Immunohistochemical observations on spinal tissue innervation

A review of hypothetical mechanisms of back pain

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Immunohistochemical studies support earlier reports of a rich nerve supply to the posterior longitudinal ligament, a less developed innervation of the anterior ligament and the outermost annular ring, and a total lack of innervation in deeper parts of the intervertebral disc. Whether this pattern of innervation is altered when the disc becomes severely degenerated is presently uncertain. Recent studies have also revealed neuropeptide-immunoreactive nerves in the outermost parts of the annulus and adjacent peridiscal ligaments. These nerves are probably involved in discogenic back pain, and may become sensitized when disc tissue is injured. This sensitization appears to be coupled to an alteration of neuropeptide pools in the nearby dorsal root ganglion, the important site of neuropeptide production. Direct influences on the dorsal root ganglion, mechanical and/or chemical, may also be important, and may be involved in spinal segment degeneration.

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Submitted 91-02-20. Accepted 91-06-25

Forty years ago, Wiberg (1949) presented in this journal his observations on silver-stained nerve-like structures, which were only present in the outer part of the ligament covering the disc, whereas no nerves were observed in either the annulus fibrosus or the nucleus pulposus. In 1963, Hirsch et al. presented a comprehensive study of different types of nerves in back tissues obtained either at back surgery or at autopsy. The tissues were either stained by intravital staining with methylene blue or impregnation with heavy metals. In another detailed study by Jackson et al. (1966) a few years later, silver and cholinesterase staining were used on autopsy material and material obtained at surgery. This study material included 2 fetuses and 2 newborn infants. According to the results of both studies, the longitudinal ligaments, especially the posterior longitudinal ligament, and the capsular tissue of facet joints were especially richly innervated spinal tissues, whereas interspinal ligaments, the flavum ligament, and the annulus fibrosus were more sparsely innervated. The nucleus pulposus lacked innervation, even when there was ingrowing granulation tissue in a degenerated disc (Jackson et al. 1966). Only in the outermost parts of the annulus, especially in the area beneath the posterior longitudinal ligament, were nerves observed. A few nerves were also observed on the outermost posterior surface of the flavum ligament. We have observed a very similar distribution of immunoreactive nerves in the rat disc (Figure 1), in accordance with the earlier observations. In addition to the longitudinal ligaments and the facet joint capsule, the vertebral periosteum was observed to be richly innervated, with both free endings and more complex unencapsulated endings (Hirsch et al. 1963, Jackson et al. 1966).

The results of these early studies form the basis for present knowledge of spinal tissue innervation, even if more sophisticated and specific methods for visualizing neural structures in spinal connective tissues are now available (Figure 1). The detailed neuroanatomic work of Bogduk (1983) has revived a keen interest in the role of spinal innervation in back pain. Immunohistochemical methodology has permitted a new look at neural elements in spinal tissues, with the added advantage of a higher specificity with respect to neural structures and the possibility to characterize neural chemical constituents, e.g., neural peptides in spinal tissues. The linkage between these basic observations and the pathophysiology of back pain is still, however, not evident. A well-chosen statement by Bogduk (1983): "... whatever it is in the lumbar spine that causes pain, it must have a nerve supply," will, however, always hold true. Understanding back pain will most probably, as recognized by the earlier

Figure 1. Peripheral part of rat disc. Protein gene product 9.5-(PGP 9.5-) immunoreactive nerves marked with arrows. Avidin-biotin-peroxidase complex (ABC) immunostaining. Bar = 10 µm.

Figure 2. Human posterior longitudinal ligament. Abundant neurofilament triplet protein-immunoreactive nerves (arrows) can be seen. ABC immunostaining. Bar = 10 µm.

investigators (Wiberg 1949, Hirsch et al. 1963, Jackson et al. 1966), depend upon a thorough knowledge of back tissue innervation and of the role of neural chemical substances in back tissue pathophysiology.

Visualizing nerves in back tissues with immunohistochemistry

The disc and peridiscal ligaments

Immunohistochemical methodology has introduced a convenient and specific means for studying the innervation of back tissue samples freshly obtained at surgery, with good preservation of neurochemicals stored within the nerves. Thus, it has even become possible to study the intricate innervation of bone with a characterization of neuronal peptides in this tissue (Bjurholm et al. 1988, Bjurholm 1989).

Having found substance P-immunoreactive nerves and nerve terminals in human periosteum (Grönblad et al. 1984), we began a search for similar nerves in spinal ligament tissue (Liesi et al. 1983, Korkala et al. 1985). We observed substance P-immunofluorescence in five of seven samples of posterior longitudinal ligament, whereas no such immunoreactivity was seen in either samples of the flavum ligament or the intervertebral disc (Korkala et al. 1985). These results were later confirmed (Konttinen et al. 1990a) using avidin-biotin-peroxidase complex (ABC) immunohistochemistry according to the method of Hsu et al. (1981), again with tissue samples obtained at operations for intervertebral disc herniation. With this method, immunoreactive nerves stain brown and are easily seen in connective tissue of experimental animals (Figure 1) and in human tissue specimens obtained at operations (Figures 2–7). It was especially noted that the posterior longitudinal ligament has a very rich innervation indeed (Figure 2), as had been suggested by the earlier workers. Interestingly, in a recent immunohistochemical study by a Dutch group (Coppes et al. 1990), there was evidence of penetration of immunohistochemically demonstrable nerves into deeper parts of degenerated discs. This could explain why degenerated discs may become increasingly painful, but other mechanisms are probably also responsible.

