Nebivolol, but Not Metoprolol, Improves Endothelial Function of the Corpus Cavernosum in Apolipoprotein E-Knockout Mice

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ABSTRACT
To determine the effects and underlying mechanisms of treatment with the β-receptor blockers nebivolol and metoprolol on penile endothelial function in apolipoprotein E (ApoE−/−) mice, wild-type (WT) and ApoE−/− mice were fed with a cholesterol-rich diet for 7 weeks. ApoE−/− mice were treated with nebivolol (10 mg/kg/day) or metoprolol (90 mg/kg/day). Endothelial function of aortic and corpora cavernosal tissue was assessed by pharmacological stimulation with carbachol (endothelium dependent) or glycerol trinitrate (endothelium independent) in organ bath experiments. Atherosclerotic lesion formation was determined with oil-red staining, and modulation of reactive oxygen species (ROS) production was determined with lipid peroxidation. Heart rate, but not blood pressure, was decreased in nebulol− and metoprolol-treated ApoE−/− mice (p < 0.01) compared with controls and WT mice without significant intergroup differences. Atherosclerotic lesion formation in the aortic root was increased in ApoE−/− mice (p < 0.01) with a more significant improvement in nebivolol− (p < 0.01) compared with metoprolol-treated mice (p < 0.05). Endothelium-dependent relaxation of the corpora cavernosa was significantly impaired in ApoE−/− mice (p < 0.05), which improved in nebulol− versus metoprolol-treated mice. Efficacy of endothelium-dependent relaxation was comparable in aortic and penile tissue. Quantification of ROS production via lipid peroxidation revealed a significant reduction of superoxide anion production in nebulol-treated (p < 0.05) but not metoprolol-treated mice compared with ApoE−/− controls. Nebulol improves penile endothelial function as a surrogate of erectile function in ApoE−/− mice. These effects may be related to a reduction of ROS production, which is independent of heart rate reduction, because metoprolol did not increase endothelial function.

Erectile dysfunction (ED) is defined as the inability to attain or maintain penile erections sufficient for satisfactory sexual performance (National Institutes of Health Consensus Statement, 1992). Prevalence of ED in Western industrialized countries is approximately 20 to 30% in the general population, but it is rising to more than 50% in patients with high-risk cardiovascular events, indicating a strong association between cardiovascular risk factors, especially hypertension, and erectile function (Feldman et al., 1994; Braun et al., 2000; Baumhäkel and Böhm, 2007; Böhm et al., 2007). Although nitric oxide (NO) plays an important role in the physiology of erectile function, impairment of the endothelial monolayer in the penile arteries and the corpus cavernosum is suggested to be the pathophysiological mechanism (Yavuzgil et al., 2005; Baumhäkel et al., 2006). Moreover, recent trials demonstrated differential effects of antihypertensive treatments on erectile function. Substances inhibiting the renin-angiotensin system are supposed to have beneficial effects on erectile function in vivo as well as in vitro (Pogari et al., 1998, 2001; Duesing, 2003). In contrast, in the last two decades especially, β-receptor blockers were reported to impair erectile function. A meta-analysis of the latest β-receptor blocker trials conducted in the 1990s with 35,000 patients showed a significant increase of sexual dysfunction in patients treated with a β-receptor blocker compared with placebo, but this analysis was limited by poor data management regarding erectile function (Ko et al., 2002). In contrast, recent randomized trials suggested that β-receptor blockade had no effect on erectile function (Franzen et al., 2001; Silvestri et al., 2003). Moreover, previous reports about increased ED may be biased by the psychological effect on patients with knowledge of being treated with β-receptor blockers as demonstrated by Silvestri et al. (2003). Consistent with these findings, a prospective randomized trial in a huge cardiovascular

ABBREVIATIONS: ED, erectile dysfunction; NO, nitric oxide; ApoE, apolipoprotein E; WT, wild-type; CCS, corpora cavernosal strips; AoR, aortic rings; ROS, reactive oxygen species.
high-risk population did not demonstrate a negative effect of β-receptor blockade on erectile function in multivariate analysis (Böhm et al., 2007).

