Nebivolol: A Highly Selective β_1-Adrenergic Receptor Blocker That Causes Vasodilation by Increasing Nitric Oxide

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Nebivolol (Bystolic®) is a cardioselective beta 1 (β_1)-adrenergic receptor blocker with endothelium-dependent vasodilating properties. The endothelium-dependent relaxation induced by nebivolol is blocked by inhibitors of nitric oxide synthase (NOS) and guanylate cyclase. Nebivolol also increases *in vitro* and *in vivo* nitric oxide (NO), which is an essential signaling molecule involved in the maintenance of cardiovascular homeostasis.

This review summarizes the data involving nebivolol and NO bioavailability. Endothelium-dependent relaxation of blood vessels, which is impaired in hypertensive animals and humans, is reversed by nebivolol treatment. Animals exhibiting endothelial dysfunction also show an improvement in NO–cyclic guanosine monophosphate (cGMP) signaling and an increase in NO bioavailability when treated with nebivolol. When blood vessel and cultured endothelial cells from hypertensive animals are treated with nebivolol, there is a decrease in superoxide production and an increase in the expression and activity of endothelial NOS (eNOS). As a result of the increased bioavailability of NO, nebivolol also increases *in vivo* arterial distensibility, glomerular filtration rate, and renal plasma flow. In normotensive volunteers, nebivolol infusion increases the forearm blood flow, an effect that is blocked by inhibitors of NOS and restored by the NOS substrate, L-arginine. In hypertensive patients, chronic treatment with nebivolol improves endothelium-dependent vasodilation induced by acetylcholine and shear stress and reverses endothelium-dependent vasoconstriction. Furthermore, nebivolol displays distinct hemodynamic properties in patients that include improvements in stroke volume and a decrease in peripheral vascular resistance.

These studies demonstrate that nebivolol produces endothelium-dependent vasodilation by increasing NO release, decreasing oxidative stress to increase NO bioavailability, or both. The NO-dependent vasodilatory action of nebivolol, coupled with its high β_1-adrenergic receptor selectivity, is unique among the clinically available β-blockers and contributes to its efficacy and improved tolerability (e.g., less fatigue and sexual dysfunction) as an antihypertensive agent.

Introduction

Beta (β)-blockers are a heterogeneous class of agents frequently used in the treatment of hypertension. They work by blocking the action of β_1-adrenergic receptors in the heart and kidney, which results in the reduction of blood pressure. The nonselective β-blockers, however, also block actions of β_2-adrenoreceptors, preventing vasodilation of blood vessels. Nebivolol (trade name Bystolic®; Forest Laboratories, New York, NY, USA) is a next-generation selective β_1-adrenergic receptor antagonist that promotes vasodilation and was recently approved in the United States for the treatment of hypertension. Unlike other selective β_1-adrenergic

receptor antagonists, such as atenolol and metoprolol, nebivolol has hemodynamic effects that cannot be ascribed to β_1-adrenergic receptor antagonism alone. For example, unlike traditional β-adrenergic receptor antagonists, such as propranolol and atenolol, nebivolol causes an immediate decrease in arterial blood pressure in spontaneously hypertensive rats (SHR) [1]. Furthermore, nebivolol is associated with negative inotropic effects in anesthetized dogs, as well as increased cardiac output and stroke volume [2]. Studies in humans have mirrored these findings, showing nebivolol to increase cardiac output and stroke volume, relative both to a predrug baseline and to an atenolol control [3].

The unique hemodynamic effects of nebivolol are related to its ability to cause endothelium-dependent vasodilation, which is an essential feature of its mode of action. Nitric oxide (NO), generated by endothelial NO synthase (eNOS), a nicotinamide dinucleotide phosphate (NAD[P]H)-dependent enzyme that converts L-arginine and oxygen (O_2) to NO and L-citrulline, is one of the most widely studied signaling molecules derived from the endothelium. The activation of eNOS is mediated by a number of naturally occurring receptor-dependent agonists, most notably acetylcholine and bradykinin, which are potent vasodilators. In addition to its important vasodilatory function, NO inhibits smooth muscle cell contraction, migration, and proliferation as well as endothelin production, platelet aggregation, and adhesion of leukocytes to the endothelium [4,5]. Hence, NO has become recognized as an essential signaling molecule in the vasculature and is responsible for the maintenance of cardiovascular homeostasis.

The vascular and hemodynamic effects of nebivolol have been extensively investigated in both clinical and preclinical studies. This review will examine these data, particularly with regard to the endothelium-dependent NO vasodilatory properties of nebivolol.

Data for this review were collected and analyzed based on published articles on preclinical and clinical studies of nebivolol, specifically focusing on those studies examining the mechanistic characteristics of nebivolol.

Nebivolol Is a Cardioselective β_1-Adrenergic Receptor Blocker: Studies in Human Myocardium

The first clinically used β-blocker, propranolol, which was discovered by Sir James Black in 1964, demonstrated both β_1-adrenergic and β_2-adrenergic receptor blockade. Propranolol represented a great advancement in the treatment of angina, hypertension, and other cardiovascular diseases. However, due to its nonselectivity, propanolol was associated with a number of adverse effects, including increased peripheral vascular resistance and aggravation of bronchospastic airway disease. Hence, a need for cardioselective agents that demonstrated a higher selectivity for the β_1-adrenergic receptor was recognized.

In the 1970s, β_1-selective agents, such as atenolol, bisoprolol, and metoprolol, were developed and brought to the market. Although still widely prescribed today, these agents are associated with high rates of adverse effects, including fatigue and sexual dysfunction. In the 1990s, agents that combined both α-adrenergic and β-adrenergic receptor blockade, such as labetalol and carvedilol, were developed. These agents blocked both β_1-adrenergic and β_2-adrenergic receptors, but also caused vasodilation, primarily through α_1-adrenergic receptor antagonism. The vasodilating properties of these β-blockers were a positive step forward; however, their lack of adrenergic receptor selectivity brought with them a number of adverse effects—most notably, bronchoconstriction, orthostatic hypotension, and dizziness.

Nebivolol is the only β-blocker that is both highly β_1-selective and promotes endothelium-dependent vasodilation. In the human myocardium, nebivolol demonstrates the highest selectivity (up to 320-fold vs β_2) among the cardioselective β-blockers, demonstrating 2- to 3-fold greater selectivity than bisoprolol and 4- to 10-fold greater selectivity than metoprolol for the β_1-adrenergic receptor (Table 1) [6,7].

