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Nebivolol: A Third-Generation β-Blocker That Augments Vascular Nitric Oxide Release

Endothelial β_2-Adrenergic Receptor–Mediated Nitric Oxide Production

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Background—Nebivolol is a β_1-selective adrenergic receptor antagonist with proposed nitric oxide (NO)–mediated vasodilating properties in humans. In this study, we explored whether nebivolol indeed induces NO production and, if so, by what mechanism. We hypothesized that not nebivolol itself but rather its metabolites augment NO production.

Methods and Results—Mouse thoracic aorta segments were bathed in an organ chamber. Administration of nebivolol did not affect NO production. When nebivolol was allowed to metabolize in vivo in mice, addition of plasma of these mice caused a sustained 2-fold increase in NO release. Interestingly, coadministration of a selective β_2-adrenergic receptor antagonist (butoxamine) prevented the response. Immunohistochemistry and Western blot analysis demonstrated the presence of β_2- but not β_1-adrenergic receptors on endothelial cells. In the absence of calcium, metabolized nebivolol failed to increase NO production, suggesting a role for calcium-dependent NO synthase. With digital fluorescence imaging, a rapid and sustained rise in endothelial cytosolic free Ca^{2+} concentration was observed after administration of metabolized nebivolol, which also was abrogated by butoxamine pretreatment.

Conclusions—In vivo metabolized nebivolol increases vascular NO production. This phenomenon involves endothelial β_2-adrenergic receptor ligation, with a subsequent rise in endothelial free $[Ca^{2+}]_i$ and endothelial NO synthase–dependent NO production. This may be an important mechanism underlying the nebivolol-induced, NO-mediated arterial dilation in humans. **(*Circulation*. 2000;102:677-684.)**

Key Words: nitric oxide ■ endothelium ■ receptors, adrenergic, beta ■ calcium

The use of β-blockers, especially the so-called third-generation blockers with vasodilating properties, in the treatment of hypertension, heart failure, and ischemic syndromes is increasing.[1,2] In addition to the third-generation blockers, the clinically used β-blockers are classified either as first-generation nonselective drugs, which block both β_1- and β_2-adrenergic receptors, or as second-generation, β_1-selective drugs. β-Blockers may also have α-receptor–blocking effects (labetolol, carvedilol), intrinsic sympathomimetic effects (pindolol), or class III antiarrhythmic effects (sotalol).[3]

Nebivolol, a newly developed β-adrenergic receptor–blocking drug, is a racemic mixture of D- and L-enantiomers, of which D-nebivolol is considered to be a highly selective β_1-adrenergic receptor antagonist.[4] In addition, nebivolol has been shown to cause vasodilatation in animals[4] and humans.[4–6] It has been suggested that this effect is mediated by increased nitric oxide (NO) production, because it can be abrogated by inhibitors of NO synthase (NOS).[5,6] NO released by endothelial cells has been shown to be a key participant in numerous biological processes, serving the maintenance of vascular integrity; one of its main actions is induction of vascular relaxation.[7,8]

The present study was performed to explore whether nebivolol indeed induces NO production and to obtain insight into the mechanism underlying this enhanced NO production,[6] if any. We hypothesized that not nebivolol itself but rather its metabolites increase NO production, because pilot experiments in our institute indicated that nebivolol itself does not induce NO release. In vitro techniques were used to test the influence of plasma metabolites formed in vivo on NO production and endothelial Ca^{2+} concentration in isolated vascular segments;

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because it is known that the stimulation of adrenergic receptors and 5-HT serotonergic receptors can activate endothelial constitutive (ec) NOS, leading to NO release,[9,10] we investigated the involvement of both α- and β-adrenergic receptors and 5-HT_{1A} serotonergic receptors.

Methods

Animal and Vessel Preparation

Experiments were performed on thoracic aortas isolated from 51 male wild-type Swiss mice. The experiments were conducted according to protocols approved by the local committee on the use of laboratory animals. Aorta segments were placed in either 1 of 2 organ chambers, both containing continuously aerated (95% O_2/5% CO_2) and warmed (37°C) Krebs solution. The first, a glass organ chamber, was specially suited for NO measurements (total volume, 10 mL; Radnoti Glass Technology). The second, an aluminum organ chamber (total volume, 5 mL; Applied Imaging), was designed to fit the stage of a fluorescent imaging inverted microscope (Nikon) to allow measurements of endothelial cytosolic free Ca^{2+} concentrations.

Use of Drugs and Mouse Plasma

The effects of a mixture of D- and L-nebivolol (50/50 mixture; 1 μmol/L final bath concentration; Menarini) and of metoprolol (1 μmol/L; CIBA-Geigy), another commonly used β_1-selective antagonist without indications of NO release–inducing activity, on vascular NO release and endothelial cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) were assessed in separate experiments. To test the hypothesis that not nebivolol itself but rather its metabolites induce NO release and increase endothelial $[Ca^{2+}]_i$, 0.5 mL of D- and L-nebivolol (50/50; 0.5 mg/mL) was injected into the tail vein of mice. In separate experiments, the effect of metabolites of metoprolol (0.5 mg/mL) was tested by the same procedure (0.5 mL IV). In these experiments, in vivo drug metabolization was allowed for 45 minutes. Then, anesthesia was induced, the abdomen opened, and blood collected. Blood samples were centrifuged (2 minutes, 12 000 rpm at 4°C; centrifuge 5412, Eppendorf) to obtain plasma, which was stored at 4°C for use in an NO or Ca^{2+} assay on the same day. Plasma of noninjected mice treated similarly was used to test the effects of plasma factors. In these experiments, plasma volume constituted 3% of the total volume of the final bath solution. Acetylcholine (1 μmol/L in NO assay; 0.1 mmol/L in Ca^{2+} assay; Sigma) was administered to investigate whether a normal response could be induced in endothelium-intact aorta segments.

