Contractile endothelin-B (ETB) receptors

M. Adner*, L.O. Cardell**, T. Sjöberg+, A. Ottosson++, L. Edvinsson*

Contractile endothelin-B (ETB) receptors in human small bronchi. M. Adner, L.O. Cardell, T. Sjöberg, A. Ottosson, L. Edvinsson. ©ERS Journals Ltd 1996.
ABSTRACT: Endothelins (ETs) are a family of novel regulatory peptides and various lines of evidence suggest an important role for ETs in regulating pulmonary function. Two receptors for endothelin, ETA and ETB, have been found in the human lung, and according to recent studies a non-ETA receptor seems to mediate the contraction of large sized human bronchi. Several studies have emphasized the importance of small bronchi in the pathogenesis of airway disease.

In the present paper, improved methodology was used which enables *in vitro* studies of small human bronchi down to a diameter of 0.5–1.0 mm. Using the new methodology we have tried to further characterize this receptor.

Small bronchi from the distal parts of the bronchial tree were obtained from pulmonary tissue removed from 15 patients with lung cancer. They were dissected and cut into ring segments, in which isometric tension was recorded.

ET-1, ET-2 and ET-3 elicited strong concentration-dependent contractions of the human small bronchus. Basically, the three peptides were equipotent with about the same maximal response. Upon reapplication, they all showed the same tachyphylaxis pattern, reaching half the initial contraction. Comparative analysis of IRL 1620, a selective ETB receptor agonist, revealed that the effect of the ETB agonist was, in all respects, similar to the responses induced by the ETs. PD 145065, a combined ETA/ETB receptor antagonist competitively inhibited the contractions induced by IRL 1620, whereas FR139317, a selective ETA receptor antagonist, was without effect.

In conclusion, the present study shows that accurate measurements can be made *in vitro* on small human bronchi and all present data are in favour of an ETB receptor mediating endothelin-induced contraction of human bronchi smaller than 1.0 mm.
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Depts of *Internal Medicine, Lund University Hospital, +Cardiothoracic Surgery, and ++Forensic Medicine, Lund University Hospital, Lund, Sweden. **Dept of Otorhinolaryngology, Malmö General Hospital, Malmo, Sweden.

Correspondence: M. Adner
Dept of Cellbiology 1
EB-blocket
Lund University Hospital
S-22185 Lund
Sweden

Most studies of isolated human and animal airways refer to experiments performed on trachea or on other relatively large airways. However, several recent reports emphasize the importance of small airways in the regulation and control of different lung functions [1, 2]. Some studies also indicate that substances like the endothelins (ETs), which can be endogenously released in lung, have different effects in peripheral and central parts of the lung [3, 4].

The human ET family consists of ET-1, ET-2 and ET-3, all derived from separate genes [5, 6]. Although the ETs are very potent vasoconstrictors, they have effects beyond their vasoconstrictor potential [7]. Aerosol administration of ET-1 induces bronchoconstriction without changes in the pulmonary perfusion pressure, lung wet weight or mediator release, whereas intra-arterial administration of ET-1 results in bronchoconstriction associated with increased pulmonary perfusion pressure, oedema formation and release of eicosanoids [8, 9]. Furthermore, cultured airway epithelial cells have the ability to secret

both ET-1 and ET-3 [10, 11]. This could indicate a role for the ETs in the airway system.

Two different ET receptors have been cloned; the ETA receptor, with binding affinity for ET-1 = ET-2 >ET-3, and the ETB receptor, with equipotent affinity for the three ET isopeptides [12, 13]. The ETA receptor is mainly situated on vascular smooth muscle cells mediating constriction, while the ETB receptor is more widely distributed [14]. Both receptor subtypes reside in the human lung [15]. Recent results suggest the involvement of a "non-ETA" receptor in the endothelin-induced contraction of isolated human major bronchi [16]. The exact nature of this receptor has not been characterized and it is not known if the same type of receptor is responsible for the endothelin-induced contraction seen in more distal parts of the bronchial tree.

