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An Insight Into Neurophysiology of Pulpal Pain: Facts and Hypotheses

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Pain and pain control are important to the dental profession because the general perception of the public is that dental treatment and pain go hand in hand. Successful dental treatment requires that the source of pain be detected. If the origin of pain is not found, inappropriate dental care and, ultimately, extraction may result. Pain experienced before, during, or after endodontic therapy is a serious concern to both patients and endodontists, and the variability of discomfort presents a challenge in terms of diagnostic methods, endodontic therapy, and endodontic knowledge. This review will help clinicians understand the basic neurophysiology of pulpal pain and other painful conditions of the dental pulp that are not well understood. (Korean J Pain 2013; 26: 347-355)

Key Words:
inflammation, mediators, pulpal pain, pulpitis, sensory nerves.

INTRODUCTION

Since dentistry was first practiced, the prime reason for seeking some form of dental treatment has been the relief of pain. For patients, understandably, the elimination of pain takes precedence over all other considerations. The relief of oral pain, therefore, is the highest priority of the profession. Although pain receives substantial attention from all healthcare providers, many patients consider pain and dentistry to be synonymous. Pain is a complex phenomenon, and dental pain, a multifactorial or multidimensional experience, involves sensory responses and emotional, conceptual, and motivational aspects [1]. One study reported that dental pain is the most common type of orofacial pain [2]. Pulpal and periapical pains are two of the reasons patients seek dental care [3].

Knowledge of factors associated with pulpal and periapical pain may provide important information for planning preventive or therapeutic strategies as well as for understanding the outcomes of urgent endodontic treatment. The mechanisms of oral pain, however, remained largely obscure until the last three decades, when advances in neurophysiology made such pain easier to understand and treat. This brief work focuses on the role of the pulpal

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nerves and the chemical mediators in the induction and maintenance of inflammation and dental pain, and it suggests possible future lines of research. The literature was systematically reviewed to offer explanations for a number of pain conditions that are not well understood.

PULP NERVES

The dental pulp has long been thought of as a richly innervated and vascularized tissue that, when stimulated, has a single sensory response: pain. The neural component of the pulp tissue consists of sensory trigeminal afferent axons [4,5]. Sympathetic efferent fibers regulate the blood flow; no consensus exists concerning the role of the parasympathetic fibers.

INTRAPULPAL SENSORY NERVE FIBERS

Sensory nerve fibers of the dental pulp are afferent endings of the trigeminal cranial nerve. These fibers reach the root canal through the apical foramen, going to the root pulp in lumps. These lumps are often associated with blood vessels in a collagen sheath, forming the neurovascular bundle. Only a few bifurcations occur in the root canal, but when these reach the coronal pulp, the nerve endings begin to divide and distribute branches to the surrounding dentin. On approaching the subodontoblastic region, the fibers form an intricate network known as the plexus of Raschkow. After this, the myelinated fibers lose their myelin sheath and emerge as free nerve endings. In studies performed by Gunji from 1982 to 1988, it was shown that many nerve fibers end in the extracellular space of the rich cell zone or in the odontoblast layer, while others extend into the predentin or the dentinal tubules, penetrating up to 150 μ. These intratubular fibers are more numerous in the region of the pulp horns (present in around 25% of the tubules), while in another part of the dentin crowns, they are present in smaller numbers (about 15%). In the root, only about 10% of the tubules contain nerve fibers, which tend to be smaller and do not extend above the predentin [6].

These sensitive fibers act as nociceptors and belong to two groups according to their diameter, conduction velocity, and function: A∂ (myelinated) and C (unmyelinated) [7]. However, about 7% of the myelinated fibers entering the human premolar pulp are of the Aβ variety [8].

