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Beta₃-Adrenoreceptor Stimulation Ameliorates Myocardial Ischemia-Reperfusion Injury Via Endothelial Nitric Oxide Synthase and Neuronal Nitric Oxide Synthase Activation

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Abstract

Objectives—This paper examined whether nebivolol protects the heart via nitric oxide (NO) synthase and NO-dependent signaling in an in vivo model of acute myocardial infarction.

Background—Beta₃-adrenergic receptor (AR) activation promotes endothelial nitric oxide synthase (eNOS) activity and NO bioavailability. We hypothesized that specific beta₃-AR agonists would attenuate myocardial ischemia-reperfusion (MI/R) injury via eNOS activation and increased NO bioavailability.

Methods—Mice were subjected to 45 min of myocardial ischemia in vivo followed by 24 h of reperfusion (R). Nebivolol (500 ng/kg), CL 316243 (1 μg/kg), BRL-37344 (1 μg/kg), or vehicle (VEH) was administered at the time of R. Myocardial area-at-risk (AAR) and infarct size (INF)/AAR was measured at 24 h of R. Cardiac tissue and plasma were collected to evaluate eNOS phosphorylation, neuronal nitric oxide synthase (nNOS), inducible nitric oxide synthase expression, and nitrite and nitrosothiol levels.

Results—Nebivolol (500 ng/kg) reduced INF/AAR by 37% ($p < 0.001$ vs. VEH) and serum troponin-I levels from 41 ± 4 ng/ml to 25 ± 4 ng/ml ($p < 0.05$ vs. VEH). CL 316243 and BRL-37344 reduced INF by 39% and 42%, respectively ($p < 0.001$ vs. VEH). Nebivolol and CL 316243 increased eNOS phosphorylation at Ser-1177 ($p < 0.05$ vs. VEH) and increased nitrite and total nitrosylated protein levels. Nebivolol and CL 316243 significantly increased myocardial nNOS expression. Nebivolol failed to reduce INF after MI/R in beta₃-AR$^{-/-}$, eNOS$^{-/-}$, and in nNOS$^{-/-}$ mice.

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Conclusions—Our results indicate that beta$_3$-AR agonists protect against MI/R injury. Furthermore, the cardioprotective effects of beta$_3$-AR agonists are mediated by rapid eNOS and nNOS activation and increased NO bioavailability.

Keywords

beta$_3$ adrenergic receptor; cardiac ischemia; endothelial nitric oxide synthase; neuronal nitric oxide synthase; nitric oxide

Acute myocardial infarction is one of the leading causes of death in the adult population. Myocardial ischemia and reperfusion (MI/R) injury involves excess formation of free radicals and calcium overload, leading to cell death, tissue scarring, and remodeling (1–3). Beta-adrenergic receptor (AR) blockers have been shown to play a key role in the management of cardiovascular disease by improvement of coronary circulation, protection from cardiomyocyte apoptosis, and reduction of infarction (4 – 6). These beta-blockers target the beta$_1$ and beta$_2$ ARs as their primary mode of action (7,8). However, the beta$_3$-AR has recently emerged as a potential target for the treatment of heart disease (9,10).

Nebivolol (alpha,alpha′-[iminodimethylene]bis[6-fluoro-2-chrommethanol]) is a third-generation beta blocker approved by the U.S. Food and Drug Administration for the treatment of hypertension. The selectivity of nebivolol for the beta$_1$-AR is considerably higher than that of bisoprolol, carvedilol, or bucindolol, and it was shown to reduce mortality and morbidity in elderly patients with heart failure (11–13). Nebivolol also exhibits nitric oxide (NO)-mediated vasodilating properties thought to result from the stimulation of the endothelial beta$_3$-AR (14,15). Stimulation of the beta$_3$-AR and activation of endothelial nitric oxide synthase (eNOS) increases NO release after nebivolol treatment to cause peripheral vasodilatation and improved endothelial function (16,17). Nebivolol might also activate the beta$_2$-AR (18 –20).

NO exerts profound cardioprotection in animal models of MI/R injury (21–23). The overexpression of eNOS decreases MI/R injury, whereas eNOS deficiency exacerbates MI/R injury (24 –26). Nitric oxide also inhibits mitochondrial respiration and prevents platelet aggregation and neutrophil adherence to vascular endothelium (27,28).

In the present study, we examined whether nebivolol or highly specific beta$_3$-AR agonists protect the heart via eNOS and NO-dependent signaling in an in vivo murine model of acute myocardial infarction.

Methods

An expanded Methods section is available in the Online Appendix.

Animals

Male C57BL6/J mice, 10 to 12 weeks of age (Jackson Labs, Bar Harbor, Maine), eNOS deficient (eNOS$^{-/-}$) mice (26) (10 to 12 weeks old), and beta$_3$ adrenergic receptor deficient (beta$_3$-AR$^{-/-}$) mice (12 to 14 weeks) were used (29,30). All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication No. 85-23, Revised 1996). Procedures were approved by the Emory University Institutional Animal Care and Use Committee.

