooth muscle to dilator and constrictor agents is increased in patients showing reversibility of airway constriction. Thus hyperresponsiveness of airway smooth muscle may be associated with a similar phenomenon in the surrounding vascular smooth muscle.

Keywords: Human pulmonary artery; endothelium; bronchodilator test; acetylcholine; noradrenaline

Introduction

Bronchial hyperresponsiveness appears to be a functional disorder characterized by an abnormal response of airway smooth muscle. Possible causes for this response include damage to the airway epithelium, an abnormality in the autonomic nervous control of the smooth muscle, or an increased responsiveness of the smooth muscle itself (Boushey *et al.*, 1980; American Thoracic Society, 1991). The abnormal response can be assessed by pharmacologically induced improvement in expiratory flow after inhalation of bronchodilator agents (Dales *et al.*, 1988) or worsening after inhalation of bronchoconstrictor agents (Eiser *et al.*, 1983). Although challenge tests with bronchodilator agents appear to be rather unsuccessful in distinguishing the disease process in the airways, they are widely used in clinical practice as well as in pathophysiological research studies to estimate the reversibility of airflow limitation (Gross, 1986; Meslier *et al.*, 1989). We postulated that hyperresponsiveness might not be restricted to airway smooth muscle but could reflect a situation in which an abnormal response of the surrounding vascular smooth muscle might be expected. To test this hypothesis we have studied responses to dilator and constrictor substances of human pulmonary arteries from patients with an increased bronchodilator response. The results were compared with those obtained from patients with a negative bronchodilator test.

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Methods

Lung segments were obtained from 14 male patients (mean age 67 ± 3 years) undergoing lobectomy for removal of lung carcinoma. The study was approved by the ethical committee of our institution and informed consent was obtained from each patient before the study. The patients were separated into two groups according to the results of a bronchodilator test performed before surgery. The bronchodilator test involved inhalation of salbutamol (0.6 mg) from a metered-dose inhaler. The improvement in forced expiratory volume in 1 s (FEV_1) was expressed as a percentage change of the initial value and as the absolute difference in ml. A positive bronchodilator response was established when at least a 15% increase and a 200 ml increase in FEV_1 following the inhalation of salbutamol was obtained (Brand *et al.*, 1990; American Thoracic Society, 1991). We studied 7 patients with increased bronchodilator response (BDR group) ($22 \pm 1.6\%$ increase and 390 ± 65 ml increase in FEV_1 after bronchodilator test) and 7 patients with negative bronchodilator test (control group) ($7 \pm 0.5\%$ increase and 112 ± 15 ml increase in FEV_1).

Segmental and subsegmental pulmonary arteries (2-3 mm external diameter) were dissected free and cut into rings 4 mm long for isometric recording of tension. Each artery ring was mounted in a 4 ml bath containing Krebs-Henseleit solution of the following composition (mM): NaCl 115, KCl 4.6, $MgCl_2.6H_2O$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11.1 and disodium EDTA, 0.01. The solution was equilibrated with 95% O_2 and 5% CO_2 to give a pH of 7.3 ± 7.4. The temperature was held at 37°C. To establish the resting tension for maximal force development, a series of preliminary experiments was performed on rubbed and unrubbed artery rings of similar length and outer diameter which were exposed repeatedly to 60 mM KCl. Basal tension was increased gradually

until contractions were maximal. The optimal resting tension was 2 g. The artery rings were allowed to attain a steady level of tension during a 2 h accommodation period before testing.