We have previously demonstrated the presence of ETA and ETB receptors in the guinea-pig lung [17, 18]. The aim of the present study was to characterize the ET subreceptors in human small airways by the use of different

pharmacological tools; IRL 1620, a selective ET_B receptor agonist [19], FR139317, a selective ET_A receptor antagonist [18, 20], and PD 145065, a newly developed combined ET_A and ET_B receptor antagonist [21].

Material and methods

In vitro *pharmacology*

Pulmonary tissues were removed from 15 patients with lung cancer. The protocol was approved by the Ethics Committee of University Hospital of Lund, Lund, Sweden. After extirpation, the fresh part of the lung (not damaged by the cancer) was immediately immersed in cold (4°C) buffer solution, aerated with carbogen gas with 5% CO_2 in O_2, resulting in a pH of 7.4. Small bronchi, from distal parts of the bronchial tree were dissected and cut into ring segments (1–1.5 mm long, with a diameter in the resting state of 0.5–1.0 mm). Each segment was mounted on two L-shaped metal prongs (0.2 mm in diameter), one of which was connected to a force displacement transducer (FT03C) attached to a MacLab unit for continuous recording of the isometric tension.

The mounted specimens were immersed in temperature-controlled (37°C) tissue baths containing a buffer solution (see below). The solution was continuously aerated with carbogen gas resulting in a physiological pH. Length-tension measurement was performed, comparing activated segments (exposed to 1 mM acetylcholine) with similar segments exposed to Ca^{2+}-free buffer solution for 24 h, by step-wise increased of the distance between the metal prongs [22]. Thus, a tension of 1–2 mN was applied to the segments and they were allowed to stabilize at this level for 90 min. The contractile capacity of each segment was examined by repetitive exposure to 1 mM acetylcholine, which was used as a reference. Only segments with strong and reproducible contractions were used, *i.e.* less than 10% difference between two acetylcholine-induced contractions. After 30 min rest, the effects of the agonists were examined by cumulative application. There were no differences in the responses to ETs when concentration-response curves were obtained by cumulative application compared to a single-dose procedure. The tachyphylaxis caused by ET-1 was investigated by the use of homologous desensitization tests. The bronchi were first contracted with ET-1, ET-2 or ET-3. After repeated washes, during a 90 min period, the initially contracted segment had returned to its resting state (baseline). A single dose of the same peptide (0.3 µM) was then reapplied to the segment [17]. In antagonist experiments the segments were preincubated with FR139317 (10 µM) or PD 145065 (1 µM) for 15–20 min before agonists were applied.

Light microscopy

The bronchial segments corresponding to the *in vitro* studies were immersed into a formalin solution (6%). After fixation and staining with haematoxylin-eosin the segments were cut into thin cross-sections for examination with a light microscope.

Solution and drugs

Buffer solution (mM): NaCl, 119; $NaHCO_3$, 15; KCl, 4.6; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $CaCl_2$, 1.5; and glucose, 11.

Drugs: ET-1, ET-2, ET-3 (Auspep, Parkville, Australia); IRL 1620 Suc[Glu^9, $Ala^{11,15}$]- endothelin-1(8-21) (Ciba Geigy, Takarazuka, Japan); FR139317 (R)2-[(R)-2-[(S)-2-[[1-(hexahydro-lH- azepinyl)]carbonyl]-amino-4-methylpentanoyl]amino-3[3-(1-methyl-1H-indolyl)]-propionyl]-amino-3-(2-pyridyl)propionic acid (Fujisawa Pharmaceuticals Co., Osaka, Japan); PD 145065, Ac-D-Bhg^{16}-Leu-Asp-IIe-Ile-Trp^{21} (Parke-Davies Pharmaceutical Research, Ann Arbour, MI, USA); acetylcholine and histamine (Sigma, St Louis, MO, USA). The drugs were dissolved and further diluted in buffer solution or saline. Concentration-dependent potassium-induced contraction was performed using the above-mentioned buffer solution, exchanging NaCl for KCl giving a step-wise increase in potassium concentration up to 123.6 mM.

