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Influence of regional differences in ET_A and ET_B receptor subtype proportions on endothelin-1-induced contractions in porcine isolated trachea and bronchus

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1 Quantitative autoradiographic studies were conducted to determine the distributions and densities of ET_A and ET_B binding site subtypes in porcine tracheal and bronchial smooth muscle. In addition, the roles of ET_A and ET_B receptors in endothelin-1-mediated contraction of these tissues were assessed.

2 Quantitative autoradiographic studies revealed that both ET_A and ET_B binding sites for [^{125}I]–endothelin-1 were present in both bronchial and tracheal airway smooth muscle. However, the proportions of these sites were markedly different at these two levels within the respiratory tract. In tracheal smooth muscle, the proportions of ET_A and ET_B sites were $30 \pm 1\%$ and $70 \pm 1\%$ respectively, whereas in bronchial smooth muscle, these proportions were virtually reversed, being $73 \pm 2\%$ and $32 \pm 8\%$ respectively.

3 Endothelin-1 induced concentration-dependent contraction of porcine tracheal and bronchial airway smooth muscle. Endothelin-1 had similar potency (concentration producing 30% of the maximum carbachol contraction, C_{max}) in trachea (22 nM; 95% confidence limits (c.l.), 9–55 nM; $n=9$) and bronchus (22 nM; c.l., 9–55 nM; $n=6$). Endothelin-1 also produced comparable maximal contractions in trachea ($59 \pm 5\%$ C_{max}; $n=9$) and bronchus ($65 \pm 4\%$ C_{max}, $n=6$).

4 In trachea, endothelin-1 induced contractions were not significantly inhibited by either the ET_A receptor-selective antagonist, BQ-123 (3 μM) or the ET_B receptor-selective antagonist, BQ-788 (1 μM). However, in the combined presence of BQ-123 and BQ-788, the concentration-effect curve to endothelin-1 was shifted to the right by 3.7 fold ($n=8$; $P=0.01$).

5 In bronchus, concentration-effect curves to endothelin-1 were shifted to the right by BQ-123 (3 μM; 4.3 fold; $P<0.05$), but not by BQ-788 (1 μM). In the presence of both antagonists, concentration-effect curves to endothelin-1 were shifted by at least 6.7 fold ($n=6$; $P=0.01$).

6 Sarafotoxin S6c induced contraction in both tissue types, although the maximum contraction was greater in trachea ($53 \pm 7\%$ C_{max}; $n=6$) than in bronchus ($21 \pm 5\%$ C_{max}; $n=6$). BQ-788 (1 μM) markedly reduced sarafotoxin S6c potency in both trachea and bronchus (e.g. by 50 fold in trachea; c.l., 14–180; $n=6$; $P<0.05$).

7 These data demonstrate that the proportions of functional endothelin receptor subtypes mediating contraction of airway smooth muscle to endothelin-1, vary significantly at different levels in the porcine respiratory tract.

Keywords: Porcine bronchus and trachea; endothelin-1; ET_A and ET_B receptors; airway smooth muscle

Introduction

Endothelin-1 is a potent and efficacious spasmogen of airway smooth muscle (Uchida *et al.*, 1988; Henry *et al.*, 1990b; Hay *et al.*, 1993a). In airway smooth muscle of some animal species, including human, endothelin-1-induced contractions are mediated via activation of the ET_B receptor subtype (Hay, 1992; Hay *et al.*, 1993b; Battistini *et al.*, 1994). These conclusions have been drawn from the finding that the ET_B receptor-selective agonists, sarafotoxin S6c and IRL 1620, induced marked contractile responses in these tissues and that endothelin-1-induced contractions were not inhibited significantly by the ET_A receptor-selective antagonist, BQ-123. However, recent studies have clearly demonstrated that endothelin-1-induced contractions are not mediated solely by ET_B receptors in all species. Most notably, in sheep tracheal smooth muscle, sarafotoxin S6c was inactive as a spasmogen and endothelin-1-induced contractions were inhibited by BQ-123, indicative of an ET_A receptor-mediated contractile response (Goldie *et al.*, 1994).

In other species, endothelin-1-induced contractions of airway smooth muscle were mediated by both ET_A and ET_B receptors. For example, in rabbit, guinea-pig, rat and murine tracheal smooth muscle, endothelin-1-induced contractions were at most, only partially attenuated either by blocking the ET_A receptor-effector system with BQ-123 or by blocking the ET_B receptor-effector system by desensitization with sarafotoxin S6c or IRL 1620 (Henry, 1993; Henry & Goldie, 1994; Yoneyama *et al.*, 1995). However, endothelin-1-induced contractions were markedly attenuated by blocking both receptor-effector systems simultaneously, demonstrating that both ET_A and ET_B receptors subserved contraction in these species (Henry, 1993; Henry & Goldie, 1994; Yoneyama *et al.*, 1995).

The conclusions from functional studies that endothelin-1-induced contractions in human, sheep and rat airways were mediated by ET_B, ET_A and by both ET_A and ET_B receptors respectively, is entirely consistent with quantitative autoradiographic assessments of the relative proportions of ET_A and ET_B receptors in these tissues. These studies using [^{125}I]–endothelin-1 in the presence and absence of receptor-selective ligands, sarafotoxin S6c and BQ-123, revealed that the ratios of ET_A:ET_B receptors in airway smooth muscle were 12:88 in human bronchus, 100:0 in sheep trachea and 50:50 in rat trachea (Henry, 1993; Goldie *et al.*, 1994; 1995).