Concentration-response curves for noradrenaline (10^{-8} to 3×10^{-5} M) and endothelin-1 (10^{-11} to 3×10^{-7} M) were determined in a cumulative manner. To test responses to acetylcholine and sodium nitroprusside, the artery rings were precontracted with noradrenaline ($10^{-6}-3 \times 10^{-6}$ M) and cumulative relaxation curves to acetylcholine (10^{-8} to 3×10^{-6} M) and sodium nitroprusside (10^{-8} to 10^{-4} M) were obtained for each ring. The responses to acetylcholine and sodium nitroprusside were carried out in intact artery rings and in rings in which the endothelium had been removed mechanically with a roughened stainless steel wire. Functional integrity of the endothelium was determined by the presence or absence of relaxation induced by acetylcholine during contraction obtained with noradrenaline. After each experiment the artery rings were carefully opened flat and stained with $AgNO_3$ to visualize the endothelium. Only results from rings with more than 70% of the endothelium were considered as control rings. Vessels in which the endothelium had been rubbed never showed more than 5% of their intima covered with endothelium.

Drugs

The following drugs were used: endothelin-1, noradrenaline hydrochloride, N^G-nitro-L-arginine methyl ester, acetylcholine chloride and sodium nitroprusside (Sigma Chemical Co, St. Louis, MO. U.S.A.).

Data analysis

All values are expressed as mean ± s.e.mean. Contractions are reported as a percentage of the response to KCl (100 mM). Relaxation is expressed as the percentage of relaxation from precontraction in response to noradrenaline ($10^{-6}-3 \times 10^{-6}$ M). EC_{50} values (concentrations of drugs producing half-maximal responses) were determined from individual concentration-response curves by non-linear regression analysis. The EC_{50} values are presented as pD_2 ($pD_2 = -\log EC_{50}$). The pD_2 values were compared by an unpaired t test and an analysis of variance. The number of rings taken from each patient varied from 16 to 24; n values are presented as the number of patients from whom the blood vessels were obtained. A value of $P<0.05$ was considered to be statistically significant.

Results

Noradrenaline (10^{-8} to 3×10^{-5} M) and endothelin-1 (10^{-11} to 3×10^{-7} M) induced concentration-dependent contractions which were of greater magnitude in artery rings from BDR patients (Figure 1). Table 1 summarizes the pD_2 and maximal contraction values for noradrenaline and endothelin-1 determined in arterial rings from control patients and from patients with a positive bronchodilator test. Maximal responses and pD_2 values were significantly increased in arteries from the BDR group of patients (all changes statistically significant; $P<0.05$). There was no significant difference in the contractile response to 100 mM KCl between arteries from the BDR group of patients and those from controls (1422 ± 233 mg versus 1288 ± 163 mg, $P>0.05$; $n=6$).

Precontracted pulmonary artery rings with endothelium from BDR patients showed greater relaxation in response to acetylcholine and sodium nitroprusside compared with controls (Figure 2). The acetylcholine and sodium nitroprusside pD_2 values were significantly higher ($P<0.05$) in the group of patients having a positive bronchodilator test (Table 1). Rings

