Brain-Derived Neurotrophic Factor Protects Against Cardiac Dysfunction After Myocardial Infarction via a Central Nervous System–Mediated Pathway

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Objective—The central nervous system is thought to influence the regulation of the cardiovascular system in response to humoral and neural signals from peripheral tissues, but our understanding of the molecular mechanisms involved is still quite limited.

Methods and Results—Here, we demonstrate a central nervous system–mediated mechanism by which brain-derived neurotrophic factor (BDNF) has a protective effect against cardiac remodeling after myocardial infarction (MI). We generated conditional BDNF knockout mice, in which expression of BDNF was systemically reduced, by using the inducible Cre-loxP system. Two weeks after MI was induced surgically in these mice, systolic function was significantly impaired and cardiac size was markedly increased in conditional BDNF knockout mice compared with controls. Cardiomyocyte death was increased in these mice, along with decreased expression of survival molecules. Deletion of the BDNF receptor (tropomyosin-related kinase B) from the heart also led to the exacerbation of cardiac dysfunction after MI. The plasma levels of BDNF were markedly increased after MI, and this increase was associated with the upregulation of BDNF expression in the brain, but not in the heart. Ablation of afferent nerves from the heart or genetic disruption of neuronal BDNF expression inhibited the increase of plasma BDNF after MI and led to the exacerbation of cardiac dysfunction. Peripheral administration of BDNF significantly restored the cardiac phenotype of neuronal BDNF-deficient mice.

Conclusion—These results suggest that BDNF expression is upregulated by neural signals from the heart after MI and then protects the myocardium against ischemic injury. (Arterioscler Thromb Vasc Biol 2012;32:1902-1909.)

Key Words: myocardial infarction ■ angiogenesis ■ heart failure ■ central nervous system

Myocardial infarction (MI) is the most common cause of cardiovascular morbidity and mortality in many countries, and left ventricular remodeling after MI is an important phenomenon because it causes progression to heart failure. Although numerous therapeutic strategies have been developed, death from heart failure is still a serious problem worldwide. Brain-derived neurotrophic factor (BDNF) was originally discovered in the brain as a member of the neurotrophin family, and is known to have neurotrophic functions that are crucial in both the embryonic and adult brain. BDNF specifically binds to the tropomyosin-related kinase receptor B (TrkB) and activates many intracellular signaling pathways, thereby affecting the development and function of the nervous system.

BDNF-deficient mice develop atrial septal defects and also die from diffuse intramyocardial hemorrhage attributable to impaired endothelial adhesion. It has been reported that transcriptional activation of TrkB is crucial for coronary vessel development. The previous studies have revealed that BDNF acts on endothelial cells and promotes neovascularization in response to hypoxic stimuli via the Akt pathway. In addition to a direct action on local endothelial cells, BDNF enhances capillary formation by recruiting proangiogenic hematopoietic cells, indicating that it has a critical role in maintaining the integrity of the vascular system in adults. However, it remains unclear whether BDNF is involved in the pathophysiology of adult cardiac diseases such as MI.

In the present study, we demonstrate a cardioprotective role of the BDNF–TrkB axis by using a variety of genetic mouse models. Systemic deletion of BDNF or disruption of TrkB in
the heart led to exacerbation of cardiac dysfunction after MI. The plasma levels of BDNF were markedly increased after MI, and this increase was associated with the upregulation of BDNF expression in the brain. Ablation of afferent nerves from the heart or genetic disruption of neuronal BDNF expression inhibited the increase of plasma BDNF after MI and led to exacerbation of cardiac dysfunction, suggesting a central nervous system–mediated mechanism by which BDNF has a protective effect against cardiac remodeling after MI. These findings also suggest that the activation of neuronal BDNF may become a therapeutic strategy for cardiac disease.

Materials and Methods

Animal Study
The animal experiments were approved by our institutional review board. All mice used in this study were males between 12 and 14 weeks’ old unless otherwise indicated. All experimental procedures were performed according to the guidelines established by Chiba University for animal experiments. Mice were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg), and MI was induced by ligation of the left anterior descending artery as described previously. Generation of conditional knockout mice is described in the online-only Data Supplement.

Physiological and Histological Analysis
Echocardiography was performed with a Vevo 770 High-Resolution Imaging System (Visual Sonics Inc, Toronto, Ontario, Canada). The heart rate was kept at ≈500 to 600 bpm to minimize data deviation when cardiac function was assessed by area length methods. Frozen cross-sections (4 µm) of the heart were fixed with 4% 10N formaldehyde and subjected to Masson trichrome staining or immunohistochemistry.

Western Blot Analysis
The lysates were resolved by SDS-PAGE. Proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA), which was incubated with the primary antibody followed by anti-rabbit or anti-mouse IgG conjugated with horseradish peroxidase (Jackson, West Grove, PA).

RNA Analysis
Real-time polymerase chain reaction was performed by using the Light Cycler (Roche) with the Taqman Universal Probe Library and the Light Cycler Master (Roche) according to the manufacturer’s instruction.

Cell Culture
Neonatal Wistar rats were purchased from Takasugi Experimental Animal Supply (Tokyo, Japan). Cardiomyocytes were prepared from these neonatal rats and cultured as described previously. RNA preparation was performed by using the RNAzol B reagent (Cinna Biosciences, Tokyo, Japan). RNA was subjected to reverse transcription using the M-MLV reverse transcriptase and oligo(dT)15 primer. cDNA was amplified using the Light Cycler PCR System (Roche) with the Taqman Universal Probe Library and the Light Cycler Master (Roche) according to the manufacturer’s instruction.

Statistical Analysis
Data are shown as the mean±SEM. Comparison among multiple groups was performed by ANOVA with a post hoc Fisher test, whereas comparisons between 2 groups were performed with the unpaired Student t test. A P value <0.05 was considered statistically significant.

Results

BDNF and TrkB Are Expressed by Cardiomyocytes and Activate the Intracellular Signaling Pathways
To investigate the role of BDNF in the adult heart, we first examined the expression of BDNF and its receptor TrkB in cardiomyocytes. Western blot analysis showed that both BDNF and TrkB were expressed by cardiomyocytes (Figure 1A). It has been reported that binding of BDNF to TrkB triggers receptor dimerization and autophosphorylation, followed by the activation of diverse signaling cascades such as the Akt and mitogen-activated protein kinase pathways in neuronal cells. Consistent with these reports, treatment of cardiomyocytes with BDNF rapidly activated these signaling cascades (Figure 1B). It was also reported that BDNF exerts a protective effect against neuronal death induced by various stresses such as hypoxia. We therefore exposed cardiomyocytes to hypoxia and examined the influence of BDNF on cell viability. As was the case for neurons, treatment with BDNF decreased hypoxic cardiomyocyte death (Figure 1C and 1D), and this effect was associated with increased expression of prosurvival molecules (Figure 1E). These results indicate that BDNF promotes the survival of cardiomyocytes by activating intracellular signaling pathways via TrkB.

