Dental Pulp Neuropathophysiology

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Mechanisms of pulpal pathophysiology are complex and the low compliance environment in which the dental pulp is allocated, further enhances the complexity of this process. Although it is known that it involves the interaction of the immune cells, pulpal cells, cytokines, chemokines and multiple neuropeptides but still there are many gaps in our current knowledge. The understanding of the biochemical and molecular pathways involved in the pulpal inflammation is important so that it can be used clinically to keep the dental pulp vital and healthy. It may thus provide an opportunity to develop potentially new treatment modalities for the inflamed dental pulp in future.

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INTRODUCTION

Infectious agents initiate a series of inflammatory and immunological responses in the dental pulp. Pulpal pathophysiology involves complex mechanisms that include the interaction of various molecular mediators and cells. The low compliance environment in which the dental pulp is allocated further enhances the complexity of its pathophysiology. Since the dental pulp is highly innervated, neuropeptides play a significant role in mediating and modulating the inflammatory response in it. Therefore, it is generally accepted that the nervous system contributes to the pathophysiology of peripheral inflammation and a neurogenic component has been implicated in pulpitis.[1] However, the neurogenic inflammatory response is complex and cannot simply be regarded as a series of neuronal events occurring in isolation. Indeed, it is known that the initiation and sustenance of neurogenic inflammation depend on a variety of factors present in the local environment.[1] The understanding of these biochemical and molecular pathways involved in the pulpal inflammation is important as it may provide an opportunity to develop potentially new treatment modalities for the inflamed dental pulp in future. Hence this review article addresses the current perspectives on pulpal pathophysiology providing an overview of neuropeptides, explaining their biological effects as related to dental pulp, and the interaction between neuropeptides, cytokines, chemokines, immune cells and pulpal cells in pulpitis is also discussed.

Neuropeptides

The nerve fibers in the pulp are components of a large peripheral nervous system that includes afferent fibers originating from the trigeminal ganglion and sympathetic fibers originating from the cervical sympathetic ganglia.[2,3]

Neuropeptides are simply peptide neurotransmitters. It is implicit that for a peptide to be defined as a neuropeptide, it is synthesized and released from neurons and has biological actions mediated via extracellular receptors on target cells.[4] Neuropeptides are widely distributed throughout the entire human body and are present in every branch of the nervous system. They have multiple and variable functions, they can act as neurotransmitters, growth factors and as immune system signaling molecules.[5] Approximately 80% of neuropeptide receptors are G-protein-coupled receptors (GPCRs).[1] Different neuropeptides and their receptors detected in the human dental pulp are:

- Tachykinins: Substance P and Neurokinin A (SP and NKA)
- Calcitonin Gene Related Peptide (CGRP)
- Neuropeptide Y (NPY)
- Vasoactive Intestinal Peptide (VIP)

Tachykinins (SP and NKA) – The vasoactive material extracted into powder form by von Euler and Gaddum[1] (1931) was named substance ‘P’ for ‘powder’ and is an 11 amino acid peptide. SP was the first neuropeptide to be

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identified in dental tissues.[6] SP fibers originate from trigeminal ganglion and travel in close proximity to blood vessels in the central pulp. In the sub-odontoblastic layer they branch towards predentin, with some SP fibers penetrating into the dentin.[7] SP is released from neurons on various types of noxious stimuli (thermal, mechanical, chemical).[8,9] NKA originating from trigeminal nerve has also been detected in the dental pulp.[10,11] There are three types of tachykinin receptors, NK1, NK2 and NK3. NK1 receptors have preference for Substance P and are expressed on most inflammatory cells such as mast cells and macrophages. SP receptor expression in human pulps is significantly greater during clinical inflammatory phenomenon.[12]

Calcitonin Gene Related Peptide (CGRP) – CGRP is a 37-amino-acid peptide.[1] Trigeminal afferent neurons expressing CGRP innervate dental pulp.[13,14] They branch extensively near pulp horn and enter into dentin up to 0.1 mm.[8] There are two known types of CGRP receptors, CGRP1 and CGRP2. CGRP1 receptors have also been demonstrated in ameloblasts suggesting their role in enamel formation.[15] CGRP receptor expression in human pulps is significantly greater during clinical inflammatory phenomenon.[16]

