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Unilateral subcutaneous bee venom but not formalin injection causes contralateral hypersensitized wind-up and after-discharge of the spinal withdrawal reflex in anesthetized spinal rats

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Abstract

This study aimed to investigate the effect of tonic nociception on spinal withdrawal reflexes including (1) long lasting spontaneous responses elicited by subcutaneous (s.c.) administration of formalin (2.5%, 50 μl) and bee venom (BV, 0.2 mg/50 μl) into the hind paw and (2) corresponding ipsilateral (primary) and contralateral (secondary) hypersensitivity to noxious pinch and repetitive supra-threshold (1.5 × *T*) electrical stimuli at different frequencies (3 Hz: wind-up; 20 Hz: after-discharge) in anesthetized spinal rats. Spinal withdrawal reflexes were studied by simultaneously assessing single motor units (SMUs) electromyographic (EMG) activities from the bilateral medial gastrocnemius (MG) muscles. Subcutaneous formalin-induced persistent spontaneous SMU EMG responses were in typical biphasic manner with an apparent silent period (about 13–18 min), but in contrast, BV elicited monophasic long lasting (about 1 h) SMU EMG responses without any resting state. The mechanically and electrically evoked responsiveness of SMUs were enhanced significantly by ipsilateral BV injection, whereas enhanced electrically, but not mechanically, evoked responses (including wind-up and after-discharge) were found at the non-injection site of the contralateral hind paw. However, s.c. administration of formalin was only able to establish ipsilateral hypersensitivity of the SMUs to repeated electrical, not mechanical, stimulation. Neither mechanically nor electrically evoked contralateral hypersensitivity of the SMUs was found during the ipsilateral formalin-induced nociception. For pharmacological intervention, intrathecal administration of the non-*N*-methyl-D−aspartate (non-NMDA) receptor antagonist CNQX (40 nmol/10 μl), but not the non-competitive NMDA receptor antagonist MK-801 (40 nmol/10 μl), significantly depressed BV-induced contralateral hypersensitivity of the SMUs to repeated 3 Hz (wind-up) and 20 Hz (after-discharge) frequencies of electrical stimulation.

Using the extracellular SMU recording technique, we found that s.c. administration of formalin and BV shows a significant difference in long lasting spontaneous firing of SMUs. This is consistent with previous observations in animal behavioral studies. Additionally, contralateral electrically evoked hypersensitivity of the SMUs was found only following BV injection, not in the formalin test. The maintenance and development of BV-induced contralateral hypersensitivity of the spinal withdrawal reflex to noxious electrical stimulation indeed depend on different central pharmacological receptors. The spinal non-NMDA, but not the NMDA, receptors may play important role in BV-induced contralateral central hyperexcitability and sensitization.

Keywords: After-discharge; Bee venom; Formalin; Inflammation; NMDA receptor antagonist; Non-NMDA receptor antagonist; Wind-up; Withdrawal reflex

Introduction

According to the contemporary theory concerning different observations of location and different quality of responsiveness or sensation (Willis, 1992), behavioral and electrophysiological studies in humans as well as in animals reveal that a peripheral tissue lesion or injury following strong natural noxious stimuli or inflammation may lead to two kinds of peripheral hyperalgesia to different noxious stimulation. Specifically, primary hyperalgesia can be found within or restricted to the injured area in response to noxious

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mechanical and thermal stimulation. Whereas, in clinical patients with some muscle pain syndromes, secondary hyperalgesia and/or referred pain usually occurs in the undamaged skin surrounding, adjacent, or even remote to the injured site to noxious mechanical stimulation, but controversially not thermal stimulation (Hardy et al., 1952; Lewis, 1942). At present, it is widely accepted that the occurrence of primary hyperalgesia is markedly related to the ongoing sensitization of peripheral nociceptors, whereas central components, in particular the mechanism involving the spinal cord, may mainly take part in the generation of dynamic or static secondary hyperalgesia (Cervero et al., 2003; Klede et al., 2003; Treede and Magerl, 2000; Treede et al., 1992; Ziegler et al., 1999).

