

A novel ET_A-receptor antagonist, FR 139317, inhibits endothelin-induced contractions of guinea-pig pulmonary arteries, but not trachea

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1 The effects of a proposed endothelin-receptor antagonist, FR 139317, on the contraction induced by endothelin-1, endothelin-2 and endothelin-3, were analysed on isolated circular segments of pulmonary arteries and rings of trachea from the guinea-pig.

2 The pharmacological profiles of endothelin-1 and endothelin-2 were almost identical in the guinea-pig pulmonary artery, whereas endothelin-3 demonstrated a weaker and less potent contractile effect. The contractions induced by endothelin-1 and endothelin-2 were competitively antagonized by FR 139317. Schild plot analysis revealed a straight line with a slope that did not differ from unity. The pA₂ value was 6.65. In contrast, the endothelin-3 induced contractile response was unaffected by FR 139317.

3 In tracheal segments endothelin-1, endothelin-2 and endothelin-3 evoked contractions of similar magnitude and sensitivity. FR 139317 had no effect on the endothelin-induced contractions in tracheal segments.

4 In ring segments of pulmonary artery and trachea, potassium, noradrenaline and histamine caused concentration-dependent contractile effects. These contractions were not modified by FR 139317 in the concentration range 10⁻⁷ to 3 × 10⁻⁶M.

5 FR 139317 seems to be a selective ET_A-receptor antagonist which competitively antagonizes the endothelin-1- and endothelin-2-induced contractions of guinea-pig isolated pulmonary arteries. Thus, the guinea-pig pulmonary artery appears to be endowed with one receptor type (ET_A) which is antagonized by FR 139317 and with another endothelin-receptor subtype which responds to endothelin-3, but is not antagonized by FR 139317. In the trachea, all three peptides act on a homogeneous population of receptors which is unaffected by FR 139317. This suggests an ET_A-receptor in the guinea-pig pulmonary artery and another receptor, probably of ET_B-type, in the guinea-pig trachea.

Keywords: ET_A-receptors; endothelin; FR 139317; pulmonary artery; trachea

Introduction

Three endothelin genes with vasoactive products have been described in the human genome (Inoue *et al.*, 1989). The products expressed by these genes are distinct from each other but display a considerable homology. The 'original' endothelin, endothelin-1, is the product originally isolated from porcine aortic endothelial cells (Yanagisawa *et al.*, 1988a). Endothelin-2 bears a close resemblance to endothelin-1, whereas endothelin-3 differs from endothelin-1 in 6 out of 21 residues (Yanagisawa *et al.*, 1988b; Inoue *et al.*, 1989). The existence of at least two distinct endothelin-receptor subtypes has been postulated, they are termed ET_A (endothelin-1-selective; Arai *et al.*, 1990) and ET_B (equally sensitive to isopeptides of the endothelin family; Sakurai *et al.*, 1990). The endothelin family of peptides and their receptors are widely distributed both in peripheral tissues and in the central nervous system, where it has been suggested that they are involved in numerous biological responses (Naylor, 1990; Whittle & Moncada, 1990; Hemsén & Lundberg, 1991; Takayanagi *et al.*, 1991; Webb, 1991; Rubanyi, 1992).

We have previously presented pharmacological evidence for different endothelin receptor populations in the guinea-pig trachea and pulmonary artery by the use of desensitization experiments (Cardell *et al.*, 1991; 1992). The recent development of endothelin antagonists (Ihara *et al.*, 1991; Saeki *et al.*, 1991) has provided suitable tools for a more strict receptor classification. In the present study, we have examined the endothelin-induced responses of pulmonary artery and tracheal segments in relation to the new

endothelin ET_A-receptor antagonist, FR 139317 (Sogabe *et al.*, 1992) in order to characterize further these receptors.

Methods

Young male guinea-pigs (200–300 g) were killed by a blow on the neck. The lungs, including the heart and trachea, were quickly removed and immersed in a cold (+4°C) buffer solution (for composition, see below). The main pulmonary artery and a distal portion of the trachea were dissected free of surrounding tissues. The vessels and the trachea were used in the experiments either immediately or, occasionally, following overnight storage in a cold buffer solution. Circular segments were mounted on two L-shaped metal prongs. One prong was connected to a force displacement transducer attached to a computer for continuous registration of isometric tension and the other to a displacement device. The mounted segments were immersed in small (2.5 ml) temperature-controlled (37°C) tissue baths containing the buffer solution. The solution was equilibrated with 5% CO₂ in O₂, giving a pH of 7.4.

