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Influence of parainfluenza-1 respiratory tract viral infection on endothelin receptor-effector systems in mouse and rat tracheal smooth muscle

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1 In this study we have compared the effects of parainfluenza-1 respiratory tract viral infection on the density and function of ET_A and ET_B receptors in rat and mouse tracheal airway smooth muscle.

2 The bronchoconstrictor effect of inhaled methacholine was significantly enhanced in virus-infected rats, at both 4 and 12 days post-inoculation. That is, the concentration of methacholine causing an increase in resistance of 100% (PC_{100} methacholine) was significantly lower in virus-infected animals at both 4 and 12 days post-inoculation ($n=6-8$; $P<0.05$).

3 Total specific binding of [^{125}I]-endothelin-1 and the relative proportions of ET_A and ET_B binding sites for [^{125}I]-endothelin-1 were assessed in tracheal airway smooth muscle in parainfluenza-1-infected rats and mice at days 2, 4 and 12 post-inoculation using the ligands BQ-123 (1 μM; ET_A receptor-selective) and sarafotoxin S6c (100 nM; ET_B receptor-selective). Total specific binding in mice was significantly reduced at day 2 post-inoculation ($n=5$; $P<0.05$) but not at days 4 and 12 post-inoculation ($n=5$). In control mice, the proportions of ET_A and ET_B binding sites were 53%:47% at day 2 and 43%:57% at day 4 and these were significantly altered by parainfluenza-1 infection such that, the ratios were 81%:19% at day 2 and 89%:11% at day 4 ($P<0.05$). By day 12 post-inoculation, the proportion of ET_A and ET_B binding sites in tracheal smooth muscle from mice infected with parainfluenza-1 was not significantly different from control. In rat tracheal airway smooth muscle, neither total specific binding nor the ET_A and ET_B binding site ratio (64%:36%) were significantly altered in virus-inoculated rats at days 2, 4 or 12 post-inoculation ($n=5$).

4 Parainfluenza-1 infection in mice had no effect on the sensitivity or maximal contractile effect of endothelin-1 in tracheal smooth muscle at days 2, 4 or 12 post-inoculation ($n=4$). In contrast, contraction in response to the ET_B receptor-selective agonist sarafotoxin S6c was attenuated by 39% at day 2 and by 93% at day 4 post-inoculation ($P<0.05$). However, by day 12 post-inoculation, contractions to sarafotoxin S6c were not significantly different between control and virus-infected mice. In parainfluenza-1-infected rats, there were small but significant reductions in the sensitivity to carbachol, endothelin-1 and sarafotoxin S6c whilst the maximal responses to the highest concentrations of these agonists were not significantly altered by virus infection ($n=8$).

5 BQ-123 (3 μM) had no significant effect on cumulative concentration-effect curves to endothelin-1 in tracheal preparations from control mice ($n=4$) or parainfluenza-1-infected rats ($n=8$). In contrast, in tissues taken from virus-infected mice at day 4 post-inoculation, BQ-123 caused a marked 9.6 fold rightward shift in the concentration-effect curve to endothelin-1 ($n=4$).

6 In summary, we have demonstrated that parainfluenza-1 infection in mice transiently reduced the density of tracheal airway smooth muscle ET_B receptors and this was reflected in reduced responsiveness to the ET_B receptor-selective agonist sarafotoxin S6c. In contrast, whilst parainfluenza-1 infection in rats was associated with the pathological features and bronchial hyperresponsiveness common to respiratory tract viral infection, there was no selective down-regulation of ET_B receptor expression or functional activity. The reasons for these species differences are not clear, but may relate to differences in the airway inflammatory response to parainfluenza-1 virus.

Keywords: Endothelin receptors; tracheal smooth muscle; parainfluenza-1 virus

Introduction

Various respiratory tract viruses, including parainfluenza-1 (Hall *et al.*, 1977) can precipitate and exacerbate asthma symptoms (Boushey *et al.*, 1980; Empey, 1983). Animal models of parainfluenza-1 infection indicate that this virus induces airway inflammation and hyperresponsiveness (Castleman, 1983; Sorkness *et al.*, 1994). These infections are transient in nature, with peak lung virus titres occurring 4-5 days after inoculation. When viral titres begin to fall, there is an accompanying resolution of inflammation as indicated by reductions in mononuclear cell and leukocyte infiltration, epithelial damage and luminal debris (Castleman, 1983).

Respiratory tract infection with influenza-A virus in mice, has been shown to attenuate significantly the tracheal airway smooth muscle endothelin$_B$ (ET_B) receptor-effector system (Henry & Goldie, 1994; Carr *et al.*, 1996). This infection caused a marked down-regulation in the density of tracheal airway smooth muscle ET_B receptors that was reflected by reduced ET_B receptor-mediated airway smooth muscle contraction. Importantly, these effects were transient and their duration appeared to mirror the time course of respiratory tract viral infection (Carr *et al.*, 1996). Cytokines including interleukin-1 and -6 that are known to be released during respiratory tract viral infections can stimulate the release of endothelin-1 from cultured airway epithelial cells (Endo *et al.*, 1992; Hennet *et al.*, 1992). It has been suggested that al-

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terations in ET_B receptor expression during respiratory tract viral infections may be a consequence of increased endothelin-1 synthesis and release (Henry & Goldie, 1994; Carr *et al.*, 1996).

