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Nerve Growth Factor Decreases in Sympathetic and Sensory Nerves of Rats with Chronic Heart Failure

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Abstract

Nerve growth factor (NGF) plays a critical role in the maintenance and survival of both sympathetic and sensory nerves. Also, NGF can regulate receptor expression and neuronal activity in the sympathetic and sensory neurons. Abnormalities in NGF regulation are observed in patients and animals with heart failure (HF). Nevertheless, the effects of chronic HF on the levels of NGF within the sympathetic and sensory nerves are not known. Thus, the ELISA method was used to assess the levels of NGF in the stellate ganglion (SG) and dorsal root ganglion (DRG) neurons of control rats and rats with chronic HF induced by myocardial infarction. Our data show for the first time that the levels of NGF were significantly decreased ($P < 0.05$) in the SG and DRG neurons 6–20 weeks after ligation of the coronary artery. In addition, a close relation was observed between the NGF levels and the left ventricular function. In conclusion, chronic HF impairs the expression of NGF in the sympathetic and sensory nerves. Given that sensory afferent nerves are engaged in the sympathetic nervous responses to somatic stimulation (i.e. muscle activity during exercise) via a reflex mechanism, our data indicate that NGF is likely responsible for the development of muscle reflex-mediated abnormal sympathetic responsiveness observed in chronic HF.

Keywords

NGF; Sensory nerve; Sympathetic nerve; Myocardial infarction

Introduction

Congestive heart failure (HF) is a chronic condition that is characterized by impaired cardiac function that leads to a decrease in blood supply to metabolizing tissues. It is well known that sympathoexcitation plays a prominent role in disease progression [1]. Moreover, sympathoexcitation is inversely related to disease prognosis [2].

In general, exercise increases sympathetic nerve activity (SNA), an effect which in turn increases arterial blood pressure (BP), heart rate (HR), myocardial contractility and peripheral vascular resistance [3, 4]. Two mechanisms, namely central command and the exercise pressor reflex, evoke this exercise-induced increase in sympathetic activity. Central command postulates a parallel and simultaneous increase in sympathetic and alpha motoneuron discharge [5, 6]. The exercise pressor reflex postulates that afferents innervating skeletal muscles are activated by contraction-induced mechanical and metabolic stimuli to elicit a reflex increase in SNA [7–9].

In chronic HF, cardiovascular regulation with exercise is abnormal. Specifically, sympathetic tone to muscle is elevated [10, 11], renal vasoconstriction is enhanced [12–14] and the rise in muscle blood flow is attenuated in HF [15, 16]. The reduced blood supply to the kidney leads to excessive stimulation of renin secretion and inappropriate salt and water retention [12, 17], whereas the reduced skeletal muscle blood flow is an important contributor to exercise intolerance [18]. Heightened SNA seen as HF worsens is well correlated with mortality [19].

The exercise pressor reflex has been implicated as a mechanism by which circulatory control is dysregulated in individuals with chronic HF, thereby, contributing to a poor clinical outcome [19, 20]. The contribution of neurally mediated peripheral mechanisms to the evolution of abnormal cardiovascular responses to exercise in HF, however, is poorly understood.

In the periphery nerve growth factor (NGF), the prototypic member of a family of neurotrophins [21, 22], has important contributions to the maintenance and survival of both sympathetic and sensory neurons. In addition, prior studies suggest that the primary role played by NGF is to initiate and maintain hypersensitivity of sensory neurons after tissue injury or inflammation [23, 24]. This hypersensitivity by sensory neurons is attributed to either a lower threshold or an increased discharge or both in response to a given stimulus. Muscle afferent nerves (neurons) contribute to the SNA and pressor responses to muscle contraction via a reflex mechanism [9], and the abnormalities in this reflex response are observed in human and animals with HF [10, 12–14, 19, 20, 25, 26]. However, little is known about the role played by NGF in modifying the exercise pressor reflex during pathological states such as chronic HF.

