Constitutive BDNF/TrkB signaling is required for normal cardiac contraction and relaxation

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Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved December 9, 2014 (received for review September 17, 2014)

BDNF and its associated tropomyosin-related kinase receptor B (TrkB) nurture vessels and nerves serving the heart. However, the direct effect of BDNF/TrkB signaling on the myocardium is poorly understood. Here we report that cardiac-specific TrkB knockout mice (TrkB^{-/-}) display impaired cardiac contraction and relaxation, showing that BDNF/TrkB signaling acts constitutively to sustain in vivo myocardial performance. BDNF enhances normal cardiomyocyte Ca²⁺ cycling, contractility, and relaxation via Ca²⁺/calmodulin-dependent protein kinase II (CaMKII). Conversely, failing myocytes, which have increased truncated TrkB lacking tyrosine kinase activity and chronically activated CaMKII, are insensitive to BDNF. Thus, BDNF/TrkB signaling represents a previously unidentified pathway by which the peripheral nervous system directly and tonically influences myocardial function in parallel with β -adrenergic control. Deficits in this system are likely additional contributors to acute and chronic cardiac dysfunction.

BDNF | TrkB receptor | cardiac contractility/relaxation | CaMKII | neurotrophins

In the brain, BDNF has a pleiotropic profile, preserving cell viability and function, preventing neuronal degeneration during stress (1), and acting as an antidepressant (2). BDNF is also expressed in various nonneuronal tissues, including smooth and skeletal muscle cells, where it enhances airway smooth muscle cell contractility (3) and acts as a metabolic enhancer (4), respectively.

In the mammalian heart, BDNF is essential for organ development because its genetic deletion leads to the thinning of cardiac chambers, microvascular leakage, and ultimately, early death in mice (5). In adult mammals, BDNF governs autonomic transmission to the heart (6) and exerts prominent angiogenic effects (7). Recent evidence also points to the presence of the BDNF receptor, tropomyosin-related kinase receptor B (TrkB), in the myocardium (8). However, the role of myocardial BDNF/TrkB signaling in cardiac physiology and myocardial response to pathologic stress, independent from its well-known trophic actions on blood vessels and autonomic efferent neurons (9), is largely unknown.

In neurons, BDNF regulates Ca²⁺ signaling, and calmodulin-dependent protein kinase II (CaMKII) is one of the downstream effectors of the BDNF/TrkB signaling pathway. Because CaMKII is one of the important regulators of excitation–contraction coupling, and because BDNF also enhances smooth muscle contraction by augmenting intracellular Ca²⁺ signaling, we first determined the effects of BDNF on cardiomyocyte contractility, relaxation, and Ca²⁺ dynamics. Then, to establish whether BDNF/TrkB stimulation is relevant in maintaining basal cardiac function in vivo, we generated cardiac-specific TrkB^{-/-} mice by crossing conditional TrkB^{-/-} mice with MHC promoter-driven Cre mice and subjected these mice and their WT littermates to pressure–

volume analysis. We further investigated which signaling pathway mediates the BDNF cardiac effects, and finally, we tested whether modulation of myocyte function by BDNF is preserved or altered in myocytes from failing hearts.

Results

BDNF Directly Increases Myocyte Function, Enhancing Cardiac Ca²⁺ Cycling. First, we determined the effect of exogenous application of BDNF on isolated rodent cardiomyocytes. These cells contain surface TrkB receptors (Fig. 1A), consistent with previous reports (8). Incubating mouse cardiac myocytes with BDNF (20 nM) resulted in increased cell contractility ($43 \pm 7.5\%$) and enhanced relaxation ($11 \pm 3\%$; $n = 19$; $P < 0.01$ for both) (Fig. 1B). This enhancement of function was accompanied by larger whole-cell Ca²⁺ transients ($10 \pm 4\%$; $P < 0.01$) (Fig. 1B). Ca²⁺ spark frequency was also increased after BDNF stimulation (1.3 ± 0.3 to 3.4 ± 0.7 sparks/100 μm^2 ; $P < 0.05$; $n = 6$) (Fig. 1C). We next asked whether this stimulatory action stems from an increase in sarcoplasmic reticulum (SR) Ca²⁺ fractional release and/or activity of L-type Ca²⁺ channels. BDNF markedly augmented SR Ca²⁺ fractional release (from $54 \pm 6\%$ to $68 \pm 6\%$; $P < 0.05$; $n = 11$)

