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Role of the cerebrospinal fluid-contacting nucleus in the descending inhibition of spinal pain transmission

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ABSTRACT

The brainstem is well recognized as a critical site for integrating descending modulatory systems that both inhibit and facilitate pain at the level of the spinal cord. The cerebrospinal fluid-contacting nucleus (CSF-contacting nucleus) distributes and localizes in the ventral periaqueductal central gray of the brainstem. Although emerging lines of evidence suggest that the CSF-contacting nucleus may be closely linked to transduction and regulation of pain signals, the definitive role of the CSF-contacting nucleus in pain modulation remains poorly understood. In the present study, we determined the role of the CSF-contacting nucleus in rat nocifensive behaviors after persistent pain by targeted ablation of the CSF-contacting nucleus in the brainstem using the cholera toxin subunit B–saporin (CB–SAP), a cytotoxin coupled to cholera toxin subunit B. Compared with CB/SAP, CB–SAP induced complete ablation of the CSF-contacting nucleus, and the CB–SAP-treated rats showed hypersensitivity in responses to acute nociceptive stimulation, and exacerbated spontaneous nocifensive responses induced by formalin, thermal hyperalgesia and mechanical allodynia induced by plantar incision. Furthermore, immunohistochemical experiments showed that the CSF-contacting nucleus was a cluster of 5-HT-containing neurons in the brainstem, and the spinal projection of serotonergic axons originating from the CSF-contacting nucleus constituted the descending 5-HT pathway to the spinal cord. CB–SAP induced significant downregulation of 5-HT in the spinal dorsal horn, and intrathecal injection of 5-HT significantly reversed hypersensitivity in responses to acute nociceptive stimulation in the CB–SAP-treated rats. These results indicate that the CSF-contacting nucleus 5-HT pathway is an important component of the endogenous descending inhibitory system in the control of spinal nociceptive transmission.

Introduction

The spinal dorsal horn being the first synapse in the pain pathway, is a key target for the control of spinal nociceptive transmission via both local segmental and supraspinal mechanisms. The descending regulation of spinal nociceptive processing originates from many brain regions, and the brainstem plays a critical role in the experience of pain. The sites in the brainstem involved in the descending control of pain include the periaqueductal gray (PAG) (Leith et al., 2007; Reynolds, 1969), rostral ventromedial medulla (RVM) (Dubner and Ren, 2004; Heinricher et al., 2009), the more lateral and caudal dorsal reticular nucleus (DRt) and the ventrolateral medulla (VLM) (Tavares and Lima, 2007). It is known that the descending regulation of these areas can be inhibitory as well as facilitatory (Ossipov et al., 2010; Staud, 2013).

Wang and Zhang (1992) first identified a group of cells with their bodies mainly located in the ventral PAG of the brainstem, with their

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dendritic processes penetrating the ependymal cells and stretching into the cerebrospinal fluid (CSF) in the cavity of the ventricle in the central nervous system. They identified these cells by injecting horseradish peroxidase-conjugated toxin subunit B (CB–HRP), as a retrograde tracer, into the lateral ventricle, and this group of cells was named the cerebrospinal fluid-contacting nucleus (CSF-contacting nucleus) (Zhang et al., 2003); their findings indicated that it may be involved in signal transmission and transport of substances between the brain parenchyma and CSF via release or absorption of bioactive substances. Previous studies, including one by the present authors, suggest that the CSF-contacting nucleus may be closely linked to transduction and regulation of pain signals via certain molecular mechanisms (Chao et al., 2010; Du et al., 2009; Geng et al., 2008; Lu et al., 2009; Wang et al., 2014; Xu et al., 2011); however, the definitive role of the CSF-contacting nucleus in pain modulation is not clear yet.

It has been well documented that a major spinal projection of serotonergic axons originating from an anatomically discrete group of serotonin (5-HT)-containing neurons mainly in the PAG, RVM, and DRt of the brainstem, constitutes the descending 5-HT pathway to the spinal cord (Millan, 2002), and accumulating evidence from anatomical, pharmacological, and electrophysiological studies has indicated that the descending 5-HT pathway is an important component of the descending modulatory system that constitutes a major mechanism underlying the control of spinal ascending conduction of nociceptive information (Campbell and Meyer, 2006; Sommer, 2006; Dogrul et al., 2009). Given its anatomical and functional characteristics, it would be reasonable to postulate that the CSF-contacting nucleus may be involved in descending pain modulation. We hypothesize that the CSF-contacting nucleus may act as a component of the descending modulatory mechanism involved in the control of spinal pain processing. To better understand a more definitive role of the CSF-contacting nucleus in spinal pain transmission, in the present study, we established rats with targeted ablation of the CSF-contacting nucleus in the brainstem using the cholera toxin subunit B–saporin (CB–SAP), a cytotoxin coupled to cholera toxin subunit B. To the best of our knowledge, this is the first such model to be reported. Using this model, we performed immunohistochemical characterization of the CSF-contacting nucleus and its fiber projection, and assessed the contribution of the CSF-contacting nucleus to descending pain modulation in different persistent pain models.

