Effects of Vasodilatory β-Adrenoceptor Antagonists on Endothelium-Derived Nitric Oxide Release in Rat Kidney

Masao Kakoki, Yasunobu Hirata, Hiroshi Hayakawa, Hiroaki Nishimatsu, Yasuko Suzuki, Daisuke Nagata, Etsu Suzuki, Kazuya Kikuchi, Tetsuo Nagano, Masao Omata

Abstract—The mechanisms for the vascular actions of vasodilatory β-blockers remain undetermined. For some kinds of β-blockers, the involvement of nitric oxide (NO) has been suggested. We studied the effects of vasodilatory β-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±2%; celiprolol 10⁻⁴ mol/L, −27±2%; nebivolol 10⁻⁵ mol/L, −35±3%; NO: bopindolol 10⁻⁶ mol/L, +33±2; celiprolol 10⁻⁴ mol/L, +41±2; nebivolol 10⁻⁵ mol/L, +45±5 fmol · min⁻¹ · g kidney⁻¹, mean±SEM). Metrogeline (10⁻⁶ mol/L), a 5-hydroxytryptamine (5-HT)₁₂ antagonist, or NAN-190 (10⁻⁶ mol/L), a 5-HT₁₄ 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, N⁵-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 · kg⁻¹ · d⁻¹ IV) restored the responses regarding RPP and NO release to acetylcholine. These results suggest that several β-blockers exert their vasodilatory action through the 5-HT₁₄ receptor/NO pathway and that treatment with these β-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 β-adrenoceptor antagonists (β-blockers) is not fully understood. It has been partly explained by intrinsic sympathomimetic activity (ISA).¹ Celiprolol² and dilevalol³ are known to stimulate β₂-adrenoceptors. Other mechanisms, such as the blockade of calcium channels by betaxolol⁴ and the blockade of α-adrenoceptors, have been reported, suggesting that the mechanisms for vasodilator β-blockers may be heterogeneous.

Although vasodilatory β-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,⁵,⁶ 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 β-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 N⁵-nitro-L-arginine, an NO synthase (NOS) inhibitor; by hemoglobin, a scavenger of NO; or by methylene blue, a soluble guanylate cyclase inhibitor.⁷⁻¹⁰ If NO were involved in the effects of vasodilatory β-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 β-blockers such as ISA, lipid solubility, cardioselectivity, and membrane-stabilizing activity do not seem to explain systematically the reported NO-releasing activity of carteolol,¹¹ nebivolol,¹² and tertatolol.⁷⁻⁹ The mechanisms have been speculated to be an α₂-agonist activity in the case of carteolol¹¹ and a 5-hydroxytryptamine (5-HT)–agonist activity in the case of tertatolol.⁹

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

Measurement of Perfusion Pressure and NO Release in Isolated Perfused Kidney

Male Wistar rats, weighing 316±7 g, were anesthetized with pentobarbital sodium (40 mg/kg IP). The kidneys were isolated and perfused as described elsewhere. 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^{-7} mol/L indomethacin to maintain renal perfusion pressure (RPP) at ~100 mm Hg. When agents used interacted with α₁-adrenoceptors, their effects were also examined using 10^{-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 to measure NO output.

Sixty minutes after the kidney was isolated, we examined the effects of celiprolol and other β-blockers on RPP and NO release in a cumulative manner at 10-minute intervals. To examine the endothelium-dependency of the action of β-blockers, we also examined the effects of pretreatments with N⁰-nitro-L-arginine methyl ester (L-NAME) or E-4021 (1-[6-chloro-4-(3,4-methylbenzyl)amino-quinazolin-2-yl]piperidine-4-carboxylate), a cGMP-specific type V phosphodiesterase inhibitor. Furthermore, we studied whether the action of β-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 F₂α (≈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 β-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-HT₁A antagonist.

Results

Effects of β-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
Effects of propranolol and bisoprolol on 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_{1A} Receptor Agonist in Isolated Kidney

We also examined the effects of clonidine (an α_{2}-agonist), dobutamine (a β_{1}-agonist), salbutamol (a β_{2}-agonist), and (±)-8-hydroxy-2-(di-n-propyl-amino)tetralin (8-OH-DPAT), a selective 5-HT_{1A} 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 β-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.

