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Acute myocardial ischemia up-regulates nociceptin/orphanin FQ in dorsal root ganglion and spinal cord of rats

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Abstract

Nociceptin/orphanin FQ (N/OFQ) possesses modulatory effects on somatic noxious signals in spinal cord, while the potential role in visceral nociception remains elusive. We designed this study to investigate the hypothesis that cardiac nociceptive signals from acute ischemic myocardium to the spinal cord are transmitted or modulated by mechanisms including N/OFQ. We examined the changes of N/OFQ and its mRNA in the dorsal root ganglia and spinal cord of upper thoracic segments innervating the heart of rats. Thoracic epidural anesthesia was performed to confirm neural mechanism underlying the changes. We observed that selective coronary artery occlusion significantly up-regulated N/OFQ and ppN/OFQ mRNA in the dorsal root ganglia and spinal cord. Thoracic epidural anesthesia abolished the changes in the expression of N/OFQ and its mRNA. The observations indicate that cardiac noxious neural afferent drive is responsible for the up-regulation of N/OFQ in the primary afferent neurons and intrinsic spinal neurons.

Keywords: Nociceptin/orphanin FQ (N/OFQ); Dorsal root ganglion; Spinal dorsal horn; Myocardial ischemia; Coronary artery occlusion

Nociceptin/orphanin FQ (N/OFQ) binds to the orphan opioid receptor-like 1 receptor, presently termed NOP receptor [13,20]. The receptor is widespread in the CNS including spinal cord. N/OFQ is an endogenous ligand of the NOP receptor [16]. Substantial evidence indicates that NOP receptor and N/OFQ in spinal cord are involved in modulation of nociception [1,19,14,11] including which mediated by polymodal substance P fibers in spinal cord [8]. However, it is still not clear whether N/OFQ plays modulatory role in visceral nociception.

Having found the up-regulation of substance P (SP) and its mRNA in the dorsal root ganglia (DRG) and spinal cord during acute myocardial ischemia [6], we think it is important to know whether N/OFQ is or is not involved in the neural reaction under the condition. We designed this study to investigate the hypothesis that cardiac nociceptive signals from ischemia-stimulated myocardium to the spinal cord are transmitted or modulated [3] by mechanisms including N/OFQ.

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) [7] and approved by the Institutional Animal Care and Use Committee of Shanxi Medical University. Efforts were made to minimize the number of animals used and their suffering in the study.

The experiments were performed on adult male Sprague–Dawley rats weighing 260–280 g. A tubing (8 μL of dead capacity) was inserted caudally into the epidural space through a small incision in the occipitoaxial ligament for the animal under anesthesia with sodium pentobarbital (65 mg/kg, i.p.), reaching at the level of T_2–T_3 of the rat. A successful implantation of epidural catheter was verified after the animal was immediately recovered from the surgery and anesthesia by detection of reversible segmental loss of response to noxious stimulation in thoracic area (T_1–T_8) without motor and sensory disturbance in hind limbs following injection of 20 μL of 2% xylocaine through the catheter. The rats were allowed to recover for further 48 h before experiment.

After completion of each experiment, the catheter position was verified in each animal by autopsy.

The procedure for preparation of the acute myocardial ischemic model was the same as previously reported [6]. The ligation of the coronary artery was carried out in animals of

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the CAO group 15 min after epidural injection of 30 μL of 1% ropivacaine.

The CAO was confirmed by changes in the ECG and by autopsy.

Samples of the DRGs and spinal cord of T_1–T_5 segments were collected from CAO group ($n = 24$, 6 animals for each subgroup) and sham animals ($n = 24$, 6 animals for each subgroup) at 0.5 h, 1 h, 3 h and 6 h of CAO or sham surgery and from control group (without any surgical procedure or CAO, $n = 6$). The samples were fixed and processed as previously reported [6], with antiserum (rabbit anti-rat, at a dilution of 1:3000, Phoenix Pharmaceuticals, USA) and secondary anti-anti-N/OFQ (mouse anti-rabbit, at a dilution of 1:200, Boster Biotechonology Inc., Wuhan, China) according to the instructions of the suppliers. The specificity of the antibodies was examined simple by omitting the primary anti-N/OFQ with the other procedure the same as described above.

