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SPECIFIC THALAMIC NUCLEI FUNCTION AS NOVEL 'NOCICEPTIVE DISCRIMINATORS' IN THE ENDOGENOUS CONTROL OF NOCICEPTION IN RATS

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Abstract—Recently, we hypothesized that supraspinal structures may have important functions in discriminating between noxious mechanically and heat mediated nociception through distinct functions: facilitation and inhibition. In this study, conducted in conscious rats, we explored the role of different thalamic nuclei: the mediodorsal (MD) nucleus, the central medial (CM) nucleus, the submedius (SM) nucleus, the ventralmedial (VM) nucleus and the ventral posterolateral (VPL) nucleus, in the descending control of secondary and contralateral mechanical hyperalgesia and heat hypoalgesia occurring in intramuscularly hypertonic (HT, 5.8%) saline-induced muscle nociception. We found that the MD nuclei participated in the descending facilitation of mechanical hyperalgesia, and that the VM nuclei were specifically involved in the descending inhibition of heat hypoalgesia. Neither descending facilitation nor descending inhibition was affected after electrolytic lesion of the thalamic CM, SM, and VPL nuclei. This descending facilitatory and inhibitory modulation of nociception was strengthened by glutamate, and weakened by GABA, microinjected into the thalamic MD and VM nuclei. It is suggested that (1) thalamic MD nucleus and VM nucleus form two distinct endogenous systems in the control of noxious mechanically and heat evoked responses, and (2) the strengthening of descending inhibition and the weakening of descending facilitation by means of up regulation and down regulation of appropriate receptor expression in the VM and MD nuclei may provide a new strategic policy in treating pathological pain.

Key words: thalamus, endogenous descending controls, hyperalgesia, hypoalgesia, pain.

INTRODUCTION

Animal experiments and clinical trials have provided much progress in the knowledge of pain physiology, e.g. the description of hyperalgesia associated with central sensitization. However, the mechanisms of nociceptive modulation are the subject of much debate. For instance, (1) it is as yet unknown whether the endogenous modulation of pain and nociception is tonic or not (Fields et al., 2006), and (2) the significance of descending modulation to the development of spinal central sensitization in pathological pain is still unclear (Woolf, 1983).

With regard to the endogenous modulation of pain and nociception, we recently proposed a novel concept; the supraspinal nociceptive discriminator, that generates and controls endogenous facilitatory and inhibitory actions (You et al., 2010). The nociceptive discriminator governs endogenous descending modulation, and is normally inactive or 'silent'. Once triggered by sufficient C-afferent input and the resultant central summation, the supraspinal nociceptive discriminator has a robust effect on pain; either descending facilitation of noxious mechanically mediated activities or descending inhibition of heat-evoked responses (You et al., 2010). This provides direct evidence to explain the existence of secondary mechanical, but not heat, hyperalgesia that occurs outside the injured area. A previous study from our laboratory has further demonstrated that the 'silent' or inactive supraspinal nociceptive discriminator has different thresholds; a lower threshold for descending facilitation and a higher threshold for descending inhibition (Lei et al., 2011). Accordingly, we hypothesize that the supraspinal nociceptive discriminator may be dependent on a multi-unit neural network involving different nuclei in supraspinal structures. Thus, there is a series of questions that need to be addressed concerning the location and function of this modulatory network.

It is generally accepted that an important pathway for sensory afferent information, including nociceptive information, from the periphery to the cortex is *via* the thalamus. As early as 1911, the importance of the thalamus for the perception of pain was reported, and lesion of the cerebral cortex alone did not affect the threshold for the painful sensation (Head and Holmes, 1911). The thalamus is not only considered to be a major nociceptive relay, but also a processing center with discriminatory and modulatory functions (Dostrovsky

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Abbreviations: ANOVA, analysis of variance; CM, central medial; HT, hypertonic; MD, mediodorsal; RVM, rostroventral medulla; SM, submedius; VM, ventralmedial; VPL, ventral posterolateral.

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and Craig, 2009). Due to its specific location in ascending sensory pathways (Davis et al., 1998), the thalamus is hypothesized to be a primary candidate for studies into the location and function of the supraspinal nociceptive discriminator.

In this study, i.m. injection of hypertonic (HT) saline into gastrocnemius muscle was employed as a model of muscle nociception. Secondary and contralateral, but not primary, mechanical hyperalgesia and heat hypoalgesia were assessed by measuring thresholds for paw withdrawal reflexes to noxious stimulation. Testing of paw withdrawal reflexes distant to the insulted muscle may provide insights into manifestations of central plasticity without direct interference from local peripheral sensitization (You et al., 2010). Here we systematically investigated and demonstrated that different thalamic nuclei are responsible for the modulation of noxious mechanically and heat evoked responses: the ventromedial (VM) nucleus is involved in descending inhibition whereas the mediodorsal (MD) nucleus participates in descending facilitation.

