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Review article

Expression of sodium channels in dental pulp

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***Background*:** Several isoforms of voltage-gated sodium channels (VGSCs) found in peripheral nerves is associated in the pathogenesis of neuropathic and inflammatory pain. Until now, there are few studies of the distribution of VGSCs in dental pulp and its relationship to dental pain.
***Objective*:** Perform literature review to provide update information of VGSCs in dental pulp.
Methods: We reviewed and discussed seventy-eight articles listed in MEDLINE (PubMed) database using keywords including "sodium channels" and "dental pain". They are articles published in English from 1978 to 2010.
***Results*:** Although several VGSCs isoforms are distributed in dental pulp, only $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ have been found to be upregulated in painful pulpitis.
***Conclusion*:** $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ seem to have key roles in inflammatory dental pain. As a result, they might be the targets to treat dental pulp inflammation.

Keywords: Dental pulp, expression, inflammation, sodium channels

List of abbreviations

CGRP	Calcitonin gene related peptide
CNS	Central nervous system
DRG	Dorsal root ganglion
ENaC	Epithelial sodium channel
NKA	Neurokinin A
PGP9.5	Protein gene product 9.5
PNS	Peripheral nervous system
SP	Substance P
TTX	Tetrodotoxin
TTX-R	Tetrodotoxin-resistant voltage-gated sodium channel
TTX-S	Tetrodotoxin-sensitive sodium channel
VGSCs	Voltage-gated sodium channels

Our review aims to focus on recent information regarding sodium channels, which are related to dental pain from both primary and permanent teeth, information which has not yet been thoroughly reviewed. We also include information on a variety of fields of the pulpodentin complex, particularly the field of neural reaction to pulpal injury. Therefore, our review is divided into three parts as follows,

1. The pulpodentin complex
   - 1.1 Innervation in permanent and primary tooth pulp
   - 1.2 Sensory neuropeptides in dental pulp
   - 1.3 Neural reactions to pulpal injuries
2. The expression of sodium channels in dental pulp
3. The expression of sodium channels related to dental pain

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1. The pulpodentin complex

The dental pulp is surrounded by the dental hard tissues, which form a physical barrier against pathogens and injury. The dental pulp and dentin are often discussed together as one functional unit, the pulpodentin complex. Dental pulp is responsible for dentin formation. The permeable properties of dentin regulate the diffusion rate of irritants that can initiate pulpal inflammation. Dental pulp contains a dense vascularity and nerve supply. The blood vessels in pulpal tissue are for nutrient supply and cellular recruitment, while the nerves in pulpal tissue are for

dental sensitivity and defense response following pulpal injury, from either dental caries or trauma. The dental pulp has a low capacity for defense or repair responses because of the lack of an adequate blood supply and cellular recruitment following dental injury [1]. Several studies have shown that pulpal innervation plays an important role in both defense and repair responses [2-4]. Therefore, this review article focuses on pulpal innervation in the response to pulpal injury.

1.1 Normal innervation in primary and permanent tooth pulp

The pulpodentin complex in both primary and permanent teeth is extremely rich in innervation [5], and the innervation influences the defense reactions in the connective tissue of the dental pulp. This innervation consists of sensory, sympathetic, and parasympathetic nerve fibers.

