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The nucleus raphe magnus OFF-cells are involved in diffuse noxious inhibitory controls

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ABSTRACT

Diffuse noxious inhibitory controls (DNIC) are very powerful long-lasting descending inhibitory controls which are pivotal in modulating the activity of spinal and trigeminal nociceptive neurons. DNIC are subserved by a loop involving supraspinal structures such as the lateral parabrachial nucleus and the subnucleus reticularis dorsalis. Surprisingly, though, whether the nucleus raphe magnus (NRM), another supraspinal area which is long known to be important in pain modulation, is involved in DNIC is still a matter of discussion. Here, we reassessed the role of the NRM neurons in DNIC by electrophysiologically recording from wide dynamic range (WDR) neurons in the trigeminal subnucleus oralis and pharmacologically manipulating the NRM OFF- and ON-cells. In control conditions, C-fiber-evoked responses in trigeminal WDR neurons are inhibited by a conditioning noxious heat stimulation applied to the hindpaw. We show that inactivating the NRM by microinjecting the $GABA_A$ receptor agonist, muscimol, both facilitates C-fiber-evoked responses of trigeminal WDR neurons and strongly attenuates their inhibition by heat applied to the hindpaw. Interestingly, selective blockade of ON-cells by microinjecting the broad-spectrum excitatory amino acid antagonist, kynurenate, into the NRM neither affects C-fiber-evoked responses nor attenuates DNIC of trigeminal WDR neurons. These results indicate that the NRM tonically inhibits trigeminal nociceptive inputs and is involved in the neuronal network underlying DNIC. Moreover, within NRM, OFF-cells might be more specifically involved in both the tonic and phasic descending inhibitory controls of trigeminal nociception.

Introduction

Pain is a complex experience that involves multiple components, including sensory-discriminative, cognitive-evaluative, and affective-emotional ones. In turn, the central nervous system modulates the transmission of nociceptive messages according to the nature of the painful stimulus and behavioral state of the individual (Fields and Basbaum, 2006; Millan, 2002). Thus, a network of descending pathways projecting from the brainstem either inhibits or facilitates the transfer of nociceptive information at the level of the spinal and medullary dorsal horn to higher centers.

One of these naturally activated descending controls is the diffuse noxious inhibitory controls (DNIC). DNIC are very powerful, long-lasting inhibitory controls which have been shown to strongly inhibit spinal (Le Bars, 2002) as well as trigeminal nociceptive neurons (Dallel et al., 1999; Dickenson et al., 1980b). They are triggered by noxious stimuli applied to any part of the body distant from the excitatory receptive field of the neuron under study. DNIC are subserved by a loop with the afferent and efferent pathways running within the ventrolateral quadrant and the dorsolateral funiculus of the spinal cord, respectively (Le Bars, 2002). Nevertheless, that sectioning the spinal cord suppresses DNIC suggests that these controls also involve supraspinal areas (Le Bars, 2002). Thus a region in the caudal medulla, the subnucleus reticularis dorsalis (SRD), was shown to be critically involved in DNIC (Bouhassira et al., 1992). More recently, we demonstrated that the spinoparabrachial (Lapirot et al., 2009) and the hypothalamic dopaminergic descending pathways (Lapirot et al., 2011) contribute to the ascending and descending part, respectively, of the loop subserving DNIC.

The nucleus raphe magnus (NRM), which has been extensively studied in many species, including the rat, is known to play a key role in the descending control of dorsal horn nociceptive processing (Fields and Basbaum, 2006; Heinricher and Ingram, 2008). Its electrical stimulation produces antinociception as well as inhibits the responses of spinal (Fields and Basbaum, 2006) and trigeminal nociceptive neurons (Chiang et al., 1995; Lambert and Zagami, 2009; Meng and Hu, 2000). In addition, the NRM sends direct massive projections to the spinal cord and trigeminal sensory complex via the dorsolateral funiculus (Fields and Basbaum, 2006; Lovick and Wolstencroft, 1983; Mason and Fields, 1989). Such

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evidences suggest that the NRM contributes to DNIC. Surprisingly, though, experiments designed to test whether the NRM is involved in DNIC have provided conflicting results. On one hand, Morton et al. (1987) showed that, in cats, DNIC are reversibly blocked by a microinjection of local anesthetic into the NRM, a finding consistent with an earlier report of reduced DNIC in rats after electrolytic lesion of this brainstem region (Dickenson et al., 1980a). On the other hand, Bouhassira et al. (1993) failed to find any effect of the ibotenic acid lesion of the NRM area on DNIC. Interestingly, though, a recent fMRI study in humans using cold pressor of the right leg as a conditioning stimulation to reduce heat-induced pain in the left arm suggests the involvement of the periaqueductal gray–rostral ventromedial medulla network into such descending controls of pain (Sprenger et al., 2011). The present experiments were designed to reexamine the issue of the involvement of the NRM in DNIC by using electrophysiological methods in the rat trigeminal system.

Methods

Animals

Adult male Sprague–Dawley rats (175–200 g) were obtained from Charles River laboratories (France) and maintained in a light- and temperature controlled environment (lights on 19.00–7.00 h, 22 °C) with food and water ad libitum. All efforts were made to minimize animal suffering and to reduce the number of animals used. Experiments followed the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and ethical guidelines of the directive 2010/63/UE of the European Parliament and of the Council on the protection of animals used for scientific purposes. Protocols applied in this study have been approved by the local animal experimentation committee: CEMEAA "Comité d'Ethique en Matière d'Expérimentation Animale Auvergne" (n° CE 26-12).