Initially, a tension of 1–2 mN was applied to the arterial segments and 2–3 mN was applied to the tracheal segments. The segments were subsequently allowed to stabilize at this level of tension for 90 min. The contractile ability of each segment was then examined by exposure to a potassium-rich (60 mM) buffer solution (for composition, see below). Only when two reproducible contractions could be elicited was the individual segment used in further studies. The integrity of the vascular endothelium was assessed at the end of the

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experiments by obtaining a dilator response to 10^{-6} M acetylcholine (Furchgott, 1984). The presence of tracheal epithelium was confirmed by staining with a 5% silver nitrate solution followed by light microscopy (Abrol *et al.*, 1984). Preparations which showed signs of being without endothelium or epithelium were rejected. There were no differences in the responses to the endothelins when concentration-response curves obtained by cumulative application were compared to those obtained by a single dose procedure (Cardell *et al.*, 1990).

The responses to potassium and to the maximally effective concentration of noradrenaline and histamine were completely reversible. The responses in the absence and presence of the endothelin antagonist, FR 139317 could consequently be carried out by successive cycles of agonist exposures in each segment. This could not be done in experiments with endothelin due to the virtually sustained responses to this vasoconstrictor. Therefore, these experiments were carried out in matched pairs of segments, one segment in each series being incubated with the vehicle (0.9% saline, control) and the others incubated with the antagonist, FR 139317.

The log concentration-response relationship was approximated by linear regression analysis of the data within the 20–80% interval and the pD_2 value (i.e. the negative logarithm of the concentration eliciting half the maximum response, EC_{50}) was calculated for each experiment. Since the linear regressions in many experiments only were reliant on 3 to 4 points, the data were also fitted to a logistic hypobolic equation with tension as a function of concentration (Acheves *et al.*, 1985; Randall *et al.*, 1989):

$$t(k) = \frac{t_{\max} \times k}{A + k}$$

where t represents the tension, k the concentration and A the concentration at half-maximal tension.

Since only small differences in the pD_2 were seen between the two methods (e.g. ET-1-induced contraction of the pulmonary artery; linear regression, $pD_2 = 8.11 \pm 0.28$ and fitted to a logistic equation, $pD_2 = 8.03 \pm 0.33$) only the values from linear regression are presented in the tables. $E_{\max}\%$ (the maximal contraction elicited by an agonist expressed as a percentage of the contraction induced by 60 mM K^+) was calculated for each experiment. The concentration ratio (CR) was defined as the ratio of the EC_{50} value in the presence and absence of a given concentration of agonist (B). The pA_2 was calculated as described by Arunlakshana & Schild (1959) and modified by Tallarida *et al.* (1979); $\log (CR-1/B)$.

Solutions and drugs

The following solutions were used: (a) standard buffer solution (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2, glucose 11. (b) 60 mM K^+ buffer

solution: as above, but substituting equimolar amounts of NaCl with KCl. Analytical grade chemicals and twice-distilled water were used for preparing all solutions.

The following drugs were used: FR 139317, ((R)-2-[(S)-2-[[1-(hexahydro-1H-azepinyl)]-carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1H-indolyl)]propionyl]amino-3-(2-pyridyl)propionic acid), an endothelin ET_A-receptor antagonist, kindly donated by Dr Jo Mori, Fujisawa Pharmaceutical Co, Osaka, Japan; acetylcholine chloride (Sigma Chemical Co., St Louis, MO, U.S.A.), endothelin-1, endothelin-2, endothelin-3 (Peninsula Laboratories, Mountain View, CA, U.S.A.), histamine dihydrochloride (Sigma, Chemical Co., St. Louis, MO, U.S.A.) and noradrenaline hydrochloride (Sigma Chemical Co., St Louis, MO, U.S.A.). All agents were dissolved in, and further diluted in, saline containing 1% bovine serum albumin (Behringwerke, Marburg, Germany) and used in the experiments within 30 min to avoid any possible degradation. The concentrations of the agents are expressed as the final molar concentration in the tissue bath.

Statistics

Statistical differences between means were tested by analysis of variance (ANOVA; Wallenstein *et al.*, 1980). Statistical significance was assumed when $P < 0.05$.

