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Effects of the endothelin ET_A receptor antagonist, TA-0201, on pulmonary arteries isolated from hypoxic rats

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Abstract

To investigate the roles of endothelin-1 in the pathogenesis of hypoxic pulmonary hypertension, we studied the effects of a selective endothelin ET_A receptor antagonist, TA-0201 (*N*-(6-(2-(5-Bromopyrimidin-2-yloxy) ethoxy)-5-(4-methylphenyl) pyrimidin-4-yl)-4-(2-hydroxy-1,1-dimethylethyl) benzensulfonamide sodium salt sesquihydrate), on helical strips of pulmonary arteries isolated from hypoxia-induced pulmonary hypertensive rats as compared with those of normoxic rats. Endothelin-1-induced maximum contractions were significantly inhibited by exposure to hypoxia in the pulmonary arterial strips, but not in the mesenteric arterial strips. The hypoxia also induced right ventricular hypertrophy in rats. Addition of TA-0201 to the bath inhibited the endothelin-1-induced contraction of pulmonary arterial strips isolated from hypoxic rats more effectively than in those of normoxic rats. Oral administration of TA-0201 to hypoxic rats inhibited the hypoxia-induced right ventricular hypertrophy, and decreased the maximum contractile response to endothelin-1 in pulmonary arterial strips isolated from these rats. Those inhibitory effects induced by the oral administration of TA-0201 were not observed in the pulmonary arteries from normoxic rats or in the mesenteric arteries from both hypoxic and normoxic rats. These results suggest that endothelin-1 has important pathophysiological roles in hypoxia-induced pulmonary hypertension, and that TA-0201 may inhibit the endothelin-1-induced contraction through a change in the function of endothelin ET_A receptor as well as competitive inhibition for endothelin ET_A receptor.

Keywords: Endothelin-1; Hypoxia; Pulmonary hypertension

1. Introduction

Pulmonary hypertension is characterized by an increase in vascular tone or an abnormal proliferation of muscle cells in the walls of pulmonary arteries that ultimately leads to right-heart failure and death. Endothelin-1 is a potent endothelium-derived vaso-constrictor peptide (Yanagisawa et al., 1988) with important mitogenic properties (Dubin et al., 1989). In patients with pulmonary hypertension, pulmonary circulating levels of endothelin-1 are increased (Stewart et al., 1991), and endothelin-1-like immunoreactivity was observed to be abundant predominantly in endothelial cells of the human pulmonary arteries (Giaid et al., 1993). Similarly in animal studies, increased intrapulmonary endothelin-1 production and endothelin-1 mRNA expression were shown in fawn hooded rats, a strain which develops idiopathic pulmonary hypertension (Stelzner et al., 1992), in rats with monocrotaline-induced pulmonary hypertension (Miyauchi et al., 1993), and in rats with hypoxia-induced pulmonary hypertension (Li et al., 1994). These findings indicated that pulmonary hypertension may be associated with an overproduction of endothelin-1 in the lung.

The vasoactive effects of endothelin-1 are mediated by at least two receptor subtypes: endothelin ET_A and ET_B receptors (Arai et al., 1990; Sakurai et al., 1990). With the development of specific antagonists for endothelin ET_A and ET_B receptors, new tools to investigate the role of endothelin-1 in the pathogenesis of pulmonary hypertension have become available. BQ123 (cyclo (D-α-aspartyl-L-prolyl-D-valyl-L-leucyl-D-tryptophyl)) is an endothelin ET_A receptor antagonist derived synthetically from the fermentation product of *Streptomyced miakiensis* (Ihara et al., 1992). Bonvallet et al. (1994) showed that BQ123 treatment attenuated hypoxic pulmonary hypertension in

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Table 1
Effect of hypoxia on right ventricular hypertrophy in rats. Values are means ± S.E.; *n*, number of rats

