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PRE-CLINICAL RESEARCH

Cardioprotective Effect of Beta-3 Adrenergic Receptor Agonism

Role of Neuronal Nitric Oxide Synthase

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Objectives The aim of this study was to determine whether activation of β3-adrenergic receptor (AR) and downstream signaling of nitric oxide synthase (NOS) isoforms protects the heart from failure and hypertrophy induced by pressure overload.

Background β3-AR and its downstream signaling pathways are recognized as novel modulators of heart function. Unlike β1- and β2-ARs, β3-ARs are stimulated at high catecholamine concentrations and induce negative inotropic effects, serving as a "brake" to protect the heart from catecholamine overstimulation.

Methods C57BL/6J and neuronal NOS (nNOS) knockout mice were assigned to receive transverse aortic constriction (TAC), BRL37344 (β3 agonist, BRL 0.1 mg/kg/h), or both.

Results Three weeks of BRL treatment in wild-type mice attenuated left ventricular dilation and systolic dysfunction, and partially reduced cardiac hypertrophy induced by TAC. This effect was associated with increased nitric oxide production and superoxide suppression. TAC decreased endothelial NOS (eNOS) dimerization, indicating eNOS uncoupling, which was not reversed by BRL treatment. However, nNOS protein expression was up-regulated 2-fold by BRL, and the suppressive effect of BRL on superoxide generation was abrogated by acute nNOS inhibition. Furthermore, BRL cardioprotective effects were actually detrimental in $nNOS^{-/-}$ mice.

Conclusions These results are the first to show in vivo cardioprotective effects of β3-AR–specific agonism in pressure overload hypertrophy and heart failure, and support nNOS as the primary downstream NOS isoform in maintaining NO and reactive oxygen species balance in the failing heart. (J Am Coll Cardiol 2012;59:1979–87) © 2012 by the American College of Cardiology Foundation

There is accumulating evidence that β3-adrenergic receptors (β3-AR) play an important role in the modulation of cardiovascular function in heart failure (1–3). In contrast to the well-characterized β1/β2-AR, stimulation of β3-AR induces a negative inotropic effect that is associated with nitric oxide (NO) release via nitric oxide synthase (NOS) activation (4). Chronic heart failure is associated with sustained overactivation of the sympathetic nervous system, which initially plays a compensatory role for depressed contractility, but worsens heart function over time. The positive inotropic response to β-AR stimulation is diminished during heart failure due to selective down-regulation and desensitization of β1/β2-AR (5). Conversely, β3-AR is up-regulated in failing hearts in both human and animal models (6–8). Whether this increase is a protective response to catecholamine overexpression or a contributor to heart failure has been controversial; however, increasing evidence suggests it functions as a "physiological brake" to

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Abbreviations and Acronyms

AR = adrenergic receptor

BRL = ((±)-(R*,R*)-[4-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl] phenoxy]acetic acid sodium

eNOS = endothelial nitric oxide synthase

EPR = electron paramagnetic resonance

LV = left ventricular

nNOS = neuronal nitric oxide synthase

NO = nitric oxide

NOS = nitric oxide synthase

ROS = reactive oxygen species

TAC = transverse aortic constriction

WT = wild-type

XOR = xanthine oxidoreductase

reduce the effects of sympathetic overstimulation. Belge et al. (9) showed that mice with cardiomyocyte-specific overexpression of β3-AR had attenuated left ventricular (LV) hypertrophy compared with wild-type (WT) mice in response to chronic isoprenaline administration. Our recent work revealed exacerbated pathological remodeling and impaired cardiac functional compensation in mice lacking β3-AR (2,10). These studies support the idea that β3-AR serves a chiefly protective role in maladaptive remodeling and in the development of heart failure.

It is well established that the negative inotropic effect of β3-AR results from the downstream production of NO by NOS signaling (4,11). The effects of a β3 agonist, BRL 37344 (BRL), were inhibited by both NO and NOS inhibitors and could be reversed by an excess of the substrate for NO production, L-arginine. Several studies have suggested that endothelial NOS (eNOS) is solely responsible for β3-induced negative inotropy (4,11–13). However, the relative involvement of neuronal NOS (nNOS), which is also constitutively expressed in cardiomyocytes, still remains unclear. Alterations of NOS-dependent regulation on cardiac function make this issue more complex. There is an apparent paradox between the observation that β3-AR stimulation induces an NO-dependent negative inotropic effect on failing ventricular myocardium despite the finding that eNOS-derived NO is decreased in this setting (14). These inconsistent findings may be reconciled by recent evidence indicating that nNOS-derived NO production was involved in altered contractile response by β3-AR stimulation in both diabetic and senescent hearts (15,16).

In the present study, we hypothesized that the β3-AR preferential agonist BRL could reduce cardiac remodeling induced by pressure overload and preserve cardiac function through NO production, which may be largely attributable to activation of nNOS.