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In addition to these marked interspecies differences, there is evidence suggesting regional differences in the relative proportions of ET_A and ET_B receptors within airway smooth muscle. Much of the evidence has come from functional studies of guinea-pig trachea and bronchus. The strongest evidence of regional variations was provided from the studies of Hay and coworkers, who reported that in descending from the upper trachea to the primary bronchus, there was an increase in the spasmogenic effectiveness of sarafotoxin S6c. Moreover, whereas BQ-123 potently antagonized endothelin-1-induced contraction in all regions (upper, central and lower) of trachea, this agent was without significant effect in bronchus (Hay *et al.*, 1993b). This is consistent with the presence of mixed populations of ET_A and ET_B receptors in guinea-pig tracheal smooth muscle cells (Inui *et al.*, 1994) and with evidence that ET_B receptors mediated contractions to endothelin-1 in guinea-pig bronchus (Hay *et al.*, 1993b; Kizawa *et al.*, 1994). Thus, there is a relatively greater involvement of ET_A receptors in endothelin-1-induced contraction of tracheal than of bronchial airway smooth muscle.

In sharp contrast, Battistini and coworkers (1994) reported that contractile responses to endothelin-1 in guinea-pig trachea and bronchus were mediated almost exclusively by ET_B receptors, whereas Maggi *et al.* (1990) reported functional data suggesting a mixed population of endothelin receptors in guinea-pig bronchus. Significantly, in none of these studies in intact airway smooth muscle, was the functional characterization of ET_A and ET_B receptors in the various regions of the airways linked to direct measurements of the relative proportions of the endothelin receptor subtypes. The present study has used a combination of autoradiographic and functional techniques to evaluate systematically the extent of regional variations in ET_A and ET_B receptors in porcine tracheal and fourth generation bronchial airway smooth muscle.

Methods

Tissue preparation

Lung and tracheal tissue was obtained from male pigs 20–25 weeks of age freshly slaughtered at a local abattoir or from similar animals anaesthetized with sodium thiopentone (25 mg kg^{-1}, i.v.). The lungs and trachea were removed and transferred to ice-cold Krebs bicarbonate solution, the composition of which was (mM): NaCl 117, KCl 5.36, $NaHCO_3$ 25.0, KH_2PO_4 1.03, $MgSO_4$. $7H_2O$ 0.57, $CaCl_2$ 2.5 and glucose 11.1.

Autoradiographic studies

Airway tissue was prepared for autoradiographic studies as described previously (Goldie *et al.*, 1994). Briefly, tracheal airway smooth muscle was removed with the epithelium and some cartilage still attached. These preparations were then submerged in Macrodex (6% dextran 70 in 5% glucose) and frozen by immersion in isopentane, quenched with liquid nitrogen. In addition, tracheal smooth muscle from 6 pigs was dissected free of adventitial tissue and cartilage and a piece (approx. 10 mm × 10 mm) from each animal stacked in layers in Macrodex and frozen as described above. Tube segments of fourth generation pig bronchus (1.5–2 mm i.d., 10 mm in length) were also placed in aluminium foil cups and emersed in Macrodex and snap frozen. Serial frozen sections (10 μm thick) of bronchial and tracheal tissue were cut at −30°C in a cryostat and thaw-mounted onto gelatin chrom-alum-coated glass slides.

Time course of [^{125}I]-endothelin-1 binding in porcine tracheal smooth muscle

These studies were conducted essentially as previously described (Goldie *et al.*, 1994). Since [^{125}I]–endothelin-1 binds pseudo-irreversibly to its specific sites (Marsault *et al.*, 1991; Waggoner *et al.*, 1992), an equilibrium binding isotherm cannot be used to derive accurately the maximum specific binding capacity (B_{max}). However, B_{max} and the time point at which B_{max} is attained can be derived from data describing the time course of [^{125}I]-endothelin-1 binding. Maximal binding to both ET_A and ET_B binding sites can then be derived by assessing specific [^{125}I]-endothelin-1 binding in the presence and absence of receptor subtype-selective ligands at a single time point at which binding plateau has been established to occur. An estimate of endothelin binding site subtype proportions can then be derived from these data.

In these experiments, slide-mounted frozen sections of stacks of six tracheal smooth muscle segments were incubated for 2 × 5 min at 22°C in 170 mM Tris-HCl buffer (pH 7.6) containing 0.25% (w/v) bovine serum albumin and the protease inhibitor phenylmethylsulphonyl fluoride (10 μM). Sections were then incubated in buffer containing 0.3 nM [^{125}I]-endothelin-1 for 10–240 min in the absence (total binding) or presence of 1 μM BQ-123 (ET_A receptor-selective ligand; Ihara *et al.*, 1992), or 100 nM sarafotoxin S6c (ET_B receptor-selective ligand; Williams *et al.*, 1991), or in the combined presence of 1 μM BQ-123 and 100 nM sarafotoxin S6c (to assess non-specific binding). In some experiments, non-specific binding was determined in the presence of non-radiolabelled endothelin-1 (100 nM). Autoradiographic grain densities over airway smooth muscle were determined with an automated grain detection and counting system (Henry *et al.*, 1990a). Six separate fields (5 over tissue and 1 over a non-tissue area) were viewed for each tracheal smooth muscle specimen and triplicate slides were analysed. Thus, a total of 2592 fields were analysed [(6 fields per tissue section) × (6 tissue sections per slide) × (3 slides per treatment) × (4 treatments per time point) × (6 time points)]. Autoradiographic grain densities were expressed as grains per 1000 μm^2.

