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Robert B. Neuman
Emory University

Salim Hayek
Emory University

Joseph C. Poole
Emory University

Ayaz Rahman
Emory University

Vivek Menon
Emory University

See next page for additional authors

Authors
Robert B. Neuman, Salim Hayek, Joseph C. Poole, Ayaz Rahman, Vivek Menon, Nino Kavtaradze, David Polhemus, Emir Veledar, David J. Lefer, and Arshed A. Quyyumi

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Nitric Oxide Contributes to Vasomotor Tone in Hypertensive African Americans Treated With Nebivolol and Metoprolol

Robert B. Neuman[1], **Salim Hayek**[1], **Joseph C. Poole**[1], **Ayaz Rahman**[1], **Vivek Menon**[1], **Nino Kavtaradze**[1], **David Polhemus**[2], **Emir Veledar**[1], **David J. Lefer**[2], and **Arshed A. Quyyumi**[1]

[1]Emory University School of Medicine, Division of Cardiology, Atlanta, GA

[2]Louisiana State University Health Sciences Center, Department of Pharmacology, New Orleans, LA

Abstract

Endothelial dysfunction is more prevalent in African Americans (AA) compared to whites. We hypothesized that nebivolol, a selective β-1 antagonist that stimulates NO, will improve endothelial function in AA with hypertension when compared to metoprolol. In a double-blind, randomized, cross-over study, 19 AA hypertensive subjects were randomized to a 12-week treatment period with either nebivolol 10mg or metoprolol succinate 100mg daily. Forearm blood flow (FBF) was measured using plethysmography at rest and after intra-arterial infusion of acetylcholine, and sodium nitroprusside to estimate endothelium-dependent and independent vasodilation, respectively. Physiologic vasodilation was assessed during hand-grip exercise. Measurements were repeated after NO blockade with L-N^G-monomethylarginine (L-NMMA), and after inhibition of endothelium-derived hyperpolarizing factor (EDHF) with tetraethylammonium chloride (TEA). NO blockade with L-NMMA produced a trend toward greater vasoconstriction during nebivolol compared to metoprolol treatment period (21% vs 12% reduction in FBF, p=0.06, respectively). This difference was more significant after combined administration of L-NMMA and TEA (p<0.001). Similarly, there was a contribution of NO to exercise-induced vasodilation during nebivolol but not during metoprolol treatment. There were significantly greater contributions of NO and EDHF to resting vasodilator tone and of NO to exercise-induced vasodilation with nebivolol compared to metoprolol in AA with hypertension.

Keywords

nebivolol; metoprolol; nitric oxide; hypertension; black; african american; vasodilation; acetylcholine; exercise

Corresponding Author: Arshed A. Quyyumi, MD, Professor of Medicine, Division of Cardiology, Co-Director, Emory Clinical Cardiovascular Research Institute, 1462 Clifton Road N.E. Suite 510, Atlanta GA 30322, Tel: 404 727 3655, Fax: 404 712 8785, aquyyum@emory.edu.

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INTRODUCTION

Endothelial dysfunction precipitated by loss of nitric oxide (NO) bioavailability is associated with exposure to cardiovascular risk factors. African Americans (AA) shoulder a higher burden of hypertension and its associated target organ damage including nephropathy, stroke, myocardial infarction and cardiovascular mortality.[1] In comparison to their white counterparts, AAs have decreased NO bioavailability and worse endothelial function that has been attributed to both decreased production and increased degradation of NO.[2–5]

Nebivolol is a third-generation, β_1-adrenergic receptor antagonist with vasodilatory properties that appear to be independent of β_1-receptor antagonism and related to β_3-receptor agonist effects.[6–8] It stimulates nitric oxide release through β3-receptor and ATP-dependent, P2Y-receptor activation. [6,9–13] In hypertensive patients, vasodilation with nebivolol is evident after a single dose and persists after chronic administration.[14,15] Furthermore, nebivolol increased endothelium-dependent vasodilation with acetylcholine when compared to atenolol in whites with hypertension.[16]

