CHAPTER II

REVIEW OF THE LITERATURE

The review is divided into four parts as follows:

2.1 The pulpodentin complex

2.1.1 Normal innervation in permanent and primary tooth pulp

2.1.2 Neural reactions to pulpal injuries

2.2 MMP-9 and its role in pulpal inflammation

2.3 VGSCs and their relation to pain sensation

2.4 Dental pain assessment in children

2.1 Pulpodentin complex

The dental pulp is surrounded by the dental hard tissue, which protects the dental pulp against pathogens and injury. Dental pulp is responsible for dentin elaboration. Dental pulp and dentin are often discussed together as a functional unit, the pulpodentin complex. Dental pulp contains blood vessels for nutrient supply and cellular recruitment, and nerve supply for dental sensitivity and defense response following dental injury, either from caries or trauma. Several studies have shown the important role of pulpal innervation in both defense and repair responses of the dental pulp (Byers and Taylor, 1993; Haug and Heyeraas, 2006; Khayat et al., 1988).

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2.1.1 Normal innervation in permanent and primary tooth pulp

Dental pulp is a liberally innervated tissue. The dental pulp innervation consists of sensory nerve fibers, sympathetic nerve fibers, and parasympathetic nerve fibers.

The sensory nerve fibers are the major component of the nerve supply in the dental pulp of both permanent and primary teeth. They originate from the trigeminal ganglion and peripherally pass through the apical foramen to innervate the dental pulp. At the coronal part of the dental pulp, they diverge, branch, and terminate as free nerve endings in the odontoblast layers, subodontoblastic plexus, predentin, the inner 0.1 mm of dentin and along blood vessels (Figure 2.1). There are three subgroups of sensory nerve fibers in dental pulp based on size, conduction velocity, and function. One subgroup, the A-β nerve fibers, which are medium-sized myelinated fibers, are the smallest population of sensory nerve fibers, responsible for sensitivity to mechanical stimuli such as hydrodynamic, percussion, and movement force. The others are the small myelinated A-δ and the unmyelinated C nerve fibers. Both A-δ and C fibers are classified as nociceptive fibers, which respond to noxious stimuli. Besides their role in sensitivity to external stimuli, the sensory nerve fibers are also involved in dentinal fluid dynamics, vasoregulation, and the protective reflex against dental injuries (Kim, 1990; Pashley, 1996; Rodd and Boissonade, 2003). They provide vitality to the dental pulp by interacting with other pulpal cells, such as odontoblasts, immunocompetent cells, and blood vessels. A previous study in rat models indicated the role of sensory nerve fibers in dental pulp tissue survival by showing that teeth with sensory denervation had greater loss of pulp tissue than those with innervation (Byers and Taylor, 1993).

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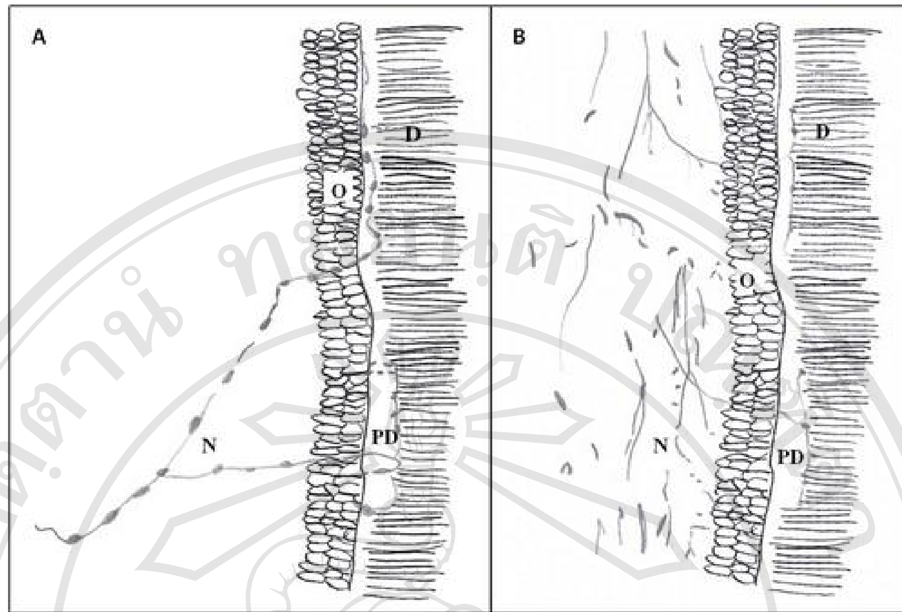

