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SPECIAL REPORT

Endothelin$_B$ (ET_B) receptor-activated potentiation of cholinergic nerve-mediated contraction in human bronchus

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In human isolated bronchial preparations, the endothelin$_B$ (ET_B) receptor-selective agonist, sarafotoxin S6c (Stx6c; 1 nM) increased nerve-mediated contraction in response to electrical field stimulation (EFS) at 0.5–1 Hz from 19±4% to 42±7% (n=9). This effect was blocked in the presence of the ET_B receptor-selective antagonist, BQ-788 (10 μM). These data are consistent with findings in some animal species that ET-1 and related peptides have marked neuromodulatory influences on the cholinergic system. Furthermore, they provide additional support for the concept that ET-1 may have a mediator role in bronchial obstruction in asthma.

Keywords: Endothelin receptors; cholinergic nerves; human bronchus; sarafotoxin S6c

Introduction Endothelin-1 (ET-1) induces various responses in the respiratory tract, including airway smooth muscle contraction and proliferation and mucus hypersecretion, that are consistent with it having a mediator role in asthma (Hay *et al.*, 1993; Goldie *et al.*, 1995). Importantly, ET-1 has also been shown to have powerful modulatory effects on cholinergic nerve-mediated contraction in isolated airway smooth muscle preparations. For example, both ET-1 and the ET_B receptor-selective agonist, sarafotoxin S6c (Stx6c), caused marked potentiation of such responses in mouse trachea via activation of a prejunctional ET_B receptor (Henry & Goldie, 1995), an effect similar to that of ET-3 in rabbit bronchus (McKay *et al.*, 1993). However, in sheep tracheal preparations, stimulation of a prejunctional ET_B receptor, inhibited cholinergic nerve-mediated contraction (Henry *et al.*, 1996). Preliminary evidence suggests that ET-1 might also inhibit cholinergic nerve-mediated contraction in human isolated bronchus (Black *et al.*, 1995). Such an effect would significantly diminish the case for a mediator role for ET-1 in asthma. The present investigation was conducted to definitively evaluate the influence of Stx6c on cholinergic nerve-mediated contraction in human bronchus.

Methods Macroscopically normal bronchial tissue was obtained from 14 patients (7 females of 61±5 years of age; 7 males 67±2 years of age) undergoing lobectomies for respiratory tract tumours. Bronchial rings (approx. 1–7 mm i.d. × 5–6 mm wide) were mounted under 1 g tension in Krebs bicarbonate solution (KBS), aerated with 5% CO_2 in oxygen and maintained at 37°C. Changes in isometric tension were measured as previously described (Henry *et al.*, 1990). KBS contained 3 μM indomethacin to inhibit cyclo-oxygenase activity, 1 μM propranolol to abolish noradrenergic effects, mepyramine (1 μM) (Sigma) and the leukotriene receptor antagonist, SKF 104353 (1 μM; gift from SmithKline Beecham Pharmaceuticals, Philadelphia) to abolish intrinsic tone (Ellis & Undem 1994) and 100 μM N^{ω}-nitro-L-arginine methyl ester hydrochloride (Sigma) to abolish neuronal nitric oxide-mediated relaxation. A non-cumulative frequency-response curve was then constructed with electrical field stimulation (EFS; 100 V, 0.5 ms, 10 s train; 0.3, 1, 3, 10 and 30 Hz) delivered via parallel platinum stimulating electrodes. Submaximal responses to EFS at 0.5 or 1 Hz were then evoked at 3 min intervals and assessed as % response to EFS at 30 Hz (EFS_{30}). Some preparations served to assess the influence of time on EFS-induced contraction. Other preparations were exposed to 10 μM BQ-788 (N-*cis*-2,6-dimethylpiperidinocarbonyl-L-γMe-Leu-D-Trp(COOMe)-D-Nle-ONa; gift from the Banyu Pharmaceutical Corporation, Tsukuba, Japan) or diluant 15 min prior to the addition of Stx6c (1 nM; Auspep, Melbourne, Australia) and the magnitude of responses assessed as % control EFS_{30}. The effects of the muscarinic cholinoceptor antagonist atropine (0.1 μM; Fluka AG), the ganglion blocker, hexamethonium (10 μM) and the neuronal sodium channel blocker, tetrodotoxin (10 μM) (Sigma) were also tested against EFS-induced responses. Cumulative concentration-effect

