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RESEARCH NOTE

Lumbar Dorsal Root Potentials Elicited by Stimulation of Nucleus Locus Coeruleus

A. VANIA APKARIAN, CHARLES J. HODGE, JR., RICHARD T. STEVENS, AND JOEL I. FRANCK[1]

Neurosurgery Laboratory, Upstate Medical Center, State University of New York, Syracuse, New York 13210

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Lumbar dorsal root potentials (DRP) were elicited by nucleus locus coeruleus (LC) stimulation in the cat. Inhibition, by LC stimulation, of dorsal horn cells responding to noxious inputs corresponded in time with the DRPs evoked by LC stimulation. Comparing cutaneous stimulation-evoked DRPs with LC stimulation-evoked DRPs and their respective effects on dorsal horn single-unit activity suggested a shared segmental underlying mechanism and the possible involvement of the coeruleospinal system with that of a diffuse noxious inhibitory suprasegmental loop.

The dorsolateral pons (DLP) of the cat, consisting of the nucleus locus coeruleus (LC), nucleus subcoeruleus, the Kolliker–Fuse nucleus, and the parabrachial nuclei, has a large concentration of noradrenaline (NA)-containing cells (4, 14, 22) and has been shown to be the primary source of noradrenergic innervation to the spinal cord (6, 17, 21, 28). The bulbospinal noradrenergic projection has been implicated in the modulation of nociceptive inputs to the spinal cord, affecting both stimulation-produced and morphine-induced analgesia (1, 2, 8, 9, 20, 23, 25). Recent investigations have demonstrated differential effects of electrical stimulation of the LC on lumbar dorsal horn interneurons (7, 11) such that cells with noxious afferent inputs

Abbreviations: DRP—dorsal root potential, LC—locus coeruleus, DNIC—diffuse noxious inhibitory control.

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have their responses to peripheral cutaneous stimulation inhibited more often and at a lower LC stimulation amplitude than cells with purely innocuous inputs. The inhibition was shown to be independent of noradrenergic stores (11), and anatomical studies demonstrated that in the cat the majority of cells projecting to the lumbar spinal cord from the region of the LC are, in fact, nonnoradrenergic (26). These results imply that LC stimulation effects, in the cat, are probably mediated by primarily nonnoradrenergic neurons. We assessed the possible role of primary afferent depolarization in coeruleospinal sensory modulation. The effects of LC stimulation on dorsal horn primary afferent fibers were investigated by recording dorsal root potentials (DRPs) induced by LC stimulation. The temporal relationship between these DRPs and the effects of LC stimulation on lumbar dorsal horn interneuron activity was also studied. The LC stimulation-evoked DRPs were also compared with DRPs evoked by cutaneous or electrical stimulation of the hind limb.

Thirteen adult cats weighing 2.5 to 3.5 kg were anesthetized with alpha-chloralose (70 mg/kg, i.v.) and paralyzed by intravenous injection of gallamine triethiodide (Flaxedil). Blood pressure, body temperature, and end expiratory CO_2 were maintained within acceptable values (10). The lumbar enlargement was exposed and the L7 or S1 dorsal roots mounted on a pair of silver–silver chloride recording wires. Extracellular single-unit activity was recorded in the lumbar dorsal horn using parylene-coated tungsten electrodes. Units were identified as dorsal horn cells by criteria described elsewhere (10) and their location within the dorsal horn was verified both by direct microdrive depth measurements and the histologic verification of electrolytic lesions. Only cells situated within lamina IV and V were used. Monopolar parylene-coated tungsten stimulating electrodes were placed in the dorsolateral pons region.

