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ROLE OF THALAMIC NUCLEI IN THE MODULATION OF FOS EXPRESSION WITHIN THE CEREBRAL CORTEX DURING HYPERTONIC SALINE-INDUCED MUSCLE NOCICEPTION

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Abstract—It has been proposed that thalamic mediodorsal (MD) and ventromedial (VM) nuclei form thalamic 'nociceptive discriminators' in discrimination of nociceptive afferents, and specifically govern endogenous descending facilitation and inhibition. The present study conducted in rats was to explore the role of thalamic MD and VM nuclei in modulation of cerebral neuronal activities by means of detection of spatiotemporal variations of Fos expression within the cerebral cortex. Following a unilateral intramuscular injection of 5.8% saline into the gastrocnemius muscle, Fos expression within the bilateral, different areas of the cerebral cortex except S2 was significantly increased ($P < 0.05$). Particularly, the increases in Fos expression within the cingulate cortex and the insular cortex occurred at 0.5 h, 4 h and reached the peak level at 4 h, 16 h, respectively. Electrolytic lesion of the contralateral thalamic MD and VM nuclei significantly blocked the 5.8% saline intramuscularly induced increases in Fos expression within the bilateral cingulate and insular cortices, respectively. Additionally, the 5.8% saline-induced Fos expression in the cingulate cortex and the insular cortex were dose-dependently attenuated by microinjection of μ-opioid antagonist β-funaltrexamine hydrochloride into the thalamic MD and VM nuclei. It is suggested that (1) the neural circuits of 'thalamic MD nucleus – cingulate cortex' and 'thalamic VM nucleus – insular cortex' form two distinct pathways in the endogenous control of nociception, (2) mirror or contralateral pain is hypothesized to be related to cross-talk of neuronal activities within the bilateral cerebral cortices modulated by μ-opioid receptors within the thalamic MD and VM nuclei.

Key words: mirror pain, cerebral cortex, Fos expression, endogenous controls, μ-opioid receptor.

INTRODUCTION

Referred pain has been realized since the late 1890s (Head, 1896), and it was used to describe pain perceived at a remote location other than the site of the painful stimulus. In clinical practice, patients with deep visceral pain always claim pain sensation at distant sites (Eyzasuirre and Fidone, 1975). In addition to referred pain, there is one specific, symmetrical pain phenomenon termed contralateral or mirror pain, during which pain or nociception could be observed in the un-injured extremities contralateral to the injured limb (Donaldson, 1999; Koltzenburg et al., 1999; Accerra and Moseley, 2005; You and Arendt-Nielsen, 2005; Bliddal, 2007; Huang and Yu, 2010; You et al., 2010; Lei and You, 2012). To date, the role of supraspinal neural circuits in bilateral symmetry of pain or nociception in humans and animals is yet unclear.

Since 1930s, intramuscular (i.m.) hypertonic saline-induced muscle nociception has been regarded as a valid model to mimic clinical muscle pain (Kellgren, 1938; Lewis, 1938). In our previous animal studies, unilateral i.m. injection of 5.8% saline into the gastrocnemius (GS) muscle elicits bilateral secondary mechanical hyperalgesia and heat hypoalgesia (You et al., 2010; Lei et al., 2011). These noxious mechanically and heat-evoked nociception have been further documented to be discriminated and controlled by the endogenous descending modulations: facilitation and inhibition, which are organized by the thalamic mediodorsal (MD) nucleus and ventromedial (VM) nucleus, respectively (You et al., 2013, 2014). Although there is much evidence on neural connections between sub-cortex structures, i.e., thalamus, and cerebral cortex, the role of different areas of the cerebral cortex in the thalamic MD and VM nuclei meditated discrimination and modulation of peripheral noxious afferents is less known.

Fos expression within the central nervous system has been used as an appropriate neuronal marker to explore dynamic variations of neuronal activities following transient or persistent nociceptive afferents (Semenenko and Lumb, 1999; Panfil et al., 2006). By detection of Fos expression, our recent studies demonstrated that activities of spinal nociceptive neurons in superficial and deep layers are differently modulated by endogenous

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Abbreviations: β-FNA, β-funaltrexamine hydrochloride; ABC, avidin–biotin-peroxidase complex; AIP, agranular insular cortex (posterior part); DI, dysgranular insular; Fos-LI, Fos-like immunoreactive; GI, granular insular; GS, gastrocnemius; HT, hypertonic; MD, mediodorsal; PBS, phosphate-buffered solution; VM, ventromedial.

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descending controls (Chen et al., 2013; Lei et al., 2015). From functional perspectives, it is suggested that Fos can be employed as a valid tracing marker to detect the functional connections between different areas of the central nervous system. In the present study, we systematically investigated spatiotemporal variations of Fos expression within the different areas of the cerebral cortex in response to i.m. 5.8% saline-induced muscle nociception. Additionally, the role of thalamic MD and VM nuclei associated with central opioid mechanisms in modulation of activities of the cerebral cortex were explored.

