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Time course of changes in ET_B receptor density and function in tracheal airway smooth muscle during respiratory tract viral infection in mice

Michael J. Carr, Roy G. Goldie & [1]Peter J. Henry

Department of Pharmacology, University of Western Australia, Nedlands, Western Australia, 6907, Australia

1 In the current study, the density and function of ET_A and ET_B receptors in mouse tracheal airway smooth muscle were determined over the time course of respiratory tract infection with influenza A/PR-8/34 virus.

2 Quantitative autoradiographic studies using [^{125}I]-endothelin-1 revealed that the tracheal airway smooth muscle from control mice contained ET_A and ET_B sites in the ratio of 49%:51% (±2%, $n=29$ mice). Respiratory tract viral infection was associated with increases in the density of ET_A sites and decreases in the density of ET_B sites at days 1, 2 and 4 post-inoculation which were reversible by day 19. For example, at day 4 post-inoculation, a time when the manifestations of viral infection were at or near their peak, the ratio of ET_A:ET_B sites was 72%:28% (±4%, $n=6$ mice, $P<0.05$). In contrast, at day 19 post-inoculation, by which time viral infection had essentially resolved, the ratio of ET_A:ET_B sites was similar to control (51%:49% (±3%), $n=6$ mice).

3 Endothelin-1 was a potent spasmogen in isolated tracheal airway smooth muscle preparations from control mice (ED_{70} = concentration producing 70% of contraction induced by 10 μM carbachol = 6.3 nM (95% confidence limits, 4.0–10; n = 6 mice)). Neither the ET_A receptor-selective antagonist, BQ-123 (3 μM), nor the ET_B receptor-selective antagonist, BQ-788 (1 μM) alone had any significant inhibitory effect on endothelin-1-induced contractions of mouse isolated tracheal smooth muscle. However, simultaneous treatment with BQ-123 (3 μM) and BQ-788 (1 μM) resulted in a 10 fold rightward shift in the concentration-effect curve to endothelin-1 (ED_{70} = 60 nM, (44–90; n = 6 mice, $P<0.05$)), indicating that contraction was mediated via both ET_A and ET_B receptors.

4 Endothelin-1 evoked similar concentration-dependent contractions of tracheal smooth muscle isolated from control and virus-inoculated mice. In the presence of the ET_B receptor-selective-antagonist, BQ-788 (1 μM), the potency and maximum response to endothelin-1 were similar in preparations from control and virus-inoculated mice at all time points investigated. However, unlike control responses, endothelin-1-induced contractions in preparations from virus-infected mice were significantly inhibited by the ET_A receptor-selective antagonist, BQ-123. For example, at day 4 post-inoculation, the contractile response to 30 nM endothelin-1, in the presence of BQ-123 (3 μM), was only 20±12% (n = 6 mice, $P<0.05$) of that produced in control preparations under similar conditions. However, at day 19 post-inoculation, contraction evoked by 30 nM endothelin-1 in the presence of BQ-123 (3 μM), was similar to that in preparations from control mice.

5 In summary, during the early stages (days 1–8 post-inoculation) of respiratory tract infection with influenza A/PR-8/34 virus, we observed decreases in the density of tracheal airway smooth muscle ET_B receptors which were reflected in decreases in ET_B receptor-mediated airway smooth muscle contraction. In addition, during the same period of viral infection we observed increases in the density of tracheal airway smooth muscle ET_A receptors which were not associated with increased function of the ET_A receptor-effector system linked to contraction. Virus-associated modulation of ET_A and ET_B receptor density and function was reversible with recovery from infection.

Keywords: Respiratory tract virus; influenza A/PR-8/34 virus; endothelin-1; ET_A receptors; ET_B receptors; airway smooth muscle; sarafotoxin S6c; BQ-123; BQ-788

Introduction

Respiratory tract viral infections exacerbate pre-existing asthma (Beasley *et al.*, 1988; Lemanske *et al.*, 1989) and can induce asthma-like symptoms in otherwise healthy individuals (Empey *et al.*, 1976; Little *et al.*, 1978; Aquilina *et al.*, 1980; Laitinen *et al.*, 1991). The underlying mechanisms of these effects are unknown although virus-induced damage to the respiratory epithelium (for review see Hegele *et al.*, 1995) and the resulting inflammation of the airways (for review, see Folkerts & Nijkamp, 1995) seem to be important contributing factors.