A FIBERS

The myelinated axons have a fast conduction speed and low stimulation threshold, are superficial (located in the pulp and dentin junction), transmit pain directly to the thalamus, and generate a sharp and stabbing pain that is easily localized. These characteristics make them the first nerve fibers to react and transmit the pain impulse even when there is no irreversible tissue damage. The principal clinical features of A-delta fibers are that they are activated by hydrodynamic stimuli, such as drilling, sweet foods, cold air, and hypertonic solutions, which lead to rapid fluid movement within the tubules [9,10], stimulating the mechanosensitive nerve endings and resulting in a short, sharp initial pain. Although outward capillary fluid movement, which is normally slow, does not stimulate the nerve endings and cause pain [11-13], more intense and rapid fluid flow, as when desiccating or drying the dentin, is likely to activate the pulpal nociceptors [14]. Consequently, any factor that might lead to an increase in fluid movement by opening dentinal tubules would be expected to lead to an increase in dentine sensitivity [12]. Clinical examples of factors leading to such activation include cutting a cavity and exposure of an open root dentine by scaling or toothbrush abrasion.

Among the A∂ fiber population, the arousal thresholds vary. Low-threshold fibers respond to stimuli such as cooling and vibration—stimuli that rarely are associated with nociception. However, there is evidence that they may be involved in reflection or other functions related to perception. The higher-threshold A fibers respond to much stronger stimuli, such as mechanical instrumentation, and they can act as nociceptors [15].

The A-beta fibers are thought to be functionally similar to the A-delta fibers [16]; however, the A-beta fibers have been reported to respond differently to vibration [17] and are stimulated at a lower electrical threshold [8]. The site that responds to stimulation, or the receptive field of these fibers, is located at the pulp-dentine border or in close proximity to the odontoblast cell body [18,19].

C FIBERS

C fibers are unmyelinated and have a low conduction velocity, a smaller diameter, and a higher excitation threshold. They are located deeper than the myelinated fi-

bers and are principally activated by heat, causing slow, diffuse, and durable pain [6]. If the pain stimulus intensity increases, the sensory C fibers are recruited and the pain becomes a burning sensation. The C fiber reaction shows that the pulp damage is irreversible. The location of the C fibers within the nerve bundles in the core or central region of the pulp may explain the diffuse pain, called *referred pain*, from a specific tooth because nerve fibers innervate multiple teeth with multiple pulps [20].

C fibers also differ from A fibers in their ability to maintain functional integrity when the tissue becomes hypoxic; this is because the oxygen consumption is higher in the thick A fibers than in the thin C fibers. Thus, when an injury results in an interruption in the pulp microcirculation, the C fibers continue to function for a longer time compared to the A fibers because the latter have been inactivated or infracted [21]. The typical clinical description of this type of pain is that it is a dull, vaguely located, aching pain that increases several seconds after a hot drink. This characteristic also underlies the familiar clinical occurrence in which a tooth that responds negatively to testing with a cold CO_2 stick (because the A-delta fibers have degenerated) is undeniably painful to mechanical instrumentation at the commencement of endodontic therapy. Therefore, to avoid pain during root canal therapy, it is always wise to administer a local anesthetic in cases where a patient reports pain in response to hot drinks (indicating viable C- fibers) but the tooth responds negatively to cold or electric sensitivity tests.

Based on this discussion of fibers and their responses, we can relate the types of fibers to various clinical pulp testing methods listed below:

• Thermal pulp testing depends on the outward and inward movement of the dentinal fluid, whereas electric pulp testing depends on ionic movement [22].

• Because of their distribution, their larger diameter compared to that of C fibers, their conduction speed, and their myelin sheath, A-delta fibers are those that are stimulated in electric pulp testing [22].

• C fibers do not respond to electric pulp testing. Because of their high threshold, a stronger electric current is needed to stimulate them [23].

• Based on the hydrodynamic effect, outward movement of dentinal fluid caused by the application of cold (contraction of fluid) produces a stronger response in A-delta fibers compared to the inward movement of fluid caused by the application of heat [9].

• Repeated application of cold will reduce the displacement rate of the fluids inside the dentinal tubules, causing a less painful response from the pulp for a short time, which is why the cold test is sometimes refractory [22].

The A-delta fibers are more affected by a reduction of the pulpal blood flow compared to the C fibers because the A-delta fibers cannot function in the case of anoxia [24].

• An uncontrolled heat test can injure the pulp and release mediators that affect the C fibers [25].

• A positive percussion test indicates that inflammation has moved from the pulp to the periodontium, which is rich in proprioceptors, causing this type of localized response.