In addition, the vasodilatory effects of nebivolol are unrelated to antagonism of the α_1-adrenergic receptor, since

Table 1 Comparative binding data for nebivolol and other β-blocking agents in human cardiac membranes

Compound	n	KH(β_1), nM	KL(β_2), nM	β_1/β_2 Selectivity
Nebivolol	10	0.70 ± 0.10	225 ± 28	321
Propranolol	18	3.63 ± 0.64	3.63 ± 0.64	1.0
Carvedilol	7	3.84 ± 1.22	3.84 ± 1.22	1.0
Metoprolol	12	43.0 ± 18.0	3186 ± 1400	74
Betaxolol	23	6.19 ± 0.92	576 ± 172	93
Bucindolol	5	2.35 ± 0.62	2.35 ± 0.62	1.0
Bisoprolol	6	36.5 ± 22.7	3751 ± 2142	103
Celiprolol	12	120 ± 24	8292 ± 2862	69

Binding to β_1-adrenergic and β_2-adrenergic receptors was investigated using 50 pM of the nonselective β-receptor radioligand ^{125}[I]CYP in the presence or absence of varying cold β-receptor ligand concentrations in membranes prepared from explanted human left ventricular myocardium. Competition curve data were analyzed with nonlinear regression curve fitting deriving the goodness of fit (2 or 1 site), the dissociation constant (K_i) of each binding site, and the fraction/total of each site. Reproduced with permission from Bristow et al. [7]. CYP, cyanopindolol; KH, high affinity binding site; KL, low affinity binding site.

it has low binding affinity for this receptor [8]. Nebivolol also does not antagonize α-adrenergic receptor-mediated responses *in vivo*, as shown in preclinical and clinical studies [6,9–13]. Its lack of α-adrenergic receptor blockade and β_1-selectivity distinguishes nebivolol from other vasodilating β-blockers, such as carvedilol and labetalol, which are nonselective β-adrenergic receptor blockers that vasodilate via α-adrenergic receptor blockade.

Nebivolol Causes Endothelium-Dependent Relaxation by Increasing Nitric Oxide: Evidence from *In Vitro* Studies

Gao and colleagues first described the endothelium-dependent vasorelaxant effects of nebivolol in isolated dog coronary artery rings [2]. In this study, the relaxation induced by nebivolol was concentration-dependent and significantly greater in the rings with endothelium, compared with those without endothelium. Furthermore, pretreatment of coronary artery rings with nebivolol (0.3 μM) potentiated adenosine diphosphate (ADP)-induced endothelium-dependent relaxation. Moreover, it was demonstrated that endothelium-dependent vasodilator responses to nebivolol in rat aortic rings involved NO and cyclic guanine monophosphate (cGMP) since inhibitors of NOS and guanylate cyclase diminished the nebivolol responses [14].

In another study, nebivolol, but not atenolol, produced a concentration-dependent relaxation (starting at 0.1 μM) of mesenteric arteries isolated from 9-week-old rats that was blocked by an NOS inhibitor, N$^{\omega'}$-nitro-L-arginine methyl ester (L-NAME). Furthermore, the addition of NOS substrate L-arginine reversed L-NAME inhibition (Fig. 1) [15]. Similarly, nebivolol has been shown to produce NO-mediated relaxation of the rat mesenteric vascular bed and activate constitutive NOS activity in cultured bovine coronary postcapillary endothelial cells [16].

Nebivolol has also been demonstrated to induce relaxation of rat renal glomerular vasculature by increasing NO release [17]. Nebivolol produced a concentration-dependent relaxation of isolated rat glomeruli precontracted with angiotensin II, with the full relaxant effect occurring at 1 μmol/L. This effect of nebivolol was abolished by an eNOS inhibitor, N^G-nitro-L-arginine (L-NNA), and also by oxadiazolo-[4,3-a]quinoxalin-1-one, a selective inhibitor of NO-sensitive guanylate cyclase.

