Aerosolized endothelin-1, but not its C-terminal hexapeptide, causes airway narrowing in the rat

G.U. Di Maria*, S. Bellofiore*, L.S. Malatino**, C.A. Maggi***, A. Torrisi*, A. Mistretta*


ABSTRACT: We investigated the effects of aerosolized endothelin-1 (ET-1) and of its C-terminal hexapeptide, ET-(16-21), on the pulmonary mechanics of anaesthetized spontaneously-breathing rats. ET-1 inhalation caused a concentration-dependent increase in pulmonary resistance (Rt) and decrease in dynamic lung compliance (Cdyn). In control conditions Rt and Cdyn were 0.17±0.04 cmH2O·mL−1·s (mean±SE; n=6) and 6.25±0.66 mL·cmH2O−1, respectively. After ET-1 4×10−5 M Rt and Cdyn averaged 1.45±0.28 cmH2O·mL−1·s (p<0.05) and 1.12±0.36 mL·cmH2O−1 (p<0.05), respectively. The pulmonary responses to ET-1 lasted up to 60 min. By contrast, ET-(16-21) up to a concentration of 10−4 M did not affect pulmonary mechanics. These results indicate that the bronchoconstrictor activity of aerosolized endothelin-1 in the rat is not dependent on its C-terminal sequence, and suggests a role for endothelin-1 in the regulation of airway calibre.


Methods

We studied 12 Sprague-Dawley female rats (aged 8–10 wks; body weight 182–204 g). Rats were anaesthetized with thiopental sodium (50 mg·kg−1 i.p.), and orally intubated with a 6 cm length of polyethylene catheter (Intramedic PE-240; i.d. 1.6 mm) introduced into their trachea. A heating pad maintained their body temperature constant throughout the experiment. Rats were placed in the left lateral decubitus posture, with the tip of the tracheal catheter protruding into a small Plexiglas cylinder. A pneumotachograph (Mercury F10 L, Mercury Electronics Ltd, Glasgow, UK), coupled to a differential pressure transducer (Statham PM97 ±3.5 cmH2O) was attached to the other end of the cylinder to measure airflow (V). Oesophageal pressure (Poes) was measured with a saline-filled catheter-transducer system. The oesophageal catheter consisted of a polyethylene tube (Intramedic PE-200; i.d. 1.4 mm) 25 cm long that was advanced into the oesophagus of the rat until a clear cardiac artifact was discerned. The other end of the catheter was connected to a pressure transducer (Bentley Trantec 800 -100 +300 cmH2O). The pressure at the airway opening (Pawo) was measured using a pressure transducer (Honeywell 143PC03D ±150 cmH2O) connected to the Plexiglas cylinder.

Electrical signals of V, Poes and Pawo were amplified, filtered by a low-pass filter with a threshold of 20 Hz and converted to digital signals by a 12-bit analogue-to-digital converter (Data Translation DT2801-A). Signals were sampled at a frequency of 60 Hz for 30 s periods and stored on diskettes using a personal computer (Epson AX2). Transpulmonary pressure (Ptp) was calculated as the difference between Pawo and Poes. Tidal volume (VT) was obtained by digital integration of the V signal. Total pulmonary resistance (Rt) and elastance (E) during spontaneous breathing were calculated using a method previously described in detail [7]. Briefly, the data of Ptp, VT and V were fitted to the equation of motion of the respiratory system:
Ptp = RL × ˙V + E × Vt + k

where k is the value of Ptp at end-expiration. The three unknown parameters (RL, E and k) were calculated by multiple linear regression analysis using the least-squares method [7]. Dynamic compliance of the lung (Cdyn) was calculated as the reciprocal of E. The resistance of the tracheal catheter was 0.11 cmH2O·ml·s at a flow rate of 25 ml·s and was almost linear over the observed flow range (±25 ml·s). The resistance of the tracheal catheter was subtracted from all RL values.

After baseline measurements, six rats inhaled aerosols of normal saline and progressively increasing molar concentrations of endothelin-1 (ET-1) dissolved in saline (from 10 to 4×10 M). Another group of six rats was challenged with saline and ET-(16-21) from 10 to 10 M. Aerosols were generated by a Hudson nebulizer that was driven by a compressed air source with an output of 0.18 ml·min and delivered for two minute periods through a side-port of the Plexiglas cylinder. During aerosolization, the pneumotachograph was occluded and airflow was diverted through a second side-port. The time elapsed between each exposure was 20 min.

