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Effects of Endothelin B Antagonist RES-701-1 on Endothelin-induced Contractile Responses In-vivo and In-vitro in Guinea-pigs

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Abstract

The effects of an endothelin (ET)-receptor B-specific antagonist, RES-701-1, on ET-induced contraction of guinea-pig trachea and on ET-induced bronchoconstriction in anaesthetized guinea-pigs were investigated.

In the epithelium-removed tracheal preparation, 1×10^{-5} M RES-701-1 inhibited contractions induced by the ET_B-specific agonist sarafotoxin S6c ($pK_B = 6{\cdot}10$). In the epithelium-intact tracheal preparation, RES-701-1 (1×10^{-5} M) inhibited the ET-3-induced contraction ($pK_B = 5{\cdot}27$), but enhanced the ET-1-induced contraction significantly and shifted the concentration-response curve to the left. The maximal responses of ET-1- and ET-3-induced contraction were augmented by epithelium removal by 1·5 and 1·8-fold, respectively. Against ET-3-induced contraction in the tracheal preparation without epithelium, RES-701-1 ($0{\cdot}3–10 \times 10^{-6}$ M) antagonized the contraction in a concentration-dependent manner ($pA_2 = 5{\cdot}9$). On the other hand, RES-701-1 (1×10^{-5} M) did not affect ET-1-evoked responses in the trachea without epithelium. The intravenous administration of ET-1 ($1{\cdot}5\,nmol\,kg^{-1}$) or ET-3 ($1{\cdot}5\,nmol\,kg^{-1}$) evoked a biphasic, fast and sustained bronchoconstriction in anaesthetized guinea-pigs pretreated with propranolol ($1{\cdot}0\,mg\,kg^{-1}$). When administered intravenously, RES-701-1 (0·3 or $1{\cdot}0\,mg\,kg^{-1}$) showed significant reduction in both phases of bronchoconstriction induced by ET-3. As in the case of ET-1-induced bronchoconstriction, RES-701-1 augmented the sustained phase although a significant reduction of the fast phase was observed.

These results indicate that RES-701-1 can inhibit the ET-3-induced airway responses not only in-vitro but also in-vivo.

ET-1 is a potent spasmogen of the airway smooth muscle (Uchida et al 1988; Henry et al 1990), and there is some evidence to suggest the important role of ETs in airway smooth muscle tone (Henry 1993; Noguchi et al 1993). Two distinct ET-receptor subtypes, ET_A (ET-1 selective) and ET_B (non-selective for the isopeptides) have been cloned (Masaki et al 1992; Sakurai et al 1992). The presence of ET_A and ET_B receptors in the guinea-pig tracheal smooth muscle has been reported (Inui et al 1994).

In the guinea-pig isolated trachea, ET_A-receptor-specific antagonist could not inhibit the ET-1-induced contraction, but ET_A/ET_B-nonspecific antagonists did (Battistini et al 1994). Recently the ET_B-receptor-selective antagonists IRL 1038 and BQ-788 were reported (Urade et al 1992; Ishikawa et al 1994). Urade et al (1992) investigated the role of ET_B receptors in ET-3-induced contraction of guinea-pig isolated trachea using IRL 1038. The authors suggested that ET-3-induced tracheal smooth-muscle contraction is mediated at least in part by an ET_B receptor. Ishikawa et al (1994) indicated that BQ-788 inhibited ET-1-induced guinea-pig bronchoconstriction. On the other hand, it was reported that the ET_A-specific antagonist failed to reduce bronchoconstriction (Noguchi et al 1993). These results indicate that ET_B receptors might be the dominant mediater in the ET-1-induced tracheal smooth-muscle contraction.

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RES-701-1 (cyclic (Gly^1-Asp^9)(Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp)) was discovered from the culture medium of *Streptomyces sp.* as a potent inhibitor of specific binding of [^{125}I]-endothelins to the membranes from bovine lung and cerebellum (Tanaka et al 1994). RES-701-1 is a specific inhibitor of ET_B receptors in the vascular system in-vitro (Karaki et al 1994), and abolishes ET-1-induced initial depressor effects and enhances the subsequent pressor response in anaesthetized rat (Tanaka et al 1994).

