ABSTRACT

Due, in part, to the unique structure of the tooth, dental pain is initiated via distinct mechanisms. Here we review recent advances in our understanding of inflammatory tooth pain and discuss 3 hypotheses proposed to explain dentinal hypersensitivity: The first hypothesis, supported by functional expression of temperature-sensitive transient receptor potential channels, emphasizes the direct transduction of noxious temperatures by dental primary afferent neurons. The second hypothesis, known as hydrodynamic theory, attributes dental pain to fluid movement within dentinal tubules, and we discuss several candidate cellular mechanical transducers for the detection of fluid movement. The third hypothesis focuses on the potential sensory function of odontoblasts in the detection of thermal or mechanical stimuli, and we discuss the accumulating evidence that supports their excitability. We also briefly update on a novel strategy for local nociceptive anesthesia via nociceptive transducer molecules in dental primary afferents with the potential to specifically silence pain fibers during dental treatment. Further understanding of the molecular mechanisms of dental pain would greatly enhance the development of therapeutics that target dental pain.

KEY WORDS: nociceptor, hydrodynamic theory, dentinal hypersensitivity, odontoblasts, neurobiology, anesthesia.
are transmitted by dental nerves that innervate the tooth pulp and dentin. In this regard, the tooth is unique as a sensory organ, in that it consists of an extensively innervated pulp encapsulated by a mineralized dentin and enamel layer. The low compliance of tooth pulp is thought to contribute to the exaggerated pain sensitivity in conditions of inflammation. In addition, several inflammatory mediators and growth factors lead to sprouting and changes in neuropeptide expression of dental afferent neurons, which could also result in increased pain sensitivity (Sessle, 2011).

Numerous chemicals, including bacterial glycoproteins, neuropeptides, cytokines, and growth factors, sensitize neurons in inflammatory conditions, and it is likely that dental afferent neurons possess activation and sensitization mechanisms similar to those of neurons innervating other tissues (Sessle, 2011). The role of the neuropeptide substance P (SP) in inflammatory dental pain, demonstrated by increased SP production and release in response to noxious stimulation of dental pulp (Caviedes-Bucheli et al., 2011), has recently garnered particular interest. Furthermore, SP is highly increased in inflamed teeth (100-fold) and irreversible pulpitis (1,000-fold) compared with normal conditions (Sacerdote and Levini, 2012). SP exerts its effect via its specific receptor, neurokinin-1 (NK-1). It has been demonstrated that SP activation of NK-1 enhances the activity of transient receptor potential vanilloid 1 (TRPV1) and purinergic P2X3, nociceptors (Zhang et al., 2007; Park et al., 2010) and therefore might sensitize peripheral sensory neurons. In addition to direct interaction with sensory neurons, SP might also activate leukocytes, leading to the production of pro-inflammatory mediators and cytokines. SP induces release of histamine from mast cells, which in turn elevates blood pressure and vascular permeability, causing pulsating inflammatory pain (Sacerdote and Levini, 2012).

The role of another neuropeptide, calcitonin gene-related peptide (CGRP), in inflammatory dental pain was suggested by reduced c-Fos expression in the brainstem after treatment of the monoclonal antibody of CGRP in inflamed teeth (Bowler et al., 2013). Chemical mediators such as histamine, serotonin (5-HT), opioids, and cytokines have been described in studies of inflammatory dental pain, with pulpal perfusion, microdialysis, and applications in human pulp (Sessle, 2011). The differential sensitivity of dental pulp to 5-HT in females was recently demonstrated, potentially explaining the higher prevalence of dental pain in women (Loyd et al., 2012). In addition, it has been suggested that inflammatory mediators may cause changes in gene expression of the trigeminal ganglia, including neuropeptide generation, intracellular molecular alteration, and increased translation of ion channels such as TRP channels, P2X, channels, and voltage-gated sodium channels, which result in increased neuronal excitability (Sessle, 2011).