• The pulp vitality electrical test performed in immature teeth tends to give unreliable results. Human pulp has a large number of C fibers during the early development of a tooth, and the A fibers increase in number with age until the root completes its apical development [19].

All functional changes to the nociceptors are reversible upon removal of the cause. For example, in the case of dentin hypersensitivity, the tubules are treated by blocking, which directly affects the A fibers (hydrodynamic cessation) and resolves the neural changes in the pulp, causing the pain to subside [26].

MOLECULAR BASIS OF PULP NOCICEPTION

Dental pain is caused by the stimulation of dental pulp nerve fibers. Both neural and vascular structures play a role in nociception. As a complex expression, this defensive mechanism involves multiple systems that contribute to its occurrence and regulation.

From a coordinated intervention between the central nervous system and endocrine system, the hypothalamus and hypophysis gland may be related to some of the events that occur in the pulp during a symptomatic period. Rutz et al. [27] described an interesting relationship between the endocrine system and the dental pulp. He explained the events that occur when corticotrophin-releasing factor (CRF) binds to its membrane receptor (CRF-Rs). Among the final actions of the activated receptor, the release of endorphins from immune cells increases the peripheral antinociception. If the dental pulp is considered to be a peripheral tissue, the above statement may be applied to a tooth. Physical and psychological stresses enhance the re-

lease of CRF from the hypothalamus. The binding of CRF to its receptor in the anterior hypophysis allows for the release of adrenocorticotropic hormone (ACTH) and endorphins into the blood stream. ACTH acts on the suprarenal gland cortex to stimulate the secretion of cortisol while endorphins produce a decrease in nociception. The synthetic use of CRF stimulates immunocompetent cells to secrete endorphins that interact with the opioid receptors on the peripheral afferent nerves, causing a significant antinociception.

Endogenous opioid peptides, which are present in large quantities in the brain (subnucleus caudalis and the substantia gelatinosa of the spinal cord) are naturally occurring, pain-suppressing neurotransmitters and neuromodulators. They reduce pain transmission by preventing the release of the excitatory neurotransmitter substance P from the primary afferent nerve terminal [8]. The immunohistochemical localization of μ type opioid receptors in human tooth pulp was discovered by Jaber et al. [28] in 2003 along the ramification of the nerve root and in the coronal pulp, suggesting a peripheral site in the dental pulp where endogenous and exogenous opioids can interact with μ opioid receptors. The hypothesis of this wok is that the μ opioid receptors may be associated with small-diameter nerve fibers (C fibers), although further studies are needed to clarify this possibility. Dionne et al. [29] in 2001 showed that a local morphine injection lessens the pulpitis pain. Later, in 2007, Chao et al. [30] demonstrated the ability of the δ opioid receptors to regulate the homeostasis of potassium ions (K^+). However, considering the role of K^+ in nerve conduction, further studies are needed to evaluate the importance of δ opioid receptors in pulpal pain.

Considering that the adrenal medulla is an important source of catecholamine (dopamine, adrenaline, and noradrenaline) production and that the pulp has receptors for these compounds located mainly in the membranes of blood vessels and some nerves [31], another relationship can be established in reference to the adrenal glands. Catecholamines, such as epinephrine and norepinephrine, exert their physiological effects on adrenoreceptors in blood vessels. The smooth muscles of pulp vessels contain α_1, α_2, and β adrenoreceptors [32,33]. The α receptors are responsible for the contraction of the vascular muscle, producing vasoconstriction. In human dental pulp, however, evidence for β-adrenergic vasodilation has not been found. Wakisaka et al. [34] showed the distribution of feline dental pulp adrenergic nerve fibers before and after cavity preparation. After cavity preparation, an inflammatory process occurs with changes in the morphology and biochemical content of the nerve fibers. Higher levels of noradrenaline, adrenaline and dopamine were found in the inflamed pulps. Nup et al. [35], managed to quantify the catecholamines in the inflamed pulp tissue and considered the possibility of using pharmacological agents that reduce their concentrations.