In the present study, we investigated the effects of RES-701-1 on the guinea-pig airway contractions induced by ETs in-vitro and in-vivo.

Methods

Tissue preparation

The trachea removed from male Hartley guinea-pig (SLC, Shizuoka, Japan), 350–550 g, and the thoracic aorta removed from male Wistar rat (Charles River, Japan), 200–300 g, were placed in modified Krebs-Henseleit solution. The composition of the Krebs-Henseleit solution, which was gassed with 95% O_2–5% CO_2 and maintained at 37°C, was (mM): NaCl, 119·0; KCl 4·7; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $NaHCO_3$, 25·0 and glucose 11·7. Following trimming of adherent fat and connective tissues, the trachea and the aorta were cut into rings about 2 and 1·5 mm in width, respectively. The preparations were

placed under approximately 0·5 g tension in a 2-mL bath containing Krebs-Henseleit solution. Mechanical responses were recorded isometrically with a force-displacement transducer (TB-612T, Nihon Kohden) and an amplifier (AP-621G, Nihon Kohden).

For tracheal preparations, experiments were conducted in the presence of the cyclo-oxygenase inhibitor indomethacin (5×10^{-6} M) to exclude the effects of prostanoids. Preparations were equilibrated for at least 30 min.

Some tracheal preparations were denuded of the epithelium and all aortic preparations were denuded of their endothelium by gently rubbing the luminal surface with a cotton-tipped applicator. Under these conditions, the majority of the epithelial cells or endothelial cells could be removed without obvious damage to the underlying mucosal and smooth muscle layers as examined microscopically.

Experimental protocol

Following an equilibration period, a concentration-response curve was obtained by addition of cumulative concentrations of ETs. To minimize the variability among preparations, responses of tracheal preparations and aortal preparations were expressed as a percentage of the contraction induced by 5×10^{-6} M histamine or by 6×10^{-2} M KCl, respectively. Each preparation was used for only one concentration-response curve for ET-1 or ET-3.

After the equilibration period, RES-701-1 or its vehicle dimethylsulphoxide (DMSO at a concentration $<0·5\%$, which had no effect on the ET-induced guinea-pig tracheal contraction) was preincubated for 5 min. Following the preincubation, cumulative concentrations of ET-1 or ET-3 were added when the effect of each preceding addition reached a steady level. Previous studies had indicated that 1×10^{-7} M ET-1 or ET-3 evoked nearly maximal contraction.

In substance P-induced tracheal contraction, the reaction induced by 3×10^{-7} M substance P was repeatedly observed. Hence, the contractile response in the absence of drug was induced, then the response in the presence of drug was induced in the identical preparation and the two responses were compared.

Assessment of the in-vivo bronchopulmonary function

Male Hartley guinea-pigs, 350–550 g, were anaesthetized with urethane ($1·4\,g\,kg^{-1}$, i.p.) and spontaneous breathing was abolished with gallamine triethiodide ($10\,mg\,kg^{-1}$, i.v.). The trachea was cannulated and the lung was mechanically ventilated with a constant volume with a respiration pump (70 strokes min^{-1}, 1 mL air/100 g). Systemic arterial blood pressure was recorded continuously from a catheter placed in the left carotid artery and connected to a pressure transducer (R-905, Takashima). The initial resistance to inflation was adjusted to 10 cm H_2O, according to the method of Konzett & Rössler (1940), and excess air volume was monitored from the lateral port of the ventilator circuit with an Ugo Basile bronchospasm transducer (Model 7020). The broncho-pulmonary response (BR) was expressed as percent change calculated over the value obtained by clamping of the trachea at the end of the experiment. To investigate the effects of ETs, the animals received $3\,mg\,kg^{-1}$ D,L-propranolol 5 min before the injection of ETs. RES-701 dissolved in 50% DMSO was administered intravenously 1 min before the injection of ETs.

