Neuroscience 209 (2012) 196–207

FUNCTIONAL INTERACTIONS BETWEEN THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS AND RAPHE MAGNUS. A COMPARATIVE STUDY OF AN INTEGRATED HOMEOSTATIC ANALGESIC MECHANISM

M. CONDÉS-LARA,[a,b]* G. ROJAS-PILONI,[a] G. MARTÍNEZ-LORENZANA,[a] D. CONDÉS DIEZ-MARTÍNEZ[c] AND J. RODRÍGUEZ-JIMÉNEZ[a]

[a]*Departamento de Neurobiología del Desarrollo y Neurofisiología, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus UNAM-Juriquilla, Querétaro, 76230, México*

[b]*INSERM/CPN, U.894 Site Pitié-Salpêtrière, Hôpital de la Pitié-Salpêtrière, París, France*

[c]*Facultad de Medicina, Universidad Autónoma de Querétaro, Querétaro, México*

Abstract—This work compares the effects of electrical stimulation of the paraventricular hypothalamic nucleus (PVN) and the raphe magnus nucleus (RMg) on the single-unit response from dorsal spinal cord neurons activated by nociceptive receptive field stimulation. We evaluated the effects of stimulating the PVN or RMg individually or simultaneously, as well as PVN stimulation after RMg electrolytic lesion. PVN or RMg stimulation suppressed the A-delta, C fiber, and postdischarge, and we demonstrated that their simultaneous stimulation increases the duration and intensity of suppressive effects. RMg lesion increased the peripheral responses, but PVN stimulation continued to be suppressive. The intrathecal administration of 20 μl of a 10^{-5} M solution of a specific oxytocin antagonist strongly reduced the PVN effects, and 20 μl of 10^{-6} M naloxone significantly reduced the RMg suppression of receptive field responses. Some spinal cord cells presented a short-latency, evoked action potential (6.8 ms and a variability of ±0.5 ms) produced by the RMg stimulation. This is interpreted as a direct postsynaptic action of the RMg on the spinal cord cells. We never found similar responses produced by the PVN, and therefore, we propose that the PVN effects are presynaptic. Finally, the immunohistochemical experiments confirmed the oxytocinergic and the vasopresinergic innervation used by the PVN projection to the RMg, and they raise the possibility that other neurotransmitters are involved. We conclude that the PVN and the RMg form part of a homeostatic analgesic mechanism acting on the same spinal cord cells to block the noxious information, but using different mechanisms. Both structures, and others, contribute to the homeostatic mechanism of endogenous analgesia.

Key words: analgesia, homeostasis, pain mechanism, oxytocin, rats.

Several structures of the central nervous system have been reported to participate in endogenous analgesic mechanisms; these include the raphe magnus nuclei (RMg) and the locus coeruleus (LC), which are among the most studied (Stamford, 1995; Kishi et al., 2006). Our group and others have recently proposed that electrical activation of the paraventricular nucleus of the hypothalamus (PVN) activates an oxytocinergic mechanism that produces analgesia and elicits antinociceptive effects in rodent models of inflammatory and acute pain (Arletti et al., 1993; Ge et al., 2002; Miranda-Cardenas et al., 2006; Yirmiya et al., 1990; Shiraishi et al., 1995; Ying et al., 2006; Pinto-Ribeiro et al., 2008) by blocking the A-delta, C fibers, and postdischarge mediated in spinal dorsal horn cells a response to noxious stimulation (Condés-Lara et al., 2006, 2007; Rojas-Piloni et al., 2007). This PVN analgesic mechanism correlates well with the presence of oxytocin (OT) and PVN projections to the spinal cord (Saper et al., 1976; Swanson and McKellar, 1979; Rousselot et al., 1990; Condés-Lara et al., 2007) and with the OT levels measured in cerebrospinal fluid and spinal cord tissue in neuropathic rats (Martínez-Lorenzana et al., 2008).