Endothelium-dependent vasodilation in response to agonists such as acetylcholine and bradykinin is due to the release of several factors including NO, endothelium-derived hyperpolarization factor (EDHF), prostaglandins, and others.[17] EDHF release may compensate for reduced NO bioavailability in certain disease states and contributes to physiologic vasodilation due to exercise.[17–20] Although there may be several EDHFs, they all relax vascular smooth muscle via activation of calcium-dependent potassium channels that can be inhibited with tetraethylammonium chloride (TEA).[20] Using these antagonists, we and others have shown that EDHF contributes to resting vasodilator tone and to bradykinin-mediated vasodilation in healthy subjects.[17] Furthermore, we found that NO but not EDHF activity is reduced in the forearm vasculature of AA compared to whites.[4]

Because of the reduction in NO bioavailability in the vasculature of AA, we sought to determine whether nebivolol, compared to metoprolol, can selectively improve endothelial function by modulating NO and EDHF activities in AAs with hypertension. We tested the hypothesis that nebivolol, by increasing NO bioavailability, would improve endothelial function compared to matched hypotensive doses of sustained release metoprolol in AA with hypertension.

METHODS

Study design

In a randomized, double-blind, crossover study, subjects with resting blood pressure (BP) >135/85 (GE Dynamap) were randomized to receive either nebivolol or metoprolol succinate in addition to their current regimen of anti-hypertensive medications (Figure 1A). Subjects with hypertension and BP<135/85 at randomization had their regimen altered by decreasing the dose of concomitant medications. The study drug was initiated as either nebivolol 5mg or metoprolol succinate 50mg daily. After 2 weeks, the dose of the study drug was increased to either nebivolol 10mg or metoprolol 100mg if BP remained >125/80. Subjects continued the highest titrated dose of study drug for an additional 10 weeks prior to

performance of vascular studies. At the end of the first treatment phase, subjects crossed over into the alternate treatment arm. BP was measured at each visit after a 10-minute rest period using a mean of 3 measurements taken 5 minutes apart. The study was reviewed and approved by the Emory University Institutional Review Board. All subjects provided written informed consent.

Subjects

Self-identified AA subjects aged 22–80 years old with a history of essential hypertension were recruited. Exclusion criteria included initiation or change in statin or anti-hypertensive therapy, occurrence of stroke or acute coronary syndrome within 2 months prior to randomization, presence of chronic stable angina, current neoplasm, symptoms of heart failure, aortic stenosis, chronic kidney or liver diseases (creatinine >2.5mg/dL, liver enzymes > twice upper limit of normal), and premenopausal females with the potential for pregnancy. Subjects with contra-indications to beta blockade (i.e. second or third degree AV block, bradycardia, severe reactive airways disease) were also excluded. Concurrent therapy with angiotensin antagonists (ACE inhibitors or ARBs) was not permitted. Allowable concurrent anti-hypertensive therapy included thiazide diuretics, calcium channel antagonists, clonidine, and vasodilators. Subjects on beta-adrenergic blockers had their drug changed to the study drug at time of enrollment. Subjects with co-morbid cardiovascular risk factors including hyperlipidemia, diabetes and smoking were included as long as there was no recent or planned change in therapy within 2 months of randomization and during the course of the study.

Materials

L-NMMA: N^G-mono- methyl-L-arginine (L-NMMA; Bachem, Laufelfingen, Switzerland) is an analogue of L-arginine which competitively and irreversibly inhibits the generation of NO from arginine by nitric oxide synthases (NOS1, NOS2 and NOS3). Given at 8 μmol/min it attenuates agonist- and exercise-stimulated FBF, respectively.[21,22] *TEA:* Tetraethylammonium (Sigma Aldrich) is a quaternary ammonium compound which selectively blocks voltage-sensitive potassium channels. When given at 1 mg/min, TEA is known to selectively inhibit K^+_{Ca} channels and inhibits bradykinin-mediated vasodilation.[23–25] Sodium nitroprusside is an endothelium-independent vasodilator that acts as a direct nitric oxide donor.[26] Its infusion serves to detect alterations in vascular smooth muscle sensitivity to nitric oxide. Acetylcholine (Novartis, East Hanover, NJ) is an endothelium-dependent vasodilator that stimulates nitric oxide release from endothelial cells.[27]

