SHORT REPORT

Endothelin-1 potentiates cholinergic nerve-mediated contraction in human isolated bronchus

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ABSTRACT: That endothelin-1 (ET-1) plays a mediator role in asthma is consistent with reports of ET-1-induced potentiation of cholinergic nerve-mediated contraction in airways from various animal species. This study examined the effect of ET-1 on cholinergic contractions in human isolated bronchus.

Macroscopically nondiseased human bronchial tissue was obtained from 23 patients with respiratory tumours. An electrical field stimulation (EFS) frequency that produced one third of the contraction at 30 Hz (EFS30) was estimated. The effect of ET-1 on these EFS-evoked contractions was assessed.

EFS-evoked contractions were frequency-dependent and abolished by either atropine or tetrodotoxin. Thus, EFS-induced contractions were mediated by acetylcholine from cholinergic nerves. ET-1 (3 nM) potentiated EFS-evoked contractions by 10±2% EFS30 (p<0.05) without any significant effect on contractions induced by exogenous acetylcholine.

Neither the ET_A receptor-selective antagonist BQ-123 (3 μM) nor the ET_B receptor-selective antagonist BQ-788 (10 μM) alone significantly altered ET-1-induced potentiation of EFS-evoked contractions. However, in the combined presence of both BQ-123 and BQ-788, ET-1-induced potentiation of EFS-evoked contractions was abolished.

Thus, prejunctional endothelin_A and endothelin_B receptors appear to mediate endothelin-1-induced potentiation of electrical field stimulation-evoked cholinergic contractions in human bronchus. This suggests another potentially important mechanism through which endothelin-1 could increase bronchial tone in asthma.


There is growing evidence to suggest that the endogenous airway smooth muscle spasmogen endothelin (ET)-1 plays an important mediator role in asthma [1]. In addition, it has been suggested that, in some cases, asthma may be accompanied by an increase in airway cholinergic tone [2, 3]. A number of agents have been shown to modulate acetylcholine release from airway nerves and there is accumulating data to indicate that ET-1 modulates cholinergic neurotransmission in the airways [4]. For example, ET peptides have been shown to markedly potentiate electrical field stimulation (EFS)-induced cholinergic contractile responses in rabbit [5, 6], mouse [7, 8] and rat [9] airways. These potentiations of EFS-induced contractions were mediated via activation of prejunctional ET receptors. Furthermore, data obtained in 3H-choline-loaded rat tracheal tissue indicates that the mechanism of ET-1-induced potentiation of these responses was enhanced acetylcholine release [9]. Evidence obtained in rabbit [6], mouse [8] and rat [9] airways showed that both ET_A and ET_B receptors were involved in this phenomenon.

A preliminary report from the authors’ laboratory showed that the ET_B receptor-selective agonist sarafotoxin S6c potentiated EFS-induced cholinergic contractions in human bronchus [10]. The present study significantly extends this earlier work by focusing on the role of the endogenously released ligand ET-1 which can activate ET_A and ET_B receptors, both of which may be linked to potentiation of EFS-induced cholinergic contractile responses in human airways.

Material and Methods

Functional studies

Human bronchial tissue from macroscopically nondiseased lung samples was obtained immediately after surgery from 23 patients (six females aged 58±4 yrs; 17 males aged 60±2 yrs) undergoing lobotomies for respiratory tumours. Bronchial rings (~1–7 mm ID × 5–6 mm wide) were mounted under an initial tension of 1 g in Krebs bicarbonate solution (KBS), aerated with 95% O_2/5% CO_2 maintained at 37°C. KBS contained indomethacin (3 μM) to inhibit cyclooxygenase activity, propranolol (1 μM) to abolish nonadrenergic effects and N^M-nitro-L-arginine methyl ester (100 μM) to abolish neuronal nitric oxide-mediated relaxation. KBS also contained mepramyl (1 μM) and the leukotriene receptor antagonist SKF 104353 (1 μM) (SKF 104353 was a gift from SmithKline Beecham Pharmaceuticals, King of Prussia, PA, USA) to abolish intrinsic tone [11].
Changes in isometric tension were recorded on a Model 7D polygraph via FT03 force displacement transducers (Grass Instruments, Quincy, MA, USA). After 90 min equilibration, bronchial rings were exposed to carbachol (10 μM) to confirm tissue viability. A noncumulative frequency response curve was then constructed to EFS (100 V, 0.5 ms, 10 s train; 0.3, 1, 3, 10 and 30 Hz) delivered via parallel platinum stimulating electrodes. EFS was delivered by a Grass 544 stimulator connected to a stimulus isolation unit (SIU5; Grass Instruments). An EFS frequency that produced approximately one third of the response obtained to EFS at 30 Hz (EFS30) was estimated (0.3, 0.5 or 1 Hz) and applied at 3 min intervals, with responses assessed as % EFS30. Preparations were exposed to the ETα receptor-selective antagonist BQ-123 (3 μM; Auspep, Parkville, Australia), the ETβ receptor-selective antagonist BQ-788 (10 μM) (BQ-788 was a gift from Banyu Pharmaceutical Corporation, Tsukuba, Japan) or both BQ-123 (3 μM) and BQ-788 (10 μM) 15 min prior to the addition of ET-1 (3 nM; Auspep) and the magnitude of the responses assessed as % EFS30.