Systemic Deletion of BDNF Leads to Exacerbation of Cardiac Dysfunction After MI
To further investigate the role of BDNF in the heart, we generated conditional BDNF knockout mice using the inducible Cre-loxP system. We prepared transgenic mice in which a transgene encoding Cre recombinase fused to the mutated estrogen receptor domain (Cre-ER) was driven by the cytomegalovirus enhancer/chicken actin (CAG) promoter. We then crossed these CAG-Cre-ER mice with mice bearing floxed Bdnf alleles (CAG-Cre-ER; Bdnflox/lox mice). In this model, BDNF expression was reduced in various tissues, including the heart and the brain, by treatment with tamoxifen (Figure 2A). Consequently, plasma BDNF levels were significantly lower in CAG-Cre-ER; Bdnflox/lox mice than in their control littermates (Figure 2B). CAG-Cre-ER; Bdnflox/lox mice had a normal appearance and viability (data not shown). We then produced MI in these mice and performed physiological and histological examinations at 2 weeks after surgery. Echocardiography revealed that the left ventricular diastolic dimension was increased and the left ventricular ejection fraction was decreased in CAG-Cre-ER; Bdnflox/lox mice compared with control littermates (Figure 2C). These mice also showed a larger fibrotic area than their control littermates (Figure 2D). Histological examination demonstrated that the number of terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling–positive cardiomyocytes was significantly increased in CAG-Cre-ER; Bdnflox/lox mice (Figure 2E). This increase was associated with impaired vascularization of the border zone of the infarcted region in CAG-Cre-ER; Bdnflox/lox mice (Figure 2F). Consistent with this observation, the expression of vascular endothelial growth factor was lower in the hearts of CAG-Cre-ER; Bdnflox/lox mice after MI (Figure 2G). Indeed, the expression of the proapoptotic molecule Bax was increased, whereas the expression of the prosurvival molecules such as Bcl-2 was decreased in the hearts of CAG-Cre-ER; Bdnflox/lox mice after MI compared with control littermates (Figure 2G). These results suggest that BDNF plays a protective role in the heart after MI by inducing angiogenesis as well as by upregulating the expression of prosurvival factors.
Disruption of Cardiac TrkB But Not BDNF Exacerbates Systolic Dysfunction After MI

Because BDNF is known to predominantly bind to and activate TrkB, its cardioprotective effect after MI may be mediated by TrkB expressed on cardiomyocytes. To investigate the role of TrkB in the MI heart, we generated cardiomyocyte-specific TrkB conditional knockout mice by using the inducible Cre-loxP system. We prepared transgenic mice in which a transgene encoding Cre recombinase fused to the mutated estrogen receptor domains (MerCreMer) was driven by the cardiomyocyte-specific α-myosin heavy chain (MHC) promoter. We then crossed these MHC-MerCreMer mice with mice bearing floxed TrkB alleles and produced MI in these mice (MHC-MerCreMer; TrkBflox/flox mice). In this model, treatment with tamoxifen induced Cre-mediated recombination of floxed TrkB alleles in cardiomyocytes, resulting in specific ablation of cardiac TrkB. Although the basal expression of TrkB was undetectable in the heart, its expression was markedly upregulated after MI in wild-type mice (Figure 3A). This upregulation was attenuated in the heart of MHC-MerCreMer; TrkBflox/flox mice after MI (Figure 3A). Similarly, the expression of TrkB was strikingly increased in the border zone of the infarcted region in control littermates, but not in MHC-MerCreMer; TrkBflox/flox mice (Figure 3B). These results indicate that myocardial ischemia mainly upregulates TrkB expression in cardiomyocytes. MHC-MerCreMer; TrkBflox/flox mice had a normal heart size and systolic function under basal conditions (Figure 3C). Two weeks after MI, however, these mice had slightly larger left ventricular diastolic dimension than control littermates (Figure 3C). Systolic function was significantly decreased in MHC-MerCreMer; TrkBflox/flox mice compared with their control littermates (Figure 3C). These mice also had a larger fibrotic area with more terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive cardiomyocytes than their control littermates (Figure 3D and 3E), indicating a crucial influence of TrkB on cardiomyocytes in the heart after MI. An increase in cardiomyocyte death was associated with impaired vascularization of the border zone of the infarcted region in MHC-MerCreMer; TrkBflox/flox mice (Figure IA in the online-only Data Supplement), suggesting a crucial role of TrkB on cardiomyocytes in inducing angiogenesis in the MI heart.

To determine the role of BDNF in the MI heart, we generated cardiomyocyte-specific BDNF conditional knockout mice by using the inducible Cre-loxP system (MHC-MerCreMer; Bdnfflox/flox mice). We created MI in these mice and then performed echocardiography and histological examination. MHC-MerCreMer; Bdnfflox/flox mice had a normal heart size and systolic function under basal conditions (Figure 4A). In contrast to MHC-MerCreMer; TrkBflox/flox mice, ablation of BDNF in cardiomyocytes did not affect cardiac remodeling.
and systolic dysfunction after MI (Figure 4A). Moreover, cardiac ablation of BDNF did not attenuate vascularization of the border zone of the infarcted region (Figure 1B in the online-only Data Supplement) nor did it increase infarct size (Figure 4B). We found that the cardiac expression of BDNF was markedly downregulated after MI (Figure 4C), whereas plasma BDNF levels were significantly increased in wild-type controls (Figure 5A). These results suggest that tissues outside the heart increase production of BDNF after MI and that circulating BDNF protects the heart against ischemic injury.

**Ablation of Afferent Nerves From the Heart Leads to Exacerbation of Cardiac Dysfunction After MI**

Given the important role of BDNF in the nervous system, we hypothesized that BDNF derived from the brain might contribute to its protective effect on the heart after MI. Consistent with this notion, the expression of BDNF was significantly increased in the forebrain including the thalamus and the hypothalamus after MI (Figure 5B). This increase was abolished in the brain of CAG-Cre-ER; Bdnflox/flox mice (systemic deletion model; Figure 5B). Similarly, plasma BDNF levels after MI were significantly lower in CAG-Cre-ER; Bdnflox/flox mice than in wild-type controls (Figure 5A). These results led us to propose a novel mechanism whereby the brain senses ischemia in the heart and increases production of BDNF, after which circulating BDNF in turn protects the heart against ischemic injury.

There is evidence that cardiac afferent nerve fibers convey mechanosensitive and chemosensitive information to the brain, leading to the activation of various signaling pathways in the CNS.12 Activation of these CNS pathways has been suggested...
to play an important role in regulating cardiac function after MI. To determine whether such neuronal pathways convey stimuli from the MI heart to the brain, we treated the heart with capsaicin (CAP), an agonist for transient receptor potential vanilloid type 1 channel that is predominantly expressed on the terminals of primary sensory neurons. CAP treatment leads to an influx of cations by activating transient receptor potential vanilloid type 1 and thereby damages afferent sensory neurons. Histological examination revealed that treatment with CAP effectively ablated primary afferent neurons from the heart (Figure IIA in the online-only Data Supplement). We then produced MI in mice treated with CAP and examined the influence of ablation of cardiac afferent fibers. Treatment with CAP did not alter the neuronal expression of BDNF in sham-operated mice compared with untreated mice (Figure 5B). In contrast, neuronal expression of BDNF after MI was significantly reduced by treatment with CAP (Figure 5B). Similarly, ablation of cardiac afferent fibers inhibited the increase of plasma BDNF after MI (Figure 5A). Histological examination demonstrated impaired vascularization of the border zone of the infarcted region in CAP-treated mice (Figure IIB in the online-only Data Supplement). Consequently, cardiac function after MI was significantly worse in CAP-treated mice than in untreated mice (Figure 5D). Peripheral administration of BDNF significantly restored the cardiac phenotype of CAP-treated mice (Figure IIC in the online-only Data Supplement). These results indicate that cardiac afferent fibers mediate upregulation of BDNF in the brain and that this upregulation protects the infarcted heart against ischemic injury.