Therefore, in the current report, we used a two-site immunoenzymatic assay (ELISA) method to assess the levels of NGF in sympathetic neurons-stellate ganglion (SG) nerves as well as sensory nerves-dorsal root ganglion (DRG) of control rats and rats with chronic HF. HF was induced by myocardial infarction (MI) following ligation of the coronary artery. We

further determined a relationship between the levels of NGF and the left ventricular function. Prior studies have demonstrated that there are significantly less levels of NGF in plasma of HF patients as well as in myocardial tissues of experimental animal models with HF [27, 28]. Thus, we hypothesized that NGF is specifically attenuated in both sympathetic and sensory nerves of rats after chronic HF.

Materials and Methods

All procedures outlined in this study were performed in compliance with the rules and regulations described in the National Institutes of Health Guide for *the Care and Use of Laboratory Animals*, and were approved by the Animal Care Committee of this institution.

Coronary Artery Ligation

Male Sprague–Dawley rats (160–200 g) were anesthetized by inhalation of an isoflurane-oxygen mixture (2–5 % isoflurane in 100 % oxygen), intubated, and artificially ventilated. A left thoracotomy between the fourth and fifth ribs was performed, exposing the left ventricular wall. The left coronary artery was ligated [25, 29, 30]. Age and body weight-matched rats that underwent the same procedure as described except that a suture was placed below the coronary artery but was not tied served as controls. The nerve tissues were taken for the measurements of NGF 6–20 weeks after the surgery.

Determination of Left Ventricular Function and Size of Infarction

Transthoracic echocardiography was performed 1–2 weeks before the experiments [25, 29, 30]. The rats were anesthetized by inhalation of an isoflurane-oxygen mixture. The transducer was positioned on the left anterior chest, and left ventricular dimensions were measured.

A Millar catheter was inserted into the right carotid artery and was threaded into the left ventricle for measurement of left ventricular end-diastolic pressure (LVEDP) before the nerve tissues were taken. The heart was excised after intravenous injection of an overdose of sodium pentobarbital (120 mg/kg) followed by 2 mL of a saturated solution of KCl, and myocardial infarct size was estimated. Briefly, the left ventricle was pressed flat. The circumference of the entire flat left ventricle and visualized infarcted area was outlined on a transparent paper sheet. The difference in weight between the two marked areas on the sheet was used to determine the size of MI that was expressed as percentage of left ventricle surface area.

Measurements of NGF

Twenty rats with severe HF, ten rats with mild HF and twelve sham control rats were deeply anesthetized and euthanized by inhalation of an isoflurane-oxygen mixture. The SGs and L4–L6 DRGs were removed quickly, weighed and frozen at –80 °C for NGF measurements. The levels of NGF were determined using the ELISA as previously described [31]. Note that the ELISA measurements were made on SGs and L4–L6 DRGs from individual animals. L4–L6 DRGs were selected because they innervate to the hind limb muscles of rats. Briefly, polystyrene 96-well microtiter immunoplates were coated with affinity-purified polyclonal

goat anti-NGF antibody (Promega Co.). Parallel wells were coated with purified goat IgG for evaluation of nonspecific signal. After overnight incubation at room temperature and 2 h of incubation with the coating buffer (50 mM carbonate buffer, pH 9.5, in 2 % BSA) plates were washed with 50 mM Tris HCl (pH 7.4; 200 mM NaCl; 0.5 % gelatin; and 0.1 % Triton X-100). After extensive washing, the diluted samples and the NGF standard solutions (Promega Co.), ranging from 0 to 1 ng/mL, were distributed in each plate and left at room temperature overnight. The plates were then washed and incubated with 4 milliunits of anti β -NGF-galactosidase per well (Boehringer Mannheim). After an incubation of 2 h at 37 °C, the plates were washed and incubated with 100 μ L of substrate solution (4 mg of chlorophenol red per ml of substrate buffer: 100 mM HEPES; 150 mM NaCl; 2 mM $MgCl_2$; 0.1 % sodium azide in 1 % BSA) that were added to each well. After an incubation of 2 h at 37 °C, the optical density was measured at 575 nm using an ELISA reader (Dynatech).

Statistics

The data of NGF measurements were analyzed using a two-way repeated-measure analysis of variance. As appropriate, Turkey post hoc tests were utilized. Values are presented as mean $\pm$ SE. For all analyses, differences were considered significant at $P < 0.05$. All statistical analyses were performed by using SPSS for Windows version 15.0 (SPSS, Chicago, IL).