Some preparations were used to assess the influence of time on EFS-induced contraction. The effects of the muscarinic cholinoceptor antagonist atropine (0.1 μM) and the Na+ channel blocker tetrodotoxin (10 μM) were also tested against EFS-induced responses. In other experiments, two consecutive concentration-effect curves to acetylcholine (30 nM–0.3 μM at 0.5 log concentration increments) were constructed in each bronchial preparation to evaluate the effect of time on EFS or ET-1 (3 nM) on contractile responses to this cholinergic neurotransmitter.

**Data analysis**

Since there was no apparent difference between lung samples in the effect of time on EFS-induced cholinergic contractile responses, data obtained from all time control experiments were pooled and used in comparisons with treatment groups. Only one estimate for either time control or drug treatment was obtained from any one patient. Thus, data are expressed as mean±SEM of n patients. Differences between EFS treatment groups were assessed by analysis of variance (SigmaStat, Jandell Corporation, San Raphael, CA, USA) using a modified t statistic [12]. Differences between changes in negative log to the base 10 median effective concentration (−log EC50=−pD2) for acetylcholine were assessed using paired t-test (SigmaStat, Jandell Corporation, San Raphael, Parkville, Australia), the ETB receptor-selective antagonist BQ-788 (10 μM), ET-1-induced potentiation was not significantly altered in the presence of either BQ-123 or BQ-788 but was abolished in the combined presence of BQ-123 and BQ-788. *p<0.05 compared with ET-1 alone. The data are expressed at mean±SEM from 5–17 patients.

EFS-induced contractions obtained at 0.3–1 Hz were 33±3% of the response to stimulation at 30 Hz (EFS30 = 1356±100 mg; n=20). The contractile response to EFS was very stable, varying from 34±4% EFS30 to 35±4% EFS30 (n=17; p>0.05) over the time course of these experiments (fig. 1 and 2).

ET-1 (3 nM) increased baseline airway smooth muscle tone (163±36 mg; n=12) and also increased EFS-evoked contractions from 30±5% EFS30 to 40±6% EFS30 (mean increase with drug treatment of 10±2% EFS30; n=12; p<0.05) (fig. 1 and 2). BQ-123 (3 μM) and BQ-788 (10 μM) were used alone and in combination to evaluate the role of ETα and ETβ receptors in mediating this potentiation. In the presence of either BQ-123 or BQ-788 alone, ET-1 potentiated EFS-evoked contractile responses by 13±5% EFS30 (n=6; p<0.05) and 10±3% EFS30 (n=5; p<0.05) respectively, values that were not significantly different from those observed in the absence of these antagonists (fig. 1 and 2). However, in the combined presence of BQ-123 (3 μM) and BQ-788 (10 μM), potentiation was abolished (mean decrease with drug treatment of 1±2% EFS30; n=4; p<0.05 compared with ET-1 alone) (fig. 1 and 2). Taken as a whole, these data indicate that both ETα and ETβ receptors located prejunctionally, mediated ET-1-induced potentiation of EFS-evoked cholinergic contractile responses in human bronchus.

**Discussion**

The aims of the present study were to assess the potentiating influence of the endogenous ligand ET-1 on EFS-induced cholinergic nerve-mediated contractions in human bronchus and to determine whether neuronal ETα and/or ETβ receptors were involved. EFS-induced contractions were abolished by atropine and by tetrodotoxin and acetylcholine-induced contractions were unaltered in the presence of ET-1. Thus, potentiation of EFS-induced contractions in human bronchus was mediated via stimulation...
of prejunctional ET receptors on postganglionic cholinergic nerves. It is conceivable that these potentiations were associated with the increase in airway tone caused by ET-1. However, this is unlikely since it has previously been shown that ET-1 caused sustained potentiation of EFS-evoked cholinergic contractions in mouse and rat tracheal preparations at concentrations that were subthreshold for airway smooth muscle contraction [9]. Furthermore, it has previously been reported that whereas no potentiation of EFS-evoked contraction was due to in

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References