

# Effects of Vasodilatory $\beta$ -Adrenoceptor Antagonists on Endothelium-Derived Nitric Oxide Release in Rat Kidney

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**Abstract**—The mechanisms for the vascular actions of vasodilatory  $\beta$ -blockers remain undetermined. For some kinds of  $\beta$ -blockers, the involvement of nitric oxide (NO) has been suggested. We studied the effects of vasodilatory  $\beta$ -blockers on renal perfusion pressure (RPP) and NO release in the rat kidney. Infusion of bopindolol, celiprolol, and nebivolol caused a dose-dependent reduction in RPP and an increase in NO release (RPP: bopindolol  $10^{-6}$  mol/L,  $-23 \pm 2\%$ ; celiprolol  $10^{-4}$  mol/L,  $-27 \pm 2\%$ ; nebivolol  $10^{-5}$  mol/L,  $-35 \pm 3\%$ ; NO: bopindolol  $10^{-6}$  mol/L,  $+33 \pm 2$ ; celiprolol  $10^{-4}$  mol/L,  $+41 \pm 2$ ; nebivolol  $10^{-5}$  mol/L,  $+45 \pm 5$  fmol  $\cdot$  min $^{-1}$   $\cdot$  g kidney $^{-1}$ , mean  $\pm$  SEM). Metergoline ( $10^{-6}$  mol/L), a 5-hydroxytryptamine (5-HT) $_{1/2}$  antagonist, or NAN-190 ( $10^{-6}$  mol/L), a 5-HT $_{1A}$  antagonist, almost completely abolished the vasorelaxation and NO release caused by bopindolol, celiprolol, and nebivolol. However, neither propranolol nor bisoprolol decreased RPP. Celiprolol and nebivolol caused vasodilation in the rat thoracic aorta, and it was markedly reduced by endothelial denudation, N $^{\omega}$ -nitro-L-arginine methyl ester ( $10^{-4}$  mol/L), or NAN-190 ( $10^{-6}$  mol/L). In deoxycorticosterone acetate-salt hypertensive rats, 4-week administration of celiprolol (50 mg  $\cdot$  kg $^{-1}$   $\cdot$  d $^{-1}$  IV) restored the responses regarding RPP and NO release to acetylcholine. These results suggest that several  $\beta$ -blockers exert their vasodilatory action through the 5-HT $_{1A}$  receptor/NO pathway and that treatment with these  $\beta$ -blockers may protect against endothelial injury in hypertension. (*Hypertension*. 1999;33[part II]:467-471.)

**Key Words:** nitric oxide ■ kidney ■ receptors, adrenergic, beta ■ 5-hydroxytryptamine

The mechanism for vasodilation by some  $\beta$ -adrenoceptor antagonists ( $\beta$ -blockers) is not fully understood. It has been partly explained by intrinsic sympathomimetic activity (ISA).<sup>1</sup> Celiprolol<sup>2</sup> and dilevalol<sup>3</sup> are known to stimulate  $\beta_2$ -adrenoceptors. Other mechanisms, such as the blockade of calcium channels by betaxolol<sup>4</sup> and the blockade of  $\alpha$ -adrenoceptors, have been reported, suggesting that the mechanisms for vasodilator  $\beta$ -blockers may be heterogeneous.

Although vasodilatory  $\beta$ -blockers have little negative chronotropic actions, previous studies have shown that the long-term administration of bucindolol or carvedilol is beneficial in terms of ventricular function and mortality rate in congestive heart failure,<sup>5,6</sup> probably because of the reduction in cardiac afterload and the increase in coronary blood flow. Recent studies have also suggested that several sorts of vasodilatory  $\beta$ -blockers, such as tertatolol and nebivolol, exert their effects partly via endothelium-derived nitric oxide (NO) release because their vasodilator effects are endothelium dependent and are suppressed by N $^{\omega}$ -nitro-L-arginine, an NO synthase (NOS) inhibitor; by hemoglobin, a scavenger of NO; or by methylene blue, a soluble guanylate cyclase inhibitor.<sup>7-10</sup> If NO were in-