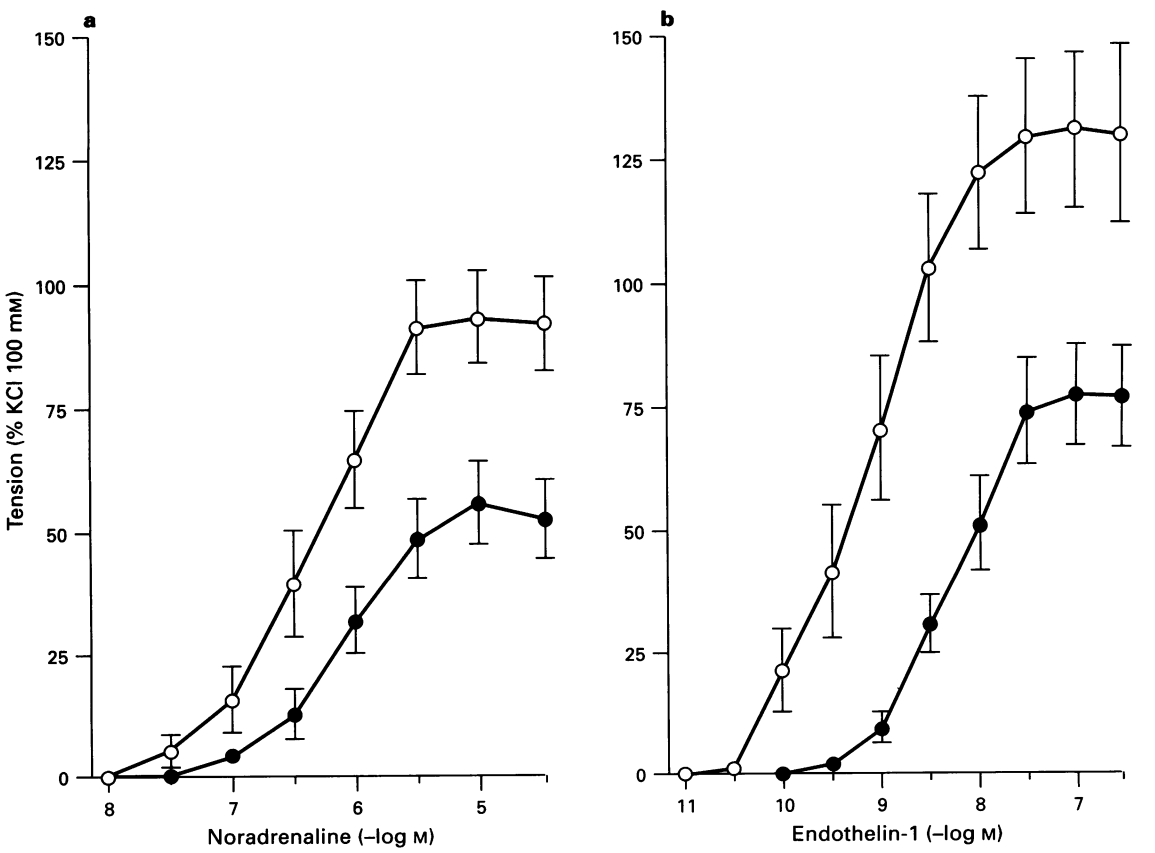

Figure 1 Concentration-response curves for (a) noradrenaline and (b) endothelin-1 determined in arterial rings from control patients (●, $n=6$) and from patients with a positive bronchodilator response (○, $n=6$). Values are means ± s.e.mean.

without endothelium from both control ($n=6$) and BDR patients ($n=5$) showed no relaxation in response to acetylcholine but the response to sodium nitroprusside was unchanged (Figure 2). Inhibition of nitric oxide synthase with L-NAME (10^{-4} M) in arterial rings with endothelium from control ($n=5$) and BDR patients ($n=4$) reduced relaxation in response to acetylcholine but did not modify relaxation to sodium nitroprusside (Table 2).

Discussion

The present study demonstrates that patients with increased bronchodilator response developed a greater tension in response to endothelin-1 and noradrenaline and achieved an increased relaxation in response to acetylcholine and sodium nitroprusside. Among the potential mechanisms that could account for this hyperresponsiveness we have taken into con-

Table 1 Responses of pulmonary arterial rings to the tested agents

	Control patients		*Patients with positive bronchodilator test*	
	pD_2	E_{max} (%)	pD_2	E_{max} (%)
Contraction				
Noradrenaline	6.04±0.08 ($n=6$)	56±8 ($n=6$)	6.44±0.10* ($n=6$)	93±9* ($n=6$)
Endothelin-1	8.29±0.1 ($n=5$)	78±10 ($n=5$)	8.92±0.1* ($n=5$)	130±16* ($n=5$)
Relaxation				
Acetylcholine	6.64±0.07 ($n=5$)	72±5 ($n=5$)	7.10±0.3* ($n=5$)	96±3* ($n=5$)
Sodium nitroprusside	6.0±0.1 ($n=6$)	98±4 ($n=6$)	8.7±0.1* ($n=6$)	98±1 ($n=6$)

Values are means±s.e.mean. Maximal contraction is expressed as a percentage of response to 100 mM KCl. Maximal relaxation is expressed as the percentage of relaxation from precontraction in response to noradrenaline. n, number of patients. *$P<0.05$ versus arterial rings from control patients.

