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Different mechanisms involved in relaxation of guinea-pig trachea by endothelin-1 and -3

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Abstract

The effects of endothelin-1 and endothelin-3 were investigated on carbachol-contracted guinea-pig isolated trachea. Endothelin-1 and endothelin-3 (0.1–100 nM) induced partial dose-dependent relaxation of the precontracted preparations. The endothelin-1-induced relaxation was markedly attenuated by haemoglobin (10 μM) and methylene blue (10 μM) and by epithelium removal. In contrast, endothelin-3-induced relaxation was not affected by haemoglobin, methylene blue or epithelium removal. The large conductance Ca^{2+}-activated K^+-channel blocker, charybdotoxin, antagonized the endothelin-1- and the endothelin-3-induced relaxation to the same extent. These results show that both endothelin-1 and endothelin-3 relaxant activities are modulated by charybdotoxin-sensitive K^+-channels, while the nitric oxide pathway is only involved in endothelin-1 relaxant effects.

Keywords: Trachea; (Guinea-pig); Smooth muscle relaxation; Endothelin; Charybdotoxin; Nitric oxide (NO)

1. Introduction

The endothelin isopeptides endothelin-1 and endothelin-3 were shown to induce potent contraction of guinea-pig (Cardell et al., 1992) and rat isolated trachea (Henry, 1993) through activation of different receptor subtypes (ET_A, ET_B) (Henry, 1993; Battistini et al., 1993). In vivo and in vitro studies have shown that endothelin-1 exerts a dual action on guinea-pig airway smooth muscle, an initial relaxation followed by a sustained contraction. White et al. (1991) reported that endothelin-1 caused a biphasic response in trachea after intravenous injection in anesthetized guinea-pigs. Uchida et al. (1991) observed a transient relaxation with endothelin-1 in vitro in isolated tracheae from sensitized guinea-pigs while Battistini et al. (1994) observed the same phenomenon in isolated tracheae from normal animals. Little is known about the relaxant effect of endothelin-3 on airway smooth muscle but endothelin-3, as well as endothelin-1, were shown to induce a biphasic effect (relaxation followed by contraction) in guinea-pig isolated ileum (Miasiro et al., 1993; Hori et al., 1994). The relaxant activity of endothelin-1 in guinea-pig isolated trachea was found to be mediated by nitric oxide (NO) release from epithelial cells (Filep et al., 1993) but other mechanisms might be involved since Hu et al. (1991) demonstrated that endothelin-1 increased the open-state probability of the large conductance Ca^{2+}-activated K^+-channels in smooth muscle cells of porcine coronary artery. The present study was undertaken to assess (1) the relaxant activity of endothelin-1 and endothelin-3 in precontracted guinea-pig isolated trachea and (2) the respective contributions of the NO pathway and charybdotoxin-sensitive K^+-channels in the relaxant effects.

2. Materials and methods

2.1. Tissue preparation

Experiments were performed using guinea-pig isolated trachea. Male guinea-pigs (450–500 g) were killed by a blow to the head. The tracheae were excised, cleaned of adhering tissues and the contractility of tracheal segments (4 tracheal rings in all cases) was measured isometrically with a myograph transducer connected to a physiograph Narco Bio-system. The tissues were suspended in 2 ml organ baths containing a Chenoweth-Koelle buffer. At the beginning of each experiment, the tissues were subjected

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to 0.5 g tension and allowed to equilibrate for 20–30 min with washing every 4 min.

2.2. Experimental protocol

After the equilibration period, all preparations were contracted with 1 μM carbachol then concentration-responses curves to endothelin-1 and endothelin-3 (0.1–100 nM) were obtained with cumulative additions in the absence or presence of 10 μM haemoglobin, 10 μM methylene blue or 60 nM charybdotoxin applied 30 min before and during the endothelin-1 or endothelin-3 stimulations. In some experiments, the epithelium was removed by gently rubbing the luminal surface (over both smooth muscle and cartilage areas) with a cotton-tipped applicator. Epithelium removal was verified pharmacologically by the lack of relaxant activity of arachidonic acid (10 μM) in rubbed preparations as compared with its relaxant activity in unrubbed preparations (Tschirhart et al., 1987) and in some cases histologically. After the concentration-response curve to endothelin-1 or endothelin-3 was completed, aminophylline (3 mM) was added to the bath to determine maximal relaxation.