**Genetic Disruption of Neuronal BDNF Expression Promotes Cardiac Remodeling After MI**

To examine the effect of reduced BDNF expression in the brain on cardiac function after MI, we generated brain-specific BDNF conditional knockout mice by crossing nestin-Cre mice with Bdnf<sup>lox/lox</sup> mice. As previously demonstrated by a study on another type of brain-specific BDNF conditional mutant, nestin-Cre; Bdnf<sup>lox/lox</sup> mice were viable and hyperactive. In these mice, the neuronal expression of BDNF was markedly reduced and its plasma levels were significantly lower than in control littermates (Figure 6A and 6B). Echocardiography revealed that the left ventricular diastolic dimension was increased and the left ventricular ejection fraction was decreased in Nestin-Cre; Bdnf<sup>lox/lox</sup> mice compared with control littermates (Figure 6D). Nestin-Cre; Bdnf<sup>lox/lox</sup> mice also had a larger area of myocardial

![Figure 3. Cardioprotective role of TrkB after MI. A, Expression of TrkB was examined in the hearts of control littermates (control) and MHC-MerCreMer; TrkB<sup>lox/lox</sup> mice by Western blot analysis. B, Immunohistochemistry for TrkB (brown) in the hearts of control littermates (control) and MHC-MerCreMer; TrkB<sup>lox/lox</sup> mice after MI. Scale bar=100 μm. C, Echocardiographic analysis of control littermates (control) and MHC-MerCreMer; TrkB<sup>lox/lox</sup> mice after sham operation (Sham) or MI. D, Masson trichrome staining of the heart after MI. Scale bar=1 mm. E, Double staining of the heart for terminal deoxynucleotidyl transferase -mediated dUTP nick-end labeling (TUNEL) (green) and lectin (red) after MI. Nuclei were stained with DAPI (blue). Scale bar=40 μm. The right graph indicates the number of TUNEL-positive cardiomyocytes in the MI heart. *P<0.05, **P<0.01; n=7 for C, D, and E. Data are shown as the means±SEM. Sham indicates sham operation; MI, myocardial infarction; LVDd, left ventricular diastolic dimension; LVEF, left ventricular ejection fraction; MHC, myosin heavy chain; TrkB, tropomyosin-related kinase receptor B.](http://atvb.ahajournals.org/issue)
fibrosis than control littermates (Figure 6E). Peripheral administration of BDNF significantly increased its plasma levels (Figure III in the online-only Data Supplement) and restored the cardiac phenotype of Nestin-Cre; Bdnfflox/flox mice (Figure 6D and 6E), suggesting a crucial role of neuronal BDNF production in protection against cardiac dysfunction after MI.

Discussion
We have demonstrated a CNS-mediated mechanism involved in the regulation of cardiac function after MI. Our results suggest that ischemic insults are transmitted from the heart to the CNS via cardiac afferent nerve fibers after MI, thereby increasing the neuronal expression of BDNF. An increase...
in circulating BDNF promotes the survival of cardiomyocytes and is associated with increased expression of prosurvival and proangiogenic factors. In agreement with our data, recent evidence has suggested the potential importance of humoral heart-brain signaling in the pathogenesis of heart failure.\textsuperscript{13,14} Accordingly, the CNS may function as a conductor that integrates input signals from a variety of sources, including the failing heart, which in turn leads to activation of certain CNS pathways. Peripheral responses elicited by CNS activation may play an important role in the progression or prevention of cardiac remodeling after MI. Several lines of evidence indicate that the forebrain, in particular the paraventricular nucleus of the hypothalamus, can sense signals generated peripherally in response to the stress of heart failure.\textsuperscript{13,18,19} Neuronal activation in this area has been shown to enhance calcium-dependent signals and increase oxidative stress.\textsuperscript{13,18,19} It has also been reported that BDNF expression is regulated by various transcription factors such as the calcium-response factor, the nuclear factor of T cells, and the nuclear factor κB, which could respond to these changes.\textsuperscript{20} Moreover, Rasmussen et al.\textsuperscript{21} demonstrated a significant output of BDNF across the human and murine brain, which was further enhanced by exercise. Taken together with these reports, our results suggest that cardiac injury activates these CNS pathways, thereby increasing the cerebral output of BDNF.

It has been suggested that decreased neural production of BDNF is a common factor in neurodegenerative diseases such as Alzheimer disease and various psychiatric disorders including major depression,\textsuperscript{3} and several studies have demonstrated that patients with such diseases have low circulating levels of BDNF.\textsuperscript{3} It has also been reported that BDNF release from the brain is attenuated by hyperglycemia in humans and that reduced plasma BDNF levels are associated with impaired glucose metabolism.\textsuperscript{22} Neuropsychiatric diseases and metabolic disorders are highly prevalent among patients with MI and are associated with a significant increase in mortality.\textsuperscript{23,24} These reports led to the hypothesis that a decrease in BDNF may contribute to a poor prognosis among patients with ischemic heart disease and that BDNF-based therapy could be particularly effective for MI patients complicated by the above-mentioned disorders.

In conclusion, our results indicate that an intriguing mechanism underlying the cardioprotective effect of BDNF involves a CNS-mediated pathway and suggest that activation of this pathway could become a novel strategy for the treatment of cardiac dysfunction after MI.

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Disclosures

None.

References

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Supplemental Information

Material and Methods

Cell Culture

Neonatal Wistar rats were purchased from Takasugi Experimental Animal Supply (Tokyo, Japan). Cardiomyocytes were prepared from these neonatal rats and cultured as described previously. Cultures were enriched with myocardial cells by preplating for 60 min. The cells were plated onto 60-mm plastic culture dishes at a concentration of $1 \times 10^5$ cells/cm$^2$ and cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin solution at 37°C in 95% air/5% CO$_2$. The culture medium was changed to fresh DMEM at 24 h before stimulation. Exposure to hypoxia was performed as described previously.

