ELSEVIER

European Journal of Pharmacology 304 (1996) 123–128

ejp

Effects of Ro 47-0203 on endothelin-1 and sarafotoxin S6c-induced contractions of human bronchus and guinea-pig trachea

Paul R. Gater *, Martin A. Wasserman [1], Louis M. Renzetti

Department of Inflammation / Autoimmune Diseases, Roche Pharmaceuticals, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley, NJ 07110-1199, USA

Received 14 December 1995; revised 1 February 1996; accepted 13 February 1996

Abstract

Endothelin exerts a variety of biological effects in the lung which indicate that this peptide may have a role in the pathophysiology of a number of pulmonary diseases. In this study, the endothelin receptors on the human bronchus were compared with those on the guinea-pig trachea using the novel, non-peptide antagonist Ro 47-0203 (4-*tert*-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzenesulphonamide, non-selective for endothelin ET_A over endothelin ET_B receptor) and the peptide antagonist BQ123 (cyclo(-D-Val-Leu-D-Trp-D-Asp-Pro), endothelin ET_A receptor selective). On the human bronchus and guinea-pig trachea, the concentration-effect curve for endothelin-1 was shifted to the right by Ro 47-0203 (100 μM) with concentration ratios of 28.2 ± 6.8 and 39.5 ± 13.9, respectively but lower concentrations of the antagonist had no effect. Although the concentration-effect curve for sarafotoxin S6c on the human bronchus was shifted to the right by Ro 47-0203 (30 and 100 μM, concentration ratio: 6.88 ± 1.72 and 69.7 ± 17.2, respectively), equivalent degrees of inhibition could be obtained on guinea-pig trachea with lower concentrations of antagonist (10 and 30 μM, concentration ratio: 6.90 ± 1.58 and 75.8 ± 14.1 respectively). The lack of effect of BQ123 (10 μM) and the high concentrations of Ro 47-0203 needed to show antagonism indicate that endothelin receptors on both tissues are probably the endothelin ET_B subtype. Although the antagonism by Ro 47-0203 is not classically competitive, the greater effect of Ro 47-0203 against sarafotoxin S6c on the guinea-pig trachea may reflect a difference between the endothelin ET_B receptors on these tissues.

Keywords: Endothelin receptor antagonist; Endothelin receptor subtype; Bronchus, human; Trachea, guinea-pig; Ro 47-0203; BQ123; Endothelin-1; Sarafotoxin S6c

1. Introduction

Endothelin-1 (Yanagisawa et al., 1988) is a potent spasmogen of isolated airway smooth muscle and a potent bronchoconstrictor in experimental animals (Hay et al., 1993a). High levels of immunoreactive endothelin-1 have been detected in bronchial epithelial cells (Springall et al., 1991) and bronchial lavage fluid from asthmatics (Sofia et al., 1993) and elevated plasma levels have been reported in adult respiratory distress syndrome patients (Druml et al., 1993). Since endothelin-1 has a number of other potent effects in the airways, including stimulation of mucus secretion (Mullol et al., 1993) and proinflammatory mediator production, it has been implicated in the pathophysiology of asthma and other airway diseases (Hay et al., 1993a). The possibility that antagonists of endothelin-1 may have therapeutic potential has prompted the development of a number of receptor antagonists and many studies of receptor subtypes in isolated tissues. Two subtypes of the endothelin receptor, designated endothelin ET_A and endothelin ET_B, have been identified from cDNA cloning and expression studies (Arai et al., 1990; Sakurai et al., 1990) and it is known that endothelin-1 is an agonist for both subtypes whereas sarafotoxin S6c is a potent and selective agonist for the endothelin ET_B receptor (Williams et al., 1991). The receptor subtypes in human airways remain to be determined, although autoradiographical analysis of [^{125}I]endothelin-1 binding indicated that the majority in the bronchi and peripheral lung are endothelin ET_B (Goldie et al., 1995; Knott et al., 1995). Since the endothelin ET_A receptor antagonist, BQ123 (Ihara et al., 1992) did not block endothelin-1- or sarafotoxin S6c-induced contractions of the isolated human bronchus, it has been concluded that the receptors are non endothelin ET_A,

* Corresponding author. Tel.: (201) 235 4120; fax: (201)235 6596.