2.3. Drugs

The drugs used were obtained from the following sources: Sigma Chemicals (USA): haemoglobin, methylene blue, arachidonic acid; Latoxan (France): purified charybdotoxin; Euromedex (France): endothelin-1 and endothelin-3.

Endothelin-1, endothelin-3, arachidonic acid, haemoglobin and methylene blue stock solutions were prepared in distilled water. Lyophilised charybdotoxin was reconstituted in saline solution (150 mM NaCl) and stored at −20°C until use. The Chenoweth-Koelle solution used had the following composition (mM): NaCl 120, KCl 5.6, $CaCl_2$ 2.4, $MgCl_2$ 2.2, $NaHCO_3$ 15 and glucose 10. It was maintained at 37°C and gassed continuously with a 95% O_2-5% CO_2 mixture.

2.4. Concentration-response curves and statistics

After carbachol-induced isometric tension had reached steady state, cumulative concentrations of endothelin-1 or endothelin-3 were added to the bath. Subsequent concentrations were added only after tension had reached a new steady state. When modifying agents were used, they were added after carbachol contraction had reached steady state and were left in the baths for 30 min before the addition of the relaxant drugs and throughout the rest of the experiment. All relaxant responses are expressed in terms of the maximal relaxation induced by aminophylline. Control tissues were treated similarly to tested tissues, but were exposed to vehicle instead of endothelin-1 or endothelin-3 to evaluate the time-dependent tone decrease in the carbachol-contracted tissues. The values are means ± S.E.M. The statistical significance of the results was assessed by a two-tailed, unpaired *t*-test. The null hypothesis was rejected when $P < 0.05$.

3. Results

The tracheal contraction induced by 1 μM carbachol (3.41 g ± 0.37, $n = 29$) represented 85–95% of the maximum contraction that could be induced by 10 μM carbachol. The spontaneous decrease in tone did not exceed 7% of the maximal relaxation induced by aminophylline and was taken into account in the expression of endothelin-1 and endothelin-3 relaxant activities.

3.1. Endothelin-1 and endothelin-3 relaxant activities

Addition of cumulative concentrations (0.1–100 nM) of endothelin-1 (Fig. 1A) and endothelin-3 (Fig. 1B) induced dose-dependent relaxation of guinea-pig isolated trachea precontracted with 1 μM carbachol. The maximal relaxations that could be achieved were 35.4 ± 3.4% and 22.4 ± 3.4% for 100 nM endothelin-1 and endothelin-3, respectively.

3.2. Effects of haemoglobin and methylene blue on endothelin-1 and endothelin-3 relaxant activities

Preincubation of tracheal rings for 30 min with haemoglobin (10 μM) and methylene blue (10 μM) did not modify the carbachol-induced baseline tone but significantly reduced the endothelin-1-induced relaxation (Fig. 1A). The concentration-response curve for endothelin-1 was shifted to the right and its maximal relaxant effect decreased from 35.4% (control) to 20.0% and 11.1% in the

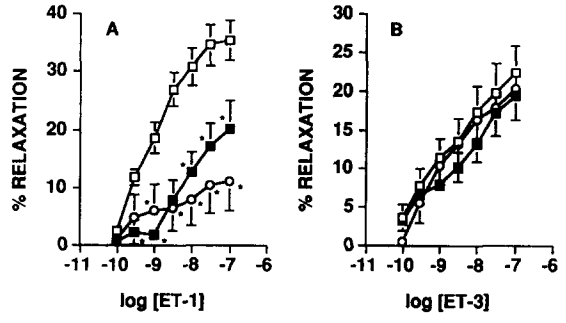

Fig. 1. Guinea-pig isolated trachea: relaxant activity of (A) endothelin-1, (B) endothelin-3 against established contraction in response to 1 μM carbachol in the absence (□) or presence of haemoglobin (10 μM) (■), methylene blue (10 μM) (○). Abscissae: log molar concentration of endothelin-1 (A) or endothelin-3 (B). Ordinate scale: percentage reduction of responses to 1 μM carbachol. Values are means with S.E.M. Control endothelin-1 $n = 16$, control endothelin-3 $n = 9$, haemoglobin $n = 6$, methylene blue $n = 5$. * Significantly different ($P < 0.05$) from the respective control values.

