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Alteration of nerve growth factor in dorsal root ganglia at early time of acute myocardial infarction and the role of spinal nerve afferents

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HIGHLIGHTS

- NGF is expressed in DRG neurons innervating hearts.
- Spinal afferents exert tonic regulation of expression of NGF in DRG.
- NGF and its mRNA are up-regulated in DRG in myocardial infarction.
- Noxious afferents of myocardial infarction were associated with increase of NGF.

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ABSTRACT

Nerve growth factor (NGF) plays important roles in transmission of nociception, neural innervation and survival in sympathetic and sensory neurons. Evidence indicates that NGF may sensitize the sensory and sympathetic reaction upon noxious stimulation. Understanding of the alterations of NGF in the sensory neurons during acute myocardial infarction and the underlying mechanism may promote clinical prognosis of the pathology. The aim of the study was to investigate the changes of NGF in the sensory neurons innervating the heart at early time after myocardial infarction and potential role of afferent nerve signals in modulation of NGF. The myocardial infarction was induced by ligation of the left anterior descending branch of coronary artery in anesthetized rats. The expressions of NGF and its coding mRNA in the sensory neurons in the dorsal root ganglia of upper thoracic segments (1–5), with and without prior blockade of the spinal nerves, were examined using immunohistochemical assay, enzyme-linked immunosorbent assay and real-time quantitative reverse transcription polymerase chain reaction assay. It was found that the immunoreactive material for NGF was significantly increased in the ganglia ($P < 0.05$) at 60 min of myocardial infarction, without change in NGF mRNA before and after the time. Blockade of the spinal nerves obviously inhibited the expression of NGF ($P < 0.05$) and the coding mRNA ($P < 0.01$). The results may indicate that the spinal nerve afferents are important in sustaining and up-regulating the expression of NGF in the sensory neurons innervating the heart in acute myocardial infarction.

1. Introduction

Nerve growth factor (NGF) in sensory neurons plays important roles in neural survival [13,20] and innervation [7,8]. NGF is also an important regulator sensitizing the sensory neurons, responding to noxious stimulation [14,16–18] or inflammation [19], and sensitizing the sympathetic reflex activity [10]. The features of NGF may be critical to the pathologic progress of acute myocardial infarction (MI), which is associated with re-balance or imbalance of the neural activities and survival of cardiac autonomic and sensory nerves [7,8,10,13,14,16–18,20]. It was reported, by this group, that cardiac sensory nerves may play an important role of cardioprotection in MI [22] via interaction with cardiac sympathetic activity [11,23]. Therefore alteration of NGF in the sensory neurons innervating the heart and the mechanism modulating the alteration during MI may be important to the development of the pathology, the prognosis and the remodeling of the infarct heart and the cardiac functions [1,2,7].

In a recent study, we found that acute myocardial infarction caused increase of NGF in the myocardium at risk of ischemia throughout the 60 min of coronary artery occlusion, in which spinal

Abbreviations: CAO, coronary artery occlusion; DRG, dorsal root ganglia; ECG, electrocardiogram; ELISA, enzyme linked immunosorbent assay; FIHC, fluorescence immunohistochemical; MI, myocardial infarction; NGF, nerve growth factor; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction.

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nerves innervating the heart play an important role of enhancement of the up-regulation of NGF [21]. To further answer the questions, what a change of NGF occurs in the innervating sensory neurons during MI and what a role of spinal afferent signals originating from the heart plays in the alteration, here we report the change of NGF in the sensory neurons of the dorsal root ganglia (DRG) in the upper thoracic segments (1–5), innervating the heart [4,6,7,15] and its potential association with afferent signals including the noxious neural afferents evoked by acute myocardial infarction. In current study, the difference of NGF in the DRG with and without blockade of the spinal nerves during the first 6 h of acute myocardial infarction (in the same sets of experiments) was examined, as we recently reported [21], with a rodent model of coronary artery occlusion (CAO).

2. Methods

The experiments were conformed to the guidelines for the care and use of laboratory animals (National Institute of Health Guide for the Care and Use of Laboratory Animals, NIH Publications No. 80-23, revised 1996) and approved by the Institutional Animal Care and Use Committee of Shanxi Medical University.