Materials and methods

Animals

Adult male Sprague–Dawley rats (weighing 250 to 300 g) were provided by the Experimental Animal Center of Xuzhou Medical College (license number: SYXK [Jiangsu] 2002–0038). Animals were housed in standard transparent plastic cages at temperatures of 23 ± 1 °C, under a 12-h light–dark cycle (lights on from 08:00 to 20:00), with food and water provided ad libitum. Prior to the experiments, the animals were allowed to acclimatize to the housing facility for at least 1 week, and during the experimental period, all efforts were made to minimize animal suffering. All experimental protocols were performed with the approval of the Animal Care and Use Committee of Xuzhou Medical College (Xuzhou, Jiangsu Province, China), and in accordance with the Declaration of the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (Publication No. 80-23, revised 1996) and the policies on the use of laboratory animals issued by the International Association for the Study of Pain (IASP).

Drugs

5-Hydroxytryptamine, serotonin hydrochloride (5-HT) and CB–HRP were purchased from Sigma-Aldrich (St. Louis, MO, USA). CB–saporin and saporin were purchased from Advanced Targeting Systems (San Diego, CA, USA), and CB was purchased from Absin Bioscience Inc. (Shanghai, China). All the drugs were dissolved in artificial cerebrospinal fluid (ACSF). The dosages of the drugs were based on the results of preliminary experiments and our previous studies (Lu et al., 2008; Zhang et al., 2003; Zhou et al., 2013). The administration protocol and dose for each drug are presented in the results and figures.

Intrathecal drug administration

Intrathecal drug administration was performed using a modified version of a previously described method (Mestre et al., 1994; Nitzan-Luques et al., 2013; Xu et al., 2006). In brief, rats were briefly anesthetized with 2% isoflurane and the lower back skin was shaved; then, the intervertebral spaces were widened by bending the spine over a cylinder. A 28-gauge stainless steel needle attached to a 50-μl Hamilton microsyringe was angled rostrally at 45° to the vertebral axis and inserted through the L2–L3 intervertebral space (at the level of the L4–L5 spinal cord lumbar enlargement), aiming about 2 mm to the left of the midline. A sudden slight flick of the tail or paw indicated successful entry into the dorsal subarachnoid space. Then, 20 μl of 5-HT or the vehicle was slowly injected over a 1-min period, and the needle was left in place for a further 15 s. In each case, the animals fully recovered from the anesthesia within 10 min after completion of the injection. No abnormal behavioral consequences of the injection were observed.

Intracerebroventricular injection

Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and then fixed in a digital stereotaxic instrument (Stoelting Co., IL, USA). A midline incision was made after infusion of 2% lidocaine in the skin. An opening was made in the skull with a dental drill to insert a microinjection needle into the target site. The stereotaxic coordinates for the rats' right lateral ventricles (LVs) were as follows: −1.2 ± 0.4 mm caudal to the Bregma, 3.2 ± 0.4 mm ventral to the surface of the skull, and 1.4 ± 0.2 mm right to the median sagittal plane (Paxinos and Watson, 2005). Then, 3 μl of CB–HRP, CB–SAP, or CB and saporin was injected slowly over a 10-min period using a 5-μl Hamilton microsyringe with a 32-gauge needle, which was left in place for a further 15 s. All the wounds were closed and covered with an antibiotic ointment, and animals were returned to their cages after they recovered from the anesthesia.

Measurement of physiologic parameters

The respiratory rate and heart rate of the rats were measured by the tail-cuff method with Softron BP98A (Softron Beijing Biotechnology Co. Ltd., China). An electronic clinical thermometer was used to measure the body temperature of the rats. Locomotor activity was measured using a video tracking system for spontaneous activity (ZH-SBS; Anhui Zheng Hua Biological Instrument Equipment Co., Ltd., China), and the distance traveled in 1 h was recorded.

Plantar incision persistent pain model

The rat hindpaw plantar incision model of postoperative persistent pain was established as previously described (Brennan et al., 1996; Spofford and Brennan, 2012), with minor modifications. Briefly, rats were anesthetized with 2% isoflurane, and the plantar surface of the right hindpaw was prepared with povidone iodine. A 1.0-cm longitudinal incision was made through the skin and fascia of the plantar aspect of the paw, starting 0.5 cm from the end of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally, allowing the muscle origin and insertion to remain intact. Following hemostasis with gentle pressure, the skin was closed with two mattress sutures using 5-0 nylon. Antibiotic ointment was applied