The odontoblast as a sensory receptor cell? The expression of TRPV1 (VR-1) channels

Reijiro Okumura1,2,3, Kaori Shima2, Takashi Muramatsu2, Kan-ichi Nakagawa3, Masaki Shimo2, Takashi Suzuki1, Henry Magloire4, and Yoshiyuki Shibukawa1

Departments of 1Physiology, 2Pathology, and 3Endodontics, Tokyo Dental College, Chiba, Japan; and 4Laboratoire du Developpement des tissus dentaires, EA 1892, IFR 62, Faculte d’Odontologie, Lyon, France

Summary. Previous reports have shown the expression of several mechanosensitive ion channels on the plasma membrane in odontoblasts, which are the cells responsible for dentin formation. The membrane characteristics of odontoblasts imply that they could play critical roles in the mechanotransduction of fluid displacement within dentinal tubules into the electrical cell signals, to carry dentin sensation to the central nervous system. However, the direct ionic mechanism underlying such a dentin nociceptive function remains unclear. In the present study, we investigated the expression of the transient receptor potential vanilloid subfamily member 1 (TRPV1) channel—which essentially contributes to the detection of pain sensation—in rat odontoblasts by immunohistochemical and nystatin perforated patch-clamp techniques. Immunohistochemical observation showed the localization of TRPV1-immunoreactions on the distal regions of odontoblast membranes. In the patch-clamp experiments, we observed capsaicin-induced inward currents that were inhibited by capsazepine, a TRPV1 channel antagonist. Our results indicate a significant expression of TRPV1 channels in odontoblasts, suggesting that odontoblasts may directly respond to noxious stimuli such as a thermal-heat stimulus, and point to the necessity for a reconsideration of the cellular mechanisms of dentin sensation based on the transmembrane ionic signals in odontoblasts.

Introduction

Odontoblasts, well-polarized columnar cells at the periphery of the dental pulp, originate from the neural crest cells. These cells are involved in dentin formation (dentinogenesis) by the synthesis and secretion of collagenous and non-collagenous matrix proteins as well as by participating in the directional Ca++ transporting pathway to the dentin mineralizing front from the circulation. These Ca++ transporting mechanisms are mediated by intracellular Ca++ signaling events via Ca++ mobilization/extrusion mechanisms in odontoblasts (Shibukawa and Suzuki, 2003; Shibukawa et al., 2006 in press).

Under the pathological inflammatory and neuronal responses in the dental pulp, intracellular Ca++ signaling events are accelerated via a Ca++ influx through store-operated Ca++ channels as well as via intracellular Ca++ released from inositol 1,4,5-trisphosphate (IP3)-sensitive Ca++ stores by the activation of phospholipase-C coupled receptors (Shibukawa and Suzuki, 2003). As a result of these cellular signaling events induced by physiological and pathophysiological responses in dental pulp, Na+/Ca++ exchangers are activated to function as a Ca++ extrusion pathway to the dentin mineralizing front, by transporting accumulated intracellular Ca++ (Shibukawa et al., 2006 in press). In addition to the Ca++ signaling cascade induced by physiological and pathophysiological chemical responses in the dental pulp, previous electrophysiological, molecular biological, and immunohistochemical studies have indicated that odontoblasts express mechanosensitive-high conductance Ca++-activated K+ channels (Allard et al., 2000), a hypo-osmotic solution induced stretch-activated Ca++ influx (Shibukawa and Suzuki, 1997),

Received November 22, 2005

Address for correspondence: Dr. Yoshiyuki Shibukawa, Department of Physiology, Tokyo Dental College, 1-2-2, Masago, Mihama-ku, Chiba 261-8502, Japan
Phone/Fax: +81-43-270-3771
E-mail: yshibu@cc.rim.or.jp
and mechanosensitive TWIK (tandem of P domains in weak inward rectifier K+ channels)-related K+ channel 1 (TREK-1) (Magloire et al., 2003). The expression of their specific phenotypes has suggested that the ionic channels in odontoblasts can mediate the mechanosensitive responses to promote pathological dentin formation by a wide range of external tooth stimuli (Allard et al., 2000). In addition, it has been hypothesized that these mechanosensitive and stretch-activated ionic channels in odontoblasts could perform critical roles in the mechanotransduction of fluid displacement within dentinal tubules into the electrical cell signals (Magloire et al., 2004). However, the direct ionic mechanism underlying dentin nociceptive function remains unclear (cf. Yamada et al., 1968; Byers, 1984).