Drugs

ET-1, ET-3 and sarafotoxin S6c were purchased from Peptide Institute Inc. (Osaka, Japan), and BQ-123 was purchased from Peninsula Laboratories (Belmont, USA). These drugs were dissolved in distilled water and stored at −20°C. BQ-788 was purchased from Peptides International, Inc. (KY, USA) and dissolved in DMSO. RES-701-1 was prepared in Kyowa Hakko Kogyo Co., Ltd., and dissolved in DMSO for in-vitro study and diluted in 50% DMSO solution for in-vivo study. Indomethacin (Sigma Chemical Co., St Louis, USA) was dissolved in ethanol. Histamine dihydrochloride (Wako Chemical Co., Osaka, Japan) was dissolved in distilled water and diluted in Krebs-Henseleit solution. Phosphoramidon (*N*-(a-L-rhamnopyranosyloxy-hydroxy-phosphinyl)-L-leucyl-L-tryptophan), substance P and DL-propranolol were purchased from Sigma Co., Ltd. Phosphoramidon and substance P were dissolved in distilled water. DL-propranolol was dissolved in saline.

Statistical analysis

Values are expressed as mean ± s.e. The pA_2 value (with 95% confidence limits) was calculated by Schild plot analysis. The pK_B value was calculated from an average of concentration curves of n preparations using single concentration of antagonist by the method of Van Rossum. Statistical analysis was performed by Dunnett's test, Scheffe test or Aspine-Welch test.

Results

Effect of RES-701-1 on the ET_A receptor- or ET_B receptor-mediated response in-vitro

RES-701-1 (1×10^{-5} M) did not influence the ET-1-induced rat thoracic aortal contractile response, which is mediated by ET_A receptors (Fig. 1a). In epithelium-removed tracheal preparations, RES-701-1 (1×10^{-5} M) inhibited the ET_B-selective agonist sarafotoxin S6c-induced contractile responses significantly ($pK_B = 6·1$) (Fig. 1b). From these results the specific inhibitory activity of RES-701-1 on the ET_B receptor was confirmed.

Effect of RES-701-1 or BQ-123 on the ET-induced guinea-pig tracheal contraction in-vitro

In the isolated tracheal preparations with epithelium, ET-1 or ET-3-evoked contraction from 1×10^{-9} M in the presence of 5×10^{-6} M indomethacin. Pretreatment with RES-701-1 (1×10^{-5} M) for 5 min, which did not influence the basal tone of preparations, significantly antagonized the contraction induced by ET-3 ($pK_B = 5·27$) (Fig. 2b). ET-1-induced contraction was enhanced by 1×10^{-5} M RES-701-1 (Fig. 2a).

ET-1-induced contraction in the trachea without epithelium was enhanced compared with the trachea with intact epithelium by 1·5-fold in the maximal responses (Table 1). In the epithelium-removed tracheal preparation, RES-701-1 (3×10^{-8} M–1×10^{-5} M) antagonized ET-3-induced contraction in a concentration-dependent manner (pA_2 5·9 (5·32–6·57), slope 1·1, $r^2 = 0·96$) (Fig. 3b), but did not affect ET-1-induced contraction at 1×10^{-5} M (Fig. 3a). An ET_A-selective antagonist BQ-123 (3×10^{-6} M) did not affect