To study the involvement of endothelium, in some experiments, endothelium-denuded aorta segments were used. The role of NOS was investigated by use of a blocker of NO synthesis (N^G-nitro-L-arginine methyl ester, L-NAME; 0.1 mmol/L; Sigma). Because ecNOS is dependent on endothelial Ca^{2+} for its activity,[7] the role of Ca^{2+} was studied by bathing aorta segments in $CaCl_2$-free solution. Finally, to test the contribution of vascular α- or β-adrenergic receptors and 5-HT serotonergic receptors to NO production, an α-adrenergic receptor blocker (phentolamine; 10 μmol/L; CIBA-Geigy), a β_2-adrenergic receptor antagonist (butoxamine; 0.1 mmol/L; Sigma), or a 5-HT_{1A} antagonist (NAN-190; 1 μmol/L; ICN) was used. A β_2-adrenergic receptor agonist (salbutamol; 10 μmol/L; Sigma) was administered to study a possible role for β_2-adrenergic receptor–mediated NO release.

Measurement of NO

NO was determined at different time points before and after administration of the reagents to be tested (t= −5, 0, 2, 5, 10, 15, 20, and 25 minutes). NO production was quantified by the Griess method, which was described extensively in an earlier study.[11] To correct for the differences in size of the vascular segments, the NO production was standardized for intimal surface area. The NO concentration ([NO]) per unit surface area was expressed in (μmol/L)/m^2, assuming that the production of NO was equal across the endothelium of the excised vascular segment.

Digital Fluorescence Imaging

ecNOS is dependent on the endothelial cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) for its activity.[7] For measurement of $[Ca^{2+}]_i$ in endothelial cells, segments of the thoracic aorta were opened longitudinally and pinned onto a silicon disk. The vessel segments were loaded with the calcium probe fura 2-AM (10 μmol/L; Molecular Probes). Subsequently, the silicon disk with the aortic segment was placed in an aluminum organ chamber containing Krebs solution, aerated with 95% O_2/5% CO_2 at 37°C. The method used to measure $[Ca^{2+}]_i$ with a digital fluorescence imaging system connected to an inverted microscope was adapted from the method described earlier by Raat and coworkers.[12] Typically, 80% to 100% of the endothelial cells responded after administration of a test agent within each microscopic field (see Figure 3). Because calibration values differed per cell, data are presented as 340/380 ratios. To obtain an impression about absolute $[Ca^{2+}]_i$, averaged 340/380 ratios of all cells in a field were translated into $[Ca^{2+}]_i$ by a calibration according to Grynkiewicz.[13] Calculated baseline $[Ca^{2+}]_i$ in the aorta endothelial cells was, on average, 45 nmol/L.

Immunohistochemistry

To investigate the presence of β_1- and/or β_2-adrenergic receptors, segments of the mouse thoracic aorta were embedded in Tissue-Tek. Samples of mouse left ventricular myocardium and brain were embedded and used as positive controls for β_1- and β_2-adrenergic receptors, respectively. Frozen tissue sections were overlaid with rabbit polyclonal anti–mouse β_1- or β_2-adrenergic receptor antibodies (30 minutes at room temperature, 1:100 in 1% BSA/0.1% Tris-HCl buffered saline; Santa Cruz Biotechnology). For negative control, the primary antibody was omitted.

Cell Culture Techniques

To investigate whether β_1- or β_2-adrenergic receptor protein could be detected in endothelial and smooth muscle cells, these cells were cultured for use in Western blot analysis. Because we, like many other groups, did not succeed in keeping mouse aorta endothelial cells viable under culture conditions, cells from rat heart endothelial cell line-50 (RHEC-50)[14] were used. The cells were used for Western blot analysis after the second passage. Smooth muscle cells were derived from 3-mm mouse aortic segments from which the endothelium was mechanically removed.

Western Blot Procedures

Cultured endothelial cells and smooth muscle cells were lysed in a standard lysis buffer. Rabbit polyclonal anti–mouse β_1- or β_2-adrenergic receptor antibodies (Santa Cruz Biotechnology), diluted 1:1000 in PBS, were used as primary antibodies and were applied for overnight incubation at 4°C. After several washing procedures, the membranes were subjected to enhanced chemiluminescence (ECL, Amersham).

Use of Mouse Hepatic Microsomes

To obtain direct evidence in favor of action via active metabolites, we performed additional experiments using hepatic microsomes. D,L-Nebivolol (0.5 mL; 50/50; 0.5 mg/mL) was incubated with freshly harvested hepatic microsomes from male Swiss mice (n=3). Incubation was allowed for 45 minutes to obtain liver metabolites of nebivolol. After incubation, hepatic microsomes were centrifuged, and the supernatant was added directly to RHEC-50 cells. NO production was quantified with a calibrated porphyrinic NO sensor (World Precision Instruments) positioned on top of a confluent layer of RHEC-50 cells.