involved in the effects of vasodilatory  $\beta$ -blockers, we could expect these to exert nitrate-like effects and prevent cardiovascular complications, including ischemic organ damage. However, the mechanisms for their NO-releasing activity are largely unknown. Differences in the pharmacological properties of these  $\beta$ -blockers such as ISA, lipid solubility, cardioselectivity, and membrane-stabilizing activity do not seem to explain systematically the reported NO-releasing activity of carteolol,<sup>11</sup> nebivolol<sup>8,10</sup> and tertatolol.<sup>7,9</sup> The mechanisms have been speculated to be an  $\alpha_2$ -agonistic activity in the case of carteolol<sup>11</sup> and a 5-hydroxytryptamine (5-HT)-agonistic activity in the case of tertatolol.<sup>9</sup>

In the present study, we investigated whether endothelium-derived NO was involved in the vasodilation caused by  $\beta$ -blockers and, if that were the case, which properties of  $\beta$ -blockers were responsible for their NO-releasing effect. Thus, we examined the endothelium-dependency of the vasodilatory effects of  $\beta$ -blockers on vascular tone and NO release in the isolated perfused rat kidney and thoracic aorta. Moreover, we investigated whether vasodilatory  $\beta$ -blockers could prevent endothelial dysfunction in hypertension.

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## Methods

### Measurement of Perfusion Pressure and NO Release in Isolated Perfused Kidney

Male Wistar rats, weighing  $316 \pm 7$  g, were anesthetized with pentobarbital sodium (40 mg/kg IP). The kidneys were isolated and perfused as described elsewhere.<sup>12</sup> The kidneys were perfused at a constant flow of 5 mL/min with a Krebs-Henseleit buffer containing  $10^{-6}$  mol/L phenylephrine and  $10^{-5}$  mol/L indomethacin to maintain renal perfusion pressure (RPP) at  $\approx 100$  mm Hg. When agents used interacted with  $\alpha_1$ -adrenoceptors, their effects were also examined using  $10^{-8.5}$  mol/L angiotensin II-containing perfusate.

The effluent perfusate from the renal vein was obtained continuously (2 mL/min), mixed with a chemiluminescence probe, and then forwarded into a chemiluminescence analyzer as previously described<sup>13</sup> to measure NO output.

Sixty minutes after the kidney was isolated, we examined the effects of celiprolol and other  $\beta$ -blockers on RPP and NO release in a cumulative manner at 10-minute intervals. To examine the endothelium-dependency of the action of  $\beta$ -blockers, we also examined the effects of pretreatments with  $N^G$ -nitro-L-arginine methyl ester (L-NAME) or E-4021 (1-[6-chloro-4-(3,4-methylbenzyl)aminoquinazolin-2-yl]piperidine-4-carboxylate), a cGMP-specific type V phosphodiesterase inhibitor.<sup>14</sup> Furthermore, we studied whether the action of  $\beta$ -blockers was exerted through adrenergic receptors or 5-HT receptors, using the antagonists and agonists of each type of receptors.

### Measurement of Tension in Aortic Ring Segments

Vascular responses of the thoracic aorta from 12-week-old, male Wistar rats were tested in organ chambers. Briefly, the rats were anesthetized by injection of sodium pentobarbital (40 mg/kg body weight IP). The thoracic aorta was excised and cut into rings (4 mm in length). Aortic rings were mounted in organ chambers filled with 25 mL of an oxygenated modified Krebs-Ringer bicarbonate solution at 37°C. Isometric tension was recorded with a force transducer (Oriental).

