Chapter

Purinergic Signaling and Dental Orofacial Pain

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Abstract

Pain is a common complaint of patients in the dental clinic. Patient with dental orofacial pain usually presents with hyperalgesia and allodynia. Its management has been a challenge, especially in the status of chronic pain or neuropathic pain. Purinergic signaling is dictated by ATP release, purinergic receptors activation, and sequential hydrolysis of ATP. Purinergic signaling participates in nociception processing in the sensory nerves by control of pain signal transduction, modulation, and sensitization. Since purinergic receptors are preferentially expressed in trigeminal nerves, purinergic singling may play a crucial role in the development of dental orofacial pain. In this chapter, we overview the expressions of purinergic receptors as well as the machinery for ATP release, ATP degradations, and adenosine generation in trigeminal nerves. Specifically, the roles of ATP signaling in dental orofacial pain generation and central sensitization via activation of P2 receptors and adenosine signaling in analgesia via activation of P1 receptors in trigeminal nerves are updated. We also discuss the affection of ecto-nucleotidases, the major enzymes responsible for extracellular ATP degradation and adenosine generation in trigeminal nerves that drive the shift from ATP-induced pain to adenosine-induced analgesia. This chapter provides advanced outlines for purinergic signaling in trigeminal nerves and unveils potential therapeutic targets for the management of dental orofacial pain.

Keywords: trigeminal nerves, orofacial pain, dentine hypersensitivity, central sensitization, analgesia, antinociception, dental pulp, P1 receptor, P2 receptor, ecto-nucleotidases, NTPDases

1. Introduction

Pain is an unpleasant sensation of subjects to harmful or potential harmful stimulations. Trigeminal nerves mediate orofacial somatosensory sensations, including dental orofacial pain. The primary trigeminal ganglia nociceptive neurons send axonal fibers innervating orofacial tissues as well as forming synapses with secondary nociceptive neurons in the brainstem. Noxious stimuli, biological insults, or pain mediators released following tissue injury and inflammations activate the nociceptors resulting in the nociceptive transduction in peripheral sensory nerves. The pain signal is conducted and further transmitted to the secondary and higher level nociceptive neurons via synaptic transmission in the brain. Nociception also depends on the condition and status of the sensory nervous system. Pain signal processing can be facilitated with maladaptive plasticity or neuropathy changes in

the nociceptive pathway that result in pain sensitization or neuropathic pain. These changes include nociceptive sensitization, malfunctioned inhibition, and circuit-level rewiring/aberrant processing [1] in both the peripheral and central nociceptive nerves. As peripheral or central sensitization occurs, slight noxious stimulation or even non-noxious stimulation induces severe pain, a phenomenon that is called hyperalgesia or allodynia, respectively. In sensitization condition, patients may also present with spontaneous and neuropathic pain without apparent stimulus. In contrast to the pain from extra-orofacial regions, dental orofacial pain is usually accompanied by the presence of hyperalgesia, allodynia. Dental orofacial pain patients often experience more severe pain and are typically emotional-distracted. Besides, patients with dental orofacial pain are prone to develop spontaneous pain, referred pain, and neuropathic pain [2].

For the past decades, studies strongly suggest a key modulatory role of purinergic signaling in pain generation and sensitization in the nociceptive nerves [3–7]. ATP induces pain via activation of P2X receptors in peripheral sensory nerve fibers. ATP is also involved in cross-talk between the primary and secondary nociceptive neurons as well as with astrocytes and microglia. Since ATP and its metabolites are pain mediators and participate in pain signal processing via activation of various purinergic receptors (P1 and P2 receptors) in the nociceptive sensory nerves [5, 8–10], one putative explanation for the unique properties in dental orofacial pain is due to the different existence or expression of purinergic signaling in trigeminal nerves. Indeed, it has been observed that purinergic receptors are preferentially expressed in trigeminal nociceptive neurons compared with that in dorsal root ganglions [11, 12]. Purinergic signaling depends on ATP release, purinergic receptors (P1, P2X, and P2Y) activation, and extracellular enzymatic ATP degradation and adenosine generation. Therefore, identification of the machinery components for purinergic signaling in the trigeminal nociceptive pathway will provide promising insight to understand the underlying nociceptive mechanisms for the pathogenesis of dental orofacial pain.

In this chapter, we overview the expression of purinergic receptors and machinery for ATP release, ATP degradation, adenosine generation in the trigeminal nociceptive nerves, and discuss the role of purinergic signaling in the pathogenesis of dentin hypersensitivity and dental orofacial pain. Understanding the role of purinergic signaling in the nociceptive mechanisms for pain signal transduction, transmission, sensitization, and modulation in trigeminal nerves will reveal multiple targets for developing more effective drugs and therapies for the management of dental orofacial pain.

2. Purinergic signaling and pain

2.1 ATP initiates pain signal via activation of peripheral P2X receptors

ATP has been recognized as a neuronal transmitter and modulator in synaptic transmission for decades [13]. ATP and its metabolites are also important pain mediators and modulators in pain signal processing [5, 8–10]. It has been proposed that ATP released from various cell types is implicated in initiating the pain signal by acting on purinoceptors on sensory nerve terminals [14]. Purinoreceptors responsible for pain transduction belong to P2X receptor family, a group of ligand-gated non-selective cation channels using ATP as a native agonist. Upon binding to P2X receptors, ATP opens the pore of channels permeable to Na^+, K^+, and Ca^{2+} that depolarize the membrane potential, enhance the excitability and induce spikes in nociceptive neurons. So far, seven distinct P2X receptor subunits (P2X1–P2X7)

have been isolated and cloned (North 2002). A total of 14 functional homo- or heterotrimers P2X receptors (P2X1–P2X7, P2X1/2, P2X1/4, P2X1/5, P2X2/3, P2X2/6, P2X4/6, and possibly P2X4/7) assembled from different subunits had been reported [15].

Using *in situ* hybridization immunohistochemistry (ISHH), mRNA expression for P2X2, 3, 4, 5, and 6 receptors had been detected in naive dorsal root ganglia (DRG) neurons, in which P2X2 and P2X3 receptors were expressed preferentially by C-fiber neurons. In contrast, the majority of P2X5 and P2X6 receptors were preferentially expressed by A-fiber neurons [16]. Specifically, expression of P2X receptors is also detected in nociceptive primary sensory neurons, peripheral nociceptive nerve fibers, and their free endings extending throughout the epidermis. Two types of P2X receptors (P2X2 and P2X3) have been particularly examined in DRG presumably nociceptive neurons [17]. P2X3 receptors appear to be exclusively expressed in a subgroup of sensory neurons that are likely to be nociceptive neurons [18]. Nociceptive neurons fall into nerve growth factor (NGF) sensitive group and glial cell-line derived neurotrophic factor (GDNF) sensitive group. It turned out that the vast majority of P2X3 positive neurons overlap with GDNF subgroup of nociceptive neurons [19]. P2X3 receptors are peripherally axonal transported and have been identified in the free end of the nerve fibers in a variety of tissues including tongue, skin, and viscera (e.g., bladder) [17, 19]. Co-localization studies suggest that many, but not all, DRG cells that express P2X2 receptors also express P2X3 receptors [17]. Furthermore, electrophysiological and pharmacological studies had demonstrated that the application of ATP or its analogs to DRG neurons results in depolarization or inward currents mediated by P2X receptors activation.