Statistical Analysis

Values are given as mean±SEM. The data were evaluated with a 2-factorial (significance over time and significance of drug effect)

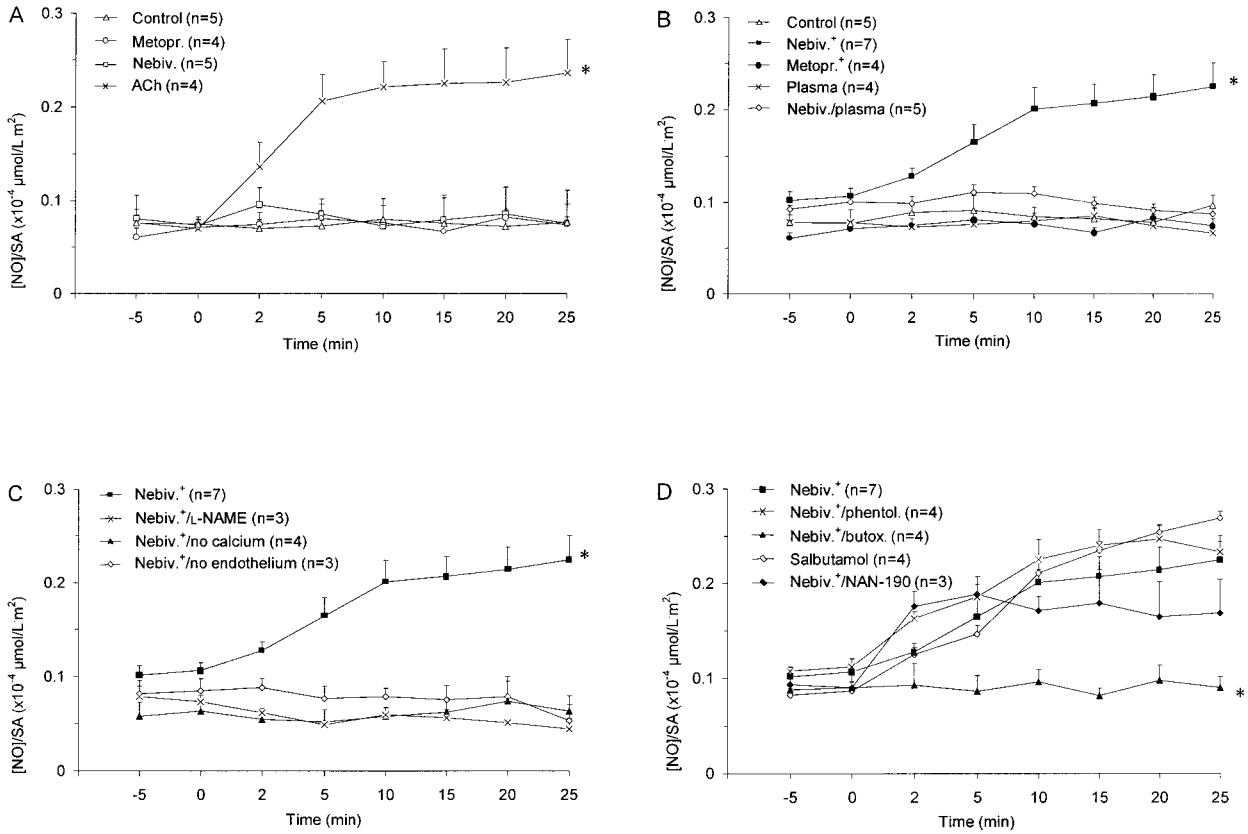

Figure 1. Effect on nitric oxide (NO) release from mouse aorta of nebivolol alone (A) or in vivo metabolized nebivolol (B); metoprolol was used as control β_1-adrenergic receptor blocker (A and B). Involvement of endothelium (A and C), Ca^{2+}-dependent ecNOS (C), and α- and β_2-adrenergic receptors and 5-HT_{1A} receptors (D). Control indicates no drugs added; n, number of mouse aorta segments; and SA, surface area. Substances added at t=0 minutes (concentrations represent final bath concentrations): Metopr., metoprolol 1 μmol/L; Nebiv., nebivolol 1 μmol/L; ACh, acetylcholine 1 μmol/L; Nebiv.$^+$, plasma from nebivolol-injected mice; Metopr.$^+$, plasma from metoprolol-injected mice; Plasma, plasma from noninjected mice; Nebiv./plasma, coadministration of 1 μmol/L nebivolol and plasma; Nebiv.$^+$/L-NAME, coadministration of Nebiv.$^+$ and 0.1 mmol/L L-NAME; Nebiv.$^+$/no calcium, Nebiv.$^+$ in $CaCl_2$-free organ bath solution; Nebiv.$^+$/no endothelium, Nebiv.$^+$ added to endothelium-denuded aorta segments; Nebiv.$^+$/phentol., coadministration of 10 μmol/L phentolamine and Nebiv.$^+$; Nebiv.$^+$/butox., coadministration of 0.1 mmol/L butoxamine and Nebiv.$^+$; Salbutamol: salbutamol 10 μmol/L; and Nebiv.$^+$/NAN-190: coadministration of 1 μmol/L NAN-190 and Nebiv.$^+$. *$P<0.05$ vs other lines in figure.