Several phenotypes of the transient receptor potential (TRP) family of ion channels are directly related to the cellular mechanisms in nociceptive and thermosensitive functions (Pedersen et al., 2005). This family comprises more than 30 cation channels, most of which are permeable for Ca$^{2+}$. On the basis of sequence homology, the TRP family has been divided into seven main subfamilies: the TRPC (‘Canonical’) family, the TRPV (‘Vanilloid’) family, the TRPM (‘Melastatin’) family, the TRPP (‘Polycystin’) family, the TRPM (‘Mucolipin’) family, the TRPA (‘Ankyrin’) family, and the TRPN (‘NOMPC’) family.

The cloned capsaicin receptor TRPV1 (vanilloid receptor 1; VR1) (Caterina et al., 1997), a member of the TRP family, is a nociceptive neuron-specific capsaicin-gated ion channel (Pedersen et al., 2005). This channel responds to heat, protons, anandamide, and lipoygenase products (Montell et al., 2002; Clapham, 2003; Corey, 2003), and is located particularly on sensory nerve endings (Pedersen et al., 2005) including trigeminal ganglion neurons (Hiura, 2000; Chaudhary et al., 2001; Yang et al., 2003). The TRPV1 channels are broadly involved in nociception, and an analysis of TRPV1 gene knock-out mice has confirmed that the channels essentially contribute to selective modalities of pain sensation and tissue injury-induced thermal hyperalgesia, suggesting a critical role for TRPV1 channels in the detection or modulation of pain (Caterina et al., 2000; Davis et al., 2000).

In order to elucidate if odontoblasts are involved in the nociceptive signal transduction mechanisms as sensory receptor cells, we have now directly identified the TRPV1 channel expression in odontoblasts using a patch-clamp and immunohistochemical techniques.

Materials and Methods

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.

Immunohistochemistry

Mandibles were dissected from 3 to 13-day-old Wistar rats under anesthesia with pentobarbital sodium (25 mg/kg, I.P.). The mandibles were immediately embedded in O.C.T. compound (TissueTek, Elkhart, IL, USA) and rapidly frozen with liquid nitrogen. Cryostat sections—without decalcification—at a 6 μm thickness were prepared by sagittal slicing of the mandibles including the incisors at −20°C, and were mounted onto glass slides (Matsunami, Osaka).

After blocking to prevent non-specific binding (10% goat serum), cryostat sections were incubated with the anti-TRPV1 polyclonal antibody (dilution 1:300) at room temperature for 1 h. The anti-TRPV1 antibody was for the epitope corresponding to amino acid residues 824–838 of rat TRPV1 (Alomone labs, Jerusalem, Israel). The sections were then incubated with Alexa Fluor 488 goat anti-rabbit IgG (1:100; Molecular probes, Eugene, OR, USA) for fluorescence staining and TO-PRO®-3 iodide (Molecular probes) for nuclear staining at room temperature for 1 h. Sections were examined with a conventional fluorescence microscope (UPM Axiophoto 2: Zeiss, Jena, Germany).

For a negative control, the sections were incubated with 10% goat serum instead of the primary antibody. They did not contain any specific immunoreaction.

Dental pulp slice preparations: whole-cell recording techniques

The dental pulp slice preparation was obtained from newborn Wistar rats using a previously described method (Shibukawa and Suzuki, 1997, 2001, 2003). Briefly, a hemimandible embedded in alginate impression material was sectioned transversely through the incisor at a 500 μm thickness using a standard vibrating tissue slicer (DTK-100, Dosaka EM, Kyoto). A section of mandible was sliced down to the required level where the dentin and enamel could be directly viewed between bone tissues and dental pulp. The surrounding impression material, bone tissue, enamel, and dentin were carefully removed from a section of the mandible and the remaining dental pulp slice was subsequently obtained. In order to form a high
Fig. 1. Immunohistochemical localization of the TRPV1 channel in rat odontoblasts. Asterisks and arrowheads show TRPV1-positive immunoreactions on the cellular processes in the dentinal tubules and on the distal ends of the odontoblast membrane, respectively. D: dentin, O: odontoblast layer, P: dental pulp. Scale bar: 50 μm

Resistance seal between the cell and patch pipette, the pulp slice obtained was treated with Krebs solution containing collagenase and trypsin at 37°C for 30 min. The treated slice was arranged on the glass bottom (precoated with poly-D-lysine) of the recording chamber for patch-clamp recordings.