Significance

BDNF plays a key role in neuron development, survival, and function, with actions occurring through the stimulation of the tropomyosin-related kinase receptor B (TrkB) receptor. Whether BDNF/TrkB signaling has any physiologic role in governing myocardial function is unknown. Here we report that intact BDNF/TrkB signaling is required for the heart to fully contract and relax. These actions occur independently from and in addition to β -adrenergic influence. BDNF-induced enhancement of myocardial performance occurs via direct modulation of Ca²⁺ cycling in a calmodulin-dependent protein kinase II-dependent manner. Thus, BDNF/TrkB signaling represents a previously unidentified way by which the peripheral nervous system controls cardiac muscle physiology. Our study suggests that loss or alterations in BDNF/TrkB stimulation may contribute to the pathogenesis of myocardial dysfunction in acute or chronic disease conditions.

Author contributions: N.F. and N.P. designed research; N.F., S.H., G.Z., C.G.T., S.S., T.A., N.K., S.E.B., J.L.M., and N.P. performed research; N.F. and N.P. contributed new reagents/analytic tools; N.F., S.H., G.Z., C.G.T., S.S., T.A., N.K., D.B.H., S.E.B., J.L.M., G.F.T., D.M.B., D.A.K., and N.P. analyzed data; and N.F. and N.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1417949112/-DCSupplemental.

Fig. 1. BDNF increases cardiomyocyte contractility and relaxation as a result of enhanced Ca²⁺ cycling, in a TrkB receptor-dependent manner. (A) TrkB protein expression is detected in isolated adult murine cardiac myocytes by Western blot (140 KD). TrkB is located on plasma membrane of cardiac myocytes by immunohistochemical study, using confocal microscopy. (B) Representative traces of sarcomere shortening and Ca²⁺ transients, with or without BDNF treatment. Incubating isolated murine cardiomyocytes with BDNF (20 nM) increases myocyte fractional shortening and whole-cell Ca²⁺ transients while accelerating myocyte relaxation; the latter is documented by decreased relaxation time ($n = 19$). (C) Representative confocal imaging of Ca²⁺ spark events in isolated rat adult cardiomyocytes: BDNF augments Ca²⁺ spark frequency in these cells. (D) Consistent with data in the mice, BDNF increases Ca²⁺ transients and SR Ca²⁺ load, measured by caffeine-induced total SR Ca²⁺ release. (E) Raw traces of BDNF's effect on L-type Ca²⁺ channel activity measured in isolated guinea pig ventricular myocytes: BDNF enhances peak L-type Ca²⁺ channel activity from baseline ($P < 0.05$). (F) BDNF inotropy is abolished in cardiomyocytes isolated from TrkB^{F616A} mice with 1-NMPP1 pretreatment.

without affecting total SR Ca²⁺ load, as assessed by caffeine transients (Fig. 1D). In isolated guinea pig myocytes, BDNF increased peak L-type Ca²⁺ current density by ~25% compared with baseline (-3.5 ± 0.2 vs. -2.8 ± 0.4 mV; $P < 0.05$; $n = 6$) (Fig. 1E). Thus, BDNF enhances myocyte Ca²⁺ cycling without altering diastolic Ca²⁺ levels or Ca²⁺ SR load (Fig. 1D).

TrkB Is Required for BDNF-Induced Enhancement of Myocyte Function.

We then tested whether the TrkB receptor is required for the inotropic/lusitropic action of BDNF. To do so, we used isolated myocytes from TrkB^{F616A} mice (kindly provided by David D. Ginty, Department of Neurobiology, Harvard Medical School, Boston) (10). In these mice, a phenylalanine-to-alanine substitution within the kinase subdomain of the TrkB receptor renders it sensitive to specific inhibition by membrane-permeable, small-molecule protein phosphatase 1 (PP1) derivatives, including derivative of the general kinase inhibitor PP1 (1NMPP1) (11). BDNF had no effect on TrkB^{F616A} myocytes pretreated with 1-NMPP1 (100 nM) (Fig. 1F), and 1-NMPP1 did not alter basal myocyte function: fractional

shortening was 3.19 ± 0.43 (baseline) vs. 2.92 ± 0.2 (after 1NMPP1) ($n = 10$; $P = 0.52$).