Methods

Experimental model. Thirty-eight C57BL/6J and 12 *nNOS*$^{-/-}$/B6129S male mice (9 to 10 weeks old; Jackson Laboratory, Bar Harbor, Maine) were arbitrarily divided into 3 groups. Two-thirds of the mice underwent transverse aortic constriction (TAC) to induce cardiac hypertrophy and heart failure via pressure overload (17), while the remaining one-third had a sham procedure (Online Appendix).

Cardiac function and geometry. In vivo cardiac geometry and function were serially assessed by transthoracic echocardiography (Acuson Sequoia C256, 13 MHz transducer, Siemens, Oceanside, California) in conscious mice at baseline, 1 week, and 3 weeks.

Histological evaluation and cellular morphometry. Myocardium was fixed in 10% formalin, processed by standard paraffin embedding, and serially sectioned at 5 to 8 μm thickness for hematoxylin and eosin and trichrome staining.

Measurement of cardiac NO production. Cardiac NO production was determined as the measurement of nitrate plus nitrite using the Griess reaction assay (Cayman Chemical, Ann Arbor, Michigan) as previously described (18).

Measurement of cardiac superoxide generation. LUCIGENIN-ENHANCED CHEMILUMINESCENCE. Fresh-frozen myocardium was homogenized in 20 mol/l HEPES buffer and diluted in lucigenin buffer. Superoxide-generated signals were recorded as counts per minute (CPM) by a liquid scintillation counter (LS6000IC, Beckman Instruments, Fullerton, California).

ELECTRON PARAMAGNETIC RESONANCE. Fresh-frozen myocardium was homogenized in 1× phosphate buffer saline (PBS) containing 0.1 mM diethylenetriaminepenta-acetic acid (DTPA) (Sigma Aldrich, St. Louis, Missouri). Samples were further diluted in 1× PBS with 1 mM 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethyl pyrrolidine (CMH) (Enzo Life Sciences, Farmingdale, New York) and assayed for superoxide signals by Bruker E-Scan (Billerica, Massachusetts) electron paramagnetic resonance (EPR) spectrometer.

Western blot analysis. Snap-frozen LV tissue was homogenized in cell lysis buffer (Cell Signaling Technology, Danvers, Massachusetts) and subjected to Western blot analysis with antibodies targeting the protein of interest. The densitometric volume of digitalized bands was evaluated by the Image J program version 1.44p (National Institutes of Health, Bethesda, Maryland).

Statistical analysis. All data are expressed as mean ± standard error of the mean. Echocardiographic data were compared using repeated measures analysis of variance. Group data were compared using 1-way analysis of variance with Tukey's post hoc test for multiple comparisons. Student *t* test with Welsh correction for multiple comparisons was used to compare the difference between BRL and vehicle-treated *nNOS*$^{-/-}$ mice. All p values <0.05 were considered to be statistically significant. GraphPad Prism 5.0 (La Jolla, California) was used for statistical analysis.

Results

β3-AR stimulation prevents deterioration of cardiac function. Mice developed increased LV chamber dilation and systolic dysfunction after 3 weeks of TAC (Fig. 1A), as evidenced by an 82% increase in LV end-systolic diameter and