Effects of β-Blockers on Rat Thoracic Aorta

To explore whether β-blockers exert vasodilatory effects on other vessels, we performed tension studies using the isolated 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.

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Effects of Celiprolol in DOCA-Salt Hypertensive Rats

Although several β-blockers actually diluted 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±3 mm Hg; DOCA-vehicle, 188±6 mm Hg; \( P<0.01 \)). Intravenous administration of celiprolol (50 mg · kg\(^{-1} \) · d\(^{-1} \)) to DOCA-salt hypertensive rats for 4 weeks slightly but significantly lowered systolic blood pressure (164±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±2\% versus control −37±4\%, \( P<0.01 \)) and NO release (10\(^{-8} \) mol/L ACh, DOCA-salt +3±1 versus control +16±2 fmol · min\(^{-1} \) · 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±2\%, NO release +19±2 fmol · min\(^{-1} \) · 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-NNAME and E-4021 resulted in attenuation and potentiation of the vasodilatory effects of these β-blockers, respectively, suggesting that the NO/cGMP pathway plays a role in renal vasorelaxation induced by these 3 β-blockers. Moreover, we observed that in the rat aorta, the vasorelaxation induced by these β-blockers was at least in part endothelium dependent.

It seemed unlikely that the 3 β-blockers had directly activated endothelial NOS via β-adrenoceptor antagonism itself because the tissues we used in the experiment were not innervated and no β-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 β-blockers activated endothelial NOS because celiprolol has been reported to stimulate β\(_2\)-adrenoceptors.\(^5\) It has been shown that the stimulation of β\(_1\)- and β\(_2\)-adrenoceptors activates endothelial NOS and that NO plays a role in the vasodilator effects of β-adrenergic stimulants.\(^{15-17}\) On the other hand, some investigators have shown that the role of NO is of little importance in β-adrenoceptor–mediated vasorelaxation.\(^{18,19}\)

In the presence of 10\(^{-6} \) mol/L propranolol, which is a nonselective β-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 β-adrenoceptors caused NO release to explain the potent endothelial NOS-activating properties of the 3 vasodilating β-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 β\(_1\)-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 β-blockers.

Although the effects of 5-HT on the cardiovascular system are complex, stimulation of 5-HT\(_1\) receptors is thought to cause endothelium-dependent relaxation, most likely via NO release.\(^{20}\) It is well established that 5-HT\(_1A\) receptors, especially 5-HT\(_1A\) receptors, can bind certain β-blockers, such as propranolol and pindolol, with high affinity.\(^{21}\) Propranolol has shown to bind stereoselectively to 5-HT\(_1\) sites and exert an antagonizing action.\(^{21}\) 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\(_1\) receptors. As for the effects of metergoline, a 5-HT\(_1B/2\) antagonist, and NAN-190, a 5-HT\(_1A\) antagonist, on the effects of the 3 β-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\(^9\) have shown that vasorelaxation caused by tertatolol in the isolated perfused rat kidney was markedly attenuated by BMY 7378, a 5-HT\(_1A\) receptor antagonist. Furthermore, 8-OH-DPAT, a 5-HT\(_1A\) agonist, increased vasodilation and NO release in a dose-dependent manner, confirming that several vasodilatory β-blockers exert their vascular action through the 5-HT\(_1A\) receptor/NO pathway. Such pharmacological cross-reactivity between β-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\(_1A\) receptor was first cloned by low stringency Southern blots using the β\(_2\)-adrenoceptor gene as a probe.\(^{22}\) It has also been demonstrated that a mutation of asparagine 385 in the seventh transmembrane domain of the human 5-HT\(_1A\) receptor decreased its affinity for pindolol and that asparagine 385 may be a critical site for the cross-reactivity.\(^{23}\)

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 subpressor dose (5 mg · kg\(^{-1} \) · d\(^{-1} \) PO), has been reported to restore endothelial dysfunction of mesenteric arteries of spontaneously hypertensive rats,\(^{24}\) 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 β-blockers are potent stimulators of endothelial NOS and exert this effect via 5-HT\(_1A\) receptors. This property may contribute to protect the endothelium from hypertension.

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References

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