Impact of parainfluenza-3 virus infection on endothelin receptor density and function in guinea pig airways

Angela C. D’APRILE*†, Lynette B. FERNANDES*†, Paul J. RIGBY*† and Roy G. GOLDIE*†

*Western Australian Institute for Medical Research, B Block, Hospital Avenue, Nedlands, WA 6009, Australia, and †Department of Pharmacology, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

ABSTRACT

We examined the impact of parainfluenza-3 (P-3) respiratory tract viral infection on the density and function of endothelin (ET) receptor subtypes (ETA and ETB) in guinea pig tracheal smooth muscle. Total specific binding of $[^{125}\text{I}]$ET-1 and the relative proportions of ETA and ETB binding sites for this ligand were assessed at day 0 (control) and at 2, 4, 8 and 16 days post-inoculation. At day 0, the proportions of ETA and ETB binding sites were 30% and 70% respectively. Total specific binding was significantly reduced at day 4 post-inoculation (32% reduction, $n=8–12$, $P<0.05$) and was largely due to a corresponding fall in ETB receptor density at this time point (38% reduction, $n=8–12$, $P<0.05$). The density of ETA receptors also fell significantly at day 8 post-inoculation (33% reduction, $n=6–12$, $P<0.05$). By day 16 post-inoculation, the densities of ETA and ETB receptors had recovered to control values. The ratio of ETA:ETB receptor subtypes did not alter with P-3 infection. While P-3 infection reduced the density of tracheal smooth muscle ETA and ETB receptors, the contractile sensitivity and maximum response to carbachol and ET-1 was not altered in tissue from day 4 post-inoculation compared with the control. There seems to be a significant functional reserve for both receptor subtypes in this species that buffers the impact of P-3 infection on airway smooth muscle responsiveness to ET-1.

INTRODUCTION

Respiratory tract viral infections are associated with up to 60% of all exacerbations and 80% of all episodes of asthma-like symptoms [1,2]. The reasons for this remain unclear, but may be linked to accompanying airway inflammation and hyperresponsiveness [3,4]. Respiratory viral infections are relatively transient, with peak lung virus titres occurring 4–5 days after initial exposure, followed by falling viral loads and resolution of inflammation, epithelial damage and luminal debris [3].

Endothelin-1 (ET-1) is a highly potent, endogenous, epithelium-derived spasmogenic and mitogenic peptide, and is an increasingly credible candidate as a significant mediator in asthma [5,6]. Asthma is associated with elevated expression and release of ETs from the bronchial epithelium [7,8], which could act as paracrine hormones to activate a range of responses, including fibroblast and airway smooth muscle proliferation, increased airway tone and mucous hypersecretion. Each of these actions is consistent with a mediator role in asthma. Importantly, we have now shown that influenza-A virus infection in mice also caused marked increases in epithelial ET production [9]. Thus, virally triggered asthma may be associated with increased ET production and activity in the bronchial wall.

Influenza-A virus infection in mice significantly reduced ET$_B$ receptor number in tracheal smooth muscle, but increased ET$_A$ receptor density [10,11]. This resulted in reduced ET$_B$ receptor-mediated contraction, although responsiveness to the dual ET$_A$/ET$_B$ receptor agonist ET-1 was not significantly altered. We were interested to

Key words: airway smooth muscle, radioligand binding, respiratory virus, smooth muscle contraction.

Abbreviations: $C_{\text{max}}$, maximal contractile capacity; ET, endothelin; P-3, parainfluenza-3; Stx, sarafotoxin; TCID$_{50}$, tissue culture infectious dose.

Correspondence: Professor R. G. Goldie (e-mail rgoldie@pharm.uwa.edu.au).
determine whether similar effects occurred in response to other respiratory tract viruses in other animal species. Thus, we have evaluated the effects of parainfluenza-3 (P-3) virus infection on ET$_\alpha$/ET$_\beta$ receptor densities and on ET-1-mediated contraction in tracheal airway smooth muscle from the guinea pig.

**METHODS**

LLCMK cells were cultured in minimal essential medium with Earle’s balanced salts and 10% foetal calf serum. At confluence, the medium was replaced with serum-free medium containing trypsin (8 $\mu$g/ml) and P-3 virus. A cytopathic effect was evident in LLCMK cells after 4 days, at which time the virus-containing medium was removed, centrifuged to remove debris and aliquots stored at –85°C. Virus infectivity was estimated using a haemadsorption assay and the tissue culture infectious dose (TCID$_{50}$) was 5 x 10$^5$ units/ml.

Male Tricolour guinea pigs (350–500 g) were anaesthetized with chloral hydrate (300 mg/kg; intraperitoneally) and inoculated intranasally on day 0 with 150 $\mu$l of either 6 x 10$^3$ TCID$_{50}$ of P-3 virus or virus-free culture medium.

Guinea pigs were sacrificed by pentobarbitone overdose (200 mg/kg; intraperitoneally) on days 0, 2, 4, 8 or 16 post-inoculation and the lungs were removed.

Tracheal tube segments were submerged in Macrodex (6% dextran 70 in 5% glucose), frozen by immersion in isopentane and quenched with liquid nitrogen. Serial transverse sections (10 $\mu$m) were cut at –20°C and thaw-mounted onto gelatin chrom-alum 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 $\mu$M), catalase (50 units/ml) and EDTA (1 mM).