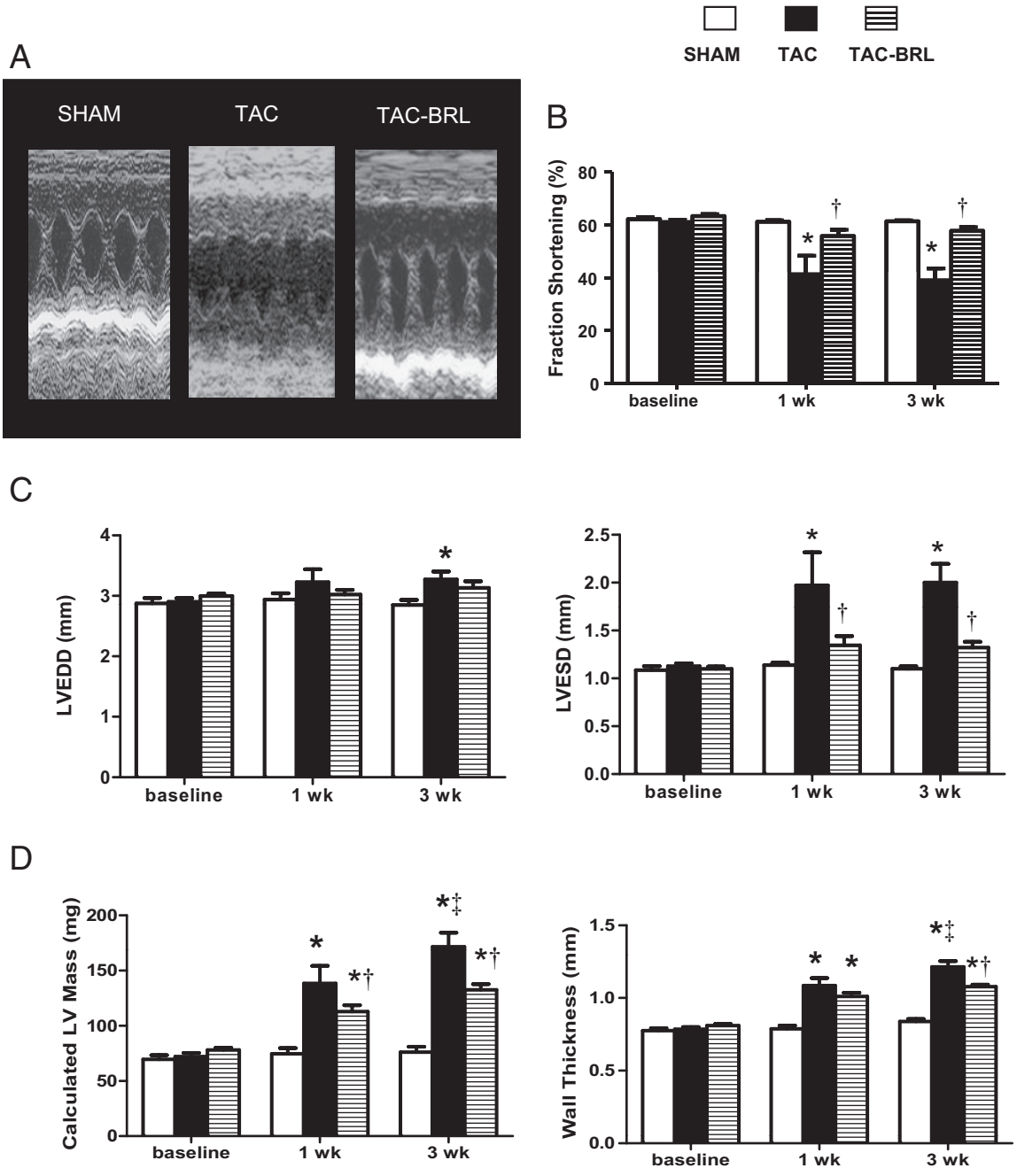

Figure 1 **Effect of BRL on LV Dilation and LV Systolic Function in TAC Mice**

(A) M-mode echocardiograms demonstrating increased left ventricular (LV) dilation and wall thickness and decreased systolic function after 3 weeks of transverse aortic constriction (TAC), which were abrogated by BRL treatment. **(B)** BRL prevented cardiac systolic dysfunction induced by TAC. **(C)** BRL reduced LV chamber dilation. **(D)** TAC causes LV hypertrophy, which was partially prevented by BRL treatment. *$p < 0.05$ vs. sham. †$p < 0.05$ vs. TAC. ‡$p < 0.05$ vs. corresponding 1-week time point. LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter.

a 36% reduction in fractional shortening (FS%) compared with sham mice by echocardiography (Figs. 1B and 1C). Calculated LV mass and average wall thickness were increased versus sham (Fig. 1D). Three weeks of BRL treatment totally prevented LV dilation, and cardiac systolic function remained normal. Calculated LV mass and average wall thickness were significantly lower in BRL-treated mice compared with vehicle-treated mice. We also treated sham mice with BRL, which had no significant effect (data not shown).

***β*3-AR stimulation reduces development of cardiac hypertrophy.** Three weeks of TAC resulted in increased cardiac hypertrophy versus sham, with a 44% higher heart weight to

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body weight ratio. BRL-treated mice developed less hypertrophy (Online Fig. 1A). These findings were paralleled by similar changes in calculated LV mass by echocardiography (Fig. 1D). Both cardiomyocyte width by hematoxylin and eosin staining and fibrosis scale (0 to 3 scale: 0 = none, 3 = severe) by trichrome staining were significantly greater in TAC versus sham. Interestingly, BRL reduced cardiomyocyte width but had no effect on fibrosis scale (Online Figs. 1B and 1C).

β3-AR stimulation increases cardiac NO production and decreases reactive oxygen species production in pressure-overloaded mice. β3-AR–induced negative inotropic effects are thought to be associated with NO release via NOS (4). Our previous data showed that mice lacking β3-AR had lower NOS activity and generated more cardiac superoxide than WT mice after pressure overload (2). We, therefore, tested NO production by measuring the sum concentrations of the NO metabolites (nitrate and nitrite) using the Griess assay and superoxide generation by lucigenin-enhanced chemiluminescence and EPR to observe the effect of β3-AR agonism on NO and reactive oxygen species (ROS) production. Total nitrate + nitrite concentrations were decreased by 50% (Fig. 2A) and superoxide was increased ~3.5-fold (Fig. 2B) in TAC hearts over sham controls. Three weeks of BRL treatment completely prevented this decrease in NO metabolite concentration and partially inhibited superoxide generation. Superoxide studies were confirmed using EPR, which showed a 1.5-fold increase in ROS generation (Fig. 2C) and a downward trend in ROS production with BRL administration.

