Peripheral Mechanisms for the Initiation of Pain Following Trigeminal Nerve Injury

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Injury to a branch of the trigeminal nerve may lead to the development of chronic pain in the affected area. The etiology of this condition is not clear, but there is strong evidence to suggest that spontaneous and mechanically induced neural discharge from the injury site plays a crucial role. In laboratory studies, we have characterized this discharge following injury to the inferior alveolar or lingual nerves and have shown a temporal association with the accumulation of neuropeptides in the damaged axons. Substance P, calcitonin gene–related peptide, and vasoactive intestinal polypeptide were all found to be capable of increasing the discharge when applied systemically, and enkephalin caused a decrease. There were also changes in the expression of specific sodium channels and nitric oxide synthase, both at the injury site and in the trigeminal ganglion. Studies on lingual nerve neuromas taken from patients undergoing nerve repair also revealed accumulation of peptides, as well as inflammatory and structural changes, but the presence of these features did not correlate directly with the reported symptoms. The application of corticosteroids to an experimental injury site decreased the mechanically induced discharge, and the anticonvulsant carbamazepine reduced the spontaneous discharge in some axons. Some of the responses that result from damage to a branch of the trigeminal nerve appear to differ from those that follow damage to other peripheral nerves. These differences will need to be taken into account when developing new therapeutic approaches for the management of injury-induced trigeminal pain. J OROFAC PAIN 2004;18:287–292

Key words: ectopic discharge, nerve injury, neuropeptides, sodium channels, trigeminal nerve

The Clinical Problem

Trigeminal nerve injuries are common. They are sometimes the result of a facial injury but most frequently result from iatrogenic damage during routine surgical procedures. For example, the surgical removal of mandibular third molars may result in damage to the inferior alveolar nerve (average incidence 4%) or the lingual nerve (average incidence of 7%).[1] Most patients recover normal sensation over the course of a few weeks or months, but a small proportion (0.5% to 1%) are left with a permanent sensory disturbance.[1] Within this group, some have chronic injury-induced neuropathic pain. As the procedures that cause injury are extremely common, there is then a significant population of patients with a condition for which there is no satisfactory treatment. The condition is particularly distressing for patients because of the importance of the trigeminal region in normal perception and in vital functions such as speech and mastication. The patient's distress may be

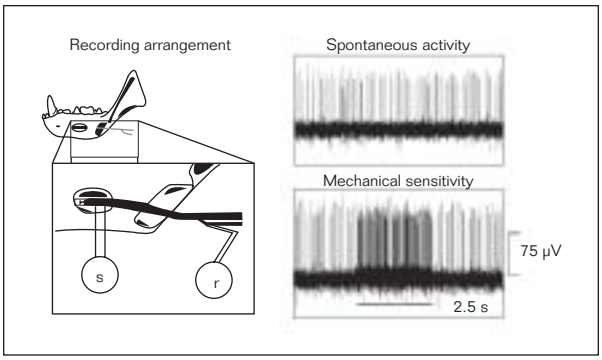

Fig 1 Spontaneous activity and mechanical sensitivity of units in the ferret inferior alveolar nerve 3 days after ligation. The recording arrangement is shown on the left. Part of the mandibular ramus was removed for the experiment, and bone was removed over the site of the injury. Recordings *(r)* made from fine filaments dissected central to the injury show spontaneous discharge *(upper right)* and increased discharge evoked by mechanical stimulation *(underlined period)* at the injury site *(lower right)*. Electrodes placed close to the injury site allow electric stimulation *(s)* to identify the number of axons in the filament.

compounded by a sense of grievance toward the surgeon who caused the iatrogenic injury.

Current management of patients who sustain an injury involves regular monitoring using sensory testing protocols and, in the case of poor recovery or persistent dysesthesia (an unpleasant, abnormal sensation), possible surgical intervention. The authors recently evaluated the outcome of lingual nerve repair in a series of 53 patients and found that postoperatively there were highly significant improvements in the results of sensory tests.[2] Moreover, patients considered the procedure to be worthwhile.[2] However, the level of success was variable; a few patients did not improve at all; in others, speech and taste sensation remained affected, and no patient recovered completely. It was particularly notable that the surgery did not reduce the number of patients suffering from dysesthesia, although the intensity of the symptoms sometimes declined. It is therefore vital that we establish alternative methods for treating injury-induced dysesthesia, and these should be based on an understanding of the etiology of the condition. The investigations reviewed in this paper have concentrated principally on the changes that occur within the peripheral components of the damaged nerve.