Neuronal activation leading to inflammatory dental pain requires a specific transducer for nociception. It is well-known that the TRPV1 ion channel plays a central role in peripheral nociception. TRPV1 is a polymodal receptor activated by various painful chemicals and by noxious temperatures (above 42°C) (Chung and Oh, 2013). It has been shown that certain inflammatory mediators can either lower the threshold of activation TRPV1 or potentiate its activity, as is the case with phospholipase C during inflammation (Chung and Oh, 2013). Interestingly, in the context of dental pain, TRPV1 is up-regulated in the trigeminal ganglion following experimental pulpitis induced by lipopolysaccharide (LPS), a product of Gram-negative bacteria (Chung et al., 2011), and it has recently been suggested that inflammation might induce up-regulation of TRPV1 in adjacent teeth, thereby causing ectopic pain in uninflamed adjacent teeth (Matsuura et al., 2013). Together, this evidence suggests that TRPV1 might serve as an integrator of chemical, physical, and inflammatory factors and that increased expression of TRPV1 in dental primary afferent neurons might contribute to the hyper-excitability to warm stimuli observed in

**Figure 1.** Three hypotheses to explain dental nociception. (A) Neural theory: Nerve endings in the dentinal tubule are directly activated by external stimuli. (B) Hydrodynamic theory: Fluid movement within dental tubules is detected by nerve endings. (C) Odontoblast transducer theory: Odontoblasts act as pain receptors, reminiscent of the role of taste receptor cells in taste buds.
patients with inflammatory conditions such as chronic pulpitis (Chung et al., 2010).

Despite numerous contributing factors, anti-inflammatory treatments such as non-steroidal anti-inflammatory drugs (NSAIDs) may help resolve many of the symptoms of inflammatory dental pain. More problematic to treat, however, are patient reports of abrupt intense pain in response to normally innocuous stimuli, known as dentinal hypersensitivity.

**DENTINAL HYPERSENSITIVITY**

**Neural Theory**

The neural theory of dentinal hypersensitivity describes the role of nociceptive trigeminal ganglion neurons that innervate dental pulp (i.e., dental primary afferents). To play a sensory role, the neuron must express a receptor that takes part in the transduction of a specific stimulus to electrical impulses. Investigation of nociceptive receptors revealed the functional expression of several temperature-sensitive (thermo-) TRP channels in trigeminal ganglion neurons and, more specifically, in dental primary afferent neurons (Chung and Oh, 2013).

Electrophysiological recordings with the application of capsaicin or noxious temperature originally suggested the functional expression of TRPV1 and TRPV2 in rodent dental primary afferent neurons. A combination of electrophysiological, single-cell RT-PCR, and immunohistochemical analyses revealed that dental afferent neurons express TRPV1 and TRPV2 more abundantly relative to the population of trigeminal ganglion neurons, consistent with the traditional view that most dental afferent neurons are nociceptors. These data must be interpreted with caution, however, since it is possible that pulp exposure during cavity preparation for the labeling of dental afferent neurons might itself induce the up-regulation of TRPV1 (Tarsa et al., 2010). In addition, the high prevalence of TRPV1 in isolated trigeminal ganglion cultures might reflect a bias toward small-sized TRPV1 positive neurons, since large-sized neurons are more prone to damage during acute isolation.

Cold stimulus is a frequent cause of the tooth pain that cannot be explained by the expression and activation of TRPV1 or TRPV2. It is known that subpopulations of Aδ- and C-fiber neurons are responsive to cooling. Calcium imaging experiments have revealed that neurons responding to cool temperatures (below 18°C) are more prevalent in the trigeminal ganglion (14%) than in the dorsal root ganglion (7%) (McKemy, 2005). TRPM8 and TRPA1 receptors are activated by low temperatures with thresholds of 25°C and 17°C, respectively. Similar to TRPV1, TRPM8 is preferentially expressed in small-sized trigeminal ganglion neurons and also activated by various chemicals, including menthol, menthone, eucalyptol, spearmint, WS-3, and icilin (Tominaga and Caterina, 2004). However, the relatively modest temperature threshold for activation and the lack of expression overlap with other nociceptive markers mean that the contribution of TRPM8 in the detection of noxious cold temperatures remains unclear. Moreover, 2 independently derived lines of Trpm8 gene knockout mice showed behavioral deficiencies only in the detection of moderately cool temperatures (Bautista et al., 2007; Dhaka et al., 2007). In contrast, the TRPA1 receptor is co-expressed in TRPV1-expressing neurons and has been proposed as a noxious cold detector (Story et al., 2003). However, extensive studies on the activation of TRPA1 over a cold temperature range suggest that cold-induced activation of TRPA1 is via an indirect mechanism (Caspiani and Heppenstall, 2009) and is a key mediator of cold hypersensitivity in pathological conditions, rather than cold noceception per se (Patapoutian et al., 2009).

