British Journal of Anaesthesia **109** (3): 345–51 (2012)
Advance Access publication 24 May 2012 · doi:10.1093/bja/aes144

BJA

Blockade of spinal nerves inhibits expression of neural growth factor in the myocardium at an early stage of acute myocardial infarction in rats

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Editor's key points

- Neural growth factor (NGF) is important for nerve regeneration after injury to the heart.
- NGF was up-regulated after coronary artery occlusion in rats.
- Blocking the spinal nerves to the heart with local anaesthetics abolished the NGF up-regulation.
- This may be important in patients.

Background. Neural growth factor (NGF) is required for healing and sprouting of cardiac sympathetic and sensory nerves and plays important roles in cardiac protection, sustaining cardiac function and regeneration in ischaemic heart disease. The overexpression or lack of the NGF could be harmful to the heart. In this study, we examined the role of spinal nerves in the modulation of expression of the NGF in the myocardium at risk of ischaemia soon after acute myocardial infarction in rats.

Methods. Coronary artery occlusion (CAO) was carried out in anaesthetized rats with and without preconditioning of blockade of the spinal nerves. The expression of the NGF protein and mRNA in the myocardium at risk of ischaemia was examined using immunohistochemical assay, enzyme-linked immunosorbent assay, and real-time quantitative reverse transcription polymerase chain reaction assay.

Results. In the left ventricle, immunoreactive cells and fibre-like structures were mainly located in the myocardium and in the epicardium. The NGF protein expression was increased by two-fold in the myocardium at risk of ischaemia during the 60 min of CAO, while the NGF mRNA was up-regulated three-fold, at 360 min after acute myocardial infarction. The blockade of the spinal nerves completely abolished the up-regulation of the NGF in the myocardium ($P<0.05$).

Conclusions. The spinal nerves innervating the heart may play an important role in sustaining the up-regulation of the NGF in the myocardium early after acute myocardial infarction, an effect which can be inhibited by the blockade of these nerves.

Keywords: epidural anaesthesia; nerve growth factor, acute myocardial infarction; spinal nerves

Accepted for publication: 8 March 2012

Nerve growth factor (NGF) is a critical neurotrophic factor required for the development, growth, and survival of peripheral autonomic and sensory neurones.[1] However, in adults, the NGF plays a key role in the regeneration of the nerves after damage.[2 3] The concentration of the NGF in tissues determines the density of innervation of sympathetic and sensory nerves.[4 5] Innervation of cardiac nerves is an important issue in heart transplantation, post-infarction remodelling, and cardiac arrhythmias. However, unbalanced regeneration of the cardiac sympathetic nerves is associated with NGF activity, which is potentially responsible for the lethal cardiac arrhythmias in the process of myocardial remodelling after acute myocardial infarction (AMI).[6] Substantial evidence indicates that increased local NGF expression may also play an important role in cardiac protection, sustaining cardiac function, and myocyte regeneration in ischaemic heart disease.[7] Therefore understanding the mechanism by which the production and release of the NGF in the myocardium is modulated during acute myocardial infarction is important to preserve the beneficial effects of the NGF while avoiding harmful consequences.

Previous studies indicated that the sympathetic nerves or neurotransmitters may influence the expression of the NGF,[6 8] implying a potential interruption of NGF expression in the heart by the blockade of spinal nerves, which mainly consist of efferent sympathetic and afferent sensory nerves. This can be achieved during anaesthesia for surgery and postoperative analgesia. The aim of this study was to examine the effects of the blockade of spinal nerves on the expression of the NGF in the myocardium of rats at risk of ischaemia using a rat model of acute myocardial infarction induced by permanent coronary artery occlusion (CAO). The blockade of

spinal nerves was carried out by epidural anaesthesia at the upper thoracic level, and the expression of NGF proteins and mRNA in the myocardium was analysed using fluorescence immunohistochemical (FIHC) assay, real-time quantitative reverse transcription polymerase chain reaction (qRT–PCR), and enzyme-linked immunosorbent assay (ELISA).

