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ORIGINAL ARTICLE

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Endothelin-1 induced bronchial hyperresponsiveness in the rabbit: an ET_A receptor-mediated phenomenon

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Abstract Endothelin-1 (ET-1) is a potent and efficacious spasmogen of airway smooth muscle. Recent observations suggest that an increased intrapulmonary production of ET-1 may occur in asthma. Our previous study showed that endothelin-1 induced bronchial hyperresponsiveness to inhaled histamine in the rabbit. The aim of this study was to investigate whether the ET_A and ET_B receptors mediate the bronchial hyperresponsiveness induced by endothelin-1 in the rabbit.

Our data showed that bronchial hyperresponsiveness induced by ET-1 was significantly inhibited ($P<0.01$) by the ET_A receptor-selective antagonist, FR 139317 (from 2.5 to 10 mg kg^{-1}). Moreover, bosentan (from 2.5 mg kg^{-1} to 10 mg kg^{-1}), an ET_A/ET_B receptor antagonist, also inhibited the bronchial hyperresponsiveness achieved 24 h following endothelin-1 challenge ($P<0.01$), but with no difference from FR 139317. The ET_B receptor agonist, sarafotoxin S6c (from 25 μg to 2.5 mg kg^{-1}) did not modify airway responsiveness to inhaled histamine in the rabbit.

These results indicate that bronchial hyperresponsiveness induced by ET-1 may be mediated by ET_A receptor activation.

Key words Endothelin-l · Bronchial hyperresponsiveness · ET-receptors

Introduction

Although the focus of the research to date on the endothelins (ET) has been on their effects and potential pathophysiological relevance in the cardiovascular system (Yanagisawa et al. 1988; Filippelli et al. 1996), they produce an array of activities in a variety of other systems (Yanagisawa and Masaki 1989a; Yanagisawa and Masaki 1989b). In fact, the ET family exerts several effects in the pulmonary system (Pons et al. 1992; Hay et al. 1993b), including contraction of human airways and vascular smooth muscle (Henry et al. 1990; Brink et al. 1991) and stimulation of prostanoids release from human bronchus (Hay et al. 1993c); these effects are mediated via an interaction with specific ET membrane receptors.

Radioligand binding and autoradiographic studies have demonstrated the presence of both ET_A and ET_B receptors in human airways (Goldie et al. 1995) and in those of various animal species (Henry and Goldie 1994; Goldie et al. 1994). Although both receptor subtypes co-exist in human bronchial smooth muscle, contraction is predominantly mediated via the ET_B receptor subtype (Goldie et al. 1995), whereas both ET_A and ET_B receptors mediate contraction in guinea-pig (Tschirhart et al. 1991; Hay et al. 1993a), rabbit (Yoneyama et al. 1995), rat (Henry 1993) and mouse tracheal smooth muscle (Henry and Goldie 1994).

Other observations suggest that human asthma is also associated with increased levels of immunoreactive ET-1 in both bronchial epithelial cells (Springall et al. 1991) and bronchial lavage fluid (Sofia et al. 1993). Moreover, in our previous study we demonstrated that ET-1 may induce airway hyperresponsiveness to inhaled histamine via the involvement of capsaicin sensitive nerves (D'Agostino et al. 1998) supporting a link between the pathogenesis of asthma and endogenous ET-1 levels in the airways (Hay et al. 1993b).

In the current study we investigated whether the ET_A and ET_B receptors mediate the airway hyperresponsiveness to inhaled histamine induced by endothelin-1 in rabbits.

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Methods

Animals. New Zealand white (NZW) rabbits (La Palma Castellammare di Stabia, Italy) of either sex were used throughout the study. Animals were housed at constant temperature (21±1°C), relative humidity (55±5%), under a regular light–dark schedule (light 7:00

a.m.–7:00 p.m.). Food and water were freely available. The experimental procedures were in accord with Italian Legislative Decree 116/92.