The expression patterns of TRPM8 and TRPA1 in dental primary afferent neurons have been well-documented (Chung and Oh, 2013). Up-regulation of TRPA1 in response to experimental tooth injury suggests that the cold receptor might play a central role in dental pain (Haas et al., 2011). However, it is interesting that the prevalence of TRPM8 and TRPA1 expression in trigeminal ganglion neurons was lower than that of TRPV1 (Park et al., 2006), despite the propensity for cold-stimuli-induced tooth pain. Moreover, the cold receptors TRPM8 and TRPA1 are co-expressed within subpopulations of TRPV1-positive dental afferent neurons. These observations might help to explain why it is difficult to discriminate between hot and cold stimuli applied to teeth; what remains unknown is the reason for cold stimuli being a more frequent cause of dental pain. Investigation of the central projection of the dental primary afferents might contribute to understanding of the detection of noxious temperatures. Nevertheless, thermo-TRP channels are in a prime position to contribute to the primary detection of hot and cold temperatures in the tooth (Fig. 2).

**Hydrodynamic Theory**

Sudden and intense tooth pain can also be elicited by normally innocuous stimuli such as water spray, an air puff, or sweet substances. This wide range of dental pains cannot be explained solely by the transduction of noxious temperature by thermo-TRP channels. Pulsating pain often described by chronic pulpitis patients suggests that tooth pain might be induced by hydrostatic pressure applied to inflamed pulp tissue encased within hard dentin structures (Heyeraas and Berggreen, 1999). The pulsating and intense pain induced by a light air puff suggests that the generation of dental pain might involve the detection of mechanical force. Indeed, in vivo single nerve fiber recordings of beagle dogs revealed that 75% of mandibular pulpal nerves responded to mechanical stimulation of exposed pulp (Chung and Oh, 2013).

These suppositions have led to a “hydrodynamic theory” which describes the cause of dental pain in terms of mechanical forces generated by the movement of dentinal fluid. A detailed description of hydrodynamic theory can be found in several recent reviews (Brederson et al., 2013; Chung and Oh, 2013). It was recently suggested that the inward and outward movement of dentinal fluid contributes differentially to dental pain (Lin et al., 2011). Since outward movement of the fluid in response to cold stimuli is faster than inward movement by hot stimuli, cold sensation is more readily detected by Aδ fibers in dentinal tubules as sharp pain in early pulpitis. As pulpitis progresses, sensitized C-fibers within deep pulpal tissue can be activated by...
the inward movement of dentinal fluid by hot stimuli and perceived as dull pain.

So far, a molecular transducer that accounts for the mechanical detection of dentinal fluid movement has yet to be found (Fig. 3). Such a molecule must first be defined and shown to be functional in a tooth before hydrodynamic theory can be validated as a model for dentinal hypersensitivity. In fact, cellular mechanical force transducer molecules have recently begun to be elucidated (Chung and Oh, 2009). Among several candidates that show mechanosensitivity are TRPV1, TRPV2, and TRPA1, which have already been shown to be expressed in dental afferents; expression of other candidate mechanotransducers, TRPV4 and TRPM3, has been reported only in trigeminal ganglion neurons, but not in labeled dental primary afferents (Chung and Oh, 2013).

