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Tobacco-associated pulmonary vascular dysfunction in smokers: role of the ET-1 pathway

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Henno P, Boitiaux J, Douvry B, Cazes A, Lévy M, Devillier P, Delclaux C, Israël-Biet D. Tobacco-associated pulmonary vascular dysfunction in smokers: role of the ET-1 pathway. Am J Physiol Lung Cell Mol Physiol 300: L831–L839, 2011. First published March 4, 2011; doi:10.1152/ajplung.00251.2010.—Pulmonary vascular remodeling and dysfunction associated to tobacco smoking might pave the way for the subsequent development of pulmonary hypertension. Its prognosis is dreadful and its underlying mechanisms are so far largely unknown in humans. To assess the potential role of endothelin-1 and its receptors in smokers’ pulmonary artery vasoactive properties. Endothelin-dependent vasodilation to ACh was assessed in pulmonary vascular rings from 34 smokers and compared with that of 10 nonsmokers. The effects of ET-A (BQ 123) or ET-B (BQ 788) blockers and that of an ET-B activator (sarafotoxin) were evaluated. Endothelin-1 was quantitated by ELISA. Expression of its receptors was quantitated by Western blotting. Smokers exhibited an impaired pulmonary endothelium-dependent vasodilation compared with that of nonsmokers (P < 0.01). In the former group, 8 of 34 subjects exhibited a marked endothelial dysfunction (ED⁺) whereas 26 (ED⁻) (P < 10⁻⁴) displayed a vasorelaxation to ACh that was comparable to that of nonsmokers. In ED⁺ subjects, ET-A was overexpressed (P < 0.05) and inversely correlated (P < 10⁻²) with the response to ACh. Sarafotoxin significantly improved vasodilation in all subjects (P < 10⁻²). In conclusion, tobacco smoking is associated to an impaired pulmonary vasoresistance at least partly mediated by an ET-1/ET-A-dependent dysfunction.

human pulmonary arteries; acetylcholine; vascular pharmacology

MARKED ALTERATIONS in pulmonary vessels, characterized by various degrees of intimal thickening, smooth muscle cell deposition, and matrix reorganization, are present in smokers in addition to airway epithelial injury and remodeling, includ-
nisms involved in the toxicity of tobacco products on vascular structures. In guinea pigs, tobacco smoke leads to a 25% increase in mean pulmonary arterial pressure, matrix reorganization, and decrease in endothelial NO synthase (eNOS) expression (10, 20, 56), whereas arterial muscularization is correlated with levels of endothelin ET-1 and of vascular endothelial growth factor (VEGF). In humans, endothelial dysfunction has long been reported in smokers with severe (14) or with mild COPD as well as in those with a normal lung function (35) and has mostly been characterized as a reduced eNOS expression (3) and/or as an increased VEGF production (44) potentially induced by a direct toxicity of cigarette smoking on the endothelium. The ET-1 pathway is undoubtedly involved in human pulmonary arterial hypertension (18), where a widely used and validated therapeutic approach in this condition now consists in blocking its receptors (23). Increased ET-1 levels are also correlated to systolic pulmonary pressure in COPD-associated pulmonary hypertension (8). Finally, it has been clearly implicated in another model of pulmonary vascular dysfunction, i.e., that associated to end-stage cystic fibrosis (25). To our knowledge, only one study has focused on this critical side of the endothelial function, i.e., the activity of ET-1 pathway, in the context of tobacco smoking, reporting comparable pulmonary artery contents in ET-1 in smokers and in nonsmokers (3). Very few if any data are available about the expression of its receptors in vivo. Our aim in this study was to confirm the role of tobacco smoking in the pulmonary endothelial dysfunction of smokers and to assess the role of ET-1 in this dysfunction by focusing on the expression and pharmacological manipulation of its receptors. In this respect, our study brings original data pointing to the potentially pathogenic role of the ET-1 pathway.