Pulmonary function measurement. For the measurement of pulmonary function, rabbits were pre-medicated with an intraperitoneal injection of diazepam (5 mg ml^{-1}, 5 ml kg^{-1}) and subsequently administered Hypnorm (0.4 ml kg^{-1}; a mixture of fentanyl citrate 0.315 mg ml^{-1} and fluanisone 10 mg ml^{-1} i.m.). This regimen produces neuroleptoanalgesia and is recommended for recovery procedures in laboratory rabbits (Flecknall 1987). Neuroleptoanalgesia was maintained throughout the course of the experiment by the administration of 0.2–0.3 ml Hypnorm i.m. approximately every 30 min. The animals were placed in the supine position on a padded animal board and intubated with a cuffed endotracheal tube (3.0 mm internal diameter; Mallinckrodt Laboratories, Athlone, Ireland). The cuff was then inflated and the tube connected to a heated (37.5°C) Fleisch pneumotachograph (size 00; BEHA, Germany). Flow was measured by using a Validyne differential pressure transducer (Model MP 45-14-871; Validyne Engineering, Northridge, Calif.). Pleural pressure was estimated by placing a polyethylene catheter with an attached latex balloon in the lower third of the oesophagus, where it remained throughout the experiment, to obtain the maximum expiratory pressure.

Transpulmonary pressure, the difference between atmospheric and pleural pressure, was recorded by a second Validyne differential pressure transducer (Model MP 45-24-871) connected between the outflow of the endotracheal tube and the oesophageal balloon. The flow was integrated to give a continuous reading of tidal volume. Total lung resistance (R_L) and dynamic compliance (C_{dyn}) values were calculated by an on-line respiratory analyser (PMS version 8.4 Mumed, London).

Experimental protocol. On day 1, after measurement of baseline lung function, the rabbits were exposed to an aerosol of sterile saline for 2 min and lung function parameters recorded. Airway responsiveness was determined by exposing animals to cumulative concentrations of aerosolised histamine (1.25–80 mg ml^{-1}; 2 min per concentration), dissolved in saline, administered directly to the lungs via an endotracheal tube. Following each 2 min aerosol of histamine, animals were disconnected from the nebuliser and attached to the Fleisch tube. The following ten breaths were recorded and the mean value of R_L and C_{dyn} was calculated.

Aerosols of saline and histamine were generated by an ultrasonic nebuliser (De Vilbiss Health Care, Heston, Middlesex).

The provocation concentrations (PC) of histamine which produced a 50% increase in lung resistance (R_L) (PC_{50}) or a 35% fall in dynamic compliance (C_{dyn}) (PC_{35}) were determined for each animal by linear interpolation and used as indices of airway responsiveness.

On day 2, a first group of animals was exposed to cumulative concentrations of aerosolised endothelin-1 (0.25 ng ml^{-1} – 2.5 mg ml^{-1}, 2 min per concentration). A second group of animals was exposed to the same concentrations of ET-1 after 10 min pre-treatment with aerosolised FR 139317 (from 2.5 mg kg^{-1} to 10 mg kg^{-1}), an ET_A receptor antagonist, or bosentan (10 mg kg^{-1}), an ET_A/ET_B receptor antagonist or vehicle. In separate set of experiments, a third group of animals were exposed to sarafotoxin S6c (from 25 μg kg^{-1} to 2.5 mg kg^{-1}).

On day 3, airway responsiveness to histamine was determined as on day 1.

Drugs and chemicals. The drugs and chemicals used were: histamine diphosphate (Carlo Erba Reagent, Milan, Italy); diazepam (Roussel Pharma, Milan, Italy); Hypnorm (Janssen Pharmaceutical, Grove, Oxfordshire, UK); Sarafotoxin S6c (American Peptide, Sunnyvale, Calif., USA); FR 139317 (R) 2-[(R)-2-[(S)-2-[[1-(hexahydro-1H-azepinyl)] carbonyl] amino-4-methylpentanoil] amino-3-[3-(1-methyl-1H-indoyl)] prop ionyl] amino-3-(2-pyridyl) propionic acid); bosentan (4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-2,2''-bipyrimidin-4-yl]-benzenesulfonamide); endothelin-1 (Novabiochem, Laufelfingen, Switzerland). All solutions were prepared in saline.