We were interested to determine whether similar effects occurred in response to other respiratory tract viruses in other animal species. Thus, we have compared the effects of parainfluenza-1 virus infection on endothelin receptor-effector systems in tracheal airway smooth muscle in rats and mice.

Methods

Virus stock

Parainfluenza-1 (Sendai; Enders strain) virus stock that had recently been passaged in mice, was propagated for our studies in 10 day-old embryonated chicken eggs. Briefly, the allantoic cavity was injected with 100 μl of a 1 in 100 dilution of virus stock in phosphate buffered saline (PBS), or diluent (control). After 3 days incubation at 35°C, the allantoic fluid was harvested, centrifuged and stored at −70°C until required. Virus titre was determined by haemagglutination using chicken red blood cells and indicated that the allantoic fluid contained 2×10^6 haemagglutination units (HAU) ml^{-1}.

Infection of animals

Male Wistar rats (7 weeks; approximately 250 g) and male CBA/CaH mice (7 weeks; approximately 25 g) were obtained from the Animal Resources Centre (Perth, Australia) and were housed in specific pathogen-free conditions at the Institute for Child Health Research holding facility. Animals were anaesthetized with chloral hydrate (300 mg kg^{-1}, i.p.) for inoculation. Virus-free (Control) and virus-infected (Virus) allantoic fluid was diluted 1 in 100 with sterile PBS and rats received 100 μl and mice 15 μl, of either Control or Virus fluid by nasal instillation. Thus, rats were infected with 2×10^3 HAU and mice with 3×10^2 HAU. Animals were killed at days 2, 4 and 12 post-inoculation. The pathological effects of infection were assessed after tissues were fixed in buffered formal saline, embedded in paraffin for cutting, stained with haematoxylin and eosin and viewed with the light microscope.

Lung function studies in vivo

Rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and the trachea intubated with a polyethylene tube (6 cm × 1.5 mm internal diameter). This tracheal tube was attached to a heated pneumotachograph (Fleisch, type 0000) through which the animals were breathing spontaneously. Estimates of transpulmonary pressure were obtained via electronic subtraction of tracheal pressure from oesophageal pressure (measured via an air-filled cannula placed in the oesophagus) measured with differential pressure transducers (Celesco, LCVR). These signals were integrated on a breath-to-breath basis using a pulmonary monitoring system (Mumed) to yield an estimate of lung resistance (R_L). After an equilibration period of 30 min, the concentration-effect relationship to inhaled methacholine was then determined. Briefly, increasing concentrations of nebulized methacholine (0.1, 0.3, 1, 3, 10 and 30 mg ml^{-1} for 30 s; De Vilbiss) were introduced through a sideport in the tracheal tube. The concentration of methacholine was increased, until a rise in R_L of 100% above baseline (PC_{100} methacholine) was seen. Control and virus-infected rats were assessed at days 4 and 12 post-inoculation.

In vitro *tissue preparation*

Mice were anaesthetized with halothane and killed by cervical dislocation. Rats were killed by sodium pentobarbitone overdose (100 mg kg^{-1}, i.p.). The trachea was removed, cleaned 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.

Contraction studies in vitro

Six ring segments were obtained from each rat trachea and two ring segments were obtained from each mouse trachea. All preparations were suspended under 0.5 g tension 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) connected to a preamplifier and computer utilizing customized data acquisition software. Tracheal segments were allowed to equilibrate for 45 min during which time changes in resting tension were readjusted to 0.5 g. Isolated tracheal preparations were exposed to cumulative additions of 0.2 μM and 10 μM carbachol to assess the viability of the preparations and then washed and rested for 30 min. In some preparations, a cumulative concentration-effect curve to carbachol (30 nM − 30 μM) was constructed. Cumulative concentration-effect curves to endothelin-1 (1 − 300 nM) were then constructed in the absence or presence of the ET_A receptor-selective antagonist BQ-123 (3 μM; 15 min pre-incubation). In separate preparations, cumulative concentration-effect curves to the ET_B receptor-selective agonist, sarafotoxin S6c (1 − 100 nM) were constructed. Endothelin-1 and sarafotoxin S6c-induced contractile responses were expressed in terms of the maximum contraction obtained to 10 μM carbachol (C_{max}) and were presented as the arithmetic mean ± s.e.mean. As the concentration-effect relationship to endothelin-1 usually did not plateau, the potency of this peptide was taken as the concentration that produced 70% of C_{max} (ED_{70}) in mice and 40% of C_{max} (ED_{40}) in rats. Complete sigmoidal concentration-effect curves were obtained to sarafotoxin S6c and thus the potency of sarafotoxin S6c was expressed as the concentration that produced 50% of the maximum response (E_{max}). ED_{40}, ED_{70} and EC_{50} (agonist concentration causing 50% E_{max} contraction) values were calculated by interpolation of concentration-effect curves and expressed as the geometric mean associated with 95% confidence limits.

Autoradiographic studies

Tracheal tube segments 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 chromalum-coated glass microscope slides. These sections were pre-incubated for 5 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 phenylmethylsulphonylfluoride (10 μM) and then for 3 h in buffer containing 0.2 nM [^{125}I]-endothelin-1 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 coverslips (Kodak NTB-2) were attached to one end of the glass slides with cyanoacrylate adhesive and exposed for 2 − 5 days at 4°C. These were developed for 3 min in Kodak Dektol diluted 1:1 with water, 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 with a computer-assisted grain detection and counting system (Henry

et al., 1990). Slides contained tissue sections from control and virus-infected rats and mice and three estimates of grain density were made per tracheal section. Background grain density was determined over a non-tissue area in the airway lumen and this was subtracted from the tissue grain densities. Autoradiographic grain densities were expressed as grains 1000 μm^{-2} and presented as the mean grain density $\pm$ s.e. mean.