Little is known about the different mechanisms producing analgesia and their interactions other than studies comparing analgesia produced by electrical stimulation of the RMg to that of analgesia produced by the stimulation of periaqueductal gray (PAG) (Willcockson et al., 1983). This PAG–RMg system involves an endogenous analgesic mechanism related to endogenous opiate liberation at the spinal cord level (for review see Oliveras et al., 1981; Cannon et al., 1982; Prieto et al., 1983). One of the most interesting pieces of evidence that central electric stimulation produces analgesia is the decrease or suppression of this effect by the administration of an opiate antagonist, such as naloxone (NALX). It has also been proposed that the descending serotoninergic mechanism is mediated by the serotoninergic 5-HT_{1A} receptor (Buritova et al., 2005). However, in contrast to the multiple serotoninergic receptor subtypes, oxytocin has only one, currently known receptor. Moreover, the descending OT system could also be affected, at least to some extent, by NALX administration (Miranda-Cardenas et al., 2006). Immunohistochemical experiments were performed to confirm the OT projection from the PVN to RMg. As spinal dorsal horn repre-

*Correspondence to: M. Condés-Lara, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus UNAM-Juriquilla, Querétaro, México. Tel/fax: +52-442-2381042.
E-mail address: condes@servidor.unam.mx (M. Condés-Lara).

Abbreviations: ConLesion, activity evoked by receptive field stimulation 20 min after RMg lesion; control, activity evoked by receptive field stimulation; FG, flurogold retrograde tracer; NALX, naloxone; OT, oxytocin; OTA, oxytocin antagonist; PAG, periaqueductal gray; PVN, paraventricular hypothalamic nucleus; RMg, raphe magnus nucleus; stPVN, PVN electric stimulation; stPVNposL, effects of PVN stimulation 20 min after the RMg lesion; stPVN–RMg, simultaneous stimulation of PVN and RMg; stRMg, RMg stimulation; VP, vasopressin; WDR, wide dynamic range cells.

0306-4522/12 $36.00 © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2012.02.032

sents the first synaptic relay for nociceptive information it has been considered a powerful target to central modulation. Descending controls generated in the midline periaqueductal gray–rostral ventromedial medulla (PAG–RVM) have been widely studied (Heinricher et al., 2009; Vanegas and Schaible, 2004). The RVM with the double system of ON-cells and OFF-cells (Fields et al., 1983; 2006) could be engaged by higher structures in order to mediate behavioral states to modulate pain (Heinricher et al., 2009). Pain states, as primary hyperalgesia and allodynia seem to be facilitated by PVM activity and inhibited by PAG (Vanegas and Schaible, 2004). It seems likely that all facilitator and inhibitory systems will be triggered simultaneously by the noxious signalization, and this particular but essential point of view was addressed by Pinto-Ribeiro et al. (2011) showing that PVN activation by direct glutamate application could hold back the excitatory responses of caudal ventromedial medulla but exclusively in arthritic rats and not in controls. With a similar point of view we addressed the question of the PVN and RMg interactions and functional convergence on spinal cord dorsal horn cells to contribute as part of a wild antinociceptive mechanism.

EXPERIMENTAL PROCEDURES

The experiments followed the recommendations of the International Association Study for Pain (Zimmermann, 1983) and the guidelines contained in the NIH Guide for the Care and Use of Laboratory Animals (80-23, revised in 1996) and were also approved by the Bioethics Committee at the Instituto de Neurobiología. We assure that we made all the efforts to minimizing the number of animals as well as their suffering. Sixty-three male Wistar rats (280–310 g) were used in this study. The rats were housed in individual cages for at least 24 h before the experiment, with water and food *at libitum* and a light-dark cycle of 12 h–12 h.

Surgical procedures

The rats were introduced into a hermetically sealed, ventilated box with an atmosphere containing 2.5% servoflorane in a mixture of three-fourths O_2 and one-fourth N_2O. A tracheotomy was performed to introduce a cannula into the trachea, providing artificial ventilation and maintaining the anesthesia throughout the experiment at 2% servoflorane. End tidal CO_2, O_2, and the electrocardiogram were monitored throughout the experiment, and the temperature was maintained at 38 °C by means of a circulating water pad. The rats were fixed in a stereotaxic apparatus, and two small holes were drilled in the skull to place silver ball electrodes for electrocorticogram recording. This allowed us to be certain about the rat's anesthesia level and to ensure that there was no electroencephalographic awakening during the noxious stimulation. Moreover, the animals were not paralyzed, and we did not observe withdrawal reactions during noxious stimulation. The animals were placed in a spinal cord unit frame, and lumbar vertebrae were fixed to improve stability at the recording site. A laminectomy was performed to expose spinal cord segments L4–L6 as described elsewhere (see Condés-Lara et al., 2003).