Figure 2.1 Innervation density in primary and permanent teeth. There is less innervation in the coronal pulp of primary (A) than permanent (B) human teeth (Modified from Egan et al., 1996; Rodd and Boissonade, 2001)

The sympathetic nerve fibers are sparse in dental pulp. They are located along the blood vessels in deeper pulp and are involved in vasoconstriction. The remaining component of pulpal innervation is parasympathetic nerve fibers, which play roles in the regulation of pulpal blood flow, but have been much less studied than the sensory and parasympathetic nerve fibers.

During maturation and aging, the dental pulp becomes narrower with the deposition of tertiary dentin and dead tracts, which normally have no innervation. With increasing loss of primary dentin, tooth innervation also decreases, as shown by the reduction in expression of neuropeptides and their receptors, which are normally expressed at nerve terminals, in the dental pulp (Byers et al., 1990a; Pashley, 1996). Previous studies showing the distribution of nerve fibers in dental pulp usually used

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PGP9.5, a soluble protein isolated from brains, as a marker of nerve fibers. The use of PGP9.5 staining appears to be reliable in reacting with nerve fibers in several studies with different techniques: immunohistochemistry (Renton et al., 2005), immunoblotting (Warren et al., 2008), immunocytochemistry (Rodd and Boissonade, 2000; 2002; Wells et al., 2007b) and immunofluorescence (Luo et al., 2008; Rodd and Boissonade, 2001; Wells et al., 2007b).

A remarkable difference between permanent and primary teeth are that primary teeth have less overall innervation density than permanent teeth (Johnsen and Johns, 1978; Rodd and Boissonade, 2001; 2002). Due to the rich sensory innervation and its prominent function in pain transmission, several investigators have hypothesized that the primary teeth have less sensitivity than the permanent teeth because of less sensory innervation in primary teeth (Rodd and Boissonade, 2001; 2002). Another difference in sensory innervation between primary and permanent teeth is that the distribution of the innervation within the crowns of primary teeth is highest in the cervical region, whereas the permanent teeth are most densely supplied in the pulpal horn dentin. Moreover, the roots of primary teeth are particularly innervated at the cervical ends, but the roots of permanent teeth are virtually uninnervated (Egan et al., 1996). Those authors also suggested that primary teeth are more sensitive at the cervical third than are permanent teeth.

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Physiologic root resorption of primary teeth is a normal phenomenon in dental development. The changes in pulpal environment during physiologic root resorption are still controversial. Sari and colleagues found no change in the histological structure of primary teeth with different levels of physiologic root resorption (Sari et al., 1999). Moreover, Monteiro and colleagues found no significant change in

numbers of immune cells in the dental pulp of primary teeth with higher degrees of root resorption and no change in the innervation density, although the quality of nerve fibers was affected by being more beaded, fragmented, and varicose (Monteiro et al., 2009).

2.1.2 Neural reaction to pulpal injuries

As stated earlier, besides their function in electrophysiological signal transmission, sensory nerve fibers are also involved in pulpal survival. The sensory nerve fibers variously react to dental injuries by anatomical and cytochemical changes depending on the level of injuries. Inflammatory mediators that are released during injury elaborate the local production of nerve growth factor (NGF) by fibroblasts near the injury site, and, therefore, result in extensive nerve sprouting (Byers et al., 1992). The sprouting causes an increase in potential sites of neuropeptides-containing fibers (Awawdeh et al., 2002; Caviedes-Bucheli et al., 2004; Khayat et al., 1988; Okiji et al., 1997; Rodd and Boissonade, 2000; 2002). Neuropeptides are synthesized proteins from neurons. They act as neurotransmitters, growth factors, hormones, vasoregulators, immunomodulators, and signaling molecules (Caviedes-Bucheli et al., 2008; Olgart and Kerezoudis, 1994; Rodd and Boissonade, 2003). These functions of neuropeptides contribute to pain transmission and neurogenic inflammation, which is a process of stimulus-induced neuropeptide release, change in vascular permeability, and the recruitment of immunocompetent cells (Caviedes-Bucheli et al., 2008). Neurogenic inflammation is part of the healing process, in order to maintain the vitality of dental pulp (Byers and Narhi, 1999; Byers and Narhi, 2002; Caviedes-Bucheli et al., 2008). Several studies have demonstrated the neurogenic inflammation