Localization of the LC was both by stereotaxy and by recording from the trigeminal mesencephalic nucleus (11, 24). The following brain stem stimulation characteristics were used: stimulus trains of 50 ms, biphasic constant current pulses of 200 μs duration with a 100 Hz frequency and amplitudes varying from 50 to 200 μA. At the end of each experiment the brain stem site eliciting the greatest DRP was marked by an electrolytic lesion (constant current, 10 μA for 10 s). Brains were removed and stored 1 week in 10% buffered Formalin. Serial transverse sections (40 μm) were collected and stained with cresyl echt violet to determine the locus of the electrolytic lesion (Fig. 1A). The site of the lesion and the path of the stimulating electrode were plotted on the appropriate sagittal section (Fig. 1B). By this means, the correspondence between the nuclei traversed and their specific stimulation effects could be determined.

The brain stem stimulating electrode, inserted at a 45° angle, passed through the cerebellum, fourth ventricle, dorsolateral pons, and the pontine reticular

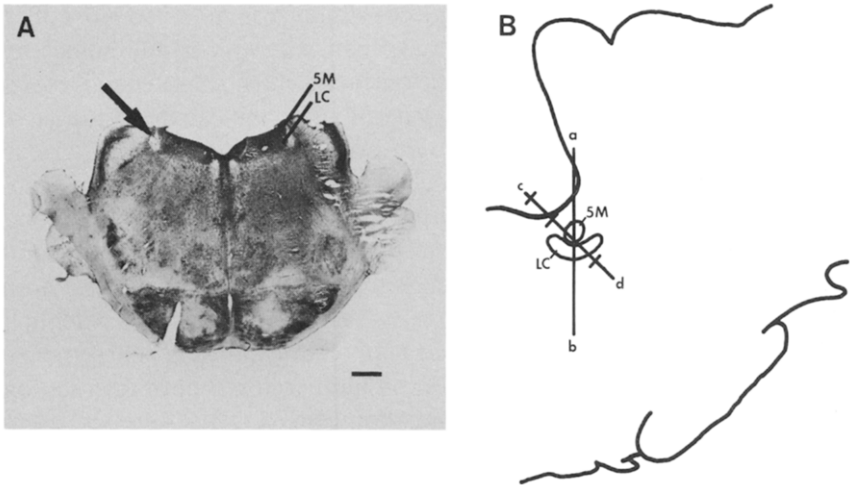

FIG. 1. Photomicrograph of the electrolytic lesion and a plot of the path travelled by the stimulating electrode. A—a Nissl-stained transverse section from the midpontine level illustrating an electrolytic lesion (large arrow) at the border between the 5M and the LC (labeled on the opposite side of the brain stem). Bar = 1.0 mm. B—sagittal plot of the brain stem at a mediolateral position where the lesion would be placed. Line ab represents the plane of the transverse section corresponding to the photomicrograph in A. Line cd shows the path travelled by the stimulating electrode. The electrolytic lesion was at the intersection of lines ab and cd. Abbreviations: 5M—mesencephalic trigeminal nucleus, LC—locus coeruleus.

formation (Fig. 2). In all animals stimulation in the region of the dorsolateral pons evoked a negative lumbar DRP. Whenever checked (N = 6), this LC stimulation-evoked DRP was bilateral (Fig. 2). As the stimulating electrode traversed the brain stem, the threshold current required for evoking a DRP varied. The minimum threshold was found when the stimulating electrode was in the LC region. Electrical stimulation within the cerebellum or the pontine reticular formation also evoked DRPs, as reported by others (3). The LC stimulation-evoked DRP was differentiated from cerebellar stimulation and pontine reticular formation stimulation-evoked DRPs by a longer latency to onset. The latency for LC-evoked DRPs was 50 to 70 ms (N = 12), compared with a latency of 20 to 30 ms (N = 4) for cerebellar and pontine reticular formation-evoked DRPs. These differences in latency and threshold suggested that the LC-evoked DRPs were generated via a pathway different from the cerebellar- or the reticular-evoked DRPs.