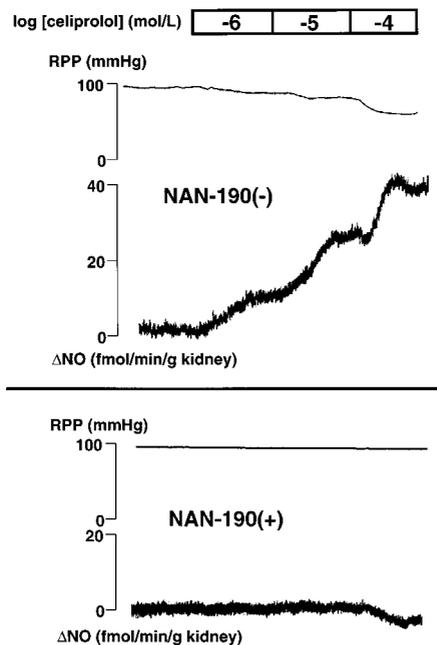
The aortic rings were precontracted with prostaglandin  $F_{2\alpha}$  ( $\approx 10^{-6}$  mol/L), and responses to celiprolol and nebivolol at 70% of the maximal contraction obtained in each individual ring were studied in the presence or absence of vascular endothelium. The endothelium of each aortic ring was removed by rubbing them gently with a stainless needle. To evaluate the role of the NO/cGMP pathway and 5-HT receptors, the responses to  $\beta$ -blockers were tested in the presence of  $10^{-4}$  mol/L L-NAME or  $10^{-6}$  mol/L NAN-190 (1-[2-methoxyphenyl]-4-[4(2-phthalimido)butyl]-piperazine), a 5-HT<sub>1A</sub> antagonist.

### Effects of Celiprolol in DOCA-Salt Hypertensive Rats

We next examined the *in vivo* effects of celiprolol in DOCA-salt hypertensive rats in which endothelial function has been suggested to be impaired.<sup>12</sup> To evaluate endothelial function, the kidneys were isolated, and the effects of acetylcholine (ACh) on RPP and NO release were examined. Six-week-old male Wistar rats were uninephrectomized, and silicone pellets containing deoxycorticosterone acetate (DOCA; 200 mg/kg) were implanted subcutaneously under ether anesthesia. The rats were then given normal saline (0.9%) for 4 weeks so that they developed hypertension (DOCA-salt hypertensive rats). Systolic blood pressure was measured by the tail-cuff method. DOCA-salt hypertensive rats were intravenously given celiprolol ( $50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) or vehicle for 4 weeks via an osmotic minipump (Alzet 2 ML4; Alza Co), which was implanted together with the DOCA pellets. All animal studies were performed in conformance with the university guidelines for animal experiments.

### Drugs and Chemicals

Bisoprolol, bopindolol, celiprolol, nebivolol, and E-4021 were kindly donated by Tanabe Seiyaku Co, Novartis Pharma KK, Nippon Shinyaku Co, Meiji Seika Co, and Eisai Co, respectively.



**Figure 1.** Representative tracings of RPP and NO release during administration of graded doses of celiprolol in the presence or absence of  $10^{-6}$  mol/L NAN-190 (5-HT<sub>1A</sub> antagonist) in the kidneys of Wistar rats.

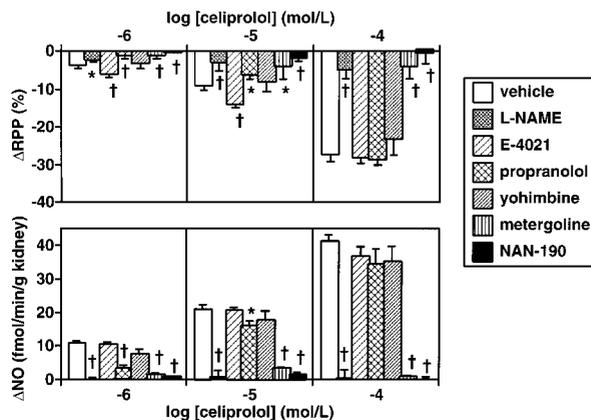
### Statistical Analysis

All values are presented as the mean  $\pm$  SEM. Data were analyzed with one-way ANOVA followed by the Student-Newman-Keuls test. Differences with  $P < 0.05$  were considered statistically significant.