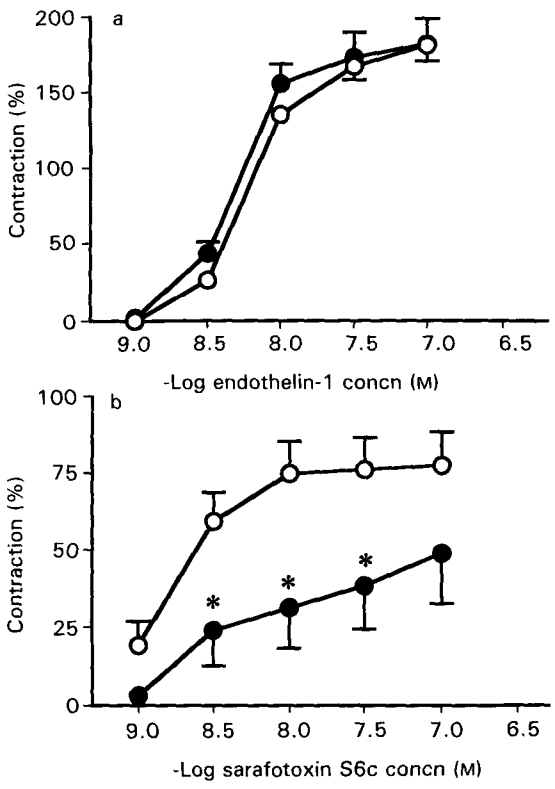

FIG. 1. Effects of RES-701-1 on ET-induced contractions in rat aortal preparation or guinea-pig tracheal preparation. Endothelium-removed aortal preparation (a) or epithelium-removed tracheal preparation (b) were preincubated with RES-701-1 (1×10^{-5} M) or vehicle for 5 min in the presence of indomethacin (5×10^{-6} M), followed by ET-1 (a) or sarafotoxin S6c (b). ○ vehicle-treated, ● RES-701-1-treated. Contractions are expressed as the percentage of the maximal response to 6×10^{-2} M KCl for aortal preparations or 5×10^{-6} M histamine for tracheal preparations. Results are shown as mean ± s.e. (a) n = 4, (b) n = 8. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with vehicle-treated preparations.

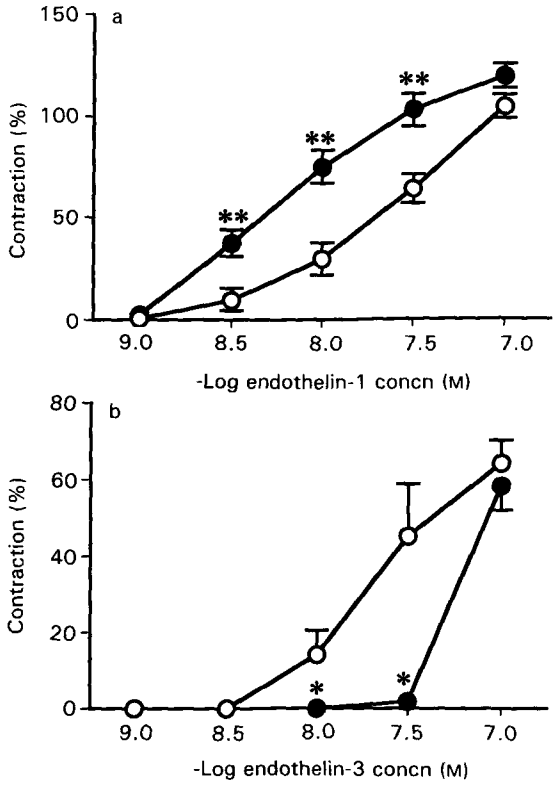

FIG. 2. Effects of RES-701-1 on ET-induced guinea-pig tracheal contraction. The trachea was preincubated with RES-701-1 (1×10^{-5} M) or vehicle for 5 min in the presence of indomethacin (5×10^{-6} M), followed by ET-1 (a) or ET-3 (b). ○ vehicle-treated, ● RES-701-1-treated. Contractions are expressed as the percentage of the maximal response to 5×10^{-6} M histamine. Results are shown as mean ± s.e. (n = 4). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with vehicle-treated preparations.

the ET-1-induced contraction in the presence of or in the absence of RES-701-1 (1×10^{-5} M) (Fig. 4).

The possibility of RES-701-1's inhibitory effect on the neutral endopeptidase in the epithelium

We investigated the effect of neutral endopeptidase (NEP) inhibitor phosphoramidon on the enhancement of ET-1-induced guinea-pig tracheal contraction by RES-701-1. Incubated with phosphoramidon (1×10^{-5} M) for 20 min, tracheal contractions induced by ET-1 were increased compared with vehicle-treated trachea by 1·4-fold in the maximal responses (Table 1). In tracheal preparations treated with phosphoramidon, RES-701-1 (1×10^{-5} M) enhanced the ET-1-induced contractions significantly (Fig. 5) and

Table 1. Effect of RES-701-1 on ET-1, ET-3 or sarafotoxin S6c-induced guinea-pig tracheal contractions.