Neuropeptide Y (NPY) –NPY is a 28-amino-acid peptide.[1] The presence of NPY has been demonstrated in the human dental pulp from sympathetic nerves originating in the superior cervical ganglion.[17] NPY fibers accompany blood vessels and characteristically encircle them. Also seen in the sub-odontoblastic plexus and as free nerves in the odontoblastic layer projecting toward the dentin.[18] Changes in the levels and distribution of NPY in human dental pulp occur during the caries process, with significantly higher levels of NPY in carious compared with non-carious adult human teeth.[18] The presence of its receptors has not yet been demonstrated in the dental pulp.

Vasoactive Intestinal Peptide (VIP) – VIP is a 36-amino-acid peptide.[1] VIP is expressed predominantly in parasympathetic neurons and its presence has been demonstrated in the dental pulp.[19] Fibers travel along large blood vessels and show a network like arrangement adjacent to the blood vessel wall.[13] Very few fibers are seen in the sub-odontoblastic layer. VIP receptors (VPAC1 and VPAC2) are expressed in osteoclasts and osteoblasts and also present in immune cells.[20,21] Noxious stimulation of dental pulp does not influence VIP release.[11]

Characteristics of Pulpal Vasculature

The arterial supply of the dental pulp has its origin from the posterior superior alveolar arteries and the infraorbital and the inferior alveolar branch of the internal maxillary arteries.[22] The main arterioles enter (diameter in the range of 100 μm) and the main venules (diameter in the range of 200 to 300μm) and lymphatics exit the dental pulp through the apical foramen. Arterioles pass through the root pulp to supply the coronal pulp. As the arterioles travel straight to the coronal area, 90^0 branching patterns develop. Near the dentin, around the odontoblastic area, they form a dense terminal capillary network in the subodontoblastic region.[23]

Before the arterioles break up into capillary beds, arteriovenous anastomosis (AVA) often arise to connect the arteriole directly to a venule.[24] The AVAs are relatively small vessels, having a diameter approximately 10 μm. Their presence is more frequent in the radicular area of the pulp. The AVAs may play a role in the regulation of blood flow.

Capillary density is highest in the subodontoblastic region with loops passing between odontoblasts. The terminal capillary network in the coronal area exhibits numerous short hairpin loops. Capillaries empty into small venules that connect with fewer and successively larger venules. At the apex multiple venules exit the pulp. Vessels of the pulp have thinner muscular walls (tunica media) than vessels of comparable diameter in other parts of the body. In most arterioles and in some venules, smooth muscle cells maintain a state of partial vasoconstriction at all times, and variety of substances such as neurotransmitters, hormones, and local factors influence this muscle tone and therefore the blood flow.[25] Nerves have intimate association with the blood vessels, with arterioles being the most densely innervated.

Neuropeptides, Neurogenic Inflammation and Painful Pulpitis

Sensory neuropeptides play important roles in neurogenic inflammation, including vasodilation, plasma extravasation, and recruitment of immune cells; however, a more extensive function for neuropeptides in the regulation of immune cell activity has also been proposed. The term neurogenic inflammation has been developed to describe the component of inflammation caused by an appropriate stimulus applied to peripheral neurons, resulting in the release of neuropeptides that alter multiple processes including vascular permeability and vasodilation at the site of injury.[26] It is now well-accepted that the pulpal innervation is not static, but shows dynamic changes; pulpal sensory nerve fibers undergo extensive sprouting reactions in response to injury.[8] During inflammation, there is a sprouting of peptidergic peripheral fibers and an increased content of neuropeptides.[10,27,28] SP, CGRP and NKA expression significantly increases in the pulp when acute irreversible pulpitis or mechanical pulp exposure occurs.[10,11] However, neuropeptides may also have a trophic role in the pulp, since innervated teeth with pulp exposures were shown to have much less tissue necrosis and periapical destruction than denervated teeth.[28]

Neuropeptides may reduce the threshold of pain in the pulp, accounting for symptoms associated with certain cases of pulpitis. Increased concentration of neuropeptides especially SP has been detected in painful pulpitis samples.[29] However not all cases of irreversible pulpitis are associated with pain. This may be explained by the action of inhibitors of neuropeptides like □-amino-butyric acid (GABA) or gastrin releasing peptide (GRP). GABA like and GRP like immunoreactivity has been identified in dental pulp.[30] Sympathetic nerves can modify pain sensation by their effects on vasculature and neuropeptides.[31] Stress-induced sympathetic vasoconstriction may also decrease pulpal pain intensity.