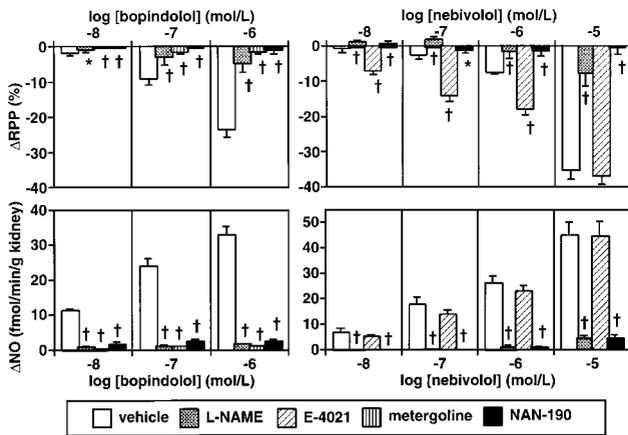
## Results

### Effects of $\beta$ -Blockers on RPP and NO in Isolated Kidney

We studied the effects of propranolol, bisoprolol, bopindolol, celiprolol, and nebivolol on RPP and NO release in the isolated perfused rat kidney. As shown in Figure 1 and 2, celiprolol decreased RPP in a dose-dependent manner. This



**Figure 2.** Effects of celiprolol on RPP and NO release in the isolated rat kidney of normal Wistar rats in the presence of vehicle, L-NAME (NOS inhibitor;  $10^{-4}$  mol/L), E-4021 (cGMP-specific phosphodiesterase inhibitor;  $10^{-8}$  mol/L), propranolol (nonselective  $\beta$ -blocker;  $10^{-6}$  mol/L), yohimbine ( $\alpha_2$ -blocker;  $10^{-7}$  mol/L), metergoline (5-HT<sub>1/2</sub> antagonist;  $10^{-6}$  mol/L), or NAN-190 (5-HT<sub>1A</sub> antagonist;  $10^{-6}$  mol/L). \* $P < 0.05$ , † $P < 0.01$  vs normal rats ( $n = 5$  for each group).



**Figure 3.** Effects of bopindolol and nebivolol on RPP and NO release in the isolated kidney of normal Wistar rats in the presence of vehicle, L-NAME (NOS inhibitor;  $10^{-4}$  mol/L), and NAN-190 (5-HT<sub>1A</sub> antagonist;  $10^{-6}$  mol/L). The effects of metergoline (5-HT<sub>1/2</sub> antagonist;  $10^{-6}$  mol/L) on the action of bopindolol and those of E-4021 (cGMP-specific phosphodiesterase inhibitor;  $10^{-8}$  mol/L) on that of nebivolol are also shown. \* $P < 0.05$ , † $P < 0.01$  vs normal rats ( $n = 5$  for each group).

vasodilation was associated with a significant increase in NO release. These effects were also observed in the kidneys pretreated with  $10^{-8.5}$  mol/L angiotensin II (data not shown). Bopindolol and nebivolol also reduced RPP and increased NO release in a dose-dependent manner (Figure 3). However, neither propranolol nor bisoprolol had such vasodilatory effects in the isolated kidney (propranolol  $10^{-6}$  mol/L,  $-1 \pm 2\%$ ; bisoprolol  $10^{-6}$  mol/L,  $-5 \pm 2\%$ ).

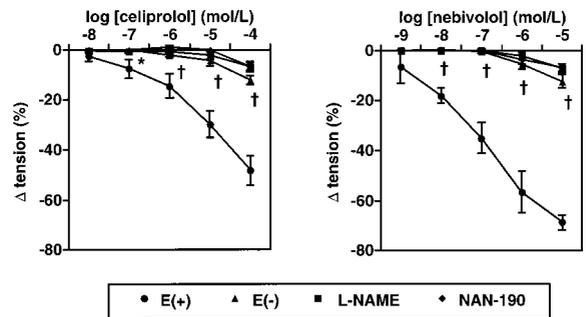
As shown in Figure 2, pretreatment with  $10^{-4}$  mol/L L-NAME markedly attenuated the responses to the infusion of celiprolol. The vasodilation in response to celiprolol, especially at the lower concentrations employed in this study, was enhanced by pretreatment with  $10^{-8}$  mol/L E-4021, but NO release induced by celiprolol and nebivolol was unaltered by E-4021.