Western blot analysis

Whole cell lysates were prepared in lysis buffer (25 mM Tris-HCl, pH 7.4, 25 mM NaCl, 0.5 mM EGTA, 1 mM PMSF, 10 mM NaF, 10 mM Na$_4$PO$_7$, 4% complete TM protease inhibitor cocktail (sc-29131, Santa Cruz), and 1 mM Na$_2$VO$_3$), and these lysates (30-50 µg) were resolved by SDS polyacrylamide gel electrophoresis (PAGE). Then proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA) and incubated with the primary antibody, followed by incubation with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G, anti-mouse immunoglobulin G, or anti-goat immunoglobulin G (all from Jackson, West Grove, PA). Target proteins were detected by enhanced chemiluminescence (Amersham, Buckinghamshire, UK). The following primary antibodies were used for Western blotting: anti-pERK antibody (#9101, Cell Signaling Technology, Danvers, MA), anti-ERK antibody (61-7400, Zymed, South San Francisco, CA), anti-pAkt antibody
(#9271, Cell Signaling Technology), anti-Akt antibody (sc-1618, Santa Cruz, Santa Cruz, CA), anti-GAPDH antibody (sc-20357, Santa Cruz), anti-actin antibody (A5060, Sigma, St. Louis, MO), anti-Bdnf antibody (sc-546, Santa Cruz) and anti-TrkB antibody (610101, BD Transduction Laboratories, Franklin Lakes, NJ). The plasma levels of Bdnf were measured by ELISA (Promega, Madison, WI) according to the manufacturer’s instructions.

**RNA analysis**

Total RNA was isolated from the hearts of rats with RNA-Bee (Molecular Research Center), after which 30 µg of the RNA was separated on formaldehyde denaturing gel and transferred to a nylon membrane (Amersham, Buckinghamshire, UK). The blot was then hybridized with a radiolabelled Bdnf cDNA probe using Quickhyb hybridization solution (Stratagene, Tokyo, Japan) according to the manufacturer’s instructions. Real-time PCR was performed with a LightCycler (Roche), employing the Taqman Universal Probe Library and the Light Cycler Master (Roche) according to the manufacturer’s instruction.

**Animal study**

The animal experiments were approved by our institutional review board. All mice used in this study were males between 12 and 14 weeks old unless otherwise indicated. All experimental procedures were performed according to the guidelines established by Chiba University for animal experiments. Mice were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg) and MI was induced by ligation of the left anterior descending artery as described previously. To ablate the primary afferent neurons, subepicardial injection of capsaicin (50 mg/ml, Sigma) was done at 2 weeks before induction of MI. Transgenic mice were prepared, in which a transgene encoding Cre recombinase fused to the mutated estrogen receptor domain (ER) was driven by the
cardiomyocyte-specific α-myosin heavy chain (MHC) promoter\textsuperscript{4-6} or the CMV enhancer/chicken actin (CAG) promoter. We then crossed these Cre recombinase-expressing strains with mice that carried floxed Bdnf or TrkB alleles. To induce Cre-mediated recombination, the mutant mice received 10 mg/kg of tamoxifen (Sigma) intraperitoneally once a day for five consecutive days. Ten days after this treatment, mutant mice underwent surgery to induce MI as described above. There were two types of littermate controls, which were transgenic floxed mice without tamoxifen treatment and non-transgenic floxed mice with tamoxifen treatment. We also crossed nestin-Cre mice (a gift from Akihiko Yoshimura, Keio University, Tokyo, Japan) with floxed Bdnf mice to establish neuron-specific Bdnf knockout mice. C57/BL6 mice were purchased from SLC Japan (Shizuoka, Japan). Floxed Bdnf mice and CAG-Cre-ER mice (with a C57/BL6 background) were purchased from the Jackson Laboratory (Bar Harbor, ME). The generation and genotyping of floxed TrkB mice (a gift from Luis F. Parada, University of Texas Southwestern Medical Center, Dallas, TX) have been described previously.\textsuperscript{7} We intraperitoneally injected BDNF recombinant protein (50 µg/kg) on Day 0 and Day 1 after operation.

**Physiological and Histological Analysis**

Echocardiography was performed with a Vevo 770 High Resolution Imaging System (Visual Sonics Inc, Toronto, Ontario, Canada). The heart rate was kept at approximately 500-600 beats per minute to minimize data deviation when cardiac function was assessed by area length methods. Frozen cross-sections (4 µm) of the heart were fixed with 4% paraformaldehyde and subjected to Masson trichrome staining or immunohistochemistry. The extent of fibrosis was measured in 3 sections from each heart and the value was expressed as the ratio of the Masson’s trichrome-stained area length to the total LV free wall length. Revascularization was assessed by measuring the number of capillary endothelial cells in microscopic sections taken from the border zone.
of each heart at 2 weeks after MI. Capillary endothelial cells and TrkB-positive cardiomyocytes were identified by immunohistochemical staining with anti-CD31 antibody (Pharmingen, San Jose, CA) or anti-TrkB antibody (BD Transduction Laboratories, Franklin Lakes, NJ), respectively. For cell death analysis the hearts were fixed with 10% formalin, embedded in paraffin, sectioned, and were subjected to TUNEL labeling according to the manufacturer's protocol (In Situ Cell Death Detection Kit, Fluorescein, Roche) in combination with immunohistochemical staining for cell markers. Photographs were taken in the border zone of the infarcted region, and 5 high-power fields (HPF) from each sample were chosen and quantified in a blinded manner. In situ hybridization for Bdnf was performed on frozen coronal brain sections (Genostaff, Tokyo).

**Statistical analysis**

Data are shown as the mean ± SEM. Comparison among multiple groups was performed by ANOVA with a post-hoc Fisher’s test, while comparisons between two groups were done with the unpaired Student’s t-test. A p value<0.05 was considered statistically significant.
References


Supplemental Figure legends

Supplemental Figure I Vascularization of the border zone of the infarcted region

The number of capillary endothelial cells were estimated by immunohistochemical staining with anti-CD31 antibody in the border zone of the MI heart of MHC-MerCreMer; TrkB<sup>flox/flox</sup> mice (A) and MHC-MerCreMer; Bdnf<sup>flox/flox</sup> mice (B). *P<0.05; n=4. Data are shown as the mean ± s.e.m.

Supplemental Figure II Effect of capsaicin (CAP) treatment

(A) Expression of calcitonin gene-related peptide (CGRP), a maker for sensory afferent nerves, in sympathetic trunk of vehicle-treated (CAP–) or capsaicin-treated mice (CAP+) was identified by immunohistochemistry with anti-CGRP antibody (upper panel, brown). Expression of tyrosine hydroxylase (TH), a maker for sympathetic efferent nerves, was also examined by immunohistochemistry with anti-TH antibody (lower panel, brown). Scale bar=20 µm. (B) The number of CD31-positive cells was estimated in the border zone of the MI heart of vehicle-treated (CAP–) or capsaicin-treated mice (CAP+). (C) Echocardiographic analysis and Masson’s trichrome staining of vehicle-treated (CAP–) or capsaicin-treated mice (CAP+) after MI with or without peripheral administration of Bdnf. Scale bar=1 mm. *P<0.05; n=4 for A, B; n=5 for C. Data are shown as the mean ± s.e.m.

