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Prolonged exposure to bradykinin and prostaglandin E2 increases TRPV1 mRNA but does not alter TRPV1 and TRPV1b protein expression in cultured rat primary sensory neurons

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HIGHLIGHTS

- BK- and PGE_2-evoked changes in TRPV1 and TRPV1b was assessed in cultured DRG neurons.
- Prolonged exposure to BK and PGE_2 increases capsaicin responsiveness of DRG neurons.
- Prolonged exposure to BK and PGE_2 increases TRPV1 but not TRPV1b mRNA expression.
- Prolonged exposure to BK and PGE_2 does not alter TRPV1 or TRPV1b protein expression.
- The role of TRPV1b in regulating channel responsiveness in DRG neurons is challenged.

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ABSTRACT

Sensitisation of the capsaicin receptor, transient receptor potential vanilloid type 1 (TRPV1) ion channel in nociceptive primary sensory neurons (PSN) underlies the development of inflammatory heat hyperalgesia. Removal of the negative-dominant splice variant of the TRPV1 molecule, TRPV1b from TRPV1/TRPV1b heterotetrameric channels, which should be associated with changes in the expression of TRPV1 and TRPV1b transcripts and proteins, has been suggested to contribute to that sensitisation. Respective reverse-transcriptase polymerase chain reaction (RT-PCR) and Western-blotting revealed that both TRPV1 and TRPV1b mRNA, and their encoded proteins are expressed in rat cultured PSN. Sequencing of the RT-PCR products showed that TRPV1b mRNA lacks the entire exon 7. Further, growing PSN for 2 days in the presence of 10 μM bradykinin (BK) and 10 μM prostaglandin E_2 (PGE_2) significantly increases TRPV1 responsiveness and TRPV1 mRNA expression, without producing any changes in TRPV1b mRNA, and TRPV1 and TRPV1b protein expression. These data challenge the hypothesis that alterations in the composition of the TRPV1 ion channel contributes to the sensitisation.

1. Introduction

Transient receptor potential vanilloid type 1 molecules, either on their own, or together with TRPV1 splice variants, or other members of the transient receptor potential vanilloid sub-family, assemble to form the non-selective cationic channel known as the capsaicin receptor (TRPV1 ion channel) [2,6,9,14,25,26]. When one of the splice variants of TRPV1, TRPV1b is co-expressed with TRPV1 in heterologous systems, an inhibitory effect on channel activity, as evoked by various TRPV1 activators, including capsaicin, is seen [14,25,26].

Nociceptive primary sensory neurons constitute the archetypical cell type that expresses TRPV1 [2,27]. In these neurons, the TRPV1 ion channel, through its increased responsiveness, is

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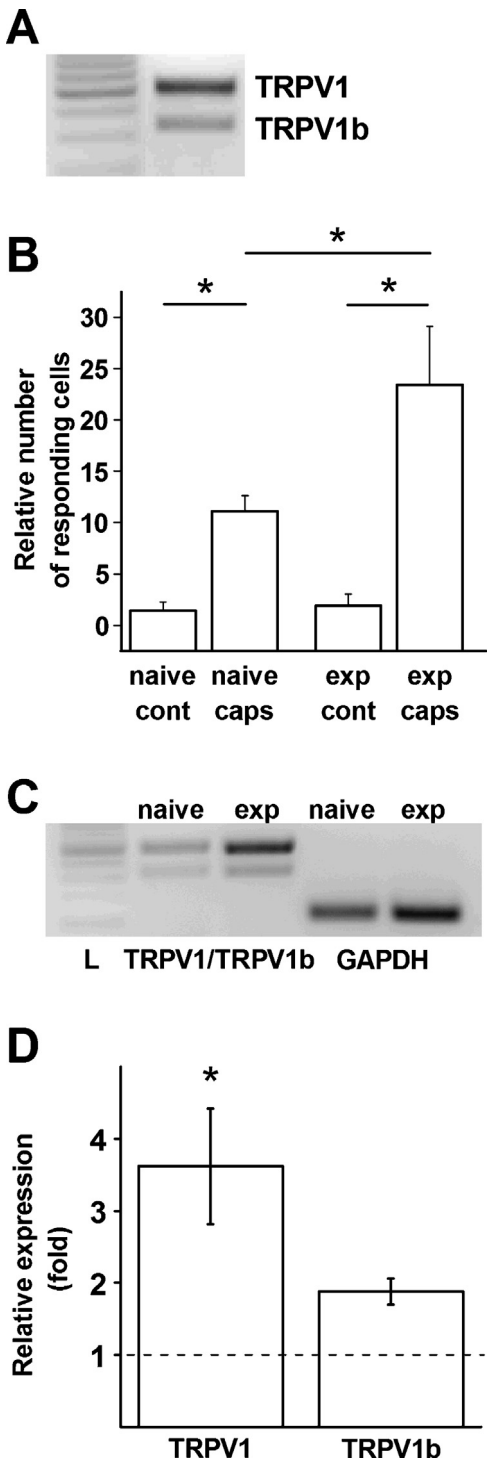