The selective expression of P2X3 and P2X2 receptors within the nociceptive nerves has inspired a variety of approaches to elucidate the potential role of ATP as a pain mediator. ATP elicits excitatory inward currents in nociceptive small diameter sensory ganglion cells. Interestingly, these inward currents resemble the currents evoked by ATP on recombinantly expressed heteromeric P2X(2/3) channels as well as homomultimers consisting of P2X2 and P2X3 [8, 14, 18, 20]. It had been observed that ATP and its analogs produce spike activity when applied to peripheral nociceptive terminals [8]. In *in vivo* pain behavioral animal models, the algogenic effects of ATP in normal conditions and models of peripheral sensitization had been confirmed. In humans, local skin delivery of ATP induces dosage-dependent pain sensation. Furthermore, it has been shown that ATP-induced algogenic responses depends on capsaicin-sensitive neurons and is augmented in the presence of inflammatory mediators. Since ATP is released in the vicinity of peripheral nociceptive terminals under a variety of conditions such as tissue injury or inflammation, the existence of purinergic signaling in peripheral sensory nervous free ends strongly links the tissue damage and inflammation to pain perception [9].

2.2 Purinergic signaling is involved in central sensitization

Noxious stimulation to the nociceptive nerves induces pain sensation in the brain. Acute pain is a warning signal for the individual to survive in response to tissue injuries or diseases. However, in nociceptive sensitization statuses, such as in chronic pain or neuropathic pain, nociception no longer relates to or depends on external noxious stimulation, and slight noxious or even no-noxious stimulation or non-stimulation at all can induce severe pain. The central sensitization theory proposed that neuronal plasticity occurred in the sensory nerve circuits that enhance the sensitivity to noxious stimuli or even turn the innocuous slight touching to pain [21]. Pain sensitization can also be induced by nerve injury (deafferentation, compression, and constriction) or neuropathy changes resulting from physical,

chemical, metabolic, or biological insults to the sensory nerves. Besides, it has been proposed the pain signal itself that is accompanied with releasing of proinflammatory cytokines, neuronal transmitters, and modulators in nociceptive neuronal circuits participate in plasticity changes and induce central sensitization [22].

Synaptic neuronal transmission is accompanied with large amount of ATP release. Accumulated evidence suggests that ATP signaling play an essential role in the development and maintenance of central sensitization (**Figure 1**) [3–7]. In the somatosensory nerves, P2X receptors are expressed in DRG nociceptive neurons and then transferred to central axonal terminals as well as peripheral free ends. For example, immunoreactivity to P2X3 subunits is detected in lamina IIi in spinal cord dorsal horn and is disappeared after axotomy or following destruction of IB4-positive afferent fibers [17]. Immunoreactivity to P2X1 and P2X2 subunits are also located on the central terminals of primary afferent neurons that innervate superficial lamina of the spinal cord dorsal horn [17]. The presence of P2X receptor subunits at the central terminals of primary afferent neurons raises the possibility that ATP may act on central terminals of primary afferent neurons to either modulate or directly evoke the release of neuronal transmitters such as glutamate and neuropeptides. Activation of central terminal P2X receptors will depolarize the membrane potential and induce Ca^{2+} influx that will enhance the release of glutamate and substance P, and subsequently increase the secondary nociceptive neuron responses and dorsal horn nociceptive output. In neuropathic pain condition, an increase in the number of P2X receptors positive DRG neurons is observed following sciatic nerve injury by chronic constriction [23]. Specifically, an increase of P2X3 receptor immunoreactivity is detected in the dorsal horn ipsilateral side of the injured nerve indicating the upregulation of P2X receptors at the central terminals of primary afferent neurons. P2X receptor expression is also upregulated following tissue injury or inflammation within the spinal cord. The upregulation of P2X receptor expression on the central terminals of primary afferent neurons would sensitize the responses to ATP, which in turn may facilitate P2X receptor-mediated nociceptive modulation.

Activation of homomeric, as well as heteromeric P2X2/3 receptors, appears to modulate longer lasting nociceptive sensation associated with nerve injury or chronic inflammation [1]. P2X3 receptor function is highly sensitive to soluble factors like neuropeptides and neurotrophins and is controlled by transduction mechanisms, protein-protein interactions, and discrete membrane compartmentalization. Recent findings have demonstrated that P2X3 receptors interact with the synaptic scaffold protein calcium/calmodulin-dependent serine protein kinase (CASK) in a state-dependent fashion, indicating that CASK plays a crucial role in the modulation of P2X3 receptor stability and efficiency [24]. Activation of P2X3 receptors within CASK/P2X3 complex has essential consequences for neuronal plasticity and possibly for the release of neuromodulators and neurotransmitters. Better understanding the interaction machinery for P2X3 receptors and their integration with other receptors and channels on pre- and postsynaptic membranes is proposed to be essential to unveil the process of nociceptive neuronal sensitization.

Multiple other purinoceptor subtypes participate in pain processing. In neuropathic pain, activation of purinergic receptors on microglia is thought to maintain nociceptive sensitization through neural-glial cell interactions [3]. Microglia expresses several P2 receptor subtypes, and of these the P2X4, 7, and P2Y12 receptor subtypes have been implicated in neuropathic pain. It has been shown that activation of P2X4, 7, and P2Y12 receptors expressed on microglia is critically involved in neuropathic pain arising from peripheral nerve injury [25], while blocking these receptor with antagonists reduces neuropathic pain [3]. The P2X4 receptor has emerged as the core microglia-neuron signaling pathway. In response

to peripheral nerve injury, P2X4 receptors are upregulated in spinal cord microglia [26]. Activation of this receptor causes the release of brain-derived neurotrophic factor (BDNF) which causes disinhibition of pain-transmission neurons in spinal lamina I. Several mechanisms have recently been implicated in the upregulation of P2X4 receptors including CCL21, interferon γ, tryptase, fibronectin, and the activation of μ-opioid receptors. Activation of P2X4 receptors leads to an influx of extracellular Ca^{2+} activating p38 MAPK that leads to SNARE-dependent release of BDNF from the microglia. BDNF is a crucial microglia-neuron signaling molecule that causes disinhibition of nociceptive dorsal horn neurons by disrupting intracellular Cl^- homeostasis of inhibitory interneurons [27–29]. Activation of P2X7 or P2Y12 receptors is also through signals of p38 MAPK. p38 MAPK signaling drives the release of interleukin-1β and cathepsin S, which contributes to the maintenance of mechanical hypersensitivity in the spinal cord. P2Y12 receptor expression is also upregulated in microglia, and activation of these receptors is involved in neuropathic pain. Recent studies have demonstrated that inhibition of microglia-expressed P2 receptors (P2X4, P2X7 or P2Y12) by the pharmacological blockade, antisense knockdown or genetic deletion suppresses both mechanical allodynia and thermal hyperalgesia in nerve-injured rats [30, 31]. Conversely, intrathecal administration of the P2Y12 receptor agonist 2Me-SADP elicits pain behaviors in naïve rats that mimic those observed in nerve-injured rats [30].