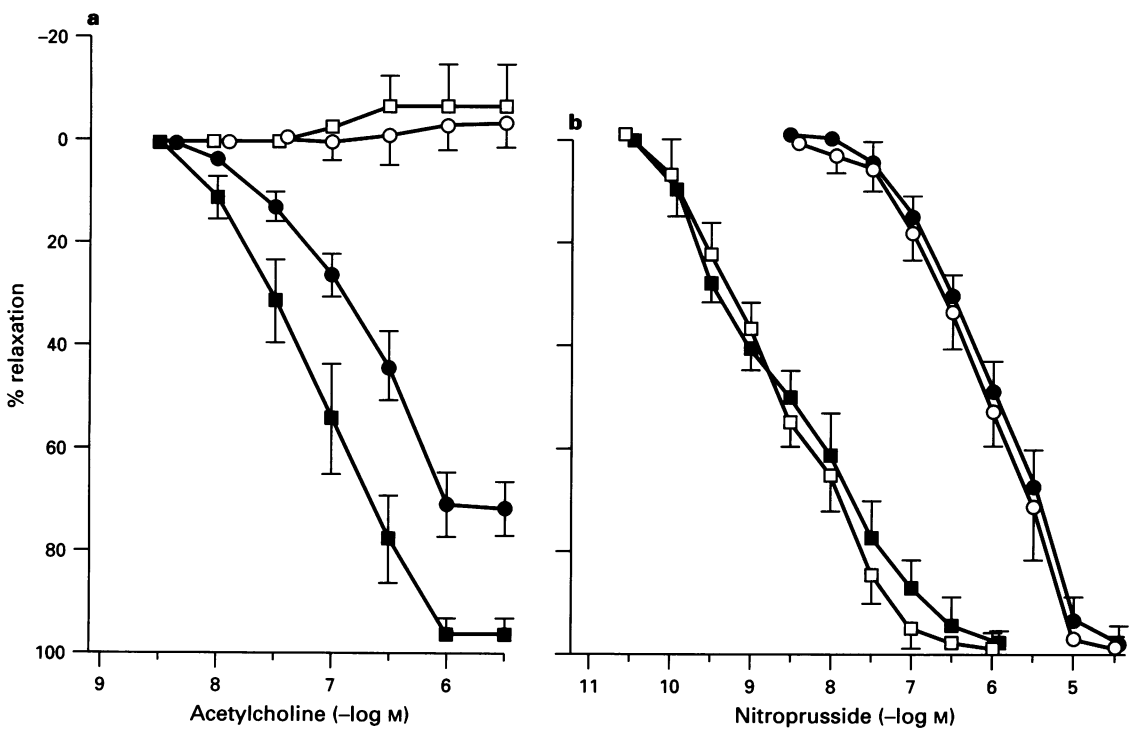

Figure 2 Concentration-response curves for (a) acetylcholine and (b) sodium nitroprusside determined in arterial rings with (solid symbols) and without (open symbols) endothelium from control patients (○, ●, $n=6$) and patients with positive bronchodilator test (□, ■, $n=5$). Values are means±s.e.mean.