The effects of nebivolol on the endothelium-dependent generation of NO and peroxynitrite (ONOO$^-$) were also studied in mesenteric resistance arteries isolated from spontaneously hypertensive and normotensive Wistar-Kyoto (WKY) rats [18]. Compared with blood vessels from WKY rats, SHR vessels showed significantly lower NO bioavailability, with a concomitant increase in ONOO$^-$ in response to the eNOS activator, acetylcholine, and the calcium ionophore, A23187. The [NO]/[ONOO$^-$] ratio in arteries from SHR was significantly reduced compared with that of WKY rats, indicating uncoupling of eNOS as well as endothelial dysfunction. Treatment with nebivolol (10 μmol/L) inhibited eNOS uncoupling and reduced endothelial dysfunction in arteries from SHR,
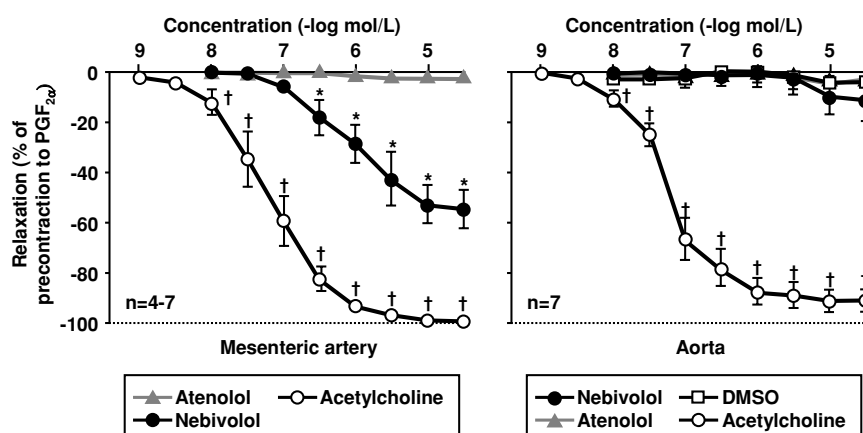
Figure 1 Left panel: responses of rat mesenteric arteries to acetylcholine, nebivolol, and atenolol. Results are given as mean ± SEM (n = 4–7), and relaxations are expressed as percentage of the increase in intraluminal diameter from half-maximal contraction to PGF$_{2\alpha}$. Right panel: effects of L-NAME (10^{-4} M) with and without L-arginine (10^{-3} M) on relaxations induced by nebivolol in rat mesenteric arteries. Values are presented as mean ± SEM (n = 4), and relaxations are expressed as percentage of the increase in intraluminal diameter from half-maximal contraction to prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$). *Significantly different from the group with atenolol; †significantly different from both preparations with nebivolol and with atenolol. SEM = standard error of the mean; PGF$_{2\alpha}$ = prostaglandin F$_{2\alpha}$; L-NAME = N$^{\omega'}$-nitro-L-arginine methyl ester. Reproduced with permission from Altwegg et al. [15].
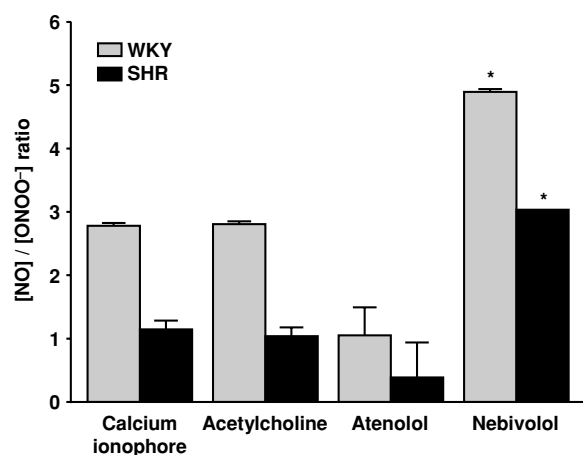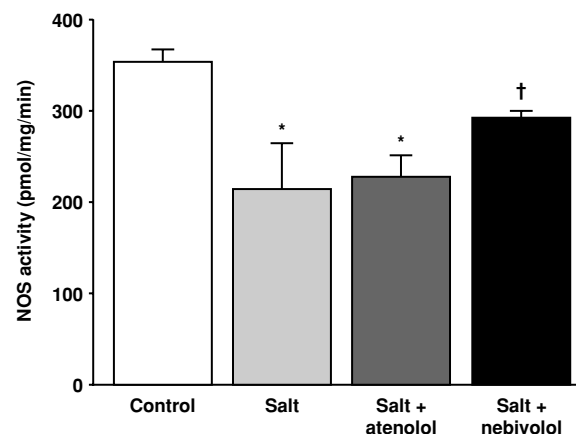
Figure 2 Ratio of maximal NO concentration to the maximal concentration of ONOO$^-$ produced by mesenteric endothelium of WKY rats (solid bars) and SHR rats (open bars) after stimulation with calcium ionophore (1 μmol/L), ACh (1 μmol/L), atenolol (10 μmol/L), and nebivolol racemate (10 μmol/L). *$P < 0.001$ versus CI, ACh, or atenolol treatment (n = 5–6). WKY = Wistar-Kyoto rat; SHR = spontaneously hypertensive rat. Reproduced with permission from Mason et al. [18].


Figure 3 Constitutive NOS activity assayed in aortic tissue from the four experimental groups of Dahl salt-sensitive rats. Values are mean $\pm$ SEM (n = 4–5 per group). *$P < 0.05$ versus control; †$P < 0.05$ versus salt + atenolol. SEM = standard error of the mean; NOS = nitric oxide synthase. Reproduced with permission from Cosentino et al. [20].

as evidenced by an increase in the [NO]/[ONOO$^-$] ratio (Fig. 2).

Nebivolol (1 μmol/L) also increased NO release from cultured rat glomerular endothelial cells, which was attenuated by an inhibitor of eNOS and P2 purinoceptor antagonists, but not by an inhibitor of inducible NOS (iNOS). Recently, nebivolol, but not metoprolol, was shown to increase NO release from human umbilical vein endothelial cells (HUVEC) by activating eNOS [19].

Nebivolol Modulates Endothelium-Dependent Responses by Increasing Nitric Oxide *In Vivo*

The findings linking nebivolol to NO-mediated endothelial-dependent vasodilatory actions were confirmed in the Dahl salt-sensitive rat model of hypertension. Lüscher and colleagues demonstrated that blood vessels of animals on a high-salt diet exhibited an impaired endothelial responsiveness to acetylcholine *ex vivo* [20]. After treatment with nebivolol (10 mg/kg/d for 8 weeks), endothelial dysfunction in aortic strips and mesenteric arteries from these rats was reversed, whereas atenolol had no effect on endothelial dysfunction, despite causing similar reductions in blood pressure. Furthermore, chronic treatment with nebivolol, but not atenolol, restored constitutive NOS activity in aortic tissues from Dahl salt-sensitive rats (Fig. 3). These findings may be clinically relevant since acute treatment with nebivolol

causes a dose-dependent decrease in arterial pressure in animal models of hypertension [effective dose at 50% of maximal response (ED$_{50}$) $\sim$3–10 mg/kg, depending upon the route of administration] [10].

In anesthetized male Sprague-Dawley rats, nebivolol (0.1–2.0 mg/kg) produced a dose-dependent increase in glomerular filtration rate, renal plasma flow, urine flow, and urinary excretion of Na$^+$ and Cl$^-$ [21]. An NOS inhibitor, N^G-monomethyl-L-arginine (L-NMMA), blocked the effect of nebivolol on the glomerular filtration rate, indicating that salutary changes in renal functioning from nebivolol had an NOS etiology. A further indication of an NOS mechanism underlying the renal physiologic effects of nebivolol was the marked increase in NO excretion (by 70.7%), an effect that was abolished by L-NMMA (1 mg/kg).