Results

The General and Echocardiographic Measurements

These measurements are shown in Fig. 1. The left ventricular fractional shortening (LVFS) was determined by echocardiographic measurements. On the basis of FS data, the animals were specifically defined as control with FS > 40 %, mild HF with 30 % $< FS < 40$ %, and severe HF with FS < 30 %. Rats with severe HF showed increases in plasma norepinephrine (NE), LVEDP and the size of MI comprising the LV area.

NGF Levels Versus Time Courses After HF

Nerve growth factor (NGF) levels in the SG and DRG of control rats and rats with 6–20 weeks of severe HF induced by the coronary artery ligation were examined (Fig. 2). The levels of NGF tended to decline with extension of time. There were no significant differences in NGF levels among different time courses between control and chronic HF. However, the levels of NGF in the SG and DRG tissues were significantly decreased in HF rats as compared with control rats ($P < 0.05$).

NGF Levels Versus Left Ventricular Functions

As compared with mild and control rats, the levels of NGF were significantly lower in the SG and DRG tissues of rats with severe HF ($P < 0.05$, Fig. 3). In addition, a linear relationship analysis shows that there was a close relation between LVFS and NGF levels of the SG ($r = 0.65$, $P < 0.01$) and DRG tissues ($r = 0.76$ and $P < 0.01$, Fig. 4).

Discussion

In this report, our results demonstrate that the levels of NGF in sympathetic and sensory nerves remained lower 6–20 weeks after MI induced by ligation of the coronary artery. In particular, a close correlation was observed between the levels of NGF and the LV function. These data suggest that NGF in sympathetic and sensory nerves is impaired in HF and this effect begins ~6 weeks after induction of MI.

Cardiac-specific NGF over-expression in the mouse causes increases in sympathetic innervation density in the heart [32]. In contrast, sympathetic ganglia fail to develop in the NGF null mouse [33]. An influence of NGF on the sympathetic nervous system is also evident in adult animals. For instance, infusion of NGF into the left SG over several weeks in dogs leads to pronounced sympathetic neuronal growth and nerve sprouting in the heart [34].

In addition, NGF injection into the SG has been reported to improve NE reuptake in the failing heart [35]. The relation that exists between myocardial NGF and the sympathetic nerves of the heart seems to involve more than just neural support by the trophic factor. There is evidence of a feedback inhibition of cardiac myocyte production of NGF at high levels of NE. The production of NGF by cultured cardiac myocytes is inhibited by NE, and chronic intravenous infusion of NE in dogs reduces myocardial NGF content [36]. Thus, high sympathetic tone in an innervated organ presumably inhibits its NGF production. These observations may well explain the concurrence in the failing heart of high sympathetic nerve firing rates, low NGF content, and sympathetic neuronal rarefaction [35–37]. In this condition, there is an inexplicable degeneration of sympathetic nerves throughout the body, leading to the abnormalities in sympathetic control of NE manifested primarily as severe HF. In a prior study, we found exaggerated NE in the interstitium of hind limb muscles of chronic HF rats with activation of the sympathetic nerve [30]. Thus, it is well reasoned that the lower levels of NGF were observed in the SG and DRG nerves of HF rats in the present report.

As demonstrated by others' work, the cardiac sympathetic nerve density is largely regulated by the NGF expression and NGF is responsive to the levels of plasma NE and/or $\alpha 1$ adrenergic stimulation [38, 39]. This suggests that in HF long-exposure of high plasma NE concentration can cause NGF reduction and thereby the sympathetic fiber loss. In our current study, we have observed the higher levels of plasma NE and less levels of NGF in sympathetic neurons (nerves). Thus, it is well reasoned that cardiac sympathetic nerve density should be reduced in HF rats.

In addition, in the current study a main focus was to determine the relationship between the levels of NGF in sympathetic and sensory nerves and ventricular function indicated by FS in chronic HF. We have demonstrated the less NGF with the worse ventricular function. It is generally thought that worse cardiac function is likely to lead to a higher level of plasma NE and brain natriuretic peptide (BNP) as markers of HF. In another word, both NGF and NE/BNP levels are dependent on cardiac function and likely correlate each other.