Supplemental Figure III Measurement of plasma Bdnf levels

Plasma Bdnf levels were measured in control littermates (Control) and nestin-Cre; Bdnf<sup>flox/flox</sup> mice after sham operation (Sham) or MI, with or without peripheral administration of Bdnf. **P<0.01; n=5. Data are shown as the mean ± s.e.m.
Supplemental figure 1

A

Control
MHC-MerCreMer; TrkB flox/flox

CD31-positive cells

Control
MHC-MerCreMer; TrkB flox/flox

B

Control
MHC-MerCreMer; Bdnf flox/flox

CD31-positive cells

Control
MHC-MerCreMer; Bdnf flox/flox

n.s.
Supplemental figure 2

A

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- 0 – +CAP
- 5
- 10
- 15

stained area (%)

- 0
- 5
- 10
- 15

stained area (%)

B

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CD31-positive cells

- 0
- 20
- 40
- 60
- 80
- 100

C

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- 0
- 10
- 20
- 30

- 0
- 20
- 40
- 60
- 80
Supplemental figure 3

![Bar graph showing plasma Bdnf (pg/ml) levels in different groups: Sham MI, Control, Nestin-Cre; Bdnf flox/flox, MI + Bdnf. Each group contains bars for Sham and MI, with the MI + Bdnf group showing a significantly higher level of Bdnf indicated by **.](image-url)
뇌-유래 신경영상인자는 심근경색 후 유발되는 심장기능 저하를 줄이는 보호효과가 있다.

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Summary

배경
중추신경계는 말초조직으로부터 유발되는 체액성, 신경성 신호들에 대한 반응을 통해서 심혈관계 조절에 영향을 미치는 것으로 여겨지고 있지만, 아직까지 이에 대한 분자생물학적 기전에 대해서는 알려져 있지 않다. 저자들은 뇌에서 분비되는 뇌-유래 신경영상인자(brain-derived neurotrophic factor, BDNF)가 심근경색 후 심장재형성에 대한 보호효과가 있는지, 있다면 이 과정에 중추신경계가 관여하는지를 알아보고자 하였다.

방법 및 결과
Cre-loxP system을 이용하여 BDNF의 발현이 감소된 BDNF 유전자제거 생쥐(knockout mice)를 만든 후, 관상동맥을 결찰하여 급성심근경색을 유발시켰다. 심근경색 유발 2주 후, BDNF 유전자제거 생쥐의 경우 정상 대조군에 비해 심장의 수축기능이 감소하였고, 심장도 더 커졌으며, 생존관련인자의 감소와 함께 심근세포사도 증가하였다. 뿐만 아니라 심장의 BDNF 수용체(tropomyosin-related kinase B, TrkB)를 제거하였을 경우 심근경색 후 심장수축기능의 저하가 더 심하게 나타났다. 심근경색 후 혈장 내 BDNF 수치는 증가하였고, 이러한 증가는 뇌에서의 BDNF 발현증가와 연관성이 있었으며, 심장과는 연관성이 없었다. 심장으로부터의 들신경(afferent nerve)을 절제해 중추신경으로의 신호를 제거하여 중추신경반응을 유발시키지 않거나 혹은 유전적으로 뇌에서의 BDNF 발현을 파괴하였을 때는 심근경색 후 관찰되었던 혈장 BDNF의 증가는 억제되었고, 심장기능은 더욱 악화되었다. 말초혈액에 BDNF를 주입한 경우는 뇌의 BDNF 결핍 생쥐에서 관찰되었던 이상의 심장 표현형으로부터 회복되는 양상을 보였다.

결론
이상의 결과들은 심근경색 후 손상된 심장으로부터 유발되는 신경성 신호에 의해 뇌에서 BDNF 과발현이 유발되고 이는 허혈성 손상으로부터 심장을 보호하며, 이 과정에 중추신경계가 관여한다는 것을 시사한다.
BDNF는 뇌에서 처음 발견되어 보고되었고, 태아와 성인의 뇌에 중요한 신경영양인자로서 neurotrophin family의 일종으로 알려져 있다. BDNF는 TrkB와 결합한 후 다양한 세포 내 신호전달 체계를 활성화하여 신경발달과 기능에 중요한 역할을 한다고 알려져 있다.

최근 심혈관계의 발달에도 BDNF가 관여한다는 증거들이 점차 늘고 있다. BDNF 결핍 생쥐는 심방 중격결손과 함께 내피세포의 부착기능 손상과 연관된 광범위한 심근 내 혈종으로 사망한다는 것이 보고되었다. 또한 수용체인 TrkB의 전사활성화 (transcriptional activation)는 관상동맥의 발달과 관여한다는 것이 보고되었다. 하지만 BDNF의 저산소중에 의해 유발되는 손상에 대해 신생혈관의 형성을 촉진하며, 혈관혈형성에 관여하는 조혈모세포의 동원과 함께 모세혈관형성도 증가시키는 것으로 보고되어, 혈관의 통합성 유지에 중요한 역할을 할 가능성이 대두되었다.

그러나 심근경색의 병태생리에 BDNF가 관여하는지에 대해서는 알려져 있지 않았는데, 이번 연구는 BDNF의 전신적 제거 혹은 TrkB의 제거 생쥐를 이용한 심근경색모델을 통해 BDNF-TrkB가 심장 보호효과에 직접 관여함을 보여주었다. 즉, 심근경색 후 손상된 심장으로부터 유발되는 맥초경성 신호(cardiac afferent nerve)에 의해서 BDNF 과발현이 뇌에서 유발되고 이로 인해 증가된 혈장 BDNF는 심장의 수축기능이상을 줄여서 결국 허혈성 손상으로부터 심장을 보호하며, 이 과정에서 중추신경계가 관여한다는 것이다.

이 결과는 심부전에서 알려진 기존의 맥초로부터의 체액성 신호에 대한 중추신경계 역할처럼 심근경색 후 심장의 재형성과정 및 심장기능의 악화에서도 맥초로부터의 신경성 신호를 통한 중추신경계 활성화가 중요한 역할을 한다는 것을 보여준 것이다. 특히 뇌하수체의 paraventricular nucleus는 심부전에 의해 나타나는 여러 맥초 신호를 감지할 수 있다는 보고가 있으며, 이 부위의 신경 활성화는 칼슘의 존재 신호와 신화 스트레스를 증가시킨다는 보고도 있다. 또한 BDNF는 칼슘의존성 신호와 신화 스트레스에 대한 반응으로 나타날 수 있는 칼슘-반응인자, NFKb의 활성에 의해서 조절된다는 보고도 있다. 최근 운동을 하면 인체와 생쥐의 뇌에서 BDNF가 증가되며, 고혈당이 BDNF의 뇌에서부터의 혈중 유리를 줄이는 것도 인체에서 보고되었다.

심근경색 후 유발되는 맥초경성 신호에 의한 뇌에서 BDNF 과발현이 허혈성 손상으로부터 심장을 보호한다는 이번 연구결과와 함께 전술한 BDNF의 여러 보고들을 같이 고려하면, BDNF가 심근경색 환자에서 새로운 치료로 이용될 수 있을 가능성을 시사한다고 할 수 있겠다.

REFERENCES
Brain-Derived Neurotrophic Factor Protects Against Cardiac Dysfunction After Myocardial Infarction via a Central Nervous System–Mediated Pathway

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Objective—The central nervous system is thought to influence the regulation of the cardiovascular system in response to humoral and neural signals from peripheral tissues, but our understanding of the molecular mechanisms involved is still quite limited.

