Brainstem Mechanisms of Persistent Pain Following Injury

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Nerve signals arising from sites of tissue or nerve injury lead to long-term changes in the central nervous system and contribute to hyperalgesia and the amplification and persistence of pain. These nociceptor activity–dependent changes are referred to as central sensitization. Central sensitization involves an increase in the excitability of medullary dorsal horn (subnucleus caudalis) and spinal dorsal horn neurons brought about by a series of events including neuronal depolarization; removal of the voltage-dependent magnesium block of the N-methyl-D-aspartate (NMDA) receptor; release of calcium from intracellular stores; phosphorylation of the NMDA, alpha amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA), and neurokinin (NK) 1 receptors via activation of protein kineses; a change in the neuron's excitability; and an increase in synaptic strength. Central sensitization occurs in trigeminal nociceptive pathways, and more robust neuronal hyperexcitability occurs following deep tissue stimulation than cutaneous stimulation. Utilizing Fos protein immunocytochemistry, it has been found that 2 distinct regions are activated in the trigeminal brainstem sensory nuclei, the subnuclei interpolaris/caudalis transition zone (Vi/Vc) and the caudal part of the subnucleus caudalis. The latter is very similar to the spinal dorsal horn and is involved in the sensory discriminative aspects of pain. In contrast, the ventral pole of the Vi/Vc is unique. In addition to its role in the nociceptive sensory processing of deep tissues, it is involved bilaterally in somatovisceral and somatoautonomic processing, activation of the pituitary-adrenal axis, and descending modulatory control. The findings support our overall hypothesis that the ventral pole of Vi/Vc is involved in the coordination of bilateral sensorimotor functions of the trigeminal system associated with the response to deep tissue injury. J OROFAC PAIN 2004;18:299–305

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Most of our knowledge about mechanisms of persistent pain is based on studies at spinal cord levels.[1–3] It is important to determine whether trigeminal mechanisms of pain are different from pain mechanisms originating from other body sites. In recent years, researchers have concentrated on developing animal models of deep pain in the craniomandibular region and pursuing studies of the function of the trigeminal brainstem sensory nuclei and surrounding structures in persistent inflammatory pain conditions originating from joint and muscle tissue. Others have developed animal models of trigeminal nerve injury to study changes in trigeminal function.[4,5] This report will review the authors' recent findings and address the following topics:

(1) behavioral hyperalgesia and allodynia; *(2)* activity-dependent plasticity in trigeminal brainstem sensory nuclei after deep tissue injury; *(3)* the role of the trigeminal subnuclei interpolaris/caudalis transition zone (Vi/Vc) in visceral and autonomic nervous system processing after deep tissue injury; *(4)* rostral projections of Vi/Vc and subnucleus caudalis neurons activated by deep tissue injury; *(5)* the heterogeneity of Vi/Vc neurons; and *(6)* the participation of Vi/Vc neurons in descending modulation after deep tissue injury.

Behavioral Hyperalgesia and Allodynia

Injection of inflammatory agents such as complete Freund's adjuvant (CFA) or carrageenan into the hindpaw of the rat produces edema, redness, and hyperalgesia limited to the injected paw that can begin as early as 5 to 10 minutes after injection and can last for up to 2 weeks.[6,7] This model has been adapted to the orofacial region, and mechanical stimulation has been used to measure hyperalgesia and allodynia.[8] The mechanical threshold was assessed with von Frey filaments. The response to suprathreshold stimuli was evaluated using a range of filament forces that were each repeated at least 5 times, and a stimulus-response function was generated.[9] A nonlinear regression analysis can be used to calculate an EF50, that filament force that produces a response 50% of the time. The development of mechanical allodynia and hyperalgesia follows a pattern similar to that found after hindpaw stimulation, peaking at 4 to 24 hours and persisting for up to 2 weeks.

