Inhibition of Endothelin ET\textsubscript{B} Receptor System Aggravates Neointimal Hyperplasia after Balloon Injury of Rat Carotid Artery

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ABSTRACT
Endothelin-1 (ET)/ET\textsubscript{A} receptor system has been known to play an important role in the pathogenesis of neointimal hyperplasia after endothelial injury. However, the pathological role of endothelin ET\textsubscript{B} receptors on neointimal hyperplasia remains to be elucidated. In the present study, we investigated the pathological role of ET\textsubscript{B} receptors on neointimal hyperplasia in balloon-injured rat carotid arteries by pharmacological blockade with use of 2R-(4-propoxyphenyl)-4S-(1,3-benzodioxol-5-yl)-1-(N,N-di(2,6-diethylyphenyl)aminocarbonyl-methyl)-pyrrolidine-3-carboxylic acid (A-192621), a selective ET\textsubscript{B} receptor antagonist, 2R-(4-methoxyphenyl)-4S-(1,3-benzodioxol-5-yl)-1-(N,N-di(2,6-diethylyphenyl)aminocarbonyl-methyl)-pyrrolidine-3-carboxylic acid (ABT-627), a selective ET\textsubscript{A} receptor antagonist, and (+)-(5S,6R,7R)-2-butyl-7-[2-((2S)-2-carboxypropyl)-4-methoxyphenyl]-5-(3,4-methylenedioxyphenyl)cyclopenteno[1,2-b]pyridine-6-carboxylic acid (J-104132), an ET\textsubscript{A}/ET\textsubscript{B} dual receptor antagonist. Moreover, the spotting-lethal rats, which carry a naturally occurring deletion in the endothelin ET\textsubscript{B} receptor gene, were used to examine the effects of genetic deficiency for this receptor subtype. Two weeks after balloon injury, the ratio of the neointimal to the medial area (neointima/media ratio) was determined. Treatment with A-192621 (30 mg/kg/day) for 2 weeks after injury significantly increased the neointima/media ratio in the injured artery. In contrast, ABT-627 (10 mg/kg/day) and J-104132 (10 mg/kg/day) markedly decreased the neointima/media ratio to the same extent. Furthermore, the neointima/media ratio in the injured artery of the ET\textsubscript{B}-deficient rat was significantly increased compared with that of the wild-type rat, and this increase was abolished by treatment with J-104132. These findings suggest that the inhibition of the ET\textsubscript{B} receptor system leads to an aggravation of neointimal hyperplasia after balloon injury, and the augmentation of ET\textsubscript{A}-mediated actions are responsible for the neointimal hyperplasia aggravated by the pharmacological blockade of ET\textsubscript{B} receptor or by its genetic deficiency. The antagonism of the ET\textsubscript{A} receptor system is essential for preventing restenosis after angioplasty.

Balloon angioplasty and stenting are now widely used for the treatment of coronary arterial disease. Although these procedures improve regional myocardial blood flow by dilating stenotic coronary vessels, one major drawback of this therapeutic approach is the restenosis after the procedure, because of the proliferation of vascular smooth muscle cells (VSMCs) and neointimal formation triggered by mechanical endothelial injury. The use drug-eluting stents releasing antimitotics (sirolimus and paclitaxel) has recently reduced the incidence of restenosis (Moses et al., 2003; Erglis et al., 2007), but has increased the risk of late-stent thrombosis and impaired endothelial regeneration, and has not contributed to an improvement of long-term prognosis (Farb and Boam, 2007; Lüscher et al., 2007; Spaulding et al., 2007). Thus, elucidation of molecular mechanisms of restenosis is very important for the establishment of a new therapeutic approach (Douglas, 2007; Weintraub, 2007).