Measurement of forearm blood flow

Subjects refrained from exercise, alcohol, tobacco and caffeine for at least 24 hours before the study admission. After an overnight fast in a quiet temperature-controlled (22 to 24°C) room, subjects received 975mg of aspirin to inhibit prostacyclin synthesis at least 1 hour prior to the study.[28] A 20-gauge catheter (Teleflex Inc, Research Triangle Park, NC) was inserted in the brachial artery of the non-dominant arm under direct ultrasound guidance for intra-arterial drug infusions, pressure monitoring, and blood sampling. Simultaneous

forearm blood flow (FBF) measurements were obtained in both arms using a dual-channel venous occlusion strain gauge plethysmograph (model EC6, DE Hokanson, Bellevue, WA) as described previously.[17,21] Flow measurements were recorded for approximately 7 seconds, every 15 seconds up to eight times and a mean FBF value in $mL \cdot min^{-1} \cdot 100\ mL^{-1}$ was computed. Forearm vascular resistance (FVR) was calculated as the mean arterial pressure ÷ FBF and expressed as mmHg per $mL \cdot min^{-1} \cdot 100\ mL^{-1}$.

All agents were administered intra-arterially. Resting FBF measurements were made after 15 minutes of normal saline infusion (1.5ml/min) and repeated during intra-arterial infusion of the acetylcholine at 7.5, 15 and 30 μg/min for 5 minutes each. Physiologic forearm vasodilation was investigated using intermittent handgrip exercise where the evaluated forearm was exercised by squeezing an inflated pneumatic bag as previously described.[21,29] Exercise was performed at 15%, 30%, and 45% of the subject's maximum voluntary grip strength. Each contraction lasted for 5 seconds followed by relaxation for 10 seconds and was repeated for 5 minutes at each workload. FBF was measured in the final 2 minutes of each dose/exercise workload.

After recovery, the acetylcholine and exercise protocol was repeated during inhibition of NO synthesis with the concomitant infusion of L-NMMA at 8 μmol/min during acetylcholine infusion and 16μmol/min during exercise. After recovery, while continuing L-NMMA, TEA was infused intra-arterially at 1 mg/min and acetylcholine and exercise protocols were repeated. Thus, we measured resting vasomotor tone, acetylcholine- and exercise-mediated vasodilation under control conditions, during NO blockade, and during combined NO and EDHF blockade allowing quantification of NO- and EDHF-dependent vasodilation. Finally, sodium nitroprusside was infused intra-arterially at 1.6 and 3.2 μg/min for 5 minutes each with FBF and FVR measured with each dose. L-NMMA and TEA have both been shown to not alter vasodilator responses to nitroprusside (Figure 1B).[17]

Analysis of inorganic nitrite and nitrate levels

Plasma nitrite and nitrate were measured from blood samples collected prior to FBF studies into distilled water-rinsed centrifuge tubes containing 100 mL of 100 mmol/L *N*-ethylmaleimide and 5 mL of 0.5 mmol/L EDTA. Extracted plasma was flash frozen and stored at −80°C and subsequently analyzed for nitrite and nitrate levels by ion chromatography (ENO20, Eicom USA, San Diego, CA) as previously described.[30]

Statistical Analysis

Descriptive subject characteristics were reported as means, standard deviations (SD), standard errors (in figures), and percentages. A paired student's t-test was used to compare BP between treatment phases. Analysis of FBF and FVR measurements was performed using linear mixed effects modeling with repeated measures, after log-transformation of the non-normal and positively skewed variables. An unstructured covariance form was assumed for the repeated measures. In the model, treatment period (metoprolol or nebivolol) and inhibitor (L-NMMA, TEA, L-NMMA and TEA) were added as fixed effects, and subject ID as a random effect. Based on previous studies, with a sample size of 20 we should detect a