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occurring in the dental pulp following dental injury. For example, the sprouting of sensory (Byers et al., 2003; Rodd and Boissonade, 2002; Taylor et al., 1988) and sympathetic (Haug and Heyeraas, 2006) nerve fibers found in inflamed dental pulp indicates the increased neuropeptide releasing sites. Byers and colleagues (Byers et al., 1990b) demonstrated the correlation between various degrees of sensory nerve fiber sprouting and various degrees of injury to the dental pulp in rat models. In that study, mild injury from shallow cavities caused a temporary increase in the number of sensory nerve fibers, which subsided within 21 days. Deep cavities led to microabscess formation with more numerous branches of sensory nerve fibers sprouting underneath. The sprouting fibers took a longer time to subside than those occurring in shallow cavities, and there was reparative dentin substitution in the microabscesses. When the dental pulp was exposed, several defensive reactions, such as pulp polyps, coagulation necrosis, and liquefying necrosis, was found. The sprouting of sensory nerve fibers was found next to the borders of defensive reactions and congregate axons were found in the core of the surviving pulp.

Due to the increased number of potential sites of neuropeptide release following pulpal injuries and the role of neuropeptides in the pain mechanism, the sprouting of sensory nerve fibers following inflammation may alter cytochemical reactions in the dental pulp and contribute to the altered efficacy of local anesthesia.

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2.2 MMP-9 and its roles in pulpal inflammation

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases. The functions of MMPs are degradation of all extracellular matrix (ECM) components, as well as modification of several non-matrix substrates, such as

cytokines and chemokines (Chakraborti et al., 2003). MMPs are classified into six subgroups according to substrate specificities: collagenases, gelatinases, stromelysins, membrane-associated MMPs, matrilysins and other MMPs (Summarized information on MMPs is shown in Table 1). Several cell types can secrete MMPs in an inactive form, which are further activated outside the secreting cells, either by other enzymes in the same family or by other proteolytic enzymes (Woessner, 1991). The activity of MMPs is controlled by tissue inhibitors of matrix metalloproteinases (TIMPs), which are highly expressed during development (Visse and Nagase, 2003). Due to the ability of MMPs to degrade ECM, MMPs have been reported to be associated with ECM turnover in embryogenesis and several physiological activities such as tissue remodeling, angiogenesis, and cell migration (Amalinei et al., 2007). In addition, the imbalance between MMPs and their inhibitors, TIMPs, leads to tissue destruction that can be found in many diseases, such as metastasis of tumors (Deryugina and Quigley, 2006; John and Tuszynski, 2001), rheumatoid arthritis (Liu et al., 2004), atherothrombosis (Back et al., 2010), multiple sclerosis (Leppert et al., 2001), respiratory diseases (Lagente and Boichot, 2010) and inflammation of many organs (Manicone and McGuire, 2008). MMPs are involved in inflammatory processes in three ways (Manicone and McGuire, 2008). First, MMPs cause physiological breakdown by direct disintegration of the epithelium and endothelium. Second, the cleavage of MMPs by cytokines or chemokines can activate or inactivate the function of inflammatory mediators. Third, MMPs facilitate the recruitment of leukocytes to the site of infection by cleaving proteins that bind chemokines in ECM, consequently resulting in chemokine gradient formation, which

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leads to an influx of leukocytes from the low to the high part of the chemokine gradient.

In the oral environment, MMP activities, under regulation, are involved in the formation of enamel and dentin during the developmental stage, whereas an increase in MMP levels has been demonstrated in caries progression, periodontal disease, growth and invasion of oral tumors, and degradation of pulpal tissue during inflammation (Hannas et al., 2007). Despite sparse evidence of the role of MMPs in dental pulp tissue breakdown (Chang et al., 2002; Gusman et al., 2002; Shin et al., 2002), there is strong evidence that the expression of MMP-9 in the dental pulp of permanent teeth is significantly higher in inflamed dental pulp than in clinically healthy pulp using several techniques: gelatin zymography (Gusman et al., 2002), reverse-transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry (Tsai et al., 2005).