Epithelial cells lining the airways produce and release the 21 amino acid peptide endothelin-1 (MacCumber *et al.*, 1989; Black *et al.*, 1989; Rozengurt *et al.*, 1990; Hay *et al.*, 1993a; Noguchi *et al.*, 1995). Endothelin-1 is known to induce various effects within the airway wall, some of which are mediated by multiple endothelin receptor subtypes. For example, endothelin-1-induced contraction of airway smooth muscle can be mediated by both ET_A and ET_B receptors (Hay *et al.*, 1993b; Henry, 1993; Inui *et al.*, 1994). Recent investigations in our laboratory demonstrated that respiratory tract viral infection was associated with a significant reduction in the density of tracheal airway smooth muscle ET_B sites which was reflected in a marked attenuation of contractile function of the ET_B receptor-effector system (Henry & Goldie, 1994). Clearly, re-

[1] Author for correspondence.

spiratory tract viral infection can significantly influence airway function by modulating endothelin receptor-effector systems within the airway wall.

In a previous investigation (Henry & Goldie, 1994) we examined the influence of respiratory tract viral infection on tracheal airway smooth muscle ET_A and ET_B receptors at a single time point (day 2 post-inoculation) in the early stages of infection. The aim of the current study was to characterize the time-dependence of influenza A/PR-8/34 virus-induced modulation of tracheal airway smooth muscle ET_A and ET_B receptor-effector systems for a period spanning initial inoculation to recovery from infection.

Methods

Respiratory tract virus stock

Influenza A/PR-8/34 virus was grown in the allantoic fluid of 10-day-old embryonated chicken eggs at 37°C for 3 days as described previously (Williams & MacKenzie, 1977). The allantoic fluid was harvested and contained 2.7×10^6 egg infectious doses (EID_{50}) of virus ml^{-1} as determined by the method of allantois-on-shell titration for infectivity (Fazekas de St. Groth & White, 1958). The virus stock was stored in 0.5 ml aliquots at −70°C.

Tissue preparation

Eight week old male CBA/CaH mice, specified pathogen-free, were obtained from the Animal Resources Centre (Perth, Australia), housed in a controlled environment (Microbiology Animal House, University of Western Australia) and received food and water *ad libitum*. Mice were anaesthetized (50 mg kg^{-1} pentobarbitone sodium, i.p.) and inoculated intranasally with 15 μl of fluid containing 1000 EID_{50} doses of influenza A/PR-8/34 virus or 15 μl of a 1 in 40 dilution of the allantoic fluid from virus-free chicken eggs (control mice). At day 1, 2, 4, 8 and 19 post-inoculation, mice were anaesthetized with halothane (Fluothane, ICI) and killed by cervical dislocation. The trachea was removed and placed in Krebs bicarbonate solution (KBS) of the following composition (in mM): NaCl 117, KCl 5.36, $NaHCO_3$ 25.0, KH_2PO_4 1.03, $MgSO_4.7H_2O$ 0.57, $CaCl_2.2H_2O$ 2.5 and glucose 11.1. The isolated trachea was carefully cleaned of adherent fat and connective tissue.

Autoradiographic studies

Tracheal tubes were submerged in Macrodex (6% dextran 70 in 5% glucose) and frozen by immersion in isopentane, quenched with liquid nitrogen. Serial transverse sections (10 μm) were cut at −20°C and thaw-mounted onto gelatin chrom-alum coated glass microscope slides. These sections were pre-incubated (2 × 10 min) at 22°C in buffer (50 mM Tris-HCl, 100 mM NaCl, 0.25% bovine serum albumin, pH 7.4) containing the protease inhibitor phenylmethysulphonylfluoride (10 μM), and then in buffer containing 0.3 nM [^{125}I]-endothelin-1 for 3 h alone (total binding) or in the presence of BQ-123 (ET_A receptor-selective ligand; 1 μM) or sarafotoxin S6c (ET_B receptor-selective ligand; 100 nM). Nonspecific binding was determined in the combined presence of BQ-123 (1 μM) and sarafotoxin S6c (100 nM). After 3 h, tissue sections were washed twice for 10 min in buffer, rinsed in distilled water and dried under a stream of cold dry air. Emulsion-coated cover slips (Kodak NTB-2) were attached to one end of the glass slides with cyanoacrylate adhesive and incubated for 4 days at 4°C. The emulsion-coated coverslips were developed (Kodak Dektol, 1:1) for 3 min, rinsed for 15 s in dilute acetic acid (2%) containing hardener (Ilford) and fixed (Ilford Hypam, 1:4) for 2.75 min. Tissue sections were then stained for 30 s with Gill's double strength haematoxylin, dehydrated in ethanol, cleared in xylene and mounted (Depex, BHD) for light microscopy.