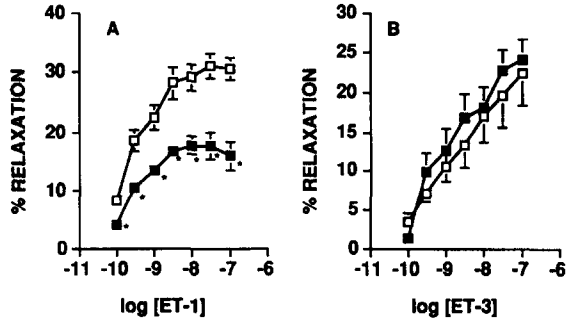

Fig. 2. Guinea-pig isolated trachea: relaxant activity of (A) endothelin-1, (B) endothelin-3 against established contraction in response to 1 μM carbachol in the presence (□) or absence of epithelium (■). Abscissae: log molar concentration of endothelin-1 (A) or endothelin-3 (B). Ordinate scale: percentage reduction of responses to 1 μM carbachol. Values are means with S.E.M. ($n = 5$). * Significantly different ($P < 0.05$) from the respective control values.

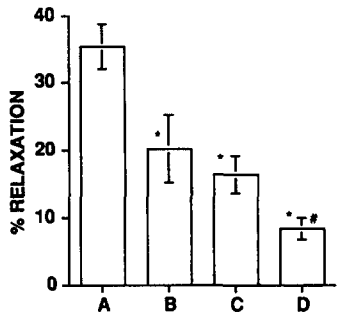

Fig. 4. Guinea-pig isolated trachea: relaxant activity of endothelin-1 (100 nM) against established contraction in response to 1 μM carbachol in the absence (A) or presence of haemoglobin (10 μM) (B), charybdotoxin (60 nM) (C), haemoglobin (10 μM) and charybdotoxin (60 nM) (D). Ordinate scale: percentage reduction of responses to 1 μM carbachol. Values are means with S.E.M., $n = 5$. * Significantly different ($P < 0.05$) from A, # significantly different ($P < 0.05$) from B and C.

presence of haemoglobin and methylene blue, respectively. In contrast, the relaxant activity of endothelin-3 was not significantly affected by haemoglobin or methylene blue pretreatment (Fig. 1B). The maximal relaxant effects of endothelin-3 were 19.4 ± 3.2% and 20.3 ± 2.0% in the presence of haemoglobin and methylene blue, respectively.

3.3. Influence of epithelium removal

The log concentration-response curves for endothelin-1 and endothelin-3 in carbachol-contracted guinea-pig trachea with and without epithelium are shown in Fig. 2A and Fig. 2B. Epithelium removal significantly reduced the endothelin-1-induced relaxation (Fig. 2A). The maximal relaxation that could be achieved with 10 nM endothelin-1 in the absence of epithelium was 17.6 ± 2.2%. At higher concentrations (30–100 nM), the extent of relaxation elicited by endothelin-1 tended to be lower (15.9 ± 2.4% at 100 nM). In contrast, epithelium removal did not affect endothelin-3 relaxant activity (Fig. 2B).