EXPERIMENTAL PROCEDURES

Ethical approval and animals

Male Sprague–Dawley rats weighing 260–300 g (10 weeks of age) were recruited and provided by the Animal Center of the College of Medicine, Xi'an Jiaotong University, and housed in pairs 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. IASP's guidelines for pain research in conscious animals were followed (Zimmermann, 1983). The animals were acclimatized to the laboratory and habituated to the test boxes for at least 1 h each day 5 days prior to testing. All efforts were made to minimize the number of animals used and their suffering.

Muscle nociception elicited by i.m. injection with HT saline

As described elsewhere (You et al., 2010; Lei et al., 2011), a volume of 0.2 ml HT (5.8%) saline was injected intramuscularly into the gastrocnemius muscle of the left (ipsilateral) hind limb in order to establish muscle nociception. The injection site was in the middle part of the gastrocnemius muscle, and the depth of the injection was about 0.5 cm. The injection procedure was performed manually and was made over more than 30 s.

Behavioral tests to measure mechanical and heat sensitivities

The experimental rats were randomly divided into several individual groups; eight rats were randomly assigned to each group for the behavioral tests.

For measurement of mechanically-evoked paw withdrawal responses, rats were placed in individual Plexiglas chambers with mesh floors and transparent covers (20 × 20 × 25 cm). A hand-held electronic von Frey device (2290 Electrovonfrey®, IITC, Woodland Hills, CA, USA) with a rigid filament was used to detect the mechanical paw withdrawal threshold. According to the map of the withdrawal field of the gastrocnemius muscle (You et al., 2003, 2010), the filament was applied to the heel part of the hind paw until a foot-withdrawal response was elicited, indicating that the mechanical threshold (g) and the cut-off force had been achieved.

Heat-evoked paw withdrawal responses were determined using a 390G plantar stimulator Analgesia Meter (IITC, Woodland Hills, CA, USA). The rats were tested individually in a Plexiglas cubicle placed onto a constant temperature-controlled transparent glass plate, which was used to avoid temperature sink from the tested hind paws. The heat stimulus was a high-intensity beam (setting = 30–40% intensity) aimed at the heel part of the hind paw. The withdrawal latency was defined as the time from the onset of noxious heat stimulation to withdrawal of the tested hind paw. The intensity of the beam was adjusted so that the latency of the paw withdrawal reflex was around 10–11 s in untreated animals. A painful, but tolerable, sensation could be elicited using this 10–11 s heat stimulation on the operator's hand. To avoid excessive tissue injury, manual cut-off of the heat stimulus was performed if no paw withdrawal reflex could be evoked during 20 s of heat stimulation.

As described elsewhere (You et al., 2010), paw withdrawal thresholds to mechanical and heat stimulation were measured for both ipsilateral and contralateral hind paws (heel part) before the muscle nociception. During the muscle nociception, variations of bilateral paw withdrawal reflexes responding to mechanical and heat stimuli were evaluated at 30 min, hourly at 1–4 h, and daily 1–7 days after the unilateral i.m. injection of 5.8% HT saline. At each time point, both hind paws received three trials each, with at least a 30-s interval between subsequent trials, for the mean of which represented the mechanical paw withdrawal threshold (g) and thermal paw withdrawal latency (s). A reduced or increased threshold for the withdrawal response compared with the threshold before the HT saline injection was defined as hyperalgesia or hypoalgesia, respectively.

Electrolytic lesion of thalamic nuclei

The anesthetized (sodium pentobarbital, 50 mg/kg, i.p.) rats were mounted in a stereotaxic frame with the head fixed by ear bars and a tooth plate (MP8003, RWD Life Science Co., China). After local lidocaine analgesia, the scalp was cut and the cranium was drilled. A thalamic nucleus located contralaterally to the i.m. HT saline injection was electrolytically lesioned. To perform the electrolytic lesion of the mediodorsal (MD), the central medial (CM), the submedius (SM), the ventralmedial (VM) or the ventral posterolateral (VPL) 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 at the following coordinates: MD nucleus: anteroposterior – (2.12–3.6) mm from bregma, lateral 0.5 mm from midline, dorsoventral 5.4 mm from the cranium; CM nucleus: anteroposterior – (2.3–3.14) mm from bregma, 0.6–0.9 mm lateral, 6.0–6.42 mm below the cranium; SM nucleus: anteroposterior – (2.3–3.0) mm from bregma, 0.6–0.9 mm lateral, 7.0 mm below the cranium; VM nucleus: anteroposterior – (2.3–3.3) mm, lateral 1.6 mm from midline, dorsoventral 7–7.4 mm from the cranium; VPL nucleus: anteroposterior – (2.3–3.3) mm, 3.0 mm lateral, 6 mm below 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. Animals showing severe permanent neurological