2.1. Protocol

The protocol of the experiments was the same as we previously reported [21]. The expression of NGF in the neurons of the dorsal root ganglia at the upper thoracic segments (1–5) was investigated using fluorescence immunohistochemical assay (FIHC). To study the temporal variation and the mechanism of NGF expression, in the sensory neurons of the dorsal root ganglia at the early times of myocardial infarction induced by ligation of left anterior descending branch of coronary artery, we quantitatively analyzed the expressions of the NGF protein at 15, 30, 60 and 360 min after acute myocardial infarction using enzyme linked immunosorbent assay (ELISA) and the encoding mRNA at 15 and 360 min of the myocardial infarction using the real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

The blockade of spinal nerves was introduced in this study as an intervention factor. After successful implantation of an epidural catheter, one hundred and two rats were divided into three groups: sham surgery group (sham), group of myocardial infarction (CAO), group of thoracic epidural anesthesia plus myocardial infarction (EA). Each group was further divided into four subgroups, according to duration of the myocardial infarction, 15, 30, 60 and 360 min (Table 1). In each subgroup, six animals were used for ELISA. FIHC was carried out at the time when high expressions of NGF were shown by ELISA in CAO group ($n=3$) and sham controls ($n=3$) respectively. Four animals were respectively assigned to each of the two subgroups for qRT-PCR assay at 15 and 360 min after the CAO (Table 1). The myocardial infarction was induced at 15 min after epidural injection of either 20 μl of 1% ropivacaine for the animals in the EA group or the same volume of 0.9% saline for the rats in the CAO and sham surgery groups, respectively. The blood pressures and heart rate were monitored via a cannula inserted in left carotid artery of the animals and the CAO was confirmed by changes in the ECG and by autopsy.

2.2. Epidural catheterization

The procedure for epidural catheterization was the same as previously reported [5,21]. After recovery from the surgery and anesthesia for 48 h, the animals exhibiting any sign of neurological impairment were excluded from the study. A successful implantation of epidural catheter was testified by detection of reversible segmental loss of response to noxious stimulation in thoracic segments (T1–T8) without motor disturbance in hind limbs following injection of 20 μl of 1% lidocaine through the catheter.

2.3. Acute myocardial ischemic model

The experimental model of acute myocardial infarction was prepared as we previously reported [4,5,21]. Briefly, the rat's pericardium was opened through an incision in the left fourth intercostal space under general anesthesia and mechanical ventilation. The ligation of the left anterior descending branch of the coronary artery was performed in animals of CAO and EA groups, while the rats in the sham surgery group underwent the same surgery as described above except without the ligation procedure.

2.4. FIHC assay

The bilateral DRG at the level of thoracic 1–5 segments were removed as scheduled, processed and embedded in optimal cutting temperature (OCT; Bioportfolio, Dorset, UK). Sections were cut at 8 μm on a cryostat (Leica CM 1850, Nussloch, Germany) and mounted on superfrost slides. The FIHC method was the same as previously reported [21].

2.5. ELISA

The samples of the spinal dorsal root ganglia at the upper thoracic segments (T_1–T_5) were quickly removed as scheduled. The dorsal root ganglia from each animal were pooled and analyzed separately in the study. The concentration of NGF was determined using the NGF Emax Immuno-Assay kit (Promega, WI, USA) as previously reported [21]. The results were presented as picograms per gram of total sample protein (TP) ($pg\,g^{-1}TP$). All samples were assayed in duplicate. (please read Supplemental data for the details).

2.6. qRT-PCR

The changes in the number of copies of NGF mRNA were examined at 15 and 360 min after coronary artery ligation or sham surgery, using the qRT-PCR assay as previously reported [4,21]. (please read Supplemental data for the details).

Table 1
Protocol of the study.

Experiments	Adult male Sprague-Dawey rats											
	Sham				CAO				EA + CAO			
	15 min	30 min	60 min	360 min	15 min	30 min	60 min	360 min	15 min	30 min	60 min	360 min
ELISA	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$
QRT-PCR	$n=4$	–	–	$n=4$	$n=4$	–	–	$n=4$	$n=4$	–	–	$n=4$
FIHC	–	–	$n=3$	–	–	–	$n=3$	–	–	–	–	–

ELISA, enzyme linked immunosorbent assay; qRT-PCR, real-time quantitative reverse transcription-polymerase chain reaction; FIHC, fluorescence immunohistochemical assay; sham, sham surgery group; CAO, coronary artery occlusion group; EA + CAO, prior blockade of spinal nerves plus coronary artery occlusion.