Endothelin-1 (ET-1) is a vasoconstrictor peptide and has a mitogenic effect on VSMCs (Yanagisawa et al., 1988; Hirata et al., 1989). ET-1 acts by binding to its two subtypes of receptors, endothelin ET\textsubscript{A} and ET\textsubscript{B} receptors. In the vessels, ET\textsubscript{A} and ET\textsubscript{B} receptors are located on VSMCs to induce vasoconstriction and cell proliferation. ET\textsubscript{A} receptors are ex-

ABBREVIATIONS: VSMCs, vascular smooth muscle cells; ET-1, endothelin-1; sl, spotting-lethal; SBP, systolic blood pressure; NO, nitric oxide; SD, Sprague-Dawley; J-104132, (+)-(5S,6R,7R)-2-butyl-7-[2-((2S)-2-carboxypropyl)-4-methoxyphenyl]-5-(3,4-methylenedioxyphenyl)cyclopenteno[1,2-b]pyridine-6-carboxylic acid; ABT-627, 2R-(4-methoxyphenyl)-4S-(1,3-benzodioxol-5-yl)-1-(N,N-di(2,6-diethylyphenyl)aminocarbonyl-methyl)-pyrrolidine-3R-carboxylic acid; A-192621, 2R-(4-propoxyphenyl)-4S-(1,3-benzodioxol-5-yl)-1-(N,N-di(2,6-diethylyphenyl)aminocarbonyl-methyl)-pyrrolidine-3R-carboxylic acid.
pressed not only on VSMCs, but also on endothelial cells. Endothelial ET$_B$ receptors induce vasodilation and antiproliferative action (Clozel et al., 1992; Miyachi and Masaki, 1999). In the clinical study, expressions of endothelin-converting enzyme, ET-1, and endothelin receptors are enhanced in neointimal VSMCs after percutaneous coronary intervention in human coronary arteries (Shirai et al., 2006). In addition, ET-1 levels are elevated in the coronary circulation after percutaneous transluminal coronary angioplasty (Takase et al., 2003). These results suggest that ET-1 system plays an important role in the pathogenesis of restenosis after angioplasty. In animal models with restenosis such as balloon injury, expressions of ET-1, endothelin-converting enzyme, ET$_A$, and ET$_B$ receptors are increased in rat carotid arteries after balloon injury (Wang et al., 1996), and ET-1 infusion worsens neointimal hyperplasia after balloon injury in the rat model (Douglas et al., 1994). Moreover, selective ET$_A$ receptor and ET$_A$/ET$_B$ dual receptor antagonists have been reported to inhibit neointimal hyperplasia (Douglas et al., 1994; McKenna et al., 1998; Ferrer et al., 1995; Sanmartin et al., 2003). Accordingly, ET$_A$ receptor-mediated ET-1 actions are contributive to the neointimal hyperplasia, and ET$_A$ receptor antagonists are expected to be a therapeutic target for restenosis. However, the pathological role of ET$_B$ receptors on neointimal hyperplasia remains unknown. Murakoshi et al. (2002) demonstrated that inhibition of ET$_B$ receptor system in a knockout mouse model or pharmacological blockade by a selective ET$_B$ receptor antagonist leads to the enhancement of vascular remodeling by the cessation of blood flow in the carotid artery. In addition, Sachidanandam et al. (2007) reported that resistance artery remodeling in the diabetic rat is worsened by a selective ET$_B$ receptor antagonist, in contrast to the beneficial effect of a selective ET$_A$ receptor antagonist. These findings indicate that ET$_B$ receptor system plays a vasoprotective role in vascular remodeling. However, the pathological role of ET-1/ET$_B$ receptor system on neointimal hyperplasia after endothelial injury has not been fully elucidated.

The purpose of the present study is to evaluate the pathological role of ET$_B$ receptors on neointimal hyperplasia after balloon injury and compare the vasoprotective efficacy of selective ET$_A$ receptor or ET$_A$/ET$_B$ dual receptor antagonist. We used A-192621, a selective ET$_B$ receptor antagonist (von Geldern et al., 1999), ABT-627, a selective ET$_A$ receptor antagonist (Von Geldern et al., 1999), and J-104132, an ET$_A$/ET$_B$ dual receptor antagonist (10 mg/kg/day). The animals were housed in a light-controlled room with a 12-h light/dark cycle and were allowed ad libitum access to food and water. Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Committee at Osaka University of Pharmaceutical Sciences.