Data analysis

Differences between treatment means were assessed by analysis of variance (SigmaStat) using a modified *t* statistic (Wallenstein *et al.*, 1980). For statistical comparisons ED_{40}, ED_{70} and EC_{50} data were log transformed to mean $-\log ED_{40}$, $-\log ED_{70}$ and mean $-\log EC_{50}$ values respectively. *P* values less than 0.05 were considered to be statistically significant.

Drugs

Endothelin-1, sarafotoxin S6c, BQ-123 (cyclo[D-Trp-D-Asp-L-Pro-D-Vel-L-Leu], [^{125}I]-endothelin-1 (Auspep, Melbourne, Australia), carbamylcholine chloride (carbachol), methacholine chloride, bovine serum albumin (Sigma Chemical Company, St. Louis, U.S.A.) and phenylmethylsulphonylfluoride (Calbiochem, La Jolla, U.S.A.) were used.

Results

Time course of parainfluenza-1 virus infection

Parainfluenza-1 virus inoculation resulted in respiratory tract infection in both rats and mice. Histological examination of paraffin sections of tracheal and peripheral lung tissue revealed luminal material consisting of inflammatory cells, mucus and cellular debris. During the period of infection, inflammatory cells were observed in the tracheal epithelium which became disrupted and subsequently underwent repair. The submucosal layer of the major conducting airways was densely infiltrated with inflammatory cells and focal areas of inflammation were seen in the lung parenchyma. These indications of the presence of a respiratory tract infection were time-dependent in both rats and mice, with inflammation of the trachea preceding that seen in the peripheral lung. The pathological effects of parainfluenza-1 infection in the trachea in rats and mice were most apparent at day 4 post-inoculation and these spontaneously resolved so that few obvious signs of infection were seen by day 12 post-inoculation.

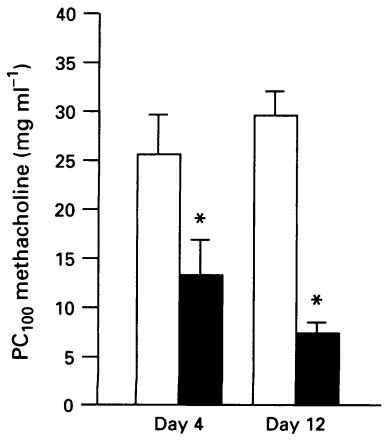

Figure 1 *In vivo* responsiveness of control (open columns) and parainfluenza-1-inoculated (solid columns) rats to inhaled methacholine at days 4 and 12 post-inoculation. Columns represent the concentration of methacholine required to increase lung resistance 100% above baseline (PC_{100} methacholine; mg ml^{-1}). Data are expressed as mean $\pm$ s.e.mean from 4–8 animals. *$P<0.05$ compared with respective control.

In vivo *airway responses to methacholine*

Baseline lung resistance (R_L) was significantly higher in virus-infected rats at day 12 post-inoculation (control, 115 ± 9 cmH_2O l^{-1} s^{-1}, $n=4$; virus, 146 ± 8 cmH_2O l^{-1} s^{-1}, $n=6$; $P<0.05$), but not at day 4 post-inoculation (control, 98 ± 5 cmH_2O l^{-1} s^{-1}, $n=8$; virus, 105 ± 14 cmH_2O l^{-1} s^{-1}, $n=8$). However, the potency of inhaled methacholine was significantly enhanced in virus-inoculated rats, at both 4 and 12 days post-inoculation. Thus, the concentration of methacholine causing an increase in resistance of 100% (PC_{100} methacholine) was significantly lower in virus-inoculated rats at both 4 and 12 days post-inoculation ($P<0.05$; Figure 1).

Effects of parainfluenza-1 virus infection on endothelin-1 receptor density in rat and mouse tracheal airway smooth muscle

The influence of parainfluenza-1 virus infection in rats and mice on [^{125}I]-endothelin-1 binding was assessed in tracheal airway smooth muscle at days 2, 4 and 12 post-inoculation. Non-specific binding determined in the combined presence of BQ-123 (1 μM) and sarafotoxin S6c (100 nM) accounted for approximately 10% of total binding in all cases. There were significantly fewer (38%) specific autoradiographic grains over airway smooth muscle in parainfluenza-1-infected mice than in controls at day 2 post-inoculation ($P<0.05$; Figure 2a). However, at days 4 and 12 post-inoculation, there was no significant difference in specific binding of [^{125}I]-endothelin-1 between control and virus-infected mice (Figure 2b and c). Similarly, in rats, there was no difference in total specific binding of [^{125}I]-endothelin-1 (grains 1000 μm^{-2}) in airway smooth muscle in trachea from control and virus-inoculated rats at day 2 (control, 424 ± 20; virus, 468 ± 13), day 4 (control, 459 ± 23; virus, 466 ± 29) or day 12 (control, 257 ± 19; virus 252 ± 14) post-inoculation ($n=5$ in all cases). In both rats and mice, binding experiments using virus and control tracheal tissues at day 12 post-inoculation were performed separately. Differences in absolute grain densities reflect variations in the specific activity of the radioligand. Thus, for our purposes, the relevant comparison is with the appropriate time control.