EXPERIMENTAL PROCEDURES

Ethical approval and animals

In total, 132 male Sprague–Dawley rats weighing 260–300 g (about 10 weeks age) were used. The animals were provided by the Animal Center of the College of Medicine, Xi'an Jiaotong University, and housed pairwise in plastic boxes under a 12:12-h light–dark cycle (lights on at 08:00 AM) at 22–26 °C with food and water available *ad libitum*. All experiments were approved by the Xi'an Jiaotong University Animal Care Committee in accordance with the Committee's guidelines for pain research in conscious animals (Zimmermann, 1983). The animals were acclimatized to the laboratory and habituated to the test boxes for at least 1 h each day five days prior to testing. All efforts were made to minimize the number of animals used and their suffering.

Muscle nociception elicited by intramuscular injection with hypertonic saline

As described elsewhere (You et al., 2010, 2013; Lei et al., 2011, 2014), a bolus of 0.2-ml hypertonic (HT, 5.8%) saline was intramuscularly injected into the GS muscle of the left hind limb to elicit muscle nociception. The injection site was in the middle part of the GS muscle, and the depth of the injection was about 0.5 cm. The injection procedure was performed manually and lasted more than 30 s.

Electrolytic lesion of thalamic nuclei

The anesthetized (sodium pentobarbital, 50 mg/kg, i.p.) rats were mounted in a stereotaxic frame with fixation of the head by ear bars and tooth plate (MP8003, RWD Life Science Co., Shenzhen, China). After local lidocaine anesthesia, the scalp was cut and the cranium was drilled. A thalamic nucleus located contralaterally to the i.m. 5.8% saline injection was electrolytically lesioned. To perform the electrolytic lesion of the mediodorsal (MD) and the VM nuclei, an insulated stainless steel electrode (shank diameter 200 μm; tip diameter 50 μm, exposed tip 50 μm) was advanced stereotactically into the different nuclei areas at the following coordinates: MD nucleus: anteroposterior −(2.56–2.8) mm from the bregma, lateral 0.6 mm from midline, dorsoventral 5.4 mm from the cranium; VM nucleus: anteroposterior −(2.56–2.8) mm, lateral 1.4–1.6 mm from midline, dorsoventral 7.3 mm from the cranium (Paxinos and Watson, 1998). An electrolytic lesion was made by means of an electrical stimulator generating a 150–200-μA anodal DC current for 30 s through the electrode tip. The lesion current was monitored continuously using an oscilloscope to measure the voltage drop across a 100 Ω resistor in series with the electrode. After the electrolytic lesion, the microelectrode was slowly withdrawn, the wound was washed with sterile saline, treated with antibiotics, and the skull was closed with dental cement.

A recovery period of 7 days was allowed, during which the animals' behavior and motor function were monitored strictly. Animals showing severe permanent neurological deficits or motor dysfunction assessed by means of the Rota-Rod treadmill were excluded from the remaining experiments.

Intracerebral microinjection of β-funaltrexamine hydrochloride (β-FNA)

A craniotomy was conducted with a dental drill in order to perform the intracerebral (i.c.) catheterization. One guide cannula (OD: 0.35 mm; ID: 0.25 mm; RWD Life Science Co., Shenzhen, China) was advanced into the target thalamic nuclei. After the catheterization, the wound was washed with sterile saline, treated with antibiotics, and the skull was closed with dental cement. The animals were then put back to the box for a 7 days' recovery during which the animals' behavior and motor function were strictly monitored. Animals showing severe permanent neurological deficits or motor dysfunction as stated above were excluded.

During the experiments, a bolus of 0.5-μl solution containing β-FNA 0.5 nmol, 1 nmol or 5 nmol (Sigma–Aldrich Chemie Gmbh, Munich, Germany) was injected through the intrathalamic catheter using a 1-μl microsyringe while the rat was gently restrained by hand. β-FNA was freshly prepared and dissolved in 0.9% saline, and slowly infused at a constant speed over a 30-s period. After the experiments, the drug injection site was marked by microinjection with Pontamine Sky Blue dye (0.25 μl; 2% in 0.5 M sodium acetate acid).

Assessment of motor function

Animals were placed on a Rota-Rod treadmill (Model 755, IITC, Woodland Hills, CA, USA) rotating at a gradually increasing speed from 5 to 30 rpm for 30 s and maintained for another 120 s at 30 rpm. Rats with motor dysfunction after the chronic electrolytic lesion or the i.c. catheterization were excluded from the remaining experiments.

Histology for identification of electrolytic thalamic lesion and microinjection

The animals receiving electrolytic lesion of a thalamic nucleus or intracerebral microinjection were anesthetized by sodium pentobarbital (50 mg/kg, i.p.) and transcardially perfused with 10% formalin. The brains were then isolated and stored in 30% sucrose for