**Materials and Methods**

**Animals.** Two series of experiments were carried out. In the first series to investigate the pathological role of ET$_B$ receptors by pharmacological blockade, male Sprague-Dawley (SD) rats (Japan SLC, Shizuoka, Japan) were used. In the second series, male ET$_B$ receptor-deficient (sl/sl) and wild-type (+/+ rats were used. The creation of transgenic sl/sl rats has been described previously (Gariepy et al., 1998). Homozygous (sl/sl) rats have dark eyes and pigmented coats only in small spots on their heads. Wild-type and heterozygous rats have pigmented heads, backs, and tails. To definitively differentiate these rats, polymerase chain reaction was performed on DNA isolated from tail biopsy specimens, as described previously (Gariepy et al., 1998). The animals were housed in a light-controlled room with a 12-h light/dark cycle and were allowed ad libitum access to food and water. Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Committee at Osaka University of Pharmaceutical Sciences.

**Experimental Protocols.** SD rats (10 weeks of age, 300–400 g), and ET$_B$-deficient and wild-type rats (12–15 weeks of age, 300–400 g) were given the balloon injury procedure. After balloon injury, SD rats were assigned to the following treatment groups: vehicle, A-192621 (a selective ET$_B$ receptor antagonist: 10 mg/kg/day), ABT-627 (a selective ET$_A$ receptor antagonist: 10 mg/kg/day), and J-104132 (an ET$_A$/ET$_B$ dual receptor antagonist: 10 mg/kg/day). ET$_B$-deficient and wild-type rats were divided into vehicle-treated and J-104132 (10 mg/kg/day)-treated groups, respectively. Rats were gavaged with a vehicle, A-192621, ABT-627, and J-104132 for 2 weeks, starting 12 h after balloon injury. These doses of A-192621, ABT-627, and J-104132 have previously been shown to almost abolish the exogenous ET-1-induced depressor and pressor effects, respectively (Ogdenorth et al., 1996; Nishikibe et al., 1999; Von Geldern et al., 1999). In separate experiments, some ET$_B$-deficient rats were given hydralazine by drinking water (40 mg/l). The administration of hydralazine was started 3 days before and continued until 2 weeks after balloon injury. In all animals, 2 weeks after balloon injury, systolic blood pressure (SBP) was measured by the tail-cuff method and a pneumatic pulse transducer (BP-98A; Softron, Tokyo, Japan).

**Balloon Injury Procedure.** Rats were anesthetized by injection of ketamine (80 mg/kg i.p.) and xylazine (5 mg/kg i.p.), and the right carotid artery was injured with a 2F Fogarty balloon catheter (Baxter International, Deerfield, IL), as described in previous article (Mori et al., 2000). The left carotid artery was not damaged. Two weeks after balloon injury, the rats were sacrificed with a sodium pentobarbital overdose (75 mg/kg), and both left and right carotid arteries were harvested.

**Plasma ET-1 Level.** In separate experiments, SD rats were gavaged with a vehicle, A-192621 (30 mg/kg/day), ABT-627 (10 mg/kg/day), or J-104132 (10 mg/kg/day) for 5 days. ET$_B$-deficient and wild-type rats were also gavaged with a vehicle or J-104132 (10 mg/kg/day) for 5 days. After 5 days of treatment with each drug, rats were anesthetized with sodium pentobarbital (50 mg/day), and blood was withdrawn from the abdominal aorta for analysis. Plasma ET-1 level was analyzed by enzyme immunoassay kit (Assay Designs, Ann Arbor, MI).