2.3 Adenosine induces antinociception by activation of P1 receptors

In contrast to the algogenic effects of ATP, adenosine, the metabolite of ATP, induces antinociception via activation of P1 receptors. P1 receptors are G protein-coupled metabotropic receptors. Four subtypes of P1 receptors (A1, A2A, A2B, and A3) have been cloned in the nervous system [32].

A1 receptor is detected in the peripheral sensory nerve fibers [33]. Local delivery of adenosine induces analgesia and blocking A1 receptors abolishes adenosine-induced antinociception in various inflammatory pain models [34]. In addition, it has been shown that adenosine also mediates the analgesic mechanism of acupuncture via peripheral A1 receptors [35]. Adenosine also produces antinociception via activation of A1 receptors in the spinal cord [33]. Intraspinal injection of adenosine or A1 receptor agonists induced antinociception in both inflammatory and neuropathic pain animal models. It has been proposed that the underlying mechanism for adenosine-induced antinociception is related to potassium channel activation-induced cell membrane hyperpolarization [36]. Activation of pre- or postsynaptic A1 receptor triggers cAMP/PKA, PLC/IP3/DAG, and nitric oxide/cGMP/PKG pathways [33, 37] that induce analgesia by reducing presynaptic vesicle release and postsynaptic excitability [36]. Indeed, it has been demonstrated that A1 receptor activation decreased the excitatory neurotransmitter release in synaptosomes isolated from the spinal cord dorsal horns [38, 39].

Endogenous adenosine also mediates antinociception by A2A, A2B, or A3 receptors expressed in nociceptive neurons, astrocytes, or immune cells [40, 41]. Considering the extensive involvements of glial cells (microglia and astrocytes) in central sensitization and chronic pain [42], activation of adenosine receptors on microglia and astrocytes are potentially involved in the antinociceptive mechanism of adenosine in the nervous system [43]. Additionally, adenosine may also mediate the antinociception by enhancing GABA inhibition and blocking neuroinflammation via activation of A3 receptors [44]. Collectively, these studies suggest the possibility of treating chronic pain by targeting specific adenosine receptor subtypes in anatomically defined regions with agonists or with ecto-nucleotidases that control the generation of adenosine.

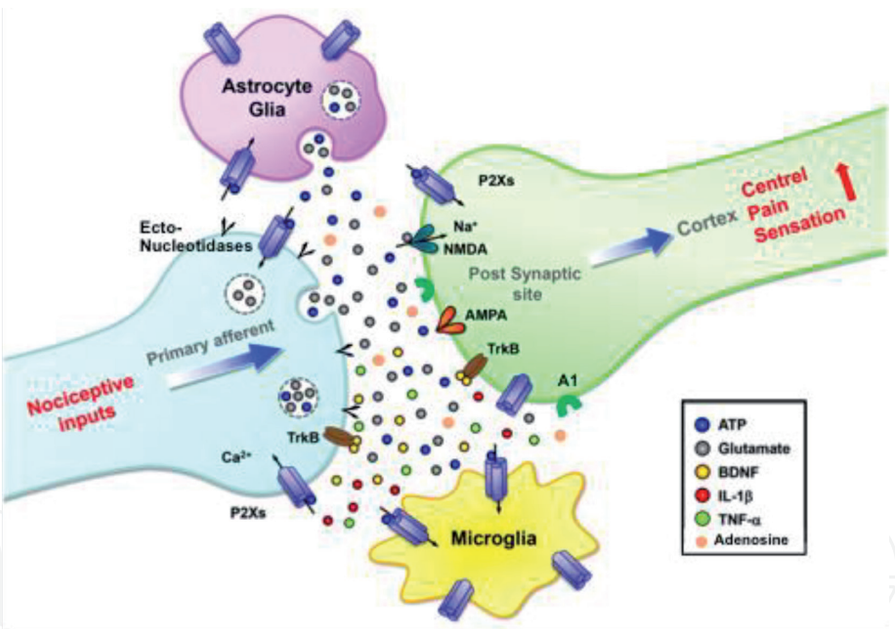
2.4 Ecto-nucleotidases drive the shift from ATP-induced pain to adenosine-induced antinociception

Purinergic signaling depends on ATP release, purinergic receptor action, and sequential hydrolysis of ATP to ADP and nucleoside adenosine [45]. Because of their dynamic catalytic activities under physiological conditions, ecto-nucleotide diphosphatases (Ecto-NTPDases) are the major enzymes responsible for the hydrolysis of extracellular ATP and ADP. Four members of ecto-NTPDse family (NTPDase1, 2, 3, and 8) have been cloned. Three of which (i.e., NTPDase1, 2, and 3) are expressed in the nervous system [46]. NTPDase1 and 3 hydrolyze both ATP and ADP, while NTPDase2 primarily hydrolyzes ATP with minimal ADP hydrolytic activity [47]. Extracellular AMP is further hydrolyzed to adenosine by ecto-5′-nucleotidase (CD73) [47, 48] and a transmembrane isoform of prostatic acid phosphatase (PAP) [47, 48] in the nervous system. By control of ATP degradation and adenosine generation, these ecto-nucleotidases affect nociception by terminating ATP-induced pain and pain sensitization and promoting adenosine-mediated analgesia.

ATPase activity had been detected in dorsal root ganglion (DRG) and spinal cord using enzymatic histochemistry staining. Nucleotidase activity is robust in spinal cord dorsal horn nociceptive lamina suggesting that nucleotide hydrolysis would play a role in nociceptive processing. In DRG, extensive staining revealed ecto-ATPase activity in a subset of neurons and non-neuronal cells. The mRNA expression and immunoreactivity for NTPDase1–3, but not NTPDase8, was detected in lumbar DRG and spinal cord. Immunoreactivity for NTPDase3 that closely matches the distribution of ecto-ATPase activity labels DRG central projections in the dorsal root and superficial dorsal horn, as well as intrinsic spinal neurons concentrated in lamina II. It has been reported that NTPDase3 is located in nociceptive and non-nociceptive neurons of DRG, in the dorsal horn of the spinal cord, and the free nerve endings in the skin [49]. These data suggest that NTPDase3 would be a negative regulator for nociceptive signaling [50]. However, studies from NTPDase3 knockout mouse show that deletion of NTPDase3 does not impair ATP hydrolysis in primary somatosensory neurons or dorsal spinal cord. Also, NTPDase3 (−/−) mice did not differ in nociceptive behaviors when compared with wild-type mice. These observations suggest the existence of multiple ecto-nucleotidases acting redundantly to hydrolyze nucleotides [49].