The effects of nebivolol were also studied in a well-characterized rat model of angiotensin II-induced hypertension, which exhibits severe endothelial dysfunction and a marked impairment of NO/cGMP signaling [22]. In this model, nebivolol (10 mg/kg/day), but not metoprolol (10 mg/kg/day), normalized endothelial function and increased plasma NO bioavailability, as demonstrated by the increases in plasma nitrite and whole blood hemoglobin-NO levels (Fig. 4). In addition, nebivolol, but not metoprolol, inhibited upregulation of the activity and expression of the vascular NAD(P)H oxidase, as well as prevented eNOS uncoupling, as evidenced by a reduced vascular superoxide formation. Collectively, this study and those earlier described with atenolol demonstrate that the effects of nebivolol on endothelial function are independent of β_1-adrenergic receptor blockade.
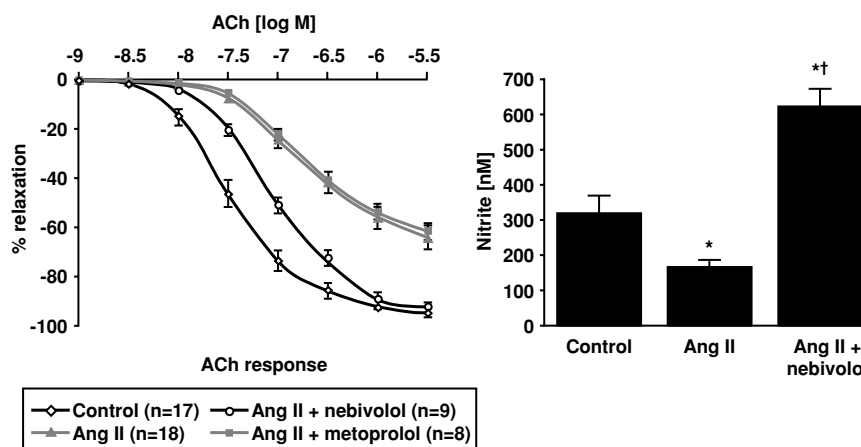
Figure 4 Left panel: effects of *in vivo* nebivolol and metoprolol treatment (Ang II + nebivolol and Ang II + metoprolol, each 10 mg/kg per day for 7 days) on the concentration–response relationship to acetylcholine in aortic rings from Ang II-infused (Ang II, 1 mg/kg per day for 7 days) rats. Data are the mean ± SEM of n = 8–18 experiments. Right panel: effects of *in vivo* nebivolol treatment on plasma nitrite levels as determined by NO analyzer (n = 6). ACh = acetylcholine; SEM = standard error of the mean; NO = nitric oxide; Ang II = angiotensin II. Reproduced with permission from Oelze et al. [22].

In Watanabe heritable hyperlipidemic (WHHL) rabbits, the endothelium-dependent and endothelium-independent relaxation of blood vessels was shown to be modulated by nebivolol [23]. Treatment of WHHL rabbits with nebivolol (10/mg/kg/day for 8 weeks) significantly improved the sensitivity of acetylcholine-induced endothelium-dependent relaxation. In addition, nebivolol significantly inhibited the increase in superoxide production in vessels from WHHL rabbits by preventing NOS uncoupling and increasing NO production. In this study, nebivolol also inhibited agonist-stimulated NAD(P)H oxidase activity and superoxide production in whole blood and neutrophils, further suggesting that nebivolol increases NO bioavailability. Recently, such nebivolol-mediated increases in NO have been linked to the prevention of atherosclerosis in cholesterol-fed rabbits [24]. Eight weeks of treatment with nebivolol inhibited the development of atherosclerotic lesions as well as increased aortic eNOS expression and enhanced relaxation to acetylcholine (ACh). Carvedilol, on the other hand, did not show significant changes in these endpoints, even though it and nebivolol both inhibited thiobarbituric acid reactive substances (TBARS) formation.

The direct effect of nebivolol on pulse wave velocity (PWV), a measure of arterial distensibility, has been studied using an anesthetized sheep hind-limb model [25]. PWV was recorded *in vivo* using a dual pressure-sensing catheter placed in the common iliac artery. An intraarterial infusion of nebivolol reduced PWV, whereas atenolol had no effect. The effect of nebivolol on PWV was significantly attenuated during the coinfusion of an NOS in-

hibitor, L-NMMA. These results support the findings that nebivolol, but not atenolol, increases arterial distensibility through the release of endothelium-derived NO [26].

In a recent study, the effects on endothelium-dependent relaxation and eNOS expression in penile corpus cavernosum tissue were evaluated in SHR rats after 6 months of treatment with 10 mg/kg nebivolol or amlodipine [27]. Nebivolol, but not amlodipine, significantly enhanced acetylcholine-induced endothelium-dependent relaxation and expression of eNOS when compared with untreated SHR controls (Fig. 5). These findings indicate that chronic administration of nebivolol reverses the basic cellular changes (e.g., eNOS expression, activity, and/or uncoupling) that affect NO bioavailability.

Nebivolol Increases Nitric Oxide Bioavailability in Humans

The role of NO in nebivolol-induced vasodilation has been studied in normotensive volunteers [26]. The infusion of nebivolol (354 μg/min) into the brachial artery produced a significant and dose-dependent increase in forearm blood flow (Fig. 6). In contrast, the infusion of an equimolar dose of atenolol had no effect on the forearm blood flow. The vasodilatory effect of nebivolol could be inhibited by the coinfusion with L-NMMA, an effect that was eventually overcome in the presence of increasing levels of the NOS substrate, L-arginine. In the same study, the vasodilatory effects of carbachol, which induces its activity through the L-arginine/NO pathway, were inhibited by L-NMMA to the same extent as nebivolol [26].
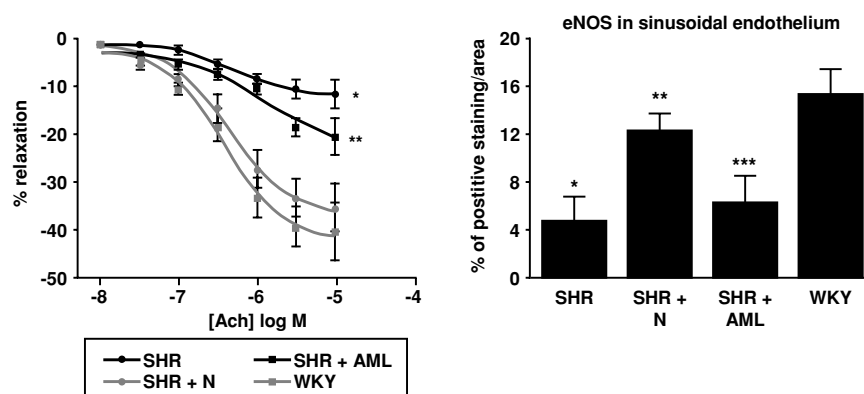
Figure 5 Left panel: concentration–response curve to acetylcholine (Ach, 10^{-8}–10^{-4} M/L) in rat corporal strips from SHR and WKY rats treated with nebivolol (10 mg/kg/day for 6 months) or amlodipine (3 mg/kg/day for 6 months). Results are expressed as a percentage of relaxation from the initial developed tension obtained with Phe 10^{-4} M/L. *$P < 0.01$ versus all groups; **$P < 0.01$ versus SHR + N and WKY. Right panel: bars indicate percentages of eNOS in sinusoidal endothelium from cav-ernous tissue in all groups. Untreated SHR and SHR + AML presented similar low immunoexpression of eNOS versus WKY rats and SHR + N. *$P < 0.01$ versus SHR + N and WKY; **$P < 0.01$ versus AML and WKY; ***$P < 0.01$ versus WKY. ACh = acetylcholine; eNOS = endothelial nitric oxide synthase; N = nebivolol; AML = amlodipine; SHR = spontaneously hypertensive rat; WKY = Wistar-Kyoto rat. Reproduced with permission from Toblli et al. [27].
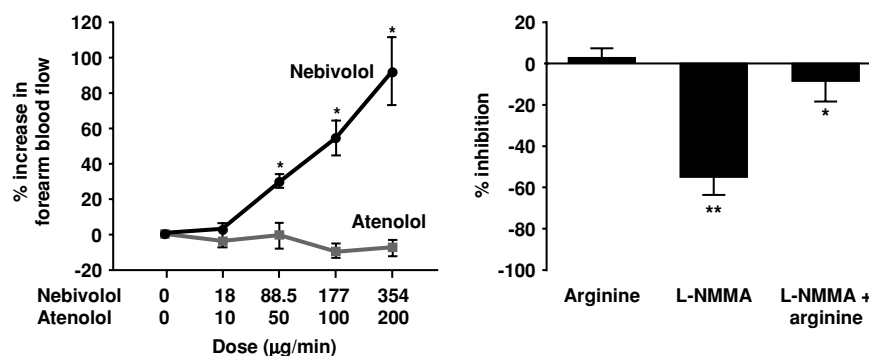