β3-AR stimulation regulates nNOS protein expression and superoxide generation. Recent studies demonstrated that nNOS-derived NO production was involved in altered contractile response by β3-AR stimulation in both diabetic and senescent hearts (15,16). We observed a 2-fold increase in nNOS protein expression in BRL-treated compared with vehicle-treated hearts (Fig. 3A), although there was no difference between sham and untreated TAC. More interestingly, pre-treating LV homogenates with 100 nM of the specific nNOS inhibitor vinyl-L-NIO (L-VNIO) completely abolished BRL suppression of superoxide generation (Fig. 3B). This suggests that β3-AR reduces superoxide generation via an nNOS-dependent mechanism in the failing heart.

BRL modulation of eNOS and inducible NOS activation. To investigate further the role of BRL on other NOS isoforms, we examined eNOS protein expression and phosphorylation. We focused on 3 enzyme phosphorylation sites that have been shown to modulate eNOS activity in the myocardium (19): $eNOS^{Ser1177}$, $eNOS^{Ser114}$, and $eNOS^{Thr495}$. After 3 weeks of TAC+BRL treatment, $eNOS^{Ser1177}$ phosphorylation, which indicates eNOS activation, was decreased by 50% as compared with TAC alone. In contrast, phosphorylation of $eNOS^{Ser114}$, which is an indication of eNOS deactivation, was increased 100% in BRL-treated mice, whereas levels were similar between sham and untreated TAC (Fig. 4A). Total eNOS protein expression (Fig. 4A) and $eNOS^{Thr495}$ phosphorylation were unchanged between groups (Online Fig. 2A). In addition, no difference in inducible NOS expression was observed between groups (Online Fig. 2B).

eNOS monomer-to-dimer ratio is an indication of eNOS uncoupling, which results in NO generation switching to

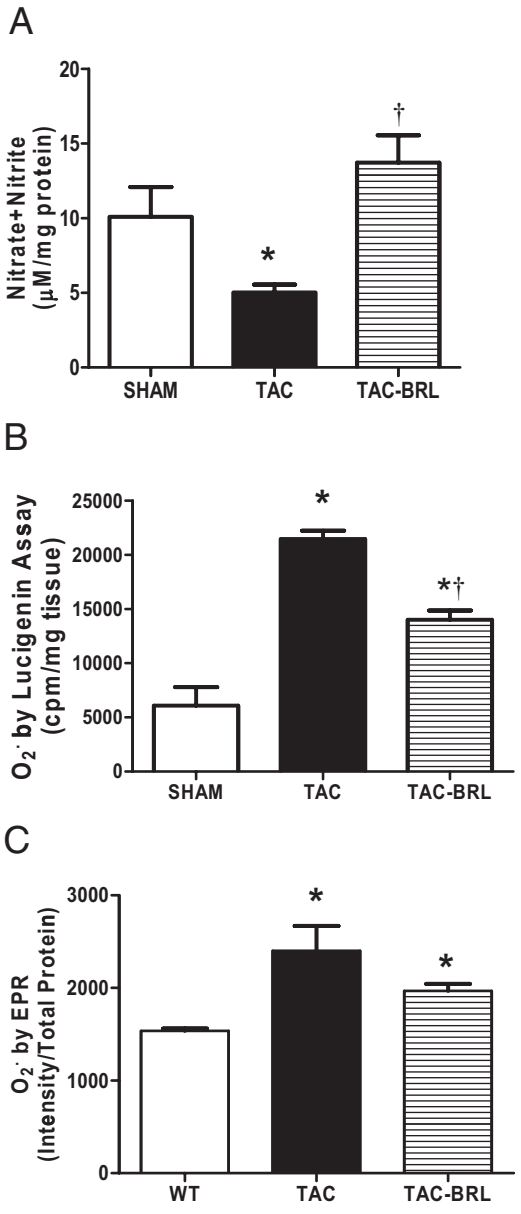

Figure 2 **Changes in NO Production and Superoxide Generation by BRL Treatment**

(A) Nitric oxide (NO) production, measured by Griess assay, was decreased by 50% after 3 weeks of transverse aortic constriction (TAC), but preserved at normal levels in the presence of BRL. **(B)** Lucigenin-enhanced chemiluminescence detected 3.5-fold increase in superoxide in 3-week TAC mice, which was significantly reduced BRL administration. **(C)** Electron paramagnetic resonance (EPR) detected a 1.5-fold increase in superoxide in TAC animals and downward trend in TAC+BRL. *$p < 0.05$ vs. sham. †$p < 0.01$ vs. TAC. WT = wild type.