Methods and Results—Here, we demonstrate a central nervous system–mediated mechanism by which brain-derived neurotrophic factor (BDNF) has a protective effect against cardiac remodeling after myocardial infarction (MI). We generated conditional BDNF knockout mice, in which expression of BDNF was systemically reduced, by using the inducible Cre-loxP system. Two weeks after MI was induced surgically in these mice, systolic function was significantly impaired and cardiac size was markedly increased in conditional BDNF knockout mice compared with controls. Cardiomyocyte death was increased in these mice, along with decreased expression of survival molecules. Deletion of the BDNF receptor (tropomyosin-related kinase B) from the heart also led to the exacerbation of cardiac dysfunction after MI. The plasma levels of BDNF were markedly increased after MI, and this increase was associated with the upregulation of BDNF expression in the brain, but not in the heart. Ablation ofafferent nerves from the heart or genetic disruption of neuronal BDNF expression inhibited the increase of plasma BDNF after MI and led to the exacerbation of cardiac dysfunction. Peripheral administration of BDNF significantly restored the cardiac phenotype of neuronal BDNF-deficient mice.

Conclusion—These results suggest that BDNF expression is upregulated by neural signals from the heart after MI and then protects the myocardium against ischemic injury. (Arterioscler Thromb Vasc Biol 2012;32:1902-1909.)

Key Words: myocardial infarction ■ angiogenesis ■ heart failure ■ central nervous system

Myocardial infarction (MI) is the most common cause of cardiac morbidity and mortality in many countries, and left ventricular remodeling after MI is an important phenomenon because it causes progression to heart failure. Although numerous therapeutic strategies have been developed, death from heart failure is still a serious problem worldwide. Brain-derived neurotrophic factor (BDNF) was originally discovered in the brain as a member of the neurotrophin family, and is known to have neurotrophic functions that are crucial in both the embryonic and adult brain. BDNF specifically binds to the tropomyosin-related kinase receptor B (TrkB) and activates many intracellular signaling pathways, thereby affecting the development and function of the nervous system. BDNF-deficient mice develop atrial septal defects and also die from diffuse intramyocardial hemorrhage attributable to impaired endothelial adhesion. It has been reported that transcriptional activation of TrkB is crucial for coronary vessel development. The previous studies have revealed that BDNF acts on endothelial cells and promotes neovascularization in response to hypoxic stimuli via the Akt pathway. In addition to a direct action on local endothelial cells, BDNF enhances capillary formation by recruiting proangiogenic hematopoietic cells, indicating that it has a critical role in maintaining the integrity of the vascular system in adults. However, it remains unclear whether BDNF is involved in the pathophysiology of adult cardiac diseases such as MI.

In the present study, we demonstrate a cardioprotective role of the BDNF–TrkB axis by using a variety of genetic mouse models. Systemic deletion of BDNF or disruption of TrkB in

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the heart led to exacerbation of cardiac dysfunction after MI. The plasma levels of BDNF were markedly increased after MI, and this increase was associated with the upregulation of BDNF expression in the brain. Ablation of afferent nerves from the heart or genetic disruption of neuronal BDNF expression inhibited the increase of plasma BDNF after MI and led to exacerbation of cardiac dysfunction, suggesting a central nervous system–mediated mechanism by which BDNF has a protective effect against cardiac remodeling after MI. These findings also suggest that the activation of neuronal BDNF may become a therapeutic strategy for cardiac disease.

**Materials and Methods**

**Animal Study**
The animal experiments were approved by our institutional review board. All mice used in this study were males between 12 and 14 weeks old unless otherwise indicated. All experimental procedures were performed according to the guidelines established by Chiba University for animal experiments. Mice were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg), and MI was induced by ligation of the left anterior descending artery as described previously. Generation of conditional knockout mice is described in the online-only Data Supplement.

**Physiological and Histological Analysis**
Echocardiography was performed with a Vevo 770 High-Resolution Imaging System (VisualSonics Inc., Toronto, Ontario, Canada). The heart rate was kept at 500 to 600 bpm to minimize data deviation when cardiac function was assessed by area length methods. Frozen cross-sections (4 µm) of the heart were fixed with 4% formaldehyde and subjected to Masson trichrome staining or immunohistochemistry.

**Western Blot Analysis**
The lysates were resolved by SDS-PAGE. Proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA), which was incubated with the primary antibody followed by anti-rabbit or anti-mouse IgG conjugated with horseradish peroxidase (Jackson, West Grove, PA).

**RNA Analysis**
Real-time polymerase chain reaction was performed by using the Light Cycler (Roche) with the Taqman Universal Probe Library and the Light Cycler Master (Roche) according to the manufacturer’s instruction.

**Cell Culture**
Neonatal Wistar rats were purchased from Takasugi Experimental Animal Supply (Tokyo, Japan). Cardiomyocytes were prepared from these neonatal rats and cultured as described previously.

**Statistical Analysis**
Data are shown as the mean±SEM. Comparison among multiple groups was performed by ANOVA with a post hoc Fisher test, whereas comparisons between 2 groups were performed with the unpaired Student t test. A P value <0.05 was considered statistically significant.

**Results**

**BDNF and TrkB Are Expressed by Cardiomyocytes and Activate the Intracellular Signaling Pathways**
To investigate the role of BDNF in the adult heart, we first examined the expression of BDNF and its receptor TrkB in cardiomyocytes. Western blot analysis showed that both BDNF and TrkB were expressed by cardiomyocytes (Figure 1A). It has been reported that binding of BDNF to TrkB triggers receptor dimerization and autophosphorylation, followed by the activation of diverse signaling cascades such as the Akt and mitogen-activated protein kinase pathways in neuronal cells. Consistent with these reports, treatment of cardiomyocytes with BDNF rapidly activated these signaling cascades (Figure 1B). It was also reported that BDNF exerts a protective effect against neuronal death induced by various stresses such as hypoxia. We therefore exposed cardiomyocytes to hypoxia and examined the influence of BDNF on cell viability. As was the case for neurons, treatment with BDNF decreased hypoxic cardiomyocyte death (Figure 1C and 1D), and this effect was associated with increased expression of prosurvival molecules (Figure 1E). These results indicate that BDNF promotes the survival of cardiomyocytes by activating intracellular signaling pathways via TrkB.