The sensory nerve fibers are the major innervation in the dental pulp of both primary and permanent teeth. They originate from the trigeminal ganglion, and peripherally pass through the apical foramen to innervate the coronal pulp. Into the coronal pulp, they diverge, branch, and terminate as free nerve endings in the odontoblast layers, sub-odontoblastic plexus, predentin, in the inner 0.1 mm of dentin, or along blood vessels, as shown in Byers's study [6]. After stimulation, sensory nerve fibers transmit signals back via the trigeminal nerves to the trigeminal ganglion. The signals from trigeminal ganglion provide input through the spinal trigeminal tract to the spinal trigeminal nucleus and then, these signals pass through the spinothalamic tract to terminate in the somatosensory cortex of brain. There are three subgroups of sensory nerve fibers in dental pulp. They are based on size, conduction velocity, and function. First, the A-β nerve fibers are medium-sized myelinated fibers. They comprise the smallest population of sensory nerve fibers and are sensitive to mechanical stimuli such as hydrodynamic, percussion, and movement force. Second, it is the small myelinated A-□ nerve fibers. Finally, the largest population is the unmyelinated, slow conducting C fibers. Both A-□ and C fibers are classified as nociceptive, which respond to noxious stimuli. The sensory nerve fibers are also involved in dentinal fluid dynamics, vasoregulation, and protective reflexes against dental injuries [7-9]. They provide the vitality of the dental pulp by interacting with other pulpal components, such as odontoblasts, immunocompetent cells, and blood vessels. A previous study in the rat model indicated that the sensory nerve fibers in dental pulp play an important role in the survival of pulpal tissue. In that study, the authors demonstrated that teeth with sensory denervation had greater loss of pulpal tissue than those with innervation [4].

Sympathetic nerve fibers are sparse in the dental pulp of both primary and permanent teeth. They originate in the superior cervical ganglion, are located along the blood vessels in the deeper pulp, and are involved in vasoconstriction.

The parasympathetic nerve fibers play roles in the regulation of pulpal blood flow but are much less important than either the sensory or the sympathetic fibers [10].

During maturation and aging in permanent teeth, dental pulp chamber becomes narrower with the deposition of tertiary dentin and dead tracts, which are normally not innervated. With increasing loss of primary dentin, tooth innervation decreases, as shown by the reduction in expression of neuropeptides and their receptors in the dental pulp [9, 11]. Several studies have shown the distribution of nerve fibers in dental pulp by using the expression of protein gene product 9.5 (PGP9.5), a soluble protein isolated from brains, as a marker of nerve fibers. PGP9.5 staining appears to be reliable in reacting with nerve fibers, in several studies using different techniques: immunohistochemistry [12], immunoblotting [13], immunocytochemistry [14-16], and immunofluorescence [5, 16, 17].

The sensory innervation of permanent teeth is greater than that of primary teeth [5, 14, 18]. Due to the prominent function of sensory nerve fibers in pain transmission, several investigators have hypothesized that the primary teeth have less sensitivity than the permanent teeth. This is because the primary teeth have less sensory innervation. However, another study revealed different results in sensory innervation between primary and permanent teeth [19]. In that study, the sensory nerve supply in human primary teeth differs from that in permanent teeth in two ways. First, the distribution of the innervation within the crowns of primary teeth was highest cervically, while the permanent teeth were densely supplied in the pulpal horn. Second, the primary teeth were particularly innervated at the cervical ends of the roots, but the roots of permanent dentin were virtually uninnervated. In addition, physiologic root resorption does not affect the histological structure [20] or overall innervation [21] of primary teeth.

1.2 Sensory neuropeptides in dental pulp

The sensory nerve fibers in dental pulp are afferent fibers involved predominantly in dental pain perception. The terminals of sensory nerve fibers contain neuropeptides, synthesized neurotransmitter proteins from neurons. These peptidergic neurons are associated with neurogenic inflammation, caused by extreme stimuli, such as dental caries, drilling, probing of the exposed dentin, or percussion of the teeth, in order to maintain the vitality of dental pulp [22]. Dynamic changes in peptidergic neurons occur during inflammation by extensive nerve fiber sprouting. These sprouting result in an increased number of potential sites of neuropeptide-containing fibers and, consequently, an increased quantity of neuropeptide release [3, 14, 15, 23-25]. Neuropeptides cannot cross cell membranes, so they trigger biological effects by activating their receptors located on the plasma membrane of the target cells and they are rapidly degraded by the enzymes in pulpal tissue after exerting the effects [26]. The functions of sensory neuropeptides are multiple and varied. They can act as neurotransmitters, growth factors, hormones, vasoregulators, and immune system signaling molecules. It is known that neuropeptides contribute to promoting neurogenic inflammation to the control of pulpal blood flow and to the pain mechanisms of the pulpodentin complex [10]. Several studies demonstrated that neuropeptides could modulate vascular smooth muscles, increase vascular permeability, and modulate the immune system [8, 10, 27]. The sensory neuropeptides in primary and permanent tooth pulp consist of calcitonin gene-related peptides (CGRP), substance P (SP) and neurokinin A (NKA) [10, 28]. The origin, localization, stimulation, and biological effects of sensory neuropeptides in dental pulp are summarized in **Table 1**.