Autoradiographic grain densities were determined by use of a computer-assisted grain detection and counting system (Henry *et al.*, 1990). In this study, a total of twelve slides were assessed (4 × total binding, 3 × BQ-123, 3 × sarafotoxin S6c and 2 × BQ-123 and sarafotoxin S6c). Sixty tracheal rings were examined on each slide: one tracheal ring from each of 30 virus-inoculated and 30 control mice (six control and six virus-inoculated mice per day post-inoculation examined). Four estimates of grain density were made per tracheal ring; three over the tracheal smooth muscle band and one over a non-tissue area in the airway lumen. Thus, a total of 2880 fields were analysed (12 slides × 60 sections × 4 fields). Autoradiographic grain densities are expressed as grains 1000 μm^{-2} and presented as the mean grain density ± s.e.mean.

Functional studies

Two ring segments (2 mm long) were obtained from each trachea and suspended under 0.5 g tension and placed in 2 ml organ baths containing KBS bubbled continuously with 5% CO_2 in O_2 at 37°C. Changes in isometric tension were recorded via FTO3 force-displacement transducers (Grass Instruments). Tracheal segments were allowed to equilibrate for 45 min and during this time preparations were washed every 15 min. During the equilibration period, changes in resting tension were adjusted to 0.5 g. Isolated tracheal preparations were exposed to cumulative additions of 0.2 and 10 μM carbachol to assess the viability of the preparations and then washed and rested for 30 min prior to the further addition of agonist. All concentration-effect curves were constructed by the cumulative addition of agonists. Concentration-effect curves to endothelin-1 (1–300 nM) were constructed in the absence or presence (15 min pre-incubation) of the ET_A receptor-selective antagonist BQ-123 (3 μM) and/or the ET_B receptor-selective antagonist, BQ-788 (1 μM). Concentration-effect curves to the ET_B receptor-selective agonist, sarafotoxin S6c (1–100 nM), were constructed in the presence or absence of BQ-788 (1 μM). Agonist-induced contractile responses were expressed in terms of the maximum contraction obtained to 10 μM carbachol (C_{max}) and are presented as the arithmetic mean ± s.e.mean. Contractions to the highest concentrations of endothelin-1 and sarafotoxin S6c were often greater than C_{max}. Furthermore, maximum responses to endothelin-1 were often not reached at these concentrations. Thus the potency of endothelin-1 was taken as that concentration of endothelin-1 that produced 70% of C_{max} (ED_{70}). Complete sigmoidal concentration-effect curves were obtained to sarafotoxin S6c over the concentration-range studied and therefore maximum contraction (E_{max}) could be determined. Thus, the potency of sarafotoxin S6c was expressed as the concentration that produced 50% of E_{max} (EC_{50}). Both ED_{70} and EC_{50} values were calculated by interpolation of concentration-effect curves and expressed as the geometric mean associated with 95% confidence limits.

Data analysis

Differences between treatment means were assessed by analysis of variance (SigmaStat) followed by a modified *t* statistic (Wallenstein *et al.*, 1980). For statistical comparisons ED_{70} and EC_{50} data were log transformed to mean −log ED_{70} and mean −log EC_{50} values respectively. *P* values less than 0.05 were considered to be statistically significant.

Drugs

The following were used: endothelin-1, sarafotoxin S6c, BQ-123 (cyclo[D-Trp-D-Asp-L-Pro-D-Val-L-Leu]), [^{125}I]-endothelin-1 (Auspep, Melbourne, Australia), BQ-788 (N-*cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine, gift from Banyu Pharmaceutical Co., Tsukuba, Japan), carbamylcholine chloride (carbachol), bovine serum albumin (Sigma Chemical Company, St. Louis, U.S.A.), phenylmethylsulphonylfluoride (Calbiochem, La Jolla, U.S.A.).

Results

General effects of influenza A/PR-8/34 virus infection

During the initial 4 days post-inoculation the general appearance and behaviour of mice inoculated with 1000 EID_{50} of influenza A/PR-8/34 virus was indistinguishable from that of control mice. Between days 5 and 10 post-inoculation, virus-inoculated mice displayed characteristic features of infection including piloerection and lethargy. Microscopic examination of tracheal ring segments indicated that respiratory tract viral infection was associated with epithelial damage and inflammation of the upper airways at days 2 to 8 post-inoculation. By day 19 post-inoculation inflammation of the upper airways had subsided and inoculated mice appeared to have recovered from infection.