TRPA1 is particularly interesting in relation to tooth pain, since it is implicated in both cold hyperalgesia and mechanosensations. The dual function of TRPA1 might explain why dental pain elicited by a light puff of air is often confused with cold nociception. The role of TRPA1 as a cellular mechanical transducer was called into question by a report of normal hearing ability in TRPA1-knockout mice (Bautista et al., 2006; Kwan et al., 2006), although it is possible that another cellular mechanism might compensate for the deleted TRPA1 function in the transgenic mice. Several lines of evidence also propose TRPV1 as a receptor for mechanotransduction or hyper-osmolarity, although these remain controversial (Chung and Oh, 2013). If TRPV1 were to play a role as a detector of hypertonicity in dental afferents, it could feasibly contribute to the generation of dental pain in response to hyperosmotic conditions such as the consumption of sweet substances. An investigation of the responses of dental primary afferent neurons to mechanical stress with appropriate receptor antagonists might elucidate a potential functional role of TRPV1 or TRPA1 in mechanically induced dental pain.

TRPV1 and TRPA1 are expressed in a subpopulation of peptidergic C-fibers (Babbsbaum et al., 2009; Cavanaugh et al., 2011). However, recent publications suggest that a non-peptidergic IB4-positive population of primary afferents plays an important role in the transduction of mechanical stimuli in skin (Abrahamsen et al., 2008; Cavanaugh et al., 2009), and, more recently, a non-peptidergic mechanosensitive neuron subpopulation was demonstrated in trigeminal ganglion neurons (Chung et al., 2012). The molecular identity of the mechanical transducer in non-peptidergic polymodal nociceptors remains to be elucidated.

It is of course possible that dental pain might not even be mediated by classic C-fiber nociceptive neurons. Because of their high mechanical threshold for activation, for example, C-fibers cannot explain the sudden and intense pain induced by low-threshold mechanical stress from a very light touch such as an air-puff. In fact, most dental afferents are medium-to-large myelinated A-fibers (Paik et al., 2009; Fried et al., 2011), which are believed to be low-threshold mechano-sensitive neurons, not normally nociceptive. In addition, all of the mechanosensitive pulpal nerve fibers in the beagle dog were identified as myelinated A-fibers, according to conduction velocities (Chung and Oh, 2013). To explain these observations, A-fiber innervating dental pulp were recently proposed as low-threshold ‘algoneurons’: low-threshold mechanoreceptors involved in nociception, in contrast to conventional low-threshold mechanoreceptors thought to transduce light touch in other parts of the body (Fried et al., 2011). It is critical therefore that the molecular identity of mechanical transducers in low-threshold ‘algoneurons’ be addressed in future studies. It is possible that TRPV2 might play an important role as a mechanical transducer in algoneurons, since TRPV2 has been preferentially detected in medium-to-large neurons (Caterina et al., 1999; Ichikawa and Sugimoto, 2000), and stretch-induced activation of TRPV2 was reported in vascular smooth muscle (Muraki et al., 2003). Putative mechanosensitive ion channels revealed by single-cell RT-PCR analysis of dental primary afferent neurons, such as TRPM3, TRPV4, ASIC3, TREK-1, TREK-2, ENaC-α, and ENaC-γ, might therefore also contribute to dental pain (Hermanstyne et al., 2008; Vandewauw et al., 2013).
Odontoblast Transducer Theory

Odontoblasts constitute a cell layer at the outermost part of dental pulp and secrete mineralized calcium matrix to form dentin. Their strategic location, along with several lines of evidence, suggests that odontoblasts might play an additional role as sensory transducers (Fig. 4). To confirm such a role in odontoblasts, the following 3 criteria must be met: functional expression of receptors for specific stimuli, ability to transmit signals following the activation of receptors, and a synaptic structure between odontoblasts and neurons.