MMP-9, or geletinase B, is an enzyme in the gelatinase subgroup, which, like other MMPs, is released in the inactive proenzyme form. The activation process is also regulated by the inhibitor. MMP-9 substrates are found in the forms of both ECM substrates and cytokines, such as collagen, gelatin, elastin and interleukin (IL)-1β (as shown in Table 1). MMP-9 is produced by neutrophils (Pugin et al., 1999), monocytes (Menshikov et al., 2001), epithelial cells (Wilson et al., 2002), osteoclasts (Tezuka et al., 1994), and keratinocytes (Harvima, 2008). Several inducers are responsible for the induction of MMP-9 activation, for example, lipopolysaccharides (LPS) from bacteria, double-stranded ribonucleic acid (RNA) from viruses, plant products, and several cytokines (Opdenakker et al., 2001). MMP-9 levels are found to increase in correlation with other inflammatory cytokines, such as IL-1α, IL-1β and

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IL-8 (Hulejova et al., 2007; Shoshani et al., 2005), which are released in the early stage of inflammation in pathologic lesions, leading to the use of MMP-9 as a marker of the induction state of inflammation.

Although MMP-9 in the oral cavity has been found to be mostly secreted by neutrophils (Tsai et al., 2003), MMP-9 in inflamed dental pulp has also been identified in odontoblasts, fibroblasts, and endothelial cells. However, MMP-9 expression in clinically healthy pulp is much less detected than in inflamed dental pulp (Tsai et al., 2005). These findings suggest that MMP-9 may be reserved in dental pulp cells and can be released during the inflammatory process. Several cytokines and bacterial toxins have been found to induce the release of MMP-9 in pulp cells. Panagakos and colleagues found that IL-1β-, tumor necrosis factor (TNF)-α- and LPS-treated rat pulp cells had increased levels of MMP-9 within 24 hours, whereas there was no change in human pulp cells (Panagakos et al., 1996). However, during long-term culture (9-16 days), IL-1 can stimulate MMP-9 production in human pulp cells (O'Boskey and Panagakos, 1998). These results suggest dissimilar effects of MMP-9 inducers on the release of MMP-9 in different species and during different periods of time, and may be the supporting evidence of the role of MMP-9 in chronic inflammation of human permanent dental pulp.

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Table 2.1 Classification of MMPs (Modified from Chakraborti et al., 2003)

Group	Nomenclature		Examples of substrates	
	Enzyme	MMP	ECM	Non-ECM
Collagenases	Collagenase-1	MMP-1	Collagens, gelatin, peoteoglycan link protein, aggrecan, versican	α1-PI, IL-1β, pro-TNF, MMP-2, MMP-9
	Collagenase-2	MMP-8	Collagens, gelatin, aggrecan	α1-PI, α2-antiplasmin, fibronectin
	Collagenase-3	MMP-13	Collagens, gelatin, aggrecan, perican, fibronectin, osteonectin	MMP-9, plasminogen activator inhibitor-2
	Collagenase-4	MMP-18	ND	ND
Gelatinases	Gelatinase-A	MMP-2	Collagens, gelatin, elastin, fibronectin, laminin-1, laminin-5, galectin-3, aggrecan, proteoglycan link protein, osteonectin	IL-1β, α1-PI, MMP-1, MMP-9, MMP-13
	Gelatinase-B	MMP-9	Collagens, gelatin, elastin, galectin-3, aggrecan, fibronectin, proteoglycan link protein, entactin, osteonectin	IL-1β, α1-PI, plasminogen
Stromelysins	Stromelysin-1	MMP-3	Collagens, gelatin, aggrecan, versican, perlecan, decorin,fibronectin	α1-PI, antithrombin-III, substance P, IL-1β, MMP-1 superactivation, MMP-2/TIMP-2 complex, MMP-7, -8, -9, -13
	Stromelysin-2	MMP-10	Collagens, gelatin, casein, aggrecan, elastin	MMP-1, -8
	Stromelysin-3	MMP-11	Casein, laminin, fibronectin, gelatin, collagen IV	α1-PI

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Table 2.1 Classification of MMPs (modified from Chakraborti et al., 2003) (continued)