The influence of parainfluenza-1 virus infection on the relative proportions of ET_A and ET_B binding sites was also assessed in rats and mice at each of these time points using the competing ligands BQ-123 (1 μM) and sarafotoxin S6c (100 nM). In mouse tracheal smooth muscle, the proportions of ET_A and ET_B binding sites in control mice were 53%:47% at day 2 and 43%:57% at day 4 and these were significantly altered by parainfluenza-1 virus infection at days 2 and 4 post-inoculation. That is, the percentage of ET_B binding sites was reduced by approximately 59% at day 2 and 81% at day 4 (Figure 2d and e). Some variation in the proportion of ET_A and ET_B receptors was seen in control mouse tracheal smooth muscle at days 2, 4 and 12 post-inoculation. The reasons for these differences are unclear, but may reflect biological variability. The reduced ET_B binding site density seen at day 4 was balanced by a concomitant increase in the density of ET_A binding sites resulting in an unchanged absolute specific binding density. However, at day 2, the absolute density of ET_A binding sites was not increased, resulting in fewer specific [^{125}I]-endothelin-1 binding sites. By day 12 post-inoculation, the proportion of ET_B binding sites in parainfluenza-1-infected mouse tracheal smooth muscle was not significantly different from control (Figure 2f), suggesting recovery from the influence of viral infection. Interestingly, in rats, there was no

significant difference in the proportions of ET_A and ET_B receptor-specific binding between control or virus-inoculated animals at any of the time points tested. Thus the ratio of endothelin receptor subtypes ($\%ET_A : \%ET_B$) at day 2 in control rats was $64\pm2 : 36\pm2$ and in virus-infected rats was $69\pm1 : 31\pm1$. At day 4 the ratio was $64\pm4 : 36\pm4$ in control rats and $64\pm5 : 36\pm5$ in virus-infected rats. Similarly at day 12, the control ratio was $68\pm2 : 32\pm2$ and that in virus-infected rats was $63\pm2 : 37\pm2$ ($n=5$ in all cases).

Effects of parainfluenza-1 virus infection on endothelin-1 and sarafotoxin S6c-induced contractions in rat and mouse isolated tracheal smooth muscle

Parainfluenza-1 virus infection in mice had no significant effect on tracheal airway smooth muscle sensitivity or maximal response to endothelin-1 at 2, 4 or 12 days post-inoculation (Figure 3a, b and c; Table 1). In contrast, at day 2 post-inoculation, responses to 100 nM sarafotoxin S6c were 39%