Analyses

The responses were characterized by pD_2 values (*i.e.* the negative logarithm of the agonist concentration eliciting half the maximum contraction) and E_{max} values (*i.e.* maximum contractile response induced by an agonist, expressed as a percentage of acetylcholine induced contraction). Statistical differences were determined with Wilcoxon signed-rank test and Mann-Whitney U-test for paired and unpaired groups, respectively. Probability values less than 0.05 were considered significant.

Results

The human bronchus segment used in this study (0.5–1.0 mm in diameter) presented discontinuous plates of cartilage and a typical pseudostratified columnar epithelium with numerous interspersed goblet cells (fig. 1). A layer of smooth muscle cells encircled the segments (fig. 1). According to the length-tension measurements these bronchi were able to develop their maximal contractile capacity at the passive level of 1–2 $mN·mm^{-1}$ (not shown).

Acetylcholine induced a concentration-dependent contraction with a pD_2 of 5.44±0.17 (number of patients (n)=2, numbers of segments tested (z)=8) and a maximal contraction of 1.92±0.18 mN (n=15, z=50). This response was without any sign of tachyphylaxis (fig. 2). Histamine (pD_2 6.03±0.13; E_{max} 95.6±9.8%; n=2, z=8) and potassium (pD_2 1.59±0.11; E_{max} 53.1±10.2%, n=2,
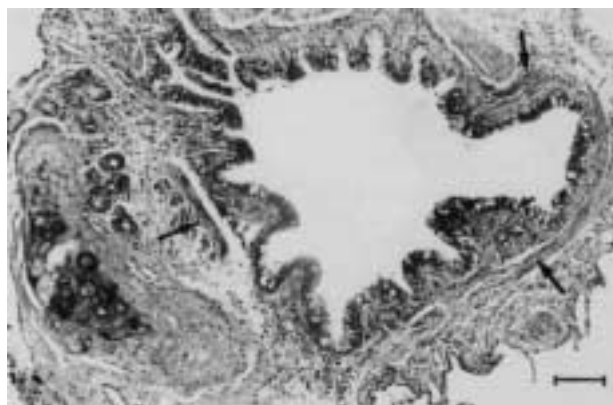
Fig. 1. – Histological section showing the cross-section of a small human bronchus. A discontinuous plate of cartilage, epithelial lining and goblet cells are visible. Arrows indicate the smooth muscle cell layer. (haematoxylin and eosin staining; internal scale bar=100 µm).

z=8) provoked concentration-dependent contractile responses with the same principal appearance as acetylcholine, *i.e.* fast developing and reproducible.

ET-1, ET-2 and ET-3 elicited similar strong, concentration-dependent contractions in human bronchus segments (table 1). The contraction was slowly developing and long-lasting (fig. 2). There were no significant differences in the tachyphylaxis for the three peptides; they all reached 57.6±8.3% of the initial contraction upon reallocation (n=3, z=7; not shown). IRL 1620, a selective ETB receptor agonist, induced a similar contractile response to the three endothelins and the Emax and pD2 values were identical with the values for the ETs (table 1).

The ET-1-induced contraction was not affected by incubation with the selective ETA receptor antagonist FR139317 (10 µM) (fig. 3a); pD2-values were 8.55±0.21 without and 8.72±0.20 with antagonist (NS) and Emax values were 102.8±8.9 and 100.6±15.7%, respectively (NS). The combined ETA/ETB receptor antagonist PD 145065 (1 µM) demonstrated a significant rightward shift of the IRL 1620-induced curve (pD2 6.79±0.36; p<0.05) without affecting the Emax (110.1±36.0%, NS) (fig. 3b).