Group	Nomenclature		Examples of substrates	
	Enzyme	**MMP**	**ECM**	**Non ECM**
Membrane type MMPs	MT1-MMP	MMP-14	Collagens, casein, elastin, fibronectin	α1-PI, MMP-2, -13
	MT2-MMP	MMP-15	Large tenascin-C, fibronectin, laminin, entactin	MMP-2
	MT3-MMP	MMP-16	Collagen type III, gelatin, casein, fibronectin	MMP-2
	MT4-MMP	MMP-17	ND	ND
	MT5-MMP	MMP-24	ND	ND
	MT6-MMP	MMP-25	ND	ND
Matrilysins	Matrilysin	MMP-7	Collagen type IV and X, gelatin, aggrecan, decorin, proteoglycan link protein, fibronectin, laminin	MMP-1, -2, -9, MMP-9/TIMP-1 complex, α1-PI, plasminogen
	Matrilysin-2	MMP-26	Collagen type IV, gelatin, fibronectin	pro MMP-9, fibrinogen, α1-PI
Others	Macrophage elastase	MMP-12	Collagen type IV, gelatin, elastin, casein, laminin, proteoglycan monomer, fibronectin, vitronectin,enactin	α1-PI, fibrinogen, fibrin, plasminogen, myelin basic protein

α1-PI, α1-Proteinase inhibitor; IGFBP, Insulin-like growth factor binding protein; IL-1, Interleukin-1; TNF, Tumor necrosis factor; ND, Not determined

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2.3 VGSCs and their relation to pain sensation

VGSCs are the transmembrane selective filter complex composed of one large, continuous protein, the α-subunit, and one or two small proteins, β-subunits. The α-subunit, a 220-260 kDa polypeptide, is a functional part, which includes a voltage sensor, an ion pore, and an activation and an inactivation gate. The β-subunits modulate the functions of the α-subunits and stabilize them to the plasma membrane. Several excitable cells, such as neurons, myocytes, and some types of glial cells present VGSCs, which are responsible for depolarization of the action potential (Eder, 2005; Goodman, 2008). To function, VGSCs open within a millisecond in response to electrical change across the membrane to allow sodium ion influx, which causes increased neuronal membrane potential, and terminate within a very short period of time to occlude the sodium ion flow. The neurons then enter a repolarization stage by the allowance of potassium ion influx at the neuronal membrane before the VGSCs return to the resting state, in which they are available to reopen in response to a new wave of electrical change. Therefore, VGSCs contribute to the determination of neuronal excitability and also play a role in the propagation of nerve impulses. During injuries or inflammation, VGSCs are continuously activated, giving rise to unprovoked, spontaneous action potential, finally causing pain (Cummins et al., 2007).

In mammals, at least nine VGSC isoforms are categorized depending on genes that encode VGSC α-subunits. Each isoform differs in distribution and function, such as electrophysiological properties, pharmacological properties, and response to nerve injury and inflammation. Moreover, each one is associated with a variety of receptor molecules to regulate the excitability of nociceptors, so there are various processes of

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nerve impulse propagation depending on the presence of sodium channel α-subunit isoforms, for example, the variation in opening thresholds, opening time length, amount of inactivation time, and rate of isoform transition from the closed inactivated state to the resting close state (Amir et al., 2006).

VGSCs can be functionally classified, according to their sensibility to tetrodotoxin (TTX) blockage, into TTX-sensitive (TTX-S) and TTX-resistant (TTX-R) VGSCs. TTX-S VGSCs consist of $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, $Na_V1.4$, $Na_V1.5$, $Na_V1.6$, and $Na_V1.7$, whereas TTX-R VGSCs consist of $Na_V1.8$ and $Na_V1.9$. The characteristics of each VGSC α-subunit isoform are summarized in Table 2.

The sensory neurons in the dorsal root ganglion (DRG) and the trigeminal ganglion normally express both TTX-S and TTX-R sodium channels. The larger group of sensory neurons is mechanoreceptive containing rapid-inactivating TTX-S sodium channels, whereas the smaller group is nociceptive, expressing a mixture of rapidly-inactivating TTX-S and slowly-inactivating TTX-R sodium channels. The VGSC α-subunit isoform 1.8 ($Na_V1.8$) and VGSC α-subunit isoform 1.9 ($Na_V1.9$) are both TTX-R isoforms but they differ in specific distribution and function. $Na_V1.8$ is remarkably found in unmyelinated and small myelinated sensory neurons that have been identified as nociceptive neurons, whereas $Na_V1.9$ is found only in unmyelinated sensory neurons (Amaya et al., 2000). $Na_V1.8$ currents have slow activation and inactivation rates. The role of $Na_V1.8$ in electrogenesis is to determine the action potential of neurons due to its slow, depolarized voltage-dependent of activation. The steady-state voltage dependence of $Na_V1.8$ inactivation contributes to the continuous firing activity in the sustained depolarization state (Renganathan et al., 2001). $Na_V1.9$ currents are unique. They can be easily activated at a voltage near the resting