Materials

Nebivolol was provided by the Forest Research Institute (Jersey City, New Jersey). The beta$_3$ receptor agonists CL 316243 and BRL-37344 were purchased from Sigma-Aldrich (St. Louis, Missouri). Nitro-L-arginine methyl ester (L-NAME) (hydrochloride) and the beta$_3$ AR antagonist L-748,337 were purchased from Cayman Chemical (Ann Arbor, Michigan).

MI/R protocol

Surgical ligation of the left coronary artery (LCA), myocardial INF determination, and troponin I measurements were performed as previously described (25,31).

Western blot analysis

Western blot analysis was performed as previously described (25).

NO metabolites

NO and NO metabolite analysis was performed as previously described (32).

Measurement of nebivolol

Liquid chromatography tandem mass spectrometry was used for determination of d/l-nebivolol in mouse plasma. See the Online Appendix for detailed methods.

Hemodynamic parameters

Mice were anesthetized with isoflurane (1.0 to 2.0 l/min) in 100% oxygen. The right carotid artery was exposed for a length of approximately 5 mm. A 1.0-F Millar pressure catheter (Millar Instruments, Houston, Texas) connected to the computerized data-acquisition system (PowerLab 4/30, AD Instruments, Colorado Springs, Colorado) was advanced into the aorta through the right carotid artery to record heart rate (HR), systolic, diastolic, and mean arterial pressures. These values were recorded with LabChart 6 PRO (AD Instruments).

Echocardiography

Baseline echocardiography images were obtained 1 week before LCA ischemia to avoid cardioprotective effects of the isoflurane, as previously described with slight modification. Mice were anesthetized with isoflurane, and transthoracic echocardiography of the left ventricle (LV) was performed with a 30-MHz RMV scanhead interfaced with a Vevo 2100 (Visualsonics, Toronto, Ontario, Canada) to obtain high-resolution M mode images at the rate of 1,000 frames/s. These were used to measure LV end-diastolic diameter, LV end-systolic diameter, and ejection fraction. Echocardiography images were obtained again 1 week after the MI/R protocol.

Statistical analysis

All data in this study are expressed as the mean ± SEM. Differences in data between the groups were compared with Prism 4 (GraphPad Software, La Jolla, California) with Student unpaired 2-tailed *t* test when 2 groups were compared or a 1-way analysis of variance, when 3 or more groups were compared. If a significant difference was found with the analysis of variance test, a Tukey's (Figs. 1 to 5, Online Fig. 2) or Dunnett's (Online Fig. 3) multiple comparison test was used for post hoc analysis. A p value <0.05 was considered significant.

Results

Nebivolol limits myocardial injury

We subjected male C57BL6/J mice to 45 min of LCA ischemia followed by reperfusion. Nebivolol (250 ng/kg to 50 μg/kg) or vehicle (VEH) was administered into the LV lumen at reperfusion (Fig. 1A). We evaluated myocardial INF at 24 h of reperfusion. Representative mid-ventricular cross-sections of VEH- and nebivolol-treated (500 ng/kg) mice are shown in Figure 1B. The area-at-risk (AAR)/LV was similar (p = NS) in all groups (Fig. 1C). Evaluation of INF revealed a dose-response curve, with 250 and 500 ng/kg displaying the most cytoprotection, as assessed by 2,3,5-triphenyltetrazolium chloride staining (Fig. 1C). Mice receiving 250 ng/kg displayed an INF/AAR of ($34.7 \pm 5.5\%$) compared with VEH ($55.3 \pm 2.8\%$), a 37% reduction. Mice receiving 500 ng/kg displayed an INF/AAR of ($35.2 \pm 4.7\%$), a 36% reduction. The dose of 500 ng/kg was used in all subsequent in vivo studies.

We evaluated circulating plasma levels of the cardiac-specific isoform of troponin-I as a marker of myocardial injury (Fig. 1D). The cardiac-specific isoform of troponin-I levels were significantly reduced in nebivolol-treated animals, containing 25.5 ± 3.8 ng/ml versus the VEH level of 40.9 ± 4.6 ng/ml.

Effects of nebivolol on hemodynamic status

We measured mean arterial blood pressure (MABP) and HR after administration of nebivolol to determine the hemodynamic effects (Table 1). The MABP and HR were measured at baseline and at increasing doses to 500 ng/kg nebivolol. There was no significant change in MABP or HR at 500 ng/kg versus baseline. The dose was then increased to 1 mg/kg, which significantly decreased MABP and slightly decreased HR.

Nebivolol activates NO synthase

We investigated the effects of acute nebivolol on expression and phosphorylation of eNOS at serine residue-1177 (eNOS-P^{Ser1177}) to determine eNOS activation (Fig. 2). Total eNOS expression remained constant (Figs. 2A and 2B). However, nebivolol significantly increased eNOS-P^{Ser1177} at 5 min after administration and returned to basal levels after 30 min (Figs. 2A and 2C).

We also evaluated neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) (Fig. 3). Interestingly, we observed a 1.7-fold increase in the expression of nNOS in the heart 15 min after treatment, which returned to baseline levels by 30 min (Figs. 3A and 3B). Cardiac levels of iNOS remained unchanged (Fig. 3C).