	Vehicle			RES-701-1 (1×10^{-5} M)		
	E_{max} (%)	EC50	n	E_{max} (%)	EC50	n
Epithelium (+)						
ET-1	104·7 ± 5·8	7·7 ± 0·1	8	119·5 ± 6·0	8·3 ± 0·1**	8
+ phosphoramidon	150·1 ± 8·2$^{\#\#}$	8·5 ± 0·1$^{\#\#}$	9	173·3 ± 19·5$^{\#}$	8·9 ± 0·1$^{\#}$	9
ET-3	63·6 ± 6·1	7·7 ± 0·2	8	57·8 ± 6·1	N.D.	8
Epithelium (−)						
ET-1	152·4 ± 7·8$^{\#\#}$	8·9 ± 0·1$^{\#\#}$	8	141·0 ± 6·1$^{\#}$	8·7 ± 0·1	8
ET-3	111·2 ± 19·1$^{\#}$	8·1 ± 0·2$^{\#}$	4	50·1 ± 5·9*	N.D.	4
sarafotoxin S6c	77·0 ± 11·3	8·9 ± 0·1	4	48·6 ± 16·0	N.D.	4

Maximal responses (E_{max} %) are expressed as a percentage of the contraction induced by 5×10^{-6} M histamine, and effective concentrations (EC50) are the negative $\log_{10}$ of agonist concentration (M) eliciting half of the response to 5×10^{-6} M histamine. The values are mean ± s.e. N.D. = not determined. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the vehicle-treated preparation. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the epithelium-intact preparation.

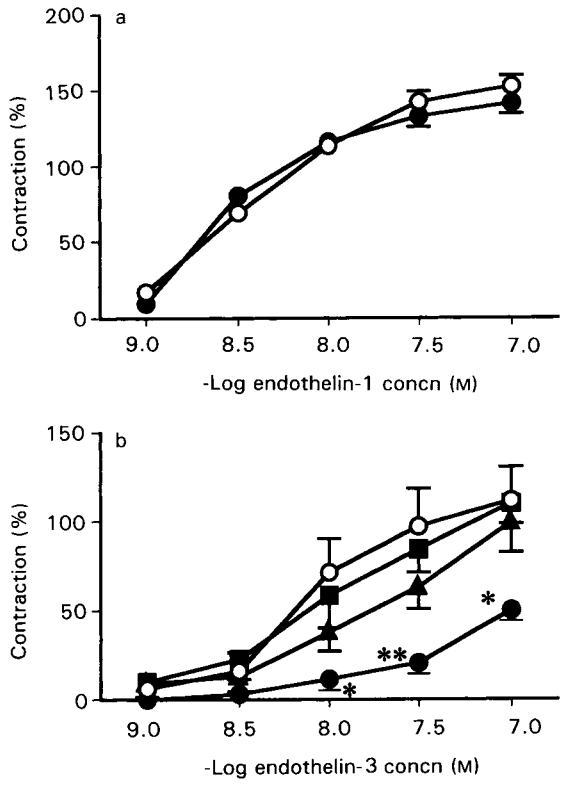

FIG. 3. Effects of RES-701-1 on ET-1-induced or ET-3-induced guinea-pig epithelium-removed tracheal contractions. The tracheae were preincubated with RES-701-1 (■ 3×10^{-7} M, ▲ 1×10^{-6} M, ● 1×10^{-5} M) or vehicle (○) for 5 min in the presence of 5×10^{-6} M indomethacin, followed by ET-1 (a) or ET-3 (b). Contractions are expressed as the percentage of the maximal response to 5×10^{-6} M histamine. Results are shown as mean ± s.e. (n = 4). $*P < 0.05$, $**P < 0.01$ significantly different from vehicle-treated preparations.

shifted the concentration-response curve leftward by 2·5-fold (Table 1). Although phosphoramidon significantly increased the substance P-induced contractile response by 2·5-fold, RES-701-1 (1×10^{-5} M) had no effect (data not shown).