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membrane potential and can remain open for a longer time than those in $Na_V1.8$. Thus, $Na_V1.9$ generates persistent currents and contributes to the setting of activation thresholds (Amir et al., 2006; Cummins et al., 2007; Devor, 2006). Previous studies in rats, using oligodeoxynucleotides as antisense for $Na_V1.8$ (Joshi et al., 2006; Khasar et al., 1998), and a study in $Na_V1.8$-null mice (Akopian et al., 1999) have shown that $Na_V1.8$ plays a role in inflammatory pain and neuropathic pain. The role of $Na_V1.9$ channels in inflammatory pain is still controversial (Amaya et al., 2006; Coggeshall et al., 2004; Porreca et al., 1999; Strickland et al., 2008). However, $Na_V1.9$ is not involved in neuropathic pain (Dib-Hajj et al., 1998; Tate et al., 1998). Both $Na_V1.8$ and $Na_V1.9$ are believed to be involved in the prolonged duration of action potential in response to painful stimuli (Amir et al., 2006). Moreover, the inflammatory mediators present during inflammation potentiate the currents and excitability of TTX-R in primary sensory neurons, contribute to continuous neuronal hyperexcitability (Maingret et al., 2008), and finally cause increased pain sensitivity. The previous studies that have shown the upregulation of $Na_V1.8$ and $Na_V1.9$ in several peripheral inflammatory pain models (Amaya et al., 2006; Joshi et al., 2006; Strickland et al., 2008) suggest the potential role of these channels in inflammatory pain. Therefore, the blockers for $Na_V1.8$ and $Na_V1.9$ may be interesting targets for the treatment of peripheral inflammatory pain.

In dental pulp, several studies of pain sensation in human permanent teeth with irreversible pulpitis reveal an increase in expression of $Na_V1.7$ (Luo et al., 2008), $Na_V1.8$ (Renton et al., 2005; Warren et al., 2008), and $Na_V1.9$ (Wells et al., 2007a) compared to the dental pulp of nonpainful teeth. In addition, the expression of $Na_V1.8$ messenger RNA in rat models was found to be increased in relation to

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increased degrees of pulpal inflammation (Esmaeili et al., 2011). $Na_V1.6$, the TTX-sensitive sodium channel, has also been found in pulpal immune cells of rats (Byers et al., 2009), but its altered expression during pulpal inflammation has not been found (Luo et al., 2010), and its function in pulpal inflammation remains unclear. Although at least three α-subunit isoforms of sodium channel are associated with dental pain, this study focuses on only $Na_V1.8$ and $Na_V1.9$ because both sodium channel subtypes are found to be expressed in specific nociceptive neurons and have been shown to increase their expression in the dental pulp of human permanent teeth with symptomatic irreversible pulpitis (Cummins et al., 2007). Although there is some evidence of $Na_V1.8$ and $Na_V1.9$ expression in the dental pulp of human permanent teeth and their changes in expression following pulpal inflammation, the expression of $Na_V1.8$ and $Na_V1.9$ in the dental pulp of primary teeth in normal or inflammatory conditions has not been studied.

The compared sensitivity of TTX-R and TTX-S sodium channels to lidocaine, a nonspecific sodium channel blocker and a commonly used anesthetic agent in dentistry, is still argued. Scholz and colleagues reported that TTX-R channels are more resistant to lidocaine than TTX-S in rat models (Scholz et al., 1998). In contrast, other studies in rat models reported that TTX-R channels are more sensitive to lidocaine than TTX-S sodium channels (Chevrier et al., 2004; Leffler et al., 2007). However, the properties of $Na_V1.8$ and $Na_V1.9$ and the increased expression of these channels in inflamed dental pulp may be the cause of lidocaine failure in dental treatment. Therefore, $Na_V1.8$ and $Na_V1.9$ are suggested as potential targets for novel treatment of pain in pulpal inflammation and novel anesthetics in the treatment of painful pulpitis (Renton et al., 2005; Warren et al., 2008; Wells et al., 2007a).

