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Histochemistry of nerve fibres double labelled with anti-TRPV2 antibodies and sensory nerve marker AM1-43 in the dental pulp of rat molars

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ABSTRACT

AM1-43 can label sensory nerve fibres and sensory neurons. Permeation of non-selective cation channels of the nerve cell membrane is suggested to be the mechanism responsible for labelling. To identify these channels, two candidates, TRPV1 and TRPV2 were examined by immunocytochemistry in the dental pulp and trigeminal ganglion of rats injected with AM1-43. A part of AM1-43-labelled nerve fibres was also positive for anti-TRPV2 antibody but negative for anti-TRPV1 antibody in the dental pulp. In the trigeminal ganglion, a part of the neuron showed both bright AM1-43 labelling and anti-TRPV2 immunolabelling, but neurons double labelled with AM1-43 and TRPV1 were rare. These results suggest that TRPV2 channels, but not TRPV1 channels, contribute to the fluorescent labelling of AM1-43 in the dental pulp.

1. Introduction

In the dental pulp, abundant sensory nerve fibres are distributed beneath the odontoblast layer forming a sub-odontoblastic plexus. In addition to the sensory fibres, sympathetic nerve fibres are distributed along the blood vessels. Sensory nerve fibres have been examined extensively by histological methods such as silver impregnation, radio-autography, or immunohistochemistry using neurofilament proteins or protein gene product 9.5 (PGP9.5). Neuropeptides such as substance P (SP), and calcitonin gene-related peptide (CGRP) have been reported in the dental pulp.[1–5] In particular, CGRP-positive nerve fibres are shown to be abundant in the dental pulp.[6]

AM1-43 injected subcutaneously to the back skin results in bright fluorescence in sensory nerves of various tissues of the body,[7] of which dental pulp tissue is one.[8,9] It is believed that the labelling of sensory nerves by the original molecule FM1-43 and fixable analogue AM1-43 is due to permeation of cation channels present in the nerve membrane.[7,10] One of these channels was shown to be capsaicin receptor TRPV1 by in vitro transfection experiments.[7] Since TRPV1 is a transducer of thermal pain for temperatures above 43 °C present in the dental pulp nerve fibres,[11–15] immunohistochemistry of TRPV1 was performed after AM1-43 injection. Another TRP family thermal pain transducer, TRPV2 with a threshold > 52 °C was also examined by immunohistochemistry. TRPV2 is also a cation channel protein abundantly detected in the dental pulp nerve fibres.[16]

2. Materials and methods

Eleven 3- to 4-week-old male and female rats (60–90 g; Jcl Wistar; Clea Japan, Tokyo, Japan) and five 5-week-old male and female rats (130–170 g; Jcl Wistar; Clea Japan) were used in this study. Six 3- to 4-week-old and three 5-week-old rats were injected subcutaneously in the skin of the back with AM1-43