Ectopic Discharge from the Injury Site

Many studies have shown that at the site of a peripheral nerve injury damaged axons develop abnormal spontaneous neural discharge, and there is compelling evidence to suggest that this activity plays a crucial role in the initiation of neuropathic pain.[3–6] In a series of laboratory animal investigations, the authors used electrophysiological techniques to quantify the ectopic discharge originating at the site of a trigeminal nerve injury. In anesthetized adult ferrets, up to 26% of the damaged myelinated axons displayed spontaneous activity after inferior alveolar nerve ligation (Fig 1), and this was usually irregular, with discharge rates of up to 13 Hz.[7] In addition, up to 36% of the damaged axons were sensitive to gentle mechanical stimulation of the injury site. The proportion of axons that were spontaneously active and mechanically sensitive was highest a few days after the injury and declined with time, but there was still a low level of abnormal activity even after 6 months.[7,8] Similar observations were made after sectioning the inferior alveolar nerve and allowing regeneration and after creating a chronic constriction injury,[8] which shows that different types of peripheral nerve injury are capable of initiating similar raised levels of afferent discharge. Interestingly, none of the fibers that had regained peripheral receptive fields were either spontaneously active or mechanically sensitive, suggesting that this abnormal behavior is only generated in the terminals of axons that have become trapped at the injury site.

In subsequent investigations on the lingual nerve, both spontaneous and mechanically evoked ectopic discharges were again found after each of these types of nerve injury, although the proportion of spontaneously active units was lower after ligation than after either section or constriction.[9] When data from the lingual nerve were compared with data from the inferior alveolar nerve, however, a significant difference in time-course of the spontaneous activity in the 2 nerves was found. In the lingual nerve there was a late rise in spontaneous discharge, with high levels recorded up to 6 months after the injury. This difference in the time-course of ectopic activity in adjacent branches of the trigeminal nerve suggests that the fiber types or anatomic relationships can affect the outcome of injury. Furthermore, it may help to explain the authors' clinical impression that persistent dysesthesia is more common after lingual nerve injury than after inferior alveolar nerve injury. A previous study[10] in rats also revealed a difference

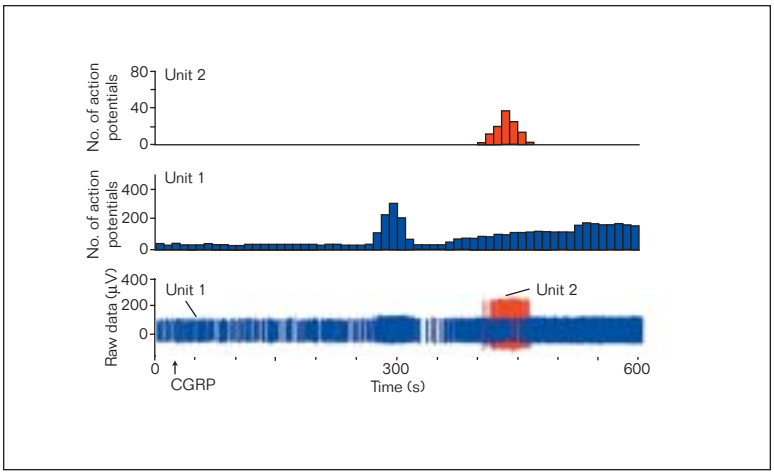

Fig 2 Activity recorded from 2 units in the inferior alveolar nerve 3 days after ligation. The bottom record shows the raw data with action potentials from unit 1 shown in blue and from unit 2 shown in red. The histograms show the number of action potentials recorded from each unit (10 s bin width) in response to topical application of CGRP (10^{-4} M) to the neuroma. The spontaneously active unit (unit 1) increased its discharge frequency (latency 250 s), and the previously silent unit (unit 2) became active (latency 375 s).

between the ectopic discharge in another trigeminal branch, the infraorbital nerve, compared with the sciatic nerve.

Previously, it has been shown that increases in circulating catecholamines or excitation of postganglionic sympathetic fibers, effects mediated via α-adrenoceptors, can increase the ectopic discharge from damaged axons.[3] Therefore, the role of sympathetic fibers in injury-induced ectopic discharge after inferior alveolar nerve injury was investigated. It was found that degeneration of these fibers by sympathectomy prior to the experimental injury had no effect on the extent or characteristics of the activity.[11] It was therefore concluded that the sympathetic nervous system does not play a significant role in the development of peripheral discharge in the trigeminal system. Interestingly, it has also been shown that while damage to a spinal nerve initiates sprouting of sympathetic fibers to form networks around the associated ganglion cells, this does not occur in the trigeminal ganglion following damage to one of its peripheral branches.[12,13]