Measurements of RL and Cdyn were obtained 5, 10, 15 and 20 min after each inhalation in both groups of rats. RL and Cdyn were also followed up to 30 and 60 min after the last concentration of drug was inhaled. In the intervals between measurements, the cylinder was flushed with fresh air to avoid accumulation of carbon dioxide. Post-saline values of RL and Cdyn were used as control values. The concentration of drug required to double RL (ECRL) was computed by linear interpolation from individual dose-response curves and was used as an index of airway reactivity. All data are expressed as mean±SEM. Statistical analysis was performed by using the one-way analysis of variance for repeated measurements and the Dunnett t-test for paired contrasts. Statistical significance was accepted for p values less than 0.05. Drugs used were thiopental sodium (Farmitalia-Carlo Erba, Milan, Italy) and endothelin-1 (Peninsula Laboratories Inc., Merseyside, UK). The C-terminal hexapeptide of endothelin-1 was synthesized by conventional solid phase methods by Dr P. Rovero (A. Menarini Pharmaceuticals, Florence, Italy).

Results

Saline inhalation did not affect either RL or Cdyn in both groups of rats. Changes in RL and Cdyn occurred within 5 min after inhalation of ET-1; peak responses were observed between 10–15 min and used for analysis. Changes in pulmonary mechanics in response to ET-1 are shown in figure 1A. Inhalation of ET-1 elicited a concentration-dependent increase of RL and decrease of Cdyn in all six rats. Control RL was 0.17±0.04 cmH2O·ml·s and rose substantially to 1.45±0.28 cmH2O·ml·s (p<0.05) after ET-1 4×10 M. Conversely, Cdyn fell from 6.25±0.66 to 1.12±0.36 ml·cmH2O·s (p<0.05). However, a wide individual variability in airway reactivity to ET-1 was observed. In fact, ECRL ranged between 10 and 2×10 M. The bronchoconstriction caused by ET-1 persisted throughout the follow-up period. Mean values of RL and Cdyn measured at 30 and 60 min after the last inhalation of ET-1 are reported in table 1.

By contrast, inhalation of ET-(16-21) did not affect pulmonary mechanics in all rats (fig. 1B). RL averaged 0.26±0.04 and 0.25±0.04 cmH2O·ml·s in control conditions and after 10 M ET-(16-21), respectively. Similarly, Cdyn did not change (3.61±0.37 vs 3.76±0.37 ml·cmH2O·s).

![Figure 1](image_url)

**Table 1.** RL and Cdyn values measured in control conditions and after the inhalation of the highest concentration of ET-1, and 30 and 60 min later

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control</th>
<th>ET-1 30 min</th>
<th>ET-1 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL cmH2O·ml·s⁻¹</td>
<td>0.17</td>
<td>1.45*</td>
<td>1.18*</td>
</tr>
<tr>
<td>Cdyn ml·cmH2O·s⁻¹</td>
<td>6.45</td>
<td>1.12*</td>
<td>2.6*</td>
</tr>
</tbody>
</table>

RL: total pulmonary resistance; Cdyn: dynamic lung compliance; ET-1: endothelin-1. Values are mean±SEM; *: p<0.05 vs control values.
Discussion

This study demonstrates that aerosolized endothelin-1 but not its C-terminal hexapeptide, affects pulmonary mechanics in anesthetized, spontaneously-breathing rats. Inhalation of ET-1 induced a prominent increase in pulmonary resistance and a marked decrease in lung compliance. The bronchopulmonary responses to inhaled ET-1 peaked between 5–15 min and persisted up to 60 min. This pattern of response is very similar to that observed in previous studies in vivo [3], but substantially differs from the in vitro response to intravenously injected ET-1 in guinea-pigs [6], in which the changes in pulmonary compliance and tracheal pressure had a rapid onset and lasted about 10 min. Different factors may account for this discrepancy. In our model the interference of the systemic effect of ET-1 was ruled out and probably the amount of drug delivered to the airways was larger than that in the studies in which ET-1 was administered intravenously [6, 8]. The route of administration could have affected the mode of action of ET-1 on airway smooth muscle, with obvious consequences on the onset and duration of its activity. It has been demonstrated that ET-1 induced airway smooth muscle contraction in vitro is highly sensitive to Ca\textsuperscript{2+}-channel blockers, and it seems to be due to a direct mechanism [3]. By contrast, it has been reported that the bronchial response induced by intravenously injected ET-1 in guinea-pigs is not affected by Ca\textsuperscript{2+}-channel blockers but is almost completely abolished by cyclooxygenase inhibitors [6, 8]. These observations strongly suggest that the mechanisms by which ET-1 causes bronchoconstriction are dependent on the route of administration and/or experimental procedures.

