Pharmacological evidence for distinct endothelin receptors in guinea-pig bronchus and aorta

D.W.P. Hay

Department of Pharmacology, SmithKline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, PA 19406-0939, U.S.A.

The ET_A receptor antagonist, BQ-123 (1 μ M) potently antagonized endothelin-1 (ET-1) concentration-response curves in guinea-pig aorta ($pK_B = 7.1$). However, 10 μ M BQ-123 was without effect on ET-1-induced contractions in guinea-pig bronchus. The ET_B-selective agonist, sarafotoxin S6c did not contract the aorta but was a potent and effective contractile agonist in the bronchus. BQ-123 (10 μ M) was without effect on sarafotoxin S6c-induced contractions in the bronchus. These data provide evidence for distinct endothelin receptors in guinea-pig aorta and bronchus, which appear to be predominantly of the ET_A and non-ET_A, perhaps ET_B, subtypes, respectively.

Keywords: Endothelin-1; guinea-pig aorta; guinea-pig bronchus; BQ-123; ET_A receptor antagonist; sarafotoxin S6c; ET_B agonist; endothelin receptor subtypes; ET_A receptor; ET_B receptor

Introduction Endothelin-1 (ET-1) belongs to a family of recently discovered 21-amino acid vasoconstrictor peptides which also includes ET-2 and ET-3 (Yanagisawa & Masaki, 1989). Researchers have provided evidence for distinct endothelin receptors in several cells and tissues including rat lung (Masuda *et al.*, 1989). Recently the cloning and expression of cDNA for a selective and also a non-selective endothelin receptor, designated ET_A and ET_B, respectively, was described (Arai *et al.*, 1990; Sakurai *et al.*, 1990; Sakamoto *et al.*, 1991).

A comparison of the contractile effects of the endothelins and fragments provided preliminary evidence for distinct endothelin receptors in airway versus vascular smooth muscle (Maggi *et al.*, 1989; Cardell *et al.*, 1992).

The endothelins bear a close structural homology with a group of snake venoms, the sarafotoxins (Yanagisawa & Masaki, 1989), of which sarafotoxin S6c exhibits selectivity for the ET_B receptor (Williams *et al.*, 1991). In addition, BQ-123, a cyclic pentapeptide (D-Asp-L-Pro-D-Val-L-Leu-D-Trp), has been identified as a potent and selective ET_A receptor antagonist (Ihara *et al.*, 1991). In the present study, sarafotoxin S6c and BQ-123 were used to explore further potential differences in endothelin receptors mediating contraction in guinea-pig airway and vascular smooth muscles.

Methods Primary bronchi and thoracic aortae were removed from male Hartley guinea-pigs (Hazelton Research Animals, Denver, PA, U.S.A.; 450–650 g body weight). The epithelium and endothelium were removed mechanically from the bronchial and aortic rings, respectively. The preparations were placed under approximately 1.5 g tension in 10-ml water-jacketed siliconized organ baths containing modified Krebs-Henseleit solution. The composition of the Krebs-Henseleit solution, which was gassed with 95% O₂:5% CO₂ and maintained at 37°C, was (mM): NaCl 113.0, KCl 4.8, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25.0 and glucose 5.5. Mechanical responses were recorded isometrically with multi-channel polygraphs. Experiments were conducted in the presence of the cyclo-oxygenase inhibitor, meclofenamic acid (1 μ M). One hour after equilibration, and also at the end of the experiment, the bronchi and aortae were exposed to 10 μ M carbachol and 10 μ M phenylephrine, respectively; subsequent responses were expressed as a percentage of the reference contractions obtained following completion of the experiment. Cumulative concentration-response curves to ET-1 and sarafotoxin S6c were constructed by 3 fold increases in agonist concentration; in some

experiments BQ-123 was added 30 min before starting the agonist concentration-response curves.

Geometric mean EC₅₀ values were calculated from linear regression analysis of data, which are given as mean $\pm$ s.e.mean.

BQ-123 was synthesized by Dr Michael Moore and colleagues in the Department of Peptide Chemistry, SmithKline Beecham Pharmaceuticals. ET-1 and sarafotoxin S6c were purchased from Peninsula Laboratories (Belmont, CA, U.S.A.), phenylephrine and carbachol from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Results The ET_A receptor antagonist BQ-123 (1 μ M) produced a marked shift to the right of ET-1 concentration-response curves in guinea-pig aorta (Figure 1a) with a $pK_B = 7.1$. In contrast, BQ-123 (1 or 10 μ M) was without effect on contractions elicited by ET-1 in guinea-pig bronchus (illustrated for 10 μ M in Figure 1b).

Sarafotoxin S6c (0.1 nM–0.1 μ M) did not contract guinea-pig aorta but was a potent and effective contractile agent in guinea-pig bronchus (Figure 2). BQ-123 (10 μ M) was without effect on sarafotoxin S6c-induced contractions in guinea-pig bronchus (Figure 2b).