10% or greater change between the two drugs in FBF with L-NMMA or TEA with α=0.05 and power=0.8. [15,16]

RESULTS

Subjects

Of the 19 subjects (13 men and 6 women) recruited, 74% were on one or more anti-hypertensive medications, most commonly diuretics and calcium channel antagonists (Table 1). Fourteen subjects (74%) reached the target doses of nebivolol 10mg and metoprolol 100mg. Of the 5 subjects who did not require up-titration, 2 were controlled on nebivolol 5mg and metoprolol 50mg, 1 required nebivolol 5mg and metoprolol 100mg, and 2 required nebivolol 10mg and metoprolol 50mg.

Resting forearm vascular tone

The heart rate and blood pressure was similar during both nebivolol and metoprolol treatment periods (Table 1). Resting vasodilator tone was also similar with the two study agents; FBF 2.8±1.3 and 2.9±1.2 $mL \cdot min^{-1} \cdot 100\ mL^{-1}$, p=0.6 and FVR 40.8±17 and 37.5±12 $mmHg/mL \cdot min^{-1} \cdot 100\ mL^{-1}$, p=0.8 with nebivolol and metoprolol, respectively (Figure 2).

NO blockade with L-NMMA reduced resting FBF by 21% with nebivolol and 12% with metoprolol (both p<0.001 compared to baseline, and p=0.06 between groups for comparison of absolute values, and p=0.053 for comparison of % change in FBF). Similarly, FVR increased by 26% with nebivolol and 18% with metoprolol (both p<0.001 compared to baseline, p=0.1 between groups for comparison of absolute values, and p=0.1 for comparison of % change in FVR) (Figure 2). Addition of, K^{+}_{Ca} channel blockade with TEA to L-NMMA resulted in further significant reduction in FBF and an increase in FVR with nebivolol but not metoprolol (p<0.001) (Figure 2). Thus, there was a greater contribution of NO and EDHF combined to resting blood flow during therapy with nebivolol compared to metoprolol with NO accounting for the majority of the difference.

Acetylcholine-mediated vasodilation

Acetylcholine produced a similar dose-dependent increase in FBF and a concurrent decrease in FVR with both nebivolol and metoprolol (both p=0.6) (Figure 3). L-NMMA co-infusion attenuated acetylcholine-mediated vasodilation during treatment with both drugs, resulting in a 23.1±27.3%, p=<0.001 and a 14.8±35.5%, p=0.002 decrease in FBF with nebivolol and metoprolol, respectively (Figure 4). The difference between the groups did not reach statistical significance (p=0.4). Addition of TEA to L-NMMA did not further impact either the FBF or FVR during either the nebivolol or metoprolol treatment periods, indicating lack of contribution of EDHF to acetylcholine-mediated vasodilation with either drug (Figure 4).

Exercise-induced vasodilation

Graded exercise produced progressive forearm vasodilation that was similar during treatment with nebivolol and metoprolol (p=0.3 and p=0.4, respectively) (Figure 3C, D). With nebivolol however, NO antagonism with L-NMMA resulted in a significant decrease

in FBF (p=0.001) and increase in FVR (p<0.001) (Figure 5). There was no significant change in FBF or FVR with L-NMMA during the metoprolol treatment period (Figure 5). This suggests a significant contribution of NO to exercise-mediated vasodilation with nebivolol, but not with metoprolol treatment. Combined administration of L-NMMA and TEA produced no further reduction in FBF or increase in FVR during either the metoprolol or nebivolol treatment periods, indicating lack of contribution of EDHF to exercise-mediated vasodilation with either beta-antagonist (Figure 5).

Sodium nitroprusside-induced vasodilation

Sodium nitroprusside infusion produced similar vasodilation during treatment with nebivolol and metoprolol (Figure 3E and 3F) indicating no differences in endothelium-independent vasodilation with these agents.