Nebivolol increases NO

We next investigated plasma and cardiac NO levels (Fig. 4). Mice treated with nebivolol displayed a 2.5-fold increase in plasma nitrite (an NO storage molecule) 30 min after intracardiac injection (Fig. 4A). Additionally, plasma total nitrosylated protein (RXNO) levels were significantly increased (p < 0.01) at 30 min and 2 h after administration (2.6-and 2.5-fold, respectively) (Fig. 4B). Cardiac nitrite levels significantly increased at 2 h (2.1-fold) (Fig. 4C). Cardiac RXNO levels did not show a significant difference after nebivolol administration (Fig. 4D). In contrast, cardiac NO-Heme levels significantly increased (4.5-fold) after 30 min (Fig. 4E).

Nebivolol protects through beta$_3$-AR and NO synthase

We investigated the effects of nebivolol therapy in eNOS$^{-/-}$ (Fig. 5A) and nNOS$^{-/-}$ mice (Fig. 5B). Myocardial INF was not reduced in eNOS$^{-/-}$ mice after administration of

nebivolol. Similar results were found in nNOS$^{-/-}$ mice. We also investigated nebivolol treatment in beta$_3$-AR$^{-/-}$ mice. Myocardial INF was not reduced in beta$_3$-AR$^{-/-}$ mice after administration of nebivolol (Fig. 5C).

Inhibition of NO synthase and beta$_3$-AR abolishes nebivolol-mediated cardioprotection

The L-NAME inhibits the 3 nitric oxide synthase (NOS) isoforms. Therefore, we evaluated myocardial INF in wild-type mice treated with both L-NAME (25 mg/ml) and nebivolol (Fig. 6A). When L-NAME was co-administered, the cardioprotective effects of nebivolol were lost. Subsequently, we studied nebivolol co-administered with a specific beta$_3$-AR antagonist, L-748,337 (33). When L-748,337 (100 μg/kg) was co-administered, the cardioprotective effects of nebivolol were also lost (Fig. 6B).

Beta$_3$-AR specific agonists limit injury after MI/R

We investigated 2 beta$_3$-AR specific agonists, CL 316243 (34) and BRL-37344 (35), to confirm the effects of beta$_3$-AR stimulation on MI/R injury (Fig. 5D). Mice receiving 1 μg/kg of CL 316243 displayed an INF/AAR of (35.0 $\pm$ 3.3%) compared with VEH (59.0 $\pm$ 1.8%), a 39% reduction. Mice receiving 1 μg/kg of BRL-37344 displayed an INF/AAR of (33.1 $\pm$ 4.7%), a 42% reduction.

CL 316243 mediates NOS activation

We also evaluated the effect of CL 316243 on the expression and phosphorylation levels of eNOS-P^{Ser1177} in sham-operated mice. Total eNOS expression remained constant (Online Fig. 2A). However, 2 h after CL 316243 administration, eNOS-P^{Ser1177} increased significantly (Online Fig. 2A), whereas eNOS-P^{Thr495} remained constant (Online Fig. 2A). The expression of nNOS and iNOS were also measured. There was a significant increase in nNOS protein expression after 15 min of CL 316243 administration (Online Fig. 2B). The expression levels of iNOS were not significantly different (Online Fig. 2B). We also measured plasma and cardiac nitrite and RXNO levels and NO-Heme levels (Online Fig. 3). We observed an apparent increase in plasma nitrite levels and a significant increase in cardiac nitrite levels after CL 316243 injection that persisted to 2 h (1.8-fold in the heart) (Online Figs. 3A and 3C). The RXNO values also exhibited a similar trend with a 3.1-fold increase in plasma RXNO after 2 h, when compared with VEH (Online Fig. 3B). Heart RXNO and NO-Heme values also increased significantly after 30 min, showing a 1.9- and 3.6-fold increase, respectively.

Circulating levels of nebivolol

We measured the circulating levels of nebivolol at different time points (1, 5, and 15 min) after intracardiac injection, to evaluate the pharmacokinetics. Nebivolol was administered to 3 different groups of mice (n = 3), and blood samples were taken to isolate plasma. The concentration of nebivolol was 1.2 $\pm$ 0.29 ng/ml after 1 min, then 0.4 $\pm$ 0.1 ng/ml after 5 min, and below detection after 15 min.

Nebivolol and LV function

We measured LV function and cardiac dimensions of mice treated with nebivolol and VEH (Table 2). Wild-type mice were subjected to 45-min myocardial ischemia and received nebivolol or VEH at reperfusion. No significant difference was observed in the ejection fraction, LV end diastolic diameter, LV end systolic diameter, and fractional shortening.

Discussion

The effects of $beta_1$- and $beta_2$-ARs are well-established in mammals, because their stimulation mediates an increase in HR, myocardial contraction force, and acceleration of relaxation (36). The $beta_3$-AR has been shown to produce a negative inotropic effect that antagonizes the activity of $beta_1$- and $beta_2$-ARs (37,38). The $beta_3$-AR is activated at higher concentrations of catecholamines than required for $beta_1$- and $beta_2$-ARs and is thought to act as a counter-mechanism during sympathetic overstimulation (39). The $beta_3$-AR–mediated signaling also results in NO generation from eNOS (40).