Weinstein and coworkers (1988a) recently identified substance P-, calcitonin gene-related peptide- (CGRP-) and vasoactive intestinal polypeptide- (VIP-) immunoreactive nerves among the outer annular fibers of the rat disc. Similar nerves are also present in surgical specimens of peridiscal ligaments (Figures 3 and 4). The measurable amount of neurochemicals in the dorsal root ganglion, the site of neuropeptide production, could be altered by external manipulation of the disc by discography (Weinstein et al. 1988a). It was suggested that local nerves within the outer layers of the disc may become sensitized by some yet unknown stimulus within a degenerated disc. In this respect, the recent observations by Saal et al. (1990) of extremely high phospholipase A_2 enzyme activity within herniated disc tissue are very interesting. Could it be that peridiscal local nerves, perhaps in a degenerated disc even nerves in deeper parts of the disc (Coppes et al. 1990), can become excited by and perhaps sensitized by locally produced inflammatory mediators (Weinstein et al. 1988a)? A study in progress with monoclonal antibodies to several types of inflammatory cells has not, however, so far revealed an overt inflammatory cell infiltration in disc tissue samples removed at operations for disc herniation (Grönblad et al. unpublished observations).

Figure 3. Varicose calcitonin gene-related peptide immunoreactive nerves (arrows) in the human anterior longitudinal ligament. Note that the nerves show no topographic relationship to blood vessels. ABC immunostaining. Bar = 10 µm.

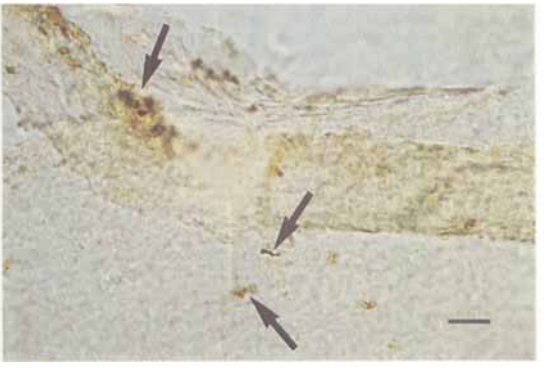

Figure 4. Substance P-immunoreactive nerves (arrows) in the periphery of human anterior longitudinal ligament. ABC immunostaining. Bar = 10 µm.

In his early studies Wiberg (1949) noted that a disc could be extremely painful upon palpation when performing spinal surgery under local anesthesia. He also suggested that a disc herniation may press on the richly innervated ligamentous tissue covering the disc, causing severe lumbago-like back pain. With the exception of the nerve root, the role of nerves in various spinal tissues still remains unknown in back pain research, requiring further study. A recent study by Parke and Watanabe (1990) suggests other neural elements in the spinal segment, e.g., branches of the sinuvertebral nerve, may also be involved, and even ruptured by an advancing disc herniation.

Immunoreactive nerves within and around the facet joints

Some types of back pain could perhaps be due to pathology within or around the facet joints (Ghormley 1933, Mooney and Robertson 1976, Shealy 1976, Giles 1989) and thus a more detailed knowledge of facet joint innervation could be of interest with respect to putative mechanisms of back pain (Giles 1989, Grönblad et al. 1991), even though the concept of a specific facet syndrome has been difficult to define (Jackson et al. 1988, van Akkerveeken 1989, Lilius et al. 1990). Recent controlled (Jackson et al. 1988, Lilius et al. 1990) and randomized (Lilius et al. 1990) injection treatment trials have, however, failed to demonstrate a specific effect on back pain.

The joint capsule of the facet joint is richly innervated, contains substance P-immunoreactive nerves (Giles and Harvey 1987, El-Bohy et al. 1988), and electrophysiologic studies suggest a presence of nociceptive nerves (Yamashita et al. 1990). In addition, within the joint, there are fatty synovial folds, which are especially large at the presacral level (Giles and Taylor 1982, Giles 1989), and which also contain peptide-immunoreactive nerves (Giles 1987, Giles and Harvey 1987, Grönblad et al. 1991), although not in abundance (Giles and Harvey 1987, Grönblad et al. 1991). Indirect histologic evidence suggesting entrapment of these folds between the joint surfaces has been presented (Giles 1986, Konttinen et al. 1990b). Nerves in the synovial folds or plicae (Grönblad et al. 1991) are mainly perivascular in location (Figure 5), but we have also observed nerves with no topographic relationship to blood vessels very near fat tissue (Figures 6 and 7). The physiologic role of such nerves is presently uncertain. They may be mechanoreceptive in nature. They are protein gene product 9.5- (PGP 9.5-) immunoreactive (Figures 5–7), but most of them lack peptide-immunoreactivity (Grönblad et al. 1991).

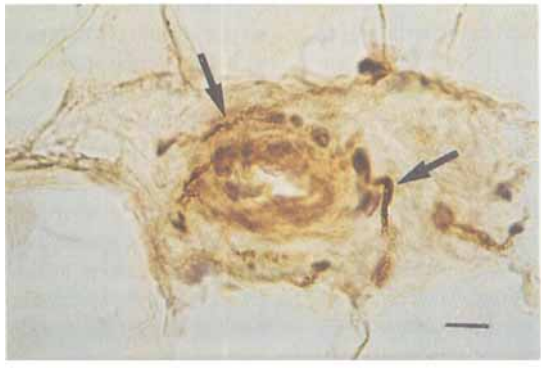

Figure 5. Intraarticular plica of human facet joint. Protein gene product 9.5- (PGP 9.5-) immunoreactive nerves (arrows) are seen around a blood vessel. ABC immunostaining. Bar = 10 µm.