A semi-quantitative analysis for the immunoreactive products was performed as previously described [6]. An average of 10 microscope fields of view (covering the distributing area of cells for each ganglion; four fields in lamina I and II, three for V and VI and three fields for VII, VIII and X, bilaterally) per slide, three slides per animal were taken as a result. The results were presented as mean optical density and immunoreactive area, relevant to the amount of the immunoreactive material and amount of the positive expressing cells, respectively.

Samples of the DRGs and the spinal cord were quickly removed from animals of CAO group ($n = 6$), thoracic epidural anesthesia group ($n = 6$) and the control ($n = 6$) and sham groups ($n = 6$) at the timing of highest expression of N/OFQ of the CAO animals, according to the results of IHch. The N/OFQ was differentially determined for dorsal and ventral parts of spinal cord (divided along the horizontal line crossing central cannel) with ELISA kit (Phoenix Pharmaceuticals, USA) according to the manufacturer's instruction.

The samples of DRGs and the spinal cord were collected from CAO groups (0.5–6 h, $n = 24$), thoracic epidural anesthesia group (at 1 h of CAO, $n = 6$) and the sham group (at 1 h of the surgery, $n = 6$). Total RNA was isolated from tissues using the Trizol-LS®TM method (Invitrogen USA), in accordance with the manufacturer's instructions and was measured using a spectrophotometer (Unico, Shanghai, China) at 260 nm/280 nm.

Real-time RT-PCR analyses were immediately performed after recovery of total RNA in a fluorescent temperature cycler (MX3005P Real Time PCR System; Stratagene, USA) as we reported before [6]. The primers for the target gene are as follows:

N/OFQ: 5′-AAC CTG AAG CTG TGC ATC CT-3′ (forward), 5′-CCT TTT CTG CAG CTG CTT CT-3′ (reverse, amplified fragment = 285 bp). GAPDH: 5′-GTG AAG GTC GGT GTG AAC GGA TTT-3′ (forward), 5′-CAC AGT CTT CTG AGT GGC AGT GAT-3′ (reverse, amplified fragment = 320 bp).

The qRT-PCR program for N/OFQ mRNA and GAPDH mRNA was set as: 50 min at 45 °C (one cycle), 10 min at 95 °C (one cycle), 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C (40 cycles) and 30 s at 60 °C followed by 30 s at 95 °C (one cycle).

Data are expressed as mean ± S.D. of the mean of n experiments. A non-repeated one-way analysis of variance and post hoc Dunnett's multiple comparison were used to compare the changes in expression of N/OFQ and ppN/OFQ mRNA detected using IHch, ELISA and qRT-PCR tests. For two groups, the comparison was made using Student's t test for paired and unpaired observations as appropriate. In each case, a P value <0.05 was considered significant.

In this study, 114 animals were fulfilled the experiments, while 3 rats died after epidural catheterization and 2 died during CAO (within 1 h of CAO).

The elevation of the ST segment could be detected at 10 min of the CAO and was in progress throughout the 360 min of CAO.

The increase in the immunoreactive material was found in some neurons of all sizes and some satellite cells in the DRG (Fig. 1A and B) and scattered neurons in the spinal cord, mainly in dorsal horn, and ventral horn as well, and epithelium of central canal (Fig. 1C).

The increase in immunoreactive material was significant at 30 min in the DRGs (Fig. 2A and B) and the spinal cord (Fig. 2C–F), and throughout the 6 h of the CAO. The climax of the expression was detected at 1 h in the DRGs and the spinal dorsal horn.

The up-regulation of ppN/OFQ mRNA was detected at 30 min of CAO and peaked at 1 h in the DRGs