Constitutive TrkB Stimulation Is Required for Optimal Cardiac Contraction and Relaxation.

To determine whether BDNF/TrkB stimulation is constitutively active in vivo and the relevance of this signaling in maintaining normal heart function, cardiac-specific TrkB knock-out mice (TrkB^{-/-}) were generated by crossing conditional TrkB^{-/-} mice (12) (from David D. Ginty) with cardiomyocyte-specific (MHC-Cre mice (Fig. S1)). BDNF is a modulator of angiogenesis (7), and thus changes in vascularity may contribute to altering cardiac performance in TrkB^{-/-} mice. Therefore, we first measured capillary density in TrkB^{-/-} mice and their control littermates. The capillary density measured by counting isolectin B4-stained capillary profiles in myocardium did not differ between WT and TrkB^{-/-} mice (Fig. S2). Next, we examined the effect of BDNF/TrkB signaling on cardiac contraction and relaxation in vivo. Pressure-volume relationships were used to distinguish intrinsic changes in myocardial contractility/relaxation from confounding effects

Fig. 2. Constitutive BDNF/TrkB signaling is required for normal cardiac contraction and relaxation. (A) Representative pressure-volume loops obtained in littermate mice and in cardiac-specific TrkB^{-/-} mice. (B) Cardiac contractility and relaxation are impaired in TrkB^{-/-} mice, as indexed by dp/dt_{max}, dpdt/EDV, dpdt/ip, prerecruitable stroke work, Ees/Ea ratio, and Tau logistic, respectively.

as a result of potential changes in vascular loading conditions (i.e., preload and afterload). TrkB^{-/-} mice displayed reduced myocardial performance, as determined by dp/dt_{max} (maximum time derivative of left ventricular pressure; a load-dependent index) and load-independent parameters of myocardial contractility such as ventricular elastance (Ees), dPdt/EDV (dPdt max against end-diastolic volume relationship), dPdt ip (dp/dt max divided by the instantaneous developed isovolumetric pressure), and prerecruitable stroke work (Fig. 2 and Table 1). All these indices were consistent with reduced systolic function in TrkB^{-/-} mice, although this was not severe enough to trigger chamber dilation or reduce ejection fraction. Cardiac relaxation was also impaired, with prolongation of the relaxation time constant (Tau) from 4.0 ± 0.2 to 5.3 ± 0.3 msec ($P = 0.0019$) (Fig. 2B

and Table 1). Total systemic vascular resistance and ventricular afterload (indexed by arterial elastance, Ea) were similar in both genotypes, so systemic load was not affected. The ventricular/arterial coupling ratio (Ees/Ea) declined solely because of the fall in contractility, which would indicate a fall in efficiency of blood transfer in TrkB^{-/-} mice. Thus, BDNF/TrkB signaling is an important independent contributor to basal cardiac contraction and relaxation in vivo. We further examined whether TrkB^{-/-} mice had altered responses to β -adrenergic receptor stimulation. Isoproterenol (from 10 to 40 ng/kg/min) infusion produced similar augmentation in both genotypes ($n = 5$ each group; Fig. 3 and Table 1). These findings indicate that a lack of BDNF/TrkB modulation does not impair β -adrenergic receptor-cAMP/PKA signaling.