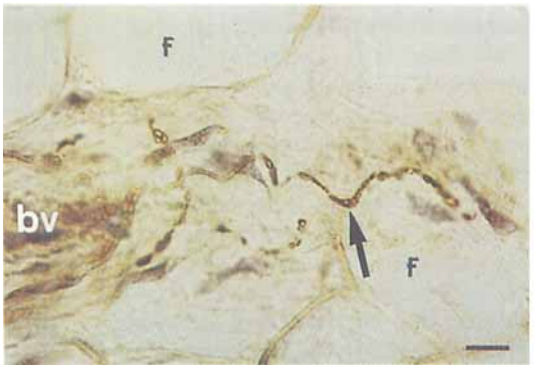

Figure 6. Intraarticular plica from human lumbar facet joint. Protein gene product 9.5- (PGP 9.5-) immunoreactive varicose nerve (arrow) further away from a blood vessel (bv). F = nearby fat lobules. ABC immunostaining. Bar = 10 µm.

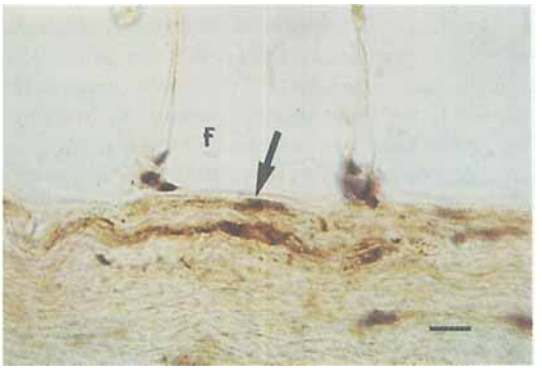

Figure 7. Intraarticular plica from human lumbar facet joint. Protein gene product 9.5- (PGP 9.5-) immunoreactive nerves (arrow) are observed near fat lobules (F). ABC immunostaining. Bar = 10 µm.

The dorsal root ganglion

As we move toward the spinal cord (central nervous system), from peripheral nerves to the spinal nerve, we encounter the dorsal root ganglion, the "brain" of the functional spinal unit (Figure 8). Lindblom and Rexed (1948) were the first to implicate the dorsal root ganglion as the modulator of low back pain. Their cadaveric studies focused on compression of the dorsal root ganglion as a result of dorsolateral lumbar disc protrusions. In some specimens, enlarged facet joints were found to be an accessory factor in causing nerve injury. Such bony enlargement no doubt can cause similar damage to the dorsal root ganglion quite independent of a disc herniation. Today, the importance of this compression is still uncertain.

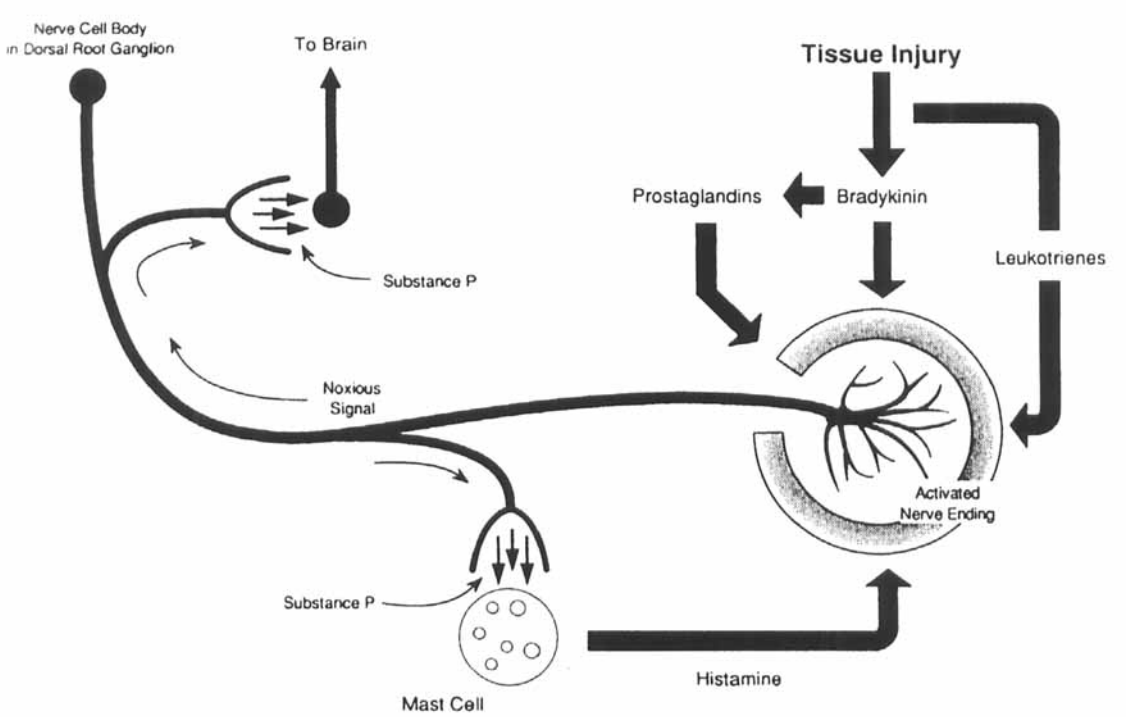

Figure 8. The interaction between peripheral tissue injury and repair and the central neurogenic components. This scheme demonstrates how neurogenic mediators can affect nonneurogenic mediators through the stimulation of mast cells by substance P.

