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Short communication

Contraction of human airway smooth muscle by endothelin-1 and IRL 1620: effect of bosentan

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Abstract

We have examined whether 4-tert-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzenesulfonamide (bosentan; endothelin $ET_{A/B}$ receptor antagonist) and (*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl] amino-4-methylpentanoil]amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl]amino-3-(2-pyridyl) propionic acid (FR 139317; endothelin ET_A receptor antagonist) inhibit contractions of human airway smooth muscle induced by endothelin-1 or Suc-[Glu^9,$Ala^{11,15}$]enthothelin-1-(8–21) (IRL 1620; endothelin ET_B receptor agonist). Endothelin-1 and IRL 1620 were equipotent. Bosentan and FR 139317 (each 10 μM) produced a small shift in response curves to endothelin-1 (1.6- and 1.5-fold, respectively). However, bosentan was more potent against contractions elicited by IRL 1620 (10 μM, 11.2-fold shift) suggesting that these agonists exhibit different kinetic interactions with endothelin receptors or implying an interaction with a novel endothelin ET_B receptor subtype in human airways.

Keywords: Endothelin-1; Bosentan (4-tert-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzene sulfonamide); IRL 1620 (Suc-[Glu^9,$Ala^{11,15}$]endothelin-1-(8–21)); FR 139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl] amino-4-methylpentanoil]amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl]amino-3-(2-pyridyl) propionic acid)

1. Introduction

The endothelins (endothelin-1, endothelin-2 and endothelin-3) are a family of 21-amino-acid peptides which are potent contractile agents, in vitro, of airway smooth muscle derived from many species including human (Advenier et al., 1990; Henry, 1993; Kizawa et al., 1994; Battistini et al., 1994a,b; Yoneyama et al., 1995; Hay et al., 1996). In mammalian tissues the endothelins appear to act through two distinct receptor subtypes, endothelin ET_A and endothelin ET_B, which have been cloned and expressed. The endothelin ET_A receptor has a higher affinity for endothelin-1 and endothelin-2 than for endothelin-3 (Arai et al., 1990) and the endothelin ET_B receptor has equal affinities for all endothelins (Sakurai et al., 1990). As increased expression of endothelin-1 has been demonstrated in inflammatory lung diseases (Barnes, 1994) there has been interest in characterising which endothelin receptor subtype mediates the effects of the endothelins within human airways. Generally, it has been found that endothelin ET_B receptor-selective agonists constrict human airways in vitro (Hay et al., 1993; Adner et al., 1996) and that such responses are antagonised by non-selective endothelin ET_A/ET_B receptor antagonists, but not by endothelin ET_A receptor-selective antagonists (Adner et al., 1996). Taken together such studies suggest that endothelin ET_B receptors are the predominant subtype mediating constrictions to the endothelins in human airways. Thus, to further characterise the endothelin receptors present on human airways, we have examined the effects of the endothelin ET_A/ET_B non-selective, non-peptide antagonist, 4-tert-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzene sulfonamide (bosentan) (Clozel et al., 1993), against contractions of

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bronchial rings induced by endothelin-1 or Suc-[Glu^{9},Ala^{11,15}]endothelin-1-(8–21) (IRL 1620).

2. Material and methods

2.1. Tissue preparation

Bronchial rings (internal diameter, 5–10 mm) were obtained from 8 donor and 3 recipient patients undergoing heart or heart-lung transplantation (age 12–56 years, 6 males). There was no evidence of long-term lung disease or airway inflammation in the 8 donors, whereas the recipients had respiratory failure caused by pulmonary emphysema. After the operation the lung tissues were immediately placed into oxygenated Krebs-Henseleit (KH) solution of the following composition (each in mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, NaH_2PO4 1.2, $NaHCO_3$ 25.5, and glucose 5.6 (pH 7.4) cooled to 4°C, and transported to the laboratory. The airways were dissected from the parenchyma with removal of all bronchial blood vessels and cut into rings. Tissues were then suspended in 10 ml organ baths, containing KH solution, for the measurement of isometric contractile responses using force-displacement transducers (Grass Instrument, Quincy, MA, USA) connected to a polygraph (Grass Instrument). The KH solution was continually gassed by a mixture of 95% O_2 and 5% CO_2, maintained at 37°C, and contained indomethacin (10 μM) to block the formation of endogenous prostaglandins.