Quantitative autoradiography

High densities of autoradiographic grains derived from [^{125}I]-endothelin-1 were associated with the airway smooth muscle band of tracheal rings from control and virus-inoculated mice. Nonspecific binding accounted for only 10–20% of total binding in tracheal airway smooth muscle from both control and virus-inoculated mice (Figure 1). Quantitative analysis of autoradiographs indicated that specific binding levels were also similar in the tracheal smooth muscle from control and virus-inoculated mice (Figure 1). Thus, respiratory tract viral infection was not associated with a significant change in the density of specific [^{125}I]-endothelin-1 binding in tracheal airway smooth muscle.

The density of specific [^{125}I]-endothelin-1 binding sites in tracheal smooth muscle from both control and virus-inoculated mice was reduced in the presence of the ET_A receptor-selective ligand, BQ-123 or the ET_B receptor-selective ligand sarafotoxin S6c, indicating the presence of both ET_A and ET_B binding site subtypes. BQ-123 and sarafotoxin S6c were used to estimate the proportions of tracheal airway smooth muscle ET_A and ET_B sites. Consistent with our previous observations (Henry & Goldie, 1994), the tracheal airway smooth muscle of control mice had approximately equal proportions of ET_A and ET_B sites (ET_A:ET_B, 49%:51% (± 2%), $n=29$). However, respiratory tract viral infection was associated with transient, but significant changes of the proportion of tracheal airway smooth muscle ET_A and ET_B sites (Figure 2). For example, at day 4, the proportion of ET_A:ET_B sites was 72%:28% (±4%, $n=6$, $P<0.05$). In contrast, at day 19 post-inoculation the proportion of ET_A:ET_B sites receptors was 51%:49% (±3%, $n=6$), i.e. similar to control ($P>0.05$). Thus, respiratory tract infection with influenza A/PR-8/34 virus induced a time-dependent, reversible increase in the density of ET_A sites and a transient decrease in the density of ET_B sites in tracheal airway smooth muscle.

Contraction studies

Characterization of endothelin receptors linked to airway smooth muscle contraction Endothelin-1 was a potent spasmogen in isolated tracheal airway smooth muscle preparations from control mice (ED_{70} = 6.3 nM (4.0–10), $n=6$, Figure 3). Pretreatment with either the ET_A receptor-selective antagonist, BQ-123 (3 μM) or the ET_B receptor-selective antagonist, BQ-788 (1 μM) had no significant inhibitory effect on endothelin-1-induced contractions (Figure 3a and 3b). However, as shown in Figure 3c, simultaneous treatment with BQ-123 (3 μM) and BQ-788 (1 μM) resulted in a 10 fold rightward shift of the endothelin-1 concentration-effect curve (ED_{70} = 60 nM (44–90), n = 6, $P<0.05$). The ET_B receptor-selective agonist, sarafotoxin S6c, was also a potent spasmogen in control mouse isolated tracheal airway smooth muscle preparations (EC_{50} = 4.0 nM (2.6–6.1), n = 4). Concentration-effect curves to sarafotoxin S6c were shifted to the right by 17 fold (10–30, n = 4) by the ET_B receptor-selective antagonist, BQ-788 (Figure 3d). Thus, both ET_A and ET_B receptors were linked to tracheal airway smooth muscle contraction.

Effects of respiratory tract viral infection on tracheal airway smooth muscle ET_A and ET_B receptor function The muscarinic cholinoceptor agonist, carbachol, evoked similar maximum contractions (C_{max}; contractile response to 10 μM carbachol) in isolated tracheal smooth muscle preparations from control (mean C_{max} calculated from days 1, 2, 4, 8 and 19 post-inoculation = 0.92±0.02 g, n = 110) and virus-inoculated mice (mean C_{max} calculated from days 1, 2, 4, 8 and 19 post-inoculation = 0.97±0.02 g, n = 125, $P>0.05$).

Endothelin-1 evoked similar concentration-dependent contractions of tracheal smooth muscle isolated from control and virus-inoculated mice (Figure 4 and Table 1). However, in contrast to control, endothelin-1-induced contractions of pre-

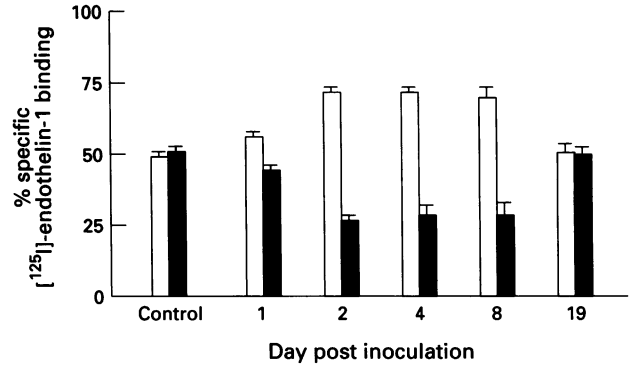

Figure 2 Time course of changes in the density of ET_A and ET_B sites in tracheal airway smooth muscle during respiratory tract viral infection. The proportion of tracheal airway smooth muscle ET_A (open columns) and ET_B (solid columns) sites were calculated from autoradiographic competition binding studies using [^{125}I]-endothelin-1, the ET_A receptor-selective ligand BQ-123 (1 μM) and the ET_B receptor-selective ligand sarafotoxin S6c (100 nM). Control is the combined control data from day 1, 2, 4, 8 and 19 post-inoculation. Values are mean with s.e.mean of n = 29 control mice and n = 6 virus-inoculated mice.