Previous studies suggest that $beta_3$-AR attenuates obesity (41). The $beta_3$-AR agonists such as BRL 37344 (34) and CL 316243 (35) were developed as a treatment for obesity but failed in clinical trials. The involvement of the $beta_3$-AR in cardiac function is primarily explained by the activation of eNOS, but the precise signaling mechanism has yet to be elucidated.

We investigated the cardioprotective actions of nebivolol and 2 other $beta_3$-AR specific agonists (i.e., BRL 37344 and CL 316243) in the setting of a murine model of acute MI/R injury. Here we show that nebivolol treatment results in significant cardioprotection in the setting of MI/R via the stimulation of the $beta_3$-AR. Nebivolol stimulates NO production in endothelial cells by activation of the $beta_3$-AR (17,33). Both exogenous NO therapy and actions that promote endogenous NO production from eNOS protect the ischemic myocardium (10,42). We have demonstrated that activation of the $beta_3$-AR in the heart by nebivolol, CL 316243, and BRL-37344 significantly reduces INF/AAR through an NO-dependent mechanism.

Several reports show inconsistencies with potency and specificity of $beta_3$-AR agonists (43–45); however, we provide evidence with multiple agonists that supports $beta_3$-AR stimulation as a novel approach to address cardiovascular disease. We also show that L-NAME, a known NOS inhibitor, abolishes the cardioprotective actions of nebivolol treatment in MI/R. Previous evidence suggests L-748,337, a specific $beta_3$-AR antagonist, is effective in reducing the effects of nebivolol (33). We also observed this in our MI/R studies.

Our study provides evidence that stimulation of the $beta_3$-AR acts as a dual activator of eNOS and nNOS. Some studies have shown that the $beta_3$-AR modulates nNOS or iNOS (46,47), whereas the role of eNOS has been confirmed (40,48). We show a rapid increase in the activation of eNOS and the expression of nNOS after administration of nebivolol or CL 316243. We also report the absence of cardioprotection by nebivolol in eNOS knockout mice, nNOS knockout mice, and $beta_3$-AR knockout mice.

Nebivolol has been shown to provide beneficial effects on LV dysfunction after myocardial infarction (49). In the present study we failed to demonstrate significant improvements in LV function at 7 days after reperfusion, which is in contrast to the Sorrentino et al. (49) study. There are, however, clear differences in methodology, because our experiments are based on a single dose of nebivolol (500 ng/kg) before reperfusion as opposed to treatment of 10 mg/kg/day for 30 days (49).

Nitric oxide is well-established as a trigger and mediator of cardioprotection and is involved in many protective signaling cascades (22,23). Activation of the $beta_3$-AR likely stimulates many cytoprotective signaling cascades, which ultimately result in the cardioprotection we have observed. Our findings demonstrate that a single dose of nebivolol confers significant cardioprotection against MI/R injury in mice when administered at the time of reperfusion. We show for the first time that a $beta_1$-blocker/$beta_3$-AR agonist and a specific $beta_3$-AR agonist attenuates myocardial INF by an NO-mediated mechanism involving both eNOS and

nNOS. We also demonstrate that the beta$_1$-blocker properties of nebivolol do not seem to be part of this cardioprotection, because hemodynamic measurements at the doses investigated in this study did not affect MABP or HR. At higher doses (twice that used in our experiments) we did observe a significant decrease in MABP but did not observe a reflex increase in HR. This might be because NO can attenuate the baroreflex HR increase (50,51). We also present data that acute administration just before reperfusion "preconditions" the heart against subsequent myocardial infarction (Online Fig. 1), whereas treatment with nebiviolol 24 h before reperfusion fails to protect. These data suggest that nebivolol can promote an "early" preconditioning state.

Taken together, our results indicate that an acute administration of nebivolol might have practical clinical use after myocardial ischemia. Future research will be aimed at further elucidation of the signaling mechanisms responsible for these cardioprotective actions. The involvement of nNOS as a redundant and additive signal for NO generation after acute administration of beta$_3$-agonists also requires additional investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations and Acronyms

AAR	area-at-risk
AR	adrenergic receptor
eNOS	endothelial nitric oxide synthase
eNOS-P^{Ser1177}	phosphorylation of endothelial nitric oxide synthase at serine residue 1177
INF	infarct size
iNOS	inducible nitric oxide synthase
HR	heart rate
LCA	left coronary artery
L-NAME	nitro-L-arginine methyl ester
LV	left ventricle/ventricular
MABP	mean arterial blood pressure
MI/R	myocardial ischemia-reperfusion
nNOS	neuronal nitric oxide synthase