The dorsal root ganglion is the producer of several neurogenic peptides, including calcitonin gene-related peptide and substance P. Calcitonin gene-related peptide is the most abundant peptide discovered to date in the dorsal root ganglion (Gibson et al. 1981). A great deal of information is available on the blood supply to the spinal cord, but little has been written about the blood supply to the dorsal root ganglion (Bergmann and Alexander 1941). Its vascular supply, both venous and arteriolar, must play a significant role in its function. Bergmann and Alexander suggested that aging and concomitant vascular changes of the dorsal root ganglion are associated with degeneration and changes in vibratory sensation. Because of the ganglion's vascular supply and tight capsule, Rydevik et al. (1988, 1989) have suggested that mechanical compression of the ganglion may result in intraneural edema and a subsequent decrease in cell-body blood supply, accounting for abnormal dorsal root ganglion activity and pain. In the spinal stenosis model of Delamarter, neurogenic claudication appeared to begin with venous congestion of the nerve roots and dorsal root ganglion, distal to the constricting band (Delamarter et al. 1990).

Anatomically, the dorsal root ganglion serves as a vital link between the internal and external environment and the spinal cord. The primary sensory role of the spinal cord is to receive afferent stimuli in the form of action potentials and to relay the information transmitted to and from the brain. This particular mechanism of pain transmission has attracted much attention. The classic hypothesis that the effects of nerves on target organs (bone, muscle, ligaments, synovium) are mediated by chemicals released from those nerves was first studied in the peripheral nervous system and has now proven to be valid in the central nervous system as well (Dale 1935, Shantha and Evans 1972). Thus, the dorsal root ganglion remains a vital link between the intrathecal spinal nerve and the extrathecal peripheral nerve. Nervi nervorum located on the dorsal root ganglion, as well as peripheral nerves, are mechanically sensitive nociceptors themselves. Therefore, the epineurium of the dorsal root ganglion may be directly activated by compression or mechanical stimulation of these nociceptors. These epineurally located nociceptors appear to respond in a similar way to cutaneous nociceptors in the peripheral nervous system.

Recently, a study to assess the role of the dorsal root ganglion in modulating the pain response associated with discography was reported (Weinstein et al. 1988a). Lumbar discography is a commonly employed diagnostic tool, but important questions about it remain unresolved. Why is an abnormal discogram painful in one patient and not in another? This study was performed to investigate the change in substance P (SP) and vasoactive intestinal polypeptide (VIP), found in the dorsal root ganglion, following discography in normal and abnormal canine lumbar intervertebral discs. The data from this experimental study suggest that dorsal root ganglion SP and VIP are indirectly affected by manipulations of the intervertebral disc. Discography increased the concentration of both peptides in the dorsal root ganglion. Discography followed by local anesthesia decreased the concentration of both peptides when the disc was normal, whereas only SP decreased significantly with a simulated herniated disc. It may be that various neurochemical changes within the intervertebral disc are expressed by sensitized (injured) annular nociceptors, and in part modulated by the dorsal root ganglion. Therefore, the concomitant pain sometimes associated with an abnormal discogram image may in part be related to the chemical environment within the intervertebral disc and the sensitized state of its annular nociceptors. In this study, immunohistochemical identification of SP, VIP, and calcitonin gene-related peptide was made, for the first time, in the outer annulus.

Chemical mediation of nociception

The site of activation of primary sensory neurons may involve the peripheral terminal endings of these neurons and tissues, such as muscles, joints, skin, periosteum, blood vessels, and meninges. Alternatively, it may involve a mechanical or chemical irritation of the dorsal root fiber or the soma within the dorsal root ganglion. Some of the endogenous chemical substances, particularly inflammatory mediators, can excite or increase the excitability of primary sensory neurons or otherwise alter their local environment.

Nociceptors are the peripheral terminal endings of sensory neurons that are selectively responsive to potentially or overtly injurious stimuli that cause pain in humans and cause affective pain-like responses in animals.

Nonneurogenic pain mediators

A variety of endogenous chemicals are released from nonneural tissues, all of which have pain-producing capabilities. These include bradykinin, histamine, prostaglandins E_1 and E_2, and leukotrienes (Ferreira 1972, Wyke 1982, Kanaka et al. 1985). Bradykinin excites heat-sensitive or mechanosensitive C- or A-fiber nociceptive afferents innervating the skin, joints, skeleton, muscle, and visceral organs. There are various interactions among these chemicals and their