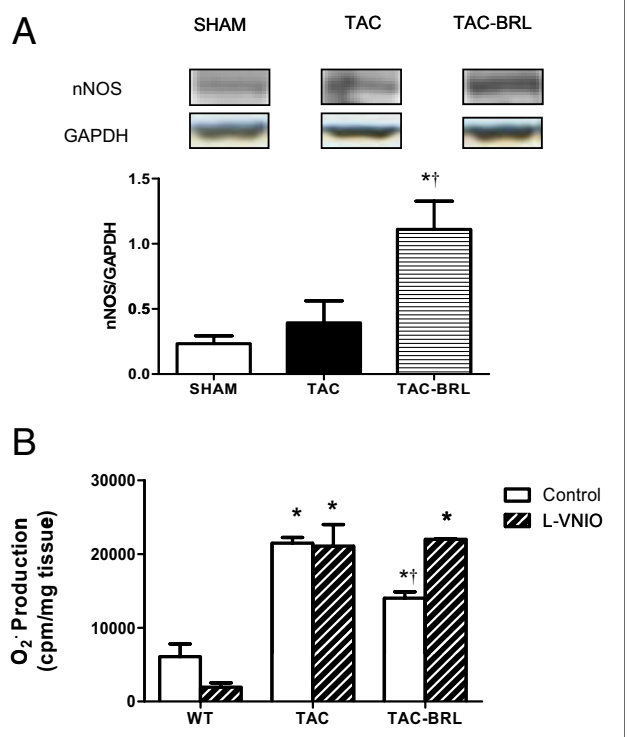

Figure 3 **Effect of BRL on nNOS Protein Expression and the Superoxide Generation by Acute nNOS Inhibition**

(A) Neuronal nitric oxide synthase (nNOS) protein expression was significantly up-regulated by BRL treatment, though similar between sham and transverse aortic constriction (TAC). **(B)** Acute nNOS inhibition **(striped bars)** abrogated BRL-induced superoxide suppression in the presence of nNOS-specific inhibitor L-VNIO, (100 uM, 30 min in cold room), as compared with non–L-VNIO treated samples **(open bars)**. *$p < 0.01$ vs. sham. †$p < 0.05$ vs. TAC. GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

superoxide generation. Our data show that 3 weeks of TAC resulted in increased eNOS uncoupling, which is consistent with previous reports (10,17,20), although 3 weeks of BRL treatment did not change the monomer-to-dimer ratio (Fig. 4B).

Lack of *nNOS* gene abolished the protective effect of β3-AR stimulation. Because nNOS protein expression was up-regulated by BRL treatment, and BRL-induced superoxide suppression was attenuated by acute nNOS inhibition, we hypothesized that nNOS is pivotal in β3-AR–induced cardioprotection. To confirm this hypothesis, we used the 3-week TAC model in the absence or presence of BRL in mice genetically lacking nNOS. After 1 week, TAC mice developed LV systolic dysfunction (Fig. 5B) and chamber dilation (Fig. 5C) as compared with sham $nNOS^{-/-}$ mice. BRL treatment not only failed to restore LV function and dilation to baseline levels after 3 weeks of pressure overload, but also exacerbated dysfunction (Fig. 5B) and LV dilation (Fig. 5C). LV mass (Fig. 5D) was 33% greater in TAC $nNOS^{-/-}$ mice as compared with sham $nNOS^{-/-}$, and worse in TAC+BRL mice (Fig. 5D) as compared with TAC-alone mice. Wall thickness was also increased in TAC $nNOS^{-/-}$ mice as compared with sham (Fig. 5D). There was no difference in wall thickness among BRL+TAC and TAC animals (Fig. 5D).

Discussion

The novel findings in this study are that β3-AR activation prevents cardiac dysfunction through generating NO and reducing ROS via an nNOS-dependent mechanism in the failing heart.

Induction of cardioprotection by β3-AR. Despite a low level of myocardial expression and physiological insignificance under resting conditions (21), accumulating literature supports a significant role for β3-AR up-regulation in the modulation of cardiac remodeling in heart failure. Until now, direct evidence in vivo has been lacking. We have previously compared the cardiac response to pressure overload in both WT and $β3^{-/-}$ mice, displaying greater hypertrophy and cardiac systolic dysfunction in $β3^{-/-}$ mice as compared with WT controls (2). More recently, Aragón et al. demonstrated that select β3-AR agonists, including the β1-blocker nebivolol, reduced cardiac infarct size in mice subjected to myocardial ischemia and reperfusion in WT, but were completely ineffective in β3-AR–deficient mice (22). In the current study, we confirm that β3-AR agonism exerts a cardioprotective role in the failing heart (6,15,22,23). We observed that administering the β3-AR–specific agonist BRL to C57BL/6 mice for 3 weeks totally prevented the deterioration of LV chamber dilation and cardiac dysfunction, and partially inhibited myocardial hypertrophy induced by chronic pressure overload. This strongly suggests that specific β3-AR agonism may constitute an interesting and novel approach in treating cardiac hypertrophy and heart failure.

Primary role of nNOS in β3-AR cardioprotection. Earlier studies have suggested that the β3-AR–induced cardiac signaling is associated with NO release via eNOS (4,11). Chronic β3-AR stimulation in our model prevented the expected decrease in NO production during pressure overload, which confirmed our previous findings that NOS activity is decreased in $β3^{-/-}$ mice after TAC (2). Although prior studies assumed that cardiac eNOS was the sole source of NO involved in the regulation of myocardial contraction (4,11), there remains great controversy over which NOS isoform is the chief player in β3-AR signaling.