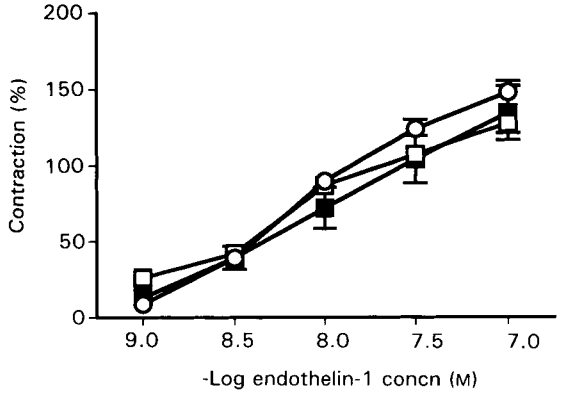

FIG. 4. Effects of BQ-123 and RES-701-1 on ET-1-induced guinea-pig epithelium-removed tracheal contractions. The trachea was preincubated with 3×10^{-6} M BQ-123 (□), 3×10^{-6} M BQ-123 and 1×10^{-6} M RES-701-1 (■) or vehicle (○) for 5 min in the presence of 5×10^{-6} M indomethacin, followed by ET-1. Contractions are expressed as the percentage of the maximal response to 5×10^{-6} M histamine. Results are shown as mean ± s.e. (n = 4).

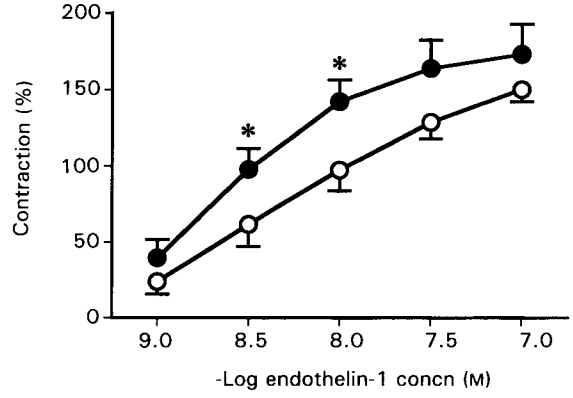

FIG. 5. Effects of RES-701-1 on ET-1-induced contraction of the guinea-pig trachea with epithelium in the presence of phosphoramidon. The trachea was preincubated with 1×10^{-5} M RES-701-1 (●) or vehicle (○) for 5 min in the presence of 5×10^{-6} M indomethacin. Each of these experiments was conducted in the presence of phosphoramidon (1×10^{-5} M) throughout. Contractions are expressed as the percentage of the maximal response to 5×10^{-6} M histamine. Results are shown as mean ± s.e. (n = 9). $*P < 0.05$ significantly different from vehicle-treated preparations.

Effects of RES-701-1 on ET-1-induced and ET-3-induced guinea-pig airway contractions in-vivo

The intravenous injection of ET-1 (1·5 nmol kg^{-1}) induced a marked and sustained increase of the excess air volume in the propranolol-treated anaesthetized guinea-pig (Fig. 6a). This airway constriction reached a peak in half a minute (fast phase), and was sustained for more than 10 min (sustained phase). ET-3 (1·5 nmol kg^{-1}) induced a marked and sustained increase in excess air volume, but with a broader sustained phase than that induced by ET-1. This sustained phase reached a peak 3 min after injection of ET-3 (Fig. 6b). RES-701-1 (0·3 or 1 mg kg^{-1}) pretreatment significantly reduced ET-3-induced airway constriction dose-dependently in both the fast phase and the sustained phase (Fig. 6b). Conversely RES-701-1 (1 mg kg^{-1}) significantly reduced the fast phase of airway constriction induced by ET-1, but enhanced the sustained phase of contraction (Fig. 6a).

Discussion

We have confirmed that RES-701-1 is an ET_B-selective antagonist, because RES-701-1 inhibited the ET_B receptor-mediated response (sarafotoxin S6c-induced guinea-pig tracheal contraction) and did not affect the ET_A receptor-mediated response (rat endothelium-removed thoracic aortal contractions). These data are concordant with the previous report (Karaki et al 1994).