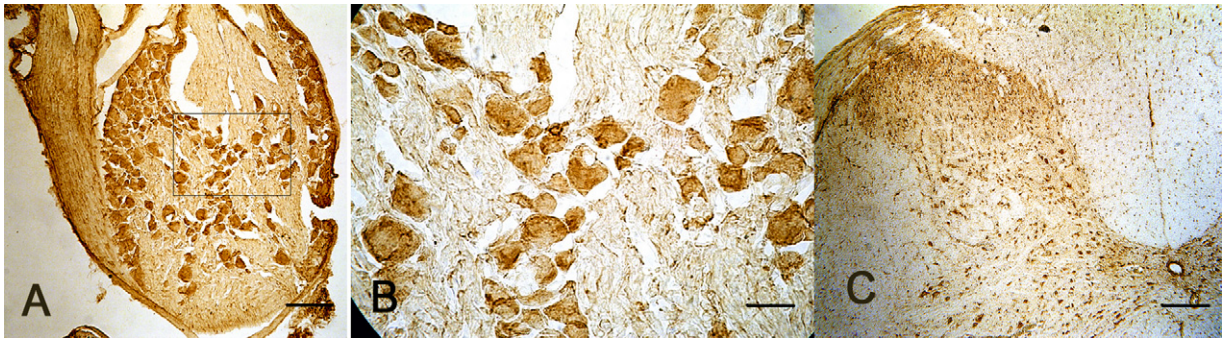

Fig. 1. Immunoreactive stain for N/OFQ located in the cells of all sizes of the DRG (A and B) and spinal cord (C), mainly in dorsal horn (laminae I–IV) and scattered neurons in the other laminae, and in the epithelium of central canal at the level of T_5. Bars represent 200 μm in A and C and 50 μm in B.

($P < 0.001$) and the spinal dorsal horn ($P < 0.01$) following the CAO (Fig. 3). There was certain expression of ppN/OFQ mRNA in DRGs and spinal cord of the animals of sham group.

The increase in N/OFQ and ppN/OFQ mRNA in the DRGs and the spinal cord could be completely abolished by pre-CAO epidural anesthesia at upper thoracic levels (Figs. 4 and 5).