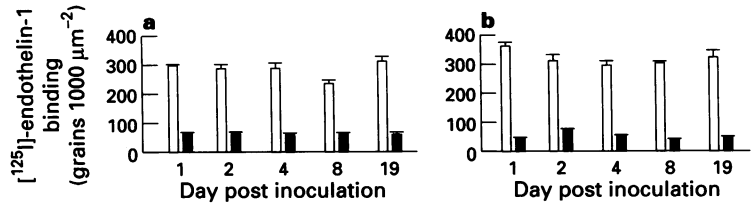

Figure 1 Quantitative autoradiographic assessment of specific (open columns) and nonspecific (solid columns) binding of 0.3 nM [^{125}I]-endothelin-1 in tracheal airway smooth muscle from (a) control and (b) virus-inoculated mice. Nonspecific binding was determined in the combined presence of 1 μM BQ-123 and 100 nM sarafotoxin S6c. Specific binding was estimated by subtracting nonspecific grain density from the corresponding total grain density. Values are mean with s.e.mean of n = 5–6 mice.

parations obtained at 1, 2, 4 and 8 days post-inoculation with influenza A/PR-8/34 virus were significantly inhibited by the ET_A receptor-selective antagonist BQ-123 (Figure 4). For example, at day 4 post-inoculation, contractions to 30 nM endothelin-1, in the presence of BQ-123 (3 μM), were only $20 \pm 12\%$ ($n = 6$, $P<0.05$) of those produced under similar conditions in control preparations (Figure 4d). Similar effects were seen at days 2 and 8 post-inoculation. However, by day 19, contractions induced by 30 nM endothelin-1 in the presence of BQ-123 (3 μM), were similar in preparations from control and virus-inoculated mice (Figure 4f). Endothelin-1-induced contractions in the presence of the ET_B receptor-selective antagonist, BQ-788 (1 μM), were similar in preparations from control and virus-inoculated mice at all time points investigated (Table 2).

As shown in Figure 5, the ET_B receptor-selective agonist, sarafotoxin S6c, also caused concentration-dependent contractions of isolated tracheal smooth muscle preparations from both control and virus-inoculated mice. The contractile potency of sarafotoxin S6c was similar in preparations from control (EC_{50} = 3.2 nM (2.7–4.0), n = 22) and virus-inoculated mice (EC_{50} = 3.2 nM (2.9–3.4), n = 22) at each time point. However, respiratory tract viral infection was associated with significant decreases in E_{max} to sarafotoxin S6c at days 4,

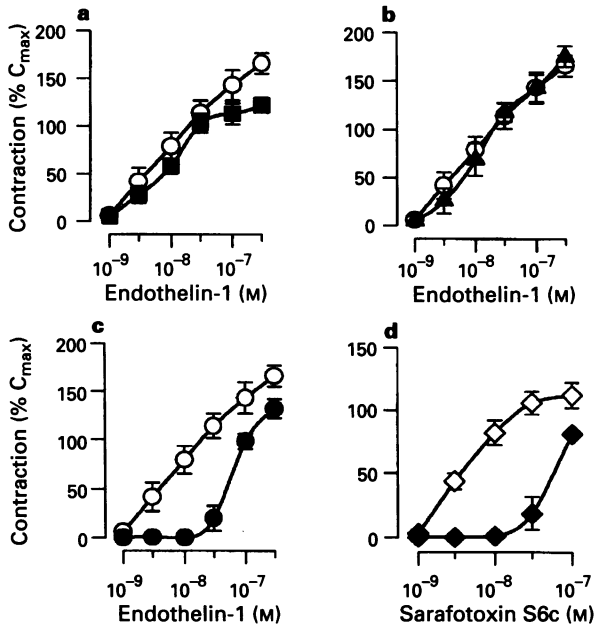

Figure 3 Concentration-effect curves to endothelin-1 in control mouse airway smooth muscle (a-c) in the absence (○) or presence of (a) the ET_A receptor-selective-antagonist, BQ-123 (3 μM, ■), (b) the ET_B receptor-selective antagonist, BQ-788 (1 μM, ▲) and (c) both BQ-123 and BQ-788 (●). Concentration-effect curves to the ET_B receptor-selective agonist sarafotoxin S6c (d) were completed in the absence (◇) or presence of BQ-788 (1 μM, ◆). Values are mean ± s.e.mean of n = 4–6 mice.