Results

Endothelin-1, endothelin-2 and endothelin-3 elicited strong concentration-dependent contractions of the pulmonary artery and the trachea. In the artery, endothelin-1 and endothelin-2 were equipotent, with endothelin-3 being significantly less potent ($P < 0.05$). In the trachea, all three isopeptides were equipotent (Table 1). In the pulmonary artery, the endothelin-1- and endothelin-2-induced responses were shifted in parallel to the right by FR 139317 in concentrations between 1×10^{-7} M and 3×10^{-6} M, without any reduction in the maximal response (Figures 1a, 2a; Table 2). An FR 139317 concentration of 1×10^{-6} M caused a rightward shift with a concentration ratio of 9.9 ± 5.3 for endothelin-1 and 10.9 ± 3.2 for endothelin-2. FR 139317 (3×10^{-6} M) caused a further parallel shift to the right with a concentration-ratio of 18.3 ± 9.7 for endothelin-1.

In contrast, FR 139317 (1×10^{-6} M, 3×10^{-6} M or 10^{-5} M) did not affect the endothelin-3 induced concentration-response curve for the pulmonary artery (Figure 2b), not did this antagonist alter the log concentration-response curves induced by endothelin-1, endothelin-2 or endothelin-3 in the trachea (Tables 1 and 2). The maximal responses as well as the pD_2 values were identical both with and without FR 139317 (Figure 1b, Table 1).

The rightward displacement of the endothelin-1 induced

Table 1 Guinea-pig pulmonary artery and trachea: maximal responses and pD_2 values for the endothelins

Pulmonary artery		Control		With FR139317 (10^{-6} M)		
	n	$E_{\max}\%$	pD_2	n	$E_{\max}\%$	pD_2
Endothelin-1	20	172 ± 29	8.11 ± 0.28	7	174 ± 42	$7.25 \pm 0.42^{**}$
Endothelin-2	6	150 ± 34	8.08 ± 0.19	6	137 ± 25	$7.10 \pm 0.23^{**}$
Endothelin-3	7	78 ± 46	$7.24 \pm 0.23^{**}$	6	92 ± 49	7.22 ± 0.19
Trachea		Control		With FR139317 (10^{-6} M)		
	n	$E_{\max}\%$	pD_2	n	$E_{\max}\%$	pD_2
Endothelin-1	11	78 ± 16	7.66 ± 0.31	8	69 ± 16	7.53 ± 0.15
Endothelin-2	7	81 ± 16	7.86 ± 0.41	7	80 ± 36	7.74 ± 0.35
Endothelin-3	8	71 ± 19	7.89 ± 0.26	6	81 ± 32	7.82 ± 0.11

Maximal responses ($E_{\max}\%$) are expressed as a percentage of the contraction induced by 60 mM potassium and sensitivity (pD_2) is expressed as the negative logarithm of the concentration eliciting half the maximum response. The values represent the mean \pm s.d. $^{**}P < 0.05$, endothelin-3 vs. endothelin-1/endothelin-2; $^{*}P < 0.05$, endothelin-1 vs. endothelin-1 + FR139317; $^{*}P < 0.05$, endothelin-2 vs. endothelin-2 + FR139317.