Table 1. Summary of sensory neuropeptides, receptors, and their functions (modified from [26])

Neuropeptide	Origin	Localization	Stimulus for release	Biologic effect
Calcitonin gene-related peptide	Trigeminal ganglion	C and Aδ fibers	- Thermal - Mechanical - Chemical - Electrical - Caries - Capsaicin - Inflammatory mediators - Bradykinin - Prostaglandins	- Vasodilation - Plasma extravasation - Chemotaxis - T lymphocyte suppression - Hard tissue formation - Repair - Mitogen for odontoblasts - Pain - Resorption control
Substance P	Trigeminal ganglion	C and A□ fibers	- Thermal - Mechanical - Chemical - Electrical - Caries - Capsaicin - Inflammatory mediators - Bradykinin - Prostaglandins	- Vasodilation - Plasma extravasation - Immune system stimulation - Chemotaxis - Enhances macrophages activity - Hard tissue formation - Tissue reparation - Mitogen for T lymphocyte
Neurokinin A	Trigeminal ganglion	C and A□ fibers	- Thermal - Mechanical - Chemical - Electrical - Caries - Capsaicin - Inflammatory mediators - Bradykinin - Prostaglandins	- Vasodilation - Plasma extravasation - Chemotaxis - Pain

1.3 Neural reactions to pulpal injuries

When dental pulp is injured, the injury activates nerve fibers to induce neurogenic inflammation. That is a process of stimuli-induced neuropeptide release, change in vascular permeability, and the recruitment of immunocompetent cells. The neurogenic inflammation can lead to the healing process [10, 29]. Several studies have demonstrated the neurogenic inflammation occurring in the dental pulp following dental injury. For example, sensory [14, 30, 31] and sympathetic [2] nerve fiber sprouting were found in inflamed dental pulp. Byers and colleagues [32] demonstrated that the variable degrees of sensory nerve fiber sprouting is correlated with various degrees of pulpal injury in the rat model. In their study, a mild injury, e.g. shallow cavities, caused an increase in CGRP-immunoreactive fibers, and those sprouting CGRP-nerve fibers subsided within 21 days. The deeper cavities caused more injury to the dental pulp and led to microabscess formation, with more numerous branches of sensory nerve fibers sprouting underneath. The sprouting fibers took a longer time to subside and reparative dentin was substituted in the microabscesses. When the dental pulp was exposed, three defensive reactions could be found, pulp polyps, coagulation necrosis and liquefying necrosis. In those severe pulpal injuries, the CGRP-immunoreactive fibers were found sprouting adjacent to the borders of defensive reactions and the axons were found to assemble in the core of surviving pulp. As we have mentioned before, due to the increased number of potential sites of neuropeptide release and the role of sensory neuropeptides in pain transmission, 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.

2. The expression of sodium channels in dental pulp

Voltage-gated sodium channels (VGSCs) are complex transmembrane pores that are responsible for depolarization of the membrane potential, or the rising phase of the action potential in the membrane. They are found in excitable cells, such as neurons, myocytes [33], and some types of glial cells [34]. VGSCs open within a millisecond in response to electrical change across the membrane to allow sodium ion influx. This causes the increased neuronal membrane potential. Then, they terminate very fast to occlude the sodium ion flow. The neurons enter a repolarization stage by the allowance of potassium ion influx at the neuronal membrane. After closing, VGSCs return to the resting state and are available to reopen in response to new waves of electrical change. Therefore, VGSCs contribute to the determination of neuronal excitability and play a role in the propagation of nerve impulses. During injuries or inflammation, VGSCs in primary sensory neurons are continuously activated and the continuous activation of VGSCs gives rise to an unprovoked, spontaneous action potential, that finally causes continuous pain [35].