	BW[a] (g)	RV/BW[b] (mg/g)	RV/LV+S[c]	*n*
Normoxic rats	226.4 ± 9.8	0.457 ± 0.019	0.226 ± 0.007	10
Hypoxic rats	192.7 ± 8.5[d]	0.731 ± 0.019[e]	0.349 ± 0.010[f]	12

[a]Body weight.
[b]Right ventricle-to-body weight ratio.
[c]Right ventricle-to-left ventricle plus septum ratio.
[d]$P < 0.05$.
[e]$P < 0.01$
[f]$P < 0.001$ (compared with normoxic rats).

rats. Bosentan, a new nonpeptide orally active compound with mixed antagonist properties for endothelin ET_A and ET_B receptors (Clozel et al., 1994), protected hypoxic rats from pulmonary hypertension (Chen et al., 1995; Eddahibi et al., 1995).

The highly potent, orally active and selective endothelin ET_A receptor antagonist TA-0201, which had been called T-0201, was recently reported by Hoshino et al. (1998). To investigate the pathophysiological roles of endothelin-1 in the pulmonary hypertension, we examined the endothelin-induced contractions of pulmonary and mesenteric arterial strips isolated from hypoxic rats, and we characterized the effects of TA-0201 on the endothelin-1-induced contractions.

2. Material and methods

2.1. Induction of hypoxic pulmonary hypertension

Rats with hypoxia-induced pulmonary hypertension were prepared according to the methods described by Maruyama and Maruyama (1994). Male Wistar rats weighing 170–220 g were used. Hypoxic rats were kept in a hypobaric chamber (air at 380 mmHg) for 10 days, and normoxic rats were housed in room air at a normal atmospheric pressure. The pressure in the hypobaric chamber was reduced using an electrically-driven vacuum pump. The chamber was opened for 15–30 min twice a day so that the cages could be cleaned and food and water replenished.

2.2. Recording of contractile activity of rat vascular strips

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The lungs and heart were removed en bloc and placed in modified Krebs–Henseleit solution at room temperature. The composition of this solution was (in mM) NaCl 115, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, and dextrose 10.0. The right ventricle of the heart was dissected from the left ventricle plus septum, and these cardiac portions were weighed separately. Intrapulmonary arteries (0.7–1.2 mm in external diameter) and mesenteric arteries (0.8–1.2 mm in external diameter) were isolated and gently cleaned of fat and connective tissue. Helically cut strips, 1.0 mm in width and 12.0 mm in length, were prepared and then suspended vertically in a 5 ml organ bath under 0.8 g tension in modified Krebs–Henseleit solution. The bath medium was maintained at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2. The vascular strips were allowed to equilibrate for 90 min before the start of the experiments. During the equilibration period, the bath solution was replaced every 20 min. Changes in muscle tension were measured isometrically through a force displacement transducer (TB612, Nihon