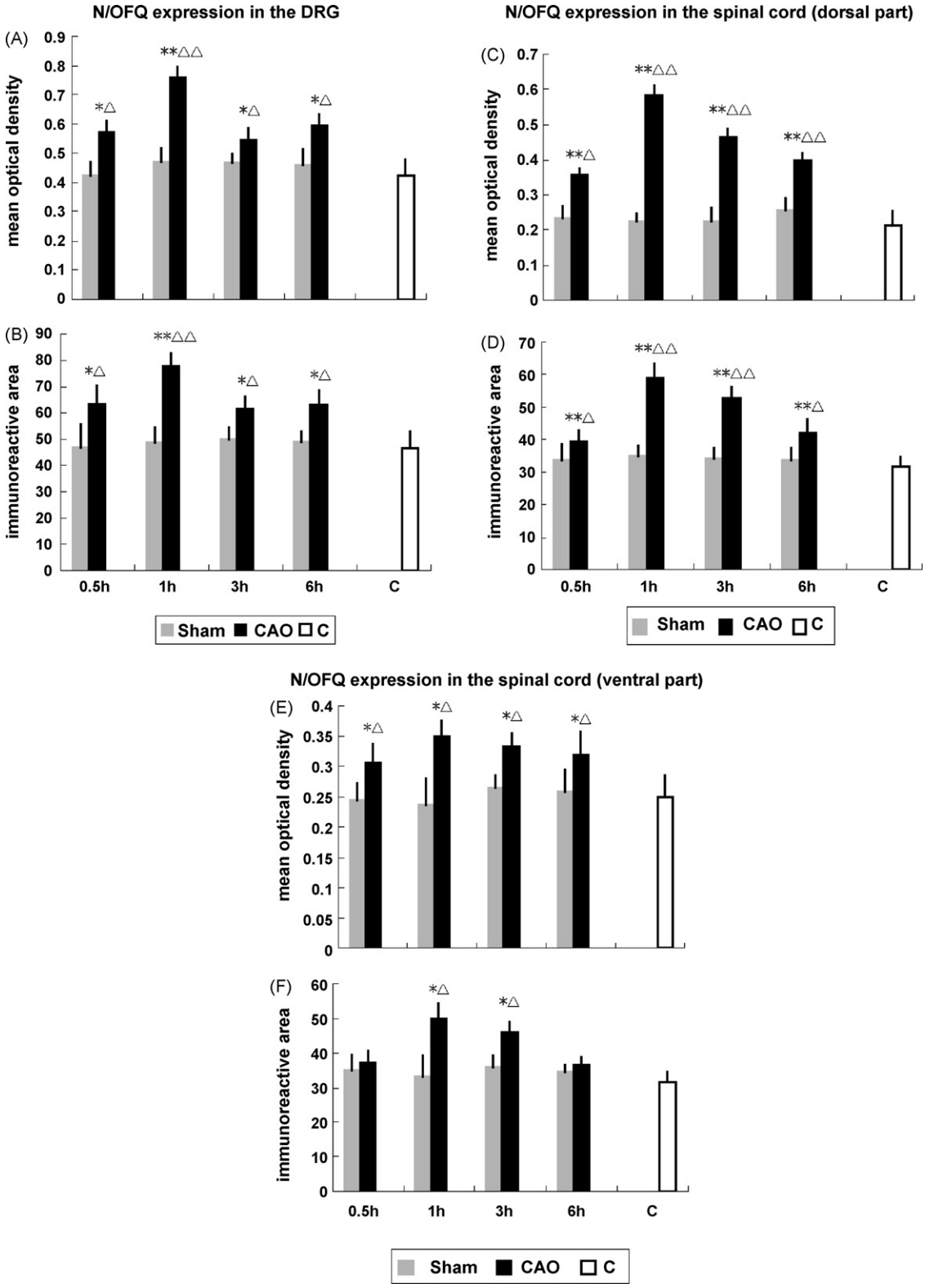

Fig. 2. Quantitative analysis of the IHch in DRGs (A and B) and spinal cord (C–F) of CAO, sham groups at 0.5 h, 1 h, 3 h and 6 h (*$P < 0.05$, **$P < 0.01$, vs. sham; $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$, vs. control).

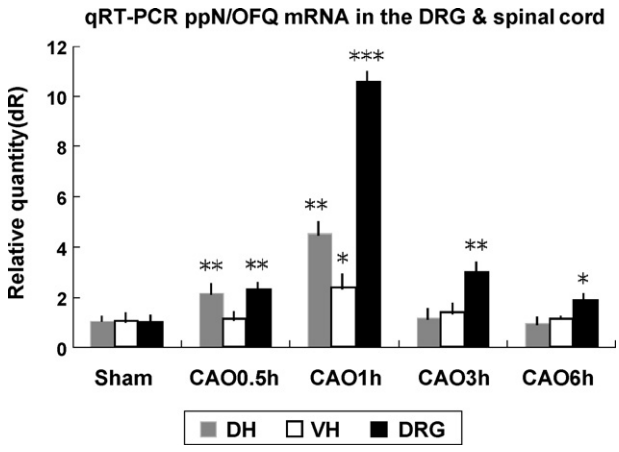

Fig. 3. Expression of ppN/OFQ mRNA in DRGs and spinal cord was significantly up-regulated at 0.5 h, 1 h, 3 h and 6 h of CAO rats (*$P<0.05$, **$P<0.01$, vs. sham).

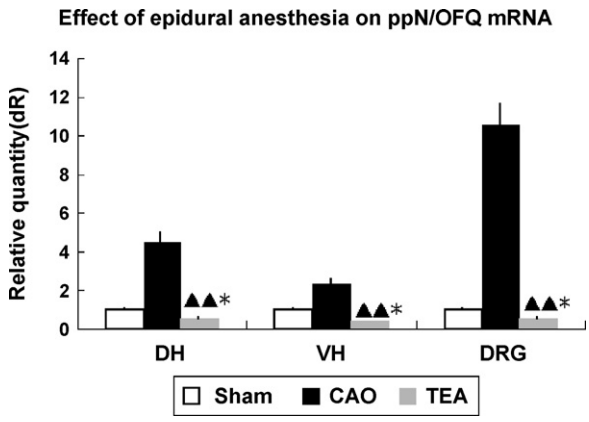

Fig. 5. Difference in the ppN/OFQ mRNA in the DRG and spinal cord of CAO animals without and with TEA was observed (*$P<0.05$, vs. sham; ▲▲$P<0.01$, vs. CAO group).

In this study, we found that in the development of acute myocardial ischemia, N/OFQ and ppN/OFQ mRNA were up-regulated in the upper thoracic spinal cord and DRGs innervating the heart. The results indicate that ppN/OFQ, precursor of N/OFQ may be synthesized in the primary sensory neurons and spinal intrinsic neurons, which are in agreement with previous findings [3,17]. However, there was report showing detection of N/OFQ in spinal cord but not in the DRGs of L_6-S_2 segments using immunohistochemical test [21]. The difference between the results of current study and previous one might be caused by settings of study.