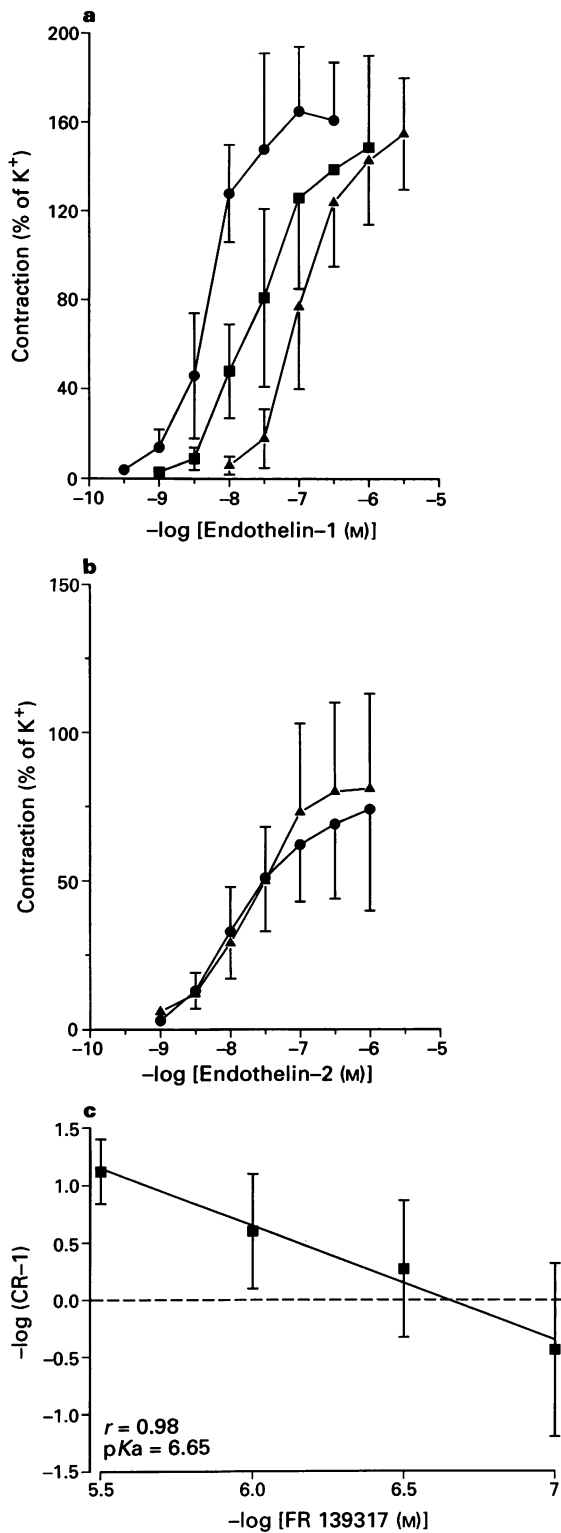


Figure 1 Concentration-response relations for endothelin-induced contractions in the presence of increasing concentrations of the endothelin ET_A receptor antagonist, FR139317. Pulmonary artery (a) and trachea (b): (●) endothelin-1/-2 control; (■) endothelin-1 + FR 139317 (3×10^{-7} M) and (▲) endothelin-1/-2 + FR 139317 (3×10^{-6} M). Responses are expressed as a percentage of the contractions induced by 60 mM potassium and each point is the mean with the s.d. shown by vertical bars ($n = 6-20$). (c) Schild plot for FR 139317 acting at the proposed ET_A-receptor in guinea-pig pulmonary artery. CR is the concentration ratio.

concentration-response curves caused by FR 130317 was used in a Schild analysis. The concentration-ratios for this antagonist yielded a line with the slope of 0.98, suggesting a