Activation of thin fiber muscle afferent nerves increases SNA, BP and HR via a reflex muscle response [40, 41]. When capsaicin [agonist to transient receptor potential vanilloid type 1 (TRPV1)] is injected into the arterial blood supply of the dog hindlimb, BP rises by 20 %, an effect abolished by sectioning afferent nerves [42]. The muscle pressor response is likely due to the stimulation of both Group III and IV fibers since capsaicin stimulates 71 % of Group IV and 26 % of Group III dog hindlimb muscle afferent fibers [40]. In a prior study, we observed that when capsaicin is injected into the arterial blood supply of the hind limb muscles of rats, BP and HR increase and the effect is mediated via the engagement of TRPV1 receptors on sensory afferents [43]. Furthermore, BP and HR responses to arterial injection of capsaicin are attenuated in MI rats [25, 26], indicating that chronic HF impairs the reflex autonomic responses that are mediated by stimulation of metabolically sensitive muscle afferent nerves. This result is consistent with the previous results observed in human study [44].

A previous report has suggested that NGF can induce TRPV1 receptors expression in the sensory DRG neurons [45]. Also, our published work shows that the magnitude of currents with activation of TRPV1 is significantly increased with NGF infusion of the hindlimb muscles [46]. In contrast, findings of the prior study further suggest that neutralization of endogenous NGF by injection of specific NGF-antibody into inflammatory tissues attenuates expression and response of TRPV1 receptors [47]. Taken together, these data indicate that NGF is likely to contribute to attenuation in TRPV1 mediated-sympathetic nerve response in HF by decreasing TRPV1 receptors and their responsiveness.

Study Limitations

In the present report, we have specifically examined the levels of NGF in both sympathetic and sensory nerves and further demonstrated the relationship between NGF and cardiac function after chronic HF. However, how the reduced NGF level is responsible for the sympathetic responsiveness seen in rats with chronic HF needs additional studies. For example, a study to examine the correlation between the magnitude of the reflex responses and the NGF levels in the SG and DRG neurons in control rats and HF rats will better address this issue. Also, to examine the effect of some interventions (i.e. administration or neutralization of NGF in the hind limb muscles) on the reflex responses in HF will address this issue.

In conclusion, our data demonstrate that chronic HF significantly decreases the levels of NGF in the SG and DRG nerves as compared with control. Also, NGF response to chronic HF is closely related with the LV function. The worse function of the LV induces less NGF in sympathetic and sensory nerves. Overall, the evidence of our study provides strong support for the proposition that NGF regulation in muscle metabolic receptor changes contributes to the abnormal SNA in chronic HF.

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Fig. 1.

a Showing the levels of plasma norepinephrine (NE), left ventricular end-diastolic pressure (LVEDP) and infarction size. Representative myocardial infarct is also demonstrated in this panel. **b** Showing data of echocardiographic measurements. Also, M-mode images are illustrated in this panel. Values are mean $\pm$ SE. * $P < 0.05$ versus control and mild HF. LVDD left ventricular diastolic dimension, LVSD left ventricular systolic dimension, and LVFS left ventricular fractional shortening. The number of rats = 12 in control; 10 in mild

HF; and 20 in severe HF. Note that NE data were obtained from six control rats, six rats with mild HF and ten rats with severe HF

Fig. 2.

NGF levels (pg/mg wet weight) in stellate ganglion (SG) and dorsal root ganglion (DRG) tissue of control rats, and rats with chronic heart failure (HF) at different time course. * $P < 0.05$, versus control. There was no significant difference in NGF levels at different time course after ligation of the coronary artery. 3–5 control rats and 6–8 HF rats were used in each group

Fig. 3.

NGF levels (pg/mg wet weight) in stellate ganglion (SG) and dorsal root ganglion (DRG) tissue of control rats, and rats with mild and severe chronic heart failure (HF). Severe HF induced a significant decrease in NGF of SG and DRG. * $P < 0.05$, versus control and mild HF. The number of rats = 12 in control; 10 in mild HF; and 20 in severe HF

Fig. 4.

There is a liner relationship between NGF and left ventricular function indicated by the left ventricular fractional shortening. The number of rats = 12 in control; 10 in mild HF; and 20 in severe HF