The sodium channel is a selective filter composed of one large, continuous protein, the β-subunit, and one or two smaller proteins, the β-subunits. The β-subunit, a 220-260 kD polypeptide, is a functional part of the sodium ion channel, and contains a voltage sensor, an ion pore, and activation and inactivation gates. The β-subunits modulate the functions of the α-subunits and stabilize them to the plasma membrane. In mammals, nine genes have been identified to encode VGSC β-s ubunits into nine isoforms, depending on amino acid sequence homology and genetic location. These isoforms include $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, $Na_V1.4$, $Na_V1.5$, $Na_V1.6$, $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$. Each isoform differs in function, such as tissue distribution, electrophysiological properties, pharmacological properties, and response to nerve injury and inflammation. Moreover, different isoforms aggregate to form a variety of macromolecules and to regulate the excitability of nociceptors. Therefore, there are diversified processes of nerve impulse propagation such as variation in opening thresholds, opening time length, amount of inactivation time, rate of isoform transition from the closed inactivated state to the resting, or closed state, depending on the presence of sodium channel α-subunit isoforms [36].

VGSCs can be functionally classified depending on the criteria used, as shown in **Table 2**, and the properties of each VGSC α-subunit isoform are summarized in **Table 3**.

In physiological, rather than pathological, conditions, the sensory neurons in the dorsal root ganglion (DRG) and trigeminal ganglion express both TTX-sensitive (TTX-S) and TTX-resistant (TTX-R) sodium channels. The population of sensory neurons is primarily mechanoreceptive, expressing rapidly-inactivating TTX-S sodium channels, with a small proportion being nociceptive, expressing a mixture of

Table 2. Classification of VGSCs depending on function [36]

Criteria	Classification of VGSCs
Threshold of activation	- Low threshold - High threshold
Rate of activation	- Fast activation - Slow activation
Rate of inactivation	- Fast inactivation - Slow inactivation
Sensitivity to tetrodotoxin (TTX), which is a toxin found in the liver of puffer fish	- TTX-sensitive (TTX-S) - TTX-resistant (TTX-R)

Table 3. Voltage-gated sodium channel □-subunit isoforms and their properties [35, 36]

α-subunit isoform	Site of expression	Inactivation rate	Sensitivity to blockade 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	Intermediately 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	Resistant
$Na_V1.9$	Small DRG sensory neurons and trigeminal ganglia	Very slow (persistent)	Resistant

rapidly-inactivating TTX-S and slowly-inactivating TTX-R sodium channels. Details of studies of the expression of sodium channels in normal dental pulp are described in **Table 4**.

During the inflammatory process, inflammatory mediators can lower the threshold of activation and increase the excitability of TTX-R in primary sensory neurons, contributing to neuronal hyperexcitability [37]. Moreover, several studies have shown the alteration in the expression of both TTX-S and TTX-R VGSCs in inflamed peripheral tissues [36, 38]. These changes may lead to increased pain states.

The rapidly inactivating, TTX-S sodium currents have been detected in cultured human dental pulp cells [39]. Davidson suggested that the main source of these sodium currents is neuronal satellite cells, not odontogenic cells, because the odontoblastic processes firmly embed the odontoblasts to the dentin and do not allow these cells to be explanted. On the other hand, an *in vitro* study of Allard and colleagues [40] found that odontoblasts expressed voltage-gated TTX-S currents which have the ability to generate action potential, but TTX-R sodium currents have not been detected.