Plasma nitrite and nitrate levels

Plasma nitrite and nitrate levels were similar during treatment with nebivolol (0.17±0.1 and 9.3±3.5 μmol/L) and metoprolol (0.17±0.1 and 8.7±4.1 μmol/L, p= 0.7 and 0.5, respectively).

DISCUSSION

Compared to whites, both healthy and hypertensive AA have diminished basal NO activity and reduced endothelium-dependent and -independent vasodilation. [4,31–36] In this study, we found evidence for NO bioavailability at rest during treatment with both nebivolol and metoprolol succinate in hypertensive AA subjects, with a clear trend for a greater contribution of NO during nebivolol therapy. Moreover, after combined blockade of NO and EDHF, there was a significantly greater vasoconstriction during nebivolol compared metoprolol therapy, suggesting greater contribution of both NO and EDHF combined to resting vasomotor tone during nebivolol treatment. Moreover, the contribution of NO to exercise-induced vasodilation was greater during treatment with nebivolol compared to metoprolol succinate. This is the first study to explore the role of nebivolol compared to metoprolol in AA hypertensives who have profound abnormalities in NO bioavailability. Specifically, we demonstrate a greater contribution of endothelium-derived vasodilators to resting FBF and greater contribution of NO to vasodilation during exercise with nebivolol compared to metoprolol. We found no differences in acetylcholine-mediated vasodilation between these agents, a finding that is different from that reported in white hypertensive subjects who received either nebivolol or atenolol.[16]

We have previously shown that there is *no* contribution of NO at rest and after acetylcholine in hypertensive subjects (no effect of L-NMMA).[37–43] Here we demonstrate that there is some contribution of NO after AA hypertensives are treated with beta adrenergic blockade, and further show that this is higher with nebivolol at rest compared to metoprolol. In healthy subjects, L-NMMA reduces resting flow by 30 to 40%. After beta blockade, we show that even in hypertensives, it is restored to approximately half of what it is in healthy subjects.

Exercise-induced vasodilation is a complex process involving multiple vasodilator mechanisms including a modest contribution of NO and various putative EDHFs.[4,21,44,45]

We have previously demonstrated that both NO and EDHF contribute individually and in concert to exercise-induced microvascular vasodilation in the healthy human forearm circulation. Nebivolol, but not metoprolol restored functional sympatholysis that is impaired in working muscle in hypertensive patients.[46] In this study, while there was no difference in the magnitude of vasodilation during exercise between treatments, we observed a significant contribution of NO to exercise-induced vasodilation during the nebivolol but not the metoprolol treatment period. This suggests that nebivolol restores contribution of NO to exercise-induced vasodilation in AA with hypertension. Interestingly, neither drug increased the contribution of EDHF to exercise-induced vasodilation.

In previous studies in hypertensive subjects from both ethnicities, we and others found that acetylcholine-mediated NO release was lower compared to normotensive controls.[4,34–36] In fact, L-NMMA did not inhibit acetylcholine responses in *untreated* hypertensive subjects. Herein, we show significant NO release in response to acetylcholine during treatment with both beta-receptor antagonists. In a previous study of European subjects with hypertension, increased acetylcholine-mediated NO activity was reported with nebivolol but not after atenolol.[16] Improved NO activity with endothelium-dependent vasodilators long-acting metoprolol may thus account for the benefits of this preparation compared to atenolol.[47]

Greater NO activity was found in internal mammary artery and vein specimens from subjects pre-treated with nebivolol compared to metoprolol.[48] In subjects with coronary artery disease or hypertension, nebivolol, but not atenolol improved flow-mediated dilation (FMD) and lowered asymmetric dimethylarginine (ADMA) levels, a naturally occurring amino acid that inhibits eNOS. [15,49] Finally, in AA with stage 1 hypertension, nebivolol monotherapy increased FMD and improved arterial wave reflections compared to untreated state.[50] In contrast, no differences between metoprolol succinate and nebivolol were observed in aortic compliance indices in a largely AA population of diabetics.[51]

Moreover, these experiments were performed in the setting of prostacyclin inhibition. The contribution of the prostacyclin pathway was thus not investigated in this study and may potentially be also affected by beta-blockade. Whether other endogenous vasodilators such as prostacyclin, adenosine, carbon monoxide and others are affected differentially by nebivolol versus other beta-blockers needs to be studied.