Figure 6 Left panel: effects of nebivolol versus atenolol on forearm blood flow in normotensive patients. Change in arterial blood flow was monitored as a function of infusion with the two compounds at successively higher concentrations. Each dose of nebivolol and atenolol was infused for 6 min prior to measurement. Values are the mean increase in the ratio of blood flow in the infused versus noninfused arm plotted as a percentage of the same ratio during baseline saline infusion. Data are reported as a mean ($n = 40$) standard error of the mean. *$P < 0.01$ versus atenolol. Right panel: effect of NOS inhibitor L-NMMA on nebivolol-induced vasodi-lation. Nebivolol (354 μg/min) was infused for 12 min on three separate occasions, alone for the first 6 min and coinfused with arginine, L-NMMA (1 mg/min), or L-NMMA with L-arginine. The bars show mean ($\pm$ SEM) percentage inhibition of blood flow response at the end of the 6-min infusion of nebivolol alone. L-NMMA inhibited the response (**$P < 0.01$). L-arginine significantly reduced the inhibition produced by L-NMMA (*$P < 0.05$). NOS = nitric oxide synthase; L-NMMA = N^G-monomethyl-L-arginine; SEM = standard error of the mean. Reproduced with permission from Cockcroft et al. [26].

The endothelium-dependent vasodilation associated with nebivolol has also been demonstrated in patients with hypertension [28]. Endothelial function, as assessed by the responses to endogenous vasodilators such as acetylcholine, is impaired in some patients with hypertension [29,30]. The infusion of nebivolol into the brachial artery of hypertensive patients increased the forearm blood flow, an effect that was antagonized by L-NMMA. These results suggest that the vasodilation produced by intraarterial nebivolol in patients with essential hypertension is caused by the activation of the L-arginine/NO pathway, as separately demonstrated in normotensive patients [28].

In an 8-week double-blind study that compared nebivolol with atenolol for treatment of hypertensive patients, nebivolol alone was shown to improve
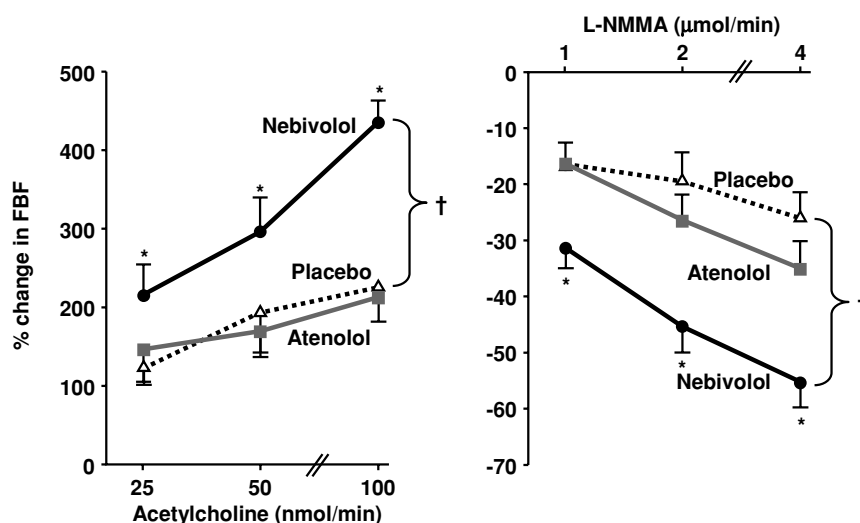
Figure 7 Effects of chronic treatment with nebivolol versus atenolol on forearm blood flow (FBF) in hypertensive patients. Change in FBF was monitored with intraarterial infusions of acetylcholine and L-NMMA to assess stimulated and basal endothelial-mediated NO release, respectively. Percentage changes in blood flow from baseline were measured following infusion with three doses of acetylcholine and sodium nitroprusside in patients treated with placebo, nebivolol, or atenolol. Data are reported as a mean (n = 12) ± standard error of the mean. *$P < 0.05$ and †$P < 0.0001$ for differences between treatments. FBF = forearm blood flow; L-NMMA = N^G-monomethyl-L-arginine. Reproduced with permission from Tzemos et al. [31].

endothelial-mediated vasodilation [31]. The forearm blood flow measurements were made via venous occlusion plethysmography, following intraarterial infusions of acetylcholine, sodium nitroprusside (an endothelial-independent stimulus of vasodilation), or L-NMMA. Nebivolol/bendrofluazide treatment was associated with a significant increase from baseline in forearm vasodilation compared with acetylcholine (by more than 400%; $P < 0.001$ vs placebo and atenolol treatment), whereas treatment with atenolol/bendrofluazide had no effect on vasodilation. Moreover, the endothelium-dependent vasoconstrictive response to L-NMMA was significantly improved only with nebivolol treatment, not atenolol, as evidenced by a 50% reduction in the L-NMMA effect on the forearm blood flow (Fig. 7), despite an equivalent blood pressure lowering. The response to sodium nitroprusside was not different between the treatment groups, suggesting that an endothelium-independent pathway was not a significant contributor to the observed responses.