On the other hand, the rate of ET-1 degradation, which has been shown to be rather fast after i.v. administration [9], could be much slower when ET-1 is inhaled. The long lasting airway narrowing observed in our study is in accordance with previous findings showing that the bronchopulmonary response induced by inhalation of ET-1 in guinea-pigs lasted more than 20 min [5]. A similar time course has not been previously reported after administration of other bronchoconstrictive agents, and it may suggest that in response to ET-1 other factors, such as mucus accumulation and airway wall oedema, were associated with airway smooth muscle contraction. This hypothesis is supported by the morphological findings of mucus plugs in the conductive airways of guinea-pigs challenged with intravenous ET-1 [6].

The large individual variability of the response to ET-1 is not surprising if we consider that the study has been carried out on outbred rats. In fact, it is well recognized that the airway responsiveness to histamine has a wide range of variability in both outbred guinea-pigs [10] and mongrel dogs [11].

The inhalation of ET-(16-21) did not affect pulmonary mechanics in the rat. This occurred despite the fact that the highest concentration of ET-(16-21) was about 100 times greater than the concentration of ET-1 causing the highest bronchopulmonary response. This lack of activity of ET-(16-21) on rat airways in vivo is in agreement with a recent report in which it has been observed that ET-(16-21) does not alter pulmonary mechanics in mechanically-ventilated cats [12].

ET-(16-21), the C-terminal sequence common to all endothelins, has been proposed to play a crucial role in the biological activity of ET-1 [1]. In this regard, it has been shown that the C-terminal free acid Trp in position 21 has a pivotal importance for the vasoconstrictor activity of ET-1 in vitro [13]. It has been also demonstrated that ET-(16-21) is a full agonist, although 30 times less potent than ET-1, in the isolated guinea-pig bronchial smooth muscle [4]. However, ET-(16-21) was not able to contract other smooth muscle preparations from different species in vitro [14, 15]. Taken together, these findings indicate that ET-(16-21) has a full agonist activity only in selected preparations.

Our study demonstrates that endothelin-1 increases pulmonary resistance in the rat and that its activity is comparable to that of other peptides, such as tachykinins, which have been shown to play a significant role in regulating airway calibre [16]. These findings, in association with the recent reports that ET-1 binding sites are largely present in rat lungs [17], and that ET-1 is produced by tracheal epithelial cells [18], suggest that this peptide may contribute to the regulation of bronchial tone.

References

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C'est l'endotheline aéroliisée, et non son hexapeptide C-terminal, qui provoque le rétrécissement des voies aériennes chez le rat. G.U. Di Maria, S. Bellofiore, L.S. Malatino, C.A. Maggi, A. Torrisi, A. Mistretta.

RÉSUMÉ: Nous avons investigué les effets de l'endotheline aéroliisée (ET) et de son hexapeptide C-terminal (ET-16-21), sur la mécanique pulmonaire de rats anesthésiés sous respiration spontanée. L'inhalation de ET a provoqué une augmentation de résistance pulmonaire (RL) dépendant de la concentration, et une diminution de la compliance pulmonaire dynamique (Cdyn). Dans les conditions de contrôle, RL et Cdyn étaient de 0.17±0.04 cmH₂O·ml·s⁻¹ (moyenne±E; n=6) et de 6.25±0.66 ml·cmH₂O, respectivement. Après ET 4×10⁻⁵ M, RL et Cdyn étaient en moyenne de 1.45±0.28 cmH₂O·ml·s⁻¹ (p<0.05) et 1.12±0.36 ml·cmH₂O (p<0.05), respectivement. Les réponse pulmonaires à ET ont duré jusqu'à 60 minutes. Au contraire, ET-16-21, inhalé jusqu'à une concentration de 10⁻³ M, n'a pas eu d'effet sur la mécanique pulmonaire. Ces résultats indiquent que l'activité bronchoconstrictrice de l'endotheline aéroliisée chez le rat ne dépend pas de sa séquence C-terminal; ils suggèrent un rôle pour l'endotheline elle-même dans la régulation du tonus musculaire lisse des voies aériennes.