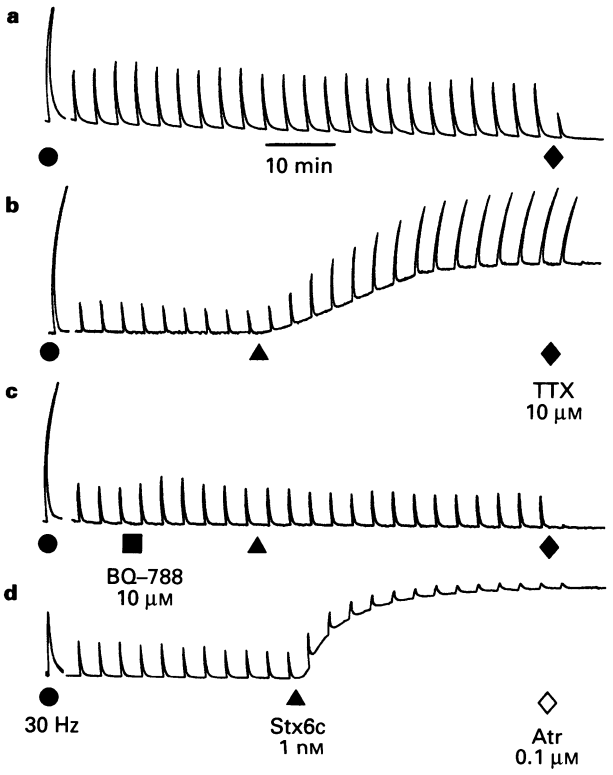

Figure 1 Representative recordings of the effects of sarafotoxin S6c (Stx6c; 1 nM) on cholinergic nerve-mediated contraction of human isolated bronchial preparations elicited by electrical field stimulation (EFS; 3 min intervals, 100 V, 0.5 ms, 10 s train, 0.5 Hz). (a) Time control; (b and d) effect of Stx6c (▲; 1 nM) alone; (c) effect of Stx6c (1 nM) in the presence of the ET_B receptor-selective antagonist, BQ-788 (■; 10 μM). EFS 30 Hz (●); TTX = tetrodotoxin, 10 μM (◆); Atr = atropine 0.1 μM (◇).

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curves to acetylcholine (Sigma) were also constructed in preparations from 2 subjects in the absence and presence of either hexamethonium (10 μM) or Stx6c (1 nM).

Results EFS caused frequency-dependent contractions that were abolished in the presence of either 0.1 μM atropine or 10 μM tetrodotoxin (Figure 1), whereas pilot studies showed that they were not altered in the presence of 10 μM hexamethonium. Furthermore, acetylcholine-induced contractions were unaltered by either hexamethonium or Stx6c (1 nM). In preparations from 9 of 12 subjects, contractions induced at 0.5 or 1 Hz were $19 \pm 4\%$ (range 6–48%) of the response to stimulation at 30 Hz ($EFS_{30} = 1175 \pm 173$ mg). In these preparations, Stx6c increased contraction height to EFS to $42 \pm 7\%$ EFS_{30} (mean increase over control $= 155 \pm 46\%$). Stx6c also increased baseline tone ($125 \pm 54\%$ EFS_{30}; $n = 9$; Figure 1b). Both the potentiation of EFS-induced contraction and the increase in baseline tone were abolished in the presence of BQ-788 (10 μM) without reducing EFS-induced contraction below control levels (Figure 1c). This relatively high but still ET_B receptor-selective concentration of BQ-788 was used to ensure abolition of Stx6c-induced potentiation of responses to EFS without nonspecific depressant effects. In bronchial ring preparations from 3 of 12 subjects, the EFS_{30} was 1587 ± 252 mg and the response at 0.5 or 1 Hz was unusually large ($53 \pm 7\%$ EFS_{30}; range = 40–65%). Although 1 nM Stx6c increased baseline tone by $113 \pm 15\%$ EFS_{30} ($n = 3$), no potentiation of responses to EFS was observed (Figure 1d).

Discussion The aim of this study was to evaluate the direct effect of the ET_B receptor-selective agonist, Stx6c, on cholinergic nerve-mediated contraction in human isolated bronchial smooth muscle. To this end, the influences of potential endogenous neuromodulators and modulators of airway smooth muscle tone, including noradrenaline, histamine, prostaglandins, leukotrienes and nitric oxide, were expressly excluded. The major finding was that Stx6c caused a BQ-788-sensitive potentiation of EFS-induced cholinergic contraction, consistent with an ET_B receptor-mediated effect. Given that contraction to exogenous acetylcholine was not affected by Stx6c, a prejunctional locus for these ET_B receptors is likely. This is in line with similar data from rabbit (McKay *et al.*, 1993), mouse (Henry & Goldie, 1995) and sheep airway smooth muscle (Henry *et al.*, 1996).

Whereas Stx6c-induced potentiation of responses to EFS was striking in most cases, no potentiation was seen in tissue from 3 subjects in which control EFS responses were unusually large. Presumably, potentiation was limited by the proximity to maximum tissue contraction, a problem exacerbated by Stx6c-induced increases in baseline airway smooth muscle tone. These latter data (Figure 1d) suggest that increases in tone *per se* do not contribute significantly to Stx6c-induced potentiation of EFS-evoked contraction.

The current evidence for cholinergic nerve ET_B receptors mediating potentiation of EFS-induced contraction in human bronchus, provides an additional mechanism through which the elevated tissue levels of ET-1 in asthma (Springall *et al.*, 1991) might promote bronchial obstruction.

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