Statistical analysis. Bartlett's test for homogeneity of variances was used on all data to determine whether parametric or non-parametric statistics were to be applied. For the lung function studies, statistical analysis was performed on log_{10} transformed data (PC_{50} and PC_{35}) in order to normalise the distribution of the data and to allow the application of parametric statistics. The paired *t*-test was used for the histamine lung function data before and after ET-l challenge. Results were considered significant if $P<0.05$.

Results

Baseline lung function

Baseline absolute values of R_L or C_{dyn} were not significantly different between the third and first days. Furthermore, no significant difference was observed between vehicle, endothelin-1, sarafotoxin S6c, FR 139317 + ET-1 or bosentan + ET-1-challenge on the third experimental day (data not shown).

Airway responsiveness to histamine

Endothelin-l challenge (0.25 ng ml^{-1} – 25 mg ml^{-1}, 2 min per concentration) to rabbits on the second experimental

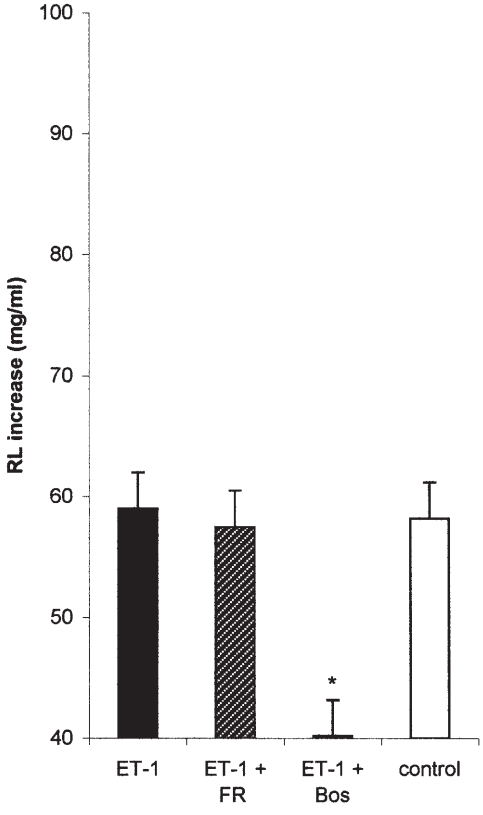

Fig. 1 Effects of the ET_A receptor-selective antagonist FR 139317 and the ET_A/ET_B receptor antagonist, bosentan, on endothelin-1 (ET-1) induced lung resistance (R_L) increase. The animals were pre-treated with FR 139317 (FR, 2.5 mg kg^{-1}), bosentan (Bos, 10 mg kg^{-1}) or vehicle (control) for 10 min before ET-1 challenge (ET-1 2.5 μg ml^{-1}). Values are means ± SEM, $n=5$. *$P<0.01$ compared to control