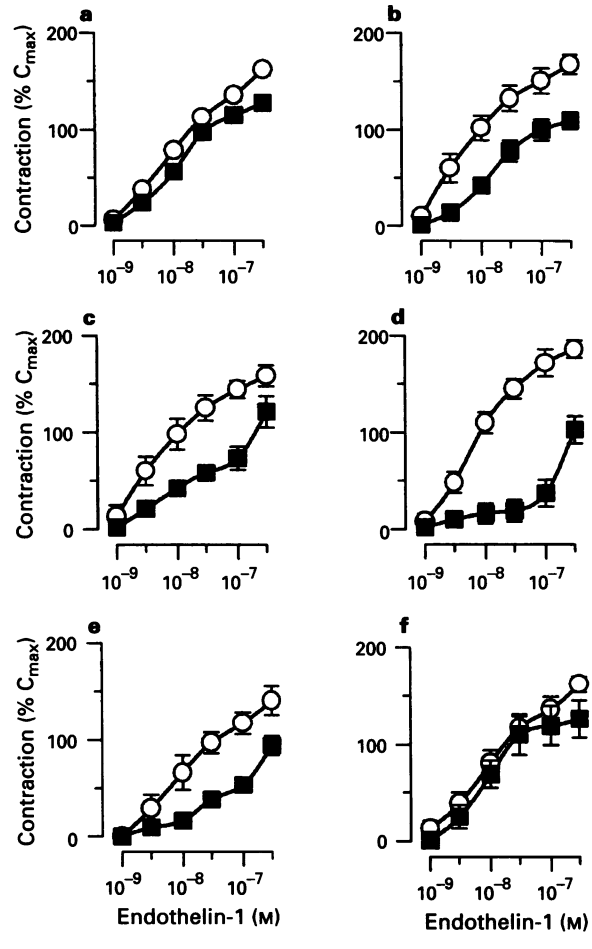

Figure 4 Cumulative concentration-effect curves to endothelin-1 in tracheal airway smooth muscle preparations from (a) control (combined data from day 1, 4, 8, and 19 post-inoculation, n = 31 mice) and (b–f) virus-inoculated mice (n = 5–8 mice) at (b) day 1, (c) day 2, (d) day 4, (e) day 8 and (f) day 19 post-inoculation. Concentration-effect curves were completed in the absence (○) or presence (■) of the ET_A receptor-selective antagonist, BQ-123 (3 μM). Values are mean ± s.e.mean.

Table 1 Comparison of endothelin-1-induced contractions in tracheal airway smooth muscle preparations from control and virus-inoculated mice

	Control			*Virus*		
	Contractile response to ET-1 (300 nM) *(% C_{max})*	*ED_{70}* (nM)	n	*Contractile response to ET-1* (300 nM) *(% C_{max})*	*ED_{70}* (nM)	n
Day 1 p.i.	167 ± 23	10 (6.3–16)	6	168 ± 10	5.0 (2.6–9.5)	6
Day 2 p.i.	166 ± 11	10 (4.0–25)	6	158 ± 11	5.0 (2.1–12)	5
Day 4 p.i.	154 ± 6	6.3 (4.0–10)	6	186 ± 9	6.3 (3.8–10)	6
Day 8 p.i.	148 ± 17	13 (5.0–32)	6	140 ± 15	13 (5.5–29)	6
Day 19 p.i.	165 ± 14	10 (6.3–16)	7	162 ± 8	10 (5.5–18)	8

Contractile response to 300 nM endothelin-1 (ET-1) is expressed as a percentage of the contractile response produced by 10 μM carbachol (C_{max}) ± s.e.mean. ED_{70} is the mean concentration of ET-1 that produced 70% of C_{max} (95% confidence limits). p.i. = post-inoculation, n = number of mice.

8 and 19 post-inoculation (Figure 5). For example, at day 4, when the lowest value was observed, E_{max} to sarafotoxin S6c (100 nM) was only 28±8% (n = 4, $P<0.05$) of that observed in control preparations (Figure 5c).