**Effects of Adrenoceptor Antagonists and 5-Hydroxytryptamine Receptor Antagonists on the Action of  $\beta$ -Blockers in Isolated Kidney**

It is possible that celiprolol may cause vasodilation through a  $\beta_2$ - or an  $\alpha_2$ -agonistic action. The vasodilation and NO release observed in response to the lower concentrations of celiprolol were significantly reduced by pretreatment with  $10^{-6}$  mol/L propranolol but not by pretreatment with  $10^{-7}$  mol/L yohimbine, an  $\alpha_2$ -adrenoceptor antagonist (Figure 2). Yohimbine alone had no effects on RPP or NO release (data not shown). We also studied the effects of 5-HT receptor antagonists on the action of the 3  $\beta$ -blockers in the isolated rat kidney. Pretreatment with  $10^{-6}$  mol/L metergoline, a 5-HT<sub>1/2</sub> antagonist, and with  $10^{-6}$  mol/L NAN-190, a 5-HT<sub>1A</sub> receptor antagonist, almost completely inhibited the changes in RPP and NO in response to celiprolol (Figures 1 and 2). Similar suppressive effects of 5-HT antagonists on the RPP reduction and NO release induced by bopindolol or nebivolol were observed (Figure 3).

**Effects of  $\beta$ -Blockers on Rat Thoracic Aorta**

To explore whether  $\beta$ -blockers exert vasodilatory effects on other vessels, we performed tension studies using the isolated

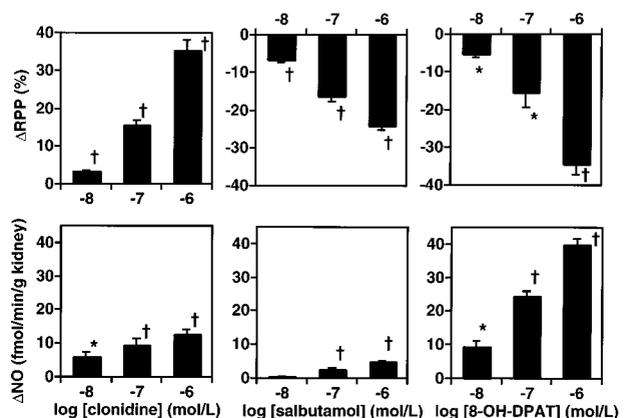


**Figure 4.** Cumulative dose-response curves for celiprolol and nebivolol in the rat thoracic aorta with endothelium [E(+)], without endothelium [E(-)], with endothelium and L-NAME (NOS inhibitor;  $10^{-4}$  mol/L), or with endothelium and NAN-190 (5-HT<sub>1A</sub> antagonist;  $10^{-6}$  mol/L). \* $P < 0.05$ , † $P < 0.01$  vs values with endothelium [E(+)] ( $n = 4$  to 8 for each group).

rat thoracic aorta. As shown in Figure 4, celiprolol and nebivolol caused vasorelaxation of the endothelium-intact aorta in a dose-dependent manner. Endothelial denudation significantly attenuated these responses. Furthermore, in the presence of L-NAME ( $10^{-4}$  mol/L) or NAN-190, vasorelaxation of the endothelium-intact aorta by celiprolol or nebivolol was markedly blunted.

**Effects of Adrenoceptor Agonists and a 5-HT<sub>1A</sub> Receptor Agonist in Isolated Kidney**

We also examined the effects of clonidine (an  $\alpha_2$ -agonist), dobutamine (a  $\beta_1$ -agonist), salbutamol (a  $\beta_2$ -agonist), and ( $\pm$ )-8-hydroxy-2-(di-*n*-propyl-amino)tetralin (8-OH-DPAT), a selective 5-HT<sub>1A</sub> receptor agonist, on vascular tone and NO release in the isolated perfused rat kidney. As shown in Figure 5, clonidine caused NO release and vasoconstriction in a dose-dependent manner (ie, it did not mimic the effects of vasodilatory  $\beta$ -blockers). Salbutamol caused vasorelaxation dose-dependently, but the dilation was associated with a very small increase of NO release. Dobutamine caused little changes in RPP or NO release (data not shown). On the other hand, infusion of 8-OH-DPAT substantially reduced RPP and increased NO in a dose-dependent manner.