ANOVA for repeated measurements. Statistical significance was inferred when $P<0.05$. In all experiments, n equals the number of aortas, corresponding with the number of mice used.

Results

NO Release by Segments of Mouse Aorta

Administration of nebivolol did not augment NO release above baseline levels, nor did metoprolol (Figure 1A). Acetylcholine, however, caused a significant increase in NO production, demonstrating a normal response of endothelium. When plasma of nebivolol-injected mice was applied, a significant increase in NO production was observed (Figure 1B). This phenomenon was not seen when plasma of metoprolol-injected mice was added. Administration of plasma alone or plasma with freshly added nebivolol did not result in an augmented NO release. The increase in NO production after the administration of metabolized nebivolol was abrogated when the endothelium was mechanically disrupted, when L-NAME was coadministered, or in the absence of calcium (Figure 1C). Interestingly, selective blockade of the vascular adrenergic β_2-receptors with butoxamine also prevented the increase in NO production after the administration of metabolized nebivolol. Coadministration of the α-blocker phentolamine or the 5-HT_{1A} antagonist NAN-190, however, had no effect (Figure 1D). Selective stimulation of the vascular β_2-receptors with salbutamol resulted in an augmentation of NO release similar to that of metabolized nebivolol (Figure 1D).

Cytosolic Free Ca^{2+} Concentration

Figure 2 illustrates the heterogeneity in response between single endothelial cells within the same vascular segment and shows the effect of metabolized nebivolol on $[Ca^{2+}]_i$ with and without butoxamine pretreatment. In Figure 3, responses in single endothelial cells are plotted. The average reaction to the same substances is presented in the Table. Figure 3A depicts the effect of fresh nebivolol together with plasma from a noninjected mouse: a short-lasting response with a maximum increase of $[Ca^{2+}]_i$ from 45 to 175 nmol/L. This effect could not be blocked by butoxamine. Administration of 10 μmol/L nebivolol resulted in a similar short-lasting response with a somewhat higher peak value (data not shown); 0.1 mmol/L nebivolol resulted in an even higher increase, but the applied concentration appeared to be toxic