MOLECULAR BASIS OF PULPAL INFLAMMATION

The two key components of pulpal inflammation are microcirculation and the activity of nerve fibers. According to the literature, the excitation of the A-delta fibers seems to have an insignificant effect on the pulp blood flow, whereas the activation of the C fibers (such as deep cavity preparation and high intensity thermal/chemical stimuli) provokes augmentation caused by the action of neurokinins, especially substance P [36]. This alteration of pulpal blood flow has a varying effect on sensory nerve activity. SP, a neuropeptide released from afferent fibers, results in neurogenic inflammation of the dental pulp by causing vasodilatation and endothelial cell contraction, which allows plasma extravasation and mastocyte degranulation. The mastocyte granules release histamine, which in turn further amplifies vascular processes and activates nociceptors. Lymphocytes, granulocytes, and macrophages have receptors for SP, and these cells can be stimulated to produce cytokines. Macrophages stimulated by SP produce the inflammatory mediators PGE2 and thromboxane, as well as the proinflammatory cytokines IL-1, IL-6, and TNF. All of these molecular events ultimately sustain the synthesis and release of new SP, thereby perpetrating the vicious cycle and further increasing pain sensitivity. These effects are mediated by G proteins associated with the NK-1 receptor, but at higher concentrations, the peptide can also activate NK2 and NK3 receptors. It can be speculated that NK1 is the receptor most involved in physiological conditions, while NK1 and NK2 become involved in pathological conditions because of the higher SP concentrations [37]. Kido et al. [38], 2005, using immunocytochemical studies in rat dental pulp, reported that NK1 receptors were present in odontoblasts and ameloblasts and were abundant on

capillaries and smaller blood vessels, and both NK1 and NK2 receptors were found on the capillary plexus subjacent to the dentin. Substance P receptors have also been reported in human pulp tissue, although the methods used for their evaluation, the radioreceptor assay, did not allow investigators to measure which type of receptor (NK1, NK2, or NK3) was primarily present [39].

Extracellular levels of Substance P are increased in symptomatic pulp tissue diagnosed with irreversible pulpitis. An 8-fold increase in SP was noted in pulp tissue diagnosed with irreversible pulpitis against clinically normal pulp tissue [40]. Thus, irreversible pulpitis is associated with significant activation of the peptidergic system. It is well known that root canal preparation generates an inflammatory process in the periapical tissues, which could explain the posttreatment pain events after root canal therapy (such as symptomatic apical periodontitis). Interestingly, it was demonstrated that SP is released in the periodontal ligaments as a result of the application of different canal preparation techniques, although the amount of released SP differs among procedures. An increase in SP could generate an inflammatory process in the periapical tissues [41]. Based on this, SP can be considered a major mediator of neurogenic inflammation and associated hyperalgesia and represents a promising target for therapies aimed at controlling pain and minimizing the deleterious consequences of tissue injury.

Capsaicin, heat, and protons activate vanilloid receptor 1 (VR1), resulting in the opening of a cation channel and increasing calcium entry through this channel and through voltage-gated calcium channels activated by sodium-induced depolarization. These effects increase the release of SP from sensory neurons [37]. Buck and Burks [42] and Ichikawa and Sugimoto [43] found a reduction in the release of SP from C fiber terminal nerve endings from the neurotoxin capsaicin, which binds to specific cell membrane receptors on neurons. Later, in 2005, Caviedes-Bucheli [44] found a reduction in SP expression with 1% capsaicin in inferior dental nerve infiltration in rat dental pulp tissue. This may provide a possible mechanism for controlling pulpal neurogenic inflammation to maintain pulp vitality when harmed by external irritants.

A variety of endogenous chemical mediators have been associated with inflammation and pain; these include histamine, bradykinin, 5-hydroxytryptamine, and prostaglandins.

Bradykinin (IBK) is a potent mediator of pain and inflammation. Bradykinin inflammatory responses include vasodilatation, plasma extravasation, and recruitment of inflammatory cells. The extracellular levels of bradykinin are significantly elevated during irreversible pulpitis, contributing to the early stages of inflammation and pain [20].