Table 1. Hemodynamic values in WT and TrkB^{-/-} mice via pressure-volume relationships

	WT		TrkB ^{-/-}		P value
	(n = 6)	(n = 5)	(n = 6)	(n = 5)	
Basal hemodynamics					
HR, min ⁻¹	611.7 ± 13.5		574 ± 25.1		0.22
SBP, mm Hg	100.1 ± 3		96 ± 5.2		0.53
SV, μL	23.9 ± 1.7		21.6 ± 1.9		0.39
CO, mL/min	14.6 ± 1		12.4 ± 1.1		0.17
EF, %	56.0 ± 2.3		56.7 ± 2.0		0.59
dPdt _{max} , mm Hg/s	10,944.7 ± 496.3		7,647.2 ± 498.9		0.0009
Ees, mm Hg/mL	15.2 ± 3.2		6.6 ± 1.0		0.041
dPdt/EDV	417.2 ± 56.4		209.2 ± 25		0.0073
dPdt ip, sec ⁻¹	185.3 ± 8.3		142.4 ± 9.8		0.0075
Prerecruitable stroke work, mm Hg	108.3 ± 2.4		85.8 ± 7.6		0.018
Tau log, msec	4.0 ± 0.2		5.3 ± 0.3		0.0019
Ea, mm Hg/mL	4.0 ± 0.4		4.3 ± 0.4		0.56
Systemic vascular resistance, mm Hg·min·mL ⁻¹	6.5 ± 0.7		7.5 ± 0.6		0.26
Ees/Ea	4.1 ± 1.1		1.5 ± 0.6		0.033
Isoproterenol, 40 ng/kg/min					
dPdt _{max} , mm Hg/s		13,973.7 ± 558.8		10,583.7 ± 655.0	0.005
Percentage increase over baseline		22.3 ± 3.0		38.8 ± 11.1	0.19

CO, cardiac output; dPdt/EDV, dPdt max against end-diastolic volume relationship; dPdt ip, dp/dt max divided by the instantaneous developed isovolumetric pressure; dPdtmax, maximum time derivative of left ventricular pressure; Ea, arterial elastance; Ees, left ventricle chamber elastance; EF, ejection fraction %; HR, heart rate; SBP, systolic blood pressure; SV, stroke volume; Tau, relaxation time constant.

Fig. 3. β -adrenergic response is intact in $\text{TrkB}^{-/-}$ mice. (A) Representative pressure-volume loops obtained before and after the infusion of the $\beta 1$ - $\beta 2$ agonist isoproterenol (40 ng/kg/min). (B) Isoproterenol increases in vivo contractility (dPdt_{max} and dPdt/IP) with the same magnitude in WT and $\text{TrkB}^{-/-}$ mice, despite the lower basal contractile values found in the $\text{TrkB}^{-/-}$ mice ($n = 5$ each group).

CaMKII Mediates BDNF-Induced Enhancement of Myocyte Function. In the brain, BDNF/TrkB is mainly coupled to CaMKII (13). We found that BDNF also increased the activated and phosphorylated state of CaMKII in isolated murine ventricular myocytes. This change was paralleled by augmented phosphorylation of CaMKII-dependent sites (14, 15) on the ryanodine receptor 2 (serine 2814), and phospholamban (PLN; threonine 17) (Fig. 4A, $n = 4$). $\text{TrkB}^{-/-}$ mice displayed decreased levels of P-CaMKII and the reduction of P-CaMKII/T-CaMKII ratio (Fig. 4A). Moreover, pharmacologic inhibition of CaMKII activity by KN93 completely abolished BDNF Ca^{2+} transients and contractility (Fig. 4B, $n = 10$). Thus, CaMKII is the main mediator of BDNF/TrkB-evoked cardiac

stimulatory actions that operates in parallel to β -adrenergic signaling to regulate myocardial contraction and relaxation.

BDNF-Induced Enhancement of Myocyte Function Is Lost in Failing Hearts. We finally tested whether BDNF-evoked cardiac enhancement is preserved in failing myocytes, using cardiomyocytes isolated from $\text{G}_{\alpha\text{q}}$ overexpressing ($\text{G}_{\alpha\text{q}}$ OE) mice that display progressive cardiac dilation and reduced ejection fraction (16). Enhanced $\text{G}_{\alpha\text{q}}$ signaling is a common pathway mediating maladaptive cardiac hypertrophy and adverse remodeling. In response to pathologic stress, $\text{G}_{\alpha\text{q}}$ signaling is activated by α -adrenergic agonist, angiotensin II, or endothelin, and so on, promoting cardiac growth, apoptosis, and