Data acquisition and analysis: whole-cell recording techniques

A nystatin perforated-patch recording configuration under voltage-clamp conditions for odontoblasts in slice preparation was conducted (Hamill et al., 1981; Horn and Marty, 1988). Patch pipettes with a resistance of 2–5 MΩ were pulled from capillary tubes using a FLAMING/BROWN micropipette puller (Model P80/PC, Sutter Instrument, Novato, CA, USA), and filled with an intracellular solution that included nystatin. Whole-cell currents activated by capsaicin were measured using a patch-clamp amplifier (L/M-EPC-7, List-Medical, Darmstadt, Germany) with a holding potential of −60 mV. Current traces were monitored and stored using a pCLAMP (Axon Instruments, Foster City, CA, USA) after digitizing the analogue signals at 0.5 kHz (DigiData 1200, Axon Instruments). The current signals were filtered at 10 Hz. Data were analyzed off-line using a pCLAMP as well as by a technical graphics/analysis program (ORIGIN, MicroCal Software, Northampton, MA, USA). All experiments were conducted at room temperature (20–22°C).

Solutions: whole-cell recording techniques

The standard extracellular solution for cell isolation consisted of the following compositions (in mM): 136 NaCl, 5 KCl, 2.5 CaCl₂, 0.5 MgCl₂, 12 NaHCO₃, 2 NaH₂PO₄ and 11 glucose. For the nystatin perforated-patch recording, an extracellular solution (ECS) consisting of the following compositions (in mM) was used: 145 sodium glutamate, 5 KCl, 2.5 CaCl₂, 0.5 MgCl₂, 10 glucose, and 10 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES). The intracellular solution (ICS) contained (in mM) 145 potassium glutamate, 5 Mg-ATP, 5 Ethylene glycol-bis (2-aminoethyl ether)-N,N,N′,N′-tetraacetic acid
(EGTA), and 20 HEPES. The pH was adjusted to 7.2 for intracellular solution with KOH and 7.4 for extracellular solutions with Tris. The $K^+$-glutamate based ICS and $Na^+$-glutamate based ECS were used to isolate cation currents (carried by $Ca^{2+}$ and $Na^+$) and minimize the contribution of $Cl^-$ currents (Guo and Davidson, 1998). Nystatin was added at a final concentration of 160 $\mu g/ml$ in the intracellular solution. Recording solutions and drugs were applied by superfusion over the dental pulp slice using a rapid gravity-fed perfusion system (VC-6, Warner Instrument, Hamden, CT, USA). Except for those noted above, all the other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

**Statistical analysis**

The results are expressed as means $\pm$ SE of mean of $n$ observations, where $n$ represents the number of tested cells. Student’s $t$-tests were used to evaluate the statistical significance. Values of $P$ less than 0.05 were considered significant.

**Results**

*Immunohistochemical localizations of TRPV1 channel in odontoblasts*

Figure 1 shows immunohistochemical localizations of TRPV1 channel (green) and nuclear staining (red) in the rat odontoblasts in a conventional fluorescence microscope. TRPV1-immunoreaction was discernable in the odontoblasts which exhibited tall columnar shapes during dentin formation (Fig. 1). Intense immunoreactivity was confined to the plasma membrane as well as cytoplasmic peri-nuclear regions (arrows in Fig. 1) in odontoblasts. In addition, distinct immunoreactions for TRPV1 were recognizable in cellular processes in the dentinal tubules (asterisks in Fig. 1) as well as the distal ends of odontoblast membranes which were adjacent to the internal dentin surface (arrow heads in Fig. 1).

*Whole-cell recordings*

Further studies were carried out to measure directly the currents passed through channel forming plasma membrane proteins of TRPV1 in odontoblasts using a nystatin perforated-patch recording configuration.

To activate TRPV1 currents in odontoblasts, we used a potent and selective TRPV1 channel activator, capsaicin. The effect of 10 $\mu M$ capsaicin is illustrated by the tracing shown in Figure 2. In odontoblasts, under the continuous

---

**Fig. 2.** Capsaicin-induced inward currents in odontoblasts. Currents were directly measured using a nystatin perforated-patch recording configuration. An application of 10 $\mu M$ capsaicin (open bar in A) elicited inward currents under a holding potential of $-60$ mV (top trace in A). An application of 10 $\mu M$ capsazepine (closed bar in B) caused a rapid termination of capsaicin-induced inward currents (open bar in B). The average current amplitudes are shown normalized to the current amplitudes observed in the 10 $\mu M$ capsaicin-induced currents (C). Data represent the mean $\pm$ S.E. of four experiments. *$P<0.05$*
voltage-clamp condition with a holding potential of −60 mV (extracellular Ca²⁺ concentration of 2.5 mM), an application of 10 μM capsaicin elicited inward currents (Fig. 2A, B), showing transient activation followed by sustained current. Since capsazepine is recognized as a TRPV1 channel antagonist (Montell et al., 2002; Clapham, 2003; Corey, 2003; Pedersen et al., 2005), we investigated the effects of this on the capsaicin-induced inward currents. As shown in Figure 2B, an application of 10 μM capsaizepine caused a rapid termination of capsaicin-induced inward currents. The amplitude of capsaizepine-induced inward currents at a membrane potential of −60 mV was −11.9 ± 5.0 pA (n = 6). Capsazepine (10 μM) significantly reduced capsaicin-induced inward currents by 21.1 ± 5.3% (n = 4, P < 0.05; Fig. 2C).