In the present study, eNOS and inducible NOS protein expressions were unchanged by BRL treatment. eNOS activity is generally modulated by either translocation or phosphorylation. Translocation has been observed by β3-AR stimulation only in the right atrium, not in the left ventricle (12,13). Ser1177 and Ser114 are 2 phosphorylation sites that can modulate eNOS activity. Phosphorylation at Ser1177 (or Ser1179 in humans) activates eNOS, whereas phosphorylation at Ser114 deactivates eNOS (24–26). We observed a decrease in Ser1177 phosphorylation and an increase in Ser114 phosphorylation after BRL treatment,

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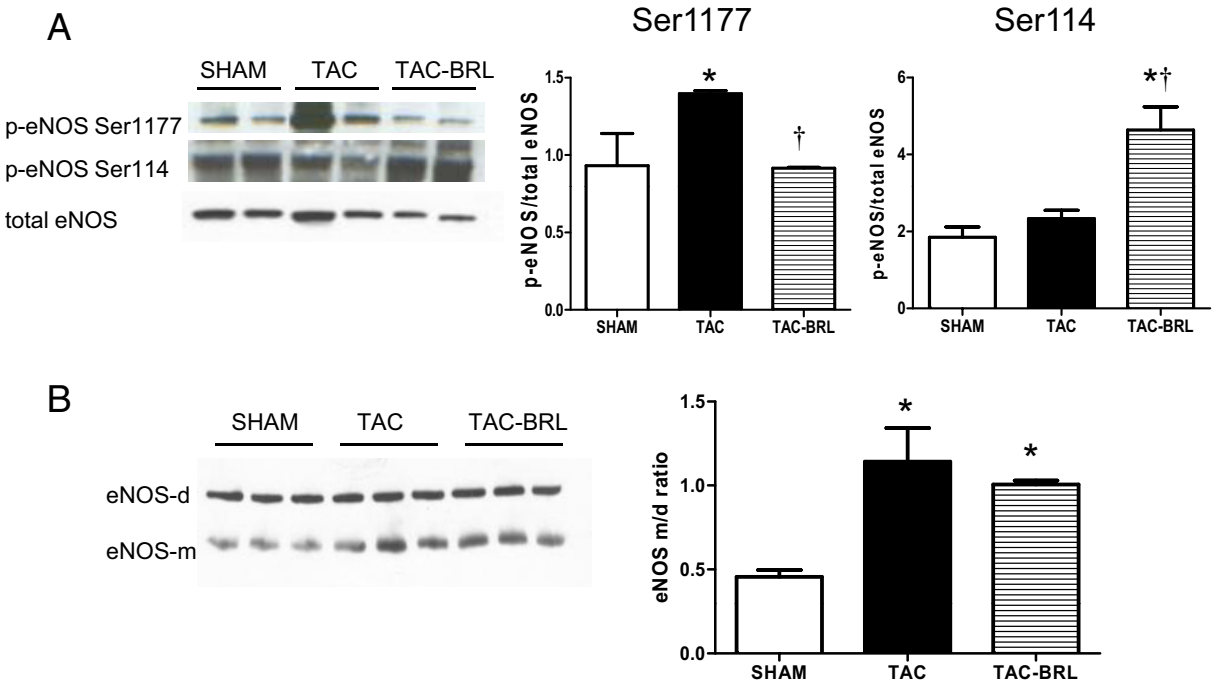

Figure 4 **Effects of BRL Treatment on eNOS Activation and Dimerization**

(A) p-Endothelial nitric oxide synthase (p-eNOS) Ser1177/eNOS was decreased and p-eNOS Ser114/eNOS was increased after BRL treatment in transverse aortic constriction (TAC) mice. **(B)** eNOS uncoupling indexed by increased eNOS monomer-to-dimer ratio was observed after 3 weeks of TAC and not reversed by BRL treatment. *$p < 0.05$ vs. sham. †$p < 0.05$ vs. TAC.

which suggested eNOS deactivation rather than activation by β3-AR stimulation. Similar results were recently observed in isolated human failing myocardium (14). The apparent paradox between the known β3-AR–mediated NO-dependent negative inotropic effect and clear eNOS deactivation in human failing myocardium could be explained by concomitant nNOS activation in cardiomyocytes. Paracrine negative inotropic effects via NO liberation from cardiac endothelial cells may be another explanation, but lacked in direct evidence until recently. The same group also reported that eNOS was activated through Ser1177 phosphorylation by BRL in human nonfailing myocardium, which identified differential downstream signaling by β3-AR stimulation between failing and nonfailing hearts (12,13).