Even though the manipulation of adenosine transport or degradation can induce antinociception [51], extracellular adenosine level is mainly controlled by extracellular AMP hydrolysis. Indeed, it has been shown that extracellular AMP hydrolysis provides the major source for endogenous adenosine in the nervous system that is essential to maintain a tonic activation of adenosine receptors in the nociceptive neurons of the spinal cord [52]. Two ecto-nucleotidases have been identified to be responsible for extracellular AMP hydrolysis in the spinal cord [48]. Ecto-5′-nucleotidase (CD73) is a membrane-anchored protein that hydrolyzes extracellular adenosine 5′-monophosphate (AMP) to adenosine in different tissues. CD73 was detected in peptidergic and nonpeptidergic nociceptive neurons in DRG and afferent terminals in lamina II of spinal cord. In addition, CD73 was also located on epidermal keratinocytes, cells of the dermis, and on nociceptive terminals in the epidermis [52]. Besides CD73, prostatic acid phosphatase (PAP) also functions as an ecto-nucleotidase and generates extracellular adenosine. PAP is expressed in nociceptive dorsal root ganglia (DRG) nociceptive neurons, it had been shown that PAP inhibits noxious thermal sensitivity and sensitization that is associated with chronic pain through sustained activation of the adenosine A1 receptor [53].

Knockout of CD73 and/or PAP reduced adenosine generation and enhanced nociception in animal models following inflammation and nerve injury [52]. It has been found that AMP hydrolysis was nearly abolished in DRG neurons and lamina II of the spinal cord from PAP/CD73 double knockout (dKO) mice. Likewise, the antinociceptive effects of AMP were reduced in PAP/CD73 dKO mice. Adenosine was maximally produced from AMP within seconds in wild-type (WT) mice but was significantly reduced in dKO mice indicating PAP and CD73 generate rapidly adenosine in lamina II. Besides, it has shown the existence of spontaneous low-frequency adenosine transients in lamina II in wild-type mice, while knockout of PAP and CD73 abolished the spontaneous adenosine transit suggesting these ecto-nucleotidases rapidly hydrolyze endogenously released nucleotides to adenosine, and there exists tonic activation of A1 receptors. Field potential recordings in dorsal horn lamina II and behavioral studies indicated that adenosine converted by these enzymes acts through the A1 receptor to inhibit excitatory neurotransmission. PAP and CD73 injected spinally produced long-lasting adenosine A1 receptor-dependent antinociceptive effects in inflammatory and neuropathic pain models [54]. Furthermore, it has been noted that following peripheral nerve injury CD73, PAP, as well as enzymatic ecto-AMPase activities were reduced in dorsal horn lamina II. Collectively, these evidences indicate that PAP and CD73 are the predominate ecto-nucleotidases that generate adenosine in the nociceptive circuits (**Figure 1**) [48].



Modified from Kuan and Shyu, Mol Brain. 2016; 9: 58.

Figure 1.
Schematic illustrates purinergic signaling responsible for the pain signal transmission and sensitization at the nociceptive synapses. (1) Primary nociceptive inputs promote glutamate and ATP co-release and synergistically cause non-selective permeability to Ca^{2+}, Na^{+}, and K^{+} cations via P2X3 receptor, leading to postsynaptic activation of NMDA or AMPA receptors and further contributing astrocytic glutamate and ATP co-release into the extracellular milieu which result in the pain signal transmission in the nociceptive synapses. (2) Activation of P2X4/7 receptors expressed on astrocytes and microglia induces a local inflammatory response with release of cytokines including IL-1β, BDNF, and TNF-α, which will lead to sensitization in pain signal transduction and conduction as well as synaptic transmission caused by enhanced excitatory and reduced inhibitory driving. (3) Activation of A1 receptor triggers multiple intracellular cAMP/PKA, PLC/IP3/DAG, and nitric oxide/cGMP/PKG signaling and induces analgesia by reducing both presynaptic vesicle release and postsynaptic excitability. Adenosine may also mediate antinociception by activation of A2A, A2B, or A3 receptors expressed on nociceptive neurons, astrocytes, or immune cells. (4) Ecto-nucleotidases (NTPDase3/CD73) are specifically expressed in primary nociceptive neurons and localized at presynaptic terminals that will drive the shift from ATP-induced pain to adenosine-mediated analgesia by control of extracellular ATP extracellular hydrolysis in the nociceptive pathway [99].

3. ATP signaling and dentin hypersensitivity

3.1 Dentin hypersensitivity

Dentin hypersensitivity (DHS) is defined as a short, sharp pain that arises from exposed dentin in response to various environmental stimuli [55–57]. Dentin exposure can be caused by physical, chemical, pathological, biological challenges, and/or developmental abnormalities that result in dental and or periodontal damage or defects. In patients with DHS, gentle touch, mild cold or hot, chemical (acidic or sweet fruits, foods, and drinks) or air-flow stimulation to the exposed dentine can induce similar short, sharp pain. DHS can affect the quality of patient's daily activities such as eating, drinking, speaking, and tooth brushing. In some cases, more severe DHS can become a constant annoyance and induce psychological and emotional distractions [55, 57]. Even though DHS is a common problem, the underlying nociceptive transduction mechanism still remains elusive, and no universally accepted or highly reliable desensitizing agents or treatment are available in dental practice.

3.2 Hydrodynamic theory

It is generally regarded that DHS is associated with dentin exposure, especially the exposure of open dentinal tubules, and dental pulp nerve responsiveness to external environmental stimuli [58]. Several theories have been proposed for the pathogenesis of DHS. The most widely accepted one is the hydrodynamic theory that is introduced by Brannstrom in 1964 [59]. It stated that environmental mechanical, thermal, or chemical changes cause the movements of fluid within dentinal tubules that stimulate the terminals of pulpal nerve fibers located at the dentin tubule inlets, thereby induces transient acute pain. The hydrodynamic theory highlights the notion that several different stimuli can evoke similar responses via dentin tubule fluid movements. The intra-dental myelinated Aβ fibers and some Aσ fibers that send terminals into the dentin tubules are thought to respond to the fluid movements resulting in the characteristic short, sharp pain of DHS. However, Aβ fibers usually mediate slight touching sensation with a lower threshold to mechanical stimulation, while Aσ fibers mediate pain, but they exhibit high threshold to noxious stimulation. How the essentially non-noxious dentin tubule fluid movements induce the nociceptive transduction in dental pulpal nerve fibers remains an enigma. Recently, the hydrodynamic theory has been challenged by emerging evidence suggesting that odontoblasts might play an essential role in the nociceptive transduction of DHS [60–62].