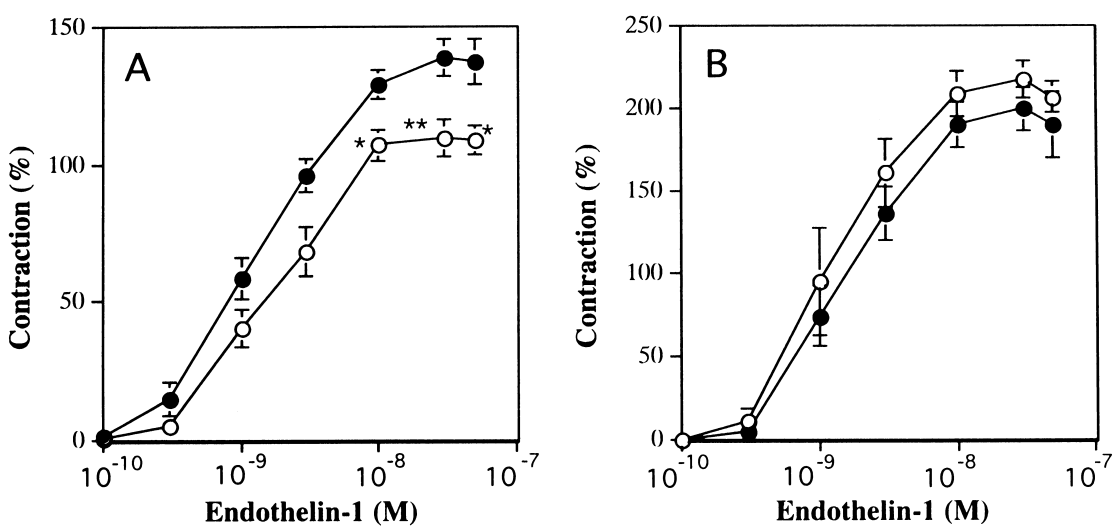

Fig. 1. Endothelin-1-induced contraction of pulmonary (A) and mesenteric (B) arterial strips from hypoxic rats (○) and normoxic rats (●). Each data point is mean ± S.E. ($n = 6–7$). The maximum contractile tension induced by 50 mM KCl was taken as 100% and the resting tension was taken as 0%. The maximum contractile tensions induced by 50 mM KCl of pulmonary arterial strips were 158.3 ± 13.5 mg (hypoxic rats), and 142.6 ± 13.1 mg (normoxic rats). The maximum contractile tension by 50 mM KCl of mesenteric arterial strips were 210.0 ± 20.5 mg (hypoxic rats), and 243.8 ± 25.7 mg (normoxic rats). $^*P < 0.05$; $^{**}P < 0.01$ compared with the response of normoxic rat arteries.

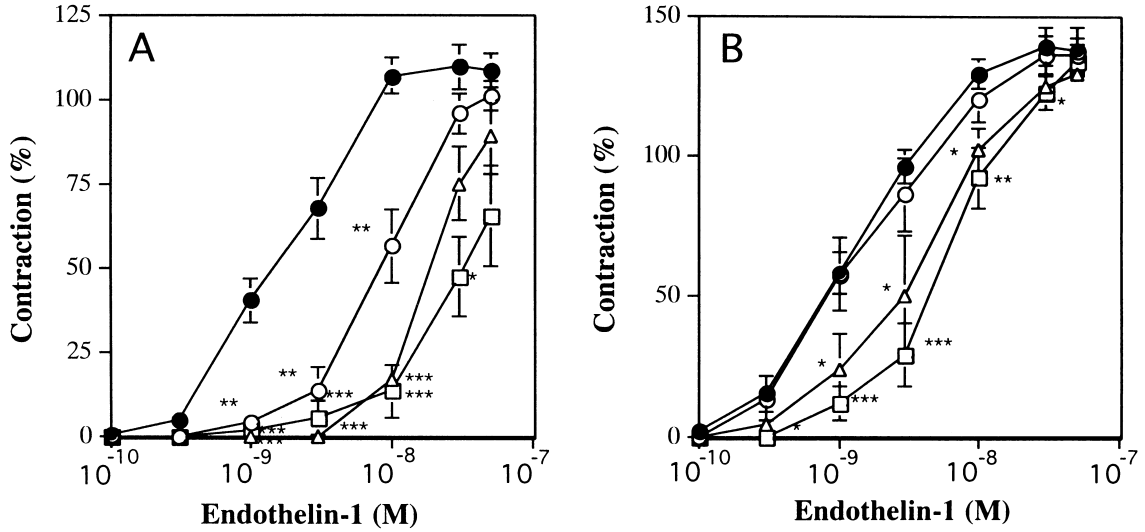

Fig. 2. Concentration–response curves for endothelin-1 of the pulmonary arterial strips from hypoxic rats (A) and normoxic rats (B) in the absence of TA-0201 (●) and in the presence of 10^{-9} M (□), 3×10^{-9} M (Δ) and 10^{-8} M (○) TA-0201. Vertical bars indicate the S.E. ($n = 5$–6). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with the response of pulmonary arterial strips to endothelin-1 in the absence of TA-0201.