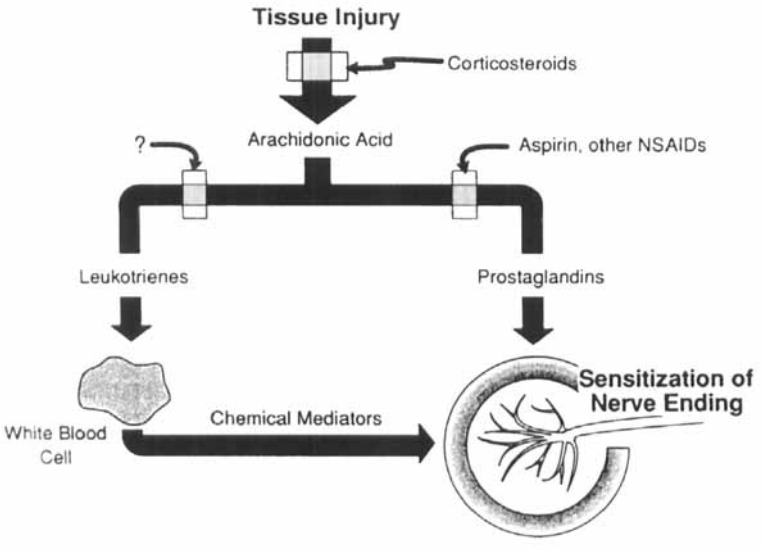

Figure 9. The leukotrienes, non-neurogenic mediators, are not affected by nonsteroidal anti-inflammatory agents but seem to be affected by steroids.

physical stimuli while affecting nociceptor responses. Prostaglandins enhance the responses of C- or A-nociceptors in skin, joint, or muscle to heat, mechanical stimuli, or bradykinin. Similarly, intradermal or subdermal injections of prostaglandin E_1 in humans produce mechanical tenderness and potentiate the pain from subdermal injections of bradykinin and the itch from histamine. Two other endogenous chemicals that have recently been shown to produce hyperalgesia are formed by the lipoxygenation of arachidonic acid: (1) dihydroxyeicosatetraenoic acid (diHETE), a 15-lipoxygenase product, and (2) leukotriene B_4, a 5-lipoxygenase product. Leukotriene B_4 is a chemotaxin for polymorphonuclear leukocytes that accumulate at inflamed sites to destroy antigens (Ford-Hutchinson et al. 1980). Leukotriene injected intradermally into the rat paw sensitizes C-nociceptors to mechanical stimuli and produces hyperalgesia. Further, this hyperalgesia is dependent upon the presence of polymorphonuclear leukocytes (Kumazawa and Mizumura 1977). The hyperalgesia resulting from these substances is not blocked by nonsteroidal anti-inflammatory drugs that block the cyclo-oxygenation of arachidonic acid (Figure 9). Levine et al. (1986a) produced evidence that diHETE injected intradermally into the rat paw produces a hyperalgesia equivalent in maximal effect to that produced by leukotriene B_4, bradykinin, or prostaglandin E_2 (Figure 8). Saal et al. (1990) have suggested that phospholipase A_2 activity may be extremely important in the presence of a clinical radiculopathy associated with a herniated nucleus pulposus.

Neurogenic pain mediators

For more than a decade, a large number of primary afferent neurons have been known to produce neuropeptides, such as substance P (SP). These neuropeptides are produced within the dorsal root ganglion in cell bodies of primary afferent neurons and delivered by axonal transport to both the central and peripheral processes of neurons. Although SP from primary afferent neurons has been demonstrated in response to intense electrical stimulation of peripheral nerves, as has the excitatory effect of SP on ascending projection neurons, it remains to be established whether SP (or any other neuropeptide within primary afferent neurons) is both necessary and sufficient as the chemical transmitter mediating nociception at the first synapse. This uncertainty stems from several sources. Capsaicin has been employed widely as a toxin for the reduction of peptides in primary afferent neurons. Studies using capsaicin have attempted to discern the role of neuropeptides in primary afferent neurons and nociception. Its limitation is that it may not destroy peptides in myelinated primary afferent nerve fibers. These residual myelinated peptidergic fibers may play an important role in nociception and, therefore, confound the interpretation of experiments in which capsaicin is used.

The number of neuropeptides now known to occur in primary afferent neurons has been steadily increasing. In addition to SP, somatostatin, cholecystokinin-like substance, vasoactive intestinal polypeptide, calcitonin gene-related peptide, gastrin-releasing peptide, dynorphin, enkephalin, and galanin are neuropeptides

