Activation of Mechanosensitive Transient Receptor Potential/Piezo Channels in Odontoblasts Generates Action Potentials in Cocultured Isolectin B_4–negative Medium-sized Trigeminal Ganglion Neurons

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Abstract

Introduction: Various stimuli to the dentin surface elicit dentinal pain by inducing dentinal fluid movement causing cellular deformation in odontoblasts. Although odontoblasts detect deformation by the activation of mechanosensitive ionic channels, it is still unclear whether odontoblasts are capable of establishing neurotransmission with myelinated A delta (Aδ) neurons. Additionally, it is still unclear whether these neurons evoke action potentials by neurotransmitters from odontoblasts to mediate sensory transduction in dentin. Thus, we investigated evoked inward currents and evoked action potentials form trigeminal ganglion (TG) neurons after odontoblast mechanical stimulation. **Methods:** We used patch clamp recordings to identify electrophysiological properties and record evoked responses in TG neurons. **Results:** We classified TG cells into small-sized and medium-sized neurons. In both types of neurons, we observed voltage-dependent inward currents. The currents from medium-sized neurons showed fast inactivation kinetics. When mechanical stimuli were applied to odontoblasts, evoked inward currents were recorded from medium-sized neurons. Antagonists for the ionotropic adenosine triphosphate receptor ($P2X_3$), transient receptor potential channel subfamilies, and Piezo1 channel significantly inhibited these inward currents. Mechanical stimulation to odontoblasts also generated action potentials in the isolectin B_4–negative medium-sized neurons. Action potentials in these isolectin B_4–negative medium-sized neurons showed a short duration. Overall, electrophysiological properties of neurons indicate that the TG neurons with recorded evoked responses after odontoblast mechanical stimulation were myelinated Aδ neurons. **Conclusions:** Odontoblasts established neurotransmission with myelinated Aδ neurons via $P2X_3$ receptor activation. The results also indicated that mechanosensitive TRP/Piezo1 channels were functionally expressed in odontoblasts. The activation of $P2X_3$ receptors induced an action potential in the Aδ neurons, underlying a sensory generation mechanism of dentinal pain. *(J Endod 2018;■:1–8)*

Key Words

Hydrodynamic theory, mechanotransduction, neurotransmission, odontoblast, pain, trigeminal ganglion

Significance

The deformation of odontoblasts by hydrodynamic forces activates mechanosensitive Piezo1/TRP channels. This induces the release of ATP and activates $P2X_3$ receptors on myelinated Aδ neurons. The activation of $P2X_3$ receptors induces an action potential in Aδ neurons and generates dentinal pain.

Noxious and innocuous stimuli applied to the orofacial area are mainly received by the trigeminal ganglion (TG) neurons. These neurons consist of subpopulations of nonnociceptive and nociceptive neurons. The nociceptive neurons are further subdivided into high-threshold mechanoreceptors, polymodal, and mechanoheat and mechanocold nociceptors in the A delta (Aδ) and C afferents (1). Ninety percent of C neurons and 70% of Aδ neurons are involved in nociception (2). Dentin and pulp are innervated by afferent axons of the TG neurons including Aβ, Aδ, and C afferents (3). The tooth pain that occurs in the dentin-pulp complex is classified into 2 types: pain occurring in the dentin (dentin sensitivity or dentinal pain (4)), which is mediated by Aδ afferents (sharp [first] pain), and pathological pain in the dental pulp (pulpal pain), which is generated by C afferents inside the dental pulp (slow [second] pain) (3–7).

Recent converging evidence (8–14) has shown that odontoblasts act as sensory receptor cells to generate dentinal pain. Ca^{2+} signaling in response to the activation of various mechanosensitive ionic channels (such as mechanosensitive transient receptor potential [TRP] channels vanilloid subfamily 1 [TRPV1], TRPV2, and TRPV4 and ankyrin subfamily 1 [TRPA1]) occurs by cell membrane deformation (ie, direct mechanical stimulation to the odontoblasts) (15–20). Not only adenosine

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triphosphate (ATP) but also glutamate is released from stimulated odontoblasts as neurotransmitters via pannexin 1 channels and glutamate-permeable anion channels, respectively (12, 13, 21). These neurotransmitters activate ionotropic ATP receptor subtype 3 ($P2X_3$ receptors), G protein–coupled nucleotide receptor subtype 1 ($P2Y_1$ receptors), G protein–coupled nucleotide receptor subtype 12 ($P2Y_{12}$ receptors), and metabotropic glutamate receptor (GluR) subtypes group I to group III in the TG neurons. Intercellular odontoblast-neuron signal transmission explains the basis of the sensory transduction mechanism of dentinal pain, named the "odontoblast hydrodynamic receptor theory" (12, 13).

Although the odontoblasts should be connected to the intradental A afferents to induce dentinal pain (dentin sensitivity), previous reports showing intercellular odontoblast-neuron signal communication has not been distinguished between A or C neurons in the TG neurons to describe the "odontoblast hydrodynamic receptor theory" (11–13). In addition, if this neurotransmission mediates the generation of dentinal pain, the trigeminal A neurons should evoke action potentials after odontoblast mechanical stimulation.