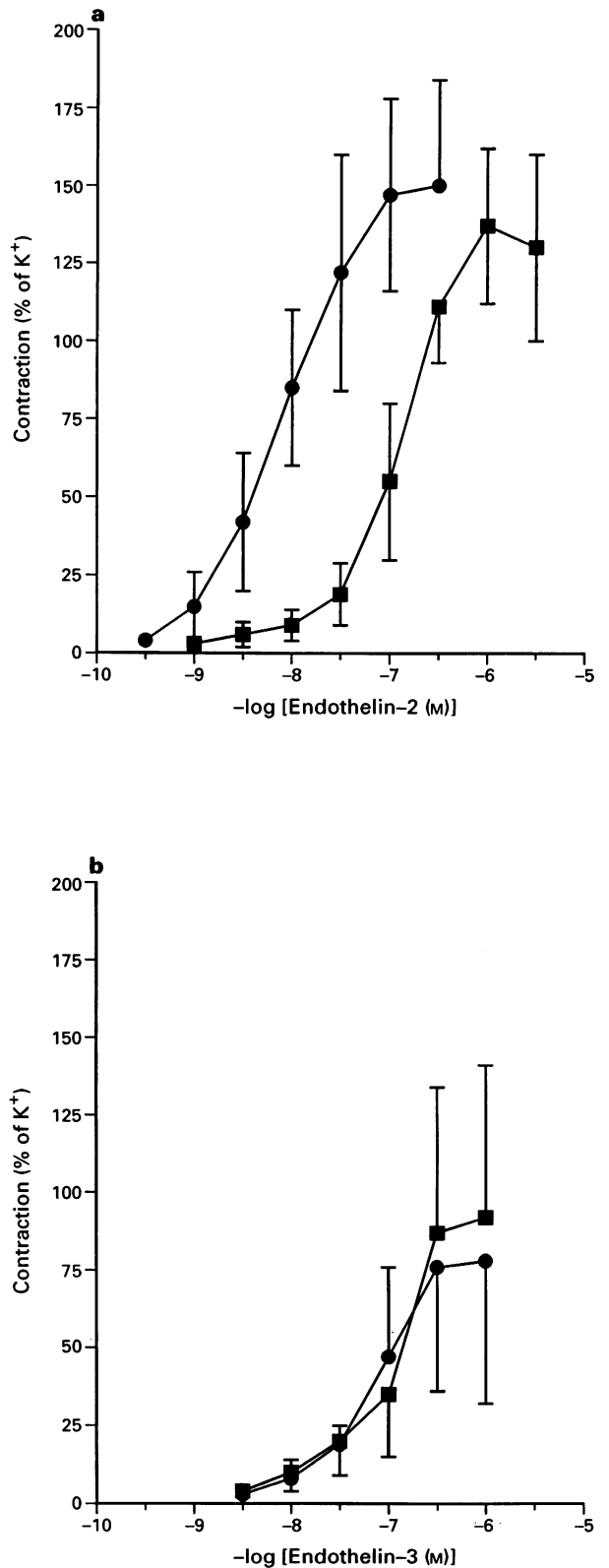


Figure 2 Concentration-response relations for endothelin-2 (a) and endothelin-3 (b) in guinea-pig pulmonary artery in the presence of the endothelin receptor antagonist FR 139317: (●) control and (■) endothelin + FR 139317 (10^{-6} M). Responses are expressed as a percentage of the contraction induced by potassium (60 mM) and each point is the mean with the s.d. shown by vertical bars ($n = 6-7$).

simple competitive antagonism at one receptor site. The resulting pA₂ for the ET_A-receptor antagonist was 6.65 (Figure 1c).

Table 2 Effects of different concentrations of FR139317 on endothelin-1 induced responses in guinea-pig pulmonary artery

Pulmonary artery		n	K ⁺	E _{max} %	pD ₂
ET	Control	20	3.81 ± 1.79	172 ± 29	8.11 ± 0.28
+ FR 139317	(10 ⁻⁷ M)	6	3.97 ± 2.45	150 ± 40	7.87 ± 0.28
+ FR 139317	(3 × 10 ⁻⁷ M)	7	3.08 ± 1.39	154 ± 45	7.58 ± 0.37
+ FR 139317	(10 ⁻⁶ M)	7	2.75 ± 0.81	174 ± 42	7.25 ± 0.42
+ FR 139317	(3 × 10 ⁻⁶ M)	6	2.79 ± 0.77	158 ± 29	6.95 ± 0.26
ANOVA			NS	NS	*

Maximal responses (E_{max} %) are expressed as a percentage of the contraction induced by 60 mM potassium and sensitivity (pD₂) is expressed as the negative logarithm of the concentration eliciting half the maximum response. The values represent the mean ± s.d.; *P < 0.05; NS, not significant.

Table 3 Guinea-pig pulmonary artery: maximal response to potassium, noradrenaline and histamine

Pulmonary artery	n	Control		With FR139317 (10 ⁻⁶ M)		
		E _{max} %	pD ₂	n	E _{max} %	pD ₂
Potassium (60 mM)	6	5.42 ± 3.82 mN		6	5.69 ± 3.66 mN	
Noradrenaline	5	139 ± 44	4.69 ± 0.13	5	140 ± 29	4.61 ± 0.14
Histamine	7	119 ± 31	5.39 ± 0.21	7	128 ± 28	5.30 ± 0.14

Maximal responses (E_{max} %) are expressed as a percentage of the contraction induced by 60 mM potassium and sensitivity (pD₂) is expressed as the negative logarithm of the concentration eliciting half the maximum response. The values represent the mean ± s.d.

The FR 139317 alone (1 × 10⁻⁶ M to 1 × 10⁻⁵ M), did not cause any contraction of isolated artery or tracheal segments. Furthermore, this antagonist, even at high concentrations (3 × 10⁻⁶ M), did not alter the vascular and tracheal constrictions induced by potassium, noradrenaline or histamine (Table 3).