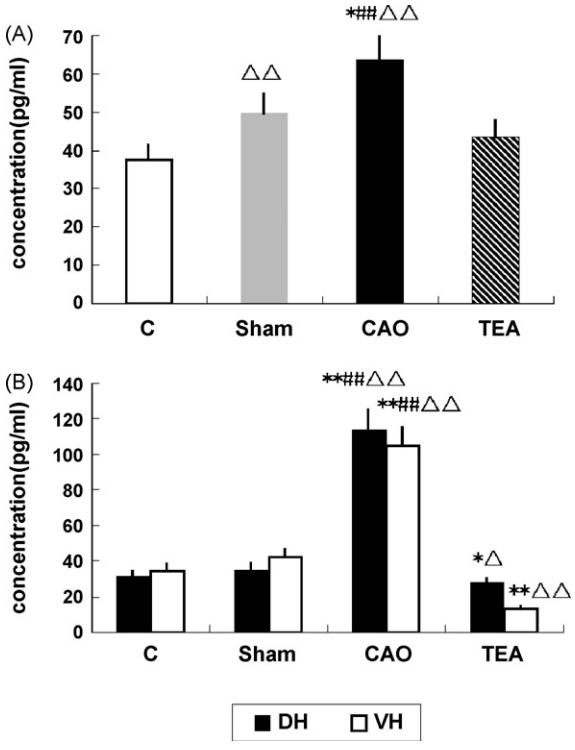

Fig. 4. Difference in the expression of N/OFQ in the DRG (A) and in the spinal cord (B) of CAO animals without and with thoracic epidural anesthesia (TEA) was observed (*$P<0.05$, vs. sham; ##$P<0.01$, vs. thoracic epidural anesthesia (TEA) group; △△$P<0.01$, vs. control).

The entire profile of the mechanism underlying the up-regulation of N/OFQ in this case is still elusive. Other neuropeptides, such as SP, contained in the afferent neuron in the DRG could be markedly increased in adjuvant-induced arthritis [9,23], which is attributed to growth factors, particularly nerve growth factor (NGF) and glial-derived neurotrophic factor (GDNF) [22,10]. It was reported that 20 min of CAO could cause a strong up-regulation of genes encoding brain-derived neurotrophic factor and vascular endothelial growth factor [18] in ischemic myocardium. We observed that in the early period (30 min) of CAO ppN/OFQ mRNA was increased in the DRG, which does not suggest the NGF mechanism is underlying the current finding because the NGF could not yet reach the cell body of the DRG neuron from myocardium within the time. Some other neural mechanism may be underlying the up-regulation.

The anaerobic metabolism in the myocardium after stop of coronary circulation could rapidly change the milieu of myocardium and results in an increase in afferent nerve traffic [12]. The neural response could be expressed as changes in chemicals in the DRG cells and release of the bioactive chemicals from their terminals [9], transmission or modulation of afferent nociception in spinal cord. N/OFQ and its receptor could play roles in modulating neural signals in spinal cord [14,11,3,15,4,26,25,24]. N/OFQ, as a neurotransmitter, could be synthesized and released by primary afferent neurons through the central axon of the DRG cell to the spinal dorsal horn conveying or modulating [3] the neural signals from peripheral to the central nervous system [28]. The results of this study showing up-regulation of N/OFQ and ppN/OFQ mRNA in the DRG neurons support the notion. The up-regulation of N/OFQ in medium and large sized cells in addition to small sized neuron in the DRG of CAO animals may imply the complexity of neuronal reaction to cardiac noxious stimulation, most of which is unknown. Together with the observation of up-regulation of N/OFQ and ppN/OFQ mRNA in spinal cord may serve as solid evidence suggesting N/OFQ as a mediator of primary afferent and spinal intrinsic neurons participates in the neural activities evoked by

acute myocardial ischemia. The effect of epidural anesthesia strongly indicates cardiac primary afferent neuronal drive may be responsible for the up-regulation of N/OFQ in the neurons of the DRG and spinal cord. But the current study could not answer the question, how the neural signals are converted into the up-regulation of the chemicals in the neurons.

The change in the ST segment of the ECG during the CAO could be evidence of myocardial injury. The injury associated neural signals could be nociceptive. The cardiac nociception could cause activation of neurons in spinal dorsal horn [2,5] and thalamus [27]. In current study control and sham surgery control were used to discriminate the changes in of N/OFQ during CAO from levels of N/OFQ under physiological condition and under influence of the surgery. The expression of N/OFQ in control animals, under the 'physiological' condition may indicate some other unknown function of N/OFQ.

The increase in the N/OFQ detected in this study either by IHch or ELISA does not mean change of the peptide at the sites of chemical synapses transmitting the neural signals. The methods used in this study only show the changes of the N/OFQ and even preproN/OFQ as a total, without discrimination of functioning fragment. The physiology and pathophysiology of N/OFQ in spinal cord in acute myocardial ischemia need to be elucidated.

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