NOS	nitric oxide synthase
RXNO	total nitrosylated proteins
VEH	vehicle

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APPENDIX

For supplementary figures and text, please see the online version of this article.
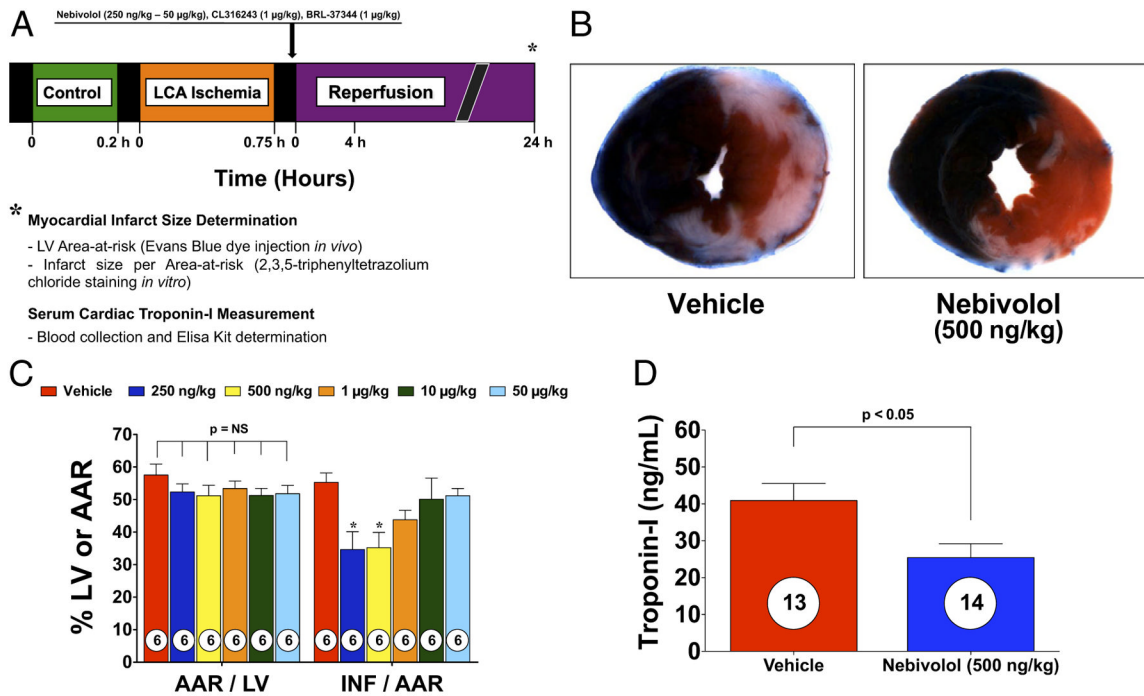
Figure 1. Nebivolol Reduces Myocardial INF and Troponin-I
(A) Experimental protocol for this study. Mice were subjected to 45 min of left coronary artery (LCA) occlusion followed by 24 h of reperfusion. Myocardial area-at-risk (AAR) and myocardial infarct size (INF) were determined at 24 h of reperfusion. In addition, blood was collected at 24 h of reperfusion, and troponin-I was measured. Nebivolol or saline were injected directly into the left ventricular (LV) cavity just before reperfusion. **(B)** Representative mid-ventricular photomicrographs of mouse hearts after 45 min of myocardial ischemia and 24 h of reperfusion treated with vehicle (VEH) or nebivolol at the time of reperfusion. **(C)** Bar graph of myocardial AAR/LV and INF/AAR in mice treated with nebivolol. **(D)** Troponin levels in VEH and nebivolol-treated mice at 24 h after reperfusion. Nebivolol (500 ng/kg) administered at the time of reperfusion decreased serum troponin I levels, compared with VEH. Values are mean ± SEM. **Numbers inside bars** indicate the number of animals investigated in each group. *p < 0.05 versus VEH.
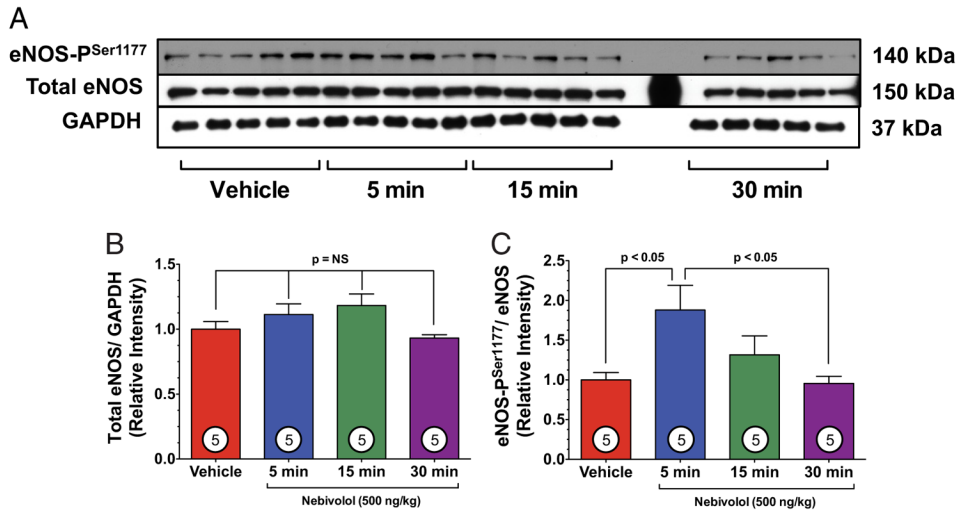
**Figure 2. Nebivolol Increases eNOS-P^{Ser1177}**