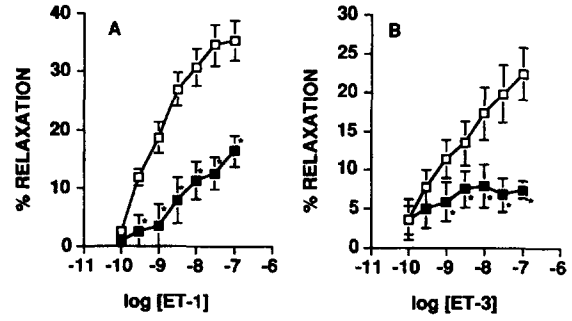

Fig. 3. Guinea-pig isolated trachea: relaxant activity of (A) endothelin-1, (B) endothelin-3 against established contraction in response to 1 μM carbachol in the absence (□) or presence of charybdotoxin (60 nM) (■). Abscissae: log molar concentration of endothelin-1 (A) or endothelin-3 (B). Ordinate scale: percentage reduction of responses to 1 μM carbachol. Values are means with S.E.M. Control endothelin-1 $n = 16$, control endothelin-3 $n = 9$, charybdotoxin $n = 5$. * Significantly different ($P < 0.05$) from the respective control values.

3.4. Effects of charybdotoxin on endothelin-1 and endothelin-3 relaxant activities

Preincubation of the tissues with charybdotoxin (60 nM), which did not produce any further change in the carbachol-induced baseline tone, resulted in a similar inhibition of endothelin-1- and endothelin-3-induced relaxation. Indeed, the maximal relaxant effect of endothelin-1 decreased from 35.4% (control) to 16.4% and that of endothelin-3 decreased from 22.4% to 7.6% in the presence of charybdotoxin (Fig. 3A and Fig. 3B).

Since the relaxant response to endothelin-1 appeared to involve both charybdotoxin-sensitive and NO-dependent components, we investigated the combined effects of charybdotoxin (60 nM) and haemoglobin (10 μM) on endothelin-1 (100 nM)-induced relaxation (Fig. 4). The relaxant activity of endothelin-1 was significantly lower in the presence of charybdotoxin and haemoglobin together than in the presence of charybdotoxin or haemoglobin alone.

4. Discussion

The present study showed that both endothelin-1 and endothelin-3 were able to induce relaxation of precontracted guinea-pig isolated trachea and, to the best of our knowledge, this is the first report of endothelin-3 relaxant activity on guinea-pig isolated trachea. These results are in agreement with those of Filep et al. (1993) in that the relaxant activity of endothelin-1 appeared to be partially mediated by NO released from epithelial cells thus leading to activation of smooth muscle guanylate cyclase. The relaxant properties of endothelin-1 were indeed antagonized by haemoglobin which presented high binding affinity for NO and by methylene blue which inhibited cytosolic guanylate cyclase. Furthermore, epithelium removal markely reduced the amplitude of the endothelin-1-induced

relaxation. In contrast, the NO pathway seemed to be a minor component of endothelin-3 relaxant activity since haemoglobin, methylene blue and epithelium removal did not significantly affect endothelin-3-induced relaxation. Taken together, these results showed that the endothelin-1-induced relaxation was partially mediated by receptors located on epithelial cells. These receptors could be of the ET_A subtype. Indeed, Battistini et al. (1994) showed that, in guinea-pig trachea, contraction in response to endothelin-1 was preceded by transient relaxation which was abolished by the selective ET_A receptor antagonist, cyclo(D-Asp-Pro-D-Val-Leu-D-Trp) (BQ-123), and by epithelium removal. Furthermore, Ninomiya et al. (1995) recently described a specific endothelin-1 binding site in cultured canine tracheal epithelial cells with characteristics similar to those of the ET_A receptor subtype.

The relaxant activities of endothelin-1 and endothelin-3 were significantly attenuated by the large conductance Ca^{2+}-activated K^+-channel blocker, charybdotoxin, suggesting that these channels are involved in both endothelin-1 and endothelin-3 relaxant activity on guinea-pig isolated trachea.

These results show that endothelin-1 and endothelin-3 are both able to induce relaxation in contracted guinea-pig isolated trachea through partially different mechanisms. It is possible that endothelin-1 causes relaxation via two mechanisms. One would be via an endothelin-1 receptor combined with NO formation, and a second mechanism would involve charybdotoxin-sensitive K^+-channels. For endothelin-3, the first mechanism could be of minor importance and cannot be observed whereas the second could dominate. Further investigations are needed to elucidate the epithelium-independent endothelin-1 and endothelin-3 relaxant effects on trachealis smooth muscle.

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