Kohden, Tokyo, Japan) connected to a carrier amplifier (AP600G, Nihon Kohden). Endothelial cells were removed by repeatedly rubbing the arterial strips with the intimal surface down over a sheet of filter paper moistened with modified Krebs–Henseleit solution (Furchgott and Zawadzki, 1980). The absence of functional endothelium was determined at the start of each experiment by the absence of relaxation response of 10^{-7} M norepinephrine-precontracted strips to acetylcholine. In the presence of endothelium, 10^{-7} M norepinephrine-precontracted pulmonary arterial strips were relaxed by 10^{-6} M acetylcholine. The acetylcholine-induced relaxation percents of pulmonary arterial strips isolated from hypoxic rats and normoxic rats were $52.8 \pm 9.6\%$ and $71.1 \pm 5.8\%$, respectively. TA-0201 was administered orally with 0.25% carboxymethyl cellulose (0.3 mg/kg, two times a day) for 12 days (2 days before exposure to hypoxia and 10 days of exposure to hypoxia) to the rats. All responses are expressed as means ± S.E. The data were analyzed by use of unpaired Student's *t*-test for two means; a value of $P < 0.05$ was considered statistically significant. To compare the inhibitory effects of TA-0201 on ET-1-induced contraction of pulmonary arterial strips isolated from hypoxic rats and normoxic rats, ANOVA with repeated measurements was performed.

2.3. Reagents

Endothelin-1 was purchased from Sigma (St. Louis, MO). TA-0201, *N*-(6-(2-(5-Bromopyrimidin-2-yloxy) ethoxy)-5-(4-methylphenyl) pyrimidin-4-yl)-4-(2-hydroxy-1,1-dimethylethyl) benzensulfonamide sodium salt sesquihydrate, was generously provided by Tanabe Seiyaku (Saitama, Japan). All other chemicals were of reagent grade or better. The solvent solutions were as follows: 0.25% acetic acid for endothelin-1 (10^{-4} M); 100% dimethyl sulphoxide (DMSO) for TA-0201 (10^{-2} M). The final concentration of DMSO in the muscle bath was never over 0.001%, a concentration which had no significant effect on the tension.

3. Results

3.1. Effects of hypoxia on right ventricular hypertrophy and endothelin-1-induced contraction of rat pulmonary arterial strips and mesenteric arterial strips

Rats exposed to hypobaric hypoxia for 10 days exhibited significant increase in the ratios of right ventricle-to-

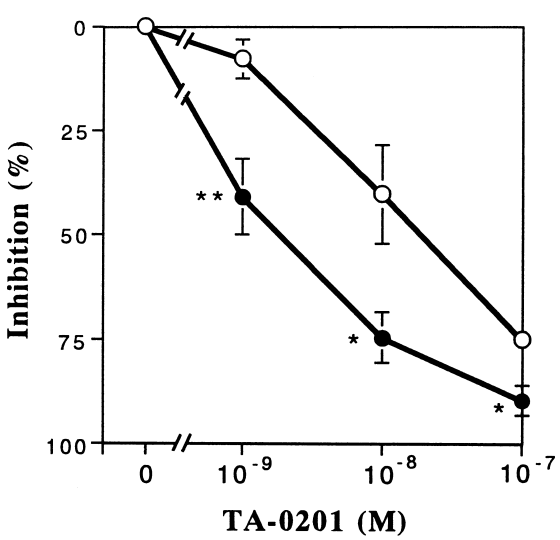

Fig. 3. Inhibitory effects of TA-0201 on 3×10^{-9} M endothelin-1-precontracted pulmonary arterial strips from hypoxic rats (●) and normoxic rats (○). Vertical bars indicate S.E. ($n = 5$–6). $^{*}P < 0.05$; $^{**}P < 0.01$ compared with the corresponding response of normoxic rat arteries.