MATERIAL AND METHODS

Subjects

To compare the impact of tobacco smoking on human pulmonary vasoactive functions, we compared the latter in lung explants from two groups of subjects undergoing surgical resection for lung cancer: smokers (n = 34, 11 current smokers and 23 ex-smokers with a range of smoking cessation period of 3–12 mo) and nonsmokers (lifetime never smokers) (n = 10). Their demographic and clinical characteristics are shown in Table 1. The smoker group consisted in 26 men and 8 women, mean aged 60 ± 1 yr (range 45–77), with a median cumulated smoking history of 50 pack·yr (range 7–120). Their pre-operative pulmonary function testing showed a mean forced expiratory volume in 1 s (FEV₁) of 83% predicted (median 79%, range 57–121%). Six of them had an obstructive lung disease [defined by a FEV₁/forced vital capacity (FVC) < 70%], with two being classified as stage I and four as stage II according to the Global Initiative for Obstructive Lung Disease (GOLD)-2006. All of the remaining 28 smokers exhibited a normal lung function. Factors with a known impact on systemic vascular function (prior chemotherapy, hypercholesterolemia, diabetes mellitus, current treatments such as statins or vasoactive drugs) were systematically evaluated and are reported in Table 1. Finally, 22 subjects in the smoker group had undergone a preoperative transthoracic echocardiography; none of them displayed a detectable pulmonary hypertension at rest, defined as a systolic pulmonary arterial pressure > 35 mmHg. Our control group consisted in 10 never smokers also undergoing surgical resection for lung cancer. Their mean age was 52 ± 16 yr. All of them exhibited a normal lung function. Their characteristics differed from those of the smoker group only in terms of smoking history (Table 1).

This study was submitted to and approved by an ethics committee, and, according to French legislation, patients gave their informed consent to this study.

Tissue Preparation

Immediately after excision, lung samples were placed in Krebs-Henseleit solution (in mM: 120 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 15 NaHCO₃, 1.2 KH₂PO₄, 11 d-glucose, and 10 HEPES, pH 7.4) and transported without delay to our laboratory. Intralobar arteries were carefully dissected free of parenchyma and adhering connective tissue, then several rings (3- to 5-mm length × 1.5- to 2-mm inside diameter) from a single artery were prepared. Some of them were used immediately for pharmacological studies, while others were snap frozen and stored in liquid nitrogen for subsequent protein extraction.

Table 1. Comparison of general characteristics and lung function measurements of patients

| Characteristics | Smokers | | | | |
|-----------------|---------|---------|---------|---------|
| Age, years (median, range) | 59 (48–74) | 60 (45–77) | 52 (41–75) | NS |
| Male/female ratio | 6/2 | 20/6 | 7/3 | NS |
| Tobacco, pack·yr (median, range) | 35 (30–120) | 60 (7–120) | NS |
| Current smokers, n | 2 | 29 | NS |
| FEV₁, % predicted | 75 (62–101) | 84 (57–121) | 87 (83–103) | NS |
| GOLD, n | 2 | 0 | 0 | NS |
| GOLD stage I, n | 1 | 1 | 0 | NS |
| GOLD stage II, n | 1 | 3 | 0 | NS |
| GOLD stage III, n | 0 | 0 | 0 | NS |
| GOLD stage IV, n | 0 | 0 | 0 | NS |
| sPAP > 35 mmHg, n | 0 | 0 | 0 | NS |
| Prior chemotherapy, n | 2 | 6 | 0 | NS |
| Hypercholesterolemia, n | 2 | 1 | 1 | NS |
| Hypertension, n | 1 | 6 | 0 | NS |
| Diabetes mellitus, n | 1 | 4 | 0 | NS |
| Treatment by statin, n | 2 | 6 | 0 | NS |
| Vasodilating treatment, n | 2 | 5 | 0 | NS |