Discussion

The present study shows immunolocalization and capsaicin-induced inward currents which were inhibited by capsaizepine, indicating the expression of TRPV1 channels in odontoblasts.

An activation of TRPV1 channels has been reported to mediate plasma membrane depolarization of noxious sensory receptors or their afferents, which then triggers the transmission of action potentials to the central nervous system (Szallas and Blumberg, 1999). A brief application of heat to the outer surface of the tooth evoked a painful response, suggesting that thermal-heat stimulation to the tooth causes the movement of dentinal fluid within dentinal tubules. This results in the activation of nerve terminals located on the dental pulp, widely described as sensory transduction mechanisms, namely hydrodynamic mechanisms of dentin sensitivity (cf. Trowbridge et al., 2002). However, the present findings indicate the localization of functional TRPV1 channels on the distal regions of odontoblasts as well as on the cellular processes in the dentinal tubules. What is the physiological role of the TRPV1 channels in odontoblasts? One possible explanation is that nociceptive stimuli (thermal-heat or low pH) applied to the external dentin surface may be directly diffused via dentinal fluid as a thermal conductor, and the resultant temperature changes are then received by TRPV1 channels on the distal membranes of odontoblasts.

A combination of recently converging evidence has pointed that a mechanical deformation of the plasma membrane in odontoblasts via dentinal fluid movement activates mechanosensitive Ca²⁺-activated K⁺ channels (Allard et al., 2000) and TREK-1 channels (Magloire et al., 2003), while chemical-inflammatory stimuli for odontoblasts activate the IP₃ signaling system (Shibukawa and Suzuki, 2003). Both of these cellular signals promote the hyperpolarizing membrane potentials by a K⁺ efflux. These hyperpolarizations further activate store-operated Ca²⁺ channels (Shibukawa and Suzuki, 2003) to allow an influx of Ca²⁺ to make signals for dentin formation with an activation of Na⁺/Ca²⁺ exchangers (Shibukawa et al., 2006 in press), as well as those for possible sensory processing (Magloire et al., 2004). In contrast, thermal stimuli to the teeth may directly activate TRPV1 channels (Fig. 3) via temperature changes in the dentinal fluid, leading to depolarizing membrane potentials by a Ca²⁺ influx. These depolarizing membrane potentials can further produce receptor potentials (Magloire and Vinaud, 1979), to possibly release transmitters to the nerve endings.

However, two important questions arise concerning
the possible direct mechanisms that odontoblasts have a nociceptive sensory receptor function: 1) whether odontoblasts produce action potential; and 2) if synaptic contacts can be detected between odontoblasts and nerve endings. In the present study, just before TRPV1 current recordings but after the whole-cell configuration obtained in odontoblasts, we could also observe depolarization-activated K+ currents showing a reversal potential of ~50 mV in the physiological external K+ concentration (not shown), suggesting that odontoblasts produce action potentials. Concerning the neuro-odontoblast interactions in dental pulp, synaptic-like contacts between neural profiles and odontoblasts have been demonstrated (Lambrichts et al., 2003) while another study reported that no synaptic structures could be detected between them (Ibuki et al., 1996).

In summary, our results provide evidence for the significant expression of TRPV1 channels in rat odontoblasts. This novel finding needs to be considered in the context of the nociceptive sensory receptor cell function of the odontoblasts. Ongoing experimental work on the cells is aimed at a biophysical identification of TRPV1 and other families of TRP channels (such as TRPM8 which responds to a cooling stimulus, and TRPP2 which is well known to localize in the primary cilia that is identified in odontoblasts) (Magloire et al., 2004; Pedersen et al., 2005). An electrophysiological study of neuro-odontoblast coupling (Maurin et al., 2005) using two separate electrodes applied to each cell forming a primary co-culture system with trigeminal ganglion cells and odontoblasts will also be of immediate interest.

References