Such NO-mediated vasodilation translates into clinically relevant hemodynamic changes. For example, in a double-blind, randomized study in patients with mild-to-moderate hypertension that compared nebivolol (5 mg) with atenolol (100 mg), both agents reduced blood pressure to a similar extent [3]. Measured via echocardiographically assessed hemodynamics, the blood pressure lowering with nebivolol was associated with a decrease in peripheral vascular resistance and a significant increase in stroke volume, with maintenance of cardiac output. In contrast, atenolol was associated with an increase in peripheral vascular resistance and a reduction in cardiac output. Similar findings have been demonstrated in hypertensive patients with diastolic heart failure [32], clearly distinguishing nebivolol from previously developed cardioselective β-blockers.

Recently, it has been shown that African Americans exhibit differences in NO bioavailability attributed to eNOS uncoupling and increased nitroxidative stress [33]. The changes in basal NO levels were measured in HUVECs obtained from African-American females and were found to be lower compared with cells from age-matched and risk factor-matched healthy Caucasian females. This reduction in NO bioavailability in HUVECs was evident despite higher levels of eNOS expression [33]. These effects were attributed to an uncoupling of eNOS resulting in $ONOO^-$ formation due to NO reactivity with O_2^-. Mason and colleagues proposed that this intrinsic biochemical difference might contribute to the greater incidence of cardiovascular disease in African-American patients relative to Caucasian patients. Substantiating these observations, in subsequent studies, nebivolol, but not atenolol, was shown to blunt excessive production of $ONOO^-$ in African Americans, thereby increasing
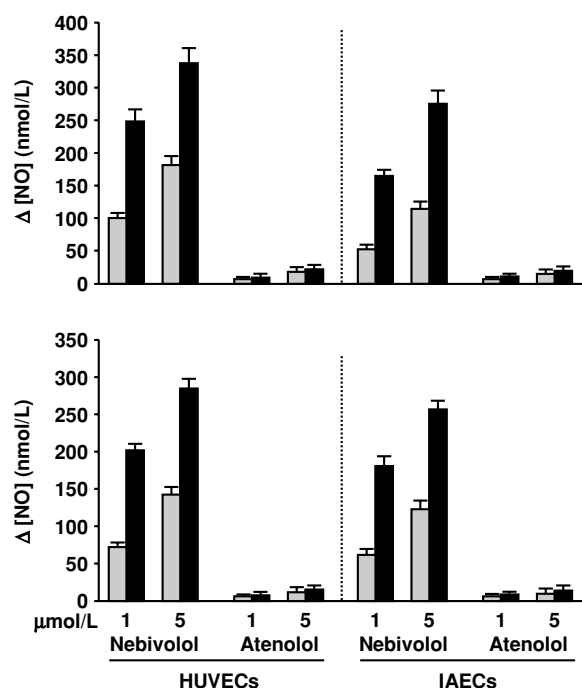
Figure 8 Changes in bioavailable NO concentration released from a single HUVEC or a single IAEC after preincubation with nebivolol or atenolol. Δ[NO] represents the difference in NO concentrations released by pretreated and control cells. The cells were harvested from Caucasian (gray bars) and African-American donors (black bars) and pretreated with different concentrations of β-blockers in the presence or absence of 0.3 mM/L L-NAME. NO release was stimulated with 1.0 μM/L Cal (top panel) or 1.0 μM/L Ach (bottom panel). Data are mean ± SEM from 12 subjects (HUVECs) or 7 subjects (IAECs) and 8 single-cell measurements per subject. *$P < 0.001$ versus Caucasians. NO = nitric oxide; HUVEC = human umbilical vein endothelial cell; IAEC = iliac artery endothelial cell; L-NAME = N$^{\omega}$-nitro-L-arginine methyl ester; Cal = calcium ionophore A23187; ACh = acetylcholine. Reproduced with permission from Mason et al. [34].

the amount of NO released from HUVECs [34]. Similar findings have been observed in endothelial cells from Hispanic Americans [35].

Nebivolol has also been shown to enhance NO release in human iliac artery endothelial cells (IAECs), supporting the findings in HUVECs. The effect of nebivolol was more pronounced in cells from African-American patients compared with those from Caucasian patients (Fig. 8). As in the HUVEC study by Mason and colleagues [34], pretreatment of IAECs with atenolol failed to modify NO bioavailability compared with the same concentrations of nebivolol, despite their similar β_1-selective antagonist properties. The degree of eNOS uncoupling in endothelial cells from African-American females was much greater than that of HUVECs from Caucasian females, due to higher O_2^- and $ONOO^-$ production, hence the lower levels of bioavailable NO [34]. These properties

may explain why nebivolol is particularly effective in patients with difficult-to-treat hypertension, such as African Americans [36,37].

Nebivolol Inhibits Platelet Aggregation by Increasing Nitric Oxide

In addition to its effects in human endothelial cells, nebivolol has been shown to inhibit aggregation of human platelets, a function modulated by NO [38]. In one study, human platelet aggregation was induced by ADP or collagen, and in both conditions, nebivolol inhibited platelet aggregation to a greater extent than carvedilol or propranolol. This effect of nebivolol occurred at clinically relevant concentrations. The evidence of NO dependency was supported by the fact that the NOS substrate, L-arginine, enhanced the inhibitory effect of nebivolol on platelet aggregation, whereas an NOS inhibitor, L-NMMA, reduced its effects (neither agent affected the response of propranolol or carvedilol). In addition to these *in vitro* studies, nebivolol's effects on platelet function have been assessed in the clinic. For instance, chronic (6-month) treatment of hypertensive patients with nebivolol, but not metoprolol, decreased the mean platelet volume and plasma soluble P-selectin, both markers of platelet activation [39]. Furthermore, in another clinical study, a 3-month treatment with nebivolol in patients with essential hypertension significantly decreased both spontaneous and ADP-induced platelet aggregation [40]. Lastly, in addition to its antiplatelet activity, nebivolol has been shown to inhibit proliferation of human coronary smooth muscle/endothelial cells and decreases endothelin-1 secretion [41]. Collectively, these data would suggest that nebivolol exhibits properties that could be particularly beneficial in patients with coronary artery disease.