(A) Representative immunoblots of phosphorylation of endothelial nitric oxide synthase at serine residue 1177 (eNOS-P^{Ser1177}) and total endothelial nitric oxide synthase (eNOS) from the left ventricle of sham-operated mice receiving either vehicle (VEH) or nebivolol (500 ng/kg) via intracardiac injection at various time points. **(B)** Relative intensity of total eNOS remained constant compared with VEH. **(C)** Nebivolol increased the eNOS-P^{Ser1177} at 15 min, compared with VEH ($p < 0.05$) and compared with 30 min ($p < 0.05$). **Numbers inside bars** indicate the number of animals studied in each group. GAPDH = glyceraldehyde-3-phosphate dehydrogenase.
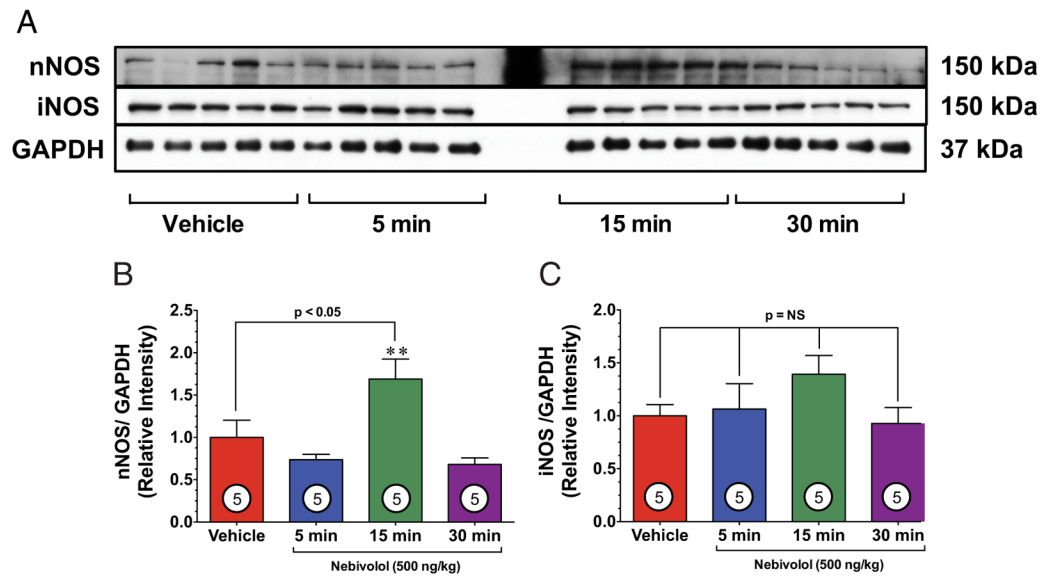
Figure 3. Nebivolol Increases nNOS Expression
(A) Representative immunoblots of neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) from the left ventricle of sham-operated mice receiving either VEH or nebivolol (500 ng/kg) via intracardiac injection at various time points. **(B)** Relative intensity of total nNOS increases after 15 min, compared with VEH ($p < 0.05$). **$p < 0.01$ versus 5 min and 30 min time points. **(C)** Relative intensity of total iNOS remained constant compared with VEH. **Numbers inside bars** indicate the number of animals studied in each group. Abbreviations as in Figure 2.
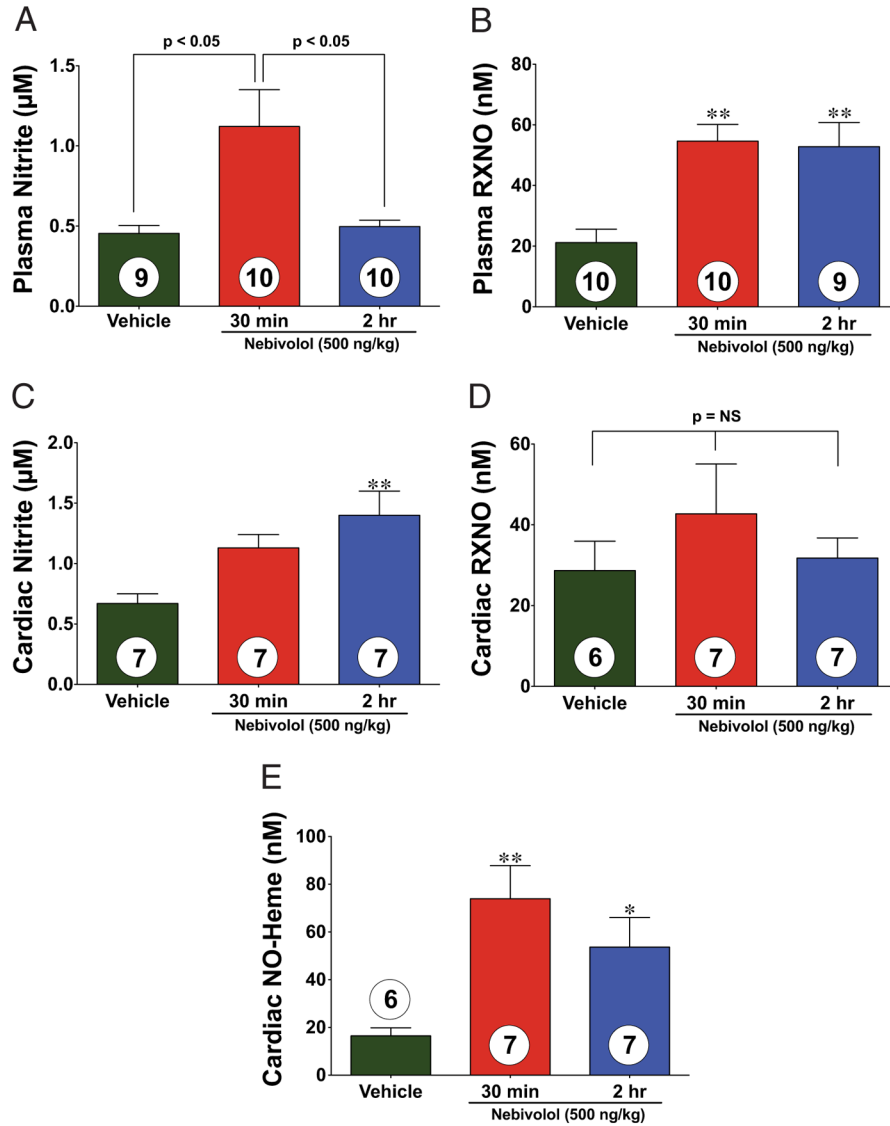
Figure 4. Nebivolol Increases NO Metabolites