To study the relation between inhibition of dorsal horn units and DRPs induced by LC stimulation, DRPs and extracellular action potentials of dorsal horn units were recorded simultaneously. The minimum threshold for both inhibiting lumbar dorsal horn cells and for evoking lumbar DRPs resulted from electrode placement in the LC. The average minimum current needed for evoking a DRP by LC stimulation was 105 μA (N = 10). This compared

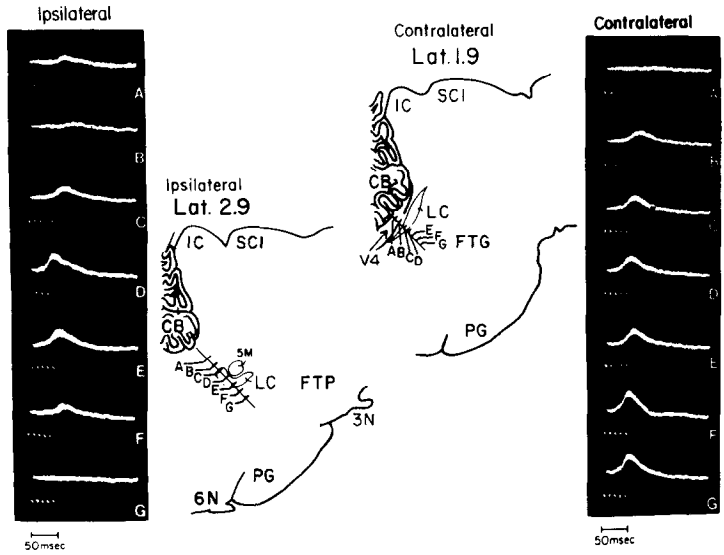

FIG. 2. Bilateral stimulation of the area of the LC results in negative (upward deflection) lumbar dorsal root potentials (DRPs). Ipsilateral—the maximum DRP was evoked by stimulation within the LC (Ipsilateral, D). Position of stimulating electrode illustrated in sagittal brainstem section Lat. 2.9, D (2.9 mm lateral to midline). As the electrode was moved away from the LC, the DRP disappeared. Contralateral—with the contralateral stimulating electrode placed more medially Lat. 1.9 (1.9 mm lateral to midline), lumbar DRPs were evoked by LC stimulation (contralateral, C), and by stimulation of the FTG, within the pontine reticular formation (contralateral F, G). Stimulation amplitude was 150 μA, 5 pulses (dots), 100 Hz. Abbreviations: 3N—third nerve, 5M—mesencephalic trigeminal nucleus, 6N—sixth nerve, FTG—gigantocellular tegmental field, FTP—parvocellular tegmental field, IC—inferior colliculus, LC—locus coeruleus, PG—pontine grey, SCI—superior colliculus.

with an average minimum current of 145 μA needed to inhibit cells with innocuous inputs ($N = 11$) and 25 μA needed to inhibit cells with noxious inputs ($N = 20$). One-way analysis of variance between the three groups of thresholds yielded a significant difference ($F = 21.2$, $P < 0.01$). To compare the differences between each of the means, the Scheffé method of post hoc comparison of the means was used. Those comparisons showed a significant difference between the mean threshold for inhibiting cells with innocuous inputs versus cells with noxious inputs ($P < 0.05$), a significant difference between the mean thresholds for inhibiting cells with noxious inputs versus evoking DRPs ($P < 0.05$), and a nonsignificant difference between the mean threshold for inhibiting cells with innocuous inputs versus evoking DRPs. The threshold for inhibiting cells with noxious inputs was significantly lower than the threshold for evoking DRPs, implying a probable causality between the two events. Therefore, the relationship between these events was further investigated.