Activity-Dependent Plasticity After Deep Tissue Injury

What are some of the underlying mechanisms of behavioral hyperalgesia and allodynia? Signals from peripheral nociceptors terminate in the spinal cord or in its trigeminal equivalent (termed the medullary dorsal horn [MDH] or subnucleus caudalis). The terminals of these nerve fibers release a number of chemical mediators, including glutamate, the major excitatory neurotransmitter in the dorsal horn, and neuropeptides such as substance P (SP) and calcitonin gene–related peptide. These chemical mediators contribute to an increase in the excitability of neurons in the dorsal horn of the spinal cord and medulla via actions at ionotropic receptors and G-protein coupled receptors, leading to what is referred to as central sensitization.[10,11] The increase in the excitability of a dorsal horn neuron is brought about by a series of events including neuronal depolarization, removal of the voltage-dependent magnesium block of the ionotropic N-methyl-D-aspartate (NMDA) receptor, calcium entry into the neurons, phosphorylation of the NMDA receptor, a change in the neuron's kinetics, and resulting hyperexcitability or an increase in synaptic strength. The critical role of NMDA receptors in central sensitization has been shown by a number of authors.[3,10–12] NMDA receptor antagonists that either act at the agonist recognition site or block ion channel permeability can almost completely reverse inflammatory thermal or mechanical hyperalgesia after intrathecal injection in a dose-dependent fashion.[12] The presence of ionotropic, calcium permeable, alpha amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors suggests that they may also play a role in central sensitization via calcium entry as well as interaction with the NMDA receptor.

The metabotropic glutamate receptors (mGluRs) are a family of large monomeric receptors that are coupled to effector systems via activation of G proteins. There are 8 mGluRs that are further classified into 3 groups (groups 1 to 3) according to their amino acid homology. Most studies indicate that the group 1 mGluR1/5 receptors are involved in nociceptive responses.[3,13] The role of mGluRs appears to be more prominent after prolonged noxious stimuli, and spinal mGluRs are required for the generation of inflammation-evoked spinal hyperexcitability.[13] Peripheral inflammation results in up-regulation of mGluR mRNA in the spinal cord.[14] Recent studies indicate that mGluR activation is involved in the initiation of behavioral hyperalgesia; it appears to lead to the mobilization of calcium from intracellular stores and the activation of kinases that phosphorylate and prime the NMDA receptor for subsequent channel activation and calcium permeability.[7] Thus, both metabotropic and ionotropic glutamate receptors play important roles in the development of central sensitization and persistent pain.

SP plays a role in dorsal horn hyperexcitability, presumably by increasing neuronal depolarization, releasing the magnesium block of NMDA receptors, and contributing to phosphorylation of the NMDA receptor via activation of second-messenger pathways. Antagonists at the NK1 or SP receptor, injected intrathecally, reverse inflammatory hyperalgesia in a dose-dependent fashion.[15] Neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) also play a role in sensitization after peripheral tissue injury.[16]

The effects of CFA-induced orofacial inflammation were tested on the response properties of neurons in the trigeminal subnucleus caudalis (the MDH) and the rostral extension of the upper cervical dorsal horn were analyzed.[16,17] Neurons were recorded extracellularly and classified as low threshold mechanoreceptive, wide dynamic range, or nociceptive-specific, similar to their categorization in the spinal dorsal horn. After inflammation of the temporomandibular joint (TMJ) with CFA, the receptive fields (RFs) of MDH nociceptive neurons were significantly enlarged. The finding confirms previous findings from other laboratories (see Ren and Dubner[16] and Hathaway et al[18] for review). The enlarged RFs were found both in neurons whose RFs included the facial skin over the TMJ as well as in neurons whose RFs were entirely outside the zone of injury. Dorsal horn neuronal hyperexcitability produced by central sensitization, manifested as an enlargement of RFs and their presence outside the zone of injury, suggests that the involvement of dorsal horn neurons in secondary hyperalgesia is likely caused by central sensitization. Responses to thermal stimuli were significantly greater in inflamed animals than in naive animals, which also suggests hyperexcitability. These findings suggest that MDH hyperexcitability and central sensitization contribute to mechanisms of persistent pain associated with orofacial deep tissue injury.

Fos Protein Activation After Tissue Injury

The stimulus-induced expression of Fos, the protein product of the immediate early gene c-fos, has been widely used as a measure of nociceptive neuronal activation (see Munglani and Hunt[19] for review). Fos protein–like immunoreactivity is induced in the trigeminal pathways after noxious stimulation.[16,18,20] The effects of persistent orofacial deep tissue injury versus cutaneous tissue injury on neuronal activation in the trigeminal brainstem sensory nuclei were systematically examined.[21] TMJ and cutaneous inflammation paralleled the intensity and course of inflammation over the 10-day observation period as measured by quantification of Evans blue plasma extravasation. Compared to skin CFA injection, the injection of CFA into the TMJ produced more intense inflammation and greater Fos protein expression in the trigeminal nuclei.

