The Caudal Ventrolateral Medulla as an Important Inhibitory Modulator of Pain Transmission in the Spinal Cord

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Abstract: The caudal ventrolateral medulla (VLM) has emerged during the last decade as one of the main components of the endogenous pain control system. Profound and long-lasting analgesia is produced by mild stimulation of the VLM. The VLMlat, the reticular formation located between the spinal trigeminal nucleus and the lateral reticular nucleus (LRt), appears to play a major role in that antinociceptive action. The projections to spinal cord laminae involved in nociceptive transmission originate exclusively in the VLMlat. The VLMlat participates in a disynaptic pathway involving spinally projecting pontine A5 noradrenergic neurons, which appears to convey α_2-adrenoreceptor–mediated analgesia produced from the VLM. Neurons in the VLMlat and in lamina I are reciprocally connected by a closed loop that is likely to mediate feedback control of supraspinal nociceptive transmission. On the other hand, the LRt, which is targeted by ventral (lamina VII) and deep dorsal (laminae IV to V) horn inputs, projects to the premotor lamina VII. Nociceptive input ascending from the cord and increases in blood pressure are discussed as possible physiologic triggers of the analgesia produced by the VLM. The overall role of the VLM as a center for integration of nociceptive, cardiovascular, and motor functions is discussed. The putative therapeutic benefits of manipulating the VLM for the control of chronic pain are envisaged.

Key words: *Pain control, supraspinal centers, stimulation-produced analgesia, nociceptive and cardiovascular integration.*

Nociceptive transmission from the spinal cord is subject to modulatory influences originating from widespread brain areas, which we will collectively refer to as the endogenous pain control system.[11,20,34,43,61] The existence of a system that selectively modulates pain was predicted by the Gate Control Theory of Melzack and Wall[81] and received support from studies showing that electrical or chemical stimulation of the periaqueductal gray (PAG) or nucleus raphe magnus and adjacent reticular formation (collectively known as the rostral ventromedial medulla, RVM) inhibited behavioral responses to noxious stimulation without affecting reactions to other environmental stimuli.[11,34,57,64,101] This so-called stimulation-produced analgesia (SPA) was later found to be also elicited from several other brain regions, providing further evidence for the wide extent and heterogeneity of the endogenous pain control system.[20,34,43,49,61,83,94,97,99]

Several studies have shown that the SPA produced from most components of the endogenous pain control system results from inhibition of nociceptive activity at the level of the spinal cord. Tract tracing studies showed direct or indirect connections with the spinal dorsal horn, whereas electrophysiologic recordings showed that local electrical or chemical stimulation in various brain areas selectively inhibited nociceptive responses of dorsal horn neurons.[61,105] Moreover, the magnitude of the analgesia obtained by manipulating the system correlated with the decrease of noxious-evoked spinal induction of the c-*fos* proto-oncogene, a reliable marker of nociceptive neuronal activation in the spinal cord.[44,51,53,98,99,128] As to the spinal dorsal horn circuitry targeted by the descending modulatory system, direct inhibition of neurons projecting in the spinothalamic and spinomesencephalic tracts has been clearly established,[61] and actions on spinal interneurons, namely, inhibition of excitatory interneurons[65] and activation of inhibitory interneurons,[82] have also been inferred.[123] With respect to their neurochemical nature, 3 main types of descending mod-

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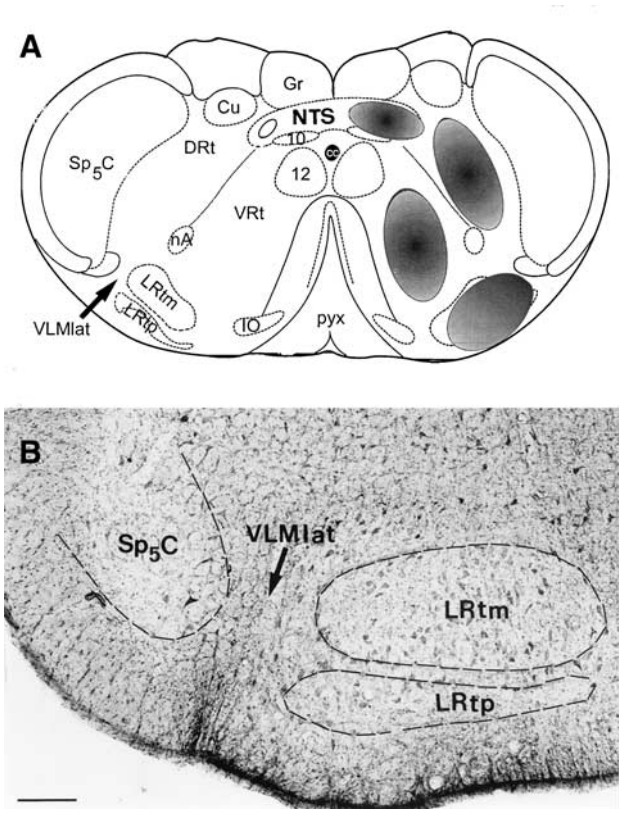