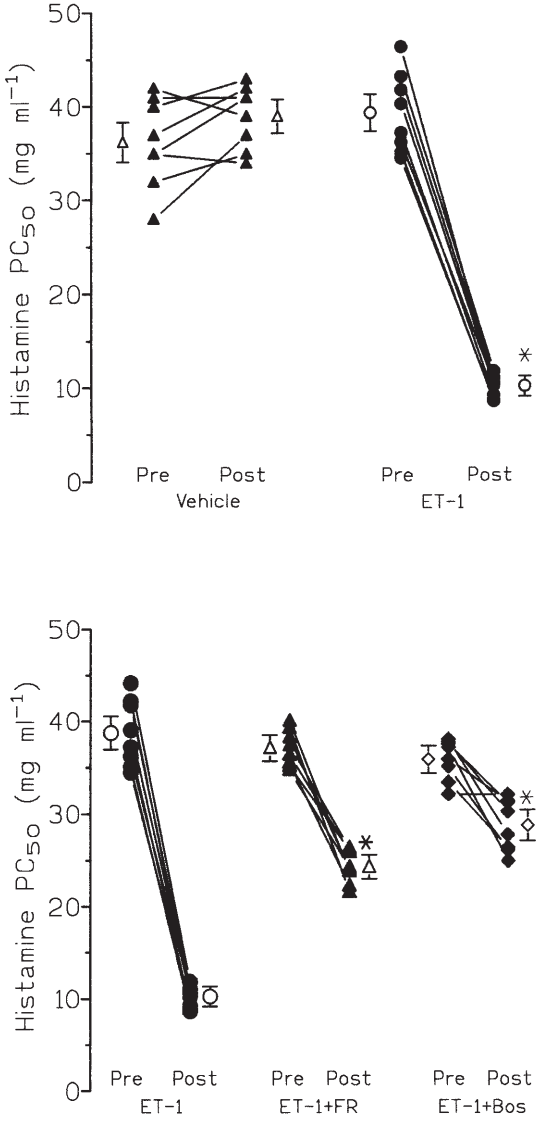

Fig. 2 Airway responsiveness to histamine 24 h prior to and 24 h following vehicle or endothelin-1 (ET-1; 0.25 ng ml^{-1}–2.5 μg ml^{-1}), or FR 139317 (FR; 2.5 mg kg^{-1}) and endothelin-1 (ET-1; 0.25 ng ml^{-1}–2.5 μg ml^{-1}), or bosentan (Bos; 10 mg kg^{-1}) and endothelin-1 (ET-1; 0.25 ng ml^{-1}–2.5 μg ml^{-1}) aerosolised challenge in normal rabbits. *Closed symbols* represent individual animal data and open symbols represent means ± SEM. Histamine PC_{50} is the concentration of histamine required to cause a 50% increase in airway resistance. **P*<0.01 compared to challenge with vehicle or endothelin alone

day, resulted in an increase of airway resistance (Fig. 1) and in an increased airway responsiveness to inhaled histamine 24 h after endothelin-1 challenge. In fact, endothelin-1-treated rabbits were 3.9-fold (P<0.01) more responsive to inhaled histamine when compared with vehicle-treated rabbits (Fig. 2).

Effect of sarafotoxin S6c

Sarafotoxin S6c (from 25 mg to 2.5 mg kg^{-1}) challenge to rabbits, on the second experimental day, resulted in an increase of airway resistance (Fig. 3), but the airway responsiveness to histamine in terms of R_L PC_{50} (Figs. 3 and 4) and C_{dyn} PC_{35} (data not shown), on the third experimental day, was not significantly altered.

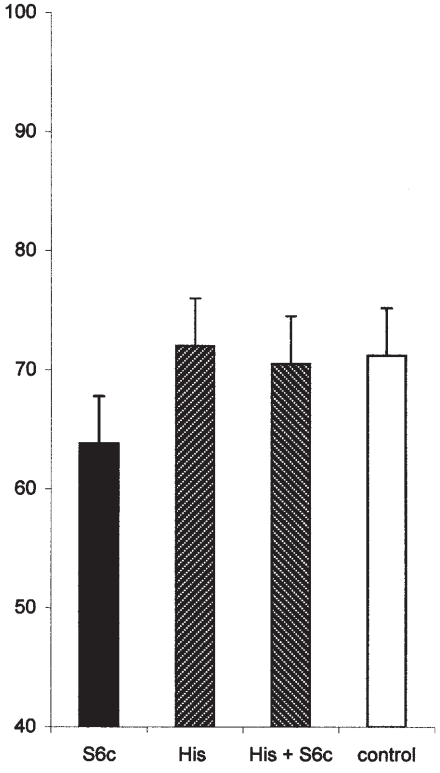

Fig. 3 Effects of ET_B receptor agonist, Sarafotoxin S6c (S6c), on lung resistance (R_L) increase (*solid columns*) and on airway responsiveness to histamine (His, 80 mg ml^{-1}) 24 h prior to and 24 h following vehicle (control, *open columns*) or S6c (10^{-7} mol Kg^{-1}) challenge in normal rabbits (*hatched columns*). Values are means ± SEM, n=5

Effect of FR 139317

Pre-treatment with FR 139317 2.5 mg kg^{-1} (10 min before of ET-1 challenge) on the second experimental day did not modify significantly the R_L increase induced by endothelin-1 (Fig. 1), but significantly inhibited the endothelin-1 induced airway hyperresponsiveness to inhaled histamine in terms of both R_L PC_{50} (Fig. 2) and C_{dyn} PC_{35} (data not shown). Increasing the dose of FR 139317 to 10 mg kg^{-1} did not cause further inhibition of the endothelin-1 induced airway hyperresponsiveness (data not shown).