Considering the strong correlation and pathophysiological link between endothelial and erectile function, substances with beneficial effects on nitric oxide synthase may improve erectile function. Nebivolol, as a third generation β-receptor blocker with NO-releasing effects, was recently shown to improve erectile function in a small trial with hypertensive men, whereas metoprolol did not (Brixius et al., 2007). Herein, possible mechanisms were not identified.

The objective of this study was to determine the effects and possible mechanisms of treatment with the β-receptor blockers, nebivolol and metoprolol, on penile endothelial function in cholesterol-fed apolipoprotein E (ApoE)−/− mice with atherosclerosis.

Materials and Methods

Animals and Procedures. Animal procedures were performed in accordance with institutional guidelines and the German animal protection law. Male C57BL/6J mice (wild-type; WT) and ApoE−/− mice (C57BL/6J ApoE−/− genetic background; Charles River Laboratories, Sulzfeld, Germany) were used for this study. The animals were maintained at 22°C with a 12-h light/dark cycle. All mice were fed with a high-fat, cholesterol-rich diet (21% fat, 19.5% casein, and 1.25% cholesterol; sniff, Soest, Germany) for 7 weeks starting at 10 weeks of age. Additional groups of male ApoE−/− mice were either treated with nebulol-HCl (orally via chow, 10 mg/kg/day) or metoprolol-succinate (orally via chow, 90 mg/kg/day). Direct effects of radicals on endothelial function of the penis (H2O2) were examined in 10-week-old, C57BL/6J wild-type mice. Systolic blood pressure and heart rate were measured noninvasively with the tail-cuff method in 10-week-old, C57BL/6J wild-type mice. Systolic blood pressure and heart rate were measured noninvasively with the tail-cuff method in conscious mice, as described previously (BP-2000; Visitech Systems, Apex, NC) (Wassmann et al., 2004). Chemicals were obtained from Sigma-Aldrich (Munich, Germany). All chemicals were dissolved in distilled water, with the exception of nebulol-HCl, which was dissolved in dimethyl sulfoxide and then further diluted, but never exceeding, 0.005% in organ bath.

Aortic Ring Preparation and Tension Recording. After excision, the descending thoracic aorta was immersed in Tyrode’s solution containing 118 mM NaCl, 2.5 mM CaCl2, 4.73 mM KCl, 1.2 mM MgCl2, 1.2 mM KH2PO4, 2.5 mM NaHCO3, 0.026 mM Na EDTA, and 5.5 mM D-(-)glucose, pH 7.4. Adventitial tissue was carefully removed. Three-millimeter rings were mounted in organ bath chambers filled with the Tyrode’s solution described above (37°C, aerated with 95% O2 and 5% CO2) and attached to a force transducer recording isometric tension. Aortic rings were stretched to a resting tension of 10 mN, which was maintained throughout the experiment. Pharmacologically induced contraction of aortic rings was performed with an α-agonist, (R)-(+)-phenylephrine-HCl (10 μM). Drugs were added in increasing concentrations to obtain cumulative concentration-response curves for carbachol (carbamylcholine-chloride, 1 nM–100 μM), as an endothelium-dependent relaxing agent, and glyceryl trinitrate (100 nM–100 μM), as a NO donor. The drugs were washed out before adding the next substance. The relaxing effect of carbachol was abolished by adding N-nitro-L-arginine methyl ester (1 μM). Relaxation of the organ bath chamber 20 min before H2O2. Both metoprolol (100 nM) and nebivolol (100 nM) were used in concentration of 50% β-receptor binding (Maack et al., 2000, 2001). To control the direct relaxing effects of nebulol, penile endothelial function was studied in C57BL/6J mice with increasing concentrations of nebivolol (0.1–10 μM) after preconstriction with phenylephrine (5 μM).