Discussion

The present study demonstrates that the contractile responses to endothelin-1 and endothelin-2 in guinea-pig isolated pulmonary arteries could be shifted in parallel to the right, in a concentration-related manner, by the endothelin receptor antagonist, FR 139317. Quantitation of the antagonism, using Schild analysis, revealed that this compound consistently exhibited a competitive type of antagonism against endothelin-1 and endothelin-2. Furthermore, FR 139317 did not alter pulmonary artery vasoconstriction induced by endothelin-3, nor did it affect the tracheal smooth muscle contraction induced by endothelin-1, endothelin-2 or endothelin-3. FR 139317 seems to be a specific endothelin antagonist since it did not affect the contractions induced by noradrenaline, histamine and potassium.

Different pharmacological profiles for endothelin-1, endothelin-2 and endothelin-3 resulted in the suggestion that there is more than one receptor subtype for endothelins (Inoue *et al.*, 1989; Yanagisawa & Masaki, 1989). This assumption gained further support from ligand binding studies (Masuda *et al.*, 1989; Watanabe *et al.*, 1989). Subsequent work with cloned receptors revealed the existence of two distinct endothelin receptor subtypes termed ET_A (selective for endothelin-1 relative to endothelin-3) (Arai *et al.*, 1990) and ET_B (non-selective with respect to endothelin-1, endothelin-2 and endothelin-3) (Sakurai *et al.*, 1990). The existence of a third 'endothelin-3-selective' receptor has also been suggested (Webb 1991; Cardell *et al.*, 1992). Definitive pharmacological confirmation of the subtypes of the endothelin receptor has to await the development of selective antagonists.

Recently two pentapeptides (BQ 123 and BQ 153), synthesized by amino acid substitutions of a novel natural endothelin-receptor antagonist BE 18257, were reported to be associated with the ET_A-receptor activity in the porcine coronary artery (Ihara *et al.*, 1991; Atkinson & Pelton, 1992). A small amount of endothelin-1 induced vasoconstriction remained resistant to this compound which led to the suggestion that both ET_A and ET_B receptors were responsible for the endothelin-1-induced vasoconstriction of isolated cor-

onary arteries of the pig. The antagonists reduced the endothelin-1-induced pressure response in a concentration-dependent manner but not the depressor responses and did not affect the blood pressure of rats *in vivo* (Ihara *et al.*, 1991). The acyclic analogue [Ala^{1,3,11,15}]endothelin-1, has been reported to be a weak, but selective ET_B-receptor ligand (Saeki *et al.*, 1991). However, other reports state that this tetra-alanyl substituted analogue is equipotent with endothelin-1 at inhibiting the binding of [¹²⁵I]-endothelin-1 (Hiley *et al.*, 1990). FR 139317 has been shown to inhibit the specific binding of [¹²⁵I]-endothelin-1 to porcine and human aortic microsomes, but exhibits only a low affinity for endothelin-1 binding sites in porcine brain (Sogabe *et al.*, 1992). In rabbit isolated aorta, FR 132317 shifts the endothelin-1-induced concentration-response to the right and, *in vivo*, this antagonist completely inhibits the pressor response to endothelin-1 in normotensive rats, without any effect on the initial depressor response (Sogabe *et al.*, 1992).

We have previously shown that endothelin-1 and endothelin-2 concentration-dependently contract guinea-pig isolated pulmonary vessels. Endothelin-3 also induces contraction but with less potency. In contrast, endothelin-1, endothelin-2 and endothelin-3 show equal potency in inducing contractions of tracheal segments. By use of a pharmacological desensitization technique, two types of functional endothelin receptors could be demonstrated. In the pulmonary artery an endothelin-1/endothelin-2 receptor was found, while a non-isopeptide-selective type of endothelin receptor was found in the trachea (Cardell *et al.*, 1991; 1992). In the same smooth muscle preparations, FR 139317 strongly inhibited endothelin-1 and endothelin-2 induced vasoconstriction whereas the endothelin-induced contractions of isolated tracheal segments were unaffected. These results are in agreement with previous desensitization experiments (Cardell *et al.*, 1991; 1992) and suggest that FR 139317 is a potent ET_A-receptor selective antagonist. Furthermore, the guinea-pig pulmonary artery is dominated by an ET_A-receptor, while another type of receptor, putatively an ET_B-receptor, is found in the trachea. The possibility of a third endothelin-3 related endothelin receptor in the guinea-pig pulmonary artery cannot be excluded.

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