**Morphometric Analysis.** The bilateral carotid arteries were fixed in 10% formalin, embedded in paraffin, and cut into 4-μm-thick sections. The tissue sections were stained by the elastica-van Gieson method. Morphometric analysis of each arterial segment was performed with a computer-based Motic Image Plus 2.0 Morphometric system (Shimadzu, Kyoto, Japan). The border of lumen, internal elastic lamina, and external elastic lamina were traced and measured the neointimal and medial area. The ratio of neointimal to medial area (neointima/media ratio) was calculated by dividing the neointimal area by the medial area.
Drugs. A-192621 and ABT-627 were provided by Abbott Laboratories (Abbott Park, IL). J-104132 was provided by Banyu Pharmaceutical Co., Ltd. (Tsukuba, Japan). Hydralazine was purchased from Wako Pure Chemicals (Osaka, Japan). A-192621 was dissolved in 0.02 N NaOH. ABT-627 was dissolved in a mixture of 10% ethanol, 40% propylene glycol, and 50% distilled water. J-104132 and hydralazine were dissolved in distilled water. Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO), Nakalai Tesque (Kyoto, Japan), and Wako Pure Chemicals.

Statistical Analysis. All values were expressed as the mean ± S.E.M. Relevant data were processed by InStat (Graph-PAD Software for Science, San Diego, CA). For statistical analysis, we used the unpaired Student’s t test for two-group comparison and one-way analysis of variance followed by the Tukey-Kramer or Dunnett multiple comparison tests. Differences were considered significant at $P < 0.05$.

Results

Neointimal Hyperplasia after Balloon Injury and Effects of Pharmacological Blockade. There were no significant differences in body weight and SBP among all groups (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (n = 6)</th>
<th>A-192621 (n = 4)</th>
<th>ABT-627 (n = 5)</th>
<th>J-104132 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>360 ± 6</td>
<td>362 ± 9</td>
<td>365 ± 9</td>
<td>353 ± 9</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>126 ± 5</td>
<td>126 ± 5</td>
<td>119 ± 7</td>
<td>125 ± 4</td>
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As shown in Figure 2, there was thickening of neointima in each group. The extent of neointimal hyperplasia was more pronounced in A-192621-treated rats. J-104132-treated rats also exhibited decreased neointimal areas and neointima/media ratios compared with vehicle-treated rats (0.056 ± 0.007 mm² versus 0.085 ± 0.008 mm², 0.421 ± 0.039 versus 0.684 ± 0.058) or A-192621-treated rats (Fig. 2, A and C). There were no significant differences in the medial area in any of the groups (Fig. 2B).

Neointimal Hyperplasia after Balloon Injury and Effects of J-104132 in ET$_B$-deficient Rats. As shown in Table 2, there was no significant difference in body weight between vehicle-treated wild-type and ET$_B$-deficient rats, and treatment with J-104132 to each group produced no significant changes. Compared with wild-type rats, ET$_B$-deficient rats tended to exhibit high SBP (142 ± 4 mm Hg versus 122 ± 3 mm Hg). J-104132 did not affect SBP in both wild-type and ET$_B$-deficient rats (Table 2).

In the uninjured arteries, neointimal formation was not observed in either wild-type or ET$_B$-deficient rats (Figs. 3, A and D). In the injured arteries, neointimal thickening was observed, and the extent of neointimal hyperplasia was more marked in ET$_B$-deficient rats than in wild-type rats (Fig. 3, B and E). Neointimal hyperplasia was markedly suppressed by the treatment with J-104132 in both wild-type and ET$_B$-deficient rats (Fig. 3, C and F).