Assessment of ET_A and ET_B binding site proportions in porcine bronchial smooth muscle

Autoradiograms were produced as described above using tissue incubated with 0.3 nM [^{125}I]-endothelin-1 for 180 min in the absence (total binding) or presence of 1 μM BQ-123 or 100 nM sarafotoxin S6c to determine the extent of ET_A and ET_B site binding respectively. Non-specific binding was assessed in the combined presence of these ligands. Autoradiographic grain densities were measured in a total of 300 fields [(5 fields per tissue section) × (5 tissue sections) × (3 slides per treatment) × (4 treatments per time point) × (1 time point)].

Airway smooth muscle contraction studies

Trachea Porcine tracheal smooth muscle strips were prepared as previously described for ovine trachea (Goldie *et al.*, 1994). Briefly, the tracheal tube was cut transversely at intervals of approximately 3 mm to provide a series of ring segments. The exterior connective tissue and other deep submucosal elements were also dissected away and the muscle band trimmed to leave a thin filament. Two ligatures approximately 5 mm apart were attached as anchorage sites in the tissue bath.

Bronchus Bronchial tubes were cut into ring preparations approximately 2 mm in width and epithelium denuded by gentle rubbing with a moist cotton swab. All airway preparations were suspended under a resting tension of 500 mg and placed in 2 ml of Krebs bicarbonate solution at 37°C, bubbled continuously with 5% CO_2 in O_2. Changes in isometric tension were measured via an FTO3C force-displacement transducer (Grass Instruments) coupled to a preamplifier and analysed and displayed using an IBM compatible personal computer with software specifically designed for the assessment of tissue bath contraction data.

Airway preparations were allowed to equilibrate for 45 min before exposure to the cumulative addition of 0.3 μM and

10 μM carbachol. Upon reaching a contraction plateau the preparations were washed in drug-free Krebs bicarbonate solution for 30 min. Concentration-effect curves were constructed to endothelin-1 or sarafotoxin S6c in the presence or absence of the ET_A receptor-selective antagonist, BQ-123 (3 μM), ET_B receptor-selective antagonist, BQ-788 (1 μM), both BQ-123 and BQ-788 or the cyclo-oxygenase inhibitor indomethacin (5 μM). In these experiments, preparations were exposed for 20 min to one of these agents or its solvent (paired control preparation) and then to cumulative additions (0.5 log-concentration increments) of endothelin-1 (1 nM–300 nM) or sarafotoxin S6c (1–300 nM). Endothelin-1- and sarafotoxin S6c-induced contractions were assessed as a percentage of the maximal response to carbachol (C_{max}). Maximal responses to the peptides were only 50–60% C_{max}. Accordingly, PC_{30} values were taken as measures of peptide potency, where $PC_{30} = -\log EC_{30}$ and EC_{30} = the concentration of agonist producing 30% C_{max}.

Drugs

Drugs used were; [^{125}I]-endothelin-1 (2000 Ci mmol^{-1}), endothelin-1, sarafotoxin S6c (Auspep, Melbourne, Australia), phenylmethylsulphonyl fluoride (Calbiochem, La Jolla, U.S.A.), BQ-123 (cyclo[D-Trp-D-Asp-L-Pro-D-Val-L-Leu]; gift from Dr D.W.P. Hay of SmithKline Beecham Pharmaceuticals, U.S.A.), BQ-788 (N-cis-2, 6,-dimethylpiperidinocarbonyl-L-γMeLeu-D-Trp (COOMe)-D-Nle-ONa; gift from Banyu Pharmaceutical Corporation, Tsukuba, Japan); carbamylcholine chloride, indomethacin (Sigma Chemical Company, St. Louis, U.S.A.). Stock solutions of endothelin-1 (50 μM) and sarafotoxin S6c (50 μM) were prepared in 0.1 M acetic acid and dilutions made in 0.9% NaCl solution (saline). BQ-123 and indomethacin were initially prepared in 100 nM Na_2CO_3 and BQ-788 was dissolved in dimethylsulphoxide (DMSO). These agents were then diluted in saline as required.

Statistical analysis

Differences between treatment means were assessed by analysis of variance followed by a modified *t* statistic (Wallenstein *et al.*, 1980) or by Student's unpaired *t* test as appropriate. *P* values less than 0.05 were considered to be statistically significant.