Figure 1. (A) Drawing depicting the components (dashed regions) of the endogenous pain modulatory system at a coronal section of the medulla oblongata obtained 5.3 mm caudal to the interaural line. Adapted from references 61 and 91. (B) Photomicrograph of the VLM. 10, Dorsal motor nucleus of the vagus; 12, hypoglossal nucleus; cc, central canal; Cu, nucleus cuneatus; Gr, nucleus gracilis; IO, inferior olive; LRtm, magnocellular part of the LRt; LRtp, parvocellular part of the LRt; pyx, pyramidal decussation; nA, nucleus ambiguus. Scale bar in (B) represents 0.20 mm.

ulatory pathways have been demonstrated by anatomic and pharmacologic studies: opioidergic, serotonergic, and noradrenergic.[34,94] The intrathecal administration of suitable agonists and antagonists has shown that the SPA frequently involves the participation of the 3 neurotransmitters, which interact in a complex manner, specific for each component of the pain control system.[61]

The existence of a similar pain modulating system in humans has been postulated on the basis of the observation that patients with chronic pain experienced analgesia after electrical stimulation of some of the pain control sites identified in animals, namely, the thalamus, the PAG, and periventricular gray areas.[12,14,104] The invasive nature of deep brain stimulation in humans has, however, precluded a complete demonstration of the phylogenetic homology of the endogenous pain modulatory system between rodents and humans. However, the distribution of the neurotransmitters involved in pain modulation is highly conserved across mammalian species, including humans.[30,92] That the opioidergic component of the system is engaged in pain control by morphine and its derivatives is evidenced by the profound hyperalgesia produced in humans on administration of the opioid antagonist naloxone.[13,46,92] Furthermore, the interactions between the opioidergic and noradrenergic components of the pain control system, demonstrated at the experimental level, are clinically relevant. The coadministration of opioid derivatives and α_2-adrenoreceptor agonists results in improved analgesia without some of the adverse effects of opioid treatment alone.[24,26,47,90]

In recent years a multitude of data has demonstrated the complexity of the endogenous pain control system. The role played by the medulla oblongata in the endogenous pain modulatory system deserves special attention. The medulla oblongata is the brain region with the higher density of areas involved in pain modulation, and it presents some peculiarities in the way it controls nociception. Besides the RVM, the medullary regions belonging to the endogenous pain modulatory system include the nucleus tractus solitarii (NTS), dorsal reticular nucleus (DRt), ventral reticular nucleus (VRt), and caudal ventrolateral medulla (VLM) (Fig 1A). Electrical or chemical stimulation of the RVM, NTS, VRT, or VLM inhibits the behavioral responses to pain and the nociceptive activity of dorsal horn neurons.[61] Contrary to the SPA produced from most areas of the endogenous pain control system, opioids do not appear to be significantly involved in descending modulation from the medulla oblongata with the exception of the RVM. Another characteristic of pain modulation from the medulla oblongata is that it is the only part of the system from which facilitation of pain transmission, ie, pronociception, can be produced along with antinociception. Neurons in the RVM facilitate nociceptive transmission in the spinal cord,[32-34,36,37] and enhanced nocifensive behavioral responses are observed after stimulation of the DRt.[5,7] Finally, it has been claimed that pain-modulating areas also control other functions that affect nociceptive transmission and are influenced by noxious events.[72,73] The medullary regions controlling autonomic functions, which are key components of the pain control system, are also likely to be responsible for the interaction between cardiovascular changes and pain.[95,127]