**Systemic Deletion of BDNF Leads to Exacerbation of Cardiac Dysfunction After MI**
To further investigate the role of BDNF in the heart, we generated conditional BDNF knockout mice using the inducible Cre-loxP system. We prepared transgenic mice in which a transgene encoding Cre recombinase fused to the mutated estrogen receptor domain (Cre-ER) was driven by the cytomegalovirus enhancer/chicken actin (CAG) promoter. We then crossed these CAG-Cre-ER mice with mice bearing floxed Bdnf alleles (CAG-Cre-ER; Bdnflox/lox mice). In this model, BDNF expression was reduced in various tissues, including the heart and the brain, by treatment with tamoxifen (Figure 2A). Consequently, plasma BDNF levels were significantly lower in CAG-Cre-ER; Bdnflox/lox mice than in their control littermates (Figure 2B). CAG-Cre-ER; Bdnflox/lox mice had a normal appearance and viability (data not shown). We then produced MI in these mice and performed physiological and histological examinations at 2 weeks after surgery. Echocardiography revealed that the left ventricular diastolic dimension was increased and the left ventricular ejection fraction was decreased in CAG-Cre-ER; Bdnflox/lox mice compared with control littermates (Figure 2C). These mice also showed a larger fibrotic area than their control littermates (Figure 2D). Histological examination demonstrated that the number of terminal deoxynucleotidyl transferase -mediated dUTP nick-end labeling-positive cardiomyocytes was significantly increased in CAG-Cre-ER; Bdnflox/lox mice (Figure 2E). This increase was associated with impaired vascularization of the border zone of the infarcted region in CAG-Cre-ER; Bdnflox/lox mice after MI (Figure 2F). Indeed, the expression of the proapoptotic molecule Bax was increased, whereas the expression of the prosurvival molecules such as Bcl-2 was decreased in the hearts of CAG-Cre-ER; Bdnflox/lox mice after MI (Figure 2G). The expression of the proapoptotic molecule Bax was increased, whereas the expression of the prosurvival factors.
Disruption of Cardiac TrkB But Not BDNF Exacerbates Systolic Dysfunction After MI

Because BDNF is known to predominantly bind to and activate TrkB, its cardioprotective effect after MI may be mediated by TrkB expressed on cardiomyocytes. To investigate the role of TrkB in the MI heart, we generated cardiomyocyte-specific TrkB conditional knockout mice by using the inducible Cre-loxP system. We prepared transgenic mice in which a transgene encoding Cre recombinase fused to the mutated estrogen receptor domains (MerCreMer) was driven by the cardiomyocyte-specific α-myosin heavy chain (MHC) promoter. We then crossed these MHC-MerCreMer mice with mice bearing floxed TrkB alleles and produced MI in these mice (MHC-MerCreMer; TrkBflox/flox mice). In this model, treatment with tamoxifen induced Cre-mediated recombination of floxed TrkB alleles in cardiomyocytes, resulting in specific ablation of cardiac TrkB. Although the basal expression of TrkB was undetectable in the heart, its expression was markedly upregulated after MI in wild-type mice (Figure 3A). This upregulation was attenuated in the heart of MHC-MerCreMer; TrkBflox/flox mice (Figure 3A). Similarly, the expression of TrkB was strikingly increased in the border zone of the infarcted region in control littermates, but not in MHC-MerCreMer; TrkBflox/flox mice (Figure 3B). These results indicate that myocardial ischemia mainly upregulates TrkB expression in cardiomyocytes. MHC-MerCreMer; TrkBflox/flox mice had a normal heart size and systolic function under basal conditions (Figure 3C). Two weeks after MI, however, these mice had slightly larger left ventricular diastolic dimension than control littermates (Figure 3C). Systolic function was significantly decreased in MHC-MerCreMer; TrkBflox/flox mice compared with their control littermates (Figure 3C). These mice also had a larger fibrotic area with more terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive cardiomyocytes than their control littermates (Figure 3D and 3E), indicating a crucial influence of TrkB on cardiomyocytes in the heart after MI. An increase in cardiomyocyte death was associated with impaired vascularization of the border zone of the infarcted region in MHC-MerCreMer; TrkBflox/flox mice (Figure IA in the online-only Data Supplement), suggesting a crucial role of TrkB on cardiomyocytes in inducing angiogenesis in the MI heart.

To determine the role of BDNF in the MI heart, we generated cardiomyocyte-specific BDNF conditional knockout mice by using the inducible Cre-loxP system (MHC-MerCreMer; Bdnfflox/flox mice). We created MI in these mice and then performed echocardiography and histological examination. MHC-MerCreMer; Bdnfflox/flox mice had a normal heart size and systolic function under basal conditions (Figure 4A). In contrast to MHC-MerCreMer; TrkBflox/flox mice, ablation of BDNF in cardiomyocytes did not affect cardiac remodeling.

Figure 1. Expression of BDNF/TrkB and BDNF-evoked signal transduction. A, Western blot analysis of BDNF and TrkB expression in cardiomyocytes (CM) and the brain. B, BDNF (100 ng/mL) induces phosphorylation and activation of Akt (pAkt) and ERK (pERK) in a time-dependent manner in cultured CM. C and D, CM infected with the mock vector (control) or the BDNF (Bdnf) expression vector were exposed to hypoxia (1%) for 12 hours. Then the number of viable cells was estimated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay (C) and by trypan blue exclusion (D). E, RNA was extracted from CM prepared as described in C and examined for Bcl-2 and Bax expression by real-time polymerase chain reaction. *P<0.05, **P<0.01; n=5 for A, B, and C; n=8 for D and E. Data are shown as the mean±SEM. BDNF indicates brain-derived neurotrophic factor; TrkB, tropomyosin-related kinase receptor B; pERK, phosphorylated extracellular signal-regulated protein kinases.
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and systolic dysfunction after MI (Figure 4A). Moreover, cardiac ablation of BDNF did not attenuate vascularization of the border zone of the infarcted region (Figure 1B in the online-only Data Supplement) nor did it increase infarct size (Figure 4B). We found that the cardiac expression of BDNF was markedly downregulated after MI (Figure 4C), whereas plasma BDNF levels were significantly increased in wild-type controls (Figure 5A). These results suggest that tissues outside the heart increase production of BDNF after MI and that circulating BDNF protects the heart against ischemic injury.

Ablation of Afferent Nerves From the Heart Leads to Exacerbation of Cardiac Dysfunction After MI

Given the important role of BDNF in the nervous system, we hypothesized that BDNF derived from the brain might contribute to its protective effect on the heart after MI. Consistent with this notion, the expression of BDNF was significantly increased in the forebrain including the thalamus and the hypothalamus after MI (Figure 5B and 5C). This increase was abolished in the brain of CAG-Cre-ER; Bdnflox/flox mice (systemic deletion model; Figure 5B). Similarly, plasma BDNF levels after MI were significantly lower in CAG-Cre-ER; Bdnflox/flox mice than in wild-type controls (Figure 5A). These results led us to propose a novel mechanism whereby the brain senses ischemia in the heart and increases production of BDNF, after which circulating BDNF in turn protects the heart against ischemic injury.