3.3 The machinery for ATP signaling in dental pulp

Odontoblasts locate at the outermost layer of the dental pulp and send odontoblastic processes to the dentin tubules. Therefore, odontoblasts are the first dental pulp cells to detect external stimuli in dentin exposure. Though a physical synaptic structure is absent, dental pulp nerve fibers are closely approached to the odontoblasts and tightly entangle these cells. This finding could provide a mechanism to explain how signals are transmitted to adjacent nerve endings through chemical mediators released from the odontoblasts. That is, a paracrine cell-cell communication is involved in signal transmission as opposed to classic neural synapses. Since purinergic P2X3 receptors are expressed in dental pulp nerve fibers [63] and activation of P2X receptors in peripheral nerve fibers induces pain [9]. ATP has

been proposed as a promising candidate that participates in cell-cell communication within the dental pulp that would be associated with pain transduction mechanism of DHS [64].

For the past decades, chemo-, mechano-, and or thermo-sensitive channels such as connexin, pannexin, TRPV1-4, TRPM3, KCa, TREK-1, beta-ENa(+) C, and ASIC2 channels have been identified in odontoblasts [60, 65–70]. Activation of these channels depolarizes the membrane potential that induces ATP release via vesicles release or channel opening in odontoblasts. Interestingly, mechanic- as well as depolarization-sensitive ATP permeable channel such as connexin 43 and pannexins had been detected in odontoblastic processes inserting into dentinal tubules [60, 64]. Indeed it has been shown that mechanical and or thermal stimulation that mimics dentin hypersensitivity in clinic induces ATP release from odontoblasts [65, 66, 71]. Besides, mechanical stimulation-induced ATP release and ATP-mediated signal transmission from odontoblasts to trigeminal neurons have been demonstrated *in vitro* using co-culture models comprising of odontoblasts and trigeminal neurons [68, 72]. The existence of autocrine/paracrine mechanisms for ATP-involved purinergic signaling in cultured odontoblast-like stem cells is also confirmed [73]. Furthermore, external mechanical and thermal stimulation that mimics dentin hypersensitivity induces ATP release in a tooth perfusion model, while pharmacological blocking connexin and pannexin channels abolished external stimulation-induced ATP release in dental pulp [66]. Based on the above observations, we proposed that, as illustrated in **Figure 2**, external stimulation-induced mechanosensitive responses and ATP release from odontoblasts and subsequently activation of purinergic receptors in dental pulpal nerves may represent a novel explanation as to how odontoblasts participate in a mechanosensory mechanism leading to the pain transduction in DHS [60, 74].

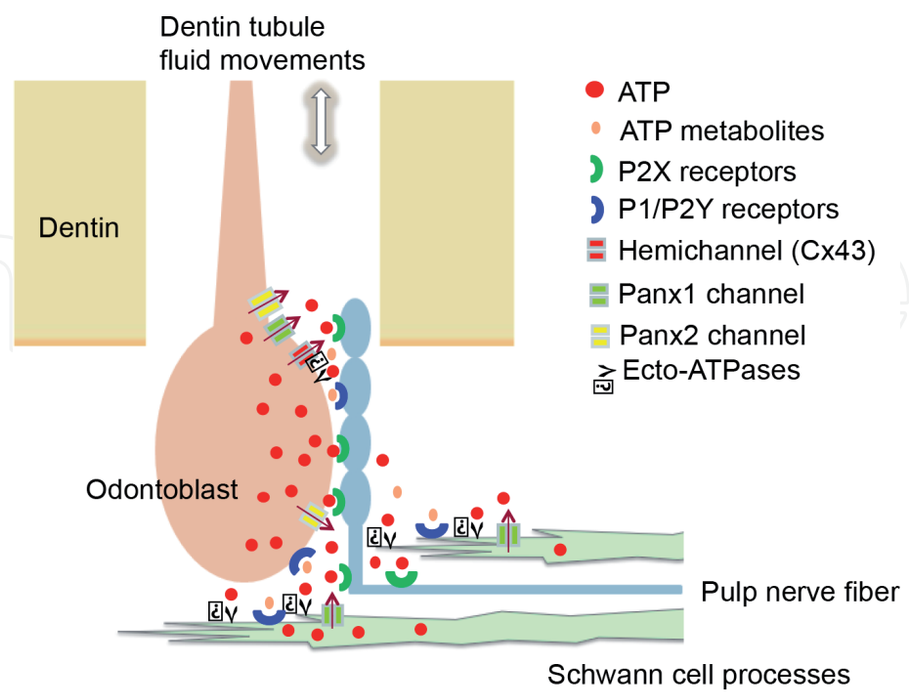


Figure 2.
A novel hypothesis for nociception transduction in DHS. External stimulation-induced dentin tubule fluid movements induces ATP release via pannexin/connexin channels in OBs (odontoblasts), ATP then activate P2X receptors on adjacent nerve fibers to trigger the transduction of pain. ATP signaling is terminated by ecto-ATPases in OBs and Schwann's cells.

3.4 Purinergic signaling and dentin hypersensitivity

Dentin-odontoblasts-nerve terminal complex represents the essential components for the process of stimulation transducing and nociceptive transduction from environmental changes to pain impulse in DHS. External stimuli promote ATP release from odontoblasts through mechanic or depolarization-sensitive channels, which then initiates pain signaling via activation of P2X3 receptors in dental pulp nerve fibers. Functional ecto-nucleotidases in odontoblasts, dental pulp nerve fibers, and Schwann cells that surround the nerve fibers modulate pain transmission by control of the local concentration of extracellular ATP and adenosine. ATP and its metabolites may also activate the P2Y and P1 receptors via a paracrine mechanism to trigger intracellular Ca^{2+} signals that further promote ATP release in odontoblasts and regulate the expression of ATP permeable channels, purinergic receptors, and ecto-nucleotidases.

The existence of mechanical-sensitive ATP permeable connexin/pannexin channels and ATP singling in dentin-odontoblast-nerve fiber complex provides a clue to explain the unique characteristic of DHS, that is, the "all" or "none" property. In patients with DHS, a common phenomenon is that external environmental stimulation induces either one sharp pain or no pain at all. A self-activated propagation of ATP signaling and calcium response in gap junction coupled cells as well as in tissues or organs was demonstrated [75]. For example, a local mechanical stimulation induces connexin/pannexin channel opening, and then result in ATP release, ATP then activates the P2X/P2Y receptors in adjacent cells inducing intracellular Ca^{2+} increase and/or cell depolarization that further promote connexin/pannexin channels opening and ATP release in further beyond adjacent cells until all the cells are activated. Since odontoblasts express connexin 43 and are functionally connected via gap junction as a syncytium [60, 76], stimulation from locally exposed dentin tubules will induce a response in the whole dental pulp odontoblasts. With this mechanism, external stimulation will cause the full dental pulp odontoblast activation and evoke the typical "all" or "none" short, sharp pain in patients with DHS.