Strengths and limitations

Strengths of our study include the blinded crossover design and investigation in AA hypertensive subjects who are at particularly high cardiovascular risk and have profound NO abnormalities. We have also examined the contribution of both NO and EDHF to resting, acetylcholine-mediated and exercise-induced vasodilation. Limitations include the small sample size, the heterogeneity of the population, which was largely driven by the requirement of repeated intra-arterial cannulation. The lack of placebo treatment phase would have allowed comparison of either agent with no therapy, however this was precluded by potential hazards of performing three invasive studies in the same subject. This study also did not include other beta-receptor antagonist comparators such as atenolol. In addition, the interaction of concomitant medications such as thiazides or diuretics with the study drugs on

NO bioavailability, while largely controlled by the cross-over design and stable dosing, could not be examined.

CONCLUSIONS

In conclusion, there was significant contribution of NO and EDHF to resting vasodilator tone and a significant contribution of NO to exercise-induced vasodilation with nebivolol compared to an equipotent dose of metoprolol.succinate. These findings demonstrate selective effects of nebivolol on NO activity in AA with hypertension and provide mechanistic insights into endothelial dysfunction in this at-risk group. Further study is needed to establish if these observations translate into clinically meaningful outcomes.

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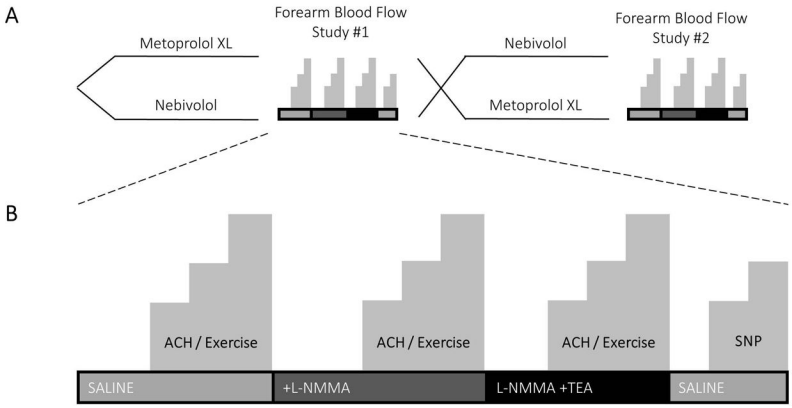

Figure 1.
Study design (A) and protocol (B)

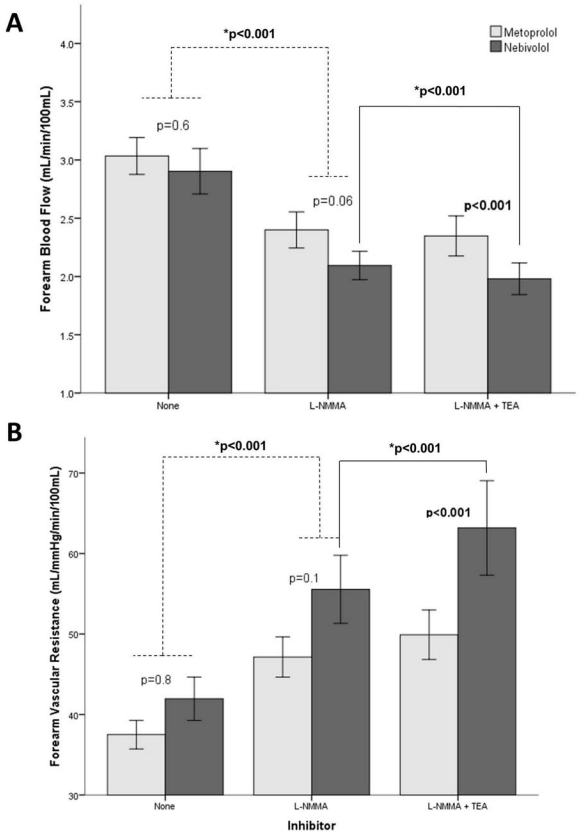

Figure 2.