Plasma and cardiac nitric oxide (NO) metabolites (nitrite and plasma total nitrosylated protein [RXNO]) measured after intracardiac injection of either vehicle (VEH) or nebivolol in sham-operated control mice. **(A)** Nitrite levels (μmol/l) increased in the plasma of nebivolol-treated mice. **(B)** The RXNO levels (nmol/l) increased after 30 min of nebivolol administration and remained elevated after 2 h. **(C)** Nitrite levels (μmol/l) increased in the heart of nebivolol-treated mice. **(D)** The RXNO levels in the heart did not significantly increase after 30 min of nebivolol administration. **(E)** The NO-Heme levels (nmol/l) significantly increased after 30 min of nebivolol administration and slightly decreased after 2 h. Values are mean ± SEM. *p < 0.05; **p < 0.01; **numbers inside bars** indicate the number of animals/group.
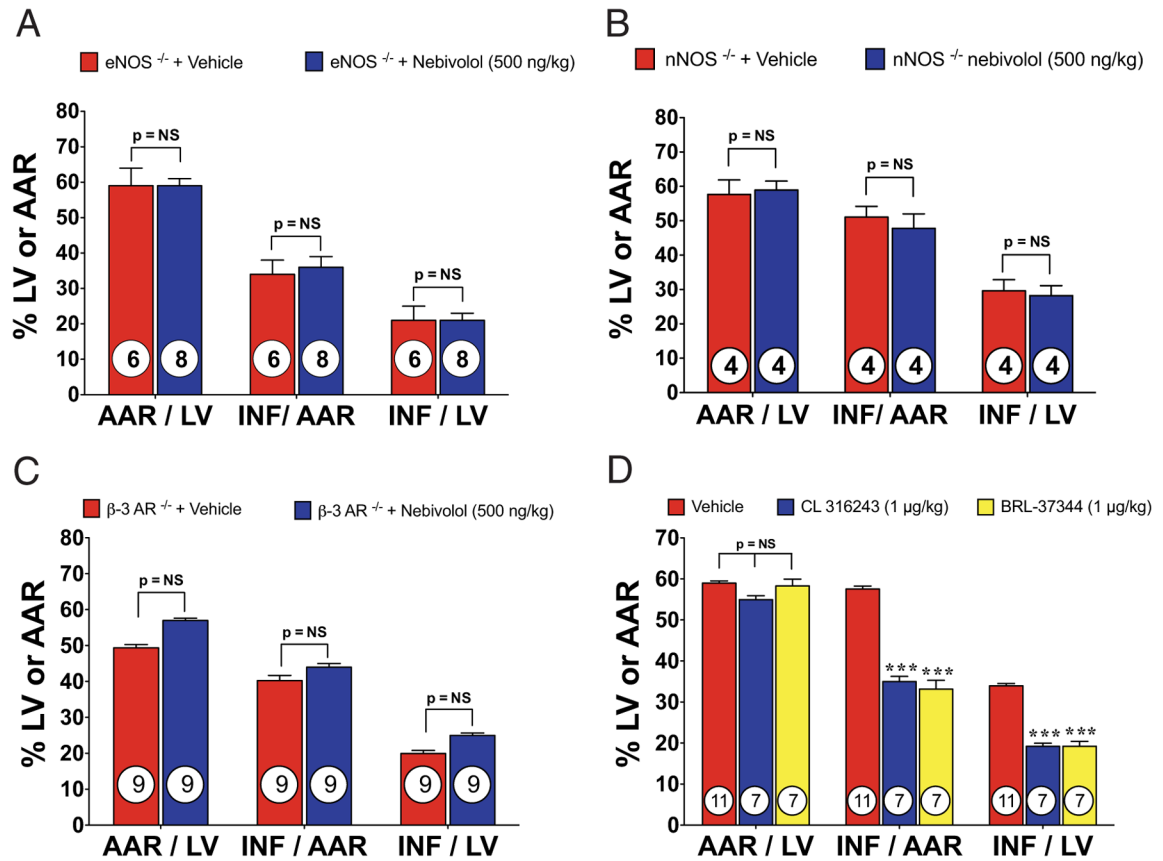
Figure 5. Nebivolol-Mediated Cardioprotection Is Lost in Pathway-Specific KO Mice
(**A**) Myocardial INF in eNOS$^{-/-}$ (eNOS knockout [KO]) mice treated with nebivolol (500 ng/kg). (**B**) Myocardial INF in nNOS$^{-/-}$ mice treated with nebivolol (500 ng/kg) at reperfusion. Bar graph of myocardial AAR/LV and INF/AAR in mice treated with nebivolol. (**C**) Myocardial INF in beta$_3$-AR$^{-/-}$ mice treated with nebivolol. Treatment with nebivolol (500 ng/kg) at the time of reperfusion failed to reduce INF/AAR or INF/LV. (**D**) Myocardial INF in mice treated with CL 316243 (1 μg/kg) or BRL-37344 (1 μg/kg). No significant differences were observed with respect to the AAR/LV. Both CL 316243 and BRL 37344 significantly reduced myocardial INF/AAR and/LV. Values are mean ± SEM. ***p < 0.001; **numbers inside bars** indicate the number of animals/group. Abbreviations as in Figures 1, 2, and 3.
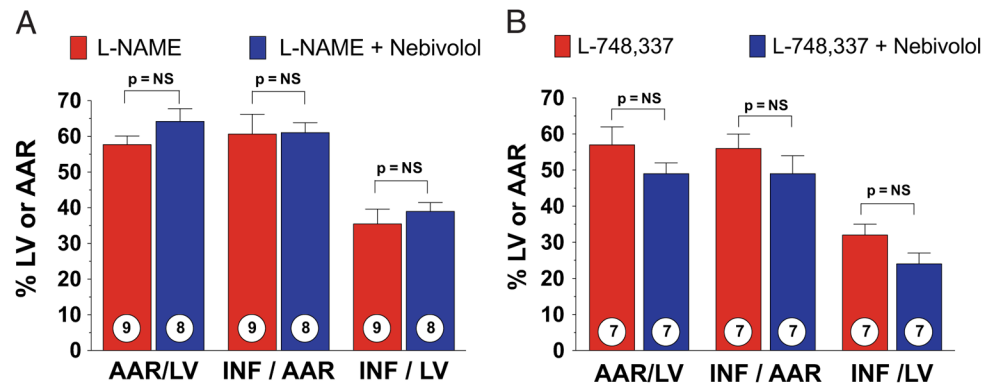
Figure 6. Nebivolol-Mediated Cardioprotection Is Lost With Pathway-Specific Pharmacological Inhibition