Compared with the formalin model, subcutaneous (s.c.) injection of bee venom (BV) in rats and cats has been developed as a 'new' experimental inflammatory tonic pain model clearly showing the monophasic persistent (about 1 h) spontaneous responses of spinal cord dorsal horn (DH) nociceptive neuron such as wide-dynamic range (WDR) neurons in electrophysiological studies or flinching, lifting, and licking reflexes in behavioral studies (Chen et al., 1999a,b; Lariviere and Melzack, 1996; You and Chen, 1999). More recently, different experiments employing multidisciplinary approaches in animals and humans have clearly demonstrated that the BV model may provide us with a useful compass for exploring, not only the long lasting spontaneous nociception in monophasic manner associated with long-term (3–4 days) toxic chemical injury-induced primary mechanical and heat hyperalgesia at injection site, but also heat hyperalgesia located in the surrounding secondary area and even the remote contralateral non-injection limb, which is mostly accompanied with some central plastic changes (Chen et al., 2000, 2003; Sumikura et al., 2003; You et al., 2002, 2003b). In contrast to the detailed different responsiveness to mechanical and thermal stimulation in primary injection site and remote non-injury site observed in behavioral investigation, no attempt using electrophysiological method has been made systematically in the BV model. The purpose would be to investigate whether some discrepancies of ipsilateral (primary) and contralateral (secondary) hypersensitivity to noxious stimuli also exist during the nociception induced by BV and other chemical agents (e.g., formalin).

The aim of the present electrophysiological study in spinalized rat was to investigate and compare the long lasting effects of s.c. administration of formalin and BV on ipsilateral and corresponding contralateral responsiveness of the spinal withdrawal reflex. The withdrawal reflex was assessed by recording single motor unit (SMU) electromyographic (EMG) response from the medial head of gastrocnemius (MG) muscles to noxious pinch and repetitive supra-threshold ($1.5 \times T$) intensity electrical stimuli at different frequencies (3 Hz: wind-up; 20 Hz: after-discharge). In addition, the possible role of the central *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors on contralateral hypersensitivity of withdrawal reflex during the BV-induced pathological nociception was investigated and revealed.

Materials and methods

Male Wistar rats weighing 250–350 g were used in the present experiment. The animals were provided by the Animal Facilities of Aalborg Hospital and housed pairwise under a 12:12 h light–dark cycle with food and water available ad libitum. The procedures were approved by the institutional Animal Ethics Committee. The International Association for the Study of Pain guidelines for experimental research were followed (Zimmermann, 1983), and efforts were made to minimize suffering and reduce the number of animals used.

Experimental preparation

The rats were initially anesthetized with pentobarbital (50 mg/kg) by intraperitoneal (i.p.) administration. During the surgical procedures, a tracheal cannula and a left jugular vein catheter were inserted in order to ensure adequate breathing and fluid circulation. The mean arterial systolic blood pressure of the right carotid artery (80–140 mm Hg) was monitored by a blood pressure amplifier (50110 V, Illinois, USA) via a catheter; and the heart rate was monitored continuously. Body temperature was maintained constant at 37.5 ± 0.5°C by means of a homeothermic circulating water blanket beneath the abdomen of the rat. All vital signs were kept within physiological range.

To exclusively exclude the influential effect from the supraspinal control on nociceptive transmission of the spinal withdrawal reflex, a mini laminectomy was performed at T8–T9, and the spinal cord was completely transected by a surgical knife under a dissection microscope. Another separate laminectomy was performed from T13 to L1 vertebrae to expose the corresponding lumbar spinal cord segments. The dura mater and the arachnoid membrane of the lumbar spinal cord were carefully removed for intrathecal (i.t.) administration of different glutamate receptor antagonists. After this procedure, a paraffin pool was made with ambient skin flaps around the exposed incision area of the lumbar spinal cord and filled with warm paraffin oil (37°C) to prevent drying.

After surgery, the animal was placed in a stereotaxic frame and artificially ventilated. The ventilation was monitored continuously; the end-tidal CO_2 was monitored by a CO_2 monitor (Normocap, Datex, Finland). The value of end-tidal CO_2 was controlled within the normal range between 25–35 mm Hg by adjusting ventilation rate (60–80 breaths/min) and tidal volume (3–5 ml). Anesthesia was continued by inhalation of halothane (2% halothane in 98% oxygen) instead of pentobarbital. Note that muscle relaxants