FEV₁, forced expiratory volume in 1 s; GOLD, obstructive lung disease, defined by FEV₁/FVC < 70% (where FVC is forced vital capacity); GOLD, Global Initiative for Chronic Lung Disease-2006; sPAP, systolic pulmonary arterial pressure; NS, not significant. Nonparametric ANOVA was used for continuous variables and χ² test for categorical variables.
Design of Pharmacological Experiments

The general experimental design was similar to that previously described (25). Briefly, arterial rings were suspended on tissue hooks in 5-ml organ baths containing Krebs-Henseleit solution at 37°C and bubbled with 95% O₂-5% CO₂. Each preparation was connected to a force displacement transducer (Statham UF-1), and changes for isometric tension were recorded as previously described (42). An initial tension of 1 g was applied to the rings, which were then left to equilibrate for 30 min until a stable resting tension (RT1) was obtained, with changes in fresh Krebs-Henseleit solution every 10 min (Fig. 1). Ring viability was verified by adding KCl (40 mM), which induced a contraction. Viable rings were then washed three times until full relaxation (resting tension 2, RT2) and were left at rest for 20 min. They were then precontracted with L-phenylephrine dichloride 10⁻⁵ M (PE) to obtain a stable plateau of contraction. Serial dilutions of ACh were then added to produce a cumulative dose-response curve (10⁻¹⁰ to 10⁻⁴ M). Relaxation to ACh is expressed as a percentage of relaxation to PE-induced tone. A contractile response to ACh is expressed as a negative value. Endothelium-independent relaxation was assessed by measuring the response to sodium nitroprusside 10⁻⁵ M (SNP) at the end of each experiment.

To assess the role of endothelium in pulmonary vasoactivity, we compared response to ACh in presence and absence of endothelium, a condition that was achieved by carefully removing the endothelium with a pipe cleaner (15).

For each patient, some rings were pretreated with various drugs for 30 min after PE precontraction. To evaluate the role of ET-1 in pulmonary vasoactivity, we assessed the effects of endothelin-A receptor (ET-A) (BQ 123, 10⁻⁵ M) or endothelin-B receptor (ET-B) (BQ 788, 10⁻⁵ M) ET-1 receptors antagonists, as well as ET-B agonist sarafotoxin 6c (S6c, 10⁻⁶ M) on dose-response curves to ACh.

All drugs were purchased from Sigma, St. Louis, MO, except for ACh, which was provided by Pharmacie Centrale des Hôpitaux, Paris, France.

All experiments were performed in duplicate, with a variability between rings less than 10%.

Western Blot Analysis

ET-A and ET-B receptors were assayed from homogenized extracts of distal lung parenchyma. Total proteins were extracted with a lysis buffer (10 mM Tris-HCl pH 7.4, 50 mM NaCl, 0.1% NP-40, and 20% antiprotease cocktail) and were measured with a bichinchoninic acid protein assay kit (Pierce, Courtaboeuf, France) on a microplate according to the manufacturer’s instructions. Total proteins (30 µg/lane) were separated by electrophoresis on a 10% sodium dodecyl sulfate-polyacylamide gel and then transferred onto nitrocellulose membranes and immunodetected. Nonspecific binding was blocked with 10% milk powder in Tris-buffered saline for 1 h at room temperature. The blots were incubated with the same antibodies as those used for immunohistochemistry against ET-A receptor (1:500 dilution) and ET-B receptor (1:500 dilution). Membranes were subsequently stripped and reprobed with β-actin to verify equal loading. The proteins were detected with an enhanced chemiluminescence (ECL) kit (GE Healthcare Europe, Aulnay sous Bois, France) and Hyperfilm ECL high-performance chemiluminescence film (GE Healthcare Europe). The intensities of protein staining on the immunoreactive Western blot bands were analyzed with ImageJ software. The relative amounts of immunoreactive proteins were obtained by dividing the scanning unit values by the respective value of β-actin protein (primary anti β-actin mouse monoclonal antibody: AAN02, 1:1,000 dilution; Cytoskeleton) and expressed in arbitrary units.