Interactions between Vasculature and Nervous System in the Dental Pulp

The presence of NPY in sympathetic terminals in dental pulp contributes to vasoconstriction. SP and CGRP are potent vasodilators whereas NKA has a much smaller effect on pulpal blood flow.[32] The initial component of the vasodialator response is mediated by SP whereas the continued long lasting rise in blood flow is dependent on CGRP. Clinically relevant stimuli such as drilling, probing of exposed dentin, application of ultrasound, or percussion of teeth cause vasodilation in the pulp which is mediated by intradental sensory nerves.[33] Both SP and CGRP are released from pulpal nociceptor terminals (unmyelinated C fibers and thinly myelinated Aδ fibers).[34] After exerting their effects, neuropeptides are rapidly degraded by enzymes in the pulp tissue. No significant differences have been found in VIP expression during inflammatory phenomenon, thus supporting a weak influence of parasympathetic system on pulpal blood flow.[11]

Neuro-immune Interactions in the Dental Pulp

The extremely rich innervation of the dental pulp may also influence the immunological reactions in the pulp. SP in general acts as an immunostimulatory agent. SP can enhance the chemotaxis and phagocytosis of macrophages.[35,36] Production by macrophages of bioactive substances, such as arachidonic acid metabolites and cytokines is also augmented by SP.[37] Moreover, SP stimulates mitogenic responses and cytokine production of T-lymphocytes.[38,39] By contrast, CGRP often acts with immunosuppression; it inhibits mitogen induced T-lymphocyte proliferation, H_20_2 production in macrophages and blocks antigen presentation of class II antigen expressing cells.[40,41,42]

SP and CGRP interact with mast cells, inducing the release of histamine and thereby causing elevated vascular permeability and increased blood pressure in tissues.[43] Neuropeptides alter the release of other inflammatory mediators. SP induces Interleukin-8 secretion from human dental pulp cells.[44] In addition to these vasodilator and immunomodulatory effects, SP and CGRP exert stimulatory effects on the growth of pulpal cells such as fibroblasts and odontoblast-like cells.[45,46] CGRP addition to human pulp cells in vitro caused a two-fold increase in the levels of bone morphogenetic protein-2 (BMP-2), a member of TGF-β superfamily, that has the capacity to induce dentin regeneration.[47]

There has been very little emphasis on the sympathetic innervation and its function in the dental pulp. Recent findings have indicated that immune responses are subjected to modulation by the sympathetic nervous system (SNS) and the SNS inhibits the production of pro-inflammatory cytokines, while stimulating the production of anti-inflammatory cytokines.[48] Sprouting of NPY nerve fibers was observed in the inflamed dental pulp and apical periodontal ligament 20 days after pulp exposure.[49]

Pulpal Dendritic Cells-An Essential Component of the Dental Pulp

There is emerging significance of pulpal dendritic cells (DCs) as an inherent immunosurveillant component of the dental pulp. Pulpal DCs have been recognized as class II molecule-expressing cells of a highly dendritic appearance, with three or more branched cytoplasmic processes (dendrites).[50] They do not show a random distribution, but exhibit prominent accumulation at the perivascular region of the inner pulp and the para-odontoblastic region of the outer pulp. It seems logical to assume that cells with potent immunosurveillance capacity are strategically concentrated at the site where the chance to encounter external antigens is the greatest.[51] Some of these cells extend their cytoplasmic processes into dentinal tubules, possibly to detect more concentrated antigens derived through the dentinal tubules.