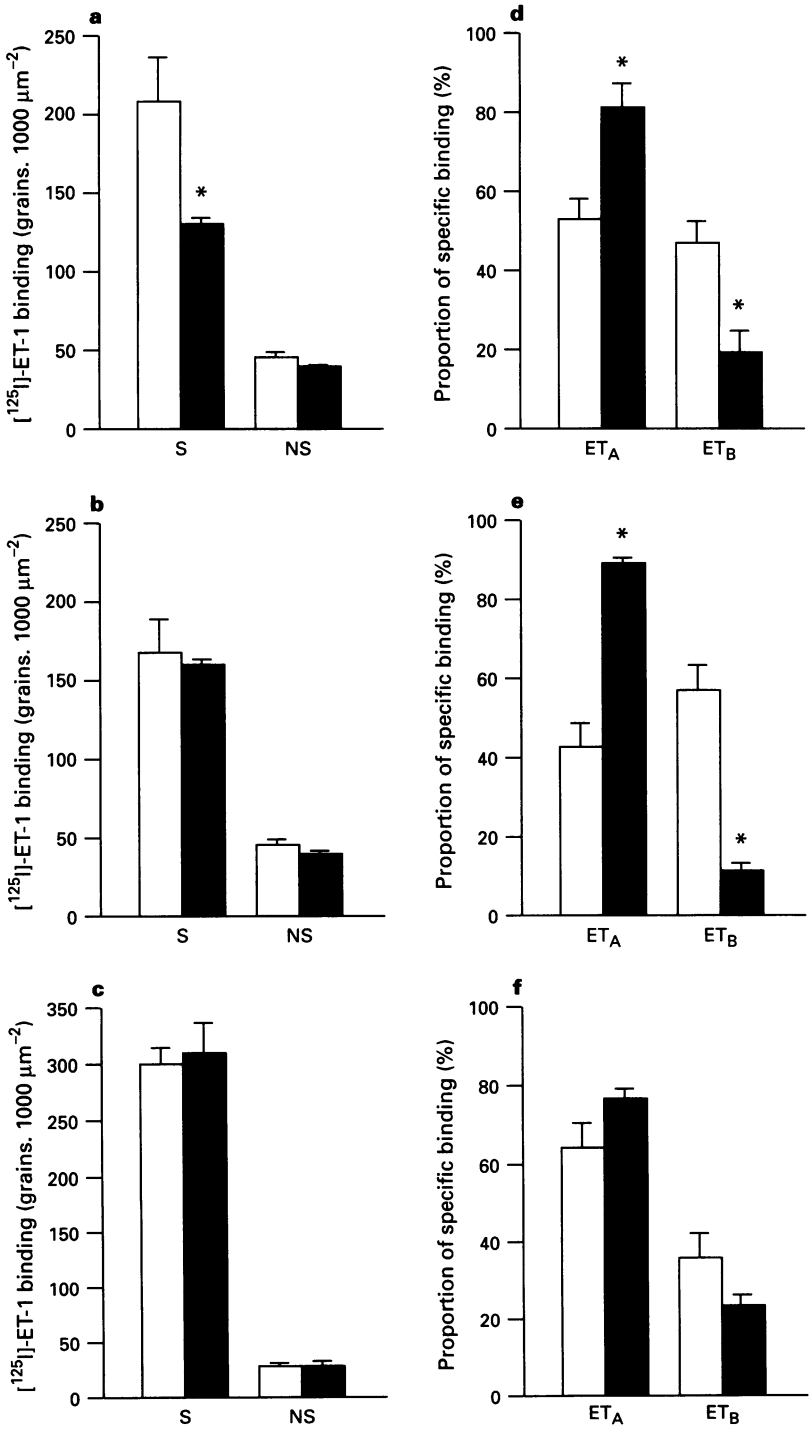

Figure 2 [^{125}I]-endothelin-1 binding in tracheal smooth muscle of parainfluenza-1-infected mice. Data were obtained from control (open columns) and virus-inoculated mice (solid columns) at days 2 (a, d), 4 (b, e) and 12 (c, f) post-inoculation. (a, b, c) Specific (S) and non-specific (NS) grain densities in control and virus-inoculated mice. (d, e, f) Relative proportions of ET_A and ET_B binding sites. Data are expressed as the mean±s.e.mean from 4 or 5 animals. *$P<0.05$ compared with respective control.

lower in virus-infected mice ($P<0.05$; Figure 3d; Table 1). This effect was more pronounced at day 4 post-inoculation where contractions to 100 nM sarafotoxin S6c were reduced by 93% in virus-inoculated animals ($P<0.05$; Figure 3e). However, by day 12 post-inoculation contractions to sarafotoxin S6c were not significantly different between control and virus-infected mice (Figure 3f). In tracheal preparations from virus-infected rats at day 4 post-inoculation, there were small but significant reductions in the sensitivity to each of carbachol (2.0 fold), endothelin-1 (3.2 fold) and sarafotoxin S6c (1.7 fold), whilst responses to the highest concentrations of carbachol, endothelin-1 and sarafotoxin S6c tested were not significantly different from control (Figure 4; Table 2).

Effects of BQ-123 on endothelin-1-mediated contractions in parainfluenza-1 virus-infected rat and mouse isolated tracheal smooth muscle

An ET_A receptor-selective antagonist, BQ-123 (3 μM), had no significant effect on cumulative concentration-effect curves to endothelin-1 in tracheal preparations from control mice (Figure 5a). In contrast, in tissues taken from virus-infected mice at day 4 post-inoculation, there was a marked 9.6 fold rightward shift in the concentration-effect relationship for endothelin-1 in the presence of 3 μM BQ-123 ($P<0.05$; Figure 5b). In tracheal smooth muscle preparations from parainfluenza-1-infected rats, 3 μM BQ-123 had no significant influence on the en-