Located at the ventrolateral quadrant of the medulla oblongata, the VLM emerged recently as an important component of the endogenous pain modulatory system. The efficacy of analgesia originated from the VLM, as assessed by the electrical thresholds for inhibition of nociceptive dorsal horn neurons and by the magnitude of duration of suppression of nociceptive reflexes,[42,54,55] is greater than that of most other areas including PAG,[19,57] the RVM,[87,102] and the locus coeruleus.[59,60] Moreover, the VLM appears to be responsible for tonic descending inhibition of spinal nociceptive neurons.[40,48] During the last decade, a large amount of anatomic and functional data on the mechanisms underlying pain modulation from the VLM has accrued, prompting the present review of the specific role of the VLM in the endogenous pain control system in relation to previously studied areas such as the PAG and RVM.[11,38,48] The anatomic data recently compiled point to the existence of subregions within the VLM in what concerns the participation in pain modulatory circuits, which could not be predicted

by physiologic and pharmacologic studies because of the characteristics of such functional studies.

Direct VLM-Spinal Pathways

Tract tracing studies have shown that the VLM is a heterogeneous region in terms of its efferent projections to the spinal cord. The projections to spinal areas containing nociceptive neurons, namely, laminae I, IV to V, and X,[35] originate in the region of the reticular formation designated as VLMlat[109,111,112] (Fig 1B) located between the spinal trigeminal nucleus, pars caudalis (Sp_5C), and the lateral reticular nucleus (LRt). On the other hand, lamina VII, which contains mainly premotor interneurons, is targeted exclusively by the LRt, a region involved in motor control.[52] That the VLMlat is the region within the VLM responsible for pain modulation is supported by the observation that the dorsolateral funiculus, which is the spinal pathway mediating the descending antinociception from the VLM,[55,56] contains fibers originating in the VLMlat, whereas the LRt projects through the ventrolateral funiculus.[114] Furthermore, although inhibition of nociceptive spinal neurons can be produced by electrical[84] or glutamate[55] stimulation of the LRt, the hypoalgesia induced from the VLMlat is more profound and long-lasting,[42] suggesting that the LRt antinociception may be due to coactivation of the VLMlat.

That the spinal laminae targeted by the VLMlat contain nociceptive neurons that project in the main ascending pathways[73] suggests that the VLMlat-spinal pathway is specifically involved in the modulation of supraspinal transmission of nociceptive information. Supporting this hypothesis, potent inhibition of nociceptive spinothalamic neurons was produced by electrical stimulation of sites encompassing the VLMlat.[55] In this regard, it is noteworthy that neurons in lamina I project back to the VLMlat,[70] forming a closed reciprocal loop that is likely to be specifically involved in pain modulation[114] (Fig 2). Terminal boutons labeled from the superficial dorsal horn (laminae I to II) contain round vesicles and establish asymmetrical synapses with VLMlat neurons projecting to those spinal layers. In lamina I, boutons labeled from the VLMlat contain either round or flattened vesicles and establish, respectively, asymmetrical or symmetrical synapses with VLMlat-projecting neurons. Considering the putative excitatory or inhibitory nature of, respectively, asymmetrical and symmetrical synapses,[23,45,103,118,120] the arrival of nociceptive input from lamina I to the VLMlat appears to trigger descending modulation of pain transmission through both excitatory and inhibitory mechanisms. Interestingly, this reciprocal loop appears to differentially involve distinct morphologic classes of VLMlat-projecting lamina I cells. Although fusiform neurons are the main source of lamina I projections to the VLMlat,[70] pyramidal cells in lamina I are contacted in much higher numbers by fibers descending from the VLMlat.[114] Incidentally, it must be noted that pyramidal neurons have a more widespread distribution of projections to supraspinal pain control centers than the other lamina I cell types and that spinofugal lamina I neurons