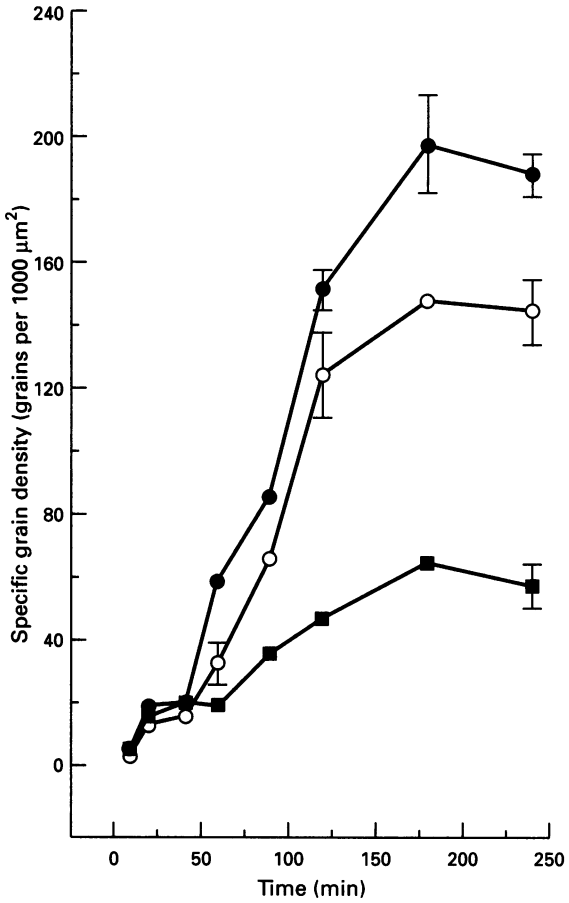

Figure 1 (a) Time-dependence of [^{125}I]-endothelin-1 (0.30 nM) binding in 10 μm transverse frozen sections of slide-mounted porcine tracheal smooth muscle. Total specific [^{125}I]-endothelin-1 binding is shown (●) as is specific binding assessed in the presence of 1 μM BQ-123 (○) (i.e. residual binding to ET_B sites) or 100 nM sarafotoxin S6c (■) (i.e. residual binding to ET_A sites). Non-specific binding (data not shown) was assessed in the combined presence of 1 μM BQ-123 and 100 nM sarafotoxin S6c. Data are presented as mean ± s.e.mean of 6 mean estimates.

Results

Autoradiographic studies

Time course of [^{125}I]-endothelin-1 binding in porcine tracheal smooth muscle Specific binding of [^{125}I]-endothelin-1 (0.3 nM) increased in a time-dependent manner to a plateau at between 120 and 240 min (Figure 1). Furthermore, specific binding clearly consisted of two components, since binding plateaus were also observed in this period in the absence and presence of either BQ-123 or sarafotoxin S6c. At 180 min, the sum of the apparent specific binding maxima for ET_B (147.4 ± 2.0 grains per 1000 μm^2) and for ET_A sites (64.5 ± 1.0 grains per 1000 μM^2), closely approximated B_{max} in the absence of endothelin receptor subtype-selective ligands (197 ± 15.4 grains per 1000 μm^2; Figure 1). These data indicate that ET_A and ET_B binding sites coexisted in porcine tracheal smooth muscle approximately in the proportion 30% and 70% respectively. Thereafter, it was assumed that B_{max} for specific [^{125}I]-endothelin-1 (0.3 nM) binding to both ET_A and ET_B binding sites occurred in all tissue at the 180 min incubation time point. Accordingly, in all subsequent autoradiographic experiments, [^{125}I]-endothelin-1 binding was assessed in the absence or presence of endothelin receptor subtype-selective ligands at this time point. Figure 2 verifies that ET_B binding sites predominated in porcine tracheal smooth muscle. Sarafotoxin S6c-sensitive ET_B sites were also detected in the airway epithelium and in submucosal gland tissue (Figure 2d), whereas BQ-123-sensitive ET_A sites were observed in association with small airway blood vessels (Figure 2c).

ET_A and ET_B binding sites in porcine bronchial smooth muscle BQ-123 (1 μM) reduced specific [^{125}I]-endothelin-1 binding at 180 min by 73 ± 2%, whereas specific binding was reduced in the presence of 100 nM sarafotoxin S6c by only 32 ± 8%. Thus, in sharp contrast with porcine tracheal smooth muscle where ET_B sites clearly predominated, the great majority of specific sites in fourth generation bronchial smooth muscle were of the ET_A subtype. Figure 3 verifies that ET_A binding sites constituted the majority of specific sites in this tissue. Mixtures of ET_A and ET_B binding sites were also present in or near the epithelium and submucosal glands, although BQ-123-sensitive ET_A sites (Figure 3c) were again in the majority in these areas.

Contraction studies

These experiments were conducted in an attempt to determine whether the ET_A and ET_B binding sites detected in autoradiographic studies were likely to be functional endothelin receptors. Endothelin-1 (Figure 4a) and the ET_B receptor-selective agonist sarafotoxin S6c (Figure 4b) caused concentration-dependent contraction of porcine tracheal and bronchial airway smooth muscle.