Table 1. – The effects of ET-1, ET-2 and ET-3 and the ETB receptor selective agonist IRL 1620 on human small bronchi

	n	pD2	Emax
ET-1	8	8.58±0.24	99.3±13.2
ET-2	5	8.73±0.19	84.9±14.2
ET-3	5	8.05±0.38	96.5±13.9
IRL 1620	12	7.97±0.24	107.0±12.8

Emax: maximum contractile response in percentage acetylcholine-induced maximum contraction; ET: endothelin. pD2: potency expressed as negative log of agonist concentration eliciting 50% of maximum contraction. The values are presented as mean±SEM, and the difference between groups are analysed by Mann Whitney U-test. No significant differences were seen between the agonists.
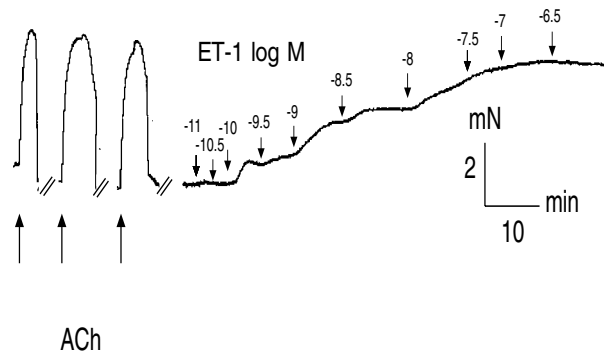

Fig. 2. – Typical example of concentration-dependent endothelin-1 (ET-1)-induced contraction of small human bronchus. At the beginning of the experiment the segment was repetitively (three times) exposed to 1 mM acetylcholine (ACh) (used as a reference). Acetylcholine was removed by washing. Time between the contractions was not recorded. ET-1 concentrations are given as log M at the curve.
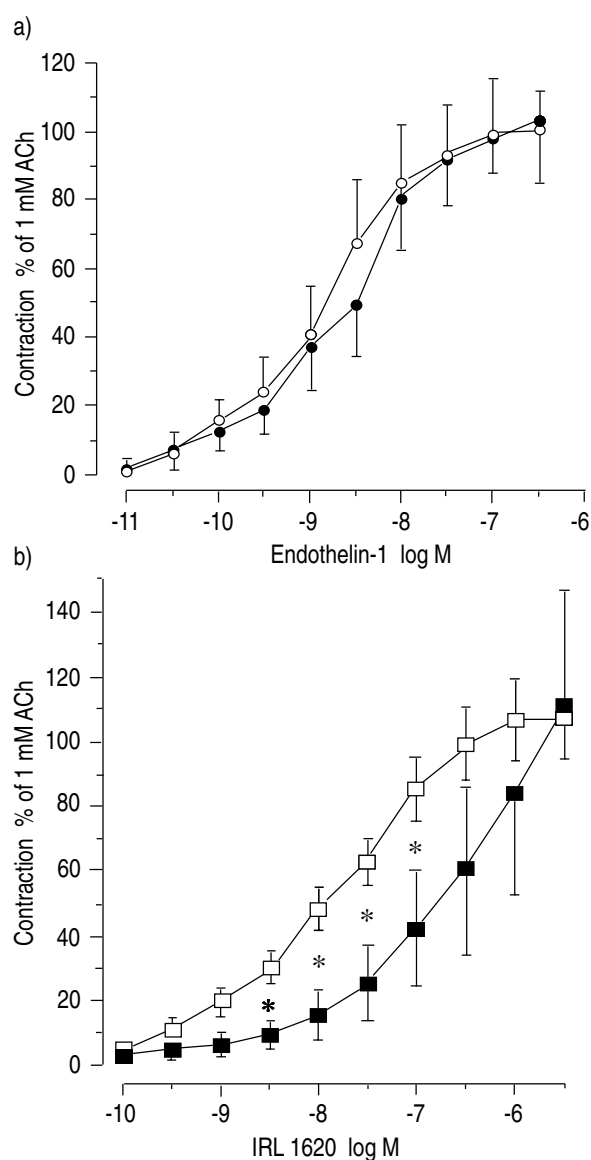
Fig. 3. – Endothelin (ET)-induced contractions in small human bronchi. a) ET-1 in absence (—●—) and presence (—○—) of 10 nM FR139317 (n=6). b) IRL 1620 in absence (—□—) and presence (—■—) of 1 nM PD 145065 (n=6–12). Responses are expressed as percentage of the contraction induced by 1 mM acetylcholine (ACh). Each point represents the mean with the SEM shown by vertical bars. *: p<0.05.