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Table 2.2 VGSC α-subunit isoforms and their properties (Amir et al., 2006; Cummins et al., 2007)

α-subunit isoform	Site of expression	Inactivation rate	Sensitivity to blockage by TTX
$Na_V1.1$	CNS and DRG sensory neurons	Fast	Sensitive
$Na_V1.2$	CNS neurons	Fast	Sensitive
$Na_V1.3$	Immature neurons	Fast	Sensitive
$Na_V1.4$	Skeletal muscle	Fast	Sensitive
$Na_V1.5$	Cardiac muscle	Slow	Intermediate sensitive
$Na_V1.6$	CNS and DRG sensory neurons	Fast	Sensitive
$Na_V1.7$	DRG sensory neurons and sympathetic ganglia	Fast	Sensitive
$Na_V1.8$	DRG sensory neurons	Slow, steady-state voltage dependent	Resistant
$Na_V1.9$	Small DRG sensory neurons and trigeminal ganglia	Very slow (Persistent)	Resistant

CNS, Central nervous system; DRG, Dorsal root gnagloin

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2.4 Dental pain assessment in children

According to the International Association for the Study of Pain (IASP), pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Classification of chronic pain 1994). Pain sensation works as a protective mechanism to warn of danger.

Both injuries to and inflammation of pulpal tissue can cause dental pain. Injuries to dental pulp, whether from physical or mechanical stimuli, cause changes in homeostasis of dentinal fluid flow in dentinal tubules and consequently lead to the activation of nociceptors. On the other hand, the inflammatory mediators that are released following pulpal inflammation act as the chemical stimuli to nociceptors. Despite differences in mechanisms for stimulating nociceptive nerve fibers in dental pulp, both dental injuries and pulpal inflammation result in nerve sprouting and increased synthesis of neuropeptides involved in pain transmission, such as calcitonin gene-related peptide (CGRP) and substance P (SP) (Awawdeh et al., 2002; Byers et al., 1990b; Rodd and Boissonade, 2000). Furthermore, the stimulative role of inflammatory mediators in TTX-R currents and excitability (England et al., 1996; Kwong and Lee, 2005) and the upregulation of $Na_V1.8$ and $Na_V1.9$ found during inflammation (Renton et al., 2005; Wells et al., 2007a) may contribute to the pathophysiology of dental pain. Nociceptive signals propagate via trigeminal nerves to the trigeminal nucleus in the medulla. Then, these inputs are transmitted to the other regions in the higher levels of the central nervous system, and finally are translated to an increased pain sensation. However, interdentition difference in the

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overall innervation density may suggest less pain sensation in primary teeth than in permanent teeth.

Pain assessment is a complex process to obtain quantitative information about pain and its effects on the person. The examination and evaluation of pain sensation can be accomplished by taking the patient's history, by performing a systems review and by the use of pain measurement tools, such as the visual analogue scale (VAS) and the McGill Pain Questionnaire (O'Rourke, 2004).

Although many tools are often used to measure pain severity in children, the VAS is the most commonly used tool. The VAS is a line, 10 centimeters long, with the number 0 at its left extremity and the number 10 at its right extremity, that is used to measure a characteristic or attitude that is believed to range across a continuum of values and cannot easily be directly measured. For pain assessment, the number 0 refers to no pain while the number 10 refers to an extreme amount of pain (as shown in Figure 2.2). The use of the VAS requires the cognition to understand serial order and to translate it into a distance measure. According to Piaget's developmental stage of intelligence, the stage at which children can perform serial ordering operations and are able to generalize along a linear dimension is the concrete operational stage, which occurs by the age of seven to 11 years (Bee and Boyd, 2007). Consequently, the VAS may not be useful for measuring pain intensity in children under seven years of age (Shields et al., 2003a; Shields et al., 2003b), but it is widely used to assess pain severity in both adults and older children. However, cognitive ability is not correlated with chronological age in all children. O'Rourke reported that the VAS can be used with children five years and older with excellent validity and reliability (O'Rourke, 2004). Excluding the consideration of age, the VAS is a powerful tool to quantify

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pain magnitude because it is more informative and more sensitive to changes pain sensation than are face scales (Powell et al., 2001).