ELISA

ET-1 was assayed from homogenized extracts of frozen distal parenchyma. Total proteins were extracted and measured as previously described. The Human ET-1 ELISA System (R&D Systems, Abingdon, UK) was used to assess ET-1 levels in protein extracts from distal parenchyma. ET-1 in the samples tested was captured by ET-1 ET-1 ELISA kit (GE Healthcare Europe, Aulnay sous Bois, France) and Hyperfilm ECL high-performance chemiluminescence film (GE Healthcare Europe). The intensities of protein staining on the immunoreactive Western blot bands were analyzed with ImageJ software. The relative amounts of immunoreactive proteins were obtained by dividing the scanning unit values by the respective value of β-actin protein (primary anti β-actin mouse monoclonal antibody: AAN02, 1:1,000 dilution; Cytoskeleton) and expressed in arbitrary units.

Statistical Analysis

Results are expressed as means ± SE except where specified. Data were analyzed with GraphPad Instat Software. For between-group comparisons (smokers and nonsmokers), the Mann-Whitney U-test was used for continuous variables. For three-group comparisons (ED⁺, ED⁻, and nonsmokers), nonparametric ANOVA followed by Dunn’s or Tukey-Kramer (where appropriate) post hoc test was used. Fischer’s exact test was used for categorical variables. For condition comparisons (within dose-response curves), continuous variables were compared with the Wilcoxon’s paired test. Spearman’s rank correlation coefficient was calculated to evaluate correlations between
pairs of variables. A $P$ value $< 0.05$ was considered statistically significant.

RESULTS

Endothelial function evaluated by the response of pulmonary arterial rings to ACh, as in systemic vessels, was endothelium and NO dependent, as demonstrated by the abrogation of the relaxation in the absence of endothelium or in the presence of $N$-nitro-L-arginine methyl ester (data not shown). In our experimental conditions, endothelial dysfunction was defined as a lack of relaxation or even a contraction response (16).

Pharmacological Evaluation of Pulmonary Vasoactivity

The vasodilative response to ACh was strongly altered in smokers compared with nonsmokers ($12 \pm 5$ vs. $42 \pm 8\%$ at ACh $10^{-4}$ M in smokers and nonsmokers, respectively; $P < 0.01$, Fig. 1A). A closer analysis of the smoker group allowed to clearly distinguish two subgroups: 8 subjects exhibited an endothelial dysfunction as defined above (group ED$^+$) whereas the remaining 26 exhibited a relaxant response (group ED$^-$) comparable to that of nonsmokers ($-26 \pm 12$ vs. $24 \pm 4$ and $42 \pm 8\%$ at ACh $10^{-4}$ M in groups ED$^+$, ED$^-$, and nonsmokers, respectively; ANOVA: $P < 0.0001$, Fig. 1B). Of note is the fact that no patient from the nonsmoker group exhibited an endothelial dysfunction. The remaining vascular tone characteristics (baseline tensions and peak vasoconstriction to PE) were otherwise similar in the ED$^+$ and ED$^-$ groups: RT1: $0.9 \pm 0.2$ g vs. $1 \pm 0.1$ g; RT2: $0.9 \pm 0.2$ g vs. $0.9 \pm 0.1$ g; PE-induced tension: $1.2 \pm 0.2$ g vs. $1.3 \pm 0.1$ g in ED$^+$ vs. ED$^-$, respectively [not significant (NS) for every comparison].

![Fig. 2](http://ajplung.physiology.org/)

Fig. 2. Effect of endothelin-1 (ET-1) receptor antagonists on dose-response curves to ACh in smokers. ET-A antagonist BQ 123 tended to improve response to ACh in patients as a whole ($n = 19$) ($P = 0.08$) (A). BQ 123 did not have a significant effect on each group taken separately: ED$^+$ ($n = 4$) and ED$^-$ ($n = 15$) (B). ET-B antagonist BQ 788 did not modify the dose-response curve to ACh in patients as a whole ($n = 16$) (C). Dual ET-1 receptor blockade had no effect on dose-response curve to ACh in patients as a whole ($n = 8$) (D).
We found no correlation in ED⁺ or ED⁻ subjects between responses to ACh and age, number of pack-yr, or FEV₁. Finally, besides striking differences between the ED⁺ and the ED⁻ groups in terms of vasodilative responses, it should be underlined that they were otherwise quite comparable in terms of spirometric and other clinical characteristics (Table 1).