Neuropeptide Changes in the Injured Nerve

In addition to making electrophysiological investigations, the authors have also used immunocytochemical techniques to examine the peripheral site of nerve injury. Using image analysis to quantify the expression of a range of neuropeptides involved in nociception, accumulations of substance P, calcitonin gene–related peptide (CGRP), vasoactive intestinal polypeptide (VIP), galanin, enkephalin, and neuropeptide Y (NPY) were found.[14,15] The level of expression was high after short recovery periods and declined with time, and this sequence of events paralleled that of the ectopic discharge seen in the electrophysiological recordings. This led the authors to speculate that these 2 observations may be linked and that, alongside other factors, the neuropeptides may be capable of initiating or modulating the ectopic discharge. Further support for this close relationship came with the observation that neuropeptide accumulation in the lingual nerve showed a late rise, comparable to the late rise in discharge.[16]

To explore this relationship further, electrophysiological recordings were made from damaged inferior alveolar nerve axons in the ferret while these neuropeptides were administered to the injury site either topically or systemically via close-arterial injections. These recordings demonstrated that 3 peptides, substance P, CGRP, and VIP, could all cause a significant increase in spontaneous discharge when given systemically. CGRP also increased discharge when applied topically.[17] Substance P and CGRP evoked changes in activity in a high proportion of units and were also capable of initiating activity in axons that were previously silent (Fig 2). Antagonists to these 3 peptides also increased the discharge rate in some units, and there was no clear indication that they were capable of reducing ongoing activity. However, close-arterial enkephalin caused a significant decrease in discharge in some spontaneously active units, which suggests that opiates may be of some use in the management of this problem.[18]

The potential role of neuropeptide accumulation at an injury site was also assessed by making observations of specimens of human lingual nerve obtained during lingual nerve repair. The clinical protocol for these patients is to resect the damaged segment of nerve, including any neuroma that has

formed, prior to mobilizing the proximal and distal nerve stumps and reanastomosing them with epineurial sutures.[2] This results in a specimen of tissue that can be examined in an attempt to correlate immunocytochemical or structural changes with the patient's clinical history. As indicated earlier, only some patients with sensory loss have dysesthesia. Thus, the specimens collected can be divided into 2 groups, those taken from patients with pain and those taken from patients without pain. Neurochemical expression in these specimens revealed that neuropeptides were present long after the initial injury; particularly high levels of CGRP were found.[19] There did not appear to be a clear correlation between CGRP expression and symptoms of dysesthesia, although the highest levels of CGRP were always associated with pain and tingling.

Changes in neuropeptide expression after injury are not confined to the injury site. Changes that occur in the trigeminal ganglion and trigeminal sensory nuclear complex in the brainstem after inferior alveolar nerve section or ligation have also been quantified in the ferret.[20,21] In these studies it was necessary to identify cells in the trigeminal ganglion that were linked to the damaged axons. The retrograde tracers fluorogold (to label all cell body types) and isolectin B4 (to identify a subpopulation with unmyelinated axons) were injected into the nerve proximal to the injury site. After nerve section there was a significant reduction in the number of trigeminal ganglion cells that contained either Substance P or CGRP. There was also a reduction in the number of labeled cells with unmyelinated axons,[20] and the expression of these peptides in the brainstem was reduced. Ligation caused different effects—a significant decrease in galanin expression in the ganglion and an increase in the expression of both enkephalin and NPY.[21] As all of the neuropeptides studied play a role in nociceptive processing, it seems likely that these changes are important in the development of neuropathic pain. The results showed that the changes in neuropeptide expression are dependent upon the nature of the peripheral injury. The results differed markedly from the results reported previously after injury to other nontrigeminal nerves. For example, previous observations on the effect of sciatic nerve injury in the rat revealed dramatic central upregulation of galanin[22] and VIP, [23] changes that were not seen in the present authors' studies.

Structural Changes in the Damaged Nerve

Light microscopic examination of the specimens of human lingual nerve obtained at the time of repair revealed a substantial increase in the number of fascicles. Furthermore, the fascicles had unusually small diameters. Metallic foreign bodies were found within the nerve; they were presumed to have resulted from damage to a metal retractor with the drill during third molar removal. Furthermore, there was evidence of a chronic inflammatory infiltrate within some neuromas; this evidence was commonly found in those patients who had pain. This infiltrate was therefore evaluated further by the use of immunocytochemistry, which revealed an accumulation of macrophages (labeled with CD68) and other leukocytes (labeled with CD45) associated with regions of viable nerve tissue. However, there was no significant correlation between the accumulation of these cells and the presence of dysesthesia.[24]

More detailed quantitative evaluation of the neuromas was carried out with electron microscopy and showed that the myelinated fibers were smaller, with thinner myelin sheaths. However, the g-ratio (axon diameter/myelinated fiber diameter) was normal, which suggests that the process of myelination in the regenerating axons was normal. The unmyelinated fibers were also smaller than normal, and there was a significantly higher incidence of both axonal exposure (where axons are separated from the surrounding connective tissue only by basement membrane and not by Schwann cell membrane) and axonal apposition (where adjacent nonmyelinated axons have closely apposed membranes without an intervening layer of Schwann cell).[25] These ultrastructural changes may account for some of the altered electrophysiological properties of nonmyelinated axons within neuromas, but again, no significant correlations were found between these characteristics and the reported symptoms.