Similar to previous reports,[18,20] there were 2 distinct regions of Fos protein activation, the subnucleus caudalis and contiguous cervical spinal cord (Vc/C1,2) and the Vi/Vc at the obex level.[22] The latter region is at the convergence of the caudal Vi and rostral Vc at the obex level. The ventral portion of the Vc is pushed dorsomedially by the Vi. The Fos protein labeling in Vc/C1,2 is quite homologous to the spinal dorsal horn, with detailed somatotopic representation. This region is involved in sensory discriminative aspects of pain originating in the trigeminal dermatomes.[16] Trigeminal Fos protein activation experiments indicate that NMDA receptors play an important role in MDH hyperexcitability. Fos activation in the MDH is attenuated by the administration of NMDA receptor antagonists,[23,24] which suggests an NMDA receptor–mediated central sensitization at the trigeminal level.

In contrast to Vc/C1,2 Fos labeling, Vi/Vc Fos activation is much more complex. Some somatotopy exists in the Vi/Vc: mandibular structures such as the TMJ and the masseter muscle send afferent terminals to the dorsomedial Vi/Vc, and corneal and frontal nerve afferents are found at the ventral Vi/Vc (see Ren and Dubner[16] and Zhou et al[21] for review). Fos protein immunoreactivity is predominantly ipsilateral only in the dorsomedial portion of Vi/Vc. The prominent features of the Vi/Vc include converging input from all trigeminal dermatomes as well as deep and cutaneous tissues and equivalent bilateral representations in the ventral portion of Vi/Vc.

The Role of the Vi/Vc in Autonomic and Somatovisceral Function

The finding that inflammation-induced Fos protein activation in the Vi/Vc is mostly not somatotopically represented has led to the hypothesis that it plays a role in autonomic and visceral processing. Trigeminal neurons send input to structures that are involved in somatovisceral and somatoautonomic processing. This hypothesis was directly tested by investigating the contribution of the adrenals and vagal afferent input to the expression of Fos protein following inflammation of the masseter muscle.[22] As previously shown, general anesthesia induced a cluster of Fos immunoreactivity bilaterally in the ventral pole of Vi/Vc. These findings are consistent with the hypothesis that this part of Vi/Vc is involved in autonomic and visceral effects associated with anesthesia. The effect of the overlying skin cut associated with the injection of CFA into the masseter muscle was also controlled for. Skin cut produced minimal activation in the ventral pole of Vi/Vc bilaterally, whereas there was significant Fos expression in caudal Vc/C1,2. In

contrast, masseter inflammation produced a robust increase in Fos-labeled neurons bilaterally in the ventral pole of Vi/Vc and ipsilateral to the CFA injection in the Vc/C1,2. The latter level of expression was greater than after skin cut. In addition, there was intense Fos activation bilaterally in nucleus tractus solitarius (NTS). Vagotomy combined with masseter inflammation and adrenalectomy combined with masseter inflammation each resulted in a significant decrease in the Fos expression in Vi/Vc and NTS produced by masseter inflammation alone. The effects of vagotomy and adrenalectomy were not additive. In contrast to the effect of these lesions at the Vi/Vc level, there were no significant changes in Fos expression at the Vc/C1,2 level.

Two important findings have emerged from these studies. First, these data support the hypothesis that stress and vagal input contribute predominantly and bilaterally to masseteric-induced Fos expression in the Vi/Vc transition zone, whereas they have minimal effect in the Vc/C1,2 region (see also Bereiter et al[23]). Second, it appears that deep and cutaneous inputs are processed differently in Vi/Vc and Vc/C1,2, with deep inputs apparently having a more robust effect in Vi/Vc and being elaborated bilaterally. The findings suggest that somatovisceral and somatoautonomic inputs are integrated at bilateral sites to enhance coordinated responses to mainly deep tissue injury of mandibular musculature and the TMJ.