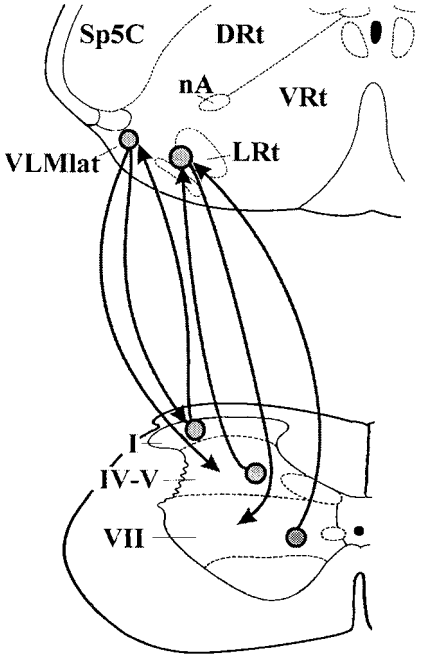

Figure 2. Diagram showing the reciprocal connections between the VLM and the spinal cord. The VLMlat–lamina I loop is closed at both the medullary and spinal levels. The LRt is reciprocally connected with lamina VII without the formation of any closed loop. The VLM projects to laminae IV-V, and this region projects to the LRt. Abbreviations as in Figure 1.

frequently have collaterals in the brain.[6,31,66-68,70,73] It is therefore possible that the pyramidal neurons may be involved in adjusting and integrating the nociceptive responses of lamina I neurons to the overall functional status of the endogenous pain control system.

Despite being targeted by the VLMlat, deep dorsal horn neurons send ascending projections only to the LRt.[70] The participation of the LRt in motor functions is well-established on the basis of its connections with the cerebellum and inferior olive.[9,22,29] Rather than being involved in feedback modulation at the spinal cord level, noxious input conveyed by deep dorsal horn neurons to the LRt[80] is more likely to trigger integrated nocifensive motor responses through connections with both motor control brain areas and spinal lamina VII.[114]

Indirect VLM-Spinal Pathways

Spinal α_2-adrenoreceptors and serotonin (5-HT) receptors mediate the antinociception produced from the VLM.[42-44,75] However, neurons of the A1 noradrenergic cell group located in VLM do not project to the cord,[111,112,121] and serotonergic cells are absent from the VLM. The widespread connections of the VLM with other components of the endogenous pain control system[61,79,124] point to the involvement of polysynaptic pathways in both noradrenergic and serotonergic antinociceptive effects from VLM. In the brainstem, VLM fibers are directly apposed to spinally projecting neurons only in the pontine A5 noradrenergic cell group and the

