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Endogenous descending facilitation and inhibition differ in control of formalin intramuscularly induced persistent muscle nociception

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ABSTRACT

In conscious rats, intramuscular injection of 2.5% formalin into the gastrocnemius muscle, at volumes between 25 and 200 μl, evoked dose-dependent biphasic persistent flinching activities: phase 1 (0–10 min) and phase 2 (10–60 min). During this intramuscular formalin-induced ipsilateral muscle nociception, bilateral secondary mechanical hyperalgesia and heat hypoalgesia assessed by measuring thresholds of paw withdrawal reflex to noxious mechanical and heat stimuli were observed ($P < 0.05$). Lesion of either the ipsilateral dorsal funiculus (DF) or contralateral thalamic mediodorsal (MD) nucleus significantly alleviated the formalin-induced flinches in both phase 1 and phase 2 of the behavioral response, and blocked the occurrence of secondary mechanical hyperalgesia, but not heat hypoalgesia. By contrast, lesion of the ipsilateral dorsal lateral funiculus (DLF) or contralateral thalamic ventromedial (VM) nucleus markedly enhanced the formalin induced flinching behavior in the late part (30–60 min) of phase 2 alone; phase 1 and early part (10–30 min) of phase 2 response were unaffected. Heat hypoalgesia, but not mechanical hyperalgesia, was markedly attenuated by this treatment ($P < 0.05$). Microinjection of GABA (0.1 μg/0.5 μl) into the thalamic MD nucleus significantly depressed the intramuscular formalin-induced biphasic persistent nociception, and the occurrence of bilateral secondary mechanical hyperalgesia was significantly delayed ($P < 0.05$). By contrast, microinjection of GABA into the thalamic VM nucleus significantly enhanced the formalin-induced nociceptive behavior in the late part (30–60 min) of phase 2, and the bilateral secondary heat hypoalgesia was temporarily prevented ($P < 0.05$). The present study demonstrates that intramuscular formalin evokes biphasic muscle nociception, and that bilateral secondary mechanical hyperalgesia and heat hypoalgesia are differentially controlled by endogenous descending facilitation and inhibition respectively. It is further suggested that thalamic MD nucleus and VM nucleus constitute an endogenous discriminative, modulatory system that exerts, via pathways in the DF and DLF, descending facilitatory and inhibitory actions on responses to peripheral afferent activity evoked by noxious mechanical and heat stimulation.

Introduction

Studies in humans and animals have extensively shown that descending controls: facilitation and inhibition, can profoundly modulate the perception of pain (Fields et al., 2006; Pertovaara, 2006). Using a muscle pain model elicited by intramuscular (i.m.) injection of 5.8% saline, our previous behavioral studies conducted in conscious rats have revealed that the actions of descending modulation have different effects on noxious mechanically and heat evoked nociception. Descending facilitation is involved in mechanically evoked nociceptive responses, whereas noxious heat evoked activities are predominantly controlled by descending inhibition (Lei et al., 2011; You et al., 2010).

The finding of differentiation in endogenous descending control of different nociceptive modalities provides new, direct, evidence in interpretation of the fact that secondary heat, but not mechanical, hyperalgesia is rarely present in the basic research and clinical setting (Ali et al., 1996; Fuchs et al., 2000; Hardy et al., 1950; Lewis, 1936; for review, see Treede et al., 1992). More recently, we systematically investigated the role and function of different thalamic nuclei in endogenous discrimination and modulation of nociception. We found that thalamic mediodorsal (MD) nucleus and ventromedial (VM) nucleus function as a novel 'nociceptive discriminator' that participates in discrimination of noxious mechanically and heat evoked responses, and are involved in descending facilitation and inhibition, respectively (You et al., 2013). The specific role of thalamic nuclei in discrimination and modulation of nociception is of particular importance as it may result in a better understanding of the dynamic function of endogenous modulation of pain, and may further assist researchers and clinicians in effectively treating intractable pathological pain.

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To date, the formalin test in animals has been validated as a 'complex' pain model showing two well-identified phases of nociceptive behaviors, which can mimic acute and tonic pain (Ambriz-Tututi et al., 2011; Cadet et al., 1993; Dubuisson and Dennis, 1977; Wheeler-Aceto and Cowan, 1991; You and Arendt-Nielsen, 2005). Subcutaneous (s.c.) injection of formalin into the hind paw of rodents induces an early phase (phase 1: about 1–5 min after injection) and a late phase (phase 2: around 15–60 min) of nociceptive behavior (Shibata et al., 1989; Wheeler-Aceto et al., 1990; for review, see Tjølsen et al., 1992). Similar to formalin-elicited behavioral responses, spinal dorsal horn nociceptive neurons, i.e. wide-dynamic range (WDR) neurons, also exhibit biphasic nociceptive firing during the exposure to s.c. formalin injection (Dickenson and Sullivan, 1987; You and Chen, 1999). With respect to the descending modulation of formalin-induced biphasic nociception, the early phase of nociceptive response and secondary hyperalgesia were reported to be controlled and maintained by descending facilitation (Ambriz-Tututi et al., 2011; Green et al., 2000; Wiertelak et al., 1997). However, others reported that descending inhibitory action involving complex signaling cascades participates in formalin-induced nociception (Omote et al., 1988). Understanding of formalin-elicited specific, biphasic nociception and its modulation from supraspinal structures, in particular the initiation and maintenance of descending facilitation and inhibition, may inform development of therapeutic strategies for the effective treatment of pathological pain.