Rostral Projections from Vi/Vc and Vc/C1,2

The aforementioned studies led to the hypothesis that identification of the rostral projections of neurons activated in the Vi/Vc transition zone after inflammation would lead to an understanding of their functional implications. Trigeminal brainstem neurons are known to project to somatosensory as well as somatovisceral and somatoautonomic regulatory centers in the brainstem, hypothalamus, and thalamus. The authors chose to study the projection of neurons to 4 sites: submedius nucleus of the thalamus (Sm), medial ventroposterior thalamic nucleus (VPM), lateral hypothalamus (LH), and the parabrachial nucleus (PB).[25] Flurogold retrograde tracing and Fos protein immunochemistry were used to identify neurons that projected to any of these sites and were activated by CFA-induced masseter inflammation. The rostral projections of trigeminal brainstem neurons often exhibit a bilateral projection pattern with a unilateral predominance. The projections to Sm and LH are mainly contralateral, VPM is exclusively contralateral, and the projection to PB is mainly ipsilateral (see Ikeda et al[25]). The authors injected Flurogold bilaterally into Sm, VPM, LH, or PB to achieve maximal labeling of projection neurons.

The majority of trigeminal Fos-activated neurons that projected to the Sm were found at the ventral pole of the bilateral Vi/Vc transition zone. The findings confirm other studies reporting SM projections from more rostral trigeminal sensory nuclei in single retrograde labeling experiments (see Ikeda et al[25]). Very few double-labeled neurons were found in Vc/C1,2. However, a region of dense double-labeling was found near this site—the bilateral caudal ventrolateral medulla. A small population of Fos-activated neurons in the Vi/Vc was labeled from PB injections, but most were located in the ipsilateral dorsal pole of Vi/Vc. In caudal Vc/C1,2, there were double-labeled neurons following PB injections, with most found ipsilateral to the site of inflammation and located in the superficial laminae. Compared to the Sm and PB groups, the major population of activated neurons labeled by Flurogold injections into LH was in the caudal ventrolateral medulla. To the authors' surprise, very few Fos-positive cells in the Vi/Vc transition zone and in Vc/C1,2 were labeled following injection of fluorogold into the VPM.

In summary, there were dramatic differences in labeling dependent on the rostral site of Flurogold injection. In the bilateral Vi/Vc, the percentage of neurons projecting to the Sm was significantly higher than the percentage projecting to LH and VPM, particularly in the ventral zone ($P < .001$). In the ipsilateral Vc/C1,2, the PB was the major site of rostral projection of activated neurons, with a secondary site in the ipsilateral dorsal Vi/Vc region. In the bilateral caudal ventrolateral medulla, there were significant projections to the Sm, PB, and LH, but not to the VPM.

The Heterogeneous Functions of Vi/Vc Neurons

These findings confirm that there are heterogeneous populations of neurons in the ventral portion of the Vi/Vc. As previously mentioned, there are neurons that are activated bilaterally by general anesthesia. Others are activated by corneal stimulation, and corneal afferents provide ipsilateral afferent input to the region. The findings also strengthen the view that the Vi/Vc plays an important role in the response to deep tissue injury. The neurons in

the dorsal pole receive primary afferent input from the TMJ and masseter muscle unilaterally and project mainly to PB and not to VPM or Sm. The neurons in the ventral pole respond to deep tissue input bilaterally. Since masseter and TMJ afferents do not terminate in this region, they likely receive this input polysynaptically. The major projections from Vi/Vc are to Sm and PB, supporting the conclusion that this region plays a role in autonomic and hormonal functions and in emotionality.[23,25,26] The bilateral neuronal activation in this transition zone may also facilitate integration of sensorimotor functions associated with recuperation from injury.