Urade et al (1992) reported an ET_B-selective antagonist IRL 1038, and its effect on ET-3-induced tracheal smooth muscle contraction was investigated. The authors suggested that ET-3-induced tracheal smooth muscle contraction is mediated by ET_B receptors at least in part. In this study, RES-701-1 inhibited ET-3-induced tracheal contraction. This observation is consistent with their findings. ET-1-induced tracheal contraction was reported not to be inhibited by ET_A antagonists, but to be inhibited by nonselective ET_A/ET_B receptor antagonists (Battistini et al 1994). The result suggested that the ET_B receptor mediates the

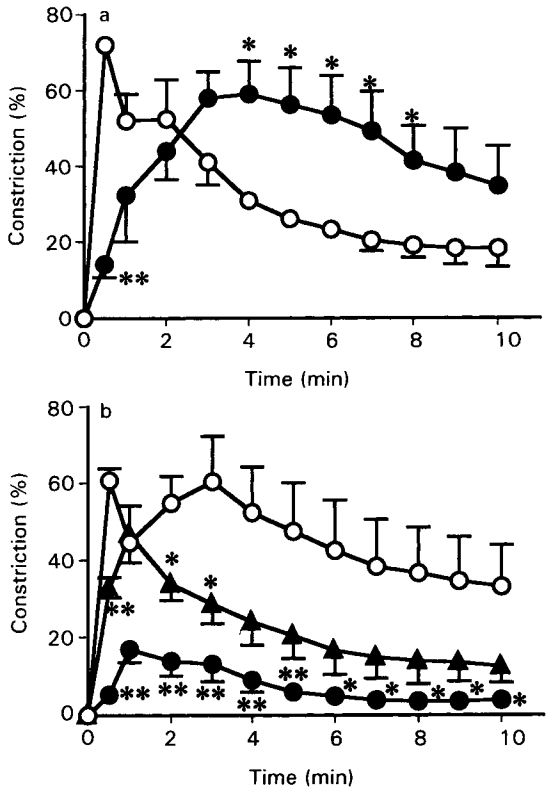

FIG. 6. Effects of RES-701-1 on ET-1-induced (a) and ET-3-induced (b) bronchoconstriction in guinea-pigs pretreated with propranolol (3 mg kg^{-1}). Vehicle (○) or RES-701-1 (▲ 0·3 mg kg^{-1}, ● 1·0 mg kg^{-1}) was administered intravenously 1 min before injection of ET-1 (1·5 nmol kg^{-1}) or ET-3 (1·5 nmol kg^{-1}). Results are expressed as percentage of maximum constriction (n = 4). *$P < 0.05$, **$P < 0.01$ significantly different from vehicle-treated animals.

ET-1-induced contractions. Contrary to expectation, RES-701-1 enhanced ET-1-induced contraction of the trachea with epithelium.

The epithelium contains the enzyme, NEP, which degrades ETs and controls the effect of ET in contracting the smooth muscle (Cadenas et al 1992). It is possible that RES-701-1 modulates NEP activity and consequently enhances ET-1-induced contraction. To investigate this possibility, we removed the epithelium of the trachea and observed the epithelial modulation on the ET-induced contraction. In epithelium-removed trachea, the contraction was increased over that of the epithelium-intact trachea as already reported (Hay 1990). In the epithelium-removed tracheal preparation, RES-701-1 inhibited the ET-3-induced contraction more than in intact preparations, but neither enhanced nor reduced the ET-1-induced contraction.