Methods

Protocol

The experiments were approved by the Institutional Animal Care and Use Committee of Shanxi Medical University and 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). Healthy male Sprague–Dawley rats weighing 260–280 g (Shanxi Medical University Experimental Animal Laboratory, Shanxi, China) were used for the experiments. The animals were allowed to acclimatize to the laboratory environment for 2 weeks. All surgical procedures were performed under general anaesthesia with urethane (25%, 1.2 g kg^{-1}, i.p.). The adequate depth of anaesthesia was ascertained by the observation of the changes in the size of pupils, and the depth and the pattern of respiration upon nociceptive stimulation. A continuous administration of 0.9% physiological saline (1 ml h^{-1}) was maintained during the experiment.

To study the temporal variation and the mechanism of NGF expression in the myocardium during ischaemia of the left ventricle after CAO, we quantitatively analysed the expression of the NGF protein at 15, 30, 60, and 360 min of CAO using ELISA, and mRNA expression at 15 and 360 min of CAO using qRT–PCR assays.

After successful implantation of an epidural catheter, 132 male Sprague–Dawley rats were randomly divided into three groups: the sham surgery group (sham, $n=36$), the CAO group ($n=36$), and the group of thoracic epidural anaesthesia plus the CAO group (EA, $n=36$). Each group was further divided into four subgroups, according to the time of observation after CAO: 15, 30, 60, and 360 min. In each subgroup, six animals were used for ELISA measurements ($n=6$) and three for FIHC ($n=3$) at the scheduled time. Four animals were, respectively, assigned to each of the two subgroups for qRT–PCR assay at 15 min ($n=4$) and 360 min ($n=4$) after CAO. The animals in the EA group were injected with 1% ropivacaine in the epidural space of the thoracic segments, and the animals in the other groups were given 0.9% saline.

Epidural catheterization

The procedure for epidural catheterization was the same as we have reported previously.[9] Briefly, after making a small incision through the occipitoaxial ligament, PE-10 tubing was inserted caudally into the epidural space reaching the level of the second or the third thoracic segment (T2–T3) of the rats. After recovery from the surgery and anaesthesia for 48 h, animals exhibiting any sign of neurological impairment were excluded from the study. Successful implantation of the epidural catheter was ascertained by the detection of reversible segmental loss of response to noxious stimulation in thoracic segments (T1 – T8) without motor disturbance in hind limbs after injection of 20 μl of 1% lidocaine through the catheter.

Acute myocardial ischaemic model

The acute myocardial infarction model was prepared as we reported previously.[9 10] Briefly, the pericardium was opened through an incision in the left fourth intercostal space under general anaesthesia and mechanical ventilation. A permanent ligation of the left anterior descending branch of the coronary artery was performed. Sham-operated rats underwent the same surgery as described above except without the ligation procedure.

The CAO was carried out 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. The arterial pressures and heart rate were monitored via a cannula inserted in the left carotid artery of the animals, and CAO was confirmed by the changes in the ECG and by autopsy.

Definition of the myocardium at risk of ischaemia

To identify the myocardium at risk of ischaemia, 1.5 ml of 1.0% Evans blue (Sigma-Aldrich, St Louis, MO, USA) was injected into the caudal vein, dyeing the perfused myocardium with a blue colour. The myocardium not stained was defined as the myocardium at risk of ischaemia (Fig. 1). Samples of the myocardium at risk were collected for further processing from animals in CAO and EA groups and from a matching position of the hearts of the animals in the sham surgery group.

FIHC assay

The hearts were removed, processed, embedded in optimal cutting temperature medium (OCT, Bioportfolio, Dorset, UK), and sectioned (8 μm) using a cryostat (Leica CM 1850, Nussloch, Germany). The FIHC method used is described in Supplementary material online.

Quantitative reverse transcription polymerase chain reaction

Samples were collected from the myocardium at risk of ischaemia at 15 and 360 min after CAO or sham surgery. The qRT–PCR assay was performed according to the manufacturer's protocol (as described in Supplementary material online), as reported previously.[10]

Enzyme-linked immunosorbent assay

The concentration of the NGF in the myocardium was determined using NGF Emax Immuno-Assay kit (Promega, WI, USA) according to the manufacturer's instructions, measured at 450 nm with a SpectraMax-Plus Microplate spectrophotometer (Thermo Electron Corporation, MA, USA). Data are presented as picograms per gram [pg g^{-1} total protein (TP)]. All samples were assayed in duplicate.