Electrical stimulation and recording procedures

Concentric stainless steel electrodes (1 MΩ resistance) were placed in the PVN at 7.0 mm AP from interaural, 0.2 mm lateral, and 2 mm height and for the RMg at −1.5 mm AP from interaural, 0 lateral, and −0.3 mm height from the Paxinos and Watson stereotaxic atlas (Paxinos and Watson, 1998). These electrodes were connected to isolated stimulation units providing the electrical stimulation of the PVN or RMg. PVN or RMg electric stimulation was delivered at 60 Hz with a pulse duration of 1 ms (Grass S88 Stimulator). In initial experiments comparing the effects of PVN and RMg electric stimulation, we varied the duration of the stimulation train; based on these results, we fixed the duration at 6 s and the intensity at 300 μA. This stimulation time was selected because it allowed us to observe an inhibitory action and a recovery of the cell responses in the minutes just after the PVN or RMg electrical stimulation. The interval between PVN, RMg, or simultaneous PVN and RMg stimulations was at least 10 min.

Single-unit recordings were made (WPI Duo773) using glass micropipettes filled with 2% Pontamine Sky Blue in a 1 M KCl solution; the electrode resistances were 8–10 MΩ. We employed a recording system that allowed us two simultaneous and independent channels of recording. However, the simultaneous recordings did not allow us to have the same receptive field for both recorded cells, and as a rule we studied only one cell because of the restricted receptive field locations. When a single-cell recording was achieved, we manually tested in the hind paw to locate the receptive field region that activated the recorded cell. In fact, all cells studied were classified as wide dynamic range (WDR) cells, as they responded to different intensities of tactile and noxious stimulations. Once the receptive field was located, we inserted thin stainless steel electrodes to deliver electrical stimulation (Grass S11 Stimulator) to the selected region with 1-ms pulses at 0.5 Hz and with increasing intensities to determine the threshold for activating A-delta and C fibers. If the cell responded with an activation corresponding to A-delta and C fibers, we increased the stimulation intensity to three times the threshold intensity. The single-unit activity was digitalized and analyzed offline using the Spike 2 system (CED, Cambridge, UK); in this way we constructed peri-stimulus histograms for 1-s duration with a bin time of 10 ms. Considering the latencies of the responding fibers, we classified the responses as A-beta between 0 and 20 ms; as A-delta for 20–90, as C fibers for 90–300, and as postdischarge when action potentials occurred with a latency of more than 300–800 ms, as described previously (Condés-Lara et al., 2005). We proceeded to test the effects of central stimulation; as a control, we used spinal cell responses to receptive field stimulation (20 stimuli) followed by the PVN or the RMg electric stimulation and then a second receptive field stimulation (another 20 stimulations). The receptive field stimulation was continuous, and Fig. 1 shows an example of the experimental procedure. There was a period of 5–10 min between each of the electric stimulating tests. These procedures allowed us to compare receptive field stimulation responses of the recorded spinal cord cells before and after PVN or RMg stimulation. We stimulated both PVN and RMg simultaneously to test for a possible additive effect. In 10 experiments the effects of PVN stimulation on the WDR cell responses were tested after electrolytic RMg lesion. Some spinal cord cells respond with short latencies to RMg stimulation, and to test if they were antidromic we used the following criteria: (1) they have fixed latency responses, (2) they can follow high stimulation frequency, and (3) there had collision between spontaneous and evoked activity. The collision time was the sum of the latency time of the responses plus the refractory period.

Additionally, we compared the effects produced after the intrathecal administration of 20 μl of 0.9% saline containing 10^{-6} NALX or a 10^{-5} M solution of the specific oxytocin antagonist (OTA, d(CH_2)5[Tyr(Me)2,Thr^4,Tyr-$NH_2$9]OVT, donated by Professor M. Manning at Medical College, OH, USA). The drugs' effects were followed for 30 min and then for another 30 min after wash. NALX or OTA action was assessed in different animals. In the case of the RMg lesion, the excitability of spinal cord cells was tested 20 min after lesion.