Henry and colleagues [41] found no change in overall sodium channel expression in painful human dental pulp. However, they found that the quantity of atypical nodal sites and the expression of sodium channels at such sites were increased but the quantity of typical nodal sites and the accumulating sodium channels at those sites were decreased. That study showed that inflammation caused the demyelinating process and the remodeling of the pattern of sodium channel accumulation. Several studies supported the study of Henry and colleagues [41], revealing, for example, an increase in the expression of $Na_V1.7$ [17], $Na_V1.8$ [12, 13] and $Na_V1.9$ [42] in permanent human dental pulp with irreversible pulpitis compared to permanent dental pulp of non-painful teeth. $Na_V1.6$ has also been found in the dental pulp of both humans and rats [43], but its function in pulpal inflammation remains unclear. The expression of multiple VGSC

isoforms in inflamed dental pulp suggests the collaborative roles of various VGSC isoforms in generating spontaneous action potential, leading to pulpal pain.

$Na_V1.1$, $Na_V1.2$, $Na_V1.3$, $Na_V1.4$, and $Na_V1.5$ have not been evidenced in dental pulp. $Na_V1.1$ and $Na_V1.2$ are predominantly expressed in adult central nervous system (CNS) neurons, in combination with $Na_V1.6$. In contrast, the expression of $Na_V1.3$ is particular in immature neurons. $Na_V1.4$ has been seen in skeletal muscle, while $Na_V1.5$ has been remarkably found in cardiac muscle [35]. Not only VGSCs isoforms, but also epithelial sodium channels, which are non-VGSCs, have been found in dental pulp [44]. The expression of each sodium channel isoform in permanent dental pulp is shown in **Table 4**.

3. The expression of sodium channels related to dental pain

$Na_V1.6$ is a TTX-sensitive VGSC isoform, remarkably expressed at the nodes of Ranvier within the myelinated PNS and CNS neurons [45] and also expressed along unmyelinated neurons of the PNS [46] and CNS [45]. Its function has been suggested to be an electrical conduction in both myelinated and unmyelinated axons [45, 46] but the role in nociception is obscure. The expression of $Na_V1.6$ in human permanent tooth pulp has been reported in the study of Luo and colleagues [47] using immunocytochemistry, in which there was no significant difference in the expression of $Na_V1.6$ in normal and painful pulp, despite an increase in the proportion of atypical nodes of Ranvier and a decrease in typical nodal sites in painful pulp. Another study of $Na_V1.6$ in dental pulp, a study in rats, using immunohistochemistry and double immuno-fluorescence [43], found that $Na_V1.6$ was expressed in non-neuronal cells, such as pulpal immune cells, dendritic pulpal cells, and odontoblasts. That finding suggests that $Na_V1.6$ play a role in those cells. Furthermore, it might be implicated in neuro-immune interactions. In contrast to the study of Luo and colleagues [47], pulpal tissue of injured rat teeth in Byers and colleagues' study [43] showed an increase in $Na_V1.6$ immunoreactive cells, predominantly around the injured pulpal tissue and dilated blood vessels. The increased expression of $Na_V1.6$ in non-neuronal dental pulp cells of injured rats [47], despite the unchanged expression of $Na_V1.6$ at the nodes of Ranvier in human inflammatory pulp [43], may reflect the different