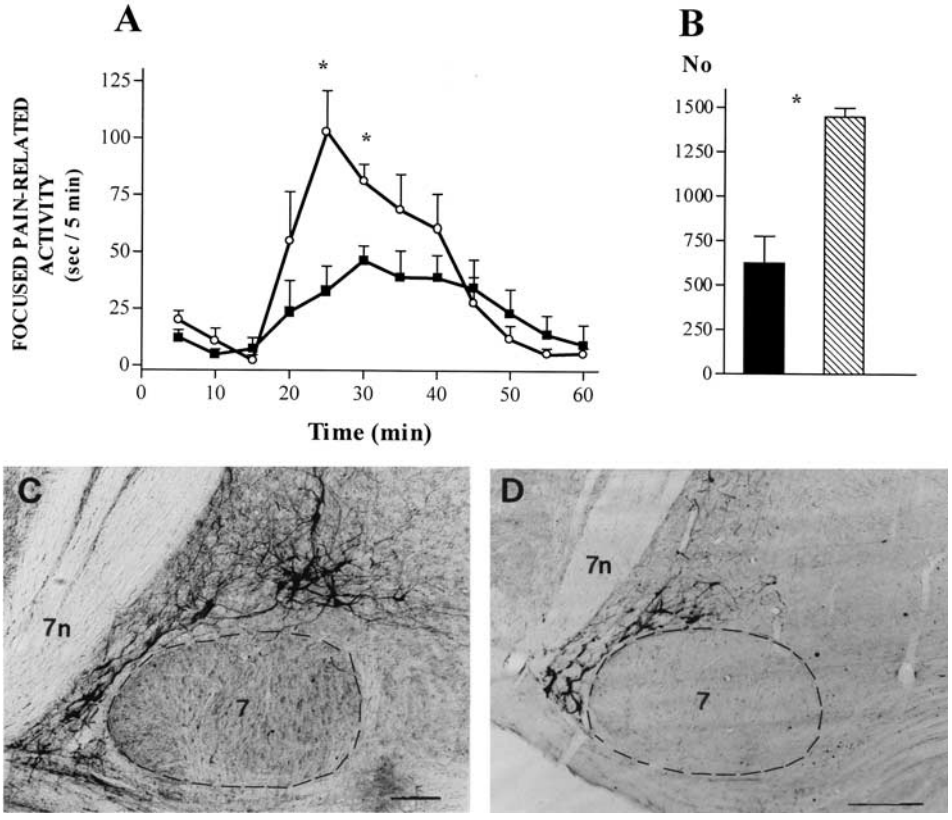

Figure 3. Effect of the administration of SAP-anti-DBH in the VLMlat on the time course of pain-related activity (A) and spinal c-*fos* expression in the dorsal horn (B) after formalin injection in the ipsilateral hindpaw. In (A), focused pain-related activity is the time (in seconds) spent in motor activity directed toward the injected paw, including licking, biting and shaking of the paw. Filled squares, control group; open circles: group with lesions. In (B), numbers of nuclei immunoreactive for the Fos protein were counted in the dorsal horn (laminae I-VI) ipsilateral to the injected paw. Filled bar, control group; dashed bar, group with lesions. In (A) and (B), numbers represent means ± standard error of mean. Asterisk represents statistical differences ($P < .05$) determined by 2-way analysis of variance with repeated measures (A) or by the paired Student *t* test (B). Photomicrographs depict DBH-immunoreactive neurons at the A5 cell group in a control animal (C) and in 1 animal with SAP-anti-DBH lesion (D). 7, Facial nucleus; 7n, facial nerve. Magnification bars, 42 μm.

RVM.[111] The VLM-A5 pathway originates exclusively from the VLMlat and establishes asymmetrical, putative excitatory, synaptic contacts with spinally projecting A5 neurons.[111,112] According to these data, the α_2-adrenoreceptor antinociception produced from the VLM is triggered in the VLMlat and mediated by the A5 noradrenergic cell group.[17,94,124] Notably, the large majority of A5 noradrenergic neurons that participate in the disynaptic pathway to the cord also have collateral projections back to the VLMlat.[112] Because noradrenaline and the α_2-adrenoreceptor agonist clonidine inhibit VLM neurons[18] and produce hyperalgesia,[89] it is possible that the A5-VLMlat projection represents the anatomic substrate for a negative feedback circuit mediating autoinhibition of the VLMlat.

Despite the lack of ultrastructural studies, it is most likely that the direct appositions between VLM fibers and spinally projecting serotonergic neurons of the RVM[111] represent synaptic contacts in a manner similar to those observed in the VLM-A5 connection.[111] The well-established involvement of 5-HT in descending antinociception from the RVM[3,15,50] suggests that the disynaptic VLM-RVM-spinal pathway conveys the serotonergic antinociceptive effects elicited from the VLM.[42,54]

Similar anatomic approaches have been used to demonstrate the existence of parallel disynaptic pathways for other components of the endogenous analgesic system. For example, terminals arising from the PAG synapse on RVM neurons that project to the cord,[62] and, in accordance, the analgesia produced by PAG stimulation are prevented by RVM lesions.[2,41,93] As to the role of the disynaptic VLMlat-A5-spinal circuit in pain modulation, ongoing experiments show that selective lesion of the A5 noradrenergic cell group by retrograde transport from the VLMlat of the neurotoxin saporin–anti-dopamine-β-hydroxylase (SAP-anti-DBH) induced moderate hyperalgesia in the second phase of the formalin test (Fig 3A; unpublished results), increased noxious-evoked spinal c-*fos* expression (Fig 3B; unpublished results), and loss of the noradrenergic neuronal population of the A5 (Fig 3C and D). The data suggest that the VLMlat input to the A5 and RVM is aimed at triggering, respectively, noradrenergic and serotonergic descending inputs. It is, however, unlikely that the VLMlat merely regulates the an-

tinociception produced from those regions. The analgesia produced from the VLMlat presents specific characteristics distinct from the antinociception elicited from the A5 noradrenergic cell group and RVM as to its intensity and duration and to the spinal receptors involved.[3,15,42,50,54,61,80] Given the degree of interconnectivity among the various components of the endogenous pain control system, it is likely that other areas besides A5 and RVM take part in the antinociception elicited from the VLMlat through more complex polysynaptic routes.