The existence of ecto-ATPase activity in dental pulp nerve fibers as well as in the odontoblast layer [60] may provide mechanisms to terminate ATP-induced pain in DHS. Studies have shown that NTPDase2 is expressed in odontoblasts as well as in Schwann's cells that encapsulate the dental pulp nerve fibers. While NTPDase3 is expressed in dental pulp nociceptive nerve fibers. The presence of these enzymes in dental pulp provides machinery responsible for ATP degradation that may provide a mechanism to terminate the pain signaling induced by purinergic receptor activation in DHS. While the existence of ecto-AMPase enzymatic activities and expression of CD73 in nociceptive nerve fibers and odontoblasts [64] will hydrolyze AMP to adenosine, the latter will activate the A1 receptors and hyperpolarize the terminals of the dental pulp nerve fibers via opening the potassium channels that will help to stop any lingering of pain impulses in nerve fibers.

4. Purinergic signaling and dental orofacial pain

4.1 P2X receptors in trigeminal nerves

Activation of P2X receptors in peripheral nociceptive nerve fibers results in pain transduction. Interestingly it has been observed that purinergic P2X receptors are preferentially expressed in trigeminal nociceptive neurons [11, 12] suggesting that purinergic signaling might play a unique role in the generation and development of dental orofacial pain. Previous studies have shown expression of P2X3 receptors

in dental pulp nerve fibers, including the iB4 positive nociceptive fibers [63]. Furthermore, it had been demonstrated that activation of P2X3 and P2X2/3 receptors in dental pulp is sufficient to elicit nociceptive behavioral as well as trigeminal brainstem neuronal activity [77]. Functional homomeric P2X3 receptor and heteromeric P2X(2/3) receptor are highly expressed on nociceptive trigeminal neurons, their contribution toward the pain mechanism in dental orofacial pain has been well established [78, 79]. Using real-time reverse transcription-PCR analysis, besides P2X3, mRNA expression for P2X1 and P2X4 was also detected in trigeminal ganglion neurons. Indeed, application of P2X receptors agonists, ATP, α,β-methylene ATP, or β, γ-methylene ATP induced neuronal Ca^{2+} influx and a series of selective antagonists for P2X1, P2X3, or P2X4 receptors inhibited these Ca^{2+} influx responses. Interestingly, expression of purinergic receptors (P2X1, 3, and 5) in trigeminal ganglion is upregulated in response to dental pulp inflammation-induced pain suggesting that these receptors may participate in the peripheral pain sensitization [80]. Expression of P2X receptors in trigeminal ganglion is also upregulated by oral facial deep tissue inflammation [11, 12]. In addition, application of P2X receptor agonist αβ-meATP to rat tooth pulp induces central sensitization in medullary nociceptive neurons, and this sensitization response can be blocked by dental pulp application of the P2X (1,2/3,3) receptor antagonist TNP-ATP as well as by medullary application of TNP-ATP. These results suggest that the activation of peripheral P2X receptors in orofacial tissues plays a critical role in producing central sensitization in medullary trigeminal subnucleus caudalis (TSNC) nociceptive neurons [81]. Therefore, trigeminal ganglion neurons preferentially express functional P2X1, 2/3, 4 receptors, and activation of these receptors attributes to generation and sensitization of dental orofacial pain [78].

Involvement of P2X receptor activation in dental orofacial pain had been demonstrated in various animal models. In the carrageenan-induced TMJ inflammatory hyperalgesia model, the P2X1, 3, and 2/3 receptor antagonist TNP-ATP, but not the selective P2X7 receptor antagonist A-438079, significantly reduced the pain behavior. These findings indicate that P2X3 and P2X2/3 receptors would be potential targets for the development of new analgesics to control TMJ inflammatory pain [82]. Interestingly, it has been found that the number of P2X3 receptor positive cells is increased in the small cell group in trigeminal ganglia, whereas there was no change in medium or large cell groups after TMJ CFA-injection. Retrograde tracing confirmed that TMJ-innervated neurons in TG exhibited P2X3 receptors. These observations provided evidence to support that P2X3 receptor play an essential role in orofacial pain induced by TMJ arthritis [83]. Pharmacological and immunohistochemical studies revealed that the P2X3 receptor also plays an essential role in the heat hyperalgesia observed in the infraorbital nerve (IoN) ligation-induced neuropathic pain model [84]. In an oral cancer pain model, injection of squamous cell carcinoma cells into the lower gingiva produces mechanical allodynia and thermal hyperalgesia. It has been observed that expression of P2X receptor, calcitonin gene-related peptide (CGRP)-, substance P (SP)-, and capsaicin receptor (TRPV1)-immunoreactive cells are strikingly upregulated in the small cell group of trigeminal ganglia (TGs) after tumor cell inoculation [85].

Whereas there is ample evidence that purinergic P2 receptors in trigeminal glial cells are altered after peripheral nerve injury, there is very little information about the changes of P2 receptors in TG satellite glial cells (SGCs), although it is well established that SGCs are endowed with P2 receptors. In submandibular inflammation with the injection of complete Freund's adjuvant, there was a marked increase in the sensitivity of SGCs to ATP, with a threshold decreasing from 5 μM to 10 nM. A similar result was observed in the intact trigeminal ganglion after infraorbital nerve axotomy. It had been demonstrated that the increased

after-inflammation response was mediated predominantly by P2X receptors. The enhanced responses to ATP after inflammation are primarily due to P2X2 and or P2X5 receptors, with a possible contribution of P2X4 receptors. It has been proposed that the over 100-fold augmented sensitivity of SGCs to ATP may contribute to the development of chronic pain status in dental orofacial pain [86].