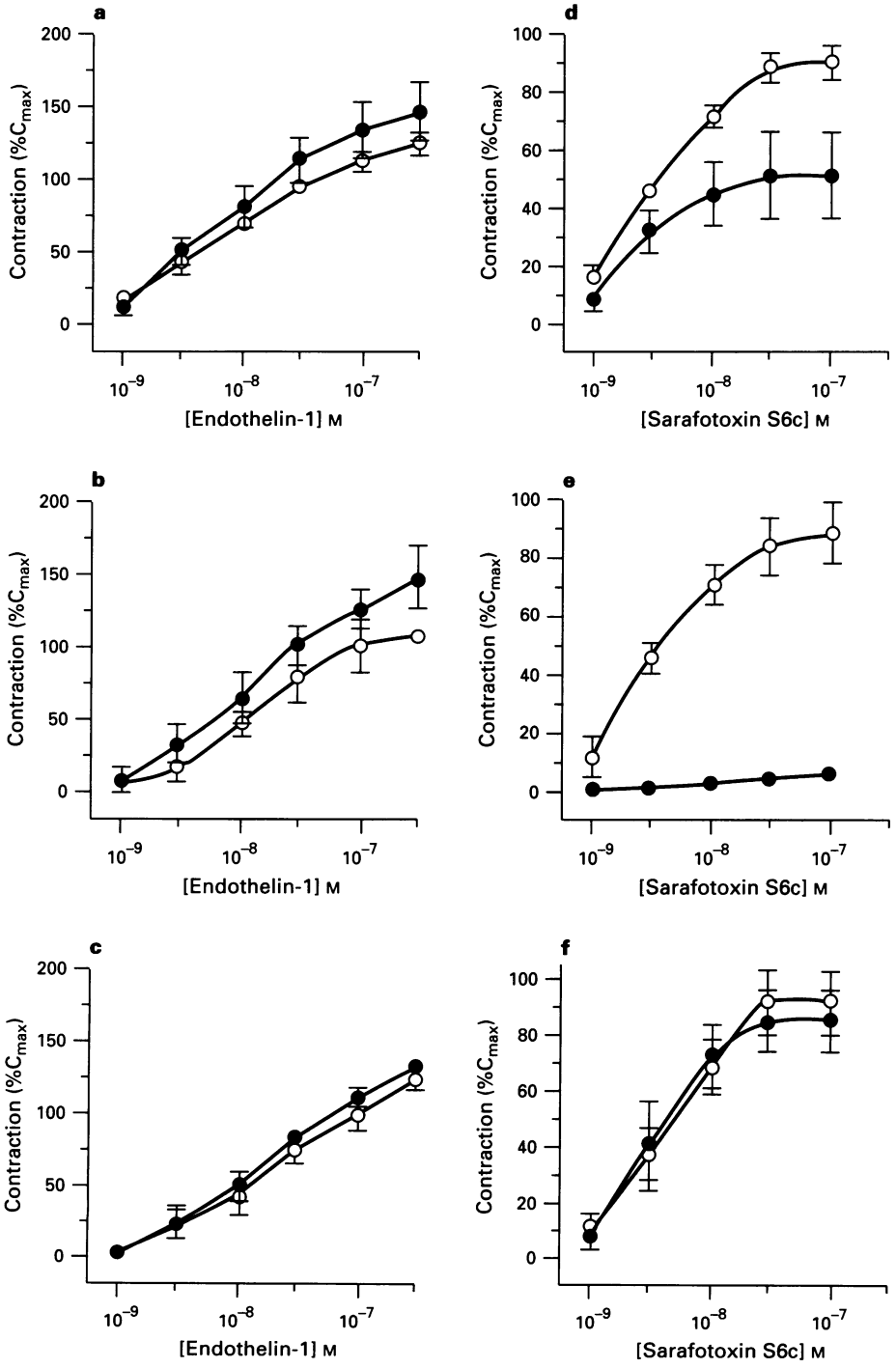

Figure 3 Cumulative concentration-effect curves to endothelin-1 (a, b, c) and sarafotoxin S6c (d, e, f) in tracheal smooth muscle preparations from parainfluenza-1-infected (●) and control (○) mice at days 2 (a, d), 4 (b, e) and 12 (c, f) post-inoculation. Data are expressed as the mean ± s.e.mean from 4 animals.

Table 1 Effect of parainfluenzea-1 virus-infection on mouse tracheal airway smooth muscle responsiveness at days 2, 4 and 12 post-inoculation

Days (p.i.)		Control	Virus
Sarafotoxin S6c			
2	E_{max}	90±5.9	51.2±14.7*
	EC_{50}	2.9 (1.3–5.6)	2.2 (1.1–4.2)
4	E_{max}	88.5±10.6	6.3±2.5*
	EC_{50}	3.1 (0.8–11.2)	ND
12	E_{max}	92±11.2	85.3±11.3
Endothelin-1	EC_{50}	4.2 (1.3–13.6)	3.5 (1.7–7.2)
2	E_{max}	123±7.1	144.8±19.8
	ED_{70}	9.8 (4.4–21.9)	7.9 (3.7–17.1)
4	E_{max}	107±4.6	148.3±21.9
	ED_{70}	24.5 (6.6–91.8)	12.0 (2.6–56.0)
12	E_{max}	122.3±5.7	130.3±5.9
	ED_{70}	24.0 (5.5–103.8)	19.5 (8.7–43.6)

E_{max} for 100 nM sarafotoxin S6c and 300 nM endothelin-1 as a percentage of the response to 10 μM carbachol (% C_{max} ± s.e.mean). EC_{50} for sarafotoxin S6c is the concentration of agonist producing 50% of its own maximal concentration (nM; 95% confidence limits). EC_{70} for endothelin-1 is the concentration of endothelin-1 causing a 70% C_{max} response (nM; 95% confidence limits). ND = not determined. p.i. = post-inoculation. *$P<0.05$ compared to respective control. Data taken from experiments using 3–5 mice.

dothelin-1 concentration-effect curve (Figure 5d), although a small shift (2.7 fold) was seen in tissue from control rats ($P<0.05$; Figure 5).

Discussion

This study has shown that parainfluenza-1 virus infection in mice was accompanied by large, time-dependent reductions in the densities of tracheal airway smooth muscle ET_B receptors that returned to control levels by day 12 post-inoculation with resolution of the infection. Carr *et al.* (1996) demonstrated a similar effect in mice in response to influenza-A infection. As with influenza-A virus, the changes seen in this study were transient, in that they were largely reversed by day 12 post-inoculation. This contrasts sharply with the data showing that parainfluenza-1 virus infection in rats was not associated with any alterations in either specific [^{125}I]-endothelin-1 binding or in the relative proportions of tracheal airway smooth muscle ET_A or ET_B receptors at days 2, 4 or 12 post-inoculation, despite histological evidence of respiratory tract viral infection that was sufficient to induce bronchial hyperresponsiveness to inhaled methacholine.

Changes in the histological appearance of tracheal tissue associated with parainfluenza-1 virus infection in rats and mice seen in this study were similar to those described previously (Robinson *et al.*, 1968; Massion *et al.*, 1993) and were similar to changes observed in other animal models of respiratory tract viral infections (Buckner *et al.*, 1985; Henry *et al.*, 1991). These included marked alterations in the structure of the epithelial cell layer and cellular infiltration into the submucosal layer of the trachea that were apparent at day 4 post-inoculation in both species. Assessment of airway responsiveness to inhaled methacholine in rats, demonstrated that the infection was associated with increased sensitivity of the airways to this spasmogen at days 4 and 12 post-inoculation. The apparently greater responsiveness seen in virus-infected rats at day 12 than at day 4, may be due in part to the elevated baseline resistance that was evident at day 12 post-inoculation.