Nebivolol Decreases Oxidative Stress *In Vivo* by Increasing Nitric Oxide

It is known that elevations in oxidized low-density lipoprotein (Ox-LDL) can lead to uncoupling of eNOS, and thus, to reductions in NO bioavailability [42,43]. Ox-LDL, in particular, is a lipoprotein that is a potent stimulator of vascular reactive oxygen species (ROS) and increases the expression of genes implicated in the formation and progression of atherosclerotic plaques. In HUVECs exposed to Ox-LDL, nebivolol has been shown to decrease intercellular adhesion molecules (ICAMs), P- and E-selectin expression, whereas β-blockers such as atenolol are ineffective [44]. Nebivolol has also been shown to normalize eNOS activity and intracellular NO
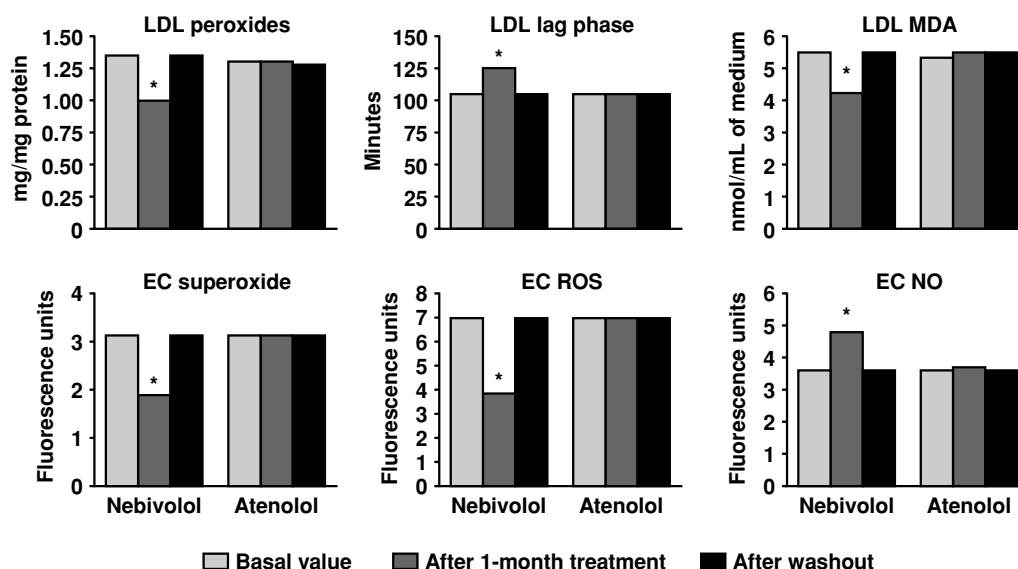
Figure 9 Effects of 1-month treatment with nebivolol (5 mg) or atenolol (100 mg) in hypertensive patients on plasma LDL oxidation status (top panels) and on plasma antioxidant activities against oxLDL-induced human umbilical vein endothelial cell activation (bottom panels). Mean values are reported. *$P < 0.0001$ versus baseline. LDL = low-density lipoprotein; oxLDL = oxidized low-density lipoprotein; MDA = malonyldialdehyde; EC = endothelial cell; ROS = reactive oxide species; NO = nitric oxide. Reproduced with permission from Zanchetti [48].

in HUVEC exposed to Ox-LDL [45] as well as prevents increases in ROS [46]. Nebivolol's antioxidant effects have also been demonstrated in the clinical setting. For example, in a randomized, double-blind, placebo-controlled, parallel-group study of mild-to-moderate hypertensive patients (n = 20), the influence of nebivolol (5 mg once daily) and atenolol (100 mg once daily) on the oxidative status of plasma (LDL) and its interaction with endothelial cells was examined [47]. The oxidative parameters were evaluated before and after 1 month of treatment, followed by a 1-week washout period. Nebivolol, but not atenolol, significantly reduced the content of hydroperoxides in LDL and their susceptibility to copper-induced oxidation (lag phase) and HUVEC-induced oxidation (malonyldialdehyde formation). Moreover, plasma from nebivolol-treated, but not atenolol-treated, patients significantly protected cultured HUVECs from the increase in superoxide and ROS concentrations, as well as from the decrease in NO content, produced by oxidized LDL (Fig. 9) [48]. In a separate study, nebivolol dosed for as little as 7 days in patients can decrease an oxidative stress marker such as urinary isoprostanes [49]. Nebivolol has also been shown to lower plasma levels of homocystine, fibrinogen, and plasminogen activation inhibitor-1 (PAI-1) after 6 months of treatment [50]. Remarkably, this effect of nebivolol was even more prominent in hypertensive smokers than nonsmokers (and to a greater extent, was more prominent than β-blockers such as celipro-

lol and carvedilol), a patient population with more pronounced endothelial dysfunction.

In summary, the effects of nebivolol on increasing NO bioavailability would suggest that treatment of patients with this novel agent should reduce endothelial dysfunction as well as convey other benefits, consequently improving cardiovascular and metabolic function.

Possible Mechanism(s) Underlying Nebivolol-Induced Nitric Oxide Increase

The exact mechanism by which nebivolol causes NO release is not yet clear. A number of receptor signaling pathways have been suggested. For example, in cellular and animal models, nebivolol has been shown to effect vasodilation through endothelial β-adrenoceptor-mediated NO production [25,51,52]. Specifically, agonism at both β_2 and β_3 receptors have been implicated. In one study, plasma from nebivolol-treated mice was able to stimulate NO release from a mouse aorta that was blocked by a β_2-adrenergic receptor antagonist, butoxamine (nebivolol itself had no effect, suggesting that its metabolite(s) was responsible) [51]. In a separate study, butoxamine was shown to block the nebivolol-induced decrease in PWV in sheep [24]. Ca^{2+}-activated K^+ channels in the smooth muscle have also been implicated in the β_2-adrenergic signaling pathway

[53]. These results are conflicting, given that nebivolol has not been shown to possess any agonist activity on β_2-adrenergic receptors (i.e., does not increase cyclic adenosine monophosphate (cAMP) in cells expressing recombinant human β_2-adrenergic receptors) [54]. On the other hand, β_3-adrenergic receptor-mediated responses have been examined with nebivolol. Nebivolol-induced relaxation of murine and human coronary vessels were not blocked by a β_1, β_2 antagonist, nadolol (relaxation of murine vessels could be blocked by a non-selective β_1, β_2, β_3 antagonist, bupranolol). Nebivolol-induced relaxation of coronary vessels was also reduced but not abolished in β_3-adrenergic receptor knockout mice [52]. Furthermore, a selective β_3 antagonist has been shown to block nebivolol-induced NO production in the mouse heart [55]. The contribution of the β_3-adrenergic receptor in human physiology is unclear. This receptor is expressed in the human vasculature, but no direct physiological role has been demonstrated [56]. Thus, it remains to be determined what role, if any, this receptor has in nebivolol-mediated increases in NO bioavailability in humans.