4.2 P2Y receptors in trigeminal nerves

Little is known about P2Y receptor expression in trigeminal nerves and their role in dental orofacial pain. It had been demonstrated that UTP, an agonist of P2Y2/P2Y4 receptors, significantly decreased the mean threshold potentials for evoking action potentials and induced a striking increase in the mean number of spikes in TG neurons [87]. Because of its vital role in the control of neuronal spike onset, fast inactivating transient K^+ channels (IA) is a key regulator of membrane excitability in sensory neurons. It has been shown that UTP significantly inhibited IA and the expression of Kv1.4, Kv3.4, and Kv4.2 subunits in TG neurons. The P2Y receptor antagonist suramin could reverse these effects. Furthermore, in ION-CCI (chronic constriction injury of the infraorbital nerve) induced neuropathic pain model, when blocking P2Y2 receptors with suramin or injection of P2Y2 receptor antisense oligodeoxynucleotides led to a long time- and dose-dependent reverse of allodynia [87]. Blocking P2Y2 receptors is accompanied with a significant increase in Kv1.4, Kv3.4, and Kv4.2 subunit expression and decrease in phosphorylated ERK expression in trigeminal ganglia. These data suggest activation of P2Y2 receptors leads to upregulation of ERK-mediated phosphorylation and decline of the expression of I(A)-related Kv channels in trigeminal ganglion neurons, which might reveal potential alternative targets for the treatment of trigeminal neuropathic pain [87].

Other type of P2Y receptors are also involved in the development of dental orofacial pain. Administration of the P2Y1, 12, and 13 receptor agonist, 2-(methylthio) adenosine 5′-diphosphate trisodium salt hydrate (2-MeSADP), in naïve rats induced neuropathic pain in the tongue, as demonstrated in lingual nerve crush rats, while co-administration of P2Y receptor antagonists (MRS2395) to naïve MRS2395 rats did not result in hypersensitivity of the tongue. P2Y12 receptor had been detected in satellite cells of the trigeminal ganglia. In an orofacial pain model after lingual nerve crush, expression of P2Y12 receptors was enhanced in pERK1/2-immunoreactive cells encircling trigeminal ganglion neurons. Administration of a selective P2Y12 receptor antagonist, MRS2395, attenuated tongue hypersensitivity to mechanical and heat stimulation and suppressed the increase in the relative numbers of calcitonin gene-related peptide (CGRP)-immunoreactive neurons and neurons encircled by pERK1/2-immunoreactive cells. These results suggest that intercellular communication between activated satellite cells and CGRP-immunoreactive neurons via P2Y12 receptors contributes to the development of orofacial neuropathic pain [88].

4.3 Purinergic signaling in trigeminal subnucleus caudalis (TSNC)

Besides purinergic receptors expressed in the central afferent terminals of primary trigeminal nociceptive neurons, multiple P2X, P2Y, and P1 receptors are also detected in the secondary nociceptive neurons, astrocytes, and microglia in TSNC. Since pain signal synaptic transmission is accompanied by a large amount of ATP release in TSNC. Activation of purinergic receptors expressed in presynaptic afferent terminals, secondary nociceptive neurons, astrocytes, and microglia in TSNC would play an essential role for the development of central sensitization [77]. Studies have shown that extracellular ATP acting on presynaptic purinergic receptors (P2X2/3 and P2X3 subunits) participate in central sensitization of dental orofacial

pain. Application of inflammatory irritant mustard oil (MO) to the tooth pulp produced a long-lasting allodynia and hyperalgesia. Intrathecal administration of the selective P2X1, P2X3, and P2X2/3 receptor antagonist, TNP-ATP, significantly and reversibly attenuated the MO-induced central sensitization. While the administration of the selective P2X1, P2X3, and P2X2/3 receptor agonist, alpha, beta-methylene ATP (alpha, beta-meATP, i.t.) produced abrupt and significant neuroplastic changes in TSNC nociceptive neurons, followed by neuronal sensitization as evidenced by the ineffectiveness of a second application of alpha, beta-meATP and subsequent MO application to the pulp. These results suggest that P2X3 and possibly also the P2X2/3 receptor subtypes in TSNC play a crucial role for the initiation and maintenance of central sensitization in brainstem nociceptive neurons [89]. Tooth pulp application of mustard oil (MO) induced a significant increase in glutamate release in TSNC. Intrathecal administration of apyrase or TNP-ATP (a P2X1, P2X3, P2X2/3 receptor antagonist) alone significantly reduced the MO-induced glutamate release in the TSNC. Furthermore, the suppressive effects of apyrase on glutamate release were reduced by DPCPX (an adenosine A1 receptor antagonist) [89].

It had been reported that P2X3 receptor expressed in astrocytes in the TSNC participates in the development of craniofacial neuropathic pain induced by chronic constriction of the infraorbital nerve (CCI-ION) [90]. The number of P2X3-positive fine astrocytic processes and the density of P2X3 receptors in these processes was increased significantly in CCI-ION model and administration of MPEP, a specific mGluR5 antagonist, alleviated the mechanical allodynia and abolished the increase of P2X3 receptor expression in the fine astrocytic processes. Specific glial cell populations become activated in both trigeminal ganglia and brainstem in CFA-injection induced temporomandibular joint (TMJ) inflammation pain model. CFA-injected animals exhibited ipsilateral mechanical allodynia that is accompanied by a substantial increase of GFAP-positive satellite glial cells and activation of resident macrophages in the trigeminal ganglia. The activated microglial cells were also observed in the ipsilateral TSNC [91]. In dental pulp, MO injection induced central sensitization model, it has been demonstrated that continuous intrathecal (i.t.) superfusion of the potent P2X7 receptor antagonists brilliant blue G or periodate-oxidized ATP could significantly attenuate the central sensitization. Specifically, central sensitization could be induced by superfusion of ATP and even more effectively produced by the P2X7 receptor agonist benzoylbenzoyl ATP. Consistent with the report that P2X7 receptors are mostly expressed on microglia, superfusion of the microglial blocker minocycline abolished the MO-induced central sensitization. These novel findings suggest that activation of P2X7 receptors in microglia cells may be involved in the development of central sensitization in acute dental orofacial pain [92].

Microglial P2Y12 receptor is also reported to be involved in the central sensitization of orofacial pain [93]. In a tongue cancer, pain model produced by squamous cell carcinoma (SCC) cell inoculation, microglia were strongly activated in TSNC, and administration of MRS2395 or minocycline reversed the associated nociceptive behavior and microglial activation in SCC-inoculated rats. The increased activity of TSNC wide dynamic range nociceptive neurons was also recorded in SCC-inoculated rats. These findings suggest that SCC inoculation results in strong activation of microglia via P2Y12 receptor signaling in the TSNC that is associated with the increased excitability of TSNC nociceptive neurons and the development of central sensitization.