An interesting question is the relative roles of those polysynaptic connections and of the direct VLMlat-spinal pathway in pain modulation. According to pharmacologic data, antagonists of α_2-adrenoreceptors and 5-HT receptors do not completely inhibit the antinociception produced from the VLMlat.[42,54] The direct pathway may, therefore, produce the additional inhibition through neurotransmitters that remain to be established. That this direct VLMlat-spinal pathway is part of a reciprocal loop with lamina I is a further indication for its effective role in pain modulation. Similar spinomedullary loops have been shown to control pain reactions through modulation of spinal nociceptive transmission.[4,5,7,8,28] The VLMlat is thus likely to modulate pain transmission at the spinal cord through direct and indirect pathways, and the former are probably specially suited to rapid control mechanisms.

Physiologic Signals Triggering Pain Modulation from the VLMlat

Despite accumulating data suggesting a role for the VLMlat in the descending modulation of nociceptive transmission, little is known about the physiologic signals that trigger those actions. Extracellular electrophysiologic recordings and c-*fos* studies have shown that VLMlat neurons are activated by noxious stimuli[16,18,39,63,74,76,106] as well as by increases in blood pressure.[58,85,115] Neurons in both laminae I and II, the main source of spinal projections to the VLMlat,[69,70,108] are likely to convey nociceptive input. Lamina I neurons responding to peripheral noxious stimulation were antidromically activated from brainstem regions encompassing the VLMlat,[80] and noxious-evoked c-*fos* induction was observed in laminae I and II neurons that were retrogradely labeled from the VLMlat.[108] Curiously, the degree of nociceptive activation of lamina I neurons projecting to antinociceptive components of the endogenous pain control system was similar but much lower than for areas involved in pronociception. The percentages of lamina I neurons that project to the VLMlat, NTS, or caudal mesencephalon and express c-*fos* in response to noxious cutaneous stimulation are similar (23%, 21%, and 16%, respectively)[5,7,54,71,73,108] and much lower than for the pronociceptive DRt (42%).[6] Collectively, these data suggest that the nociceptive responses of lamina I neurons may be controlled by their supraspinal target.[72] In accordance, nociceptive lamina I neurons projecting to the VLMlat have recently been shown to be directly targeted by the 2 neurotransmitters