Emerging evidence indicates that nNOS-derived NO production plays a substantial part in the regulation of basal and β-AR–induced myocardial contraction (27,28). nNOS was up-regulated in senescent rat hearts after myocardial infarction and in human failing hearts (22,27,29,30). Interestingly, it was shown that the positive inotropic response to nonspecific β-AR stimulation was impaired in diabetic and aged rat hearts and restored by a β3-AR antagonist, a nonselective NOS inhibitor, and the selective nNOS inhibitor L-VNIO (15,16). *nNOS* gene deletion has been associated with more severe LV remodeling and functional deterioration in murine models of myocardial infarction, suggesting that nNOS-derived NO may also be involved in a protective myocardial response to injury (18,31). The present study revealed exclusive nNOS protein up-regulation and activation by β3-AR agonism, suggesting nNOS-derived NO production is the primary source in the cardioprotective effect of β3-AR agonism. Importantly, cardiac hypertrophy, LV dysfunction, and reduced ejection fraction induced by TAC were actually worsened by BRL treatment in $nNOS^{-/-}$ mice, further confirming this mechanism. An ex vivo study from Idigo et al. also showed that the negative inotropic response to BRL in cardiomyocytes was absent in both $nNOS^{-/-}$ cardiomyocytes and WT cardiomyocytes with pharmacological inhibition of nNOS (32). These studies support nNOS-derived NO production as a primary factor in altered contractile response by β3-AR stimulation of the heart. We speculate that the pathway regulating cardiac contractility may be associated with nNOS post-translational modification such as translocation from sarcoplasmic reticulum to sarcolemma (27) or phosphorylation of select residues (33). This merits further investigation, and studies are ongoing in our laboratory.

Inhibition of oxidative stress. A significant number of animal studies and several clinical observations have demonstrated ROS activation in the cardiovascular system in response to various stressors and in the genesis of the

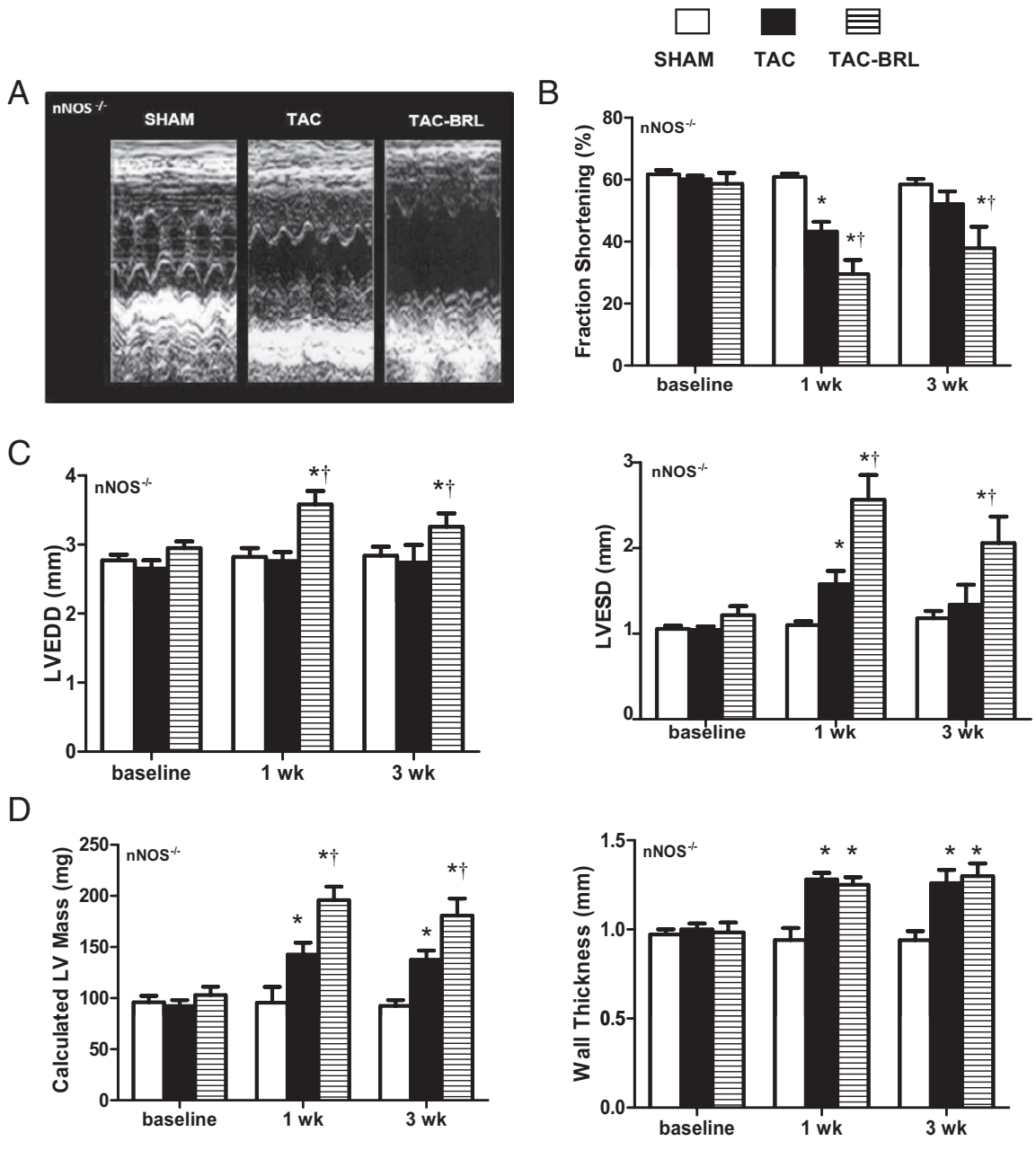

Figure 5 **Effect of BRL on LV Hypertrophy and Function in TAC Mice Lacking *nNOS* Gene**