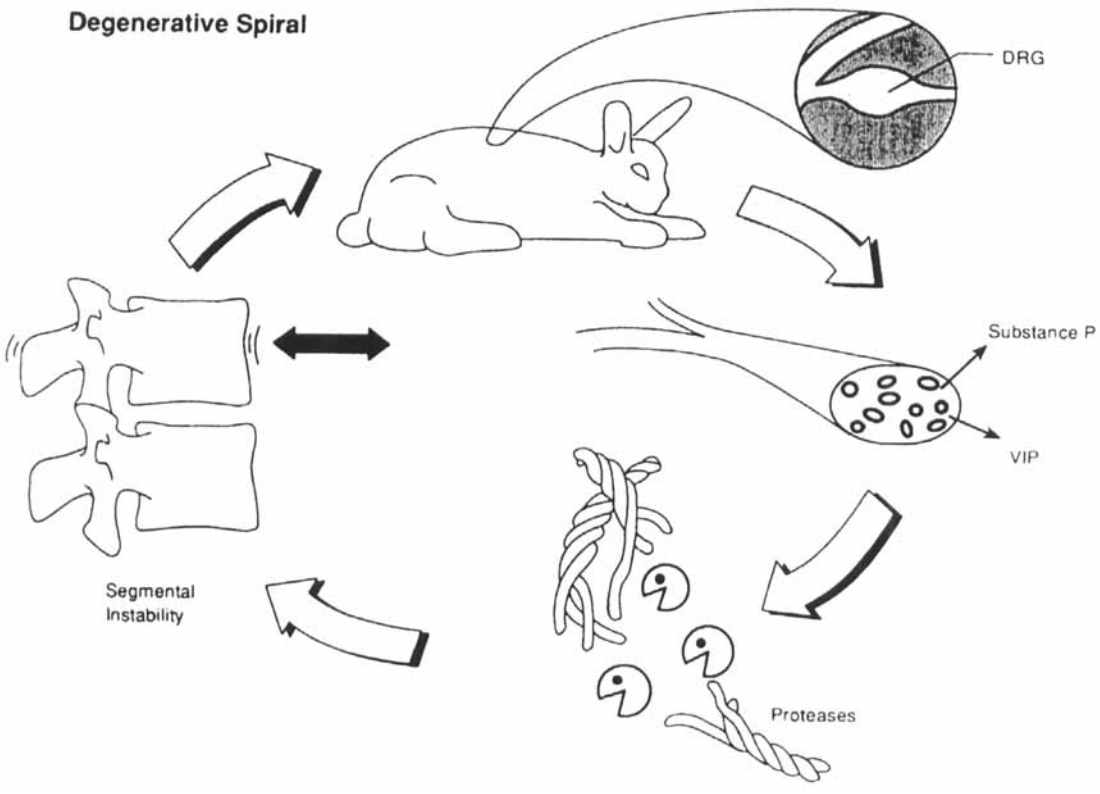

Figure 10. "Degenerative spiral": The functional spinal unit may undergo degeneration as a result of the interaction of mechanical and chemical stimuli seen in an injured or environmentally stimulated functional spinal unit (Weinstein).

predominantly produced by primary afferent neurons. Each of these peptides is likely to be produced by a biosynthetic precursor that may, through posttranslational processing, give rise to additional biologically active peptides. Thus, the potential number of biologically active peptides produced by primary afferent neurons is high and confounds this simple examination of their role or roles in nociception. Anatomic studies of neuropeptides in the dorsal root ganglion cells have found that these neurons contain enzymes that, by their presence, implicate their substrates as playing a role in neurotransmission or neuromodulation (e.g., adenosine deaminase, which implicates purines, and fluoride-resistant acid phosphatase, whose substrate is unknown) (Nagy and Daddona 1985). Neuropeptides are released from peripheral endings of nociceptive afferents as a result of noxious chemical or physical stimulation and can influence the inflammatory process (Payan et al. 1986). Antidromically induced release of neuropeptides by electrical stimulation of C-fibers can increase blood flow and vascular permeability. Substance P is believed to act directly on the blood vessels to produce plasma extravasation and indirectly to produce vascular dilatation by releasing histamine. Antihistamines and SP antibodies block the flare induced by histamine; however, it seems that SP antagonists do not block the flare produced by capsaicin, suggesting that the final vasodilator is not histamine. Another candidate mediator is calcitonin gene-related peptide, which is a potent vasodilator and is co-localized with SP (Lee et al. 1985).

Neuropeptides are also known to stimulate the release from mast cells of leukotrienes and other factors that attract and stimulate polymorphonuclear leukocytes and monocytes (Payan et al. 1987). Certain pathologic conditions are accompanied by an increased SP; for example, increased SP is seen in peripheral nerves supplying arthritic joints and in cerebral spinal fluid of patients with low back pain and chronic arachnoiditis (Howe et al. 1976). Substance P is released into joint tissues and stimulates proliferation of rheumatoid synovial sites and their release of prostaglandin E_2 and collagenase, thereby implicating this peptide in the pathogenesis of rheumatoid arthritis (Lotz et al. 1987). In addition, it has been demonstrated that VIP can cause a dose-dependent increase

in bone resorption by a cAMP-dependent mechanism. Neuropeptides, such as calcitonin gene-related peptide and SP, can also contribute to the repair of injured tissue by stimulating the proliferation of smooth muscle cells and fibroblasts.

There is also increasing evidence of an involvement of the autonomic nervous system and related transmitters, such as noradrenaline and neuropeptide Y, in various pain syndromes (Levine et al. 1986b, Coderre et al. 1989, Roberts and Kramis 1990). Such an involvement in discogenic back pain has recently been suggested (Jinkins et al. 1989).

Recently, it has been demonstrated that degeneration of the lumbar spine secondary to vibration, a known epidemiologic cause of low back pain, may in part be related to neurogenic pain modulators (Weinstein et al. 1988a, b, Frymoyer 1989). Work designed to establish and develop animal-based experimental paradigms and techniques for studying degeneration of the components of the functional spinal unit has established that low frequency vibration causes changes in the amounts of SP and vasoactive intestinal polypeptide (VIP) in the dorsal root ganglion. The presence of these neuropeptides in the dorsal root ganglion—as well as in peripheral areas, such as in the disc annulus, facet joints, and blood plasma—suggests exciting possibilities for explaining chronic degeneration of the spinal motion segment. Results from preliminary studies have motivated the development of a working model explaining chronic functional spinal unit degeneration that hypothesizes causal links between environmental factors (e.g., vibration), and functional spinal unit degeneration mediated by biological events. The model is as follows: the release of neuropeptides from the dorsal root ganglion, induced by environmental and structural factors (i.e., vibrations), mediates a progressive degeneration of the functional spinal unit structures by stimulating the synthesis of inflammatory agents (e.g., prostaglandin E_2) and degradative enzymes (e.g., collagenase). The weakened functional spinal unit structures increase the susceptibility of the dorsal root ganglion to environmental factors, which, in turn, lowers the threshold necessary to stimulate neuropeptide activity, thereby creating a degenerative spiral (Figure 10).