**Figure 5.** Effects of clonidine ( $\alpha_2$ -agonist), salbutamol ( $\beta_2$ -agonist), and 8-OH-DPAT (5-HT<sub>1A</sub> agonist) on RPP and NO release in the isolated rat kidney. \* $P < 0.05$ , † $P < 0.01$  vs the baseline value ( $n = 5$  for each group).

### Effects of Celiprolol in DOCA-Salt Hypertensive Rats

Although several  $\beta$ -blockers actually dilated renal vessels at least in part via an NO-dependent mechanism, it remains to be determined whether such an NO-releasing property protects the vascular endothelium from various insults such as hypertension. We therefore examined the in vivo effects of celiprolol in DOCA-salt hypertensive rats. Four-week treatment with DOCA and saline significantly elevated systolic blood pressure in uninephrectomized rats (control,  $124 \pm 3$  mm Hg; DOCA-vehicle,  $188 \pm 6$  mm Hg;  $P < 0.01$ ). Intravenous administration of celiprolol ( $50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) to DOCA-salt hypertensive rats for 4 weeks slightly but significantly lowered systolic blood pressure ( $164 \pm 6$  mm Hg,  $P < 0.05$  versus DOCA-vehicle). In the isolated perfused kidney of DOCA-salt-treated rats, ACh-evoked vasorelaxation ( $10^{-8}$  mol/L ACh, DOCA-salt  $-20 \pm 2\%$  versus control  $-37 \pm 4\%$ ,  $P < 0.01$ ) and NO release ( $10^{-8}$  mol/L ACh, DOCA-salt  $+3 \pm 1$  versus control  $+16 \pm 2$   $\text{fmol} \cdot \text{min}^{-1} \cdot \text{g kidney}^{-1}$ ,  $P < 0.01$ ) were markedly attenuated compared with control rats. However, administration of celiprolol to DOCA-salt rats significantly increased the responses to ACh ( $10^{-8}$  mol/L ACh, RPP  $-41 \pm 2\%$ , NO release  $+19 \pm 2$   $\text{fmol} \cdot \text{min}^{-1} \cdot \text{g kidney}^{-1}$ , both  $P < 0.01$  versus DOCA-salt rats treated with vehicle).

### Discussion

In the present study, bopindolol, celiprolol, and nebivolol caused vasodilation of the rat renal vasculature, which was associated with an increase of NO release in a dose-dependent manner. Pretreatment with L-NAME and E-4021 resulted in attenuation and potentiation of the vasodilatory effects of these  $\beta$ -blockers, respectively, suggesting that the NO/cGMP pathway plays a role in renal vasorelaxation induced by these 3  $\beta$ -blockers. Moreover, we observed that in the rat aorta, the vasorelaxation induced by these  $\beta$ -blockers was at least in part endothelium dependent.

It seemed unlikely that the 3  $\beta$ -blockers had directly activated endothelial NOS via  $\beta$ -adrenoceptor antagonism itself because the tissues we used in the experiment were not innervated and no  $\beta$ -adrenoceptor agonists were contained in the perfusate. We speculated at first that partial agonistic activity was involved in the mechanism or mechanisms by which these 3  $\beta$ -blockers activated endothelial NOS because celiprolol has been reported to stimulate  $\beta_2$ -adrenoceptors.<sup>2</sup> It has been shown that the stimulation of  $\beta_1$ - and  $\beta_2$ -adrenoceptors activates endothelial NOS and that NO plays a role in the vasodilator effects of  $\beta$ -adrenergic stimulants.<sup>15-17</sup> On the other hand, some investigators have shown that the role of NO is of little importance in  $\beta$ -adrenoceptor-mediated vasorelaxation.<sup>18,19</sup>