Through a current signature method in whole-cell patch clamp recordings, acutely isolated TG neurons in rats have been precisely subclassified into 9 cell types: 1 to 5, 7 to 9, and 13 (1). These cells have been divided into 2 groups: small-sized (types 1, 3, and 7, with diameters from 15–24 μm) and medium-sized (types 4, 5, 8, 9, and 13, with diameters from 25–38 μm), whereas type 2 was a mixed group of both cell sizes. The medium-sized type 4, 8, 9, and 13 TG neurons are classified as Aδ neurons (1). Among the sensory neurons that include dental pulp afferent of the TG neurons, unmyelinated C neurons show action potentials with a deflection (hump) during the repolarization phase, showing long-duration action potentials, whereas myelinated Aδ neurons exhibit short-duration action potentials without a deflection (1, 22). According to the current signature classification of TG neurons, medium-sized type 4, not types 8, 9, and 13, neurons show short-duration action potential. In addition, among 9 subclassified cell types of TG neurons, only small-sized type 3 and medium-sized type 4 neurons show voltage-dependent inward currents with fast inactivation properties (1). Type 4 medium-sized neurons (31–38 μm in diameter) are also isolectin B_4 (IB_4, as an unmyelinated marker) binding negative, tetrodotoxin (TTX) sensitive, and capsaicin insensitive (1). These properties indicated that type 4 TG neurons are typical Aδ neurons according to the current signature classification.

Recently, Piezo proteins, Piezo1 and Piezo2, have been identified as essential components of mechanosensitive ionic channels (23–25). Piezo2 proteins are expressed in the Merkel cells, which mediate touch sensation (26), whereas Piezo1 proteins play important roles in various cellular processes, including sensing shear stress, and form a trimeric propellerlike structure with 3 distal blades and a central cap in the extracellular domain (27).

To further confirm the mechanism of neurotransmission from the odontoblasts to TG neurons to mediate dentinal pain (dentin sensitivity), we recorded evoked current and evoked action potentials from medium-sized type 4 Aδ neurons after odontoblast mechanical stimulation in the odontoblast–TG neuron coculture system. We also examined the contribution of Piezo1 in mechanosensitive processes in odontoblasts.

Materials and Methods

Ethical Approval

This study was approved by the Ethics Committee of Tokyo Dental College, Tokyo, Japan (no. 270301, no. 270302, and no. 270303). All animals were treated in accordance with the Guiding Principles for the Care and Use of Animals in the field of physiological sciences approved by the Council of the Physiological Society of Japan and the American Physiological Society.

Solutions and Reagents

Solution containing 136 mmol/L NaCl, 5 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 0.5 mmol/L $MgCl_2$, 10 mmol/L 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), 10 mmol/L glucose, and 12 mmol/L $NaHCO_3$ (pH = 7.4 with tris[hydroxymethyl]aminomethane) was used as the standard extracellular solution (ECS). Details are described in Supplemental Appendix S1. We applied ECS or ECS-containing drugs using a gravity-fed perfusion system. The solution temperature was maintained at room temperature (30°C ± 1.0°C) (TC324 heater controller; Warner Instruments, Hamden, CT) to avoid unexpected thermal stimulation of the cells during recordings in all experiments.

Dental Pulp Slice Preparation

Dental pulp slice preparations were obtained from newborn Wistar rats (aged 3–10 days) (12, 20, 28, 29). Detailed methods are described in Supplemental Appendix S1. The primary cultured odontoblasts in the dental pulp slices were positive for the markers dentin matrix protein 1, dentin sialoprotein, and nestin (17) and were therefore identified as odontoblasts.

Isolation of the TG Neurons

TG neurons were dissected and isolated from neonatal Wistar rats (aged 4–7 days) of both sexes under isoflurane (3%) and pentobarbital sodium anesthesia (50 mg/kg, intraperitoneal injection) (12, 13, 30–32). Detailed methods are described in Supplemental Appendix S1.

Odontoblast–TG Neuron Coculture

Preparation of the odontoblast–TG neuron coculture was followed by a previously described method (12, 13) with a modification. Primary cultured TG neurons in Hank's balanced salt solution (Life Technologies, Grand Island, NY) were resuspended; incubated with 1 μg/mL fluorescent lipophilic tracer 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate ($DiIC_{18}$[3], Life Technologies) for 10 minutes at 37°C; and then rinsed with fresh standard ECS. $DiIC_{18}$[3]-labeled TG neurons were immediately added to dishes of primary cultured dental pulp slices. The coculture was incubated in fresh standard ECS for 15 minutes before the experiments, and then voltage clamp recordings were performed. For current clamp recordings, the odontoblast–TG neuron coculture was further incubated with IB_4 conjugated to fluorescein isothiocyanate (10 μg/mL) at room temperature for 10 minutes before the recordings and then rinsed with fresh standard ECS. The TG neurons in the coculture are typically spherical and are devoid of branching structures, such as dendrites, which influence patch clamp recordings in the cells.

Mechanical Stimulation of Single Odontoblasts

Mechanical stimulation (11, 12) was applied using a fire-polished glass micropipette with a tip diameter of 2–3 μm that was filled with standard ECS. The micropipette was pulled from capillary tubes using a DMZ Universal Puller (Zeitz Instruments, Martinsried, Germany). To apply precise stimulation, we used a 3-dimensional electric micromanipulator (Eppendorf, Hamburg, Germany). After the tip was positioned just above the target odontoblast, the pipette was moved downward by 8.5 μm at a velocity of 2.2 μm/s to apply focal mechanical stimulation and depress the cell membrane. The stimulation was applied for 20 seconds; after which, the pipette was retracted at the