Figure 4 shows results of morphometric analysis in the injured arteries. Compared with vehicle-treated wild-type rats, the neointimal area (0.216 ± 0.015 mm² versus 0.149 ± 0.009 mm²) and neointima/media ratio (1.528 ± 0.079 versus 1.134 ± 0.061) of vehicle-treated ET$_B$-deficient rats were significantly increased. J-104132-treated wild-type rats exhibited significantly decreased neointimal area and neointima/media ratios compared with vehicle-treated wild-type rats (0.052 ± 0.008 mm² versus 0.149 ± 0.009 mm², 0.451 ± 0.066 versus 1.134 ± 0.061). J-104132-treated ET$_B$-deficient rats also exhibited significantly decreased neointimal area and neointima/media ratio compared with vehicle-treated ET$_B$-deficient rats (0.056 ± 0.020 mm² versus 0.216 ± 0.015 mm², 0.487 ± 0.168 versus 1.528 ± 0.079) (Fig. 4, A and C). There were no significant differences in the medial area between vehicle-treated wild-type and J-104132-treated ET$_B$-deficient rats (Fig. 4, D and H).

**Fig. 1.** Light micrographs of the uninjured carotid artery (A–D) and injured carotid artery (E–H) in SD rats treated with vehicle (A and E), 30 mg/kg/day A-192621 (B and F), 10 mg/kg/day ABT-627 (C and G), or 10 mg/kg/day J-104132 (D and H) at 2 weeks after balloon injury (elastica-van Gieson staining; magnification, ×100).
vehicle-treated wild-type and ETB-deficient rats. Compared with vehicle-treated group. ††, P < 0.01, compared with vehicle-treated wild-type rats, the ET-1 level of A-192621-treated rats was significantly increased (0.560 ± 0.092 pg/ml versus 5.920 ± 1.197 pg/ml). J-104132-treated wild-type rats exhibited significantly increased ET-1 levels compared with vehicle-treated wild-type rats (0.402 ± 0.079 pg/ml versus 22.019 ± 1.751 pg/ml) (Fig. 5A).

Figure 5B shows results of plasma ET-1 level in wild-type and ETB-deficient rats. Compared with vehicle-treated wild-type rats, the ET-1 level of vehicle-treated ETB-deficient rats was significantly increased (0.560 ± 0.092 pg/ml versus 5.920 ± 1.197 pg/ml). J-104132-treated wild-type rats exhibited significantly increased ET-1 levels compared with vehicle-treated wild-type rats (0.560 ± 0.092 pg/ml versus 71.161 ± 7.110 pg/ml). J-104132-treated ETB-deficient rats also exhibited significantly increased ET-1 level compared with vehicle-treated ETB-deficient rats (5.920 ± 1.197 pg/ml versus 85.151 ± 5.280 pg/ml) (Figs. 5B).

**Discussion**

Neointimal hyperplasia is the most major factor of restenosis after balloon angioplasty and stent insertion. Neointimal formation occurs as a result of mechanical damage to endothelial cells and subsequent proliferation of VSMCs. Several growth factors or vasoactive peptides are related to the process of neointimal formation. It has been reported that ET-1 has a proliferative action for VSMCs (Hirata et al., 1989), and plays an important role in the pathogenesis of neointimal hyperplasia (Kirchengast and Münter, 1998; Takahashi, 2006). Both selective ETA receptor and ETB receptor dual receptor antagonists have been indicated to suppress effects of ET Antagonists on Plasma ET-1 Level. Figure 5A shows results of plasma ET-1 level in wild-type and ETB-deficient rats. Compared with vehicle-treated wild-type rats, the ET-1 level of vehicle-treated ETB-deficient rats was significantly increased (0.560 ± 0.092 pg/ml versus 5.920 ± 1.197 pg/ml). J-104132-treated wild-type rats exhibited significantly increased ET-1 levels compared with vehicle-treated wild-type rats (0.402 ± 0.079 pg/ml versus 22.019 ± 1.751 pg/ml) (Fig. 5A).

Figure 5B shows results of plasma ET-1 level in wild-type and ETB-deficient rats. Compared with vehicle-treated wild-type rats, the ET-1 level of vehicle-treated ETB-deficient rats was significantly increased (0.560 ± 0.092 pg/ml versus 5.920 ± 1.197 pg/ml). J-104132-treated wild-type rats exhibited significantly increased ET-1 levels compared with vehicle-treated wild-type rats (0.560 ± 0.092 pg/ml versus 71.161 ± 7.110 pg/ml). J-104132-treated ETB-deficient rats also exhibited significantly increased ET-1 level compared with vehicle-treated ETB-deficient rats (5.920 ± 1.197 pg/ml versus 85.151 ± 5.280 pg/ml) (Figs. 5B).