Fig. 1. (A) Agarose gel electrophoresis of RT-PCR products for TRPV1 and TRPV1b from cDNA made to RNA isolated from cultured primary sensory neurons. Note that with the TRPV1 primers we used, two products were detected: while one of the products was ~500 bp, the other was ~325 bp. (B) Relative number (number of labeled cell/number of total cells) of primary sensory neurons showing capsaicin-independent or 30 nM capsaicin-evoked cobalt accumulation following growing in control medium (naive) or in the presence of 10 μM bradykinin and 10 μM prostaglandin E_2 (exp) for two days. Note that addition of capsaicin to the cobalt buffer (see Methods) produced a significant increase in the number of cells accumulating cobalt in both conditions. Note also that the capsaicin-induced increase in the relative number of neurons showing cobalt accumulation is higher in the inflamed than in the naive condition. $n = 4$ at each data point. (C) Agarose gel electrophoresis of RT-PCR products for GAPDH, TRPV1 and TRPV1b from cDNA made to RNA isolated from cultured primary sensory neurons, which were grown either in control medium (naive) or in the presence of 10 μM bradykinin and 10 μM prostaglandin E_2 (exp) for 2 days. Note that exposure of neurons to bradykinin and prostaglandin E_2 induced changes in TRPV1 and TRPV1b mRNA expression. (D) Relative changes in the expression of TRPV1 and TRPV1b mRNA in primary sensory neuron cultures produced by the inflammatory mediators. Note that the relative expression of TRPV1 mRNA increased significantly, whereas the expression of TRPV1b mRNA was not changed. $n = 4$ at each data point.

essential in signalling peripheral inflammatory events to the central nervous system, and subsequently it plays a pivotal role in the development and maintenance of inflammatory heat hyperalgesia and visceral hyper-reflexia [1,4,8]. The co-expression of TRPV1 and TRPV1b molecules in PSN, and the negative dominant effect of TRPV1b on the channel's responsiveness found in heterologous systems [14,25,26] suggest that alterations in the composition of the TRPV1 ion channel might contribute to the inflammation associated increase in the responsiveness of this receptor. Such an alteration, in a long term, should be reflected by altered ratio in the expression of TRPV1 and TRPV1b transcripts and proteins. Hence, we hypothesised that culturing primary sensory neurons in an "inflammation-associated milieu" results in changes in the ratio of TRPV1 and TRPV1b mRNA and protein expressed by those cells.

2. Materials and methods

All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986, the revised National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, the Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes and the guidelines of the Committee for Research and Ethical Issues of IASP published in Pain, 16 (1983) 109–110. Every effort was taken to minimize the number of animals used.

2.1. Primary sensory neuron cultures

Cultures were prepared as described previously [17]. Briefly, DRG from the first cervical to the first sacral segment were dissected from 16 terminally anaesthetised Sprague-Dawley rats (80–100 grams) and collected in Ham's nutrient F12 culture medium (Sigma, Poole, UK) that was supplemented with 50 IU/mL penicillin (Invitrogen, Paisley, UK), 50 μg/mL streptomycin (Invitrogen) and 2% Ultroser G (Pall France, St-Germain-en-Laye Cedex France). Ganglia were incubated in 0.125% collagenase (Lorne Diagnostics, reading UK) for 3 h at 37 °C, then triturated and plated on poly-DL-ornithine (Sigma)-coated glass coverslips in the supplemented culture medium. Cells were cultured either in the absence (naive) or presence (exposed) of the inflammatory mediators, prostaglandin E_2 (PGE_2; Sigma; 10 μM) and bradykinin (BK; Sigma; 10 μM) for 2 days at 37 °C in the presence of 5% carbon dioxide.

2.2. Reverse transcription-polymerase chain reaction (RT-PCR)

The total RNA content was extracted both from naive and exposed cultures using the RNeasy Protect Mini Kit (Qiagen, Crawley, UK). The extracted RNA was reverse-transcribed using SuperScript II cDNA synthesis reagents (Invitrogen). The primer sequences were as follows: 5′-TGGAGGTGGCAGATAACACA -3′ forward and 5′ CCTTCCACAGGCCGATAGTA -3′ reverse. GAPDH primers were provided by Primerdesign Ltd. Amplification was performed in 3 mM $MgCl_2$, 5 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide mix, pH 8.3 and 1.25 U of Go-Taq Flexi DNA polymerase (Promega, UK). The amplification reaction consisted of 30 cycles with 30 s of denaturation at 96 °C, 1 min annealing at 59 °C, and 3 min extension at 72 °C in a thermal cycler (Eppendorf-Mastercycler Personal, UK). PCR products