[1] Present address: Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215-6300, USA.

0014-2999/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved.
PII S0014-2999(96)00125-2

perhaps endothelin ET_B (Hay et al., 1993b). However, definitive characterization of the receptors in human airways requires the use of a range of antagonists especially as it is becoming increasingly clear that there may be endothelin receptor subtypes that do not meet the criteria for classification as endothelin ET_A or endothelin ET_B (Bax and Saxena, 1994). The properties of Ro 47-0203, an orally active, non-peptide endothelin antagonist, were recently described showing that this compound has high affinity for endothelin receptors in binding studies and, on isolated tissues, has pA_2 values of 7.2 and 6.0 on endothelin ET_A and endothelin ET_B receptors, respectively (Clozel et al., 1994). In the present study, Ro 47-0203 has been used to attempt to further characterize the endothelin receptors on human bronchus in comparison with those on the guinea-pig trachea. A preliminary account of this work was presented at the XIIth International Congress of Pharmacology (Gater et al., 1994).

2. Materials and methods

2.1. Tissue preparation for measurement of contraction

Human lung tissues from 16 donors (9 male, 7 female, age range 18–65) with no known history of respiratory disorders were obtained from the International Institute for the Advancement of Medicine (Exton, PA, USA). Causes of death were severe head trauma following motor vehicle accidents, cerebral ischaemia or gunshot wounds. Lungs were transported in physiological medium at 4°C and used within 36 h of removal from the donor. Rings of bronchus (internal diameter = 4–8 mm; width = 3–6 mm) were prepared by insertion of a wooden probe into the lumen and dissection from the surrounding tissue. Guinea-pig tracheal rings containing 2–4 bands of cartilage were prepared and the epithelium removed by gently rubbing the luminal surface with a moistened cotton swab. Histological examination confirmed that these procedures removed the epithelium from human bronchial and guinea-pig tracheal rings. Tissues were paired and suspended in organ baths for isometric tension recording in warmed (37°C), aerated (95% O_2-5% CO_2) Krebs solution of the following composition (mM): NaCl 120, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, $NaHCO_3$ 25, K_2HPO_4 monobasic 1.2, dextrose 10, pH 7.4. Indomethacin (3 μM) was included in the Krebs solution throughout the experiment to eliminate the effects of endogenous prostaglandins. All tissues were maintained at a resting tension of 1.5 g and allowed to equilibrate for at least 90 min.

2.2. Experimental protocol

At the end of the initial equilibration period, tissue viability was assessed by the response to acetylcholine (1 mM) with preparations which did not generate more than 0.75 g tension being discarded from the study. After a further 30–45 min period, during which acetylcholine was washed out, Ro 47-0203 (3–100 μM), BQ123 (10 μM) or vehicle were added for 30 min prior to establishing cumulative concentration-effect curves for endothelin-1 (0.1–300 nM) or sarafotoxin S6c (0.01–300 nM). Responses to endothelin-1 or sarafotoxin S6c were expressed as a percentage of the maximal contraction to acetylcholine (1 mM) which was added at the end of the experiment.