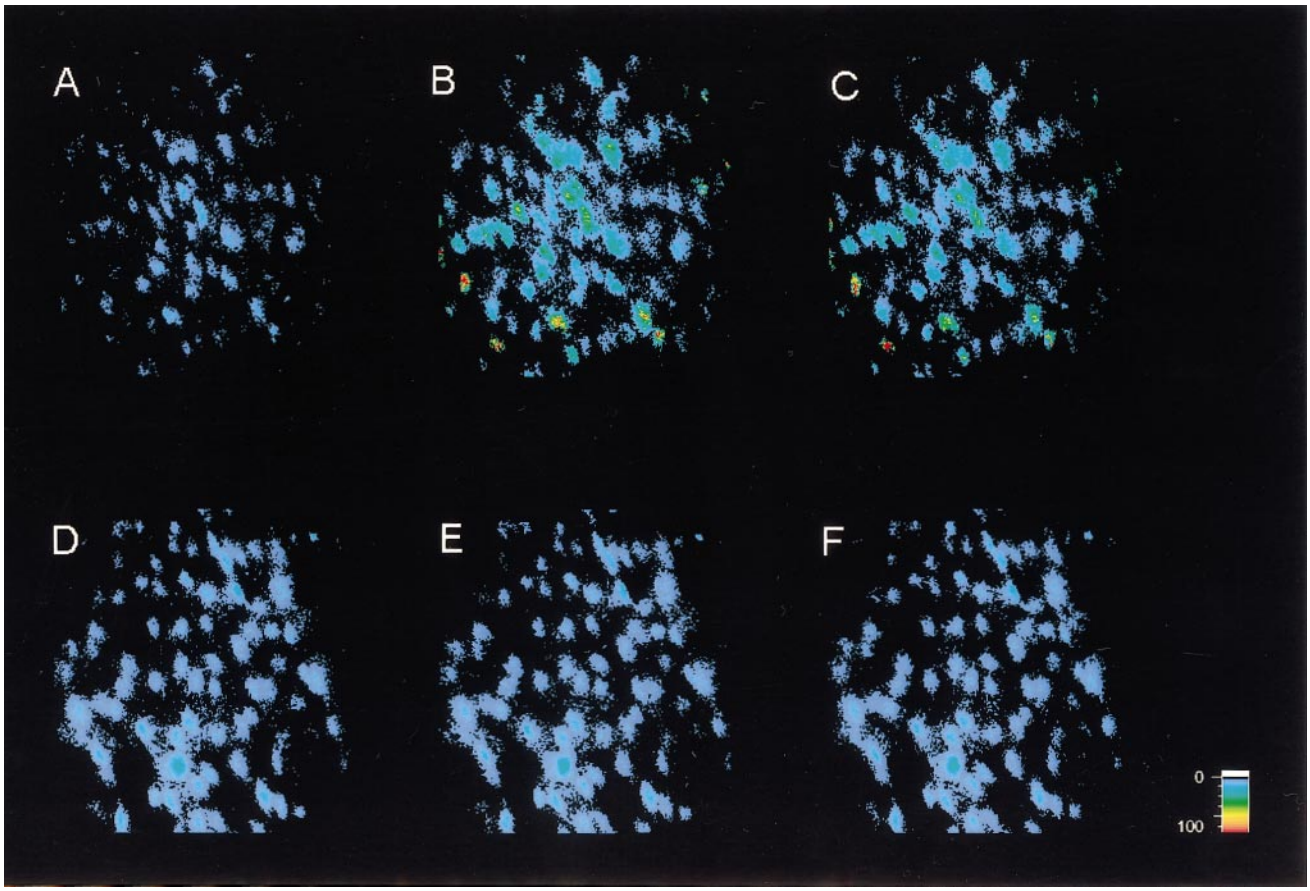

Figure 2. Examples of 2 representative experiments showing typical changes in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in mouse thoracic aorta endothelium as depicted by digital fluorescent microscopy using fluorescent Ca^{2+}-probe fura 2; 340/380-nm fura 2 ratio images are presented. Each image represents area of ≈200×200 μm, in which individual endothelial cells can be identified. Every color corresponds to a certain $[Ca^{2+}]_i$, as indicated by color bar (in nmol/L). A, Baseline, 300 seconds before administration of plasma. B and C, 60 and 300 seconds, respectively, after administration of plasma from a nebivolol-injected mouse. In this experiment, ≈95% of cells reacted (overall: 80% to 100%). D, Baseline of a butoxamine-pretreated (final bath concentration: 0.1 mmol/L) aorta segment, 300 seconds before plasma administration. E and F, 60 and 300 seconds, respectively, after administration of plasma from a nebivolol-injected mouse.

and resulted in lysis of the cell membrane with subsequent leakage of fura 2 (data not shown). Plasma from nebivolol-injected mice caused a rapid and sustained increase in $[Ca^{2+}]_i$ from 45 to 65 nmol/L (Figure 3B). Blockade of the adrenergic β_2-receptors with butoxamine abrogated this increase in $[Ca^{2+}]_i$ (Figure 3C). As a positive control, acetylcholine was administered (Figure 3D).

Immunohistochemistry and Western Blotting

Both immunohistochemistry and Western blot analysis indicated that mice aortic vascular smooth muscle cells contained mainly β_1-adrenergic receptors (Figure 4A, Figure 5). In contrast, endothelial cells selectively express β_2-adrenergic receptors (Figure 4C, Figure 5). Mouse myocardium was used as a positive control for β_1-staining (Figure 4B), whereas the choroid plexus in mouse brain tissue was used as a positive control for β_2-staining (Figure 4D).

Hepatic Microsomes

After nebivolol had been incubated with mouse hepatic microsomes for 45 minutes, addition of these nebivolol-incubates to 3 separate dishes containing RHEC-50 cells resulted in a significant rise in NO concentration in each of the dishes (Figure 6). Baseline NO concentration was on average 40 nmol/L, which increased to a peak value of 88 nmol/L after administration of nebivolol-incubate. After the endothelial cell monolayer had been washed, addition of acetylcholine caused a significant increase in NO production, from 50 to 98 nmol/L (Figure 6). Addition of nebivolol itself to RHEC-50 cells, however, had no effect.

Discussion

Plasma of nebivolol-injected mice (metabolized nebivolol) induces the release of NO from mouse aorta and augments the free $[Ca^{2+}]_i$ in endothelial cells, processes that are β_2-adrenergic receptor–mediated and endothelium-dependent. Immunohistochemistry and Western blot analysis demonstrated that β_2-adrenergic receptors are present predominantly on endothelial cells. Nebivolol alone or bound to plasma has no such effects. The effects seem to be specific for metabolized nebivolol, because metoprolol, pure or metabolized, is devoid of these effects.

The metabolism of nebivolol is complex, and many metabolites have been described.[4] The results of the hepatic