Table 2
Effects of pretreatment with TA-0201 on right ventricular hypertrophy. Values are means ± S.E.; *n*, number of rats

		RV/BW[a](mg/g)	RV/LV+S[b]	*n*
Normoxic rats	nontreated	0.457 ± 0.019	0.226 ± 0.007	10
	TA-0201-treated	0.465 ± 0.003[c]	0.220 ± 0.003[c]	5
Hypoxic rats	nontreated	0.731 ± 0.019	0.349 ± 0.010	12
	TA-0201-treated	0.645 ± 0.035[d]	0.280 ± 0.011[d]	6

[a]Right ventricle-to-body weight ratio.
[b]Right ventricle-to-left ventricle plus septum ratio.
[c]Not significant.
[d]$P < 0.01$ (compared with TA-0201-nontreated rats).

body weight and right ventricle-to-left ventricle plus septum (Table 1). Endothelin-1-induced maximum contractions were significantly inhibited by exposure to hypoxia in the pulmonary arterial strips from these rats, but not in the mesenteric arterial strips (Fig. 1).

3.2. Effects of TA-0201 on endothelin-1-induced contraction of rat pulmonary arterial strips

The preincubation of pulmonary arterial strips with TA-0201 shifted the concentration–response curves for endothelin-1 to the right in a concentration-dependent manner (Fig. 2). The pA_2 values for TA-0201 analyzed by Schild plot in pulmonary arterial strips isolated from normoxic rats and hypoxic rats were 9.05 ± 0.15 and 10.22 ± 0.26 ($p < 0.01$, compared with normoxic rats), respectively. The cumulative addition of TA-0201 to the bath also inhibited the contractions of pulmonary arterial strips induced by 3×10^{-9} M endothelin-1 (Fig. 3). The $-\log IC_{50}$ (concentration of drug producing 50% inhibition) values for TA-0201 in pulmonary arterial strips isolated from normoxic rats and hypoxic rats were 7.878 ± 0.228 and 8.822 ± 0.112 ($p < 0.05$, compared with normoxic rats), respectively. These results indicated that the inhibitory effects of TA-0201 on the endothelin-1-induced contraction of pulmonary arterial strips isolated from hypoxic rats were more potent than that from normoxic rats.

3.3. Effects of chronic pretreatment with TA-0201 on right ventricle hypertrophy and endothelin-1-induced contraction of arterial strips

The oral administration of TA-0201 for 12 days attenuated the hypoxia-induced increases in the right ventricle-to-body weight ratio and right ventricle-to-left ventricle plus septum ratio. The same chronic pretreatment with TA-0201 did not show any significant effect on the right ventricle-to-body weight ratio and right ventricle-to-left ventricle plus septum ratio in normoxic rats. These results are summarized in Table 2. The endothelin-1-induced maximum contraction of pulmonary arterial strips isolated from hypoxic rats was significantly attenuated by the oral administration of TA-0201 (Fig. 4). In contrast, the inhibitory effect induced by the chronic pretreatment with TA-0201 was not observed in pulmonary arterial strips from normoxic rats and mesenteric arterial strips from hypoxic and normoxic rats (Figs. 4 and 5).

4. Discussion

In the present study, we demonstrated that the contractile response to endothelin-1 of rat pulmonary arterial