**TABLE 2**

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<tr>
<th></th>
<th>Wild-type</th>
<th>ETB-Deficient</th>
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<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>J-104132</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>374 ± 11</td>
<td>369 ± 7</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>122 ± 3</td>
<td>126 ± 4</td>
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**Fig. 2.** Effects of A-192621, ABT-627, or J-104132 on neointimal hyperplasia of the injured arteries at 2 weeks after balloon injury. A, neointimal area; B, medial area; and C, ratio of neointimal to medial area. Data are expressed as the mean ± S.E.M. *, P < 0.05; **, P < 0.01, compared with vehicle-treated group. ††, P < 0.01, compared with A-192621-treated group.

**Fig. 3.** Light micrographs of the uninjured carotid artery (A and D) and injured carotid artery (B, C, E, and F) in wild-type (A–C) and ETB-deficient rats (D–F) treated with vehicle (B and E) or 10 mg/kg/day J-104132 (C and F) at 2 weeks after balloon injury (elastica-van Gieson staining; magnification, ×100).

**Fig. 4.** Effects of ET Antagonists on neointimal hyperplasia of the injured arteries at 2 weeks after balloon injury. A, neointimal area; B, medial area; and C, ratio of neointimal to medial area. Data are expressed as the mean ± S.E.M. **, P < 0.01, compared with vehicle-treated wild-type rat; ††, P < 0.01, compared with vehicle-treated ETB-deficient rat.
ETB-deficient compared with wild-type neointimal formation compared with wild-type rats. These findings suggest that high blood pressure itself in ET\textsubscript{B}-deficient rats does not contribute to the enhanced neointimal hyperplasia.

It has been reported that the ET\textsubscript{B} receptor is related to the clearance of ET-1 from the circulation (Fukuroda et al., 1994). In addition, it has been reported that treatment with A-192621 and the deficiency of ET\textsubscript{B} receptors increase plasma ET-1 level (Elmarakby et al., 2004; Williams et al., 2004). In the present study, we measured plasma ET-1 level at 5 days after treatment with each drug, because it is important to evaluate the change in ET-1 level ongoing during neointimal hyperplasia. The plasma ET-1 level of A-192621-treated rats was markedly increased compared with vehicle-treated rats. In addition, the plasma ET-1 level of ET\textsubscript{B}-deficient rats was also increased compared with wild-type rats. We have not directly evaluated the concentration and localization of ET-1 in the injured arteries of A-192621-treated and ET\textsubscript{B}-deficient rats, but the aggravated neointimal hyperplasia in the ET\textsubscript{B} receptor-inhibited condition seems to result, at least in part, from an increase of circulating ET-1 levels. Evaluation of ET-1 of injured artery should be performed in future studies.

Previously, we demonstrated that enhanced ET-1 production and ET\textsubscript{A}-mediated actions are responsible for the increased susceptibility to deoxycorticosterone acetate-salt-induced hypertension and tissue injury in ET\textsubscript{B}-deficient rats (Matsumura et al., 2000). Moreover, we reported that the monocrotaline-induced pulmonary hypertension is aggravated by the treatment with A-192621 and the deficiency of ET\textsubscript{B} receptors, and this aggravation is abolished by ET\textsubscript{A} receptor antagonist (Nishida et al., 2004a, 2004b). Others using salt-loaded rats have also proposed that hypertension induced by chronic ET\textsubscript{B} receptor blockade is due to the indirect activation of ET\textsubscript{A} receptors, based on findings that selective ET\textsubscript{A} receptor antagonists abolish the above hyperten-

High blood pressure is a major factor affecting vascular remodeling (Intengan and Schiffrin, 2001). In the present study, we observed that SBP was significantly higher in ET\textsubscript{B}-deficient rats compared with wild-type rats. Although SBP of ET\textsubscript{B}-deficient rats was decreased by treatment with hydralazine, increments in neointimal area and neointima/media ratio observed in ET\textsubscript{B}-deficient rats were not affected by the hydralazine treatment. Thus, these findings suggest that high blood pressure itself in ET\textsubscript{B}-deficient rats does not contribute to the enhanced neointimal hyperplasia.