Supporting the first criteria are data demonstrating the expression of several members of TRP channels in rat and human odontoblasts (Chung and Oh, 2013). Odontoblasts from neonate rats were shown to express TRPV1, TRPV2, TRPV3, TRPV4, and TRPM3 by single-cell RT-PCR, immunohistochemistry, and calcium imaging analysis (Son et al., 2009); however, in a separate study, odontoblasts isolated from adult rats did not display evidence of TRPV1 and TRPV2 (Yeon et al., 2009). Whether these differences in TRPV1 and TRPV2 expression between adult and neonate rats are due to developmental changes or simply due to differing experimental conditions remains to be confirmed. The cold receptors TRPM8 and TRPA1 were not detected in either study, suggesting that any potential sensory role of odontoblasts might not involve TRPM8 and TRPA1, at least in rats (Chung and Oh, 2013). However, calcium imaging experiments showed human odontoblasts to express TRPM8 and TRPA1 as well as TRPV1 (El Karim et al., 2011). Interestingly, of the thermo-TRP channels identified in odontoblasts, TRPV1, TRPV2, TRPV4, and TRPM3 are also thought to possess mechanosensitivity as well. Thus, it is equally possible that these receptors might contribute to a sensory role of odontoblasts by mechano-transduction (Magloire et al., 2010; Gibbs et al., 2011). In addition, the expression of mechanosensitive K⁺ channels and N-type Ca²⁺ channels was also shown in odontoblasts (Magloire et al., 2010). Recently, it was demonstrated that cooperation of TRP channels with mechanosensitive K⁺ channels participated in the perception of temperature (Noël et al., 2009), which further suggests the role of odontoblasts as sensory cells.

Evidence is now also accumulating that supports the direct excitability of odontoblasts. Such data include the functional expression of voltage-gated Na⁺ channels, voltage-gated K⁺ channels, calcium-activated K⁺ channels, store-operated calcium channels, Na⁺/Ca²⁺ exchanger, and TREK-1 channels; action potentials have also been evoked in odontoblasts by electrical stimulation in vitro (Lundquist et al., 2000; Shibukawa and Suzuki, 2003; Magloire et al., 2010). Although further functional studies are required, analysis of these data together suggests fulfillment of the second criterion for a role of odontoblasts as sensory transducers.

Finally, a mechanism of signaling between odontoblasts and underlying dental primary afferents must be demonstrated to support the sensory role of odontoblasts. Recent findings suggest that the activation of purinergic P2X receptors in orofacial tissues is sufficient to produce central sensitization in the medullary dorsal horn (Cherkas et al., 2012). Expression of P2X₃ (Alavi et al., 2001) and P2X₇ receptors (Itoh et al., 2011) in nociceptive dental primary afferents, together with the intimate relationship of odontoblasts to trigeminal sensory axons

Figure 3. Molecular mechanisms of hydrodynamic theory. Fluid movement initiated by diverse external stimuli eventually activates mechanoreceptors in dental primary afferents. Candidates of mechanosensitive molecules are listed. However, little is known about how the activation of the low-threshold mechanoreceptor is eventually perceived as pain in the central nervous system.

Figure 4. Molecular mechanisms of odontoblast transducer theory. Listed are candidates of temperature-sensitive molecules and mechanosensitive molecules, including primary cilia, which allow odontoblasts to function as sensory receptors. Activation of these molecules may produce pain following transmitting signals to dental primary afferents or contribute to the formation of tertiary dentin.
(Magloire et al., 2010), suggests that ATP could mediate painful signaling between odontoblasts and neurons (Lim and Mitchell, 2012). The question remains as to how such a transmitter is released from odontoblasts to stimulate adjacent neurons.

ATP has long been proposed as an excitatory neurotransmitter (Evans et al., 1992), but not until the past decade have the mechanisms of vesicular uptake of ATP been clarified (Sawada et al., 2008). In addition, evidence for multiple mechanisms of non-exocytotic ATP release has been documented (Lazarowski et al., 2003). In support of the latter mechanisms is a lack of evidence for neurotransmitter-containing vesicles in odontoblasts (Magloire et al., 2010). ATP release mechanisms from non-neuronal cells include ATP-binding cassette transporters, connexin/pannexin hemichannels, voltage-dependent anion channels, and P2X, channels (Burnstock, 2007). Investigation of these mechanisms in odontoblasts, along with functional analysis of neurons co-cultured with odontoblasts, may shed light on chemical transmission between odontoblasts and neurons.