Staining Procedures. The aortic sinus and corpora cavernosa were snap-frozen at −80°C and sectioned on a Leica cryostat (10 μM). At least five consecutive sections per animal per staining were used for analysis. To detect atherosclerotic lesions in the aortic sinus, Oil Red O staining was performed as described previously (Laufs et al., 2005). Morphometric analysis was performed with the Lucia Measurement Software 4.6 (Nikon, Melville, NY), measuring the lipid-staining plaque area and related vessel diameter. To assess penile collagen content, sirius-red staining of corpora cavernosal sections was performed. Corpora cavernosal strips were snap-frozen and stored at −80°C. Segments were sectioned on a Leica cryostat (10 μm) that were placed on glass slides and incubated with the Sirius Red agent. Collagen content was quantified using fluorescence microscopy. CCS from each treatment group were processed in parallel, and images were acquired with identical acquisition parameters and stored digitally.

Measurement of Lipid Peroxidation. Aortic tissue/corpus cavernosa was homogenized in phosphate-buffered saline, pH 7.4, containing butylated hydroxytoluene (4 mM). Lipid hydroperoxides were determined using the Lipid Peroxidation Assay Kit II (Caltbiochem, Darmstadt, Germany) and expressed as micromoles per milligram of protein (Laufs et al., 2005).

Statistical Analysis. All data are expressed as the mean ± S.E.M. Statistical significance was assumed at p < 0.05. Intergroup differences were assessed with the analysis of variance test using Newman-Keuls post hoc analysis (GraphPad Prism 4.03; GraphPad Software, San Diego, CA).

Results

Vital Parameters. The body weight of mice was not different in all treatment groups (WT, 28.0 ± 4.3 g; ApoE−/−, 26.5 ± 4.6 g; ApoE−/−-metoprolol, 26.5 ± 3.9 g; and ApoE−/−-nebulol, 26.7 ± 2.4 g; N.S. between all groups). The heart rates of all animals after treatment with a high-cholesterol diet for 7 weeks are shown in Fig. 1. There were no significant differences in the heart rate of WT and ApoE−/− mice. Treatment with metoprolol and nebivolol decreased heart rate significantly to a similar extent (p < 0.01). Systolic blood pressure was not significantly different in all treatment groups (WT, 116 ± 2 mm Hg; ApoE−/−, 115 ± 3 mm Hg; ApoE−/−-metoprolol, 114 ± 2 mm Hg; and ApoE−/−-nebulol, 112 ± 3 mm Hg; N.S. between all groups).
Atherosclerotic Lesion Formation. After 7 weeks of a cholesterol-rich diet, atherosclerotic lesion formation was quantified in the aortic sinus in all groups by histological analysis of Oil Red O staining. WT mice showed no signs of atherosclerotic changes. In ApoE\(^{-/-}\) mice, atherosclerosis was severely displayed with a significant reduction in nebul-

Vascular/Penile Oxidative Stress. As a global parameter of oxidative stress, lipid peroxidation of the aorta wall (\(n = 5\)) and corpora cavernosa (\(n = 7\)) was measured. Lipid hydroperoxides were significantly increased in both tissues (aorta and corpora cavernosa) in ApoE\(^{-/-}\) mice compared with WT mice with a restoration in nebivolol but not metoprolol-treated mice (Fig. 4).

Acute Antioxidative Effects of Nebivolol. A cumulative concentration-response curve of nebivolol revealed direct relaxant effects on endothelial function of the corpus cavernosum (Fig. 5). The nebivolol concentration used in additional experiments of acute antioxidative effects (100 nM) was demonstrated to be subeffective regarding relaxation of CCS. Acute oxidative stress with H\(_2\)O\(_2\) decreased endothelial-dependent relaxation of CCS in wild-type mice (Fig. 6). Pre-

**Fig. 1.** Heart rate. Heart rate was measured in \(n = 7–10\) animals per group. Mean ± S.E.M. *, \(p < 0.01\) versus ApoE\(^{-/-}\)/WT.

