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ACTIVATION OF CB1 INHIBITS NGF-INDUCED SENSITIZATION OF TRPV1 IN ADULT MOUSE AFFERENT NEURONS

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Abstract—Transient receptor potential vanilloid 1 (TRPV1)-containing afferent neurons convey nociceptive signals and play an essential role in pain sensation. Exposure to nerve growth factor (NGF) rapidly increases TRPV1 activity (sensitization). In the present study, we investigated whether treatment with the selective cannabinoid receptor 1 (CB1) agonist arachidonyl-2′-chloroethylamide (ACEA) affects NGF-induced sensitization of TRPV1 in adult mouse dorsal root ganglion (DRG) afferent neurons. We found that CB1, NGF receptor tyrosine kinase A (trkA), and TRPV1 are present in cultured adult mouse small- to medium-sized afferent neurons and treatment with NGF (100 ng/ml) for 30 min significantly increased the number of neurons that responded to capsaicin (as indicated by increased intracellular Ca^{2+} concentration). Pretreatment with the CB1 agonist ACEA (10 nM) inhibited the NGF-induced response, and this effect of ACEA was reversed by a selective CB1 antagonist. Further, pretreatment with ACEA inhibited NGF-induced phosphorylation of AKT. Blocking PI3 kinase activity also attenuated the NGF-induced increase in the number of neurons that responded to capsaicin. Our results indicate that the analgesic effect of CB1 activation may in part be due to inhibition of NGF-induced sensitization of TRPV1 and also that the effect of CB1 activation is at least partly mediated by attenuation of NGF-induced increased PI3 signaling.

Key words: transient receptor potential vanilloid 1, capsaicin, nerve growth factor, sensitization, cannabinoid receptor 1, phosphorylation of AKT.

INTRODUCTION

Transient receptor potential vanilloid 1 (TRPV1) is a voltage-gated ion channel that allows influx of cations in response to noxious stimuli, including heat, acid (protons), and capsaicin (Caterina et al., 2000; Davis et al., 2000; White et al., 2011a; Sousa-Valente et al., 2014a). TRPV1 are expressed primarily by small- to medium-sized afferent neurons that are putative nociceptors (Immke and Gavva, 2006; Woolf and Ma, 2007; White et al., 2011b). The majority of TRPV1-expressing neurons contain nociceptive neuropeptides, such as calcitonin-gene-related peptide (CGRP) and substance P, and also express the nerve growth factor (NGF) receptor, tyrosine kinase A (trkA) (Averill et al., 1995; Guo et al., 1999; Immke and Gavva, 2006; Ernsberger, 2009). Expression of TRPV1 in afferent neurons is increased by inflammatory pain (Carlton and Coggeshall, 2001; Ji et al., 2002; Luo et al., 2004; Schicho et al., 2004; Immke and Gavva, 2006; Miranda et al., 2007; Schwartz et al., 2013). Genetic deletion of TRPV1 (Caterina et al., 2000; Davis et al., 2000) and studies using selective TRPV1 antagonists have demonstrated that TRPV1 is essential for the development of referred hyperalgesia associated with certain types of tissue injury and inflammation (Ji et al., 2002; Luo et al., 2004; Schicho et al., 2004; Immke and Gavva, 2006; Charrua et al., 2009; Sousa-Valente et al., 2014a,b).

Tissue injury and inflammation generate an array of chemical mediators, including NGF, that activate and sensitize primary afferent neurons (Ji et al., 2002; Woolf and Ma, 2007; Stemkowski and Smith, 2012). NGF has been well-characterized as essential for development, growth, and function of afferent neurons (Woolf and Ma, 2007). Treatment with NGF (hours to days) increases the expression of TRPV1 in cultured afferent neurons (Winston et al., 2001; Anand et al., 2006). Acute exposure to NGF has also been shown to rapidly increase TRPV1 activity (sensitization) (Winter et al., 1988; Shu and Mendell, 2001; Zhuang et al., 2004; Jankowski and Koerber, 2010). Multiple intracellular signaling pathways are involved in sensitization of TRPV1 by NGF, including ERK 1/2 (extracellular signal-regulated protein kinase, a member of the MAPK or mitogen-activated protein kinase family), and PI3 (phosphatidylinositol 3) kinase

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Abbreviations: ACEA, arachidonyl-2′-chloroethylamide; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; CGRP, calcitonin-gene-related peptide; DRG, dorsal root ganglion; NGF, nerve growth factor; trkA, tyrosine kinase A; TRPV1, transient receptor potential vanilloid 1.