In the present study, a bolus of 25–200 μl 2.5% formalin was intramuscularly injected into the gastrocnemius (GS) muscle of conscious rats to elicit tonic muscle nociception. Bilateral thresholds of paw withdrawal reflexes to noxious mechanical and heat stimulation were tested before and after the formalin injection. We report that descending modulations: facilitation and inhibition, were differentially involved in i.m. formalin-induced long-lasting biphasic muscle nociception associated with bilateral secondary mechanical hyperalgesia and heat hypoalgesia. These endogenous facilitatory and inhibitory controls were initiated and maintained by activities of thalamic MD nucleus and VM nucleus via descending pathways of dorsal funiculus (DF) and dorsal lateral funiculus (DLF), respectively.

A preliminary report of the present study was presented in abstract form.

Materials and methods

Animals

Male Sprague–Dawley rats weighing 260–300 g (10 weeks of age) 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 Animal Care and Use Committee of the University in accordance with IASP's guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). The animals were acclimatized to the laboratory and habituated to the test boxes for at least 1 h each day for five days prior to the testing. The rats were used only once and sacrificed at the end of the experiment by intraperitoneal (i.p.) injection of an overdose of sodium pentobarbital (200 mg/kg). All efforts were made to minimize the number of animals used and their suffering.

Muscle nociception elicited by intramuscular injection with formalin

A volume of 25–200 μl 2.5% formalin (0.925% formaldehyde diluted in 0.9% saline) was injected into the gastrocnemius (GS) muscle of the left (ipsilateral) hind limb in order to establish 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 20–30 s.

Lesion of dorsal funiculus (DF) and dorsal lateral funiculus (DLF)

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and then moved to a stereotaxic frame (MP8003, RWD Life Science Co., China), followed by a laminectomy performed at T3–4 for lesion of either DF or DLF. In brief, the surgical site was aseptically treated, a sagittal incision was made after local lidocaine anesthesia at the mid-thoracic level, and blunt dissection revealed the dorsal surface of T3–4 vertebrae. After that, the *dura* and arachnoid membrane of the spinal cord were carefully opened under a dissection microscope (Nikon-SMZ645, Japan). According to the rat atlas (Paxinos and Watson, 1998), ipsilateral lesion of either DF (lateral 0–1 mm from midline, dorsoventral 0–1.2 mm from the *pia mater*) or DLF (lateral 1.5–2.7 mm from midline, dorsoventral 0–1.2 mm from the *pia mater*) was made using a sharp needle blade controlled precisely by a manual hydraulic micro drive (MO-81, Narishige Co., Japan). Special cautions were taken in the surgery to avoid damage of blood vessels or spinal roots, in particular dorsal root entry zone. After the lesion of either DF or DLF, the *dura* was patched with Gelfoam, and overlying muscles were closed in a simple continuous pattern and skin was closed in a simple interrupted pattern. In sham lesion rats, the *dura* was opened and a Gelfoam patch was placed without damaging the spinal cord. After the surgery, the animals were immediately intraperitoneally injected with 3 ml warm 0.9% saline, and benzylpenicillin sodium (80 mg/kg, Harbin Pharmaceutical Group Holding Co. Ltd, China) was subcutaneously injected to prevent inflammation. After that, the animals were returned to the box for a 1-week recovery, during which the animals' physiological signs were monitored. After a 1-week recovery, body movement and coordination were evaluated using the Rota-Rod test. Animals with motor dysfunction, i.e. loss of limb movement, alterations of stepping patterns and interlimb coordination, were only involved in histological identification, and were excluded from the remaining behavioral testing experiments.

Electrolytic lesion of contralateral thalamic MD and VM nuclei

The anesthetized (sodium pentobarbital, 50 mg/kg, i.p.) rats were mounted in the stereotaxic frame with fixation of the head by ear bars and tooth plate. After local lidocaine analgesia, the scalp was cut and the cranium was drilled. A thalamic nucleus located contralaterally to the i.m. formalin injection was electrolytically lesioned. To perform the electrolytic lesion of the thalamic mediodorsal (MD) and the ventromedial (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 thalamic nuclei areas at the following coordinates: MD nucleus: anteroposterior − (2.3–2.8) mm from bregma, lateral 0.75 mm from midline, dorsoventral 5.2–5.4 mm from the cranium; and VM nucleus: anteroposterior − (2.3–2.8) mm, lateral 1.2–1.5 mm from midline, dorsoventral 7.1–7.2 mm from the cranium (You et al., 2013). An electrolytic lesion was made by means of an electrical stimulator generating a 150 μA anodal DC current for 30 s through the electrode tip. The lesion current was continuously monitored by 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 and 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 motor dysfunction assessed by means of the Rota-Rod treadmill were excluded from the remaining experiments.