To gain insights into the mechanisms of the vascular dysfunction present in a subgroup of smokers, we further explored it focusing on both endothelium-dependent and -independent relaxation.

**Endothelin-1 Pathway and Vascular Reactivity**

We repeated the dose-response curves to ACh in the presence of specific blockers of ET-1 receptors, BQ 123 (anti-ET-A) in 19 patients (ED⁻, n = 15; ED⁺, n = 4) and BQ 788 (anti-ET-B) in 16 patients (ED⁻, n = 13; ED⁺, n = 3) or of an ET-B agonist (sarafotoxin, S6c) in 10 patients (ED⁻, n = 9; ED⁺, n = 1).

**ET-A Blockade**

In the smoker group as a whole, BQ 123 tended to improve vasorelaxation (28 ± 6 vs. 18 ± 4%, with and without BQ 123, respectively; \( P = 0.08 \) at ACh \( 10^{-7} \) M) (Fig. 2A), although this did not reach significance in either group when analyzed separately (in ED⁺: 9 ± 27% vs. −10 ± 4%, with and without BQ 123, respectively, NS; in ED⁻: 33 ± 5 vs. 26 ± 5%, with and without BQ 123, respectively, NS) (Fig. 2B). Although this difference was not statistically significant in terms of magnitude, it should be noted that in the ED⁺ group, BQ 123 allowed to abrogate the endothelial dysfunction phenotype since a slight vasodilatation could be obtained when using this ET-A blocker (9 ± 27 with vs. −10 ± 4% without BQ 123).

**ET-B blockade.** No consistent modification of the response was observed when using BQ 788 in smokers as a whole (21 ± 6 vs. 16 ± 7% with and without BQ 788, respectively; NS) nor in either group analyzed separately (Fig. 2C).

**Combined ET-A and ET-B blockades.** We repeated dose-response curves to ACh in the presence of combined ET-1 receptors blockers in 8 subjects (ED⁺, n = 2; ED⁻, n = 6) and did not observe any significant effect of this combination (Fig. 2D).

**ET-B activation.** In contrast, a marked dilating effect was noted when using sarafotoxin, which significantly enhanced ACh-induced vasorelaxation in all of the 10 subjects we could evaluate (37 ± 7 vs. 11 ± 10% with and without sarafotoxin, respectively, \( P = 0.0098 \)) (Fig. 3). However, because of the small size of this series (ED⁺: n = 1 and ED⁻: n = 9) we cannot draw any definite conclusion about this observation.

**Endothelin-1 Receptor Expression**

ET-A expression was assessed by Western blotting in the distal lung parenchyma of 16 subjects (ED⁺: n = 4 and ED⁻: n = 12). It was significantly higher in group ED⁺ (3.2 ± 0.6 vs. 1 ± 0.2 densitometric units in ED⁺ and ED⁻, respectively; \( P = 0.004 \)) (Fig. 4A). Furthermore, ET-A expression was inversely correlated to the degree of relaxation to ACh at \( 10^{-6} \) M (\( r = -0.74, P = 0.0011 \); Fig. 4B). Finally, we found no correlation between ET-A expression and FEV₁, FEV₁/FVC, number of pack-yr, or smoking status (current or ex-smoker). ET-B expression was assessed in 22 subjects (ED⁺: n = 6 and ED⁻: n = 16). It tended to be higher in group ED⁻ although not significantly (1.46 ± 0.3 vs. 0.98 ± 0.2 in ED⁺ and ED⁻, respectively; NS) (Fig. 4C). In contrast to ET-A, ET-B expression was not correlated to the degree of relaxation to ACh (Fig. 4D).