Irritation of the dental pulp produced by bacteria, mechanical stimuli, or chemical stimuli can cause inflammation. These stimuli can cause the enzymatic conversion of arachidonic acid in biologically active group mediators such as leukotrienes, PGs, and thromboxane acid. Lin et al. [45] reported the presence of PGE2 in pulp disease. Sundqvist et al. [46] pointed out the ability of pulp cells to secrete PGE2, thus indicating their role in tissue destruction in pulpal disease. PGE2 and F2 can be identified in inflamed and non-inflamed pulp tissues. PGE2 may be able to produce pulp pain in two different ways. First, it presents hyperalgesic qualities, which sensitize the nociceptive nerve endings. Second, it can increase the pain response to other pain mediators, such as IBK, histamine and 5-hydroxytryptamine [15].

PGF2-α may have a modulating effect on the tissue response to PGE2. The introduction of PGE2 in tissues causes an accumulation of AMPc. PGF2-α has no effect on AMPc except at very high concentrations. However, PGF2-α can increase the GMPc levels in various tissues. The second intracellular messengers, AMPc and GMPc, seem to be responsible for a number of contrary effects, but, overall, they play an important role during the inflammatory response and odontogenic pain. PGE2 is a potent stimulator of bone resorption. Coon et al. [47] showed the increased production of PGE2 in periradicular lesions, partly explaining bone resorbing activity. PGE2 levels in root canal periradicular exudates are associated with the clinical symptoms of endodontic pathology, especially with the onset of pain.

IL-1 and TNF-α regulate COX-2 expression in human pulp cells. Tani-Ishii et al. [48] showed that increased amounts of proinflammatory cytokines such as IL-1 and TNF-α can induce pulpitis in rats. Studies have also demonstrated that IL-1 and TNF-α regulate matrix metalloproteinases and the tissue plasminogen activator of human dental pulp cells. These conclusions highlight the importance of proinflammatory cytokines in pulp injury [49,50]. IL-1 is heavily expressed in pulpal inflammation. D'Souza et al. [51] found significantly higher IL-1 activity in human dental pulps with carious lesions than in asymp-

tomatic carious teeth or teeth with periodontal disease. In the same study, impacted third molars with pain but no caries showed elevated pulpal IL-1 activity. IL-1 evokes hyperalgesia after peripheral injection, primarily by increasing PGE2 synthesis. The cytokines IL-6 and IL-8 modulate the white series of the immune cell response. IL-6 is a cytokine that can be produced by various cells including T and B lymphocytes, monocytes/macrophages, fibroblasts, endothelial cells, and osteoblasts. A study by Lin et al. [45] suggested that fibroblasts are involved in the development of pulpitis via production of IL-6 and COX-2 because excessive IL6 and PGs levels have been linked to several inflammatory disease pathogeneses.

IL-6 also increases the plasminogen activity in pulpal cells, which then can activate collagenase enzymes and lead to tissue injury in inflamed sites [52]. High levels of IL-6 have also been associated with the pathogenesis of several inflammatory diseases such as periodontitis. Barkhordar et al. [53] found that inflamed pulp tissue contains a more than 3000-fold greater amount of IL-6 compared to healthy pulps. Fibroblasts from human dental pulp participate in the development and progression of pulpitis via IL-6 synthesis, which is regulated by cytokines via prostaglandin. IL-6 causes the formation of acute phase proteins such as fibrinogen and CRP (C- reactive protein) in the liver. Dental pulp with irreversible pulpitis displays significantly higher CRP values compared to unin-flamed pulp, independent of serum CRP values [54].

Recent data from the medical literature indicate that COX-2 plays a key role in the production of vascular endothelial growth factor (VEGF), a glycoprotein that increases the permeability of blood vessels and induces angiogenesis. Güven et al. [55] investigated the immunohistological co-expression of COX-2 and VEGF in inflamed pulp, in conjunction with the expression of CD34, a transmembrane glycoprotein expressed in endothelial cells. Recently, VEGF has been found at increased levels in inflamed pulp tissues and periapical lesions. In addition, COX-2 expression has been observed only in inflamed pulp tissue, with no expression in healthy pulp, while CD34 expression has been found in the endothelium of both normal and inflamed pulp tissues. Co-expression of COX-2 and VEGF in all inflamed pulps could be suggestive of a possible release of VEGF via the COX-2 dependent pathway. Holt et al. [56] concluded that because there is COX-2 induction at the inflammation sites, the NSAID therapeutic properties are primary factors involved in COX-2 inhibition.