Estrogen receptor (ER) activation [57,58] has been reported with nebivolol; however, these effects are observed at high concentrations of the drug. For example, binding of nebivolol to ER was evident only at concentrations of 500 μM, which are greater than 500-fold what are reached clinically. Furthermore, nebivolol has been screened against multiple steroid receptors (e.g., estrogen, glucocorticoid, progesterone, and testosterone) at concentrations as high as 10 μM and none have demonstrated any affinity [59]. One other hypothesis for how nebivolol might stimulate NO release is a study involving ATP efflux and stimulation of P2Y purinoceptors [17]. This study showed that nebivolol stimulates NO release from rat glomerular endothelial cells, and this effect could not only be blocked by L-NAME but also by apyrase (which degrades extracellular ATP) and suramin or reactive blue 2 (both block P2Y purinoceptors). Moreover, addition of Gd^{3+} inhibited nebivolol-dependent ATP release, suggesting that mechanosensitive ion channels might be involved in its action.

In addition to receptor-mediated mechanisms, nebivolol has been reported to regulate NO bioavailability by modulating proteins downstream of these signaling pathways. Nebivolol has been shown to increase eNOS expression and activity [19,60] as well as iNOS expression [55]. These effects on NOS expression show some tissue dependence, with eNOS mediating nebivolol's actions in the vasculature and smooth muscle, and iNOS in the heart. Nebivolol is also reported to decrease asymmetric dimethylarginine (ADMA) levels in HUVECs [61] and has a neutral effect on serum ADMA

levels in hypertensive, type 2 diabetic patients treated with the drug, whereas metoprolol treatment increases ADMA in these patients [62]. ADMA is an endogenous L-arginine metabolite that inhibits cellular L-arginine uptake and eNOS activity competitively. Furthermore, as previously described, nebivolol inhibits eNOS uncoupling [23] and produces systemic antioxidant effects by reducing superoxide production via inhibition of NAD(P)H oxidase activity [22,63]. Similarly, a number of cardiovascular agents, including angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, have been shown to modify endothelial function and vascular NO levels, although often through mechanisms unrelated to their recognized mechanism of action. Hence, more investigation is necessary to clearly define the mechanism(s) of action for nebivolol. However, it is evident that these actions result in a net increase in NO bioavailability, and thus, contribute to many of the positive facets of nebivolol *in vivo*.

Conclusion

Vascular endothelial dysfunction, with loss of NO production, is a common feature of cardiovascular diseases, including hypertension [31,64]. A number of clinical studies have demonstrated that a loss of endothelial-dependent NO release exacerbates cardiovascular diseases such as hypertension and dyslipidemia [30,65,66]. Nebivolol is a novel antihypertensive agent that has a unique dual mechanism of action, combining highly cardioselective β_1-adrenergic receptor blocking activity with direct vasodilating properties. The mechanism by which nebivolol produces vasodilation can be attributed, in part, to its ability to enhance endothelial-dependent NO release and NO bioavailability. Nebivolol induces endothelium-dependent relaxation that is blocked by inhibitors of NOS and guanylate cyclase. Treatment of hypertensive and hyperlipidemic animals with nebivolol reverses the impairment of endothelium-dependent relaxation, improves NO–cGMP signaling, increases NO bioavailability, and enhances eNOS expression and activity in blood vessels and endothelial cells. Nebivolol also decreases superoxide production in blood vessels and tissues isolated from hypertensive animals. In addition, nebivolol increases arterial distensibility, glomerular filtration rate, and renal plasma flow *in vivo* by stimulating the release of endothelium-derived NO.

In normotensive patients, nebivolol enhances NO-mediated forearm blood flow. In addition, chronic nebivolol treatment improves endothelium-dependent vasodilation in hypertensive patients. Such an NO-mediated vasodilation results in positive and distinct

hemodynamic changes, such as increased stroke volume and decreased peripheral vascular resistance [3]. One would predict that these distinct hemodynamic properties would be especially beneficial in heart failure patients. Indeed, this was demonstrated in a randomized clinical trial of elderly patients with a history of heart failure [67]. Nebivolol treatment was shown to be well tolerated and effective in reducing mortality and morbidity in patients of age ≥ 70 years with heart failure, regardless of their initial ejection fraction.

Hence, the high β_1-adrenergic receptor selectivity of nebivolol, coupled with its endothelium/NO-mediated vasodilation, contributes to its efficacy in hypertensive patients (especially in patient populations with difficult-to-treat hypertension, such as African Americans and the obese), as well as accounts for fewer reported adverse events [36,37,67,68]—an important point, because common β-blocker-related effects, such as fatigue, dyspnea, and sexual dysfunction, limit or lead to discontinuation of such therapies. There is a low incidence of these adverse effects with nebivolol therapy [37]. In fact, nebivolol has been compared with both angiotensin II receptor blockers (ARB) [69] and angiotensin converting enzyme (ACE) inhibitors [70] and has demonstrated comparable or better efficacy with excellent tolerability. Furthermore, in the case of one of the more frequent complaints of β-blocker treatment, erectile dysfunction, it has been shown that hypertensive men on nebivolol do not demonstrate decreases in their International Index of Erectile Function (IIEF) scores and actually show improvements in secondary sexual activity scores and other IIEF subscores, when compared with that of metoprolol [71]. Similar positive findings have been demonstrated when compared with atenolol [72]. Furthermore, nebivolol's exceptional efficacy/tolerability profile has been confirmed in a recent meta-analysis that showed that nebivolol achieved similar or better rates of treatment response and blood pressure normalization than other drug classes and other antihypertensive drugs combined, with placebo-like tolerability and significantly better tolerability than losartan, calcium channel antagonists, other β-blockers, and all antihypertensive drugs combined [73].

In conclusion, nebivolol's high β_1 selectivity, coupled with its NO-dependent vasodilatory properties, distinguishes it among the β-blocker class. None of the available β-blockers such as atenolol, metoprolol, and carvedilol exhibit such a selectivity profile, and none are reported to increase NO bioavailability. All together, these data demonstrate the clinical relevance of the effect of nebivolol on NO bioavailability, which contributes to its efficacy and tolerability as an antihypertensive agent.

Conflict of Interest

Both authors are employed by Forest Research Institute, a subsidiary of Forest Laboratories, Inc.

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