Finally, the ET-A-to-ET-B ratio was fourfold increased in ED⁺ subjects (n = 3) vs. ED⁻ (n = 12) (4.1 ± 2.5 vs. 1 ± 0.2 in ED⁺ and ED⁻, respectively; \( P = 0.04 \)) (Fig. 5A) and inversely correlated to the degree of relaxation to ACh at \( 10^{-6} \) M (\( r = -0.53, P = 0.04 \)) (Fig. 5B).

**ET-1 Levels in Lung Parenchyma**

ET-1 was quantitated in homogenized extracts of frozen distal parenchyma in ED⁺ (n = 4) and ED⁻ (n = 16) patients. Tissue content in ET-1 was comparable in both groups (25.7 ± 4 vs. 33.5 ± 3 pg/mg protein in ED⁺ and ED⁻, respectively; NS) (data not shown).

**DISCUSSION**

We have shown here that 8 of 34 (24%) current or ex-smokers with no or minimal airway obstruction displayed strongly impaired pulmonary vasodilative capacities. The underlying mechanisms at least partly include an endothelium-dependent vascular dysfunction in which the ET-1 pathway is likely to be involved.

These data are of importance considering the fact that endothelial dysfunction might pave the way for the remodeling of vascular structures and the increase of pulmonary vascular resistance, ultimately leading to an irreversible pulmonary hypertension. Indeed, endothelium plays a crucial role in the regulation of vascular homeostasis through its highly regulated and balanced secretion of mediators with opposite effects on vascular tone and structure. The predominance of vasoconstrictor and proremodeling mediators (cytokines, growth factors, free radicals) might have dreadful consequences such as the development of pulmonary hypertension, now largely identi-
fied as a critical prognostic marker in COPD (9, 27). Its underlying molecular mechanisms are still poorly elucidated and no targeted treatment is presently recommended in this condition. Getting pathogenic clues to the early steps of tobacco-associated vascular derangement therefore appears to be of strong clinical relevance.

That patients with COPD, particularly with an advanced lung disease, exhibit a marked endothelial dysfunction has long been known (14, 35). Comparable findings in mild COPD have been reported more recently with significant structural and functional vascular abnormalities occurring even in smokers with normal lung function (4, 35, 36, 43). The deleterious effects of tobacco products on the systemic vascular endothelial function are now well known (32, 38, 39). In animal models, tobacco smoking has been clearly established as responsible for pulmonary vascular remodeling (51), which can largely antecede emphysema development (57). In the human lung, cigarette smoke is also associated with marked structural derangements such as inflammation and remodeling (34, 36) and with some degree of vascular dysfunction (35). We have shown here that the impairment of pulmonary vasodilation was associated with no factor with a known impact on vascular function other than tobacco smoking. This is in keeping with the human observations cited above (35). The fact that inside the smoker group ED+ and ED− subjects had a comparable smoking history (no difference in the current or ex-smoking status nor in the number of pack-yr) suggests that tobacco products might selectively induce endothelial injury in certain subjects. Epidemiological studies are needed to clarify the role of as yet unidentified factors in their susceptibility to tobacco-induced vascular injury.

Numerous animal studies have reported on the pathogenic mechanisms that underlie vascular inflammation, remodeling and dysfunction related to short-term (52) or long-term (20, 55, 56) exposure to tobacco products such as the modulation of vasoactive mediators (VEGF, ET-1, eNOS). Very few studies if any have focused on the role of the ET-1 pathway in the context of tobacco smoking in humans. It had only been reported that ET-1 in the pulmonary artery did not differ in smokers and nonsmokers (3), but few if any data are available about the expression of its receptors in vivo and about the effects of their pharmacological modulation.

