Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

Single-cell RT-PCR and immunocytochemical detection of mechanosensitive transient receptor potential channels in acutely isolated rat odontoblasts

Minsoo Kwon [a,1], Sang Hoon Baek [a,1], Chul-Kyu Park [d], Gehoon Chung [a,b,*], Seog Bae Oh [a,b,c,**]

[a] Dental Research Institute and Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul, Republic of Korea
[b] Pain Cognitive Function Research Center, Seoul National University, Seoul, Republic of Korea
[c] Department of Brain and Cognitive Sciences, College of Natural Sciences, Seoul National University, Seoul, Republic of Korea
[d] Department of Physiology, Graduate School of Medicine, Gachon University, Incheon 406-799, Republic of Korea

ARTICLE INFO

Article history:
Accepted 19 July 2014

Keywords:
TRP channels
Odontoblasts
TRPM7
Single-cell RT-PCR
Dental pain
Mineralization

ABSTRACT

Objective: Hydrostatic force applied to tooth pulp has long been suspected to be the direct cause of dental pain. However, the molecular and cellular identity of the transducer of the mechanical force in teeth is not clear. Growing number of literatures suggested that odontoblasts, secondary to its primary role as formation of tooth structure, might function as a cellular mechanical transducer in teeth.

Design: In order to determine whether odontoblasts could play a crucial role in transduction of hydrostatic force applied to dental pulp into electrical impulses, current study investigated the expression of stretch-activated transient receptor potential (TRP) channels in acutely isolated odontoblasts from adult rats by single cell reverse transcriptase polymerase chain reaction and immunocytochemical analysis.

Results: As the result, expression of TRPM7 (melastatin 7) was observed in majority (87%) of odontoblasts while mRNAs for TRPC1 (canonical 1), TRPC6 (canonical 6) and TRPV4 (vanilloid 4) were detected in small subpopulations of odontoblasts. TRPM3 (melastatin 3) was not detected in our experimental set-up. Immunocytochemical analysis further revealed TRPM7 expression at protein level.

Conclusion: Expression of the mechanosensitive TRP channels provides additional evidence that supports the sensory roles of odontoblasts. Given that TRPM7 is a mechanosensitive ion channel with a kinase activity that plays a role in Mg^{2+} homeostasis, it is possible that TRPM7 expressed in odontoblasts might play a central role in mineralization during dentin formation.

* Corresponding author at: Seoul National University, School of Dentistry, Department of Physiology, 101 Daehakro, Jongno, Seoul 110-744, Republic of Korea. Tel.: +82 2 740 8660; fax: +82 2 762 5107.
** Corresponding author at: Seoul National University, College of Natural Sciences, Department of Brain and Cognitive Sciences, 1 Gwanak-ro, Gwanak, Seoul 151-742, Republic of Korea. Tel.: +82 2 740 8656; fax: +82 2 762 5107.
E-mail addresses: gehoon@snu.ac.kr (G. Chung), odolbae@snu.ac.kr (S.B. Oh).
[1] These authors contributed equally to this work.
http://dx.doi.org/10.1016/j.archoralbio.2014.07.016
0003–9969/

1. Introduction

Despite the severe orofacial discomfort and deterioration of the life quality caused by dental pain, the molecular and cellular pathophysiology underlying dental pain is still yet to be elucidated.[1] Because of the lack of understanding of the molecular mechanism, treatment of dental pain merely involves symptomatic procedures such as removal of infected tooth structures, often extending to intact sound dentin or pulp. Considering that no other tissues in human body are subject to routine removal because of inflammatory pain, elucidation of molecular mechanisms and development of therapeutic means that specifically target the cause of dental pain is crucial.

We have reported that the temperature sensitive transient receptor potential (TRP) channels are expressed and function as nociceptive temperature detector in dental primary afferent neurons thereby contributing to dental pain.[2] However, transduction of noxious temperature alone could not explain the sudden and intense pain induced by innocuous mechanical stimuli such as air puff or dental explorer probing.[3] Moreover, pulsating pain of chronic pulpitis patients that aggravates with increasing hydrostatic pressure suggests that dental pain might involve detection of mechanical pressure.[4]