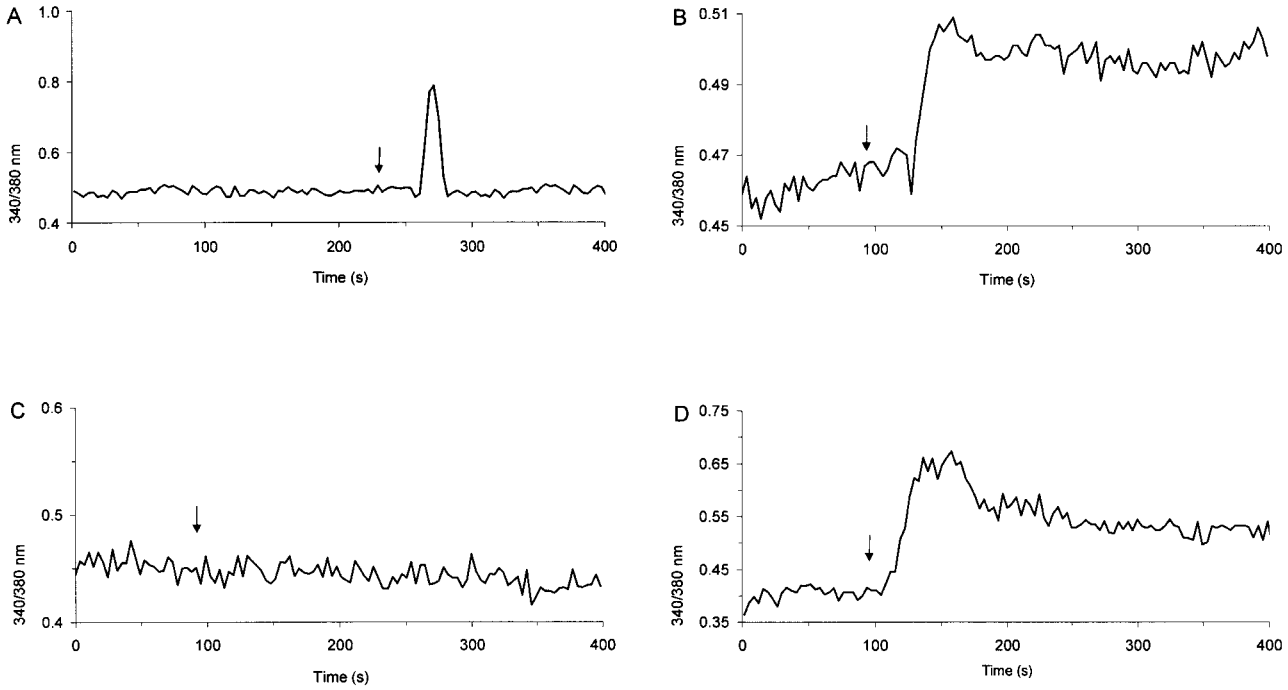

Figure 3. Cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) changes in mouse thoracic aorta endothelium. Typical changes in fura 2 ratios in single endothelial cells. Other cells in same segments showed similar responses. Substances used (with concentrations representing final bath concentrations): A, 1 μmol/L nebivolol together with plasma from noninjected mice; B, plasma from nebivolol-injected mice; C, plasma from nebivolol-injected mice 300 seconds after pretreatment with 0.1 mmol/L butoxamine; D, acetylcholine 0.1 mmol/L. Arrows indicate time of drug administration. 340/380 nm indicates fura 2 fluorescence ratio, proportional to $[Ca^{2+}]_i$.

microsome experiments show that mouse liver cells metabolize nebivolol, during which process at least 1 substance is formed that is capable of activating NO production in endothelial cells. These findings strongly suggest that 1 or more active metabolite(s) of nebivolol is/are responsible for the observed effects of plasma from nebivolol-injected animals on endothelial NO release and $[Ca^{2+}]_i$. The alternative that a nebivolol/plasma complex is responsible for the observed effects can be ruled out by our observation that addition of nonmetabolized nebivolol together with plasma from noninjected mice to cultured endothelium does not lead to stimulation of NO release. The other possibility, that nebivolol releases some endogenous mediator with β_2-agonist activity (eg, epinephrine), is unlikely in the light of the present results.

The rise in NO production in isolated segments of mouse aorta in response to metabolized nebivolol was prevented by mechanical removal of the endothelium. Further evidence for the exclusive role of endothelial cells in this response is provided by the involvement of β_2-adrenergic receptors, which are shown to be present on endothelial cells and not on vascular smooth muscle cells. The involvement of β_2-adrenergic receptors is supported by our observations that the β_2-adrenergic receptor antagonist butoxamine prevented the rise in NO production in response to metabolized nebivolol and that the β_2-adrenergic receptor agonist salbutamol induced a rise in NO production similar to the one induced by metabolized nebivolol. Interestingly, Dawes et al[15] recently showed vasodilator responses in the human forearm that were mediated predominantly through β_2-adrenergic receptors and were dependent on NO synthesis. α-Adrenergic receptors are not involved in the vasodilating effect of metabolized nebivolol, because phentolamine failed to affect the augmented NO production induced by metabolized nebivolol. Administration

$[Ca^{2+}]_i$ Changes in Mouse Aorta Endothelial Cells

	340/380 nm			$[Ca^{2+}]_i$, nmol/L		
Group	Baseline	Maximum	Persistent	Baseline	Maximum	Persistent
Nebiv./plasma (n=3)	0.47 (±0.01)	0.77 (±0.06)	0.47 (±0.00)	45	175	45
Nebiv.$^+$ (n=4)	0.47 (±0.01)	0.53 (±0.00)*	0.52 (±0.00)*	45	69	65
Nebiv.$^+$/butox. (n=3)	0.43 (±0.00)	0.43 (±0.00)	0.43 (±0.00)	30	30	30
ACh (n=6)	0.43 (±0.00)	0.71 (±0.02)	0.59 (±0.02)	30	147	94

$[Ca^{2+}]_i$ indicates average cytosolic free Ca^{2+} concentration changes in endothelium of mouse thoracic aorta; 340/380 nm, fura 2 fluorescence ratio; Baseline, baseline values obtained over 300 seconds before drug administration; Maximum, maximal response to the applied substance; Persistent, a plateau lasting for ≥300 seconds after addition of substances; n, number of mouse aorta segments. Other abbreviations as in Figure 1.

*$P<0.05$, Nebiv.$^+$ vs the other 3 groups. Data represent mean±SEM.