Table 2 Effects of L-NAME on relaxation of pulmonary arterial rings to acetylcholine and sodium nitroprusside

	Control patients		*Patients with positive bronchodilator test*	
Maximal relaxation (%)	*Untreated rings*	*L-NAME-treated rings*	*Untreated rings*	*L-NAME-treated rings*
Acetylcholine	72±5 ($n=5$)	34±3* ($n=5$)	96±3 ($n=5$)	33±5* ($n=4$)
Sodium nitroprusside	98±4 ($n=6$)	99±1 ($n=5$)	98±1 ($n=6$)	98±3 ($n=4$)

Maximal relaxation is expressed as the percentage of relaxation from precontraction in response to noradrenaline. n, number of patients. *$P<0.05$ versus untreated rings.

sideration the changes of mechanical properties of the vessel wall and the possible intervention of the endothelial cell layer. Concerning the first possibility, because histological changes in the vessel wall were not studied, we cannot rule out the possibility of involvement of hypertrophy of the vessel wall. However, the fact that maximal contraction to KCl did not differ between control and hyperreactive artery rings, suggests that a change in the mechanical properties did not play an important role.

We observed that the endothelium-dependent relaxation induced by acetylcholine was significantly increased in patients with positive bronchodilator response. The intervention of endothelium-derived nitric oxide in these effects is indicated by the absence of relaxation to acetylcholine in arterial rings without endothelium and in arterial rings treated with the inhibitor of nitric oxide synthase, L-NAME (Rees *et al.*, 1989). Possible mechanisms which may be involved in the increased relaxation to acetylcholine include an increased responsiveness of smooth muscle to endothelial nitric oxide or an enhancement of the synthesis and/or release of nitric oxide by the endothelium. The latter seems unlikely since the relaxation induced by sodium nitroprusside, an endothelium-independent vasodilator, was also increased in arterial rings from these patients. Sodium nitroprusside relaxes smooth muscle by releasing nitric oxide within smooth muscle cells and therefore the physical or functional integrity of the endothelium is not an important determinant of its vascular effects (Ignarro *et al.*, 1981; Murad, 1986). Thus our findings suggest that the increased dilator responses to acetylcholine and sodium nitroprusside is probably dependent upon an increased responsiveness of smooth muscle to nitric oxide either derived from endothelial cells by acetylcholine or generated by sodium nitroprusside in smooth muscle. In contrast to the present findings, it has been demonstrated that human pulmonary artery relaxation to acetylcholine is impaired in chronic lung disease (Dinh-Xuan *et al.*, 1991). Such an impairment correlated directly with the preoperative levels of hypoxaemia, an alteration which was not observed in the present group of patients ($PaO_2 = 84 \pm 4$ mmHg in control patients and 88 ± 2 mmHg in BDR group). Hypoxia has been observed to block the synthesis of endothelium-derived nitric oxide rather than inhibiting its action on smooth muscle cells (Warren *et al.*, 1989; Adnot *et al*, 1991).

The increased relaxation induced by acetylcholine and sodium nitroprusside is associated with an exaggerated contractile response to noradrenaline and endothelin-1. The mechanisms involved in these responses are unknown at present but probably reflect an increase in sensitivity of smooth muscle to these agonists, as was observed for nitric oxide. There appears to be no endothelial receptors for endothelin and noradrenaline in human pulmonary arteries (McKay *et al.*, 1991; Martínez *et al.*, 1995). The increased responsiveness to noradrenaline observed in chronic lung disease appears to be due to the impairment of nitric oxide-mediated relaxation which occurs in these patients (Dinh-Xuan 1991). This circumstance cannot account for the present experiments, since responses to acetylcholine, which are mediated by endothelial nitric oxide, were also potentiated.

In summary, this study demonstrates an increased responsiveness of pulmonary arteries from patients with an increased bronchodilator test. Direct assessment of the functional importance of the present results awaits the evaluation of other vasoactive agents which are found in plasma or released from perivascular nerve endings. However, it may be postulated that the vascular hyperresponsiveness observed in the present experiments should be taken into consideration in the treatment of patients having bronchial hyperresponsiveness.

This work was supported by the Comision Interministerial de Ciencia y Technología, Ministerio de Sanidad y Generalitat Valenciana.

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(Received August 1, 1996
Accepted August 30, 1996)