2.3. Analysis of data

For each individual tissue, effective concentration (EC_{50}) values were calculated using regression analysis as the concentration of endothelin-1 or sarafotoxin S6c which caused 50% of the maximum response to the respective agonist. In cases where a maximum response to endothelin-1 or sarafotoxin S6c could not be achieved, effective concentration (EC_{30}) values were calculated by regression as the concentration which caused 30% of the maximum response to acetylcholine (1 mM) added at the end of the experiment. The degree of rightward shift of the concentration-effect curve for endothelin-1 or sarafotoxin S6c in tissues treated with Ro 47-0203 or BQ123 was determined as a concentration ratio by comparing EC_{50} (or, where appropriate, EC_{30}) values relative to a paired, vehicle-treated control tissue. On the guinea-pig trachea, the effect of Ro 47-0203 on the concentration-effect curve for S6c was also subjected to further analysis to obtain a pA_2 value as described by Arunlakshana and Schild (1959). Data were expressed as mean ± S.E.M. or as mean with 95% confidence limits and were compared using paired or unpaired Student's *t*-test. Significance was accepted at $P < 0.05$.

2.4. Materials

Endothelin-1 and sarafotoxin S6c were obtained from Bachem (Torrance, CA, USA). Ro 47-0203 (bosentan; 4-*tert*-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzenesulphonamide) and BQ123 (cyclo(-D-Val-Leu-D-Trp-D-Asp-Pro)) were synthesised at Hoffmann-La Roche, Basel, Switzerland. Acetylcholine chloride and indomethacin were purchased from Sigma Chemical Co. (St. Louis, MA, USA). Ro 47-0203 was dissolved in distilled water; solutions of indomethacin and BQ123 were prepared in dimethylsulphoxide.

3. Results

3.1. Effect of Ro 47-0203 on endothelin-1-induced contractions

On both the human bronchus and guinea-pig trachea, Ro 47-0203 (100 μM) caused significant ($P < 0.05$) right-

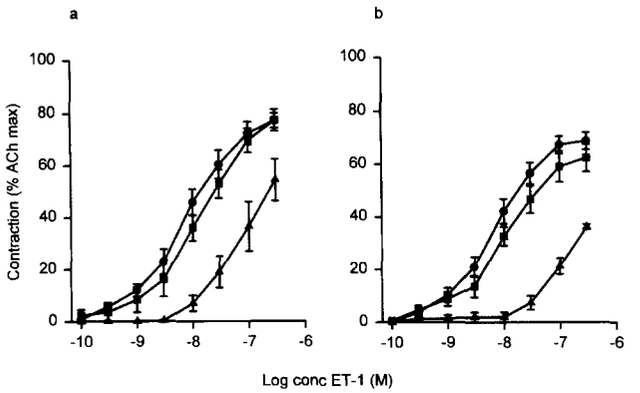

Fig. 1. Effect of Ro 47-0203 on log concentration-effect curves for endothelin-1 on (a) human bronchus and (b) guinea-pig trachea. (●) Control; (■) Ro 47-0203, 30 μM; (▲) Ro 47-0203, 100 μM. Contractions are expressed as a percentage of the maximal response to acetylcholine (1 mM) and are given as means ± S.E.M. from 4–10 preparations.

ward shifts of the endothelin-1 concentration-effect curves (Fig. 1). There was no significant ($P > 0.05$) difference between the concentration ratio for Ro 47-0203 on human bronchus (28.2 ± 6.8, $n = 4$) and on guinea-pig trachea (39.5 ± 13.9, $n = 5$). At lower concentrations, Ro 47-0203 (10 and 30 μM) had no significant ($P > 0.05$) effect on the concentration-effect curve for endothelin-1 on either human bronchus (concentration ratio = 1.12 ± 0.52 and 1.13 ± 0.41, respectively, $n = 4$; Fig. 1) or guinea-pig trachea (concentration ratio = 2.22 ± 0.42 and 2.02 ± 0.54, respectively, $n = 4$–5; Fig. 1).