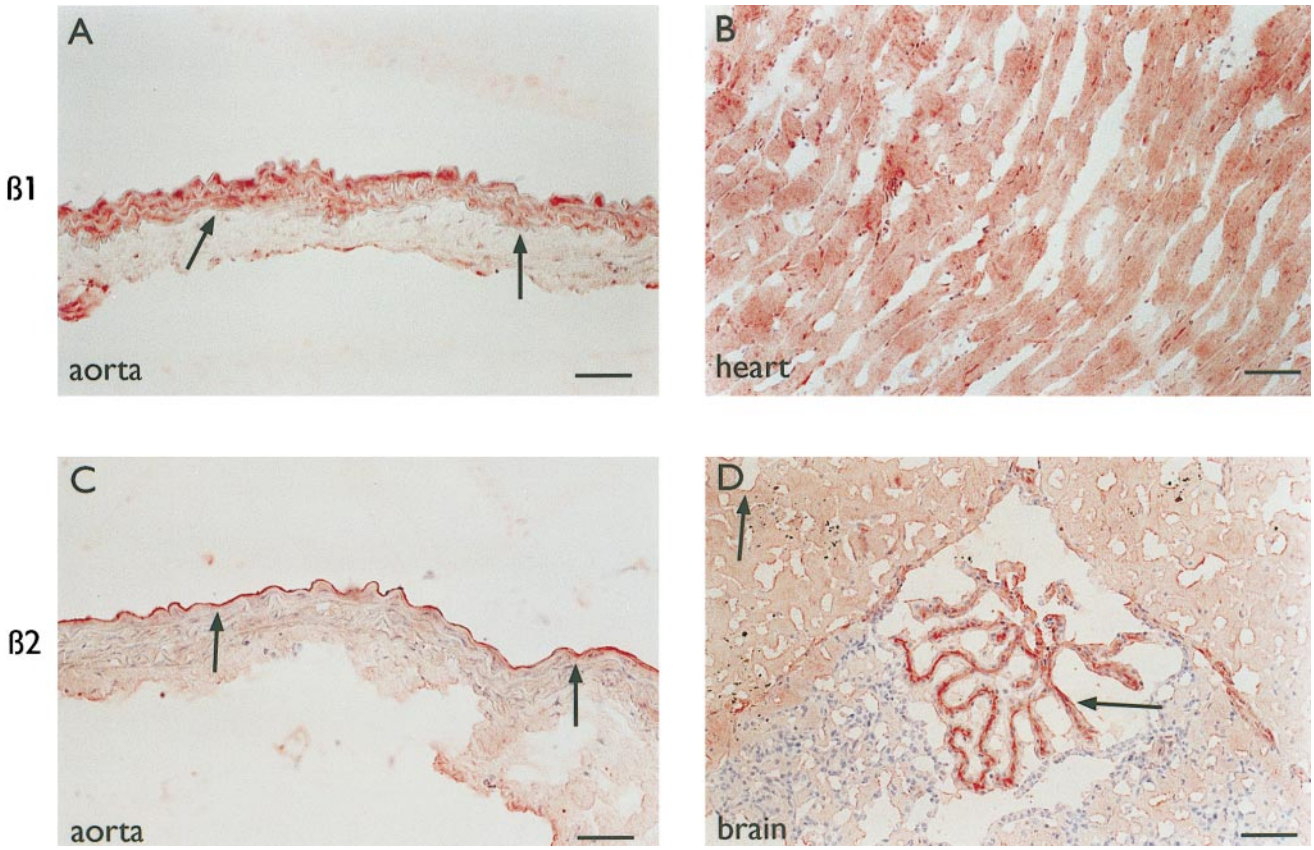

Figure 4. Expression of β_1- and β_2-adrenergic receptors on mouse thoracic aorta. Red indicates signal specific for primary antibody used. A, β_1-Adrenergic receptors on smooth muscle cells of mouse thoracic aorta; arrows indicate external elastic lamina. B, β_1-Adrenergic receptors in mouse myocardium of left ventricle. C, β_2-Adrenergic receptors on endothelial cells of mouse thoracic aorta; arrows indicate internal elastic lamina. D, β_2-Adrenergic receptors in mouse brain; heavy arrow indicates epithelium of choroid plexus; light arrow indicates endothelium of a brain capillary. Bars=100 μm.

of phentolamine alone also did not affect NO production (data not shown).

Recently, Kakoki et al[10] observed that nebivolol induces NO-mediated vasorelaxation in isolated rat aorta, which could be blocked with a 5-HT_{1A} antagonist. We show that NO production in response to metabolized nebivolol was completely blocked by butoxamine, a β_2-adrenergic receptor antagonist with no known affinity for 5-HT receptors,[16] but not by the 5-HT_{1A} antagonist NAN-190. This is in agreement with the results obtained in earlier studies showing that the documented affinity of nebivolol for the 5-HT_{1A} receptor has no functional consequences.[17] Moreover, in contrast to the results of Kakoki et al, Gao and coworkers[18] found that the nebivolol-induced relaxation of isolated rings of canine left anterior descending coronary arteries was not affected by methysergide, a nonspecific blocker of 5-HT_{1A} and other 5-HT serotonergic receptors.[19]

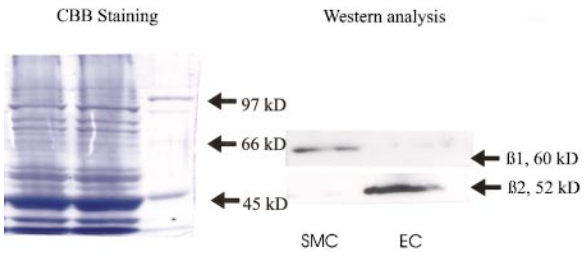

Figure 5. Western blot analysis showing expression of β_1- and β_2-adrenergic receptor protein (β_1, β_2) in cultured mouse thoracic aorta smooth muscle cells (SMC) and cultured RHEC-50 cells (EC). Rabbit polyclonal anti–mouse β_1- or β_2-adrenergic receptor antibodies, cross-reactive with rat, were used. CBB indicates Coomassie brilliant blue.