Table 4. Sodium channel expression in normal dental pulp

In vitro/In vivo	**Models**	**Major findings**	**References**
In vitro Whole-cell, patch-clamp methods	Human dental pulp cells	Detection of TTX-S current in human dental pulp cells	Davidson, 1994 [39]
In vitro Immunohistochemistry	Human dental pulp	Expression of NaV1.8 immunoreactive nerve fibers in the sub-odontoblastic layers of dental pulp	Renton, et al 2005 [11]
In vitro Immunocytochemistry	Human dental pulp	Expression of NaV1.8 in 16.5% of nodes of Ranvier in radicular tooth pulp	Henry, et al 2005 [61]
In vitro Immuno-electron microscopic methods	Rat dental pulp	Expression of □ and □ENaC in mechanoreceptive myelinated nerve fibers	Ichikawa, et al 2005 [44]
In vitro Immunohistochemistry	Rat dental pulp	Expression of NaV1.9 in unmyelinated nerve fibers and suggested role of NaV1.9 in thermal pain stimuli	Padilla, et al 2007 [62]
In vitro immunohistochemistry	Rat dental pulp	Expression of NaV1.6 in dental pulp cells and odontoblasts	Byers et al, 2009 [43]
In vitro immunocytochemistry	Human dental pulp	Prominent expression of NaV1.6 at nodes of Ranvier, particularly at typical nodal sites	Luo et al, 2010 [47]

function of $Na_V1.6$ in different cell types. However, the difference in the expression and response mechanism of $Na_V1.6$ in various species and different types of pulpal tissue damage should not be ignored.

$Na_V1.7$ is a TTX-sensitive VGSC isoform that has been widely studied. It has been identified in the sympathetic neurons and small and medium sized sensory neurons of the DRG, including nociceptive neurons. $Na_V1.7$ is rapidly activated, rapidly inactivated and slowly recovers from fast activation, so it plays an important role in setting the threshold for the generation of action potentials in peripheral nociceptive neurons [35]. $Na_V1.7$ is markedly involved in perceiving pain sensations, as evidenced in patients with the loss-of-function mutation in the SCN9A gene, a gene that encodes $Na_V1.7$, or meaning that those who have loss of $Na_V1.7$ function are unable to experience pain [48, 49]. In addition, patients with congenital pain syndrome, who have an alteration in $Na_V1.7$ function, have increased pain sensitivity associated with edema, redness, and warmth, suggesting the role of $Na_V1.7$ in chronic inflammatory pain [50]. In the dental pulp of human permanent teeth, the upregulation of $Na_V1.7$ expression has also been reported in painful pulpitis studied using either immunohistochemistry [51], or immunocytochemistry [17], demonstrating the increased expression of the $Na_V1.7$ isoform at both typical and atypical nodal sites.

The VGSC α-subunit isoform 1.8 ($Na_V1.8$) and VGSC α-subunit isoform 1.9 ($Na_V1.9$), the slower TTX-R components, are remarkably found in small unmyelinated sensory neurons that have been identified as nociceptive neurons [36]. $Na_V1.8$ has a high activation threshold, slow inactivation kinetics and contributes to the electrogenesis of an action potential in C-type peripheral neurons of mice [52]. $Na_V1.9$ is activated at potentials near resting membrane potential and generates relatively persistent current [53]. Both TTX-R isoforms, $Na_V1.8$ and $Na_V1.9$, are believed to be involved in the prolonged duration of the action potential in response to painful stimuli and have been found to upregulate during inflammatory pain in rat [38, 54] and mouse [55] models. Therefore, both sodium channel isoforms might be new targets for treatment of inflammatory pain. The different properties of $Na_V1.8$ and $Na_V1.9$ are as follows. $Na_V1.8$ currents have a slow activation and inactivation rate. The slower inactivation rate of $Na_V1.8$ compared to those of other isoforms prolongs the action potential of neurons and may cause chronic pain. The steady-state voltage dependence of inactivation contributes to generating an action potential even in the depolarized state. $Na_V1.9$ currents are unique. They can be activated at voltages near the resting membrane potential. Furthermore, they can generate persistent currents. $Na_V1.9$ can be easily activated. It can contribute to the setting of the threshold of activation. Finally, it can remain opening for a longer time than $Na_V1.8$ [36, 56]. Previous studies in rats, using oligodeoxynucleotides as antisense for $Na_V1.8$ [55, 57] and a study in $Na_V1.8$-null mice have shown that $Na_V1.8$ plays a role in inflammatory pain and neuropathic pain [58]. $Na_V1.9$ channels also have a role in inflammatory pain, but not in neuropathic pain [59, 60].