4.4 Adenosine signaling and dental orofacial pain

Purinergic P1 receptor signaling may also exist in trigeminal nerves and affects nociception processing. In 12 healthy female volunteers randomized, double-blind,

placebo-controlled, cross-over trial, the effect of A1 receptor agonist GR79236 on trigeminal nociception processing was investigated. Activation of A1 receptor with GR79236 inhibits trigeminal nociception in humans [94]. In a model of trigeminovascular nociceptive transmission, the superior sagittal sinus (SSS) was stimulated electrically, and the responding nociceptive units were recorded. It has been shown that intravenous administration of the highly selective adenosine A1 receptor agonist, GR79236 had a dose-dependent inhibitory effect on SSS-evoked trigeminal nociceptive activity. Selective adenosine A1 receptor antagonist DPCPX abolished the neuronal inhibitory effect of GR79236 [95]. In another animal experiment, adenosine decreased the amplitude of glutamatergic excitatory postsynaptic currents and increased the unpaired-pulse ratio suggesting that adenosine acts presynaptically to reduce glutamate release from primary afferents. Besides, the adenosine-induced inhibition of excitatory postsynaptic currents was impaired by a selective A1 receptor antagonist, DPCPX, and was mimicked by a selective A1 receptor agonist CPA. These findings suggest that presynaptic A1 receptors decrease action potential-dependent glutamate release from primary trigeminal afferents onto TSNC neurons, and thus adenosine A1 receptors could be a potential target for the treatment of pain of orofacial tissues [96].

4.5 Ecto-nucleotidases in trigeminal nerves and orofacial pain

By the control of ATP degradation and adenosine generation, ecto-nucleotidases drive the shift from ATP-induced nociception to adenosine-induced analgesia [97]. Since ATP induces pain and pain sensitization via activation of P2X receptors and adenosine mediates analgesia via activation of P1 receptors, existence of ecto-nucleotidases and their enzymatic activities in the trigeminal nociceptive pathway will affect the development and maintenance of dental orofacial pain. Recently we have demonstrated the expression and central terminal localization of ecto-nucleotidases (NTPDase3/CD73) in the trigeminal ganglia nociceptive neurons [64, 98]. Considering the pivotal role of purinergic singling in the pathogenesis of neuropathic pain and the preference expression and upregulation of purinergic receptors in the trigeminal nervous system, ecto-nucleotidase expression, and localization in trigeminal nerves might participate in the development of orofacial neuropathic pain.

Using histochemistry staining, ecto-ATPase and AMPase activities were detected in dental pulp odontoblast layer, Raschkow's nerve plexus, and nerve bundles. Interestingly, in inflammatory dental pulp with pulpitis, enzymatic ecto-ATPase activity was significantly upregulated. Specifically, using immunohistochemistry and immunofluorescence staining, NTPDase2 is expressed in Shwann's cells that encapsulate the Aβ and Aσ fibers, while that NTPDase3 and CD73 are detected in nociceptive nerve fibers in dental pulp [60, 64, 98].

Trigeminal ganglia contain both primary sensory neurons and satellite glial cells. Satellite glial cells encapsulate the ganglia neurons and are gap junction channel connected. Via intercellular interaction satellite glia cells affect neuronal excitability and impulse conduction in TG neurons. The ecto-ATPase activity was detected in TG cells. Specifically, NTPDase3 is expressed in TG neurons, including the nociceptive neurons, while NTPDase2 is expressed in TG satellite glial cells that encapsulated the TG neurons [60, 64, 98]. In addition, ecto-AMPases activity is also detected in TG cells and TG nerve fibers. It reveals that CD73 is expressed in TG neurons, including the nociceptive neurons [64]. By control of extracellular ATP degradation and adenosine generation, these enzymes would play a crucial role in orofacial pain signal processing by affecting the excitability, inhibition, and interaction of TG neurons.

TG nociceptive neurons project central nerve fibers to the brainstem and form synapses with the secondary nociceptive neurons in the nociceptive lamina of the TSNC. It has been well established that the nociceptive lamina in the brainstem or spinal cord is a pivotal region for pain signaling transmission, inhibition, modulation, and sensitization. Interestingly, striking ecto-ATPase and ecto-AMPase activities were detected in brainstem TSNC nociceptive lamina. Immunohistochemistry studies confirmed the existence of immunoreactivity for NTPDase3 and CD73 in the nociceptive lamina. Furthermore, it has been demonstrated that incubation with specific anti-NTPDase3 or anti-CD73 antibodies, significantly reduced ecto-ATPase and acto-AMPase activities in TNSC nociceptive lamina, respectively [64, 98]. These findings suggest that NTPDase 3 and CD73 are the major enzymes responsible for ATP degradation and adenosine generation in TSNC nociceptive lamina. Since the neuronal plasticity and central sensitization mainly occurs at the central nociceptive lamina in neuropathic pain, the presence of NTPDase3 and CD73 in TNSC nociceptive lamina may also participate in the central sensitization mechanism in orofacial neuropathic pain.

The characteristic staining patterns for NTPDase3 and CD73 in the nociceptive lamia of TSNC indicate the presynaptic localization of these enzymes [64, 98]. This observation suggests that NTPDase3 and CD73 are produced at TG nociceptive neurons and then are transferred to the central presynaptic membranes along the afferent trigeminal nerves. Disruption of ecto-nucleotidase expression and presynaptic localization caused by biological, chemical, or physical trigeminal insults such as virus infection, nerve fiber differentiation, and physical constriction//compression may attribute to pathogenesis mechanism in trigeminal neuralgia and other orofacial neuropathic pain [64, 98].

5. Conclusion

Purinergic signaling plays essential role in pain signal processing in the nociceptive pathway from peripheral to central nerves. Via activation of P2X, P2Y, and P1 receptors, ATP and its metabolites induced purinergic signaling participates in the nociceptive transduction, conduction, transmission, modulation, sensitization, and development of neuropathic pain. Ecto-nucleotidases are the predominant enzymes responsible for extracellular ATP degradation and adenosine generation that play essential role to drive the shift from ATP-induced pain to adenosine-induced analgesia. In order to identify the role of purinergic signaling in dental orofacial pain, the existence of purinergic signaling and their regulation in the trigeminal nociceptive pathway has yet to be identified.

Several ion channels and receptors that are prominent in craniofacial nociceptive mechanisms have been identified on trigeminal primary afferent neurons. Many of these receptors and channels exhibit unusual distributions compared with extracranial regions. For example, expression of the ATP receptor P2X3 is strongly implicated in nociception and is more abundant on trigeminal primary afferent neurons than analogous extracranial neurons. P2X3 receptors are often co-expressed with the nociceptive neuropeptides CGRP and SP in trigeminal ganglia neurons. Co-expression of P2X3 receptor and other nociceptors (TRPV1, and ASIC3) in trigeminal neurons imply the existence of functional complexes that allow craniofacial nociceptive neurons to respond synergistically to altered ATP and other pain mediators. These observations indicate that trigeminal P2X3 receptor expression pattern differs markedly from dorsal root ganglion that may provide a clue to explain the unique properties of dental orofacial pain. Different expression and or regulation of purinergic signaling in the trigeminal nociceptive pathway may

attribute to a nociceptive mechanism of dentin hypersensitivity and dental orofacial pain. Identification of the underlying nociceptive mechanism will unveil potential targets for better treatment and management of dental orofacial pain.

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