The Participation of Vi/Vc in Descending Modulation

Recent studies indicate that hyperalgesia in animal models of inflammatory and neuropathic pain are closely linked to activation of descending modulatory circuits involving both inhibition and facilitation. There is now considerable evidence that net descending inhibition is enhanced after inflammation at sites of primary hyperalgesia. In cats with knee joint inflammation, descending inhibition is greater in spinal dorsal horn neurons with input from the inflamed knee, as revealed by reversible spinalization with a cold block.[27] In rats with hindpaw inflammation, thoracic lidocaine block leads to an enhanced activity of dorsal horn nociceptive neurons that is greater in inflamed rats than in noninflamed rats.[28] A similar conclusion can be reached by using Fos protein expression as a marker of neuronal activation. There are more inflammation-induced Fos-immunoreactive neurons in the spinal dorsal horn in spinally transected or dorsolateral funiculus-lesioned rats compared to sham-operated inflamed rats.[29,30] These studies reveal the net descending inhibitory effects of activation of multiple supraspinal sites. The findings suggest that injury-induced dorsal horn hyperexcitability and hyperalgesia are dampened by descending pathways because of enhancement of descending net inhibition. The source of the enhanced inhibition can be traced back to brainstem structures. Local anesthesia of the rostral ventromedial medulla (RVM) results in a further increase in spinal dorsal horn nociceptive neuronal activity in rats with inflamed hindpaws.[28] Focal lesions of the RVM and locus coeruleus are followed by an increase in spinal Fos expression and hyperalgesia after inflammation.[30,31]

Descending facilitation not only parallels inhibition but also can be an active and dominant effect. The selective destruction of the medullary nucleus gigantocellularis (NGC) with a soma-selective neurotoxin, ibotenic acid, leads to an attenuation of hyperalgesia and a reduction of inflammation-induced spinal Fos expression.[30] A descending facilitatory drive also contributes to the pathogenesis of certain types of persistent pain, particularly those associated with secondary hyperalgesia or nerve injury.[32]

Trigeminal nociceptive transmission is also subject to descending modulation from rostral brainstem structures, including the RVM. The authors examined the hypothesis that the Vi/Vc plays a prominent role in the activation of RVM modulatory circuitry, since it has major projections to rostral brain sites involved in autonomic function, stress, and emotionality.[26,33] Using the same double-label methodology described earlier in this article, the authors injected Flurogold into the RVM and produced Fos activation by injection of CFA into the masseter muscle. Consistent with the aforementioned previous spinal studies, Fos protein was expressed in the Vi/Vc zone and the laminated parts of Vc/C1,2. Double-labeled neurons were found bilaterally in the ventral portion of the Vi/Vc but not in the dorsal portion. In the Vc/C1,2, Fos was expressed mainly in the superficial laminae, but double-labeled neurons were mainly found in the deep laminae. The hypothesis that Vi/Vc neurons had reciprocal connections with RVM was then tested using an anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHA-L), or the retrograde tracer Flurogold. As in earlier studies, the authors found that Fos-labeled Vi/Vc neurons sent axons to RVM. Clusters of axon temminals labeled with PHA-L injected into the RVM were observed at the level of the Vi/Vc, including a dense labeling in the ventral pole of Vi/Vc.

What is the functional significance of these connections? To directly test the role of these connections in behavioral hyperalgesia and allodynia, the authors produced in rats excitoxic lesions of RVM neurons with ibotenic acid injections into the RVM in a first set of experiments and lesions of the ventral pole of Vi/Vc in a second set.[33] Unilateral CFA-induced inflammation of the masseter muscle produced mechanical allodynia and hyperalgesia in the orofacial region overlapping the masseter muscle. The allodynia/hyperalgesia was significantly attenuated in rats receiving the ibotenic injection into the RVM 5 days prior to the inflammation. These findings indicate that modulatory inputs from the RVM enhance the hyperalgesia/allodynia found after masseter inflammation. It appears that descending facilitation contributes significantly to the hyperexcitability in this model of inflammatory

hyperalgesia. Ibotenic acid lesions of the ventral Vi/Vc resulted in similar attenuation of the hyperalgesia/allodynia, suggesting that the Vi/Vc is a component of an upstream sensory pathway leading to RVM activation and resulting descending facilitation. Bilateral descending facilitatory effects appear to originate from different brainstem sites than those involved in descending inhibition.[30] The net descending effect appears to be dependent on the total activity originating from these multiple sites after inflammation.

The findings support the authors' overall hypothesis that the ventral pole of Vi/Vc is involved in the coordination of bilateral sensorimotor functions of the trigeminal system associated with the response to deep tissue injury. This response includes roles in nociceptive sensory processing, somatovisceral and somatoautonomic function, and descending modulation. These results indicate that deep tissue injury leads to activation of Vi/Vc and alters diverse neural functions in the craniofacial and oral regions. Since nerve injury also affects Vc neural properties,[4] it seems likely that both types of injury can lead to these alterations.

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