2.2. Protocol

Tissues were allowed to equilibrate for 2 h with washing every 20 min under a resting tension of 2.0 g. Tissues were then incubated with bosentan (1–100 μM), (*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methyl-pentanoil]amino-3-[3-(1-methyl-1*H*-indoyl)]-propionyl]amino-3-(2-pyridyl) propionic acid (FR 139317; 10 μM), or vehicle for 20 min before addition of cumulative concentrations of endothelin-1 or IRL 1620 (both 1–300 nM). The response to each concentration of agonist was allowed to reach plateau before the addition of the next concentration. At the beginning and end of all experiments, maximal contractile responses were obtained to 1 mM acetylcholine.

2.3. Drugs and chemicals

Indomethacin and acetylcholine chloride were obtained from Sigma (Poole, UK). Endothelin-1 was purchased from Peptide Institute (Osaka, Japan). IRL 1620 (Suc-[Glu^{9},Ala^{11,15}]endothelin-1-(8–21)) was a gift from International Research Laboratories, Ciba-Geigy (Takarazuka, Japan). FR 139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methylpentanoil]amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl]amino-3-(2-pyridyl) propionic acid) was provided by the Chemistry Department of Parke-Davis Pharmaceutical Division of Warner-Lambert (Ann Arbor, MI, USA) and bosentan (4-tert-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzenesulfonamide} was a gift from Hoffmann-La Roche (Basel, Switzerland). All drugs were dissolved in distilled water and made up daily.

2.4. Analysis of the results

Data are expressed as mean ± standard error (S.E.) and expressed as a percentage of the maximum contraction to 1 mM acetylcholine. pD_2 values (−log of the concentration of agonist required to produce 50% of maximum response) were calculated by iterative curve fitting using Graphpad Inplot (Graphpad, San Diego, CA, USA).

3. Results

Endothelin-1 and IRL 1620 (both 1–300 nM) produced concentration-dependent contractions of the human

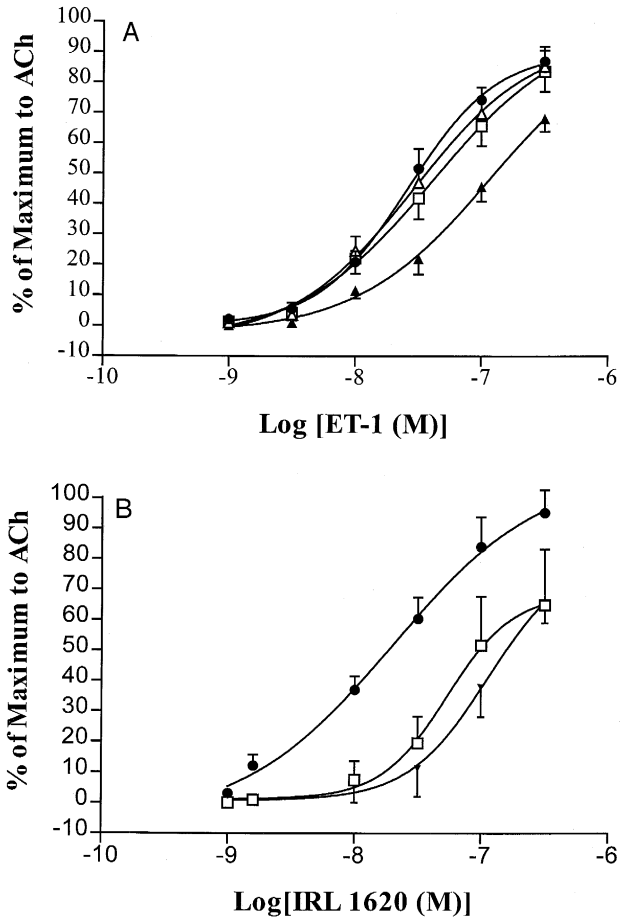

Fig. 1. (A) Effects of bosentan (control, closed circle; bosentan 1 μM, open triangle; bosentan 10 μM, open square; bosentan 100 μM, closed triangle) on contractile responses of rings of human bronchi induced by endothelin-1. (B) Effects of bosentan (control, closed circle; bosentan 1 μM, open square; bosentan 10 μM, inverted closed triangle) on contractile responses evoked by IRL 1620. Responses are displayed as a mean percentage of maximum contractions to acetylcholine (1 mM). Points represent mean ± S.E. of at least three experiments.