Localization of $Na_V1.8$ in human teeth with painful pulpitis has been investigated using immunohistochemistry [12]. It has been found that $Na_V1.8$-immunoreactive nerve fibers were localized in the sub-odontoblastic layer of both healthy and inflamed pulp tissue. However, the detection of $Na_V1.8$-immunoreactive fibers was much greater in the inflamed dental pulp. Moreover, the upregulation of $Na_V1.8$ has been reported using the immmunoblotting method in inflamed human permanent tooth pulp compared to healthy pulp [13]. An immunocytochemical study has revealed that not only the predominant $Na_V1.6$, but also $Na_V1.8$ has presented at the nodes of Ranvier in the radicular part of healthy human permanent tooth pulp [61]. This finding suggests the coexistence of multiple sodium channel isoforms in those areas where the levels of expression may change during the inflammatory period and may contribute to an increased pain status.

For $Na_V1.9$, an investigation in rats has revealed the innervation of $Na_V1.9$-immunoreactive fibers in the lip skin and in the dental pulp of non-painful teeth, suggesting the role of this VGSC isoform in orofacial pain [62]. As well as the other sodium channel mentioned above, the immunocytochemical method has reported the increased expression of $Na_V1.9$ in the axons of symptomatic pulpitis of human permanent teeth [42].

Epithelial sodium channel (ENaC) protein is a member of the degenerins family (DEG), which is a large protein family of diverse functions, such as sodium ion transport, acid sensation, proprioception, and mechanosensation [63]. Differing from VGSCs, which consist of α- and β- subunits, ENaC consists of four subunits: α, β, γ and subunits [64]. Only

α, β and γ subunits of ENaC have been indicated in mechanoreceptors in the trigeminal ganglion of rat models with a possible function in mechanotransduction [65]. βENaC has been identified in the terminal Schwann cells associated with the periodontal Ruffini endings in the periodontal ligament of rat incisors and is believed to be a key molecule for mechanosensation in mastication [66]. ENaC has also been found in rat dental pulp tissue, by using immunohistochemistry [44]. In that study, the βENaC and γENaC-immunoreactive fibers have appeared in trigeminal ganglion neurons, periodontal ligament, and deep layer of oral mucosa, inferior alveolar nerve fibers, radicular pulp, and sub-odontoblastic plexus of rat molars pulp tissue. γENaC in dental pulp was mostly around myelinated nerve fibers, which are sensitive to mechanical stimuli, whereas it was mostly absent around unmyelinated nociceptive axons.

Those studies of changes in sodium channel expression within painful dental pulp are summarized in **Table 5**.

There have been attempts to discover new substances to act as sodium channel blockers for the treatment of both neuropathic and inflammatory pain. Lidocaine, a commonly used anesthetic, is a sodium channel blocker with a non-specific blocking property that can block TTX-R and TTX-S channels. Scholz and colleagues reported that TTX-R channels are more resistant to lidocaine than are TTX-S channels in A-□ and C type neurons from the dorsal root ganglion of rats [67]. In contrast, other studies reported that TTX-R channels are more sensitive to lidocaine than are TTX-S sodium channels in rat models [68] and in the mammalian dorsal root ganglion neuroblastoma hybridoma cell line0 [69]. The differences in the results of these studies may be the result of several factors. First, the ability of lidocaine to bind sodium channels depends on the status of the sodium channels. TTX-R currents were found to be blocked by lidocaine in the inactivated state more than in the resting state [67]. It was also found that TTX-S and TTX-R currents were equally sensitive to lidocaine in the