3.2. Effect of Ro 47-0203 on sarafotoxin S6c-induced contractions

On the human bronchus, there were significant ($P < 0.05$) rightward shifts of the concentration-effect curves for

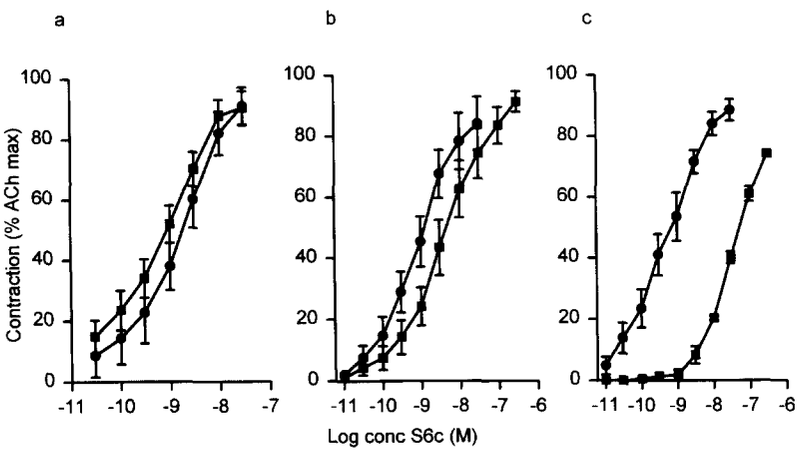

Fig. 2. Effect of Ro 47-0203 on log concentration-effect curves for sarafotoxin S6c on human bronchus. (●) Control; (■) Ro 47-0203: (a) 10 μM, (b) 30 μM, (c) 100 μM. Contractions are expressed as a percentage of the maximal response to acetylcholine (1 mM) and are given as means ± S.E.M. from 4 preparations.

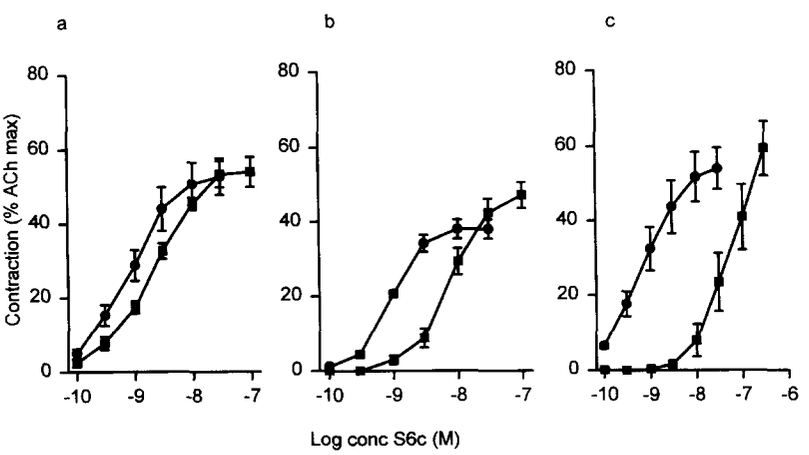

Fig. 3. Effect of Ro 47-0203 on log concentration-effect curves for sarafotoxin S6c on guinea-pig trachea. (●) Control; (■) Ro 47-0203: (a) 3 μM, (b) 10 μM, (c) 30 μM. Contractions are expressed as a percentage of the maximal response to acetylcholine (1 mM) and are given as means ± S.E.M. from 5–6 preparations.

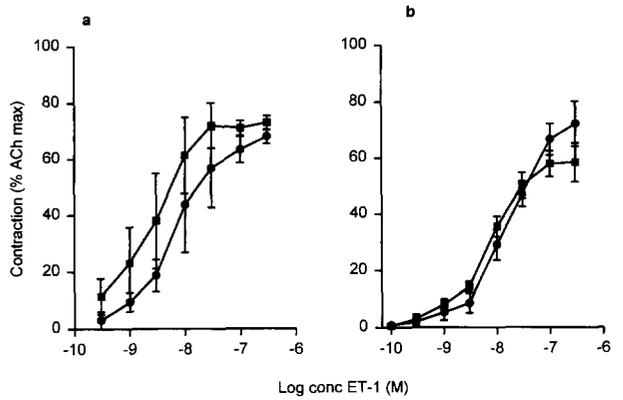

Fig. 4. Effect of BQ123 on log concentration-effect curves for endothelin-1 on (a) human bronchus and (b) guinea-pig trachea. (●) Control; (■) BQ123, 10 μM. Contractions are expressed as a percentage of the maximal response to acetylcholine (1 mM) and are given as means ± S.E.M. from 3–6 preparations.