As stated above, pulmonary vascular tone is regulated by interactions between vasoactive mediators produced by the

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**Fig. 4. ET-1 receptors expression in smokers’ distal lung parenchyma and correlation to response to ACh (10−6 M).** ET-A expression was significantly enhanced in ED+ patients (n = 4) vs. ED− patients (n = 12), P = 0.004 (A). The first 3 ED+ bands from the left were run on the same gel, whereas the 4th ED+ band was run on a separate gel. All 4 ED− bands were run on the same gel. ET-A expression was significantly inversely correlated to response to ACh (P = 0.0011) (B). ET-B expression was not different in ED+ (n = 6) and ED− (n = 16) groups (C). The first 3 ED+ bands from the left were run on the same gel but were not contiguous so that the gel has been spliced, whereas the 4th ED+ band was run on a separate gel. All 4 ED− patients were run on the same gel. ET-B expression was not correlated to response to ACh (D).
endothelium (21). Among these, ET-1 is one of the most potent vasoconstrictor and proremodeling mediators known (12, 24). However, it can have contrasting vasoactive properties through its two distinct types of receptors (18). In humans, the ET-1 pathway is undoubtedly involved in pulmonary arterial hypertension and a widely used and validated therapeutic approach in this condition now consists in blocking its receptors (23). ET-1 is also involved in at least two models of secondary pulmonary derangements such as COPD-associated pulmonary hypertension (8) and end-stage cystic fibrosis (25). To our knowledge, it had never been clearly incriminated in the pulmonary vasculopathy of asymptomatic smokers.

We have shown here that subjects with an impaired relaxation (ED) exhibited a marked increase in ET-A expression vs. those with a normal response (ED) (P < 0.005). This resulted in a strong imbalance in the ET-A-to-ET-B ratio, which was fourfold increased in ED+ vs. ED− subjects and 2.5-fold increased compared with the 1.5 normal value cited in the literature for the human lung (13). Although tissue content in ET-1 was comparable in ED+ and ED− subjects, we hypothesize that its signaling through highly overexpressed ET-A might be responsible for the impaired vasorelaxation displayed by the former. In addition, the constrictive activity of ET-A receptors might be enhanced in smokers as demonstrated in a rat model in which soluble smoking particles were able to change vascular contractility phenotypes toward an increased efficacy of ET-A receptors (58). The fact that in our study vasodilation to ACh was strongly and inversely correlated with ET-A expression (P = 0.0011) might reflect the relevance of these observations in humans. Although we could not significantly improve the vasodilative response of ED+ subjects using BQ 123, an ET-A specific blocking agent, of note is the fact that this intervention changed the phenotype of this group from dysfunctional (−10 ± 4%) to slightly vasorelaxant (9 ± 27%). The absence of statistical significance might result from the very small size of the group in which highly variable responses were observed. The confirmation of these data in a consistent cohort could undoubtedly be used for future therapeutic reflections.