Endothelin-1 had similar potency (concentration producing 30% of the maximum carbachol contraction, C_{max}) in trachea (22 nM; 95% confidence limits (c.l.), 9–55 nM; $n = 9$) and bronchus (22 nM; c.l., 9–55 nM; $n = 6$). Endothelin-1 also produced comparable maximal contractions in trachea

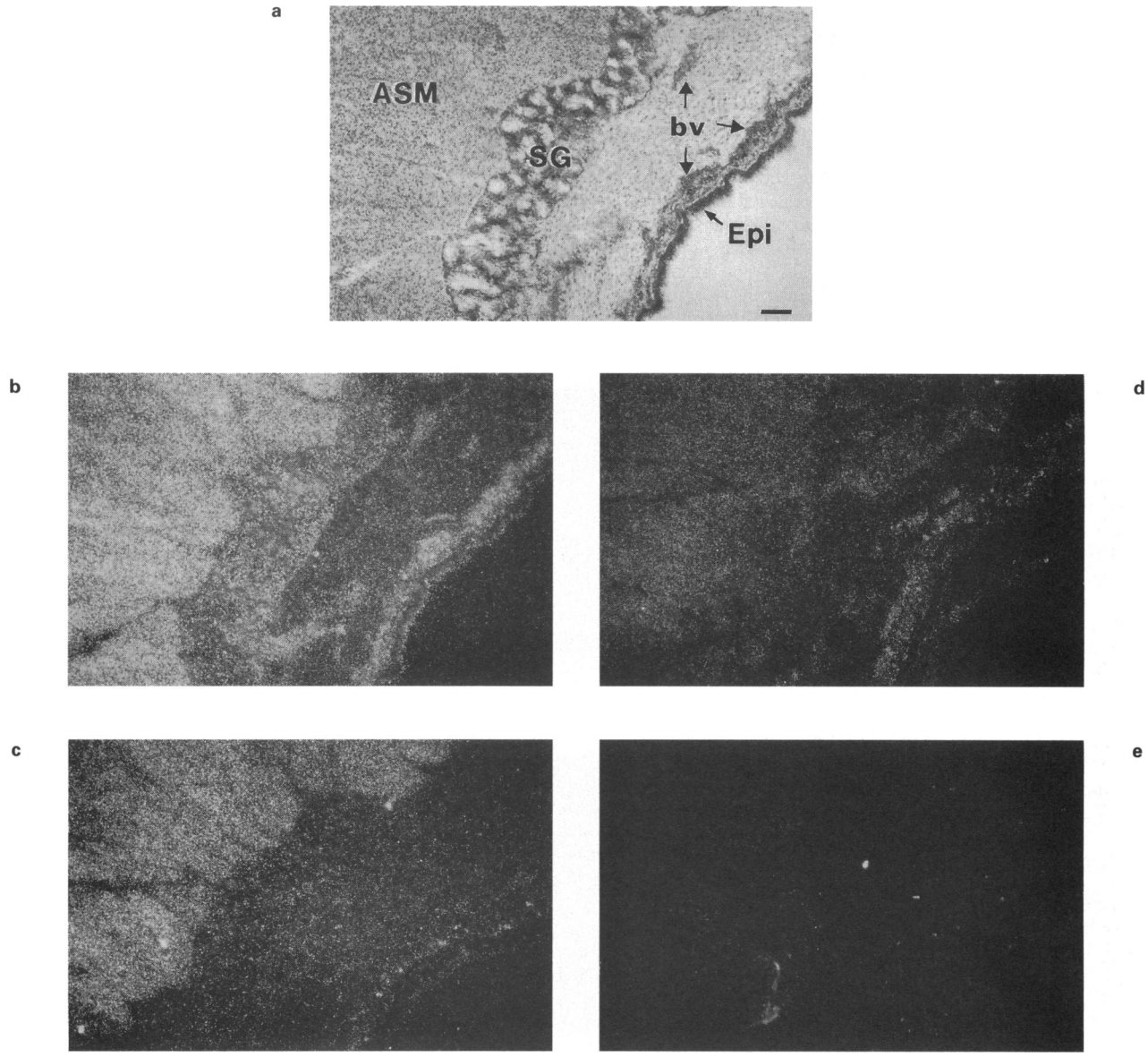

Figure 2 (a) Bright-field photomicrograph of a 10 μm transverse frozen section of porcine tracheal tissue. ASM = airway smooth muscle; Epi = epithelium; SG = submucosal bland; bv = blood vessels. (b-e) Dark-field photomicrographs showing the distribution of autoradiographic grains derived from [^{125}I]-endothelin-1 ([^{125}I]-endothelin-1, 0.30 nM, 180 min). (b) total [^{125}I]-endothelin-1 binding in the section shown in the light-field photomicrograph. (c-e) Serial sections showing [^{125}I]-endothelin-1 binding in the presence of (c) the ET_A receptor-selective ligand, BQ-123 (1 μM), (d) the ET_B receptor-selective ligand, sarafotoxin S6c (100 nM) and (e) in the combined presence of 1 μM BQ-123 and 100 nM sarafotoxin S6c (i.e. nonspecific binding). Bar = 100 μm.