Table 5. Sodium channel expression related to dental pain

In vitro/In vivo	Models	Major findings	References
In vitro Immunohistochemistry	Inflamed human permanent tooth pulp	Significant increase in $Na_V1.8$ immunoreactive nerve fibers in painful pulpitis	Renton, et al 2005 [11]
In vitro immunocytochemistry	Inflamed human permanent tooth pulp	Increase expression of $Na_V1.9$ in axons of painful pulp	Wells et al, 2007 [42]
In vitro immunocytochemistry	Inflamed human permanent tooth pulp	Increased expression of $Na_V1.7$ at both intact and remodeling nodal sites within the painful human dental pulp	Luo et al, 2008 [16]
In vitro Western blot	Inflamed human permanent tooth pulp	Increase in $Na_V1.8$ density in inflamed dental pulp	Warren, et al 2008 [12]
In vitro immunohistochemistry	Injured rat dental pulp	Increased expression of $Na_V1.6$ near dilated blood vessels beneath the injured site and nearly affected pulp	Byers et al, 2009 [43]
In vitro immunocytochemistry	Inflamed human dental pulp	1. Increased expression of NaCh at atypical nodal sites, but decreased expression at typical nodal sites within painful pulpitis 2. Decrease in density of NaCh expression in painful pulp but no significant difference when compared to normal pulp 3. Accumulation of NaCh at atypical nodal sites within A-□ fibers	Henry, et al 2009 [41]
In vitro immunocytochemistry	Inflamed human dental pulp	No difference in $Na_V1.6$ expression at nodal sites of painful and non-painful pulp	Luo et al, 2010 [47]
In vitro immunohistochemistry	Inflamed human dental pulp	Increase in $Na_V1.7$ immunoreactive area within sub-odontoblastic plexus of painful dental pulp	Beneng et al, 2010 [51]

resting state, while in the activated or opened state, TTX-S currents were more sensitive to lidocaine [69]. Another reason for different findings in the sensitivity of sodium channels to lidocaine may be the blocking methods used in the studies. Drug-bound TTX-R channels have a slower recovery period than do TTX-S channels [69]. Then, the use of frequency-dependent and tonic blockade of the channels by lidocaine leads to dissimilar results in comparing the sensitivity of TTX-S and TTX-R. Until now, the specific VGSC isoforms that are the problems in anesthetic failure are still controversial. The use of a combination of permanently charged lidocaine (N-ethyl-lidocaine) and capsaicin, an agonist for the transient receptor potential vanilloid 1 (TRPV1), in injured rats has been reported in the study of Kim and colleagues [70]. Those authors claimed that the advantage of that regimen over the use of plain local anesthetic agents is that it does not cause a deficit in motor and autonomic nerve function, but the authors claimed that it requires further study for clinical application. Isoflurane, an inhalation anesthetic agent, was also proved to block TTX-S and $Na_V1.8$ currents in rats [71]. Eugenol, a widely used agent in dentistry, has an ability to inhibit both TTX-R and TTX-S sodium ion currents in rats and has effect on nociceptive, as well as non-nociceptive, fibers [72, 73]. Therefore, eugenol may be another good choice to be an analgesic and anesthetic agent in dental treatment. In addition to the sodium channel blockers mentioned above, the sodium channel blocking efficacy of variety opioid derivatives has been studied and it has been found that tramadol, fentanyl and sufentanil have sodium channel blocking ability, especially in slow-activation sodium channel isoforms, while morphine does not [74]. The specific sodium channel blockers have been improved but they are limited to specific $Na_V1.8$ blockers, such as □O-conotoxin MrVIB from *Conus Marmoreus* [75], a small molecule antisense oligonucleotide (A-803467) [76, 77] and 5-Aryl-2-furfuramides [78]. Unfortunately, despite much research on sodium channel blockers, none of the sodium channel blocking agents is considered to be effective and safe enough to use in humans. Further studies on the new generation of pain treatments, particularly in the field of dentistry, are still needed.

Conclusions

Dental pain is a significant health problem. Although several voltage-gated sodium channel isoforms, as well as an epithelial sodium channel, have been identified in dental pulp with different location and function, only $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ play a key role in inflamed pulp. These sodium channel isoforms are suggested as potential targets for novel treatments of pain from pulpal inflammation and as options for novel anesthetics in the treatment of painful pulpitis.

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