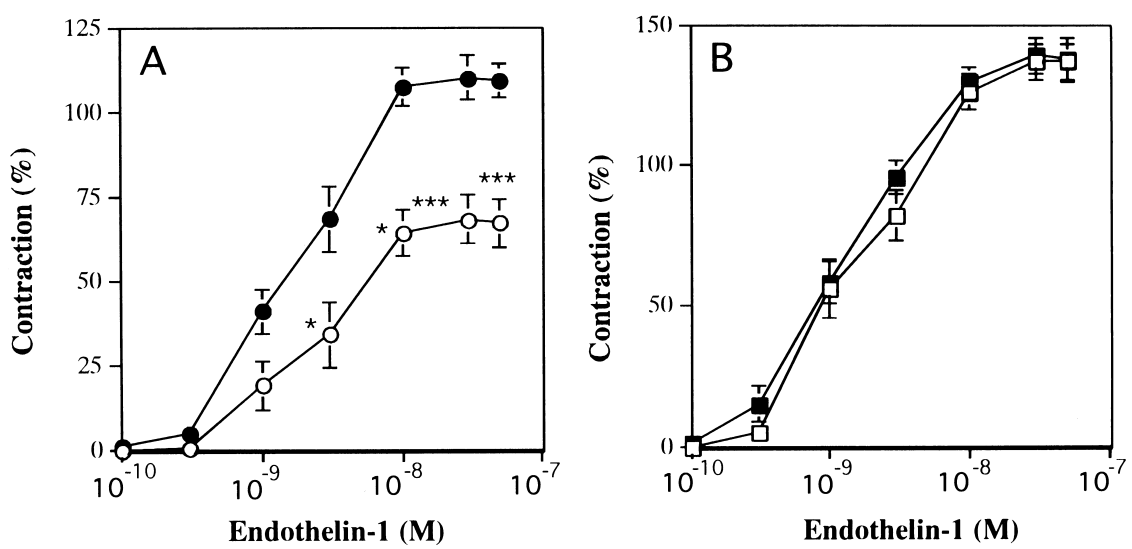

Fig. 4. Effects of oral pretreatment with TA-0201 on the endothelin-1-induced contraction of pulmonary arterial strips from hypoxic rats (A) and normoxic rats (B). Vertical bars indicate S.E. ($n = 5$–6). ○ or □, oral pretreatment with TA-0201; ● or ■, oral pretreatment with vehicle. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with the response of normoxic rat arteries.

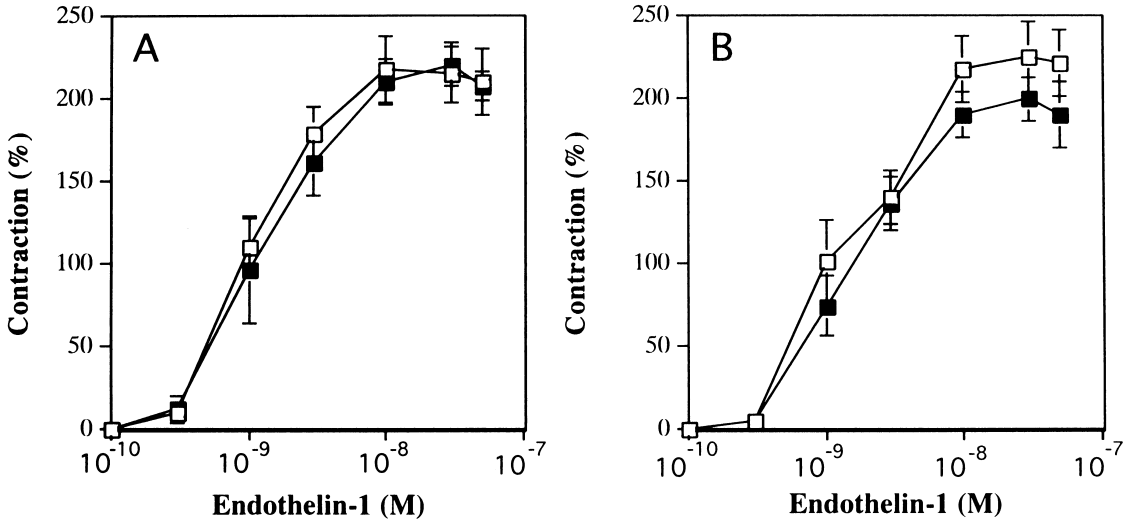

Fig. 5. Effects of oral pretreatment with TA-0201 on the endothelin-1-induced contraction of mesenteric arterial strips from hypoxic rats (A) and normoxic rats (B). Vertical bars indicate S.E. ($n = 5–6$), □ oral pretreatment with TA-0201; ■, oral pretreatment with vehicle.