DCs are a heterogeneous leukocyte population.[52] A hallmark of DC physiology is the functional duality represented by two states of maturation, which are tightly linked to tissue homeostasis and inflammation. At the site of injury, rapid recruitment of immature DCs to acute inflammatory sites is observed in response to chemotaxis by neuropeptides such as CGRP and VIP. Host derived factors such as inflammatory mediators, cytokines (e.g.IL-1,TNF-α) and VIP in pulpitis can promote DC maturation.[53] Mature DCs may play a role in continuously recruiting effector leukocytes to infection sites. The origin of pulpal DCs has not been determined. It is plausible that circulating monocytes could be the common precursors of immature DCs and macrophages in the dental pulp. After capturing antigens, immature DCs undergo maturation and migrate to lymph nodes to present antigens to T cells. The number of pulpal DCs and macrophages increases greatly in early pulpitis samples and participate in antigen presentation.[54] Harmon et al investigated the presence of mature DCs in pulp samples of human teeth with no caries, shallow dentinal caries and deep caries and demonstrated that mature DCs were frequently found only beneath deep caries pulp samples.[55]

Macrophages in Pulpitis

At the inflammatory site macrophages have three major functions: antigen presentation, phagocytosis and immunomodulation through production of cytokines and growth factors. Macrophages are generally less efficient at antigen presentation and T-cell stimulation than DCs.[56] Macrophages are activated and deactivated during the inflammatory process.[57] Activated macrophages remove dead tissue and secrete growth factors to stimulate fibroblast proliferation and revascularization. Botero *et al* found that Vascular Endothelial Growth Factor (VEGF) expression was upregulated in macrophages and odontoblast-like cells in response to lipopolysaccharide (LPS) stimulus.[58] Activated macrophages produce TNF-α, IL-1, IL-12, IL-10, chemokines and short lived lipid mediators such as platelet activating factor (PAF), prostaglandins and leukotrienes to orchestrate a local inflammation. In irreversible pulpitis, significantly increased titers of TNF-α and IL-1 were reported

and IL-1 was mainly localized in macrophages and pulp connective tissue stroma.[59,60]

T Cells and their Cytokines in Pulpitis

Cytokines are low molecular weight proteins that stimulate or inhibit proliferation, differentiation or function of immune cells and may exert their action through autocrine, paracrine or endocrine modes. After T cells are activated by antigens, they secrete cytokines and differentiate into various effector cells: CD4+ T helper cells, Cytotoxic CD8+ T cells, T regulatory cells and memory cells. Immature DCs are able to polarize T helper cells into functional subsets (according to their cytokine pattern) of Th1, Th2 cells depending on the dose, affinity, and nature of the antigens and the type and concentration of cytokines in the tissue microenvironment.[61] Hahn *et al* demonstrated that *S Mutans* associated with the initial caries lesions is a Th1 inducer, whereas Pseudoramibacter alactolyticus usually isolated from deep caries, is a Th2 inducer.[62] Th1 (type-1) and Th2 (type-2) cytokines are mutually inhibitory at the T-cell level.[63]

Type-1 cytokines including IFN-γ, IL-2, IL-12, TNF-α orchestrate strong cellular immune responses and inhibit synthesis of type-2 cytokines.

Type-2 cytokines include IL-10 and IL-4, which suppress macrophage activation and stimulate B-cell differentiation into plasma cell, indicating homeostasis or chronicity of the disease state.[64] Concentration of both types of cytokines is elevated in inflamed pulp.[65,66]

Host-derived Anti-inflammatory Factors

Cytokines with anti-inflammatory effects include IL-10, IL-4, IL-6 and TGF-β. IL-10, mainly secreted by activated lymphocytes and macrophages, is the most anti-inflammatory cytokine known.[67] It inhibits the production of many pro-inflammatory cytokines (IL-1, IL-6, IL-8, IL-12, TNF-□, IFN-γ). Its prevalence is significantly higher in deep caries pulp samples than in pulp samples from shallow caries.[65]

IL-4 suppresses IL-2 production and promotes expression of anti-inflammatory cytokines such as TGF-β_1. Expression of IL-4 and IL-10 is elevated in the later stages of pulpitis.[65] IL-6 is considered both a pro-inflammatory and an anti-inflammatory cytokine. It is secreted during inflammation and after TNF-α and IL-1 secretion. IL-6 subsequently inhibits the secretion of TNF-□ and IL-1. [68]