Discussion

This study has demonstrated the time-dependent nature of the modulation of endothelin receptor-effector systems in mouse tracheal airway smooth muscle in response to respiratory tract infection with influenza A/PR-8/34 virus. The early stages of viral infection were associated with a significant reduction in ET_B but not ET_A, receptor density and function. Importantly, upon resolution of the viral infection (within 3 weeks), both ET_B receptor density and ET_B receptor-mediated contraction had recovered towards control values.

In line with a previous study (Henry & Goldie, 1994) the present data demonstrate that ET_A and ET_B sites co-exist in mouse tracheal airway smooth muscle in approximately equal proportions. Functional studies indicated that both of these subtypes were functional receptors linked to tracheal airway smooth muscle contraction. Although endothelin-1-induced contraction was not significantly inhibited by BQ-788, sarafotoxin S6c caused BQ-788-sensitive, concentration-dependent contractions, consistent with the presence of functional ET_B receptors in mouse tracheal airway smooth muscle. Similarly, endothelin-1-induced contractions of control mouse tracheal airway smooth muscle were not significantly inhibited by the ET_A receptor-selective antagonist, BQ-123. However, ET_A receptors appeared to be linked to contraction, since simultaneous treatment with BQ-123 and the ET_B receptor-selective antagonist BQ-788 resulted in significant inhibition of endothelin-1-induced contraction. These data are consistent with previous experiments using ET_B receptor desensitization which also suggested that both ET_A and ET_B receptors subserved contraction in mouse airway smooth muscle (Henry & Goldie, 1994).

In the current study, marked decreases in ET_B receptor density were observed at 2, 4 and 8 days post-inoculation. This correlated well with significant decreases in ET_B receptor-mediated function, as assessed by the enhanced inhibitory effects of the ET_A receptor-selective antagonist BQ-123 against endothelin-1-induced contraction. Furthermore, recovery of ET_B receptor-mediated function observed at day 19 post-inoculation was consistent with the densities of ET_B receptors returning to control levels at this time. This reversal almost certainly reflected the time course of respiratory tract viral infection, since we have previously shown that tracheal virus titres fall between day 2 post-inoculation, to baseline at days 8–10 post-inoculation (Henry *et al.*, 1991). ET_B receptor function was also assessed during the time course of viral infection by monitoring contractions induced by the ET_B receptor-selective agonist, sarafotoxin S6c. As expected, sarafotoxin S6c contractions were also markedly attenuated during the early stages of virus infection when ET_B receptor densities were reduced. However, although endothelin-1-induced contractions in the presence of BQ-123 were fully restored by day 19 post-inoculation, maximum contractile responses to sarafotoxin S6c were still partially suppressed. The precise reason for these disparate findings is unclear; however, as different groups of mice were used for each protocol, these findings may be explained by variability in the rate of recovery from respiratory tract viral infection between different groups of mice. Overall, the results of the current study

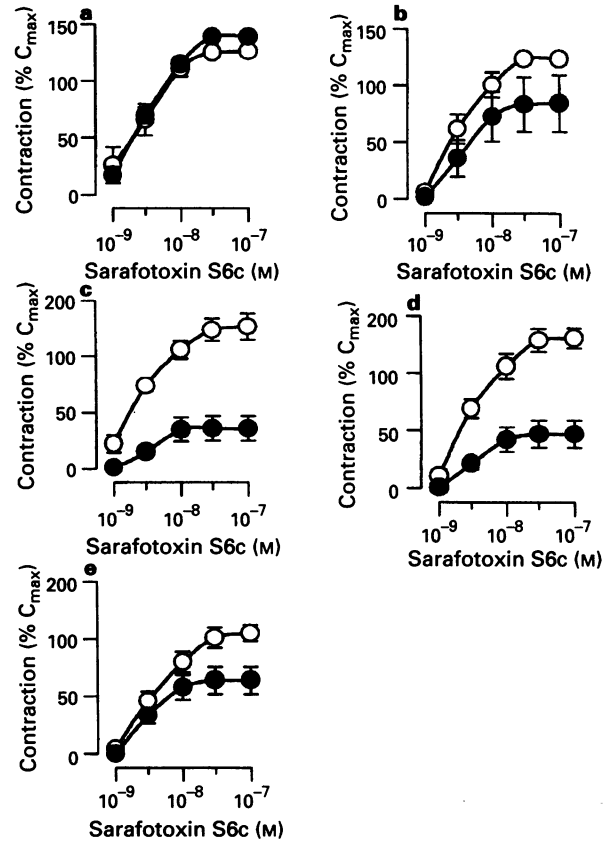

Figure 5 Cumulative concentration-effect curves to the ET_B receptor-selective agonist, sarafotoxin S6c, in tracheal airway smooth muscle preparations from control (○) and virus-inoculated (●) mice at (a) day 1, (b) day 2, (c) day 4, (d) day 8 and (e) day 19 post-inoculation. Values are mean±s.e.mean of n= 4–6 mice.