Decades of investigation led to the hydrodynamic theory, which proposed the role of dental pulp as putative mechanical transducers.[5] According to the hydrodynamic theory, temperature changes cause movement of tissue fluid within dentinal tubules, which is detected by mechanical transducers in dental pulp. When dentinal tubules are exposed by dental caries or abrasion, a subtle change in temperature can induce exaggerated movement of dentinal fluid thereby provoking sudden intense pain.[6] It is also reported that increased intrapulpal pressure in inflammatory state lowered the pain threshold of teeth.[7] However, the cellular components responsible for detection of mechanical stress in dental pulp is not fully understood yet.[1]

Odontoblasts are cells that form dentin by depositing calcium matrix at the outermost surface of dental pulp. The locational advantage, together with the odontoblastic process that extends well into the dentinal tubule, grants odontoblasts an opportunity to interact directly with dentin fluid movements.[1] In addition, the single layer of odontoblasts connected to each other via gap junctions at the outer surface of dental pulp provides an adequate location to monitor intra-pulpal hydrostatic pressure. Therefore, researchers have shown an increased interest in the sensory role of the odontoblasts in recent years.[8] The excitability of odontoblasts was supported by studies showing expression of voltage-gated and ligand-gated ion channels.[9–12] A recent paper suggested mechanosensitivity of odontoblasts by showing expression of primary cilia,[13] which functions in bone cells as detector of fluidic sheer stress.[14] However, the molecular identity that plays a role as transducer of mechanical force in odontoblasts is yet to be found. Therefore, in this study, we set out to investigate whether odontoblasts express the mechanosensitive TRP channel subtypes, such as TRPC1 (canonical 1), TRPC6 (canonical 6), TRPV4 (vanilloid 4), TRPM3 (melastatin 3) and TRPM7 (melastatin 7), which could play a role as mechanical transducer in acutely isolated rat odontoblasts.

2. Material and methods

All procedures for animal use were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Dentistry, Seoul National University.

2.1. *Preparation of odontoblasts*

Odontoblast cells were prepared from adult (200–400 g) Sprague-Dawley rats (Orient Bio Inc., Sungnam, Korea) with methods described previously.[15] Briefly, upper and lower incisors were extracted within 5–10 min of sacrifice of the animal and were kept in cold (3–5 °C) Extracellular saline (ECS). After the surrounding soft tissues were all removed, the teeth were sectioned transversely with 500 μm thickness with a diamond disc for plaster. The tooth slices were incubated in 2 ml of a standard enzyme solution for 30 min at 37 °C, and then the suspension with tooth slices was triturated with a series of Pasteur pipettes. The triturated suspension was centrifuged (1000 RPM, 5 min), re-suspended, and placed on poly-L-lysine-coated glass coverslips, and then maintained at 3–5 °C until used. ECS consisted of (in mM) 140 NaCl, 3 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, adjusted to pH 7.3–7.4 with NaOH. The standard enzyme solution consisted of collagenase IA (3 mg/ml) and protease I (0.25 mg/ml) in Ca^{2+} and Mg^{2+}-free ECS.

2.2. *Single-cell reverse transcription-polymerase chain reaction (scRT-PCR)*

Single-cell reverse transcription-polymerase chain reaction (scRT-PCR) was performed as previously described.[2,15] Briefly, the targeted cell was aspirated into a patch pipette with tip diameter of about 20 μm and put into reaction tube containing reverse transcription agents. Reverse transcription was for 1 h at 37 °C. Subsequently, the cDNA was divided into three or four 2 μl aliquots that were used in separate PCRs. All PCR amplifications were performed with nested primers (Table 1). The first round of PCR was performed in 50 μl of PCR buffer containing 0.2 mM dNTPs, 0.2 μM "outer" primers, 4 μl RT product, and 0.2 μl platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA). For the second round of amplification, the reaction buffer (20 μl) contained 0.2 mM dNTPs, 0.2 μM "inner" primers, 5 μl products from the first round, and 0.1 μl platinum Taq DNA polymerase. The PCR products were then displayed on the ethidium bromide-stained 2% agarose gel.

2.3. *Immunocytochemistry*

Odontoblasts plated on glass coverslips were fixed with 4% paraformaldehyde for 10 min and rinsed three times with phosphate buffered saline (PBS) for 10 min. Cells were treated with blocking solution (5% NDS, 5% FBS, 2% BSA) for 1 h at room temperature. Samples were then incubated overnight at 4 °C with goat polyclonal anti-TRPM7 (1:1000; Abcam, Cambridge,