strips was significantly reduced by exposure to chronic hypoxia. Miyauchi et al. (1993) reported that the isolated pulmonary artery exhibited a significantly weaker response to endothelin-1 in monocrotaline-treated rats. A study of vascular smooth muscle cells demonstrated that endothelin-1 pretreatment caused a substantial decrease in ^{125}I endothelin-1-binding sites and at the same time attenuated the ability of endothelin-1 to increase intracellular calcium (Hirata et al., 1988). A homologous downregulation of endothelin-1 binding sites in smooth muscle occurred within 30 min of endothelin-1 exposure and persisted for up to 18 h (Miasiro and Paiva, 1990; Roubert et al., 1990). The reduction of contractile response to endothelin-1 in isolated strips of hypoxic rat pulmonary artery may be due to a ligand-induced downregulation of endothelin ET_A receptors. In the present study, we observed that TA-0201, a selective endothelin ET_A receptor antagonist, inhibited the endothelin-1-induced contraction of pulmonary arterial strips isolated from hypoxic rats more effectively than in such strips from normoxic rats (Figs. 2 and 3). These data also support the downregulation of endothelin ET_A receptors of hypoxic rat pulmonary artery. In fact, chronic hypoxic exposure was found to be associated with a significant increase in endothelin-1 mRNA in the lungs and with a significant decrease in endothelin ET_A receptor mRNA in rat pulmonary artery (Li et al., 1994). Those results suggested that endothelin-1 has pathophysiological roles in hypoxia-induced pulmonary hypertension. Thus, inhibiting endothelin-1 receptors could be very effective for protect against the development of pulmonary hypertension.

BQ123, a highly specific endothelin ET_A receptor antagonist, attenuated hypoxia- and monocrotaline-induced pulmonary hypertension in rats (Miyauchi et al., 1993; Bonvallet et al., 1994; Eddahibi et al., 1995). Bosentan, an orally active endothelin receptor antagonist, protected against the development of pulmonary hypertension (Chen et al., 1995; Eddahibi et al., 1995). The present data demonstrated that the oral administration of TA-0201 inhibited the hypoxia-induced increase in right ventricle-to-left ventricle plus septum ratio (Table 2). BQ123 is a selective endothelin ET_A receptor antagonist but is not orally active (Ihara et al., 1992). Bosentan is an orally active compound with mixed antagonist properties for endothelin ET_A and ET_B receptors (Clozel et al., 1994). TA-0201, which had been called T-0201, is an orally active and highly selective endothelin ET_A receptor antagonist (Hoshino et al., 1998). TA-0201 should, therefore, be considered a valuable tool for clarifying the roles of endothelins, and may be useful as a therapeutic agent for pulmonary hypertension.

In the course of our studies, we found an interesting inhibitory effect of TA-0201 on the endothelin-1-induced contractions of pulmonary arterial strips isolated from hypoxic rats. Since TA-0201 is a selective endothelin ET_A receptor antagonist, we expected that oral pretreatment with TA-0201 would induce a recovery of the downregulation of endothelin ET_A receptor induced by hypoxia. However, the oral administration of TA-0201 to hypoxic rat produced further decrease in the maximum contractile response to endothelin-1 of pulmonary arterial strips. The TA-0201-induced decrease in endothelin-1-induced contraction was not observed in the normoxic rat pulmonary arteries or hypoxic rat mesenteric arteries. These findings suggest that the oral pretreatment with TA-0201 may cause a change in the function of endothelin ET_A receptors in the pulmonary artery of hypoxic rats.

In summary, we demonstrated that exposure to hypoxia induced right ventricular hypertrophy in rats and decreased the endothelin-1-induced contractions of rat pulmonary artery, and that an oral administration of TA-0201 inhibited the hypoxia-induced right ventricular hypertrophy and

reduced the endothelin-1-induced maximum contraction of pulmonary artery preparations from hypoxic rats. The present results not only indicate that endothelin-1 plays an important role in the development of pulmonary hypertension, but also suggest a new therapeutic approach in the management of pulmonary hypertension. However, the mechanism of these changes in the response of hypoxia-adapted pulmonary arteries to endothelin-1 remains to be determined in future investigation.

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