References

van Akkerveeken P. Facet joint "syndromes." Presented at *Back pain: Current concepts and recent advances.* Third Intern Congress 1989: 17.

Bergmann L, Alexander L. Vascular supply of the spinal ganglia. *Arch Neurol Psych* 1941; 46: 761–82.

Bjurholm A. *Neuroendocrine peptides in bone.* Thesis, Karolinska Institute, Stockholm, Sweden 1989.

Bjurholm A, Kreicbergs A, Brodin E, Schultzberg M. Substance P and CGRP immunoreactive nerves in bone. *Peptides* 1988; 9 (1): 165–71.

Bogduk N. The innervation of the lumbar spine. *Spine* 1983; 8 (3): 286–93.

Coderre T J, Basbaum A I, Levine J D. Neural control of vascular permeability: interactions between primary afferents, mast cells, and sympathetic efferents. *J Neurophysiol* 1989; 62 (1): 48–58.

Coppes M H, Marani E, Thomeer R T, Oudega M, Groen G J. Innervation of annulus fibrosis in low back pain (letter) (published erratum appears in the *Lancet* 1990; 336 (8710): 324). *Lancet* 1990; 336 (8708): 189–90.

Dale H. Pharmcology and nerve endings. *Proc Royal Soc Med Ther* 1935; 28: 319–32.

Delamarter R B, Bohlman H H, Dodge L D, Biro C. Experimental lumbar spinal stenosis. Analysis of the cortical evoked potentials, microvasculature, and histopathology. *J Bone Joint Surg* (Am) 1990; 72 (1): 110–20.

El-Bohy A, Cavanaugh J M, Getchell M L, Bulas T, Getchell T V, King A I. Localization of substance P and neurofilament immunoreactive fibers in the lumbar facet joint capsule and supraspinous ligament of the rabbit. *Brain Res* 1988; 460 (2): 379–82.

Ferreira S H. Prostaglandins, aspirin-like drugs and analgesia. *Nature New Biol* 1972; 240 (102): 200–3.

Ford-Hutchinson A W, Bray M A, Doig M V, Shipley M E, Smith M J H. Leukotriene B a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* 1980; 286: 264–5.

Frymoyer J. *New perspectives on low back pain. A workshop.* Airlie Virgina, AAOS Publishers, May 1989.

Ghormley R K. Low back pain with special reference to the articular facets with presentation of an operative procedure. *JAMA* 1933; 101: 1773–7.

Gibson S J, Polak J M, Bloom S R, Wall P D. The distribution of nine peptides in rat spinal cord with special emphasis on the substantia gelatinosa and on the area around the central canal (lamina X). *J Comp Neurol* 1981; 201 (1): 65–79.

Giles L G F. Pressure related changes in human lumbosacral zygopophyseal joint articular cartilage. *J Rheumatol* 1986; 13: 1093–5.

Giles L G F. Innervation of zygapophyseal joint synovial folds in low-back pain (letter). *Lancet* 1987; 2 (8560): 692.

Giles L G F. *Anatomical basis of low back pain.* Williams & Wilkins, Baltimore 1989.

Giles L G, Taylor J R. Intra-articular synovial protrusions in the lower lumbar apophyseal joints. *Bull Hosp Jt Dis Orthop Inst* 1982; 42 (2): 248–55.

Giles L G, Harvey A R. Immunohistochemical demonstration of nociceptors in the capsule and synovial folds of human zygapophyseal joints. *Br J Rheumatol* 1987; 26 (5): 362–4.

Grönblad M, Liesi P, Korkala O, Karaharju E, Polak J. Innervation of human bone periosteum by peptidergic nerves. *Anat Rec* 1984 ; 209 (3): 297–9.

Grönblad M, Korkala O, Konttinen Y T, Nederström A, Hukkanen M, Tolvanen E, Polak J M. Silver impregnation and immunohistochemical study of nerves in lumbar facet joint plical tissue. *Spine* 1991; 16 (1): 34–8.

Hirsch C, Ingelmark B E, Miller M. The anatomical basis for low back pain. *Acta Orthop Scand* 1963; 33: 1–17.

Howe J F, Calvin W H, Loeser J D. Impulses reflected from dorsal root ganglia and from focal nerve injuries. *Brain Res* 1976 ; 116 (1): 139–44.

Hsu S M, Raine L, Fanger H. Use of avidin biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 1981; 29 (4): 577–80.

Jackson H C, Winkelmann R K, Bickel W H. Nerve endings in the human lumbar spinal column and related structures. *J Bone Joint Surg* (Am) 1966; 48 (7): 1272–81.

Jackson R P, Jacobs R R, Montesano P X. 1988 Volvo award in clinical sciences. Facet joint injection in low back pain. A prospective statistical study. *Spine* 1988; 13 (9): 966–71.

Jinkins J R, Whittemore A R, Bradley W G. The anatomic basis of vertebrogenic pain and the autonomic syndrome associated with lumbar disk extrusion. *Am J Roentgenol* 1989; 152 (6): 1277–89.

Kanaka R, Schaible H G, Schmidt R F. Activation of fine articular afferent units by bradykinin. *Brain Res* 1985; 327 (1–2): 81–90.