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The development of neointimal formation after vascular injury has been a major focus of research (Douglas et al., 1994; Ferrer et al., 1995; McKenna et al., 2004). In the present study, we measured plasma ET-1 level of wild-type and ET\textsubscript{B}-deficient rats at 2 weeks after balloon injury, and effects of J-104132. A, neointimal area; B, medial area; and C, ratio of neointimal to medial area. Data are expressed as the mean ± S.E.M. *, P < 0.05; **, P < 0.01, compared with wild-type + vehicle group. ††, P < 0.01, compared with ET\textsubscript{B}-deficient + vehicle group.

The neointimal area and neointima/media ratio of wild-type treated rats was markedly increased compared with vehicle-treated rats. In addition, the plasma ET-1 level of ET\textsubscript{B}-deficient rats was also increased compared with wild-type rats. We have not directly evaluated the concentration and localization of ET-1 in the injured arteries of A-192621-treated and ET\textsubscript{B}-deficient rats, but the aggravated neointimal hyperplasia in the ET\textsubscript{B} receptor-inhibited condition seems to result, at least in part, from an increase of circulating ET-1 levels. Evaluation of ET-1 of injured artery should be performed in future studies.

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sion (Pollock and Pollock, 2001). Elmarakby et al. (2004) found that ET<sub>A</sub> receptor antagonist attenuates salt-induced hypertension and vascular superoxide production in ET<sub>B</sub>-deficient rats. These findings suggest that chronic inhibition of ET<sub>B</sub> receptor leads to augment the ET<sub>A</sub>-mediated action, and the antagonism of ET<sub>A</sub> receptors may be essential for the protection from cardiovascular disease, irrespective of the presence of the ET<sub>B</sub> receptor-mediated action. In the present study, pharmacological blockade of ET<sub>B</sub> receptors aggravated neointimal hyperplasia after balloon injury. On the other hand, neointimal hyperplasia was markedly attenuated by the treatment with a selective ET<sub>A</sub> receptor antagonist. Treatment with an ET<sub>A</sub>/ET<sub>B</sub> dual receptor antagonist also suppressed neointimal hyperplasia, and the efficacy of treatment was comparable with that of a selective ET<sub>A</sub> receptor antagonist. Consequently, the antagonism of ET<sub>B</sub> receptors does not seem to impair the positive effects of concomitant ET<sub>A</sub> receptor antagonism. Moreover, the worsened neointimal hyperplasia observed in ET<sub>B</sub>-deficient rats was markedly improved by the treatment with J-104132. These results indicate that chronic inhibition of ET<sub>B</sub> receptors leads to an overstimulation of ET<sub>A</sub> receptors and that aggravated neointimal hyperplasia after balloon injury in the ET<sub>B</sub> receptor-inhibited condition is prevented by the blockade of ET<sub>A</sub> receptors. Therefore, it seems likely that the augmentation of ET<sub>A</sub> receptor-mediated ET-1 action mainly contributes to the enhancement of neointimal hyperplasia observed in A-192621-treated and ET<sub>B</sub>-deficient rats. Accordingly, the antagonism of the ET<sub>B</sub> receptor is essential for the preventing neointimal hyperplasia after balloon injury, irrespective of the presence of the ET<sub>A</sub> receptor-mediated action. This hypothesis may elucidate that both the selective ET<sub>A</sub> receptor and ET<sub>A</sub>/ET<sub>B</sub> dual receptor antagonists are effective to prevent neointimal hyperplasia (Douglas et al., 1994; Ferrer et al., 1995; McKenna et al., 1998; Sanmartin et al., 2003).