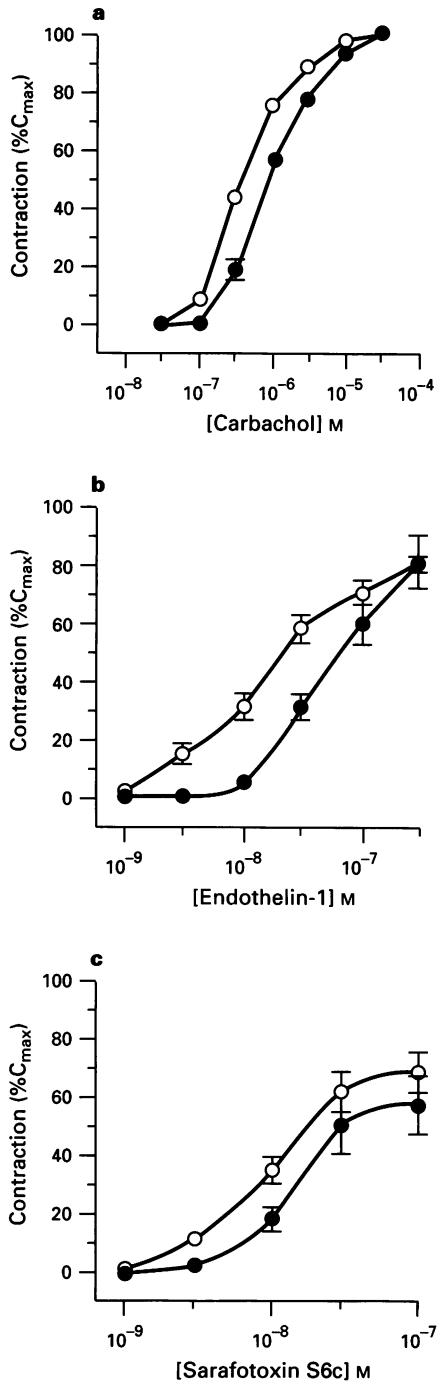

Figure 4 Cumulative concentration-effect curves to (a) carbachol (b) endothelin-1 and (c) sarafotoxin S6c in tracheal smooth muscle preparations from parainfluenza-1-infected (●) and control (○) rats at day 4 post-inoculation. Data are expressed as the mean ± s.e.mean from 9 or 10 animals.

Alteration in the proportion of ET_B receptors in airway smooth muscle caused by parainfluenza-1 virus infection in mice was accompanied by a reduction in tissue responsiveness to the ET_B receptor-selective agonist, sarafotoxin S6c, at day 2. By day 4 post-inoculation, sarafotoxin S6c was virtually inactive as a spasmogen. However, this massive attenuation of contractile function was completely reversed by day 12 post-inoculation, at which time, ET_B receptor binding levels were also similar to those observed in control tissue. Thus, resolution of the infection by day 12 post-inoculation was accompanied by recovery of airway smooth muscle ET_B receptor expression and ET_B receptor-mediated contraction as was observed and is similar to that seen in influenza-A-infected mice (Carr *et al.*, 1996).

Although parainfluenza-1 infection in rats did not apparently involve any significant alterations in endothelin receptor expression, attenuation of signal transduction pathways in rat tracheal airway smooth muscle remained a possibility. This was investigated at day 4 post-inoculation, at which time the rats were hyperresponsive to inhaled methacholine. Indeed, at day 4 there was a significant loss in tracheal airway smooth muscle sensitivity to endothelin-1 and sarafotoxin S6c. However, in contrast to the situation in mouse trachea, there was no virus-associated reduction in the magnitude of contraction induced by 100 nM sarafotoxin S6c in rat trachea. Given that there was a significant loss in airway smooth muscle sensitivity to all agonists tested including carbachol, a non-specific loss in airway smooth muscle contractility may have occurred.

Table 2 Effect of parainfluenzea-1 virus-infection on rat tracheal airway smooth muscle responsiveness at day 4 post-inoculation

	Control	*Virus*
Carbachol		
E_{max} (mg)	2462±108	2359±130
EC_{50} (μM)	0.46 (0.36–0.58)	0.93 (0.81–1.08)*
Sarafotoxin S6c		
E_{max} (%C_{max})	64.2±7.0	53.4±10.2
EC_{50} (nM)	7.4 (5.7–9.6)	12.3 (10.0–15.2)*
Endothelin-1		
E_{max} (%C_{max})	81.2±3.1	81.6±9.3
EC_{50} (nM)	10.5 (6.8–16.0)	33.1 (21.7–50.6)*

E_{max} for carbachol expressed in mg tension and that for 100 nM sarafotoxin S6c and 300 nM endothelin-1 as a percentage of the response to 10μM carbachol (mean± s.e.mean). EC_{50}s for carbachol and sarafotoxin S6c are the concentrations of agonist producing 50% contraction of their own maximal responses (95% confidence limits). EC_{40} for endothelin-1 is the concentration of endothelin-1 causing a 40% C_{max} response (95% confidence limits). *$P<0.05$ compared to respective control. Data taken from experiments using 9–10 rats.

We have previously shown that in rat trachea (Henry, 1993) and in mouse trachea (Carr *et al.*, 1996), where both ET_A and ET_B receptors are involved in the contractile response to endothelin-1, it is necessary to inhibit both receptor subtypes before significant attenuation of endothelin-1-induced contraction becomes apparent. Virus infection caused a selective attenuation of ET_B receptor function. Predictably then, virus infection was not associated with significant attenuation of endothelin-1-induced contraction which is normally mediated via both ET_A and ET_B receptors. However, when the response to endothelin-1 was examined in virus-infected mouse tracheal preparations in the presence of the ET_A receptor-selective antagonist BQ-123, there was now a marked rightward shift in the endothelin-1 concentration-effect curve. This is consistent with virus-infected mouse trachea having a selective impairment of the ET_B receptor-effector system, leaving only the ET_A receptor system fully functional. BQ-123-induced inhibition of these ET_A sites now predictably resulted in significantly attenuated contraction to endothelin-1.