Discussion Studies exploring the activity of various agonists provided preliminary evidence for the existence of different endothelin receptors responsible for contraction in vascular versus airway smooth muscle (Maggi *et al.*, 1989; Cardell *et al.*, 1992). The present study extends these observations and provides strong evidence for distinct endothelin receptors mediating ET-1-induced contraction in guinea-pig aorta and bronchus. Thus, the ET_A receptor antagonist, BQ-123 (Ihara *et al.*, 1991), potently antagonized ET-1 concentration-response curves in the aorta but was without effect on responses in the bronchus. Furthermore, sarafotoxin S6c, the ET_B-selective agonist (Williams *et al.*, 1991), did not contract the aorta but was a potent and effective contractile agonist in the bronchus. Selectivity of action of BQ-123 for ET_A-mediated responses was confirmed by its lack of effect against sarafotoxin S6c-induced contraction in bronchus. The potency of BQ-123 in guinea-pig aorta ($pK_B = 7.1$) is similar to that reported previously for inhibition of ET-1-induced contraction in porcine coronary artery ($pA_2 = 7.4$) (Ihara *et al.*, 1991).

The present data indicate that contraction of guinea-pig aorta induced by ET-1 is produced by activation of ET_A

Figure 1 Effect of BQ-123 on endothelin-1 (concentration-response curves in (a) guinea-pig aorta and (b) guinea-pig bronchus. Results are expressed as a percentage of the response to (a) 10 μ M phenylephrine or (b) 10 μ M carbachol and are the mean with s.e.mean shown by vertical bars. (a) (●) Control; (○) 1 μ M BQ-123, $n = 6$; (b) (●) control; (○) 10 μ M BQ-123, $n = 4$.

receptors. Without the availability of a selective ET_B receptor antagonist, it is uncertain whether the ET-1-induced contraction of guinea-pig bronchus is due to stimulation of ET_B receptors or perhaps a non- ET_A , non- ET_B receptor subtype similar to that recently proposed following functional studies in porcine coronary artery with various members of the endothelin and sarafotoxin families (Harrison *et al.*, 1992). The functional identification of endothelin receptor subtypes will be important in the development of selective endothelin receptor antagonists for the treatment of diseases in which

Figure 2 Concentration-response curves to sarafotoxin S6c in (a) guinea-pig aorta and (b) guinea-pig bronchus; also indicated in (b) is the effect of BQ-123 (10 μ M) on sarafotoxin S6c-induced contractions. Results are expressed as a percentage of the response to (a) 10 μ M phenylephrine or (b) 10 μ M carbachol and are given as the means with s.e.mean (vertical bars) of 4 tissues: (b) (●) control; (○) 10 μ M BQ-123.

endothelin has been implicated.

In summary, the results of this study, in addition to previous findings, suggest that ET-1-induced contractions of guinea-pig aorta and bronchus are mediated predominantly via an ET_A -type and a non- ET_A -type, perhaps ET_B -type, receptor, respectively.

The author thanks Mark Luttmann for excellent technical assistance, Dr Eliot Ohlstein for helpful discussion and Dotti Lavan for typing this material.

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.
- CARDELL, L.O., UDDMAN, R. & EDVINSSON, L. (1992). Evidence for multiple endothelin receptors in the guinea-pig pulmonary artery and trachea. *Br. J. Pharmacol.*, **105**, 376–380.
- HARRISON, V.J., RANDRIANTSOA, A. & SCHOEFFTER, P. (1992). Heterogeneity of endothelin-sarafotoxin receptors mediating contraction of pig coronary artery. *Br. J. Pharmacol.*, **105**, 511–513.
- IHARA, M., NOGUCHI, K., SAEKI, T., FUKURODA, T., TSUCHIDA, S., KIMURA, S., FUKAMI, T., ISHIKAWA, K., NISHIKIBE, M. & YANO, M. (1991). Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. *Life Sci.*, **50**, 247–255.
- MAGGI, C.A., GIULIANI, S., PATACCHINI, R., SANTICIOLI, P., ROVERO, P., GIACHETTI, A. & MELI, A. (1989). The C-terminal hexapeptide, endothelin-(16–21), discriminates between different endothelin receptors. *Eur. J. Pharmacol.*, **166**, 121–122.
- MASUDA, Y., MIYAZAKI, H., KONDOH, M., WATANABE, H., YANAGISAWA, M., MASAKI, T. & MURAKAMI, K. (1989). Two different forms of endothelin receptors in rat lung. *F.E.B.S. Lett.*, **257**, 208–210.
- SAKURAI, T., YANAGISAWA, M., TAKUWA, Y., MIYAZAKI, 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.

- SAKAMOTO, A., YANAGISAWA, M., SAKURAI, T., TAKUWA, Y., YANAGISAWA, H. & MASAKI, T. (1991). Cloning and functional expression of human cDNA for the ET_B endothelin receptor. *Biochem. Biophys. Res. Commun.*, **178**, 656–663.
- WILLIAMS, D.L., Jr., JONES, K.L., PETTIBONE, D.J., LIS, E.V. & CLINESCHMIDT, B.V. (1991). Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes. *Biochem. Biophys. Res. Commun.*, **175**, 556–561.
- YANAGISAWA, M. & MASAKI, T. (1989). Endothelin, a novel endothelium-derived peptide. Pharmacological activities, regulation and possible roles in cardiovascular control. *Biochem. Pharmacol.*, **38**, 1877–1883.

(Received March 19, 1992)

Accepted April 16, 1992)