Ten of 12 cells responding to noxious stimuli were inhibited by LC stimulation (50 ms, 20 to 100 μA). In all 10 cells, the time course of LC-induced inhibition of their noxiously evoked responses began approximately 50 ms after onset of LC stimulation and lasted for 200 to 250 ms. The duration of the simultaneously recorded LC-induced DRP was between 50 to 150 ms from onset of LC stimulations. This indicated a time correspondence between LC-evoked DRPs and LC-evoked inhibition; however, the LC-evoked inhibition, in all cases, outlasted the DRP. Because the amount of the current needed to elicit a DRP was significantly higher than the current needed to inhibit dorsal horn cells with noxious inputs, and because the LC-induced inhibitory response outlasted the DRP, it follows that one can produce inhibition of dorsal horn cells with noxious inputs by electrical stimulation of LC without eliciting DRPs. Therefore, there must be other mechanisms (i.e., postsynaptic mechanisms or multisynaptic loops), in addition to the presynaptic system, involved in the LC stimulation-produced inhibition of cells with noxious inputs.

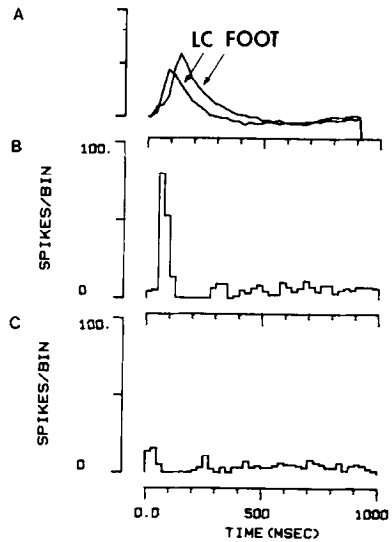

FIG. 3. Comparison of DRPs and lumbar single-unit activity evoked by LC or peripheral skin stimulation. A—the trace illustrates DRPs (averaged over 20 runs), evoked either by LC stimulation (LC) or electrical stimulation of the receptive field of a unit responding to toe pad pinch (FOOT). The peak of the LC-evoked DRP had a shorter latency than the toe-evoked DRP. B—average lumbar dorsal horn unit response to peripheral electrical stimulation (N = 20), recorded simultaneously with its DRP (A, FOOT). C—response of the same unit to LC stimulation (N = 20), recorded simultaneously with its DRP (A, LC). Both peripheral- and LC-evoked DRPs were preceded by a phasic excitation of the lumbar cell. The peak of the DRPs corresponded to the time of the inhibition of the unit. LC stimulation amplitude was 200 μA, 5 pulses, 50 ms. Cutaneous stimulation amplitude was 200 μA, single pulse of 1 ms duration. Cutaneous and LC stimuli start at time 0.0.

Stimulation of LC elicits an initial phasic excitation of dorsal horn units with noxious inputs (11). Similar results were reported by Iggo *et al.* (12, 13). When the LC is stimulated, the initial phasic excitation (Fig. 3C) precedes both the evoked DRP (Fig. 3A) and the evoked inhibition (Fig. 3C), seen in four of four recordings of the on-going spontaneous activity of cells with noxious inputs. A similar pattern of response is exhibited when cells responding to noxious inputs are inhibited by stimulation of the face or tail (11). Recently, studies by LeBars *et al.* (16, 18, 19) showed that dorsal horn cells with A and C fiber afferent inputs are inhibited by noxious stimuli applied to various parts of the body. This process was termed diffuse noxious inhibitory control (DNIC). The effect of DNIC is eliminated in the spinal animal (19), implying the involvement of supraspinal systems. The similarity of the pattern of response caused by LC stimulation and cutaneous electrical stimulation (Fig. 3B), i.e., excitation and then DRP and inhibition, suggests that the coeruleospinal innervation may be a part of the descending aspect of the DNIC loop. This is further supported by the fact that LC neurons are excited by sciatic nerve stimulation (27) and noxious stimulation throughout the body (5, 15).

In conclusion, stimulation of LC evokes negative DRPs which correspond in time with the inhibition of dorsal horn cells with noxious inputs. The underlying mechanism for this pathway is one that may be shared by peripheral afferent fibers and has a presynaptic component. It is proposed that the coeruleospinal system might be the descending part of the suprasegmental diffuse noxious inhibitory control loop.

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