sarafotoxin S6c following Ro 47-0203 (30 and 100 μM, Fig. 2) which corresponded to concentration ratios of 6.88 ± 1.66 ($n = 4$) and 69.7 ± 17.2 ($n = 4$) respectively. Ro 47-0203 (10 μM) had no significant ($P > 0.05$) effect against sarafotoxin S6c on the human bronchus (concentration ratio = 1.02 ± 0.52, $n = 4$; Fig. 2) but caused a significant rightward shift of the concentration-effect curve on the guinea-pig trachea (concentration ratio: 6.90 ± 1.58, $n = 6$). Indeed, even at a lower concentration, Ro 47-0203 (3 μM) caused a small, but significant ($P < 0.05$), inhibition of sarafotoxin S6c-induced contractions of guinea-pig trachea (concentration ratio: 2.70 ± 0.55, $n = 4$). At the highest concentration of Ro 47-0203 (30 μM), the concentration ratio against sarafotoxin S6c was 75.8 ± 14.1 ($n = 6$) on this tissue (Fig. 3) which was significantly ($P < 0.05$) greater than that observed for the same concentration on human bronchus. Schild plot analysis of the data for Ro 47-0203 on guinea-pig trachea yielded a pA_2 value of 5.62 (95% confidence limits, 5.40–5.85) but the line had a slope which was significantly different from unity (slope = −1.63; 95% confidence limits, −1.16 to −2.11).

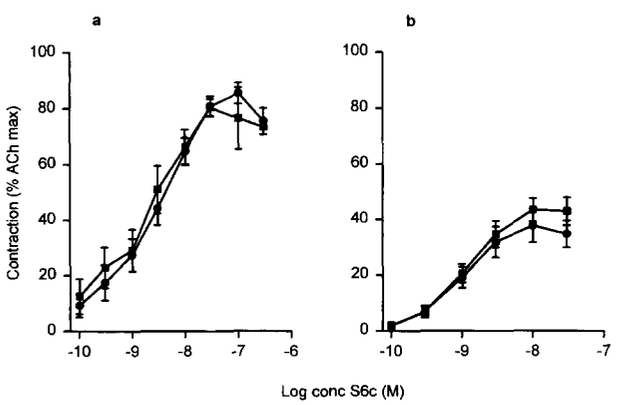

Fig. 5. Effect of BQ123 on log concentration-effect curves for sarafotoxin S6c on (a) human bronchus and (b) guinea-pig trachea. (●) Control; (■) BQ123, 10 μM. Contractions are expressed as a percentage of the maximal response to acetylcholine (1 mM) and are given as means ± S.E.M. from 4–6 preparations.

3.3. Effect of BQ123 on endothelin-1 and sarafotoxin S6c-induced contractions

BQ123 (10 μM) had no significant ($P > 0.05$) effect on the concentration-effect curve for endothelin-1 on either human bronchus (concentration ratio: 1.28 ± 1.16, Fig. 4) or guinea-pig trachea (concentration ratio: 0.70 ± 0.30, Fig. 4). BQ123 also had no significant ($P > 0.05$) effect on responses to sarafotoxin S6c on either tissue (human bronchus: concentration ratio = 0.91 ± 0.25; guinea-pig trachea: concentration ratio = 1.21 ± 0.18; Fig. 5).