Contribution of nitric oxide and K^{+}_{Ca} channel activation to resting forearm blood flow and vascular resistance during treatment with nebivolol (A and C) and metoprolol (B and D). Responses to infusion of L-NMMA and combined infusions of L-NMMA and TEA are shown. P values for effect of L-NMMA and TEA on FBF and FVR. Data presented as mean ±SEM.

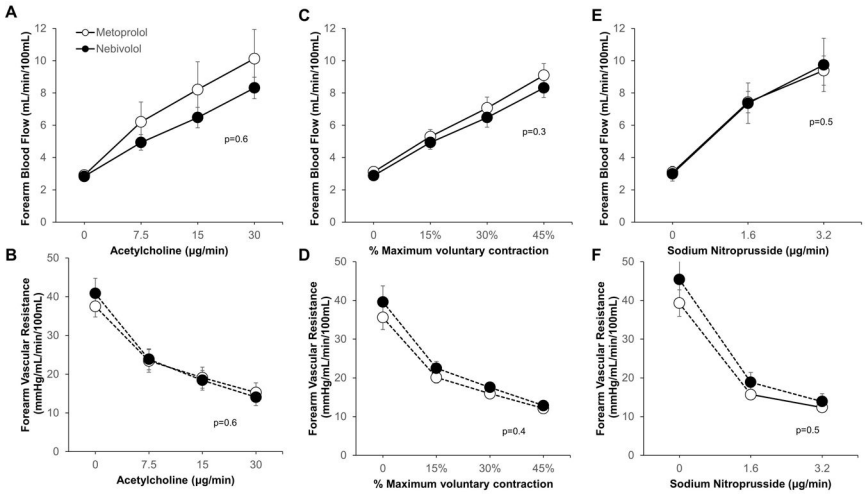

Figure 3.

Endothelium-dependent FBF (A) and FVR (B) changes with acetylcholine, endothelium-independent changes in FBF (C) and FVR (D) with sodium nitroprusside, and forearm exercise induced changes in FBF (E) and FVR (F) during treatment with nebivolol and metoprolol. P values are for treatment effect of nebivolol/metoprolol by mixed model. Data presented as mean±SEM.

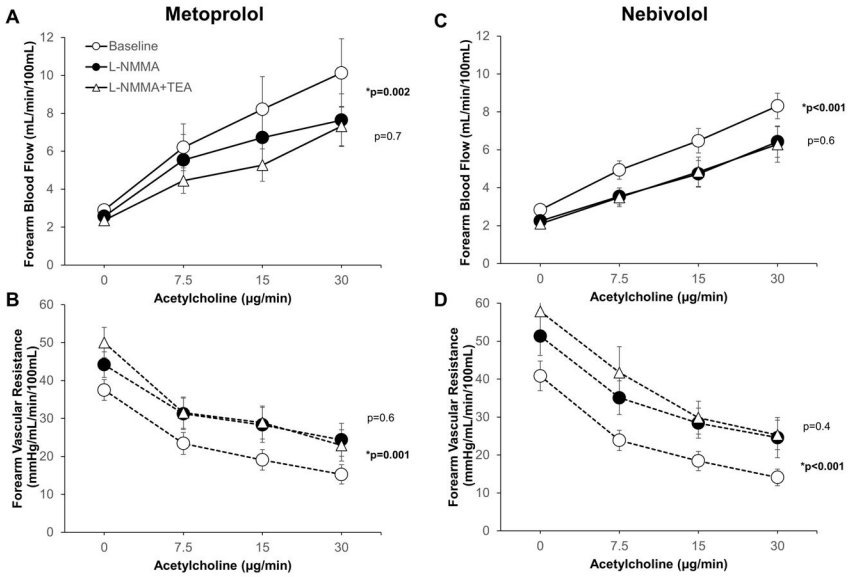

Figure 4.