The active ET-1 signaling through increased ET-A receptors might induce a vascular dysfunction through several mechanisms. One of them is the generation of reactive oxygen species (ROS) with their direct and deleterious effects on endothelial functions. The ET-1/ET-A pathway plays indeed a major role in ROS production (37, 48), which in addition to modulating proliferation and survival of vascular smooth muscle cells (41) also limits NO bioavailability in endothelial as well as in smooth muscle cells (17, 49). ROS production is increased in COPD patients and in smokers with normal lung function. Their role on vascular function is strongly argued for by the normalization of SNP-induced vasodilation of pulmonary arteries in chronically ET-1 exposed rats by the antioxidant N-acetyl-l-cysteine (31). We could hypothesize that ROS might decrease pulmonary eNOS expression in our ED+ subjects as they have been shown to play a role in smoking-mediated dysfunction of NO biosynthesis in human coronary endothelial cells (6) as well in rat aorta (40). Furthermore, eNOS might also be directly downregulated through the ET-1/ET-A pathway. The latter has indeed been shown to be involved in human persistent pulmonary hypertension in the newborn through an ET-A-induced generation of hydrogen peroxide (48). Altogether, eNOS expression might indeed be decreased in our ED+ subjects. This would be a tempting hypothesis to account for the impaired vasodilation in these subjects. However, although we did not assess eNOS expression in the present series, we think that there might be alternative explanations. Indeed, in another study carried out in a series of COPD stages 1 and 2 smokers we found comparable arterial rings contents in eNOS between ED+ and ED− subjects (unpublished observations). Importantly, this is quite in keeping with a recent report (7) showing that eNOS was similar in terms of mRNA and protein in smokers with COPD stages 1 and 2 and in nonsmokers, and that a reduction of eNOS was found only in stages 3 and 4. In addition, eNOS is not always decreased by smoke exposure but can rather be increased as has been shown in some animal models (47, 53, 56). Furthermore, eNOS expression does necessarily reflect its function. Cigarette smoke can indeed induce a decrease in NO synthase type 3 activity while protein expression is increased (5). Altogether, there is no consensus about the exact relationship between cigarette smoke and eNOS expression and activity, which strongly depends on the model under consideration. In the present study, we cannot rule out the possibility that the highly increased ET-A expression found in ED+ subjects might impair vasodilation through a decreased eNOS expression and/or activity and therefore a diminished NO bioavailability. Alternatively, the local intravascular production of
ET-1 and its binding to highly expressed ET-A might directly increase the vascular tone. The tendency of BQ 123, selective blocker of ET-A, to improve vasodilation in ED+ subjects is in keeping with this hypothesis. Although this effect lacked statistical significance, it might be of importance for pathogenesis of smoke-induced pulmonary vasculopathy and potentially for future therapeutic considerations. Further experiments comparing the effects of extrinsic ET-1 added to the organ baths in the presence or absence of BQ 123 will help us to get deeper insights into the role of the ET-1/ET-A pathway in this condition.

If the constrictive role of ET-A is clearly established (12, 29, 45) that of ET-B is more ambiguous and at least partly depends on its cellular location. Essentially constrictive when expressed on smooth muscle cells (30, 46), ET-B can exert vasodilative effects when expressed on endothelial cells through the release of NO and prostacyclin (26, 28). Endothelial ET-B does not appear to contribute significantly to pulmonary vascular tone in normal conditions but its vasodilatory role can be unmasked under conditions of pulmonary hypertension (19, 45). Its role has been emphasized in experimental models showing that endothelial ET-B-deficient mice have an endothelial dysfunction through a reduced NO synthesis and an increased ET-1 (1). Furthermore, a decreased expression of ET-B mRNA and protein has been reported in an animal model of pulmonary hypertension (46). In our study, the specific activation of ET-B using sarafotoxin leading to a marked vasodilation is in keeping with these observations. Sarafotoxin is a compound with complex effects (28, 50) among which the capacity to increase ET-B-induced NO endothelial bioavailability. It significantly increased vasodilation to ACh in all the cases tested (37 ± 7 vs. 11 ± 10% at 10-4 M ACh, n = 10, in presence and absence of sarafotoxin, respectively; P < 0.05) including in the one ED+ subject we could evaluate who exhibited a dramatic response (−6 vs. −77% in presence and absence of sarafotoxin, respectively). Although we cannot draw any conclusion from this single observation, it certainly warrants further investigation to gain more insights into the role of ET-B in smoking-induced pulmonary vasculopathy and into the relevance of its pharmacological modulation in this condition.

In conclusion, we have shown here that the tobacco-associated impairment of pulmonary relaxation involves at least partly endothelium-dependent mechanisms. Among the latter, the ET-1 pathway is likely to be implicated through a marked overexpression of ET-A with its direct impact on vascular remodeling and tone. It might also enhance the production of free radicals and contribute to a relatively decreased vascular sensitivity to NO. Further insights are needed into these complex interactions to conceive a potential role for ET-1 receptor modulation in the management of the vascular impact of tobacco smoking.

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DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the author(s).

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