Table 2 Effect of the ET_B receptor-selective antagonist, BQ-788 (1 μM), on endothelin-1-induced contractions of tracheal airway smooth muscle preparations from control and virus-inoculated mice

	Control			*Virus*		
	Contractile response to ET-1 (300 nM) (% C_{max})	ED_{70} (nM)	n	*Contractile response to ET-1* (300 nM) (% C_{max})	ED_{70} (nM)	n
Day 1 p.i.	156±15	16 (10–25)	6	165±5	10 (6.3–16)	6
Day 2 p.i.	176±11	13 (7.6–21)	6	166±6	7.9 (3.0–21)	5
Day 4 p.i.	176±18	13 (7.9–20)	6	164±11	10 (4.0–16)	6
Day 8 p.i.	161±8	16 (6.3–40)	6	159±12	13 (5.0–32)	6
Day 19 p.i.	170±8	20 (12–32)	6	150±16	16 (10–25)	7

Contractile response to 300 nM endothelin-1 (ET-1) is expressed as a percentage of the contractile response produced by 10 μM carbachol (C_{max})±s.e.mean. ED_{70} is the mean concentration of ET-1 that produced 70% of C_{max} (95% confidence limits). p.i.=post-inoculation, n=number of mice.

were consistent with the time-dependent nature of the inhibitory effects of influenza A/PR-8/34 virus on ET_B receptor density.

The transient decreases in ET_B receptor density observed during the early stages of viral infection were accompanied by significant compensatory increases in ET_A receptor density leaving total specific binding levels in the muscle essentially constant. We evaluated the possibility of increased function of the ET_A receptor-effector system by selectively stimulating the tracheal smooth muscle ET_A receptor-effector system with endothelin-1 in the presence of the ET_B receptor-selective antagonist BQ-788. Under these conditions, endothelin-1 induced similar contractions of tracheal airway smooth muscle preparations from control and virus-inoculated mice throughout the period of infection. Thus, the observed increases in the densities of ET_A receptors were not reflected in hyperfunction of the ET_A receptor-effector system linked to tracheal airway smooth muscle contraction.

It is clear that contractile responsiveness to endothelin-1 is apparently unaffected in the presence of either BQ-788 or influenza A/PR-8/34 virus infection, both of which markedly attenuate ET_B receptor activity. Similarly, inhibition of ET_A receptor function with BQ-123 failed to alter markedly responses to endothelin-1 in control tissue with a fully functional ET_B receptor-effector system. Thus, activation of either the ET_A or the ET_B receptor-effector system alone provides sufficient stimulus to evoke maximal contraction and maintain tissue sensitivity to endothelin-1. Accordingly, endothelin-1-induced contractions in control mouse tracheal airway smooth muscle were only inhibited when both ET_A and ET_B receptor-effector systems were blocked.

The mechanism of viral infection-induced down-regulation of tracheal airway smooth muscle ET_B receptors remains unknown. However, a variety of circumstantial evidence suggests that increased levels of endothelin-1 within the airway wall may be a contributing factor. Firstly, various inflammatory mediators known to be released during respiratory tract viral infection (Hennet *et al.*, 1992) caused enhanced release of endothelin-1 from airway epithelial cells (Endo *et al.*, 1992). Secondly, prolonged exposure to elevated levels of endothelin-1 or sarafotoxin S6c caused marked down-regulation of the ET_B receptor-effector system in airway smooth muscle from mice (Henry & Goldie, 1994) and rats (Henry, 1993; 1994). Thus, increased levels of endothelin-1 within the airway wall may at least be partially responsible for the down-regulation of the tracheal airway smooth muscle ET_B receptor-effector system associated with respiratory tract viral infection.

Endothelin receptors are located on a number of cell types within the airways and may therefore influence airway function via multiple effector systems. For example, recent investigations have demonstrated that endothelin-1 regulates airway tone directly, via stimulation of airway smooth muscle endothelin receptors and indirectly via prejunctional modulation of cholinergic neurotransmission (McKay *et al.*, 1993; Henry & Goldie, 1995) and via the release of secondary mediators such as platelet-activating factor and thromboxane (Filep *et al.*, 1991). We have clearly demonstrated that respiratory tract viral infection modulates the function of ET_B receptors linked directly to airway smooth muscle contraction (current study; Henry & Goldie, 1994). However, the influence of respiratory tract viral infection on the function of endothelin receptor-effector systems within the airways, other than those linked directly to contraction, remains to be established.

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