We then studied the effect of RES-701-1 using the NEP inhibitor phosphoramidon. If RES-701-1 inhibites the NEP activity and consequently enhances the ET-1 activity, the enhancing effect of RES-701-1 would become weaker in the presence of the NEP inhibitor. We found that, phosphoramidon enhanced ET-1-induced contraction as previously reported (Hay 1990), and RES-701-1 enhanced ET-1-induced contraction in the presence of phosphoramidon. Additionally, RES-701-1 did not affect the substance P-induced contraction, which is modulated by NEP. These results indicate that RES-701-1 has no direct inhibitory effect on NEP activity and on other non-selective peptidases. ET-1 produces epithelium-derived relaxation factor (EpDRF) in the epithelium (Filep et al 1993). The possibility was thus considered that ETs release EpDRF through mediating ET_B receptors. ET can relax the tracheal smooth muscle (Uchida et al 1991; Filep et al 1993), but those authors did not indicate which receptor was involved. We considered that the ET_B receptor on the epithelium may mediate the smooth-muscle relaxation and that RES-701-1 inhibited ET-1 binding to that receptor, as ET-1-induced contraction was enhanced. The ET_B antagonist RES-701-1 could not inhibit the ET-1-induced contractile responses of the epithelium-removed preparations. Battistini et al (1993) indicated the existence of non-ET_A receptors in the guinea-pig trachea using ET-1 analogues, which represent an ET_B subtype different from those reported in ET-1-binding or activities in the rabbit pulmonary artery or in the rat cerebellum or vasculature. Recently it has been reported that the ET_B receptor may be classified into subtypes ET_{B1} and ET_{B2} (Sudjarwo et al 1993, 1994). It is considered that ET_{B1} mediates the release of nitric oxide in the vascular system, and ET_{B2} mediates the guinea-pig ileum transient relaxation induced by ET-3. Karaki et al (1994) reported that RES-701-1 selectively inhibits ET_{B1} which is expressed in the endothelial cells. Taken together, ET-3- and sarafotoxin S6c-induced contractions are predominantly mediated by ET_{B1} and inhibited by RES-701-1. On the other hand, ET-1 may contract trachea binding to the ET_B receptor, which is not inhibited by RES-701-1, probably to the ET_{B2} receptor. To confirm this hypothesis, additional experiments are needed.

In in-vivo studies, ET-3 and ET-1 evoked biphasic bronchocontraction in guinea-pigs. The ET-3-induced bronchoconstriction was larger than the ET-1-induced contraction. This suggested that ET-3 is more important than ET-1 in-vivo. ET_A antagonists can not reduce the ET-1-induced guinea-pig bronchoconstriction (Noguchi et al 1993), whereas the ET_B-selective antagonist BQ-788 can inhibit it (Ishikawa et al 1994). However in the study of Ishikawa et al (1994), guinea-pig was not treated with propranolol and a lower dose of ET-1 to induce bronchoconstriction was used, so these authors probably could not observe the sustained phase of bronchoconstriction. RES-701-1 inhibited the ET-3-induced bronchoconstriction of both the fast phase and sustained phase. The result confirmed that ET_B-receptor activation is involved in the ET-3-induced airway smooth-muscle contraction. RES-701-1, however, inhibited the fast phase of ET-1-induced bronchoconstriction but enhanced the late phase. The inhibitory effect of RES-701-1 on the early phase is consistent with the previous report using an ET_B antagonist (Ishikawa et al 1994). It is speculated that ET-1 evokes the bronchoconstriction initially via production of arachidonate metabolites in inflammatory cells such as mast cells and later via direct action on the smooth muscle (Uchida et al 1992). It may be that the production of arachidonic acid metabolites involves ET_B-receptor activation in inflammatory cells and that RES-701-1 inhibits ET-1 binding to ET_B receptors on the cells. The enhancing effect of RES-701-1 was consistent with the results

obtained in-vitro and suggest that the existence of ET_B mediates relaxation and that RES-701-1 blocks that effect.

Our study shows that RES-701-1 prevents the ET-3-induced guinea-pig tracheal contraction and epithelium-dependently enhances ET-1-induced contraction. We suggest that the ET_B receptor mediates ET-3-induced guinea-pig tracheal smooth-muscle contraction, that ET-1 contracts the trachea through binding to receptors other than ET_A or ET_{B1} which is recognized by the ET_B-antagonist RES-701-1, but probably through binding to ET_{B2}, and that the ET_B receptor of the epithelium regulates ET-induced smooth muscle contraction, which might mediate the relaxation. This antagonist can inhibit ET_B-mediated responses not only in-vitro but also in-vivo, and hence is a useful tool to explore the function of ET_B receptors.

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