TGF-β, another family of host-derived anti-inflammatory factors, also counteracts the effects of pro-inflammatory cytokines by inhibiting lymphocyte proliferation and macrophage functions. IL-10 and TGF-β deactivate macrophages which results in matrix deposition and tissue remodeling.[69] TGF-β1 secreted by antigen-activated T cells, T regulatory cells, LPS-activated monocytes, and odontoblasts, causes synthesis of extracellular matrix proteins such as collagens and integrins.[70] But their anti-inflammatory efforts in irreversible pulpitis may be too late to be beneficial.

Non-neuropeptide Modulators of Neurogenic Inflammation

Cytokine-neuropeptide interactions are bidirectional—that is, cytokines and other products of the immune cells can modulate the action, differentiation, and survival of neuronal cells, while neuropeptides released from neurons play pivotal roles in influencing the immune response.[26]

Cytokines and Suppressors of Cytokine Signaling: The pro- and anti-inflammatory actions of cytokines in inflammation are well known. Historically, LPS has been associated with potent activation of the inflammatory response through the induction of cytokine production. However, it was not until the identification of the Toll-like receptors (TLRs) that specific receptors were linked to this response. As sentinels of microbial invasion, TLRs are found at sites of host-microbe interaction. To date, a wide variety of cell types- including intestinal epithelial cells, dermal endothelial cells, and peripheral blood leukocytes- have been shown to express TLRs. It is currently thought that varied expression of TLRs together with differential responsiveness to TLR ligands allows for a specific type of cytokine response, depending on the unique microenvironment.[71] Signaling pathways from the TLRs ultimately regulate the activity of the genes that produce cytokines. Therefore, LPS acting through TLRs has an indirect effect on neurogenic inflammation by stimulating the production of pro-inflammatory cytokines, which are neuromodulators capable of directly influencing neuropeptides.[1]

LPS signaling via TLRs has been identified as a pivotal mechanism for cytokine production; however, less is known about the inactivation of such signal transduction pathways. Recently, a family of proteins, known as suppressors of cytokine signaling (SOCS), has been identified as inhibitors of the actions of cytokines.[72] It has been shown that LPS induces SOCS-1 and SOCS-3 expression, and it has been postulated that such expression can be induced after the triggering of TLR signal pathways as part of the innate immune response.[73] If further experimental data confirm that LPS activation of TLRs and, subsequently, of inflammatory cells is counter-regulated by LPS activation of SOCS, then this would suggest substantial cross-talk between signaling pathways within cells, highlighting the complex nature of the neuro immune regulatory responses to LPS.

Chemokines in Leukocyte Trafficking

The name chemokine is a contraction of chemotactic cytokine. Since IL-8 was identified as a leukocyte chemotactic factor two decades ago, more than 40 chemokines have been identified in humans.[74] Inflammatory chemokines are produced by activated leukocytes and tissue cells during inflammation and they, together with the upregulation of adhesion molecules, determine the composition of inflammatory infiltrates.[75] They recruit leukocytes to sites of infection by increasing the affinity of leukocyte integrins and stimulation of their migration extravascularly.[76] Chemokines not only direct the migration of neutrophils and monocytes but also attract immature DCs and activate effector and

memory lymphocytes during infection.[76] Chemokines were originally named after their functions, such as IL-8 and monocyte chemotactic protein-1 (MCP-1). A standard nomenclature based in part on their chemical structure was later developed, with "L" (for ligand) and the number of the respective gene, such as CXCL8 for IL-8 and CCL12 for MCP-1. Chemokines are divided into four groups (CC, CXC, C, and CX3C) based on the number and arrangement of conserved cysteine motifs.[77]