4. Discussion

This study represents the first characterisation of the endothelin receptor on the human bronchus using Ro 47-0203, a non-peptide, mixed endothelin receptor antagonist. It has previously been reported that Ro 47-0203 inhibits specific binding of [^{125}I]endothelin-1 to endothelin ET_A and ET_B receptors ($K_i = 4.7$ and 95 nM respectively) and is a competitive antagonist of endothelin ET_A (rat aorta, $pA_2 = 7.2$) and endothelin ET_B (rat trachea, $pA_2 = 6.0$) receptor-mediated contractile responses (Clozel et al., 1994). We have demonstrated that high concentrations of Ro 47-0203 are required to inhibit endothelin-1 and sarafotoxin S6c contractions of the human bronchus and guinea-pig trachea and that the selective endothelin ET_A receptor antagonist, BQ123, had no effect. Although these results suggest that the receptor on both tissues is probably the endothelin ET_B subtype, the inhibitory effects of Ro 47-0203 do not meet the criteria for reversible, competitive antagonism and may indicate some complexities in the interaction between ligands and the endothelin receptors on these tissues.

Recent studies have indicated the presence of both endothelin ET_A and endothelin ET_B receptors on the human bronchus (Goldie et al., 1995) although detailed information on the distribution of endothelin receptors throughout the human airways is not currently available. The results from the present study confirm and extend the observation that the receptor subtype mediating contraction of human bronchus is probably endothelin ET_B (Hay et al., 1993b). The finding that a high concentration of BQ123 (Ihara et al., 1992) had no effect on responses to either endothelin-1 or sarafotoxin S6c supports previously reported data (Hay et al., 1993b) suggesting that endothelin ET_A receptors do not have a role in endothelin-induced contraction of this tissue. Further evidence for a functional endothelin ET_B receptor is provided by the finding that Ro 47-0203 had no effect on endothelin-1 or sarafotoxin S6c

responses until a concentration 100-fold in excess of that needed to inhibit the endothelin ET_A-receptor mediated contractile response to endothelin-1 on the rat aorta was reached (Clozel et al., 1994). The concentrations of Ro 47-0203 which were effective on the human bronchus were also in excess of that shown to inhibit sarafotoxin S6c contractions of rat trachea (Clozel et al., 1994) or guinea-pig trachea (present study). Since Ro 47-0203 is a mixed endothelin receptor antagonist, it remains possible that the effects at these high concentrations may be a consequence of interaction with an endothelin ET_A receptor which is insensitive to BQ123, in addition to an endothelin ET_B receptor. Interestingly, an endothelin-1-induced contraction of human bronchus that is resistant to blockade by BQ123 has recently been described under conditions in which endothelin ET_B receptors were desensitised (Goldie et al., 1995). Interaction with both endothelin ET_A and endothelin ET_B receptors may also account for the fact that the antagonism by Ro 47-0203 did not appear to be classically competitive. As significant shifts of the agonist concentration-effect curves were only observed at one or two concentrations of antagonist, it was not possible to conduct Schild plot analysis of this data. However, for both endothelin-1 and sarafotoxin S6c on the human bronchus, an increase in concentration of Ro 47-0203 did not elicit the equivalent increment in concentration ratio which would be predicted for competitive antagonism. Studies with antagonists which are more selective for the endothelin ET_B receptor subtype (Ishikawa et al., 1994; Tanaka et al., 1994) are needed to further characterise the receptor on human bronchus.