(59 $\pm$ 5% C_{max}; $n=9$) and bronchus (65 $\pm$ 4% C_{max}, $n=6$; Figure 4a). However, the maximum contraction induced by sarafotoxin S6c was greater in trachea (53 $\pm$ 7% C_{max}; $n=6$) than in bronchus (21 $\pm$ 5% C_{max}; $n=6$; Figure 4b). Furthermore, BQ-123 (3 μM) shifted the endothelin-1 concentration-effect curve to the right in bronchus (4.3 fold; $P<0.05$; Figure 5b), but not trachea (Figure 5a). The ET_B receptor-selective antagonist, BQ-788 (1 μM) reduced sarafotoxin S6c potency (e.g. by 50 fold in trachea; c.l., 14–180; $n=6$; $P<0.05$; Figure 6) but did not affect the potency of endothelin-1 in either tissue (Figure 5). In the combined presence of BQ-123 (3 μM) and BQ-788 (1 μM), the potency of endothelin-1 was reduced by 3.7 fold in trachea ($n=8$, $P=0.01$, Figure 5a) and by at least 6.7 fold in bronchus ($n=6$, $P=0.01$, Figure 5b). Indomethacin (5 μM) had no detectable influence on contraction to either peptide (data not shown).

Discussion

In this investigation of porcine airways, quantitative autoradiographic studies using [^{125}I]-endothelin-1 and receptor selective ligands revealed clear differences in the relative proportions of ET_A and ET_B receptor subtypes present in tracheal and bronchial airway smooth muscle, with ET_B receptors predominating in the trachea and ET_A receptors in greater numbers in fourth generation bronchus. Importantly, these regional differences in the relative densities of ET_A and ET_B receptors were found to correlate closely with changes in the apparent contributions of the ET_A and ET_B receptor systems to airway smooth muscle contraction at these sites.

In porcine tracheal smooth muscle, the relative proportions of ET_A:ET_B receptors were approximately 30:70. Functional studies using endothelin-1 and receptor selective agonists and