The two most important enzymes involved in inflammatory and pain processes are aspartate aminotransferase (AST) and alkaline phosphatase (ALP). AST is increased considerably in the early stages of the inflammatory process. Spoto et al. [57] observed an increase of AST enzyme in the early stages of the inflammatory process, related to early cell necrosis of the pulp. The authors noted that its decrease in irreversible pulpitis could be related to a depletion or destruction of this enzyme.

ALP, an enzyme present in the vesicles of mineralized tissue matrix, seems to have a significant role in their initial formation. High levels of ALP activity have been demonstrated in dental pulp cells, with the fibroblasts from the isolated pulp showing high levels of ALP activity. Spoto et al. [58] observed a decrease in ALP activity in irreversible pulpitis, a finding that could be related to a massive release of inflammatory mediators from immune cells, causing an inhibitory effect on ALP synthesis.

ROLE OF SODIUM CHANNELS

The generation and propagation of action potentials in sensory neurons depend on the activity of voltage-gated sodium ion channels. Pulpal inflammation induces alterations in primary afferent neurons, causing an increase in excitability and thereby participating in the generation of allodynia and hyperalgesia. Warren et al. reported painful pulp inflammation associated with an approximately 6-fold increase of the subtype of Na + NaV1.8 channels [59].

ROLE OF POTASSIUM CHANNELS (KV)

Kv1.4 subunits, which are found in myelinated sensory fibers, are also the main determinant of C fiber excitability. There is a significant decrease in Kv1.4 expression in symptomatic human pulp axons compared with asymptomatic healthy pulp axons. This provides evidence that Kv1.4 may contribute to hyperalgesia and allodynia pulp generation [60].

ODONTOBLATS AND PULPAL PAIN

Dental pain arises from exposed dentin following bacterial, chemical, or mechanical erosion of enamel and/or

recession of gingival. Thus, patent dentinal tubules represent the first structure involved in dentin sensitivity. Interestingly, the information is transferred to the underlying dental pulp via odontoblasts, via their apical extension bathed into the dentinal fluid running in the tubules, or via a dense network of trigeminal sensory axons intimately related to odontoblasts. Therefore, external stimuli causing dentinal fluid movements and odontoblasts and/or nerve complex responses may represent a unique mechanosensory system, giving odontoblasts a new role as sensor cells. Several lines of evidence have demonstrated that odontoblasts express mechano- and/or thermo-sensitive transient receptor potential ion channels (TRPV1, TRPV2, TRPV3, TRPV4, TRPM3, KCa, TREK-1) that are likely to sense heat and/or cold as well as movements of dentinal fluid within tubules. In addition, voltage-gated sodium channels confer excitable properties of odontoblasts *in vitro* in response to the injection of depolarizing currents. Sodium channels have been observed *in vivo*, to co-localize with nerve terminals at the apical pole of odontoblasts and to correlate with the spatial distribution of stretch-activated KCa channels. Magloire et al. (2010) highlighted the role of odontoblasts as the pivotal zone of the pulp/dentin complex for sensing external stimuli. Signaling between odontoblasts and axons may take place by the release of mediators in the gap between odontoblasts and axons, with nociception-transducing receptors on the trigeminal afferent fibers, and by the expression of putative effectors by odontoblasts.

CONCLUSION

Pain represents a major stimulus for seeking emergency dental treatment. On the other hand, fear of pain represents a significant barrier that discourages patients from seeking routine dental care. Knowledge of the physiology and clinical characteristics associated with pulpal and periapical pain may provide important information for the planning of preventive or therapeutic strategies, as well as for understanding the outcomes of urgent endodontic treatment. This article has attempted to provide an overview of research developments in this field. Endodontists as well as clinicians are encouraged to give more attention to the topic and undertake more randomized clinical trials. Further study will help elucidate the correct methods to measure, evaluate, and manage pain, as well as strategies to reduce the distress experienced by endodontic patients. Such research will help to improve not only the living standards of patients but also the practices of all endodontic clinicians.

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