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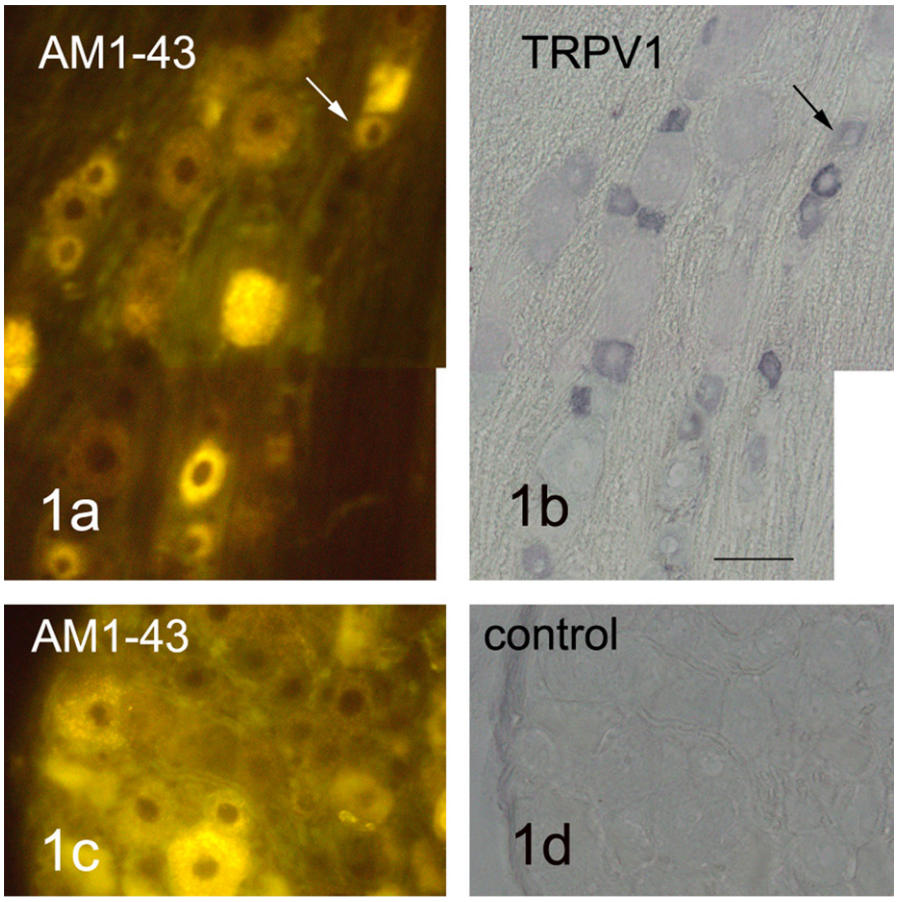
(Biotium, Hayward, CA, USA; 2 μg/g bodyweight) dissolved in phosphate-buffered saline (PBS), pH 7.3, at a concentration of 1 mg/ml. Five 3- to 4-week-old and two 5-week-old rats were injected with the same amount of PBS alone as control. One day after injection, animals were anesthetised with an injection of sodium pentobarbital (Nembutal; Abbot, North Chicago, IL, USA) and sacrificed by decapitation. Institutional guidelines for animal care were followed for all experimental procedures.

The maxilla and mandible were dissected and fixed with 4% paraformaldehyde in PBS at 4 °C for 9–48 h. They were demineralised for 4 weeks with 5% EDTA, pH 7.2 (adjusted with NaOH) at 4 °C. The tissues were washed with PBS, infused



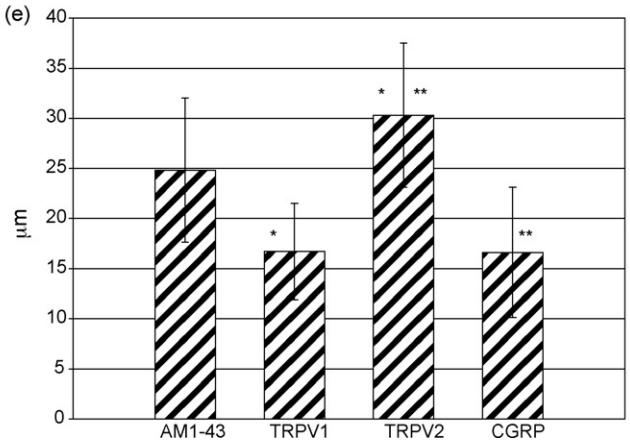


Fig. 1 – Double labelling of trigeminal ganglion in the same sections with AM1-43 and anti-TRPV1 (a and b), and AM1-43 and control (c and d). (e) Size of neurons labelled with AM1-43, anti-TRPV1, anti-TRPV2, and anti-CGRP (mean ± S.D. in cell diameter, * and **$p < 0.001$, Student's t test). AM1-43 labels both small and large neurons, thus average is intermediate (e). Fluorescent images of AM1-43 injected rats were obtained first (a and c) and then the same sections were immunolabelled or processed as control (b and d). TRPV1-positive neurons were mostly different from the bright AM1-43-positive neurons (a and b). Doubly positive neurons (arrows in (a) and (b)) were rare. Control section showed no specific labelling (d) in spite of bright AM1-43 labelling (c). Bar = 50 μm.