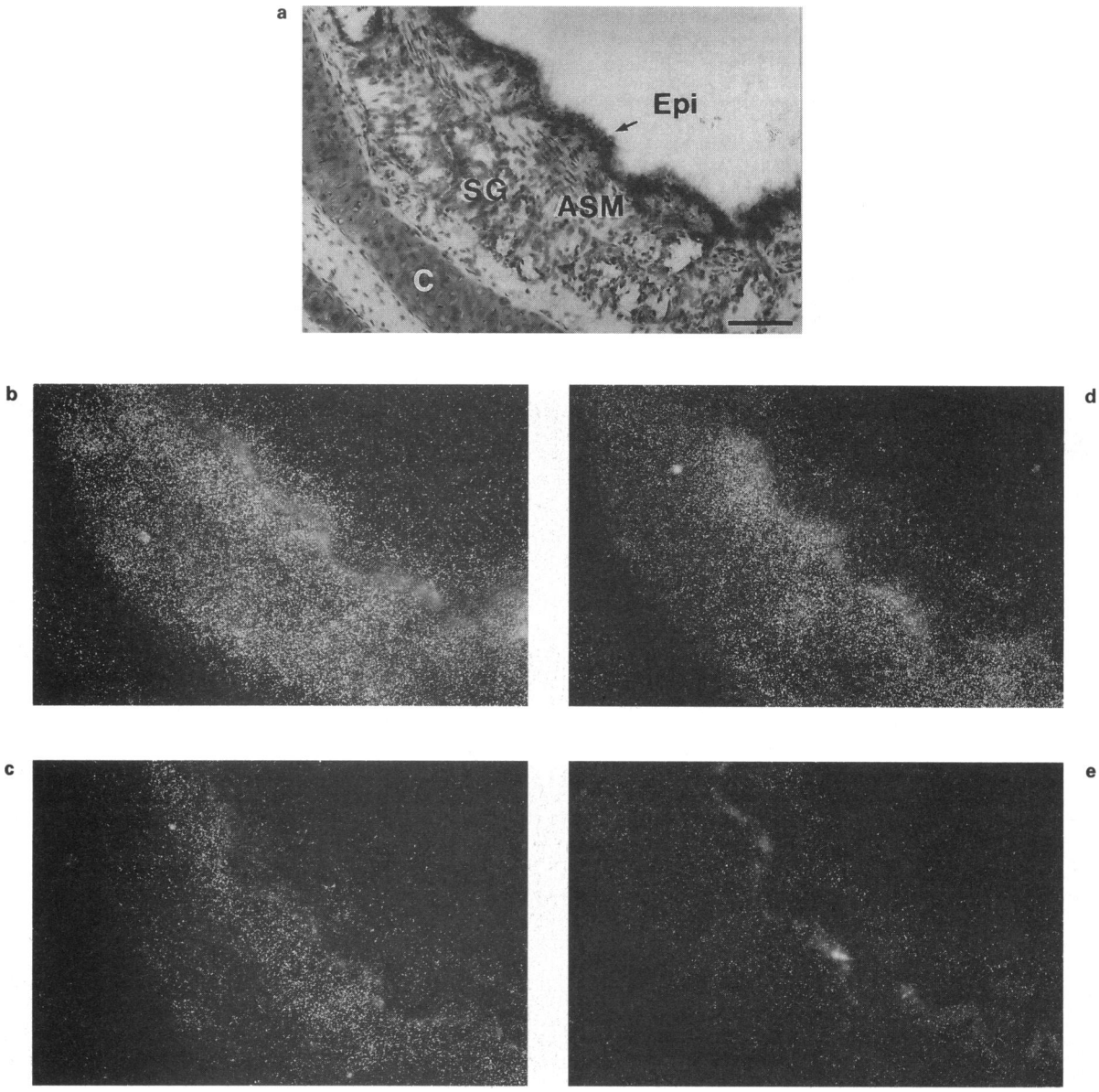

Figure 3 (a) Bright-field photomicrograph of a 10 μm transverse frozen section of porcine bronchial tissue. C = cartilage; ASM = airway smooth muscle; Epi = epithelium; SG = submucosal gland. (b-e) Dark-field photomicrographs showing the distribution of autoradiographic grains derived from [^{125}I]-endothelin-1 ([^{125}I]-endothelin-1, 0.30 nM, 180 min). (b) Total [^{125}I]-endothelin-1 binding in the section shown in the light-field photomicrograph. (c-e) Serial sections showing [^{125}I]-endothelin-1 binding in the presence of (c) the ET_A receptor-selective ligand, BQ-123 (1 μM), (d) the ET_B receptor-selective ligand, sarafotoxin S6c (100 nM) and (e) in the combined presence of 1 μM BQ-123 and 100 nM sarafotoxin S6c (i.e. nonspecific binding). Bar = 100 μm.

antagonists for ET_A and ET_B receptors indicated that both receptor subtypes mediated smooth muscle contraction. Evidence supporting ET_B receptor-mediated contraction was obtained from the findings that the ET_B receptor-selective agonist, sarafotoxin S6c, was a potent and efficacious spasmogen and that endothelin-1-induced contractions were not inhibited by the ET_A receptor-selective antagonist, BQ-123. ET_A receptors also appeared to be linked to contraction, since the ET_B receptor-selective antagonist BQ-788 did not inhibit endothelin-1-induced contractions. Although the extent of the rightward shift in the concentration-effect curve to endothelin-1 in porcine tracheal smooth muscle was modest in the combined presence of BQ-123 and BQ-788, these data support the concept that both ET_A and ET_B receptors subserved contraction in this tissue and are consistent with similar findings in rabbit, guinea-pig, rat and murine tracheal smooth muscle (Henry, 1993; Hay *et al.*, 1993a, b; Henry & Goldie, 1994; Yoneyama *et al.*, 1995). However, whether ET_A and ET_B receptors mediated contraction of porcine tracheal smooth muscle via activation of different signal transduction systems, as has been proposed for rat airways (Henry, 1993), remains to be established.

In porcine fourth generation bronchial smooth muscle, the relative proportion of ET_A:ET_B receptors was approximately 70:30, similar to values obtained by Hislop *et al.* (1995) and a virtual reversal of the ratio observed in trachea. Both receptor subtypes were linked to bronchial smooth muscle contraction, although ET_A receptors appeared to make a greater contribution than ET_B receptors to endothelin-1-induced contraction. Evidence for this was provided by the findings that endothelin-1-induced contractions were inhibited by BQ-123, but not by BQ-788. Consistent with the suggestion of a relatively minor ET_B receptor-effector system in bronchial smooth muscle, sarafotoxin S6c was a much weaker spasmogen than endothelin-1 in this tissue. Together, these functional data indicate a greater influence of ET_A receptors on endothelin-1-induced contraction in porcine bronchus than in trachea and are in accord with autoradiogrphic data which demonstrated

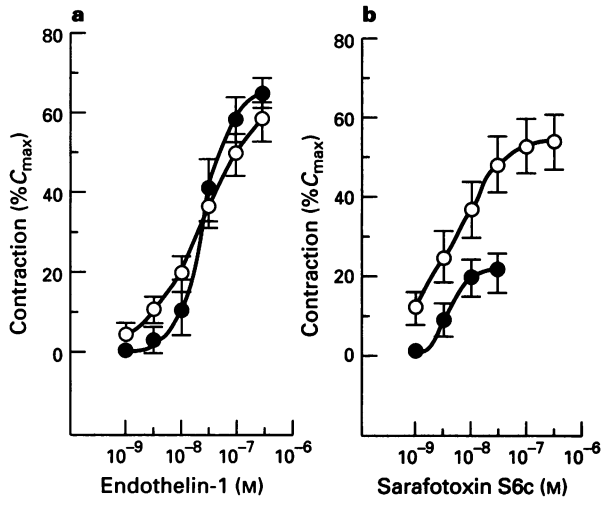

Figure 4 Cumulative concentration-effect curves to (a) endothelin-1 and (b) the ET_B receptor-selective agonist, sarafotoxin S6c, for contraction in porcine tracheal smooth muscle (○) and porcine bronchial smooth muscle (●). Four preparations were derived from each of 6 (*n*) animals. Data are presented as mean ± s.e.mean of *n* mean estimates.