The endothelin receptor population in guinea-pig trachea is not homogeneous and there are some regional variations in endothelin receptor distribution throughout guinea-pig airways (Hay et al., 1993b; Battistini et al., 1994) which may further complicate interpretation of results with agonists or antagonists possessing affinity for more than one endothelin receptor subtype. In common with the human bronchus, high concentrations of Ro 47-0203 were needed to antagonise endothelin-1 on the guinea-pig trachea and BQ123 had no effect. The observations with BQ123 support the view that most of the contractile response to endothelin-1 in this tissue is mediated through endothelin ET_B receptors (see Battistini et al., 1994). These findings are at variance with another study which indicated that part of the endothelin-1-induced contraction of guinea-pig trachea may be mediated through endothelin ET_A receptors (Hay et al., 1993b) since endothelin-1 was antagonised by BQ123. The reason for this difference between the present study and that of Hay and co-workers (Hay et al., 1993b) is not readily apparent. However, at the high concentrations of Ro 47-0203 used in the present study, it remains possible that inhibition of endothelin-1 contractions may be a consequence of antagonism at both endothelin ET_A and endothelin ET_B receptors. Sarafotoxin S6c was a potent spasmogen on guinea-pig trachea but, in common with other studies (Hay et al., 1993b), caused a smaller maximum response than endothelin-1. Given the selectivity of BQ 123 and sarafotoxin S6c for the endothelin ET_A and endothelin ET_B receptor respectively, it was not surprising that this response was not inhibited by BQ123. Interestingly, Ro 47-0203 inhibited sarafotoxin S6c contractions of the guinea-pig trachea at concentrations that had no effect on the human bronchus. However, analysis of the data for Ro 47-0203 against sarafotoxin S6c on the guinea-pig trachea revealed a Schild plot with a slope that was significantly greater than unity indicating that the antagonism was not classically competitive. Thus, while it is possible that the difference in potency of Ro 47-0203 against sarafotoxin S6c on these tissues may suggest that there are some differences between the endothelin ET_B receptors, other factors should also be considered.

There are a number of reports of complexities in the binding kinetics of endothelin receptors that have made it difficult to interpret the effects of endothelin receptor antagonists in isolated tissue systems. Kinetic analysis has shown that [^{125}I]endothelin-1 binding to membrane preparations is not readily reversible with a dissociation half-life of at least 30 h (Waggoner et al., 1992). It is also clear that the rate of association of endothelin with the receptor is slow and that the endothelin receptor can be internalised, down regulated or desensitised (Huggins et al., 1993; Bax and Saxena, 1994). In addition, a recent study of endothelin ET_B receptors on porcine cerebellum showed that binding of endothelin antagonists was more readily reversible than agonist binding which may result in a time-dependent reduction in antagonist potency (Wu-Wong et al., 1994). Further studies are needed to determine the importance of these properties to agonist and antagonist interactions with endothelin receptors on airway tissues. Furthermore, it is possible that Ro 47-0203 may have non-specific effects either on receptors other than those for endothelin or on smooth muscle contractility. However, both explanations seem unlikely since Ro 47-0203 had no affinity for a wide range of receptors and ion channels in binding studies (Clozel et al., 1994) and, in the present study, there was no reduction in the magnitude of the contractile response to acetylcholine at the end of the experiment relative to the initial response (data not shown).

In conclusion, using Ro 47-0203, a non-peptide endothelin receptor antagonist, we have provided further evidence that endothelin-induced contraction of the human bronchus and guinea-pig trachea is probably mediated through an endothelin ET_B receptor subtype. We have also shown that there may be a difference between the endothelin ET_B receptor subtypes on these two tissues which merits further exploration with more selective antagonists. However, as the inhibitory effects of Ro 47-0203 were not consistent with classical, competitive antagonism there may be a complex interaction between ligand and endothelin receptors on airway smooth muscle.

References

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

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. Chemother. 14, 48-58.

Battistini, B., T.D. Warner, A. Fournier and J.R. Vane, 1994, Characterization of ET_B receptors mediating contractions induced by endothelin-1 or IRL 1620 in guinea-pig isolated airways: effects of BQ-123, FR139317 or PD 145065, Br. J. Pharmacol. 111, 1009.

Bax, W.A. and P.R. Saxena, 1994, The current endothelin receptor classification: time for reconsideration?, Trends Pharmacol. Sci. 15, 379.