In deep caries samples, chemokine ligands such as monocyte chemotactic protein-1 (CCL2/MCP-1), macrophage inflammatory protein 3-alpha (CCL20/MIP-3□), and interleukin-8 (CXCL8/IL-8) are immuno histochemically localized mainly in macrophages.[78,79] CCL2/MCP-1, which is also produced by immature dendritic cells (DCs), endothelial cells, and lipoteichoic acid (LTA)- stimulated odontoblasts, recruits monocytes, immature DCs, memory T cells, and NK cells to amplify the inflammatory response.[80] The secretion of CCL20/MIP-3α by macrophages in the inflamed pulp may account for the recruitment of CCR6+ memory T cells, particularly Th2 cells and immature DCs in irreversible pulpitis.[61]

Adachi *et al* demonstrated that CXCL10 expression in inflamed dental pulp tissues was significantly increased compared with that in healthy dental pulp.[81] Immuno staining results revealed that CXCL10 was detected in macrophages, endothelial cells, and fibroblasts in inflamed dental pulp, and that CXCR3 expression was observed mainly on T-cells. Moreover, dental pulp fibroblasts had the capacity to produce CXCL10 in response to caries related bacteria and pro-inflammatory cytokines. Their findings suggested that CXCL10 may act as a key chemokine in the accumulation of activated lymphocytes in pulpitis and the CXCL10-CXCR3 system may be involved in the pathogenesis of pulpitis.

Takahashi et al concluded that CCL20 expression is induced by stimulation with caries-related bacteria that have invaded deeply into the dentinal tubules as well as by pro inflammatory cytokines in the inflamed pulpal lesions. It may be involved in the progression of pulpitis via accumulation of inflammatory cells.[82]

Future Directions

The diversity of nerve-pulp interactive systems and the high density of sensory innervation in teeth indicate a dominant role for dental innervation in pulpal biology and the response to dental injury. The vast evidence regarding neuropeptide interactions with normal pulp and inflammatory cells via receptors demands further research regarding potentially novel therapeutic approaches for management of pulpitis. Understanding of these complex regulatory systems, in particular that governing neuropeptide inactivation should extend our knowledge of the inflammatory process. Especially enzymes that inactivate neuropeptides are highly relevant targets for the development of inhibitors and could become candidates for drug development.

Topical SP or CGRP receptor antagonist may be used therapeutically in future in the management of inflamed pulp by blocking pulpal vasodilation. Because neuropeptides have been implied in the pain mechanism of the pulpodentin complex, the use of neuropeptide antagonists may serve as prototype for future class of analgesic drugs. Reduced hyperalgesia and nociceptive transmission after the administration of neuropeptide antagonists has already been demonstrated in animal studies.[83]

Further studies need to be conducted to assess and quantify the presence and distribution of VEGF and its receptors in the pulp of sound teeth and in teeth with active carious lesions. In this way, therapies may be instituted to modulate VEGF in the dental pulp by its expression and/or the expression of its receptors.

There is evidence that immature DCs are attracted into the odontoblast layer by TGF-ß1 originating from dentin.[84] This factor could thus direct DC trafficking in pathological conditions resulting from dentin injury. From a clinical point of view, the use of TGF-ß1 as a dentin/pulp-capping agent might represent a therapeutic strategy for modulating the early immune response and favoring healing in inflamed pulps, with the accumulation of immature DC at the pulp periphery further minimizing the risk of occurrence of a novel infection. Immunosuppressive mediators (IL-10, TGF-β and VEGF) can alter DC maturation to restore them to their immature phenotype to participate in tissue healing.[85] The expression of these mediators is elevated in irreversible pulpitis tissues.[70,86] Recent studies have demonstrated that immature DCs can capture tissue antigens from apoptotic cells and become migratory semimature DCs (tolerogenic DCs) that induce T regulatory cells to promote tolerance in draining lymph nodes.[87] It has been speculated that tolerogenic DCs can be induced in pulpitis to promote healing. Functional studies of the heterogenic dendritic cell population are necessary to elucidate its roles in pulpitis. Moreover approximately two-thirds of the known chemokines exhibit antimicrobial properties and the importance of this antimicrobial activity in early pulpitis is yet to be determined.[88] Better understanding of the intricate immune responses in the inflamed pulp and quantitative analysis of inflammatory cytokine and chemokine profiles in relation to microbes may result in more efficacious and predictable, immunology based vital pulp therapy in future.

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