Effect of bosentan

Pre-treatment with bosentan 10 mg kg^{-1} (10 min before of ET-1 challenge) on the second experimental day significantly inhibited the R_L increase induced by endothelin-1 (Fig. 1) and the endothelin-1 induced airway hyperresponsiveness to inhaled histamine in terms of both R_L PC_{50}

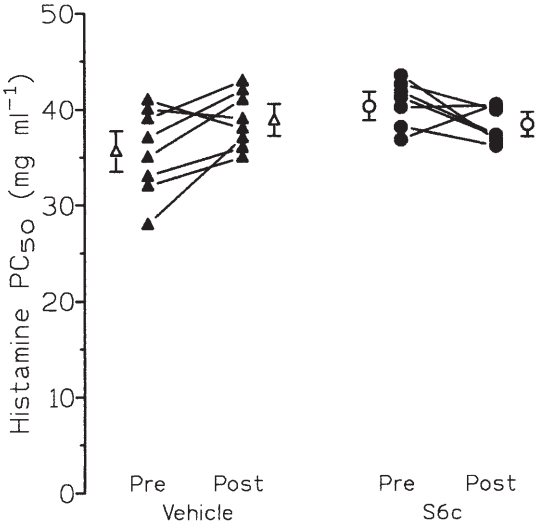

Fig. 4 Airway responsiveness to histamine 24 h prior to and 24 h following vehicle or sarafotoxin S6c (10^{-7} mol kg^{-1}) aerosolised challenge in normal rabbits. *Closed symbols* represent individual animal data and *open symbols* represent means ± SEM. Histamine PC_{50} is the concentration of histamine required to cause a 50% increase in airway resistance

(Fig. 2) and C_{dyn} PC_{35} (data not shown), but did not modify significantly the airway responsiveness to inhaled histamine respect to FR 139317 (Fig. 2).

Discussion

In agreement with our previous paper (D'Agostino et al. 1998*)*, the current study shows that ET-1 induces bronchial hyperresponsiveness to inhaled histamine.

Several reports suggest that an increased intrapulmonary production of ET-1 may specifically occur in asthma (Vittori et al. 1992). To date, the existence of at least two distinct subtypes of endothelin receptors has been demonstrated in mammalian cells (Arai et al. 1990; Sakurai et al. 1990): one is highly selective for ET-1 (ET_A), and the other is equally sensitive to isopeptides of the endothelin family (ET_B).

The current study shows that ET-1 may induce bronchial hyperresponsiveness to inhaled histamine, 24 h after ET-1 challenge, through the activation of ET_A receptors. In fact, the airway hyperresponsiveness to histamine induced by ET-1 was substantially inhibited by FR 139317, a highly selective ET_A receptor antagonist, that has been reported to be 7000 times more potent in inhibiting in vitro the binding of ET-1 to ET_A than ET_B receptors (Aramori et al. 1993; Sogabe et al. 1993). At the dose used, FR139317 appeared to be a selective ET_A receptor antagonist in vivo, as it did not inhibit in the guinea pig the responses to sarafotoxin S6c, an ET_B receptor-selective agonist (Williams et al. 1991) with greater than 10,000 fold higher affinity for the ET_B versus ET_A receptors.

Bosentan, an ET_A/ET_B receptor antagonist, also inhibited the bronchial hyperresponsiveness achieved 24 h following endothelin-1 challenge, but with no difference from FR 139317. Bosentan has been shown to antagonise the specific binding of ET-1 on ET_A (human umbilical vein vascular smooth muscle cells) as well as on ET_B receptors (microsomal membranes from human placenta) with similar Ki values (Clozel et al. 1994). These observations indicate that ET_A receptors are involved in the airway hyperresponsiveness to inhaled histamine induced by ET-1.

The findings that sarafotoxin S6c, an ET_B receptor agonist, was able to induce a substantial bronchoconstriction per se, but did not modify airway responsiveness to inhaled histamine in the rabbit lend further support to this notion.