Metabolized nebivolol caused a small but significant increase in free $[Ca^{2+}]_i$ in endothelial cells of ≈20 nmol/L. It cannot be concluded from the present study whether the measured rise in free $[Ca^{2+}]_i$ is sufficient to stimulate the activity of ecNOS. In this respect, it is interesting to note that a relatively high concentration of acetylcholine was needed to induce a measurable rise in endothelial free $[Ca^{2+}]_i$. This may be the consequence either of a relative insensitivity of the fluorescence imaging method used to quantify free $[Ca^{2+}]_i$ or of the stimulation of ecNOS in a calcium-independent way.[20] The first possibility could explain why the increase in free $[Ca^{2+}]_i$ as caused by metabolized nebivolol or acetylcholine was relatively low in proportion to their more pronounced effects on NO production. Regarding the second possibility, it is interesting to note that metabolized nebivolol was not able to induce a rise in NO release in the absence of extracellular calcium. It may well be that both intracellular and extracellular Ca^{2+} are necessary to stimulate ecNOS. After all, it has been shown by other groups that the delayed component contributing to the plateau phase of the elevated $[Ca^{2+}]_i$ is

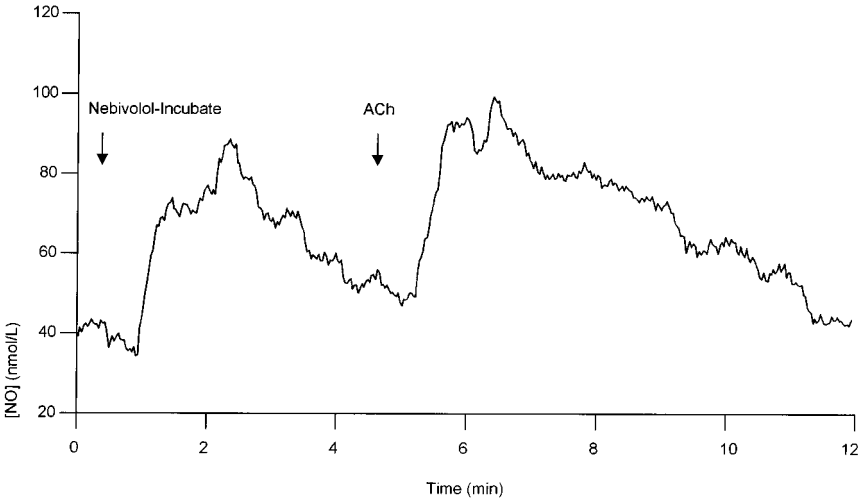

Figure 6. Effect of nebivolol (50/50; 0.5 mg/mL) incubated for 45 minutes with mouse hepatic microsomes on NO release from cultured rat heart endothelial cells. ACh indicates acetylcholine.

dependent on Ca^{2+} influx from the extracellular space, whereas the initial rise may be the result of release from intracellular stores.[21]

In separate experiments, plasma of human volunteers on nebivolol 5 mg/d did not evoke a similar rise in NO production in cultured human umbilical vein endothelial cells. The dose of nebivolol used in this study was much higher than the ones used in oral treatment in humans. One should realize, however, that the mouse aortic endothelium is only moderately sensitive to agonists, because relatively high concentrations of salbutamol and acetylcholine were needed to augment endothelial $[Ca^{2+}]_i$ and NO production.

The clinical finding that endogenous NO appears to be involved in the acute arterial and venous dilation after local nebivolol infusion is not necessarily in disagreement with the observations in the present study.[5,6] The dilating effect of nebivolol in the human forearm could be a result of nebivolol-induced peripheral arterial dilation,[4] inducing shear stress–related endothelium/NO-mediated dilation. The observation of Bowman et al[5] that nebivolol induces limited vasodilation in hand veins is also not necessarily in disagreement with our observations in mouse aorta, because venous and arterial mechanisms of vasodilation are known to be different.[22] However, the results of the present study do not exclude the possibility of local nebivolol metabolite production with subsequent NO release in the peripheral vasculature of the forearm.

The finding that metabolization of nebivolol provides a stimulatory ligand for vascular endothelial β_2-adrenergic receptors, which results in increased endothelial cell NO production in mice, may have several potential implications for the clinical use of this β-blocker, provided that the mechanism described is representative of the human situation. Beneficial effects of third-generation β-blocking drugs in hypertension and heart failure have been demonstrated.[23] It has been established that reduced bioavailability of vascular NO is a key factor in endothelial dysfunction associated with these disorders.[8] The results of the present study indicate that selective β_2-adrenergic receptor–mediated increase of endothelial NO production might become an additional therapeutic target in disorders associated with endothelial dysfunction and with preserved β-receptor–mediated NO-dependent vasodilation.

In conclusion, the findings of the present study indicate that metabolized nebivolol induces a β_2-adrenergic receptor–mediated rise in endothelial $[Ca^{2+}]_i$ and consequently, augmented NO production.

Acknowledgments

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