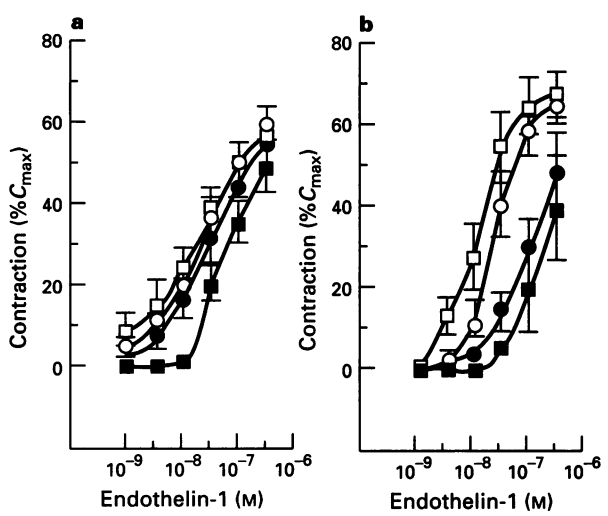

Figure 5 Cumulative concentration-effect curves for porcine (a) tracheal and (b) bronchial smooth muscle contraction to endothelin-1 in the absence (○) or presence of 3 μM BQ-123 (●) or 1 μM BQ-788 (□) or both (■). Four preparations were derived from each of 6 (*n*) animals. Data are presented as mean ± s.e.mean of *n* mean estimates.

that this tissue contained a greater density of ET_A than ET_B receptors. The present data in porcine bronchus are also consistent with a previous report of the predominance of ET_A sites in this tissue (Nakamichi *et al.*, 1992) and are similar to results in sheep bronchial smooth muscle where only ET_A receptors mediated airway smooth muscle contraction (Goldie *et al.*, 1994). However, they contrast sharply with those in human and guinea-pig bronchial smooth muscle where the predominant receptor mediating contraction to endothelin-1 was the ET_B subtype (Hay *et al.*, 1993b; Goldie *et al.*, 1995). Moreover, these data demonstrate that the regional differences in endothelin receptor subtype density between tracheal and bronchial smooth muscle have functional consequences. For example, whereas BQ-123 inhibited endothelin-1-induced contraction in bronchial smooth muscle, it was ineffective against endothelin-1-induced contraction in tracheal smooth muscle.

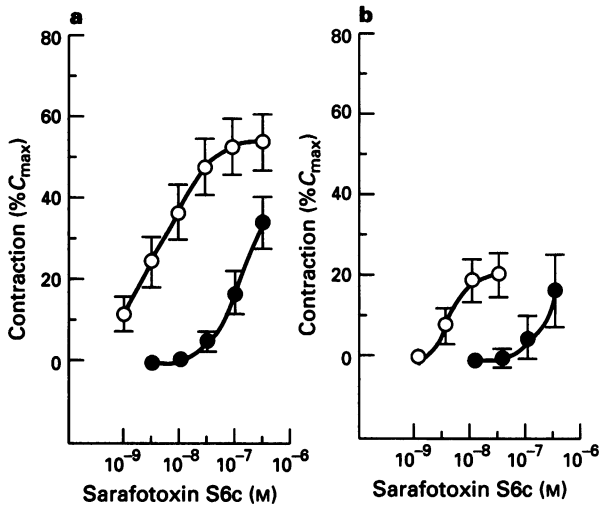

Figure 6 Cumulative concentration-effect curves for porcine (a) tracheal and (b) bronchial smooth muscle contraction to sarafotoxin S6c in the absence (○) or presence of 1 μM BQ-788 (●). Data are presented as mean ± s.e.mean of 5 estimates from different animals.

It is apparent from the present study that an established trend in endothelin receptor subtype proportions down the respiratory tract is not an indicator of the likely subtype predominance in alveoli. For example, while the trend was towards increasing numbers of ET_A receptors between tracheal and fourth generation porcine bronchial smooth muscle, this was reversed at the most peripheral sites such that ET_B sites predominated, accounting for 65% of specific [^{125}I]-endothelin-1 binding in alveolar wall tissue in this species (Goldie *et al.*, 1996).

Autoradiographic studies in several species have shown that both ET_A and ET_B receptors co-exist in airway smooth muscle (Henry, 1993; Henry & Goldie, 1994; current study). Complementary isometric tension recording studies confirmed that each subtype can independently mediate endothelin-1-induced contractions. Moreover, selective blockade of only one receptor subtype does not necessarily result in marked inhibition of endothelin-1-induced contraction. Thus, conclusions regarding the predominant endothelin receptor subtype in airway smooth muscle based solely upon the effects of subtype-selective endothelin receptor antagonists in contraction studies, must be interpreted with caution in the absence of the complementary radioligand binding or autoradiographic data.

In the current study of pig airways, marked regional differences in the relative proportions of ET_A and ET_B receptors and their contributions to endothelin-1-induced contraction were observed between tracheal and bronchial smooth muscle. In descending from the tracheal to fourth generation bronchi, the relative contribution of ET_B receptor-effector system to contraction was diminished; sarafotoxin S6c was less efficacious and endothelin-1-induced contractions were inhibited by BQ-123. This profile in pig airways of reduced ET_B receptor-effector function in the more peripheral airway smooth muscle contrasts sharply with the increased involvement of the ET_B receptor-effector system observed in moving down the respiratory tract in guinea-pig (Hay *et al.*, 1993b). Whether regional differences in the relative proportion of ET_A and ET_B receptors exist in human airways is not known.

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