In the presence of  $10^{-6}$  mol/L propranolol, which is a nonselective  $\beta$ -blocker, the vasodilatory effects of celiprolol at the lower doses used in this study were significantly attenuated in the isolated rat kidney, suggesting that ISA is ascribable at least in part for the vasodilatory effects of celiprolol. Thus, we also investigated whether stimulation of  $\beta$ -adrenoceptors caused NO release to explain the potent endothelial NOS-activating properties of the 3 vasodilating  $\beta$ -blockers. Salbutamol augmented NO release significantly,

but the extent was very small. The lack of effect of dobutamine on RPP and NO may have been due to the scarcity of  $\beta_1$ -receptors in the renal vasculature. Clonidine augmented NO release but caused vasoconstriction. Furthermore,  $10^{-5}$  mol/L yohimbine did not affect the celiprolol-induced vasorelaxation and NO release. These findings suggest that partial agonistic actions of adrenergic receptors do not explain the endothelium-dependent vasorelaxant activity of these  $\beta$ -blockers.

Although the effects of 5-HT on the cardiovascular system are complex, stimulation of 5-HT<sub>1</sub> receptors is thought to cause endothelium-dependent relaxation, most likely via NO release.<sup>20</sup> It is well established that 5-HT receptors, especially 5-HT<sub>1A</sub> receptors, can bind certain  $\beta$ -blockers, such as propranolol and pindolol, with high affinity.<sup>21</sup> Propranolol has shown to bind stereoselectively to 5-HT<sub>1</sub> sites and exert an antagonizing action.<sup>21</sup> Therefore, the blocking effects of propranolol on the celiprolol-induced changes in RPP and NO may be due to another property of propranolol: the blockade of 5-HT<sub>1</sub> receptors. As for the effects of metergoline, a 5-HT<sub>1/2</sub> antagonist, and NAN-190, a 5-HT<sub>1A</sub> antagonist, on the effects of the 3  $\beta$ -blockers on the renal vasculature, metergoline and NAN-190 almost completely abolished vasodilation and NO release induced by celiprolol, bopindolol, and nebivolol. Verbeuren et al<sup>9</sup> have shown that vasorelaxation caused by tertatolol in the isolated perfused rat kidney was markedly attenuated by BMY 7378, a 5-HT<sub>1A</sub> receptor antagonist. Furthermore, 8-OH-DPAT, a 5-HT<sub>1A</sub> agonist, increased vasodilation and NO release in a dose-dependent manner, confirming that several vasodilatory  $\beta$ -blockers exert their vascular action through the 5-HT<sub>1A</sub> receptor/NO pathway. Such pharmacological cross-reactivity between  $\beta$ -adrenoceptors and 5-HT receptors suggests a structural similarity of the ligand-binding site between the 2 kinds of receptors. The gene for the human 5-HT<sub>1A</sub> receptor was first cloned by low stringency Southern blots using the  $\beta_2$ -adrenoceptor gene as a probe.<sup>22</sup> It has also been demonstrated that a mutation of asparagine 385 in the seventh transmembrane domain of the human 5-HT<sub>1A</sub> receptor decreased its affinity for pindolol and that asparagine 385 may be a critical site for the cross-reactivity.<sup>23</sup>

Moreover, the present study has shown that celiprolol exerts beneficial effects in rats with endothelial dysfunction in the early phase of hypertension. Consistent with our results, long-term treatment with celiprolol, even at its subdepressor dose ( $5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  PO), has been reported to restore endothelial dysfunction of mesenteric arteries of spontaneously hypertensive rats,<sup>24</sup> suggesting that the beneficial effects of celiprolol in hypertension are not only due to reduction in blood pressure. Therefore, stimulation of NO release caused by celiprolol may be a mechanism underlying its vascular action.

In conclusion, several kinds of  $\beta$ -blockers are potent stimulators of endothelial NOS and exert this effect via 5-HT<sub>1A</sub> receptors. This property may contribute to protect the endothelium from hypertension.

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