In contrast, in rat tracheal airway smooth muscle, the ET_B receptor density in airway smooth muscle in virus-infected

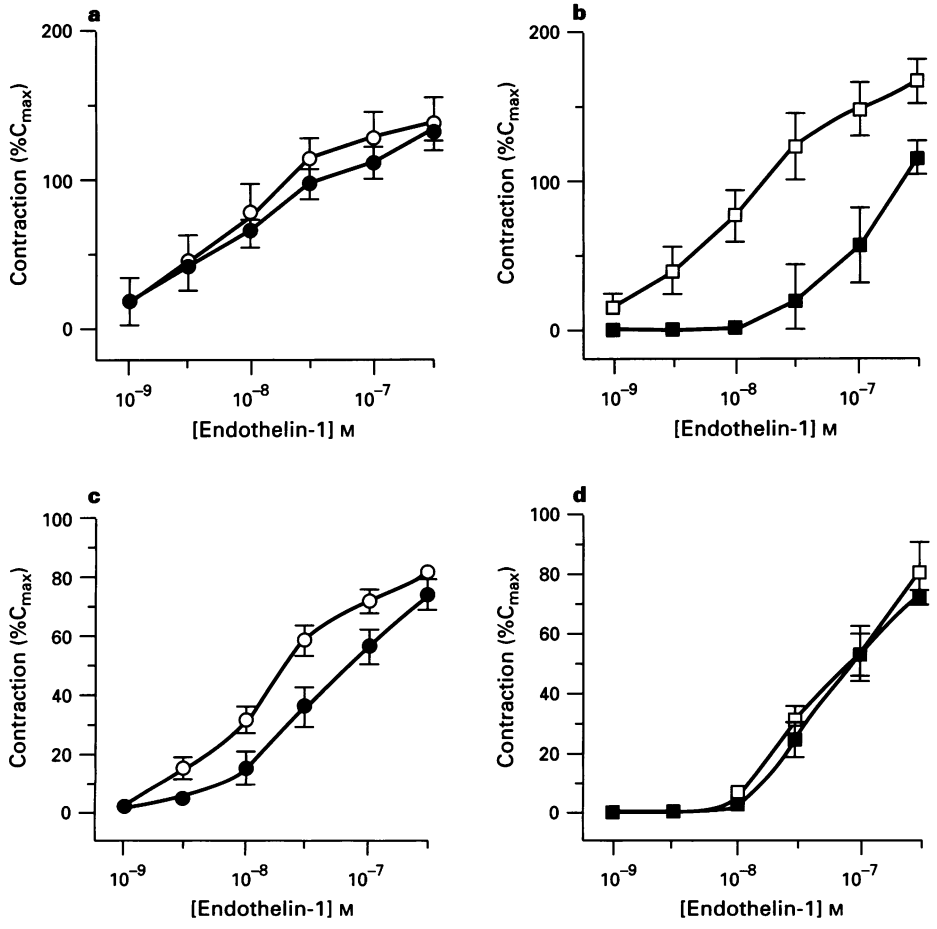

Figure 5 Cumulative concentration-effect curves to endothelin-1 in tracheal smooth muscle preparations from parainfluenza-1-infected (□, ■) or control (○, ●) mice (a, b) and rats (c, d) at day 4 post-inoculation in the presence (solid symbols) and absence (open symbols) of the ET_A receptor-selective antagonist BQ-123 (1 μM). Data are expressed as the mean±s.e.mean from 4 to 8 animals.

tissue was similar to that seen in control tissue. Consistent with this, BQ-123 had no effect on endothelin-1-induced contractions in tracheal preparations obtained from virus-inoculated rats. Functional data therefore supports the autoradiographic studies and suggests that parainfluenza-1 infection dramatically modulated the ET_B receptor-effector system in mice and not in rats. The reasons for this marked species difference are unclear, particularly as parainfluenza-1 virus infection was clearly strongly established in both species.

Various cytokines, which have been shown to be expressed during respiratory tract viral infection (Hennet *et al.*, 1992), induce the release of endothelin-1 from cultured airway epithelial cells (Endo *et al.*, 1992). It has been proposed that increased airway endothelin levels may modulate the expression of endothelin receptors (Henry & Goldie, 1994; Carr *et al.*, 11996). The nature of the inflammatory response to viral infection will determine the tissue cytokine profile, which may be critical in determining the effects of infection on the expression of airway endothelin receptors. Responses to parainfluenza-1 virus in the rat and mouse may be sufficiently different so that airway endothelin-1 levels and consequently receptor expression are differentially influenced.

In summary, we have demonstrated that parainfluenza-1 virus infection in mice reduced the density of tracheal airway smooth muscle ET_B receptors. This was reflected in reduced responsiveness to sarafotoxin S6c. In contrast, whilst parainfluenza-1 infection in rats was associated with the histological features and bronchial hyperresponsiveness common to respiratory tract viral infection, there was no selective down-regulation in ET_B receptor expression or functional activity. The reasons for these differences are not clear, but may relate to differences in the airway inflammatory responses to parainfluenza-1 virus in these species.

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