**Fig. 2.** Atherosclerotic lesion size and endothelial function. After 7 weeks of treatment with a cholesterol-rich diet, staining of aortic segments was performed in five sections of the aortic sinus per animal (\(n = 5\) animals per group by Oil Red O staining (top panel). Functional performance was assessed in organ chamber experiments. Endothelium-dependent vasodilation induced by carbachol, expressed as a percentage of maximal phenylephrine-induced vasoconstriction, is shown (bottom panel: mean ± S.E.M., \(n = 6–8\) animals per group). *, \(p < 0.01\) versus ApoE\(^{-/-}\); **, \(p < 0.01\) versus ApoE\(^{-/-}\) metoprolol; and ***, \(p < 0.05\) versus ApoE\(^{-/-}\).

**Fig. 3.** Erectile function. After 7 weeks of treatment with a cholesterol-rich diet, corpora cavernosal strips were isolated, and functional performance was assessed in organ chamber experiments. Endothelium-dependent relaxation induced by carbachol, expressed as a percentage of maximal phenylephrine-induced contraction, is shown (mean ± S.E.M., \(n = 9–10\) animals per group): *, \(p < 0.01\) versus ApoE\(^{-/-}\); **, \(p < 0.05\) versus ApoE\(^{-/-}\); and ***, \(p < 0.05\) versus ApoE\(^{-/-}\) metoprolol.
treatment with nebivolol, but not metoprolol, restored impaired penile endothelial function significantly (Fig. 6).

Collagen Content. Collagen content as a parameter of fibrotic changes in the corpus cavernosum was calculated as described above. The content of collagen fibers was significantly enhanced in ApoE−/− mice with a restoration in nebivolol and in part in metoprolol-treated ApoE−/− mice (Fig. 7).

Discussion

The results of this study indicate an impairment of aortic and penile endothelial function in atherosclerotic ApoE−/− mice compared with WT mice independent of blood pressure or heart rate. Moreover, plaque formation as well as production of reactive oxygen species was significantly increased in ApoE−/− mice. Plaque formation, oxidative stress, and subsequent endothelial and erectile function could be restored by treatment with the β-receptor blocker nebivolol, but not with metoprolol.

Erectile function is crucially dependent on nitric oxide synthesis of the endothelial monolayer of the penile arteries and the corpus cavernosum, and it is consequently associated with known cardiovascular risk factors, especially hypertension (Feldman et al., 1994; Yavuzgil et al., 2005). Moreover, ED is suggested to be an early symptom of generalized atherosclerosis preceding major cardiovascular events (Baumhâkel and Böhm, 2007). Thus, cardiovascular evaluation has been recommended for patients with ED, providing the opportunity for optimized preventative treatment, but cardiovascular drugs are often related to a decrease in erectile function, especially in patients with hypertension (Modobe, 1990). In particular, β-receptor blockers have been reported to impair erectile function, but randomized clinical trials could not confirm this effect (Grimm et al., 1997; Rosenkranz et al., 1990). In contrast, a recent trial suggests that β-receptor blocker-related ED may be dependent on knowledge of the drug class of the patient, indicating a major role of psychological factors (Silvestri et al., 2003).

In our study, endothelial function was significantly increased in nebivolol-treated ApoE−/− mice. In metoprolol-treated mice, improvement of endothelial function was significant at a carbachol concentration of 1 μM only. Moreover, atherosclerotic plaque formation in the aortic root as well as reactive oxygen species (ROS) production was decreased or even normalized by nebivolol, whereas metoprolol had less or no effects. These results are consistent with recent findings indicating the beneficial role of nebivolol on endothelial function in different animal models (Oelze et al., 2006; Wolf et al., 2007). Comparable effects could be observed in the penile tissue. Endothelial function of the corpus cavernosum was improved in nebivolol-treated mice, whereas metoprolol treatment improved endothelial function at a carbachol concentration of 30 nM only. This result emphasizes the strong association between aortic and penile endothelial function.