On the other hand, Porter et al. (1998) showed that ET<sub>B</sub> receptors mediate intimal hyperplasia in an organ culture of human saphenous vein, and a specific ET<sub>B</sub> antagonist may have a therapeutic potential for the prevention of vein graft stenosis. Their findings are in conflict with ours. We cannot elucidate the difference of these results although the difference between artery injury and vein graft intimal hyperplasia may be responsible for the conflicting findings. Further investigations are required to clarify these different results.

It has been reported that ET<sub>B</sub> receptor-mediated ET-1 action is related to vasoprotective effect such as antiproliferative action through a production of endothelium-derived nitric oxide (NO) (Garg and Hassid, 1989; Tsukahara et al., 1994). On the other hand, ET-1 can inhibit NO synthesis in vascular smooth muscle cells via ET<sub>A</sub> receptor/protein kinase C-dependent pathway (Ikedaa et al., 1997). Taken together, the possibility that the change of NO production in vasculature is involved in the aggravated neointimal hyperplasia in the ET<sub>B</sub> receptor-inhibited condition and its suppression by ET<sub>B</sub> receptor blockade cannot be ruled out. The proliferation of VSMCs and intimal thickening in response to ET-1 stimulation play a key role not only in the formation of restenosis but also in several vascular lesions, such as atherosclerosis, arterial hypertrophy by hypertension or diabetes, thereby indicating that ET receptor antagonists are useful for the treatment of vascular disease (Kirchengast and Münter, 1998; Takahashi, 2006). In the current study, we showed that the selective blockade of ET<sub>B</sub> receptors is harmful in the balloon injury model. ET<sub>B</sub> receptor-mediated vasoprotective effects have been observed in other models of vascular remodeling. Vascular remodeling caused by the cessation of blood flow was markedly accelerated in the carotid artery of ET<sub>B</sub> receptor-knockout mice, and long-term treatment with an ET<sub>B</sub> receptor antagonist worsened the vascular remodeling in wild-type mice. In contrast, the selective ET<sub>A</sub> receptor blockade could attenuate this vascular remodeling in the same animals (Murakoshi et al., 2002). In addition, Sachidanandam et al. (2007) reported that ET-1 contributes to the remodeling of mesenteric resistance arteries in diabetes via activation of ET<sub>A</sub> receptors, and ET<sub>B</sub> receptor-mediated action provides vasoprotective effects. Thereby, the blockade of ET<sub>B</sub> receptors may be harmful in vascular disease, and ET<sub>A</sub> receptor antagonists may be superior to ET<sub>A</sub>/ET<sub>B</sub> dual receptor antagonists in the treatment of vascular disease.

In summary, neointimal hyperplasia after balloon injury was worsened by the treatment with selective ET<sub>B</sub> Receptor antagonist, whereas selective ET<sub>A</sub> receptor or ET<sub>A</sub>/ET<sub>B</sub> dual receptor blockade improved neointimal hyperplasia to the same extent. Neointimal hyperplasia after balloon injury was also aggravated in the injured artery of ET<sub>B</sub>-deficient rats, and this aggravation was markedly suppressed by ET<sub>A</sub>/ET<sub>B</sub> dual receptor blockade. Moreover, plasma ET-1 levels were increased in A-192621-treated and ET<sub>B</sub>-deficient animals. These results suggest that the inhibition of ET<sub>B</sub> receptor system leads to an aggravation of neointimal hyperplasia after balloon injury. Increased circulating ET-1 levels and the augmentation of ET<sub>A</sub>-mediated actions are mainly responsible for the aggravated neointimal hyperplasia in the ET<sub>B</sub> receptor-inhibited condition. Therefore, the antagonism of the ET<sub>A</sub> receptor system is essential for preventing the restenosis after angioplasty, irrespective of the presence of the ET<sub>B</sub> receptor-mediated action.

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References