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Discussion

The present results indicate that the contractile capacity of human small bronchi located in the distal part of the tracheobronchial tree can be examined *in vitro* by the use of a modified tissue-bath technique. Reproducible values for acetylcholine, histamine and potassium were obtained. Furthermore, the three endothelins, ET-1, ET-2 and ET-3, as well as the ETB receptor agonist IRL 1620, induced concentration-dependent, strong contractions of isolated small segments.

A multitude of techniques have been utilized for studying mechanical activity in small isolated vascular smooth muscle preparations, but with a few exceptions most established *in vitro* methods for studying airways are confined to relatively large bronchi. However, the distal parts of the tracheobronchial tree are of considerable pathophysiological importance and several studies have shown that peripheral airways represent the most important part of the airways in the genesis of asthma and chronic obstructive diseases [1, 2]. Furthermore, it is in bronchi smaller than 2 mm that morphological changes as a result of smoking have been demonstrated [23]. The present method allows studies of mechanical activity to be performed on small bronchi with lumen diameter down to 0.5 mm. At this level, contraction of the thin smooth muscle cell layer that encircle the airway wall could result in a narrow obstruction [24]. In order to evaluate the optimal preload for detection of bronchial smooth muscle responses, length-tension measurements were performed according to HÖGESTÄTT *et al*. [22]. These measurements revealed that the preload should be about 1–2 mN·mm^{-1}. It is reasonable to assume that this preload reflects a diameter at which the interaction between the contractile filaments is the optimum [25].

The effect of well-known contractile agonists were tested on the isolated bronchial segments. Acetylcholine and histamine showed a very similar contractile profile without any significant differences in E_{max} or pD_2. The results are consistent with the results of ADVENIER *et al*. [26] in bronchi with a diameter of 3–5 mm. The buffer solution with a high potassium concentration showed contractions which were only 53% of the acetylcholine-induced contraction. When exposed to a single dose (1 mM) of acetylcholine, these small bronchi showed a clear-cut contraction which was stable as well as reproducible, and therefore used as a reference [27].

Earlier investigations have shown that ETs are potent and strong contractors of human bronchi [16, 26, 27]. These studies were performed on large bronchi with a diameter of 3–15 mm. In the present study, we examined smaller (0.5–1 mm) bronchi without semilunar cartilage. The ET-1-induced response at our distal level appeared to be notably stronger than the corresponding response previously reported for larger more central airways [16, 26, 27].

The ET-induced contractions were slowly developing and long lasting. The contracted bronchus could easily be relaxed by washing, during which the segment quickly returned to the "resting" state and reapplication of an ET-agonist resulted in a contraction corresponding to more than 50% of the initial tension level. These findings are consistent with previous findings in guinea-pig airways [17], and indicate the presence of an ETB receptor. In contrast, the ETA receptors is in most *in vitro* systems characterized by difficulties in "wash out", and by a complete tachyphylaxis upon reapplication [17]. The three ETs demonstrated equal potency which pharmacologically supports the presence of a contractile ETB receptor [28].

To further analyse the presence of another contractile subtype than the ETA receptor, recently developed pharmacological tools were used. IRL 1620, a selective ETB receptor agonist [19], showed a contractile response similar to that induced by the ETs. The ETA receptor antagonist FR139317 did not affect the ET-1-induced contraction, whereas the combined ET-receptor antagonist PD 145065 (1 μM) shifted the IRL 1620-induced concentration-response curve to the right in a competitive manner, suggesting that the contraction of human small bronchi is mediated mainly by ETB receptors.

The present study shows that accurate *in vitro* studies can be performed on small human bronchi down to a diameter of 0.5 mm. At this level ET-1, ET-2 and ET-3 induce strong and potent contractions, which can be mimicked by an ETB agonist (IRL 1620) and blocked by a combined ETA/ETB receptor antagonist (PD 145065), whereas a selective ETA receptor antagonist (FR139317) has no effect. Our pharmacological results, indicating the presence of a contractile ETB receptor on small human bronchi are in good agreement with recent autoradiographic findings, indicating dominance of ETB receptors in the human peripheral lung [29].

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