Metoprolol and nebivolol have a similar potency to bind to β1- and β2-adrenergic receptors, but nebivolol, in particular, is suggested to exert NO-releasing effects, probably via reduction of reactive oxygen species, whereas metoprolol is lacking this pleiotropic effect on vascular function (Oelze et al., 2006). Thus, antioxidative activity of nebivolol with a consecutive increase of nitric oxide release may be the mechanism needed to improve erectile function. In penile tissue,
only nebivolol, but not metoprolol, reduced oxidative stress significantly. Effects of the treatment with nebivolol on superoxide production in the erectile tissue are consistent with previous reports on vascular tissues and could explain decreased inhibition of endothelial NO synthase activity in the corpora cavernosa as recently demonstrated (Oelze et al., 2006; Reidenbach et al., 2007). Moreover, nebivolol decreased collagen content of the penile tissue to a greater extent than did metoprolol. These effects of nebivolol treatment on structural changes in the penis are in line with recent data comparing nebivolol and amlodipine in hypertensive rats (Toblli et al., 2006). Decrease of fibrotic changes is also probably related to a decrease of oxidative stress.

β-blockers currently used have at least one chiral center, and beneficial cardiovascular effects may reside in the S-enantiomer (Siebert et al., 2008). Nebivolol is a racemic mixture and differs from all β-blockers with a hydroxypropanolamine structure with cardiovascular activity at the R-enantiomer at the hydroxy group. Moreover, hydroxylated nebivolol metabolites could play a role in beneficial effects beyond blood pressure reduction (Siebert et al., 2008). Recent results in rat aorta suggested that nebivolol is in part degraded by its reaction with ROS (de Groot et al., 2004). Thus, antioxidative effects of nebivolol could, at least in part, be explained by acute scavenging of ROS. Therefore, even improvement of penile endothelial function might be dependent on these effects. Preincubation with nebivolol, but not metoprolol, improved diminished endothelial function of the corpus cavernosum after stimulation with ROS. These effects are probably independent of receptor binding, because the nebivolol concentrations used were ineffective in a cumulative concentration response regarding direct relaxing effects. The nebivolol-induced relaxation of penile smooth muscle cells could be demonstrated in concentrations higher than 100 nM. However, these effects are not likely to play a major role in chronic treatment regarding plasma concentrations of nebivolol, which are one order of magnitude less (Selvan et al., 2007). In summary, direct and acute antioxidative effects of nebivolol probably contribute to the presented results, but conclusions about the extent remain speculative.

Thus, for the first time, treatment with β-receptor blockers is shown to improve penile endothelial function as a surrogate of erectile function in an atherosclerotic animal model. Hence, these results support recent clinical trials, which failed to demonstrate negative effects of β-receptor blockade on erectile function in patients with high-risk cardiovascular events (Silvestri et al., 2003; Böhm et al., 2007). In particular, in hypertensive men, nebivolol did not change the frequency of sexual contacts as a surrogate of erectile function, whereas in patients treated with atenolol, the frequency of sexual contacts decreased significantly (Boydak et al., 2005). In contrast, a recent trial in male hypertensive patients comparing metoprolol and nebivolol indicated beneficial effects of nebivolol, but not metoprolol, on erectile function (Brixius et al., 2007). These clinical results are consistent with the presented mechanistic study. Moreover, in clinical trials, the effects of β-receptor blockade on erectile function are, in part, suggested to be dependent on blood pressure reduction. The results of our study demonstrate the effects, which are independent on the decrease of blood pressure, considering similar blood pressure levels in all treatment groups. Despite the absence of blood pressure reduction, metoprolol did not decrease erectile function, which is also an important finding that supports the thesis that selective β-receptor blockade does not affect erectile function. Nebivolol improved erectile function by a decrease of oxidative stress. This effect is probably dependent on increased nitric oxide release and a decreased heart rate, which is known to improve endothelial function (Beere et al., 1984).

In conclusion, treatment with the β-receptor blocker nebivolol improved endothelial function of the corpus cavernosum in ApoE/−/− mice. These beneficial effects may be dependent on a reduction of oxidative stress in the corpus cavernosum. In addition, the effects of treatment with nebivolol and metoprolol on endothelial function were comparable in penile and aortic tissue, indicating the strong association of endothelial and erectile function.

References


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