Other Changes That Could Affect Neuronal Excitability

There are other factors that regulate neuronal excitability and could be involved in the initiation of ectopic discharge from damaged fibers, eg, remodeling of transmembrane ion channels, including specific sodium channel subtypes.[26] One study showed that 3 days after inferior alveolar nerve injury in the rat, there was a downregulation of SNS/PN3 ($Na_v1.8$) mRNA transcripts in small trigeminal ganglion neurons.[27] At the site of an inferior alveolar

nerve injury in the ferret, increases in the expression of $Na_v1.8$ and $Na_v1.9$ were found, and in the ganglion there were decreases in the expression of $Na_v1.3$, $Na_v1.7$, and $Na_v1.9$ (Fig 3).[28] This contrasts with reported changes in sodium channel expression in the rat dorsal root ganglion after sciatic nerve section, where a significant increase in expression of $Na_v1.3$ has been reported.[29] The present authors have also found an increase in expression of neuronal nitric oxide synthase at the injury site, and a decrease in the associated ganglion cells.[28] All of these factors could contribute to membrane instability at the injury site and the initiation of action potentials.[3]

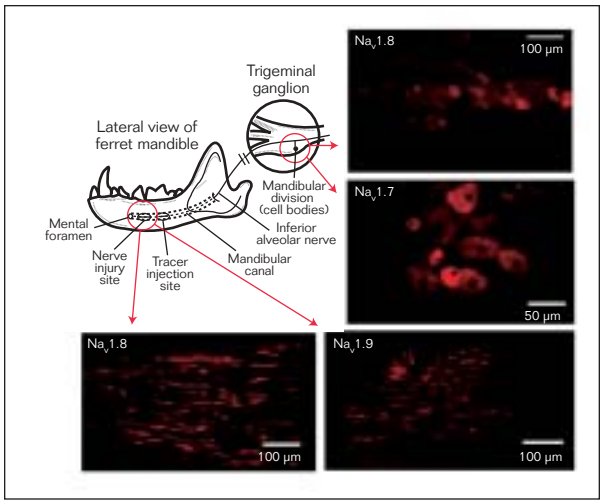

Fig 3 Sodium channel expression in the inferior alveolar nerve and trigeminal ganglion of the ferret 3 days after nerve section. The diagram shows the site of injury within the mandibular canal. As indicated, 2 photomicrographs *(left, bottom right)* show increased expression of Na_v 1.8 and Na_v 1.9 in the nerve proximal to the injury site. The other 2 photomicrographs *(upper right, middle right)* show expression of Na_v 1.8 and Na_v 1.7 in associated cells in the trigeminal ganglion.

Pharmacological Approaches to Modulating Ectopic Discharges

Assuming the importance of the ectopic activity at the injury site in the initiation of dysesthesia, it follows that any means of modulating this activity could be of therapeutic benefit. First the effect of corticosteroids applied at the injury site was investigated, as these agents are known to reduce inflammation and scarring. Studies on ferrets showed that triamcinolone hexacetonide (Lederspan; Wyeth Lederle) injected into and around the lingual nerve at the time of repair significantly reduced the mechanically induced ectopic discharge but had no effect on the level of spontaneous activity.[30] This suggests that local corticosteroids could reduce the level of dysesthesia initiated by pressure or movement at the injury site but may not affect spontaneous symptoms.

In another study the effect of the systemic anticonvulsant carbamazepine was investigated, as it is known to have membrane-stabilizing properties.[31] Recordings were made from spontaneously active axons 3 days after lingual nerve section in ferrets, and the effect of a progressively increasing systemic level of carbamazepine β-cyclodextrine (95 mg/mL; Sigma/RBI) was determined. Spontaneous activity ceased in approximately 60% of the units, which suggests that systemic carbamazepine could have a limited effect on spontaneous dysesthesia in some patients. This is consistent with the authors' clinical observations.

Comparisons with Studies on Other Nerves

Many of the studies described have revealed differences between the responses that result from damage to a branch of the trigeminal nerve and those that follow damage to other peripheral nerves. These differences include the time-course of the ectopic discharge, role of the sympathetic fibers, and some of the changes in neuropeptide and ion channel expression at the site of injury and within ganglion cells.[32] They may be the result of the specific fiber types within the damaged nerve, the anatomic location of the nerve, species variations, or differences between nerves of spinal or cranial origin. Thus, in view of these differences, it is essential that future management of patients suffering from nerve injury–induced pain is based on studies undertaken within the trigeminal system.

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