Konttinen Y T, Grönblad M, Antti-Poika I, Seitsalo S, Santavirta S, Hukkanen M, Polak J M. Neuroimmunohistochemical analysis of peridiscal nociceptive neural elements. *Spine* 1990a; 15 (5): 383–6.

Konttinen Y T, Grönblad M, Korkala O, Tolvanen E, Polak J M. Immunohistochemical demonstration of subclasses of inflammatory cells and active, collagen producing fibroblasts in the synovial plicae of lumbar facet joints. *Spine* 1990b; 15 (5): 387–90.

Korkala O, Grönblad M, Liesi P, Karaharju E. Immunohistochemical demonstration of nociceptors in the ligamentous structures of the lumbar spine. *Spine* 1985; 10 (2): 156–7.

Kumazawa T, Mizumura K. The polymodal receptors in the testis of dog. *Brain Res* 1977; 136 (3): 553–8.

Lee Y, Takami K, Kawai Y, Girgis S, Hillyard C J, MacIntyre I, Emson P C, Tohyama M. Distribution of calcitonin gene-related peptide in the rat peripheral nervous system with reference to its coexistence with substance P. *Neuroscience* 1985; 15 (4): 1227–37.

Levine J D, Lam D, Taiwo Y O, Donatoni P, Goetzl E J. Hyperalgesic properties of 15 lipoxygenase products of arachidonic acid. *Proc Natl Acad Sci U S A* 1986a; 83 (14): 5331–4.

Levine J D, Fye K, Heller P, Basbaum A I, Whiting-O'Keefe Q. Clinical response to regional intravenous guanethidine in patients with rheumatoid arthritis. *J Rheumatol* 1986b; 13 (6): 1040–3.

Liesi P, Grönblad M, Korkala O, Karaharju E, Rusanen M. Substance P: a neuropeptide involved in low back pain? (letter). *Lancet* 1983; 1 (8337): 1328–9.

Lilius G, Laasonen E M, Myllynen P, Harilainen A, Grönlund G. Lumbar facet joint syndrome. A randomised clinical trial. *J Bone Joint Surg* (Br) 1989; 71 (4): 681–4.

Lindblom K, Rexed B. Spinal nerve injury in dorso-lateral protrusions of lumbar disks. *J Neurosurg* 1948; 5: 413–32.

Lotz M, Carson D A, Vaughan J H. Substance P activation of rheumatoid synoviocytes: neural pathway in pathogenesis of arthritis. *Science* 1987 ; 235 (4791): 893–5.

Mooney V, Robertson J. The facet syndrome. *Clin Orthop* 1976; 115: 149–56.

Nagy J I, Daddona P E. Anatomical and cytochemical relationships of adenosine deaminase containing primary afferent neurons in the rat. *Neuroscience* 1985; 15 (3): 799–813.

Parke W W, Watanabe R. Adhesions of the ventral lumbar dura. An adjunct source of discogenic pain? *Spine* 1990; 15 (4): 300–3.

Payan D G, McGillis J P, Goetzl E J. Neuroimmunology. *Adv Immunol* 1986; 39: 299–323.

Payan D G, McGillis J P, Renold F K, Mitsuhashi M, Goetzl E J. Neuropeptide modulation of leukocyte function. *Ann N Y Acad Sci* 1987; 496: 182–91.

Roberts W J, Kramis R C. Sympathetic nervous system influence on acute and chronic pain. In: *Pain syndromes in neurology* (Ed. Fields H L) Butterworths, London 1990: 85–106.

Rydevik B L, Myers R R, Powell H C. Tissue fluid pressure in the dorsal root ganglion: An experimental study on the effects of compression. *Trans Orthop Res Soc* 1988; 13: 135.

Rydevik B L, Myers R R, Powell H C. Tissue fluid pressure in the dorsal root ganglion: An experimental study on the effects of compression. *Spine* 1989; 16: 574–6.

Saal J S, Franson R C, Dobrow R, Saal J A, White A H, Goldthwaite N. High levels of inflammatory phospholipase A2 activity in lumbar disc herniations. *Spine* 1990; 15 (7): 674–8.

Shantha T R, Evans J A. The relationship of epidural anesthesia to neural membranes and arachnoid villi. *Anesthesiology* 1972; 37 (5): 543–57.

Shealy C N. Facet denervation in the management of back and sciatic pain. *Clin Orthop* 1976; 115: 157–64.

Weinstein J, Claverie W, Gibson S. The pain of discography. *Spine* 1988a; 13 (12): 1344–8.

Weinstein J, Pope M, Schmidt R, Seroussi R. Neuropharmacologic effects of vibration on the dorsal root ganglion. An animal model. *Spine* 1988b; 13 (5): 521–5.

Wiberg G. Back pain in relation to the nerve supply of the intervertebral disc. *Acta Orthop Scand* 1949; 19: 211–21.

Wyke B. Receptor systems in lumbosacral tissues in relation to the production of low back pain. In: *American Academy of Orthopaedic Surgeons symposium on idiopathic low back pain* (Eds. White A A III, Gordon S L) Mosby, St Louis 1982: 97–107.

Yamashita T, Cavanaugh J M, El-Bohy A A, Getchell T V, King A I. Mechanosensitive afferent units in the lumbar facet joint. *J Bone Joint Surg* (Am) 1990; 72 (6): 865–70.