There is evidence that cardiac afferent nerve fibers convey mechanosensitive and chemosensitive information to the brain, leading to the activation of various signaling pathways in the CNS. Activation of these CNS pathways has been suggested

Figure 2. Cardioprotective role of BDNF after MI. A, Expression of BDNF was examined in the hearts and brains of control littermates (control) and CAG-Cre-ER; Bdnflox/flox mice by Western blot analysis. B, Plasma BDNF levels were measured in control littermates (control) and CAG-Cre-ER; Bdnflox/flox mice by ELISA. C, Echocardiographic analysis of control littermates (control) and CAG-Cre-ER; Bdnflox/flox mice. D, Masson trichrome staining shows that the area of fibrosis is larger in CAG-Cre-ER; Bdnflox/flox mice than in control littermates (control). Scale bar=1 mm. E, Double staining of the heart for terminal deoxynucleotidyl transferase -mediated dUTP nick-end labeling (TUNEL) (green) and lectin (red) after MI. Nuclei were stained with DAPI (blue). Scale bar=40 µm. The right graph indicates the number of TUNEL-positive cardiomyocytes in the MI heart. F, Capillary endothelial cells were identified by immunohistochemical staining with anti-CD31 antibody (brown) in the border zone of the myocardium after MI. Scale bar=40 µm. G, Western blot analysis for cardiac expression of Bax, Bcl-2, and VEGF in control littermates (control) and CAG-Cre-ER; Bdnflox/flox mice. *P < 0.05, **P < 0.01; n=5 for B and E; n=7 for C, D, and F. Data are shown as the means±SEM. Sham indicates sham operation; MI, myocardial infarction; LVDd, left ventricular diastolic dimension; LVEF, left ventricular ejection fraction; BDNF, brain-derived neurotrophic factor; VEGF, vascular endothelial growth factor; CAG, chicken actin promoter.
to play an important role in regulating cardiac function after MI.\textsuperscript{13,14} To determine whether such neuronal pathways convey stimuli from the MI heart to the brain, we treated the heart with capsaicin (CAP), an agonist for transient receptor potential vanilloid type 1 channel that is predominantly expressed on the terminals of primary sensory neurons.\textsuperscript{15} CAP treatment leads to an influx of cations by activating transient receptor potential vanilloid type 1 and thereby damages afferent sensory neurons.\textsuperscript{15,16} Histological examination revealed that treatment with CAP effectively ablated primary afferent neurons from the heart (Figure IIA in the online-only Data Supplement). We then produced MI in mice treated with CAP and examined the influence of ablation of cardiac afferent fibers. Treatment with CAP did not alter the neuronal expression of BDNF in sham-operated mice compared with untreated mice (Figure 5B). In contrast, neuronal expression of BDNF after MI was significantly reduced by treatment with CAP (Figure 5B). Similarly, ablation of cardiac afferent fibers inhibited the increase of neuronal expression of BDNF and the plasma BDNF levels after MI (Figure 5A). Echocardiography revealed that the left ventricular diastolic dimension was increased and the left ventricular ejection fraction was decreased in Nestin-Cre; Bdnf\textsuperscript{floxflo} mice compared with control littermates (Figure 6D). Nestin-Cre; Bdnf\textsuperscript{floxflo} mice were also hyperactive. In these mice, the neuronal expression of BDNF was markedly reduced and its plasma levels were significantly lower than in control littermates (Figure 6A and 6B). Moreover, upregulation of neuronal expression of BDNF and the plasma BDNF levels after MI did not occur in these mice (Figure 6B and 6C). Echocardiography revealed that the left ventricular diastolic dimension was increased and the left ventricular ejection fraction was decreased in Nestin-Cre; Bdnf\textsuperscript{floxflo} mice compared with control littermates (Figure 6D). Nestin-Cre; Bdnf\textsuperscript{floxflo} mice also had a larger area of myocardial fibrosis than untreated mice (Figure 5D). Peripheral administration of BDNF significantly restored the cardiac phenotype of CAP-treated mice (Figure IIC in the online-only Data Supplement). These results indicate that cardiac afferent fibers mediate upregulation of BDNF in the brain and that this upregulation protects the infarcted heart against ischemic injury.

**Figure 3.** Cardioprotective role of TrkB after MI. A, Expression of TrkB was examined in the hearts of control littermates (control) and MHC-MerCreMer; TrkB\textsuperscript{floxflo} mice by Western blot analysis. B, Immunohistochemistry for TrkB (brown) in the hearts of control littermates (control) and MHC-MerCreMer; TrkB\textsuperscript{floxflo} mice after MI. Scale bar=100 µm. C, Echocardiographic analysis of control littermates (control) and MHC-MerCreMer; TrkB\textsuperscript{floxflo} mice after sham operation (Sham) or MI. D, Masson trichrome staining of the heart after MI. Scale bar=1 mm. E, Double staining of the heart for terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) (green) and lectin (red) after MI. Nuclei were stained with DAPI (blue). Scale bar=40 µm. The right graph indicates the number of TUNEL-positive cardiomyocytes in the MI heart. *P<0.05, **P<0.01; n=7 for C, D, and E. Data are shown as the mean±SEM. Sham indicates sham operation; MI, myocardial infarction; LVDd, left ventricular diastolic dimension; LVEF, left ventricular ejection fraction; MHC, myosin heavy chain; TrkB, tropomyosin-related kinase receptor B.
fibrosis than control littermates (Figure 6E). Peripheral administration of BDNF significantly increased its plasma levels (Figure III in the online-only Data Supplement) and restored the cardiac phenotype of Nestin-Cre; Bdnflox/flox mice (Figure 6D and 6E), suggesting a crucial role of neuronal BDNF production in protection against cardiac dysfunction after MI.

**Discussion**

We have demonstrated a CNS-mediated mechanism involved in the regulation of cardiac function after MI. Our results suggest that ischemic insults are transmitted from the heart to the CNS via cardiac afferent nerve fibers after MI, thereby increasing the neuronal expression of BDNF. An increase
in circulating BDNF promotes the survival of cardiomyocytes and is associated with increased expression of prosurvival and proangiogenic factors. In agreement with our data, recent evidence has suggested the potential importance of humoral heart-brain signaling in the pathogenesis of heart failure.13,14 Accordingly, the CNS may function as a conductor that integrates input signals from a variety of sources, including the failing heart, which in turn leads to activation of certain CNS pathways. Peripheral responses elicited by CNS activation may play an important role in the progression or prevention of cardiac remodeling after MI. Several lines of evidence indicate that the forebrain, in particular the paraventricular nucleus of the hypothalamus, can sense signals generated peripherally in response to the stress of heart failure.13,18,19 Neuronal activation in this area has been shown to enhance calcium-dependent signals and increase oxidative stress.13,18,19 It has also been reported that the BDNF expression is regulated by various transcription factors such as the calcium-response factor, the nuclear factor of T cells, and the nuclear factor κB, which could respond to these changes.20 Moreover, Rasmussen et al.21 demonstrated a significant output of BDNF across the human and murine brain, which was further enhanced by exercise. Taken together with these reports, our results suggest that cardiac injury activates these CNS pathways, thereby increasing the cerebral output of BDNF.

It has been suggested that decreased neural production of BDNF is a common factor in neurodegenerative diseases such as Alzheimer disease and various psychiatric disorders including major depression,3 and several studies have demonstrated that patients with such diseases have low circulating levels of BDNF.3 It has also been reported that BDNF release from the brain is attenuated by hyperglycemia in humans and that reduced plasma BDNF levels are associated with impaired glucose metabolism.22 Neuropsychiatric diseases and metabolic disorders are highly prevalent among patients with MI and are associated with a significant increase in mortality.23,24 These reports led to the hypothesis that a decrease in BDNF may contribute to a poor prognosis among patients with ischemic heart disease and that BDNF-based therapy could be particularly effective for MI patients complicated by the above-mentioned disorders.

In conclusion, our results indicate that an intriguing mechanism underlying the cardioprotective effect of BDNF involves a CNS-mediated pathway and suggest that activation of this pathway could become a novel strategy for the treatment of cardiac dysfunction after MI.

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Disclosures

None.

References