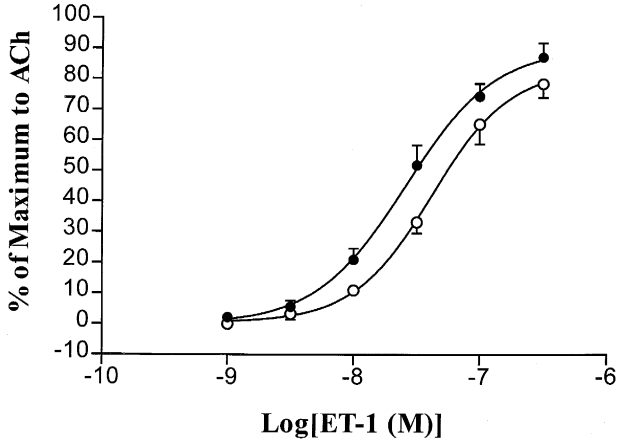

Fig. 2. Effect of FK 139317 (10 μM) (control, closed circle; FK 139317, open circle) on contractile responses of rings of human bronchi induced by endothelin-1. Points represent mean ± S.E. of at least three experiments.

bronchial rings (Fig. 1A and B) with similar pD_2 values (endothelin-1, 7.56 ± 0.08; IRL 1620, 7.7 ± 0.1).

Bosentan (1 and 10 μM) was without effect on the contractile responses induced by endothelin-1. Bosentan (100 μM) did, however, cause a rightward shift (3.4-fold) in the concentration–response curve to endothelin-1 (Fig. 1A). In contrast, 1 or 10 μM bosentan significantly inhibited constrictions induced by IRL 1620, reducing the maximum response and producing rightward shifts of the IRL 1620 concentration–response curve (6.6- and 11.2-fold with 1 and 10 μM bosentan, respectively) (Fig. 1B). FR 139317 (10 μM) had no effect on the contractile responses induced by endothelin-1 (Fig. 2).

Neither bosentan nor FR 139317 affected the basal tone of the bronchial rings or their maximal contractile responses to 1 mM acetylcholine (control, 2.5 ± 0.7 g; + bosentan, 10 μM, 2.5 ± 0.3 g; + FR 139317, 10 μM, 2.0 ± 0.5 g).

4. Discussion

Here we show that endothelin-1 induces constrictions of human bronchial rings which are unaffected by the endothelin ET_A receptor antagonist, FR 139317, depressed by the non-peptide, non-selective endothelin ET_A/ET_B antagonist, bosentan, and mimicked by the endothelin ET_B receptor-selective agonist, IRL 1620. Clearly, this suggests that endothelin ET_B receptors are the predominant mediators of the constrictor effects of the endothelins in human bronchi. This is in agreement with previous reports that have shown endothelin ET_B receptors to be the predominant receptor subtype present in human bronchial smooth muscle, making up approximately 80% of the receptors for endothelin-1 (Goldie et al., 1995). Furthermore, endothelin ET_B receptor-selective agonists, such as sarafotoxin 6c, BQ 3020 and IRL 1620, are potent contractile agents of human bronchial smooth muscle (Hay et al., 1993; Goldie et al., 1995; Adner et al., 1996) and contractile responses induced by endothelin-1 are not sensitive to antagonism by FR 139317 (Adner et al., 1996). However, the failure of the endothelin ET_A receptor antagonist FR 139317 to inhibit responses to endothelin-1 cannot be used, in isolation, as evidence supporting the existence of mainly endothelin ET_B receptors in human bronchi as responses evoked by endothelin-1 have also been shown to be unaffected by the endothelin ET_B receptor antagonist BQ-788. This is because it appears to be necessary to block both the endothelin ET_A and ET_B receptor subtypes before significant attenuation of endothelin-1-induced contractions is achieved (Fukuroda et al., 1996).

Interestingly, in this study we found the non-selective endothelin ET_A/ET_B receptor antagonist, bosentan, to be considerably more potent at inhibiting constrictions induced by IRL 1620 than those induced by endothelin-1. This is unlikely to be due to endothelin-1 acting at a sub-population of endothelin ET_A receptors, for bosentan is more potent as an inhibitor of responses mediated by endothelin ET_A receptors than those mediated by endothelin ET_B receptors (Clozel et al., 1994). Notably, such differences have been found before in studies employing animal tissues. For instance, the bosentan-related compound Ro 46-2005 at 100 μM had no effect on contractions of the guinea-pig bronchus induced by endothelin-1, but at 10 μM shifted the concentration–response curve to IRL 1620 by 9-fold (Battistini et al., 1994b). Currently the reason for the difference in potencies of bosentan as an antagonist of contractile responses induced by endothelin-1 and IRL 1620 is unclear but could be connected to the different ways, and sites, in which these two agonists bind to the endothelin ET_B receptor (Nambi et al., 1994). Whatever the reason, it makes clear that in determining the potency of agents as antagonists of endothelin ET receptors it is important to test their effectiveness against the naturally occurring ligand, endothelin-1, which may be involved in disease states.

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