(A) M-mode echocardiograms demonstrated increased LV dilation and wall thickness, and decreased systolic function after 3 weeks of TAC, which was worsened by BRL administration. **(B)** LV systolic function was decreased by TAC and worsened with BRL in *nNOS*$^{-/-}$ mice. **(C)** LV dilation was unaffected by TAC, but significantly increased by TAC with BRL treatment. **(D)** TAC resulted in LV hypertrophy, which was exacerbated by BRL. *$p < 0.05$ versus sham. †$p < 0.05$ versus TAC. nNOS = neuronal nitric oxide synthase; other abbreviations as in Figure 1.

hypertrophic and failing heart (34–36). Biomarkers for ROS have been detected in the pericardial fluid as well as in the peripheral blood of heart failure patients (37). Further experiments showed that ROS is increased in cardiac hypertrophy and adverse remodeling (38,39). However, the mechanism by which β3-AR stimulation affects ROS generation has not been clearly delineated. In our previous study using TAC in *β3-AR*$^{-/-}$ mice, we observed increased NOS-dependent generation of superoxide, implying that NOS-dependent ROS may be one of the downstream signaling pathways of β3-AR (2).

This was confirmed by the current study, which demonstrated a substantial reduction of TAC-induced superoxide generation by BRL treatment. β3-AR–induced suppression of ROS generation was abolished by acute inhibition of nNOS by preferential nNOS inhibitor, L-VNIO, at a

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concentration that only inhibits nNOS without affecting other NOS isoforms. These results revealed the antioxidant effect of β3-AR agonism is dependent on nNOS activation. Recently, Khan et al. (40) and Kinugawa et al. (41) have shown that deficiency of nNOS leads to profound increase in xanthine oxidoreductase (XOR)–mediated superoxide production without affecting XOR mRNA or protein abundance, which depresses myocardial excitation-contraction coupling in a manner reversible by XOR inhibitor. This suggests constrained XOR activity by nNOS as a possible connection between myocardial NOS and ROS systems.

We observed that eNOS was uncoupled after 3 weeks of TAC, which is in agreement with previous reports (17,20). However, eNOS was not recoupled by BRL treatment, providing further evidence that eNOS is not the sole pathway for β3-AR, as previously thought. In addition, BRL-induced eNOS-Ser114 phosphorylation could indicate uncoupling and may explain the reason for exacerbated dysfunction seen in TAC *nNOS*$^{-/-}$ animals treated with BRL. We speculate that β3-AR regulation occurs via balancing 2 opposing pathways when the heart starts to fail: a deleterious eNOS-dependent pathway and a protective nNOS-dependent pathway. Thus, the cardioprotective effect of β3-AR agonism on cardiac hypertrophy and heart failure could be attributed to nNOS activation, which maintains the equilibrium of myocardial NO and ROS production, despite uncoupled eNOS in this setting.

Clinical implication. β1-blockers have become the standard treatment for chronic heart failure since 1990. We propose that β3-AR activation can be considered similar to an endogenous β1-blocker, due to its protective and negative inotropic effect on human myocardium. This study strongly supports the notion that β3-AR plays a beneficial role in heart and highlights the potential therapeutic utility of β3-AR agonism. Although low basal expression levels of β3-AR in some tissues have resulted in disappointing outcomes from animal studies to clinical trials evaluating β3-AR agonists for obesity, type 2 diabetes, and irritable bowel syndrome treatment (42–45), heart failure represents a more realistic promising therapeutic target for 3 main reasons. First, β3-AR has been demonstrated to be expressed at levels that can mediate physiological responses in healthy human myocardium (1). Second, β3-AR is up-regulated 2- to 3-fold in the progression of heart failure, whereas β1-AR is down-regulated (6). This increase in β3-AR/β1-AR ratio likely plays a substantial role in activating β3-AR signaling. Lastly, cotreatment with conventional β-blockers can further increase the expression of β3-AR. A study in diabetic rats demonstrated that chronic treatment with metoprolol markedly increased the expression of cardiac β3-AR (46). Recent studies reported that the hemodynamic improvement obtained from third-generation β-blocker, nebivolol, in mice subjected to myocardial ischemia-reperfusion and heart failure patients is partially due to its NO-dependent negative inotropic effect by β3-AR stimulation (22,47).

Conclusions

We have shown that β3-specific agonism in vivo has substantial cardioprotective effects, and that these effects are largely attributable to nNOS activation. These findings have direct therapeutic implications for treating heart failure patients and for improving our understanding of the pathophysiology of heart failure.

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Key Words: *β*3-adrenergic receptor ■ heart failure ■ hypertrophy ■ nitric oxide synthase ■ oxidative stress.

APPENDIX

For an expanded Methods section including supplemental figures, please see the online version of this article.