Recently, it was shown that hydrodynamic pressure facilitates the differentiation of human dental pulp stem cells into odontoblastic cells (Yu et al., 2009), which suggested that mechanical force applied to the dento-pulp complex might contribute to the formation of dentin instead of initiating nerve impulses. This could be the mechanism underlying the formation of tertiary dentin. The primary cilium, present in almost every eukaryotic cell and essential for mechanical sensation (Praetorius and Spring, 2005), may take part in the detection of mechanical force in odontoblasts (Magloire et al., 2010). Since the primary cilium in bone cells has been shown to play a critical role in the detection of dynamic fluid flow and synthesis of calcium matrix in response (Malone et al., 2007), it is possible that the primary cilium in odontoblasts might play a similar role (Thivichon-Prince et al., 2009). In vitro studies of odontoblasts with various fluid flow conditions might provide a definite answer to this question.

**SILENCING OF DENTAL NOCICEPTIVE NEURONS**

Lidocaine is a widely used local anesthetic agent that inhibits the activation of voltage-gated sodium channels and abolishes the propagation of action potentials. However, the non-specific blockade of nerve impulses may result in anesthesia (numbness), paresthesia (abnormal tingling or swollen feeling), and, in rare cases, immobility, thus causing discomfort to patients. Recently, it was demonstrated that a permanently charged derivative of lidocaine (QX-314) can be used to abolish neuronal transmission specifically in nociceptive neurons. Specific delivery of QX-314 into nociceptive nerve fibers was achieved by concurrent activation of the TRPV1 receptor with capsaicin (Binshtok et al., 2007). We have further demonstrated that selective silencing of nociceptive responses can be achieved in the trigeminal system (Kim et al., 2010) (Fig. 5), and that gingival injection of the same nociception anesthetic can reduce c-fos-related neuronal activation in the brainstem following tooth extraction injury (author’s unpublished observations). Further development of such approaches may provide a new possibility for achieving pain-selective local anesthesia in the dental clinic.

A lingering concern of such an approach, however, is the necessary use of a potentially algogenic substance such as capsaicin. Interestingly, lidocaine itself may represent a less irritative agonist for TRPV1 (Leffler et al., 2008) capable of inducing selective analgesia (Binshtok et al., 2009). In addition, other potential conduits for QX-314 entry, such as TRPA1 and other transient receptor potential channels, may offer new avenues for pain-selective local anesthesia.

**Figure 5.** Selective silencing of dental nociceptive neurons. Entry of a permanently charged derivative of sodium channel blocker through TRPV1 ion channels prevents the generation of action potential firings only in TRPV1-expressing nociceptors, but not in motor neurons and proprioceptive neurons (Kim et al., 2010). This strategy allows for the production of pain-specific local anesthesia and may have potential clinical utility for specifically treating dental pain, leaving non-nociceptive and motor functions untouched.
P2X receptors, may offer a route to target nociceptive neurons that do not express TRPV1 (Kim et al., 2011).

CONCLUSIONS

Dental pain arguably affects the life quality of patients more than pain in any other part of the body, yet the mechanisms underlying the transduction of nociceptive information from tooth structures are far less well-known than other somatic signaling mechanisms. Tooth is a unique tissue with dense innervation of a pulp encased in a hard shell. Many questions remain to be answered, such as how we commonly perceive sensory stimulation of teeth as cold, or perceive a seemingly innocuous air-puff or cool drink as a sudden intense pain. The research summarized in this review suggests that nociceptive processing in the trigeminal system might differ from that in other parts of the body. Low-threshold mechanoreceptors that are responsible for the transduction of a light touch in other body areas might mediate nociception in teeth. Elucidation of the differential molecular mechanisms underlying nociception in teeth will provide an invaluable basis for the development of therapeutics for tooth-related pain.

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