Contribution of nitric oxide and K^+_{Ca} channel activation to acetylcholine-mediated vasodilation during treatment with nebivolol (A and C) and metoprolol (B and D). FBF and FVR responses to increasing doses of acetylcholine alone after initial infusion of L-NMMA, and combined blockade with L-NMMA and TEA are shown. P values are for effect of L-NMMA and TEA by mixed model. *p denotes p-value for comparison of L-NMMA and control, non-starred p reflects p-value for comparison of L-NMMA and L-NMMA+TEA. Data presented as mean±SEM.

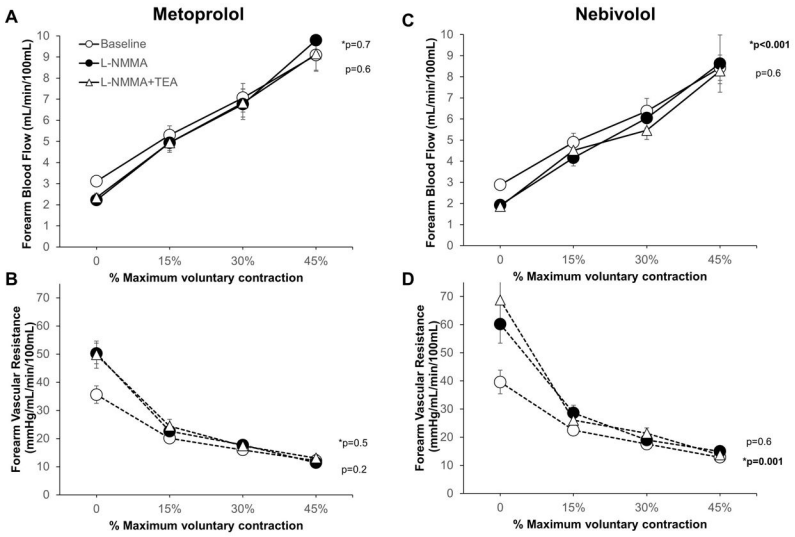

Figure 5.

Contribution of nitric oxide and K+Ca channel activation to forearm exercise mediated vasodilation during treatment with nebivolol (A and C) and metoprolol (B and D). FBF and FVR responses to increasing doses of acetylcholine alone, after initial infusion of L-NMMA, and combined blockade with L-NMMA and TEA are shown. P values are for effect of L-NMMA and TEA by mixed model. *p denotes p-value for comparison of L-NMMA and control, non-starred p reflects p-value for comparison of L-NMMA and L-NMMA +TEA. Data presented as mean±SEM.

Table 1

Characteristics of Study Subjects

Hypertensive African-Americans (n=19)			
Age, years		51±8.6	
Male/Female		13/6	
Diabetes mellitus, n (%)		1(5.1%)	
Smoker, n (%)		7 (36.8%)	
Hypercholesterolemia, n (%)		6 (31.5%)	
Family History of CAD, n (%)		6 (31.5%)	
Statin Therapy, n (%)		4 (21%)	
Weight, kg		96.5±22.3	
Body mass index, kg/m2		32.5±7.4	
Concurrent anti-hypertensive drugs			
Diuretic (thiazide)		8 (42.1%)	
Calcium Channel Antagonist		5 (26.3%)	
On-Treatment Hemodynamic Data	Nebivolol	Metoprolol succinate	p-value
Systolic Blood Pressure, mmHg	135±15	134±15	0.8
Diastolic blood pressure, mmHg	81±14	81±21	0.8
Resting heart rate, bpm	63±8	64±9	0.8

*Data are Mean ±SD