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

Druml, W., H. Steltzer, W. Waldhäusl, K. Lenz, A. Hammerle, H. Vierhapper, S. Gasic and O.F. Wagner, 1993, Endothelin-1 in adult respiratory distress syndrome, Am. Rev. Respir. Dis. 148, 1169.

Gater, P.R., M.A. Wasserman and L.M. Renzetti, 1994, Contraction of human bronchus to endothelin is mediated by an atypical receptor, Can. J. Physiol. Pharmacol. 72, (Suppl. 1), 495.

Goldie, R.G., P.J. Henry, P.G. Knott, G.J. Self, M.A. Luttmann and D.W.P. Hay, 1995, Endothelin-1 receptor density, distribution and function in human isolated asthmatic airways, Am. J. Respir. Crit. Care Med. 152, 1653.

Hay, D.W.P., P.J. Henry and R.G. Goldie, 1993a, Endothelin and the respiratory system, Trends Pharmacol. Sci. 14, 29.

Hay, D.W.P., M.A. Luttmann, W.C. Hubbard and B.J. Undem, 1993b, Endothelin receptor subtypes in human and guinea-pig pulmonary tissues, Br. J. Pharmacol. 110, 1175.

Huggins, J.P., J.T. Pelton and R.C. Miller, 1993, The structure and specificity of endothelin receptors: their importance in physiology and medicine, Pharmacol. Ther. 59, 55.

Ihara, M., K. Noguchi, T. Saeki, T. Fukuroda, S. Tsuchida, S. Kimura, T. Fukami, K. Ishikawa, M. Nishikibe and M. Yano, 1992, Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor, Life Sci. 50, 247.

Ishikawa, K., M. Ihara, K. Noguchi, T. Mase, N. Mino, T. Saeki, T. Fukuroda, T. Fukami, S. Ozakari, T. Nagase, M. Nishikibe and M. Yano, 1994, Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788, Proc. Natl. Acad. Sci. USA 91, 4892.

Knott, P.G., A.C. D'Aprile, P.J. Henry, D.W.P. Hay and R.G. Goldie, 1995, Receptors for endothelin-1 in asthmatic human peripheral lung, Br. J. Pharmacol. 114, 1.

Mullol, J., B.A. Chowdhury and M.V. White, 1993, Endothelin in human nasal mucosa, Am. J. Respir. Cell Mol. Biol. 8, 393.

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

Sofia, M., M. Mormile, S. Faraone, S. Zofra, L. Romano and L. Carratù, 1993, Increased endothelin-like immunoreactive material in bronchoalveolar lavage fluid from patients with bronchial asthma and patients with interstitial lung disease, Respiration 60, 89.

Springall, D.R., P.H. Howarth, H. Counihan, R. Djukanovic, S.T. Holgate and J.M. Polak, 1991, Endothelin immunoreactivity of airway epithelium in asthmatic patients, Lancet 337, 697.

Tanaka, T., E. Tsukuda, M. Nozawa, H. Nonaka, T. Ohno, H. Kase, K. Yamada and Y. Matsuda, 1994, RES-701-1, a novel, potent, endothelin type B receptor-selective antagonist of microbial origin, Mol. Pharmacol. 45, 724.

Waggoner, W.G., S.L. Genova and V.A. Rash, 1992, Kinetic analyses demonstrate that the equilibrium assumption does not apply to [^{125}I]endothelin-1 binding data, Life Sci. 51, 1869.

Williams, D.L. Jr., K.L. Jones, D.J. Pettibone, E.V. Lis and B.V. Clineschmidt, 1991, Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes, Biochem. Biophys. Res. Commun. 162, 371.

Wu-Wong, J.R., W.J. Chiou, S.R. Magnuson and T.J. Opgenorth, 1994, Endothelin receptor agonists and antagonists exhibit different dissociation characteristics, Biochim. Biophys. Acta 1224, 288.

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