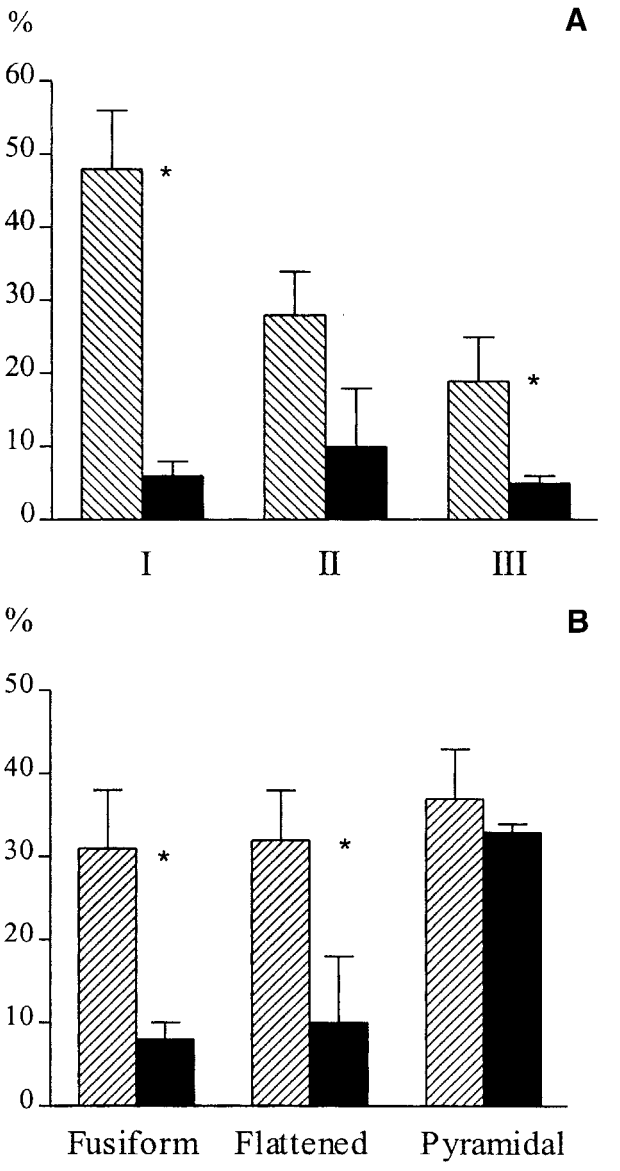

Figure 4. Proportions of spinal neurons retrogradely labeled from the VLMlat and receiving appositions from fibers immunoreactive for DBH (hatched bars) or 5-HT (filled bars). The percentages of laminae I-III receiving appositions from fibers immunoreactive for DBH were higher than those receiving appositions from 5-HT (A). In lamina I, fusiform and flattened neurons also received higher number of appositions from fibers immunoreactive for DBH (B). Asterisks represent statistical differences ($P < .05$) between percentages determined by 1-way analysis of variance with repeated measures.

involved in the descending antinociception elicited from the VLMlat, namely, noradrenaline and 5-HT.[42,54] Moreover, the noradrenergic input to superficial dorsal horn neurons, as measured by the numbers of cells receiving contacts from immunostained boutons, is much higher than the serotonergic (Fig 4A; unpublished results), which matches the stronger involvement of spinal α_2-adrenoreceptors in the analgesia elicited from the VLM.[42,54] The descending modulation of lamina I neurons appears to obey to a refined control mechanism

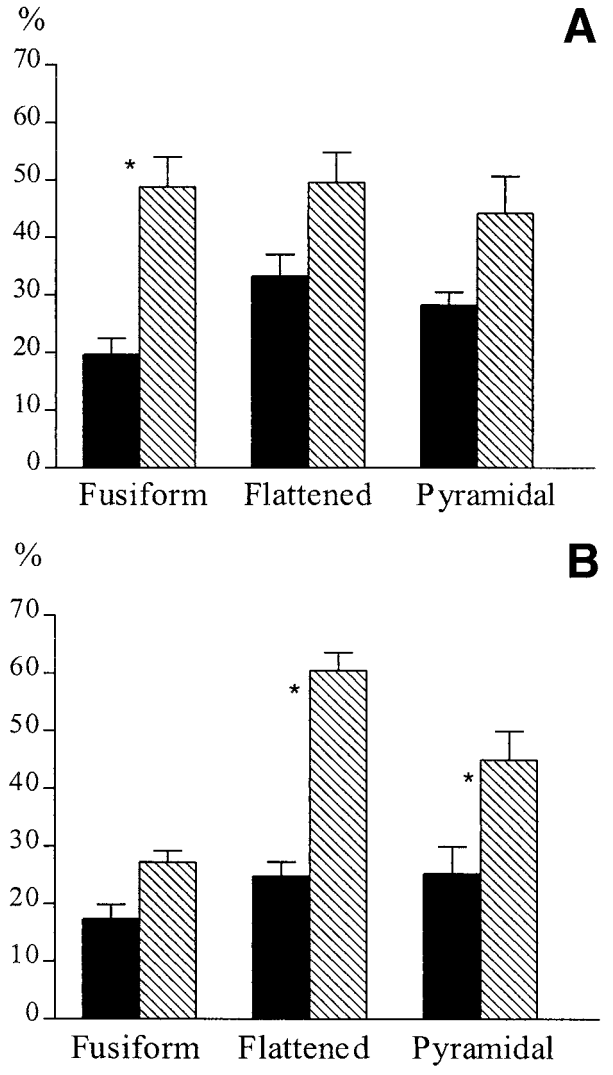

Figure 5. Effect of lesion in VLMlat on c-*fos* expression in spinal lamina I neurons retrogradely labeled with cholera toxin subunit b and with nuclei immunoreactive for the Fos protein (double-labeled neurons) from the same medullary region. The lesion was produced by injection of 0.3 μL of quinolinic acid (180 nmol/μL) after injection of the tracer. The activation of the c-*fos* proto-oncogene was induced by noxious stimulation of the internal proximal skin of the hindpaw. The % on the y-axis represent the percentages of double-labeled neurons in the total of retrogradely labeled cells. In (A), mechanical stimulation was performed by pinching the skin with a rat-tooth forceps every 2 minutes during 2 hours. In (B), chemical stimulation consisted of subcutaneous injection of 50 μL of 5% formalin and perfusion after 2 hours. Asterisks represent statistical differences ($P < .05$) in the percentages of retrogradely labeled neurons expressing c-*fos* between control animals (filled bars) and rats with lesions (hatched bars). Statistical differences were determined by 2-way analysis of variance for repeated measures.