These latter findings are consistent with previous observations that in airways more frequently the activation of ET_A-receptors induced by ET-1 produces either mitogenic effects (Panettieri et al. 1996) or release of prostanoid mediators from afferent sensory nerve endings (Hay et al. 1993c; Hay et al. 1993d). In fact, Filep et al. (1995) showed that in guinea pig, in addition to evoking airway contractions, ET-1 exerts pro-inflammatory actions via activation of the ET_A and, to a lesser extent, the ET_B receptors and, therefore, might contribute to the airway inflammation present in asthma.

Moreover, although McKay et al. (1996) demonstrated that in guinea-pig trachea and lung parenchyma contractions to ET-1 are mediated in part by ET_A receptors through the release of cyclo-oxygenase metabolites, Battistini et al. (1994) showed that in rabbit airways ET-1 induced airway contraction through ET_B receptors.

Together these studies suggest that the direct action of endothelin-1 to induce airway contraction, might be mediated mainly by ET_B receptors; while ET_A receptors may have a role in the indirect effects of endothelin-1 such as pro-inflammatory and mitogenic activities.

In conclusion, the present study showed that ET-l induced bronchial hyperresponsiveness to inhaled histamine may be mediated by ET_A receptors activation.

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References

Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S (1990) Cloning and expression of a cDNA encoding an endothelin receptor. Nature 348:730–732

Aramori I, Nirei H, Shoubo M, Sogabe K, Nakamura K, Kojo H, Notsu Y, Ono T, Nakanishi S (1993) Subtype selectivity of a novel endothelin antagonist, FR 139317, for the two endothelin receptors in transfected Chinese hamster ovary cells. Mol Pharmacol 43:127–131

Battistini B, Warner TD, Fournier A, Vane JR (1994) Characterization of ET_B receptors mediating contractions induced by endothelin-1 or IRL 1620 in guinea-pig isolated airways: effects of BQ-123, FR 139317 or PD 145065. Br J Pharmacol 111: 1009–1016

Brink C, Gillard V, Roubert P, Mencia-Huerta JM, Chabrier PE, Braquet P, Varley J (1991) Effects and specific binding sites of endothelin in human lung preparations. Pulm Pharmacol 4:54–59

Clozel M, Breu V, Gray GA, Kalina B, Loffler BM, Burri K, Cassal JM, Hirth G, Muller M, Neidhart W, Ramuz H (1994) Pharmacological characterization of bosentan, a new potent orally active non-peptide endothelin receptor antagonist. J Pharmacol Exp Ther 270:228–235

D'Agostino B, Filippelli A, Falciani M, Rossi F, Rossi F (1998) Endothelin-1 and bronchial hyperresponsiveness in the rabbit. Naunyn-Schmiedeberg's Arch Pharmacol 356:561–566

Filep JG, Fournier A, Foldes-Filep E (1995) Acute pro-inflammatory actions of endothelin-l in the guinea-pig lung: involvement of ET_A and ET_B receptors. Br J Pharmacol 115:227–236

Filippelli A, Falciani M, Palla A, D'Amico M, Vacca C, Rossi F (1996) Distribution of endothelin-1 receptor subtypes in rat portal vein. J Cardiovasc Pharmacol 27:113–118

Flecknall PA (1987) Laboratory animal anaesthesia: an introduction for research workers. Academic London Press, pp 98–100

Goldie RG, Grayson PS, Knott PG, Self GJ, Henry PJ (1994) Predominance of endothelin$_A$ (ET_A) receptors in ovine airway smooth muscle and their mediation of ET-1 induced contraction. Br J Pharmacol 112:749–756

Goldie RG, Henry PJ, Knott PG, Self GJ, Luttmann MA, Hay DW (1995) Endothelin-1 receptor density, distribution and functions in human isolated asthmatic airways. Am J Respir Crit Care Med 152:1653–1658

Hay DW, Luttmann MA, Hubbard WC, Undem BJ (1993a) Endothelin receptor subtypes in human and guinea-pig pulmonary tissues. Br J Pharmacol 110:1175–1183