Figure 2.2 Visual Analog Scale (VAS). VAS is a line of 10 centimeters long. The number 0 on the left represents no pain and number 10 on the right represents the worst pain that patient can imagine.

Face scales are another popular method for pain severity measurement, commonly used with children, consisting of a series of faces that express discriminate degrees of pain intensity, from smiling to crying (as shown in Figure 2.3). Face scales are handled by matching the face that corresponds most closely to the child's feeling. Thus, they are assumed to be easier to use than the VAS because less cognitive ability is needed. The Wong-Baker Faces Pain Scale (WBFPS) is one of the face scales that have been shown to be valid and reliable in pediatric settings. It is a scale with six faces representing different levels of pain with measures of 0, 2, 4, 6, 8, and 10 (as shown in Figure 2.3A). Garra and colleagues (Garra et al., 2010) found that both the VAS and the WBFPS had excellent agreement when used in pediatric emergency patients. The same finding was found in Thai children aged four years and older (Newman et al., 2005).

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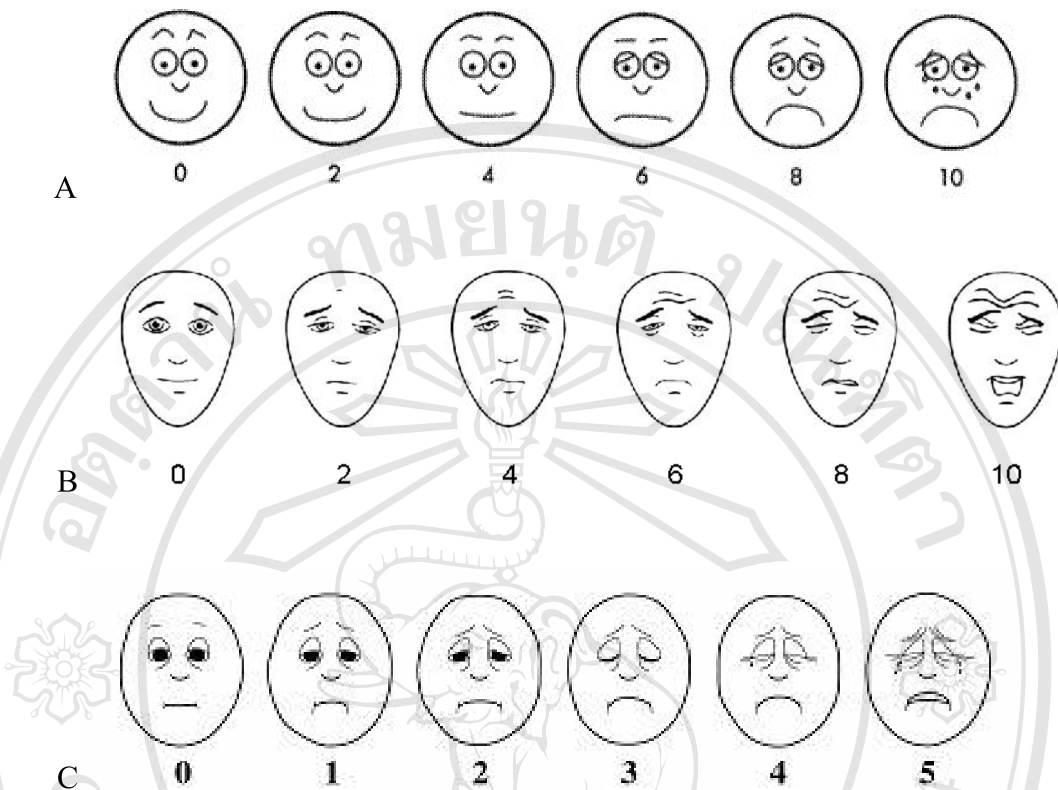

Figure 2.3 A variety of facial pain scales. (A) Wong-Baker Faces Pain Scale (WBFPS) (Garra et al., 2010) (B) Faces Pain Scale-Revised (FPS-R) (Hicks et al., 2001) (C) University of Wisconsin Children's Hospital Pain Face Scale (Soetenga et al., 1999). In this study, WBFPS was used to determine the pain intensity because of its simplicity and widely acceptance.

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