that is likely to discriminate the morphologic cell type. The percentages of VLM-projecting fusiform and flattened neurons in laminae I that are apposed by noradrenergic boutons were much higher than for 5-HT, whereas for pyramidal cells no differences were detected (Fig 4B; unpublished results). Ongoing experiments further indicate that the supraspinal target may exert an even more refined control over the nociceptive responses of lamina I neurons, depending on the nature of the noxious stimulus and the morphologic cell class. The increase in the numbers of VLMlat-projecting lamina I neurons that express c-*fos* after VLMlat lesion is more pronounced in neurons of the fusiform type after noxious mechanical cutaneous stimulation (Fig 5A; unpublished results) and of the pyramidal and flattened classes on chemical stimulation (Fig 5B; unpublished results).

The spinal input arriving at the VLMlat differs from all the other spinofugal pathways by the high incidence of lamina II cells,[70,108] which are classically considered to be local circuit neurons.[107] These neurons appear to be highly involved in the transmission of nociceptive information to the VLMlat because the proportions of VLMlat-projecting lamina II neurons that are c-*fos* activated after noxious stimulation are even higher than those of lamina I.[85] In spite of the prevalence of inhibitory neurotransmitters in lamina II, namely, gamma-aminobutyric acid (GABA), glycine, and dynorphin,[21,117-119] it is unlikely that the lamina II–VLMlat projection is inhibitory. Lamina II neurons projecting to the VLMlat do not contain GABA, and glycine occurs exclusively in neurons containing GABA.[118,119] On the other hand, glutamate and aspartate are present in lamina II,[10,100] and the ultrastructural features of VLMlat terminals labeled from lamina I-II are consistent with excitatory synaptic transmission.

Increases in blood pressure have been shown to produce hypoalgesia.[27,77,78,95,125,126] This hypertension-induced analgesia results from inhibition of nociceptive responses of spinal dorsal horn neurons[96,110] through descending noradrenergic mechanisms.[116] The supraspinal origin of this noradrenergic inhibition was recently ascribed to the VLMlat presumably through the noradrenergic A5 relay circuit described above. The decrease in noxious-evoked spinal c-*fos* induction observed in hypertensive animals is prevented by lesions of the VLMlat.[113]

Stimulation of the VLMlat produces transient decreases in blood pressure and heart rate, whereas the opposite effect is observed after local lesion,[113] indicating that this specific region participates in the vasodepressor actions ascribed to the VLM.[25,86,122] Vasodepression has been shown to result from monosynaptic, GABA-mediated inhibition of the vasopressor rostral ventrolateral medulla,[1] whereas noradrenergic antinociception is due to monosynaptic activation of the A5 noradrenergic neurons, as described above. The VLMlat activation by increases in blood pressure is thus likely to promote integrated responses of distinct neuronal populations aimed at both decreasing blood pressure through inhibition of the vasopressor nucleus and decreasing pain sensitivity through activation of the VLMlat-A5 antinociceptive circuit. According to these data, the antinociception produced from the VLMlat is not a result of the autonomic effects. On VLMlat stimulation, the analgesia was maintained for longer than cardiovascular changes, and the latter consisted of blood pressure decreases, the opposite to what should occur if this parameter was affecting pain perception.

Conclusions and Perspectives

The VLM is an important antinociceptive center that appears to integrate nociceptive, cardiovascular, and motor functions. Although data on its performance during chronic pain are still missing, potent analgesic effects have been demonstrated for acute pain. The increase in VLM metabolic activity observed during monoarthritis[88] is, however, indicative of a strong engagement of those medullary neurons during chronic pain states. The possibility of achieving therapeutic benefits from VLM manipulation by sustained and invasive stimulation strategies is precluded because of its parallel involvement in autonomic and motor regulation. However, according to the data compiled in this review, antinociception is probably promoted in a region (VLMlat) distinct from that involved in motor control (LRt). Moreover, there are strong indications that within VLMlat, the neurons involved in analgesia are distinct from those involved in autonomic control. On this basis, the possibility to selectively manipulate the VLMlat neurons involved in antinociception by recent techniques of gene therapy should be evaluated. To achieve this purpose, we recommend further detailed investigations of the anatomy and neurochemistry of the neuronal VLMlat populations involved in pain modulation or autonomic control.

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