Sections were then incubated for 180 min in buffer (100 nM). Non-specific binding was determined in the presence of both BQ-123 (1 $\mu$M) and Stx S6c (100 nM). Specific binding, determined in the presence of both BQ-123 (1 $\mu$M) and Stx S6c (100 nM), accounted for approx.

radiographic grain densities are expressed as grains/1000 $\mu$m$^2$ and are presented as the mean grain density ± S.E.M.

Tracheal rings, obtained from guinea pigs on day 4 post-inoculation, were suspended under an initial tension of 0.5 g in Krebs’ bicarbonate solution (117 mM NaCl, 5.36 mM KCl, 25.0 mM NaHCO$_3$, 1.03 mM KH$_2$PO$_4$, 0.57 mM MgSO$_4$, 7H$_2$O, 2.5 mM CaCl$_2$, 2H$_2$O and 11.1 mM glucose). Krebs’ bicarbonate solution contained indomethacin (3 $\mu$M) and was aerated with 95% O$_2$/5% CO$_2$ and maintained at 37°C. Isometric tension measurements were recorded via FTO3 force displacement transducers (Grass Instruments) connected to a preamplifier and computer utilizing customized data acquisition software. Carbachol (10 $\mu$M) was used to confirm tissue viability and provide an estimate of maximal contractile capacity ($C_{max}$). Cumulative dose–response curves were constructed for carbachol as well as for ET-1. Contractile responses to ET-1 were expressed as a percentage (%$S.E.M.$) of $C_{max}$. The potency of ET-1 was calculated as the concentration required to produce 40% of $C_{max}$ ($pEC_{40}$).

Differences between treatment means were assessed by analysis of variance (SigmaStat) using a modified t statistic [13]. For statistical purposes, mean pEC40 data were transformed to $-\log_{10} EC_{40}$ and $P < 0.05$ was considered to be statistically significant.

**RESULTS**

The influence of P-3 virus infection on [$^{125}$I]ET-1 binding was assessed in guinea pig tracheal airway smooth muscle at days 0 (control), 2, 4, 8 and 16 post-inoculation. Non-specific binding, determined in the presence of both BQ-123 (1 $\mu$M) and Stx S6c (100 nM), accounted for approx.

![Figure 1](image.png) **Figure 1** Time-related effect of P-3 virus infection on total specific [$^{125}$I]ET-1 binding in guinea-pig tracheal smooth muscle

Values are means and error bars represent the S.E.M. of observations in tissue from 6–12 animals.

© 2002 The Biochemical Society and the Medical Research Society
Table 1  Time-related effect of P-3 virus infection on ET\(_{\text{A}}\) and ET\(_{\text{B}}\) receptor proportions in guinea pig tracheal smooth muscle

<table>
<thead>
<tr>
<th>Day</th>
<th>ET(<em>{\text{A}}):ET(</em>{\text{B}}) ratio</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30 ± 2:70 ± 2</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>28 ± 2:71 ± 2</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>34 ± 1:66 ± 2</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>24 ± 2:77 ± 2</td>
<td>6</td>
</tr>
<tr>
<td>16</td>
<td>26 ± 3:74 ± 5</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 1 - Time-related effect of P-3 virus infection on ET\(_{\text{A}}\) and ET\(_{\text{B}}\) receptor proportions in guinea pig tracheal smooth muscle. Values are mean ± S.E.M. of observations in tissue from 6–12 animals.

DISCUSSION

Autoradiographic studies revealed the presence of both ET\(_{\text{A}}\) (30%) and ET\(_{\text{B}}\) (70%) receptors in guinea-pig tracheal smooth muscle. Respiratory tract viral infection with P-3 caused a marked fall in total specific binding in airway smooth muscle at day 4 post-inoculation. This suggests that P-3 virus caused a decrease in ET receptor expression in this tissue. The fall in total specific binding was mostly owing to a transient decrease in ET\(_{\text{A}}\) receptor density at this day 4 post-inoculation time. In addition, ET\(_{\text{A}}\) receptor density was also reduced at day 8 post-inoculation, with full recovery by day 16. While we have shown previously that influenza A infection in mice is accompanied by decreased ET\(_{\text{B}}\) receptor density in tracheal airway smooth muscle [10,11], a compensatory increase in ET\(_{\text{A}}\) receptor density also shown previously contrasts sharply with the decrease in ET\(_{\text{A}}\) receptor density observed in the present study. Furthermore, although total specific binding levels fell at day 4 post-inoculation, the proportions of ET\(_{\text{A}}\) and ET\(_{\text{B}}\) binding sites did not significantly alter at any time point.

P-3 viral infection did not significantly alter contractile responsiveness of guinea pig tracheal smooth muscle to ET-1, when measured 4 days post-inoculation. This is in spite of the significant decrease in ET receptor expression at this time point. This suggests that in spite of a 32% decrease in ET receptor density, there is sufficient receptor reserve in guinea pig tracheal smooth muscle for responsiveness not to be compromised. Further studies should determine the effect of P-3 infection on the function of ET receptor subtypes and on ET levels in the airway wall.

ACKNOWLEDGMENTS

This research was supported by the National Health and Medical Research Council (Australia).

REFERENCES

8 Sofia, M., Mormile, M., Faraone, S. et al. (1993) Increased endothelin-like immunoreactive material on bronchoalveolar lavage fluid from patients with bronchial asthma and patients with interstitial lung disease. Respiration 60, 89–95
10 Henry, P. J. and Goldie, R. G. (1994) ET\textsubscript{B} but not ET\textsubscript{A} receptor-mediated contractions to endothelin-1 attenuated by respiratory tract viral infection in mouse airways. Br. J. Pharmacol. 112, 1188–1194