(A) Myocardial INF in wild-type mice treated with nitro-L-arginine methyl ester (L-NAME) (25 mg/kg) and mice treated with L-NAME (25 mg/kg) and nebivolol (500 ng/kg) at reperfusion. **(B)** Myocardial INF in wild-type mice treated with L-748,337 (100 μg/kg) and mice treated with L-748,337 (100 μg/kg) and nebivolol (500 ng/kg). Values are mean ± SEM. **Numbers inside bars** indicate the number of animals/group. Abbreviations as in Figure 1.

Table 1

Hemodynamic Effects of Nebivolol on Anesthetized Mice

Group (n = 4)	Mean Arterial Blood Pressure (mm Hg)	Heart Rate (beats/min)
Baseline	78.03 ± 0.70	492 ± 22
500 ng/kg	76.56 ± 0.49	495 ± 25
50 μg/kg	72.22 ± 0.61	489 ± 18
500 μg/kg	72.70 ± 1.19	477 ± 16
1 mg/kg	67.63 ± 3.66[*]	459 ± 4

Values are mean ± SE.

[*] $p < 0.01$ versus baseline.

Table 2

Effects of Nebivolol on Left Ventricular Function and Dimensions

	Baseline		7-Day Post-MI/R	
	VEH (n = 10)	Nebivolol (n = 9)	VEH (n = 10)	Nebivolol (n = 9)
EF (%)	66.0 ± 1.87	64.4 ± 2.43	41.7 ± 3.49	41.3 ± 4.11
LVEDD (mm)	3.4 ± 0.04	3.5 ± 0.06	4.0 ± 0.14	4.1 ± 0.14
LVESD (mm)	2.2 ± 0.06	2.3 ± 0.08	3.1 ± 0.16	3.3 ± 0.20
FS	35.6 ± 1.39	34.6 ± 1.92	20.3 ± 1.89	20.3 ± 2.28

Values are mean ± SE. p = NS (nebivolol vs. vehicle [VEH]).

EF = ejection fraction; FS = fractional shortening; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; MI/R = myocardial ischemia-reperfusion.