Fig. 4. CaMKII is the major mediator of BDNF influence on myocardial mechanics. (A) BDNF induces CaMKII phosphorylation in adult murine cardiac myocytes ($n = 3$) and increases the phosphorylation of CaMKII-dependent sites on RyR (serine 2814) and PLN (threonine 17), respectively ($n = 4$); $\text{TrkB}^{-/-}$ mice display significantly reduced levels of constitutive phosphorylation of CaMKII, as indexed by the P-CaMKII/T-CaMKII ratio ($n = 4$). (B) Representative traces of BDNF's effect on isolated mouse cardiomyocytes in the absence and the presence of the CaMKII inhibitor KN93: Pretreating cells with KN93 prevents BDNF enhancement of myocyte contraction ($n = 10$).

function. Several physiologic and pathophysiologic implications arise from these findings. For example, BDNF/TrkB signaling could contribute to enhanced cardiac performance during exercise. In fact, exercise augments BDNF levels in the brain, the skeletal muscle, and plasma, enhancing function and improving energy metabolism in these organs (21, 22). Our present findings may also provide a potential explanation for recent clinical reports showing that in heart failure patients, a correlation exists between low circulating levels of BDNF and worsening of symptoms (23, 24). Although it is known that growth factors such as insulin-like growth factor 1 and vascular endothelial growth factor have pharmacologic actions to modulate cardiac contractility (25, 26), no studies have established their relevance in vivo or their signaling in this respect. Here we report that a tyrosine kinase-associated receptor such as TrkB, and its endogenous ligand BDNF, directly control the cardiac excitation–contraction coupling process, independently and in parallel to G protein-coupled receptor signaling. Thus, disruption of tyrosine kinase-based signaling and consequent perturbations in cardiac mechanical work can largely contribute to loss in ventricular systolic function that accompanies the use of tyrosine kinase inhibitors during anticancer therapies (27).

In the present study, we also found the BDNF-induced enhancement of contraction was abolished in failing myocytes. The loss of efficacy is probably a result of the increased truncated form of TrkB, which is the dominant negative form of BDNF/TrkB signaling. The underlying mechanism of elevated TrkB-T1 remains to be elucidated. In contrast, CaMKII is chronically activated, despite the diminished TrkB activation. Indeed, CaMKII is also a key downstream signal pathway of both α - and β -adrenergic receptors. In heart failure, this kinase is constitutively activated by

the neurohormone overdrive that characterizes this syndrome and that contributes to the development of adverse cardiac hypertrophy and remodeling (28). Thus, in addition to the decreased TrkB activity, the constitutive activation of CaMKII presented in failing hearts can be another major reason accounting for the loss in efficacy of BDNF/TrkB signaling in failing hearts.

In conclusion, by binding to TrkB receptors and triggering a CaMKII-dependent signaling cascade, endogenous BDNF acts in parallel with the β -adrenergic system to maintain or enhance myocardial Ca^{2+} cycling, and thus cardiac contraction and relaxation. Our study suggests that loss or decreases in circulating BDNF levels and alterations in TrkB receptor structure/function, along with abnormal CaMKII signaling, could further contribute to cardiac dysfunction under acute and chronic diseased conditions.

Materials and Methods

Detailed methods are available in the *SI Materials and Methods*.

Statistics. Results are expressed as means $\pm$ SEM. Significance was estimated by one-way repeated measures ANOVA, Student's *t* test for paired observations, or Mann–Whitney *U* test, as appropriate; $P \leq 0.05$ was considered significant.

Studies Approval. All animal protocols were approved by the Animal Care and Use Committee of the Johns Hopkins University in Baltimore, MD, and the Loyola University in Chicago, IL, following established NIH guidelines.

ACKNOWLEDGMENTS. We are grateful to Dr. David D. Ginty for providing us with TrkB^{F616A} and TrkB conditional knockout mice. We also thank Dr. Brian O'Rourke for valuable scientific discussion. This work was supported by American Heart Association Grant-in-Aid GRNT17070027, Johns Hopkins University Magic That Matters Funds, and NIH T32 Training Grant T32HL-0227.

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