Hay DW, Henry PJ, Goldie RG (1993b) Endothelin and the respiratory system. Trends Pharmacol Sci 14:29–32

Hay DW, Hubbard WC, Undem BJ (1993c) Endothelin-induced contraction and mediator release in human bronchus. Br J Pharmacol 110:392–398

Hay DW, Hubbard WC, Undem BJ (1993d) Relative contributions of direct and indirect mechanisms mediating endothelin-induced contraction of guinea-pig trachea. Br J Pharmacol 110: 955–962

Henry PJ (1993) Endothelin-1 (ET-1)-induced contraction in rat isolated trachea: involvement of ET_A and ET_B receptors and multiple signal transduction systems. Br J Pharmacol 110:435–441

Henry PJ, Goldie RG (1994) ET_B but not ET_A receptor-mediated contractions to endothelin-1 attenuated by respiratory tract viral infection in mouse airways. Br J Pharmacol 112: 1188–1194

Henry PJ, Rigby PJ, Self GJ, Preuss JM, Goldie RG (1990) Relationship between endothelin-1 binding site densities and constrictor activities in human and animal airway smooth muscle. Br J Pharmacol 100:786–792

McKay KO, Armour CL, Black JL (1996) Endothelin receptors and activity differ in human, dog and rabbit lung. Am J Physiol 270:L37–L43

Panettieri RA Jr, Goldie RG, Rigby PJ, Eszterhas AJ, Hay DW (1996) Endothelin-1-induced potentiation of human airway smooth muscle proliferation: an ET_A receptor-mediated phenomenon. Br J Pharmacol 118:191–197

Pons F, Boichot E, Lagente V, Touvay C, Mencia-Huerta JM, Braquet P (1992) Role of endothelin in pulmonary function. Pulm Pharmacol 5:123–219

Sakurai T, Yanagisawa M, Takuwa Y, Miyasaki H, Kimura S, Goto K, Masaki T (1990) Cloning of a cDNA encoding a non-isopeptide selective subtype of the endothelin receptor. Nature 348:732–735

Sofia M, Mormile M, Faraone S, Zofra S, Alifano M, Romano L, Carratù L (1993) Increased endothelin-1 like immunoreactive material on bronchoalveolar lavage fluid from patients with bronchial asthma and patients with interstitial lung disease. Respiration 60:89–95

Sogabe K, Nirei H, Shoubo M, Nomoto A, Ao S, Notsu Y, Ono T (1993) Pharmacological profile of FR 139317, a novel, potent endothelin ET_A receptor antagonist. J Pharmacol Exp Ther 264:1040–1046

Springall DR, Howarth PH, Counihan H, Djukanovic R, Holgate ST, Polak JM (1991) Endothelin immunoreactivity of airway epithelium in asthmatic patients. Lancet 337:697–701

Tschirhart EJ, Drijhout JW, Pelton JT, Miller RC, Jones CR (1991) Endothelins: functional and autoradiographic studies in guinea-pig trachea. J Pharmacol Exp Ther 258:381–387

Vittori E, Marini M, Fasoli A, De Franchis R, Mattoli S (1992) Increased expression of endothelin in bronchial epithelial cells of asthmatic patients and effects of corticosteroids. Am Rev Respir Dis 146:1320–1325

Williams DL JR, Jones KL, Pettibone DJ, Lis EV, Clineschmidt BV (1991) Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes. Biochem Biophys Res Commun 176:556–561

Yanagisawa M, Masaki T (1989a) Endothelin, a novel endothelium-derived peptide. Pharmacological activities, regulation and possible roles in cardiovascular control. Biochem Pharmacol 38:1877–1883

Yanagisawa M, Masaki T (1989b) Molecular biology and biochemistry of the endothelins. Trends Pharmacol Sci 10:374–378

Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui T, Yazaki Y, Goto K, Masaki T (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332:411–415

Yoneyama T, Hori M, Makatani M, Yamamura T, Tanaka T, Matsuda Y, Karaki H (1995) Subtypes of endothelin ET_A and ET_B receptors mediating tracheal smooth muscle contraction. Biochem Biophys Res Commun 207:668–674