Animal preparation

The experiments always started in the morning during the dark phase of the rats' light cycle. As previously described (Lapirot et al., 2009; Normandin et al., 2013), the animals were anesthetized with 2% halothane in a NO_2/O_2 mixture (2:1). After intramuscular injection of 100 μg atropine sulfate, the trachea was cannulated and the carotid artery and external jugular vein catheterized. The animals were then paralyzed by an intravenous perfusion of vecuronium bromide (2.4 mg/h) and artificially ventilated with a volume-controlled pump (54–55 strokes/min). Levels of halothane, O_2, N_2O and end-tidal CO_2 (3.5–4.5%) were measured by an anesthetic gas analyzer (Drager Vamos) during the entire experimental period. These parameters were digitally displayed and under the control of alarms. The arterial catheter was attached to a calibrated pressure transducer (UFI, Morro Bay, CA, USA) connected to an amplifier (Stoelting, Wood Dale, IL, USA) for continuous monitoring of the mean arterial blood pressure (90 to 110 mm Hg) and heart rate. The analog output from the blood pressure amplifier was connected to a computer data sampling system (Cambridge Electronics Design 1401 computer interface; Cambridge, UK). The colorectal temperature was kept constant at 38 ± 0.5 °C by means of a feedback-controlled heating blanket. A sufficient depth of anesthesia was confirmed periodically by the lack of spontaneous movements or arousal responses to noxious stimuli, and a good cutaneous vascularization, by assessing the color of the paw extremities and the delay with which they regained normal color after pressure application.

The animals were placed in a stereotaxic frame with the head fixed in a ventroflexed position (incisor bar dropped 5 mm under the standard position) by means of an adapted metallic bar. A hole was drilled into the skull over the cerebellum, and the dura removed to allow placement of a micropipette within the NRM. A second small craniotomy was performed on the right side at the level of the occipitoparietalis suture to allow the placement of a recording electrode in the spinal trigeminal subnucleus oralis (Sp5O). After surgery, the level of halothane was reduced to 0.6–0.7% and maintained at this level during the recording period.

Microinjections

Drugs were delivered into the NRM by two-barrel glass micropipettes (3GC120F-15; Clark Electromedical Instruments, Pangbourne, UK) fixed on the micromanipulator and attached to two Hamilton syringes (0.5 μl) with a length of polyethylene tubing (Dualé et al., 1998; Lapirot et al., 2009). The micropipette was broken back maximally to a diameter of 70–100 μm. It was positioned stereotaxically above the targeted brainstem site 1 h before the injection. The coordinates used for microinjection sites were 1.0 to 2.6 mm caudal to the interaural plane and 0.0 mm mediolateral (Paxinos and Watson, 1997). Micropipettes and tubings were filled with either the $GABA_A$ receptor agonist, muscimol (0.25 nmol in 100 nl or 0.75 nmol in 300 nl), the broad-spectrum excitatory amino acid antagonist kynurenate (1 nmol in 200 nl), or saline with pontamine sky blue, respectively (for location of the injection site). We varied the dose of muscimol by injecting different volumes of the same concentration rather than different concentrations within the same volume. Since we used a concentration known to effectively inactivate NRM neurons (see below), we speculated that we would obtain a stronger effect if we could reach more NRM neurons through a larger injected volume. Injections of drugs were performed with a manual injector over a period of 2 min and monitored by observing the movement of an air bubble in the tubing. The rate of injection was slow to minimize the chance of tissue damage. The micropipettes remained in place throughout the experiment. The doses of drugs were selected based on published reports (da Silva et al., 2010; Gilbert and Franklin, 2001; Heinricher and McGaraughty, 1998; Lapirot et al., 2009). The dose of kynurenate was chosen as sufficient to suppress ON-cell activity selectively with no effect on OFF- or NEUTRAL-cell discharge in normal animals (Heinricher and McGaraughty, 1998; Heinricher and Roychowdhury, 1997). Muscimol and kynurenate acid (Sigma Aldrich, France) were dissolved into saline and prepared every testing day.

Unitary extracellular recordings

Using glass micropipettes (8–10 MΩ) filled with pontamine sky blue solution, we explored the right Sp5O (1.1 to 2.6 mm caudal to the interaural plane, 2.4 to 3.0 mm lateral). Single unit activities were amplified and displayed on oscilloscopes and led into a window discriminator connected to a CED 1401plus interface and a computer (Spike 2.05 software), to allow sampling and analysis of the spontaneous and evoked neuronal activities. Wide dynamic range (WDR) neurons were recognized based on their responses to mechanical and percutaneous electrical stimulations of their receptive field (Dallel et al., 1999). Specifically, neurons that responded in a graded manner with increasing firing rates to the stimulus range from non-noxious to noxious intensity were classified as WDR neurons. Once a neuron had been identified, its receptive field was mapped. Electrical square-wave stimuli (2 ms duration) were applied through a pair of stainless steel needle electrodes subcutaneously placed into the center of the previously delineated receptive field and thresholds for eliciting A- and C-fiber-evoked responses determined. In post-stimulus time histograms (PSTHs), C- and A-fiber evoked responses were distinguished according to their latencies: all spikes between 30 and 300 ms post-stimulus were considered as C-fiber-evoked (Dallel et al., 1999; Hu, 1990). Only one cell was tested in each animal, and only cells showing no change in spike amplitude or waveform during the complete experimental procedure were considered.