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(Bonnington and McNaughton, 2003; Zhuang et al., 2004; Malik-Hall et al., 2005).

Cannabinoids have been shown to have analgesic and anti-inflammatory effects, and the effects of cannabinoids are mediated primarily by cannabinoid receptors 1 and 2 (CB1 and CB2), both coupled to inhibitory G proteins (Richardson et al., 1998; Clayton et al., 2002; Sagar et al., 2005; Demuth and Molleman, 2006). CB1 are predominantly present in neural tissues, including afferent neurons (Ahluwalia et al., 2000; Ross et al., 2001; Clayton et al., 2002; Demuth and Molleman, 2006; Agarwal et al., 2007; Hu et al., 2012). Conditional deletion of CB1 specifically in nociceptive afferent neurons in mice prevented cannabinoid-induced analgesia and exaggerated mechanical hyperalgesia induced by intraplantar injection of complete Freund's adjuvant (CFA) (inflammatory pain) or in the spared nerve injury model of neuropathic pain (axotomy and ligation of 2 of the 3 terminal branches of the sciatic nerve) (Agarwal et al., 2007). In the present study, we investigated whether treatment with the selective CB1 agonist arachidonyl-2′-chloroethylamide (ACEA) affects NGF-induced sensitization of TRPV1 in adult mouse dorsal root ganglion (DRG) afferent neurons. We also investigated the effects of CB1 activation on intracellular signaling pathways involved in NGF-induced sensitization of TRPV1.

EXPERIMENTAL PROCEDURES

Animals

Forty-six male C57BL/NH mice (10–12 weeks old) were obtained from Harlan (Indianapolis, IN). Experiments were conducted in accordance with National Institutes of Health Guidelines, and all protocols were reviewed and approved by the Animal Care and Use Committee of the University of Wisconsin.

Culture of DRG neurons

Mice were deeply anesthetized with pentobarbital (50-mg/kg, ip) and perfused with saline (0.9% NaCl) through a canula inserted into the left ventricle. Approximately 40–45 DRGs were removed from each individual mouse, and nerve trunks and connective tissue were dissected and discarded. DRGs were transferred to Dulbecco's Modified Eagle Medium (Life Technologies, Grand Island, NY) and treated with trypsin (2.5 mg/ml) and collagenase (2 mg/ml) for 60 min at 37 °C. After enzyme treatment, DRG neurons were dissociated by trituration with fire-polished Pasteur pipettes in Dulbecco's Modified Eagle Medium containing fetal bovine serum (10%), penicillin (50 U/ml), and streptomycin (50 μg/ml). The cell suspension was loaded on 30% Percoll density solution (Sigma, St. Louis, MO), and DRG neurons were collected by low-speed (800 × g) centrifugation for 10 min. Neurons were resuspended in Neurobasal medium containing B27 supplements (Life Technologies), penicillin (50 U/ml), and streptomycin (50 μg/ml), and allowed to settle on 35-mm glass-bottom dishes (MatTek, Asland, MA, for Ca^{2+} imaging) or 18-mm cover slips (for immunoblotting), both coated with poly-^{d}-lysine (Sigma). Cells from each animal were plated on 6–8 Ca^{2+} imaging dishes or cover slips and maintained at 37 °C in a humidified incubator with a gas mixture containing 5% CO_2. The neurons were used within 16–28 h of isolation.

Immunohistochemistry

Neurons were cultured on poly-^{d}-lysine-coated cover slips overnight. Cells were rinsed in phosphate-buffered saline (PBS), fixed with 2% paraformaldehyde for 30 min, and permeabilized with cold methanol for 10 min. They were rinsed and blocked with 10% normal donkey serum. Each specific antibody was then applied and incubated in a humid chamber overnight to 48 h at 4 °C. Staining was revealed using secondary donkey anti- rabbit IgG, conjugated with FITC or donkey anti-goat IgG, conjugated with rhodamine red (1:500, Jackson ImmunoResearch, West Grove, PA). Cover slips containing neurons were rinsed and mounted to slides with an anti-fading solution (Vector Labs, Burlingame, CA). Negative staining controls were prepared using normal rabbit or goat IgG instead of the specific antibody. Each staining was performed on 6 coverslips from 3 cultures. Staining was examined with a Nikon E600 microscope and photoimages from stained and control cells were acquired with the same acquisition set-up. For staining on each coverslip, 5–6 optical fields (18–37 neurons per field, about 120–200 neurons per coverslip) were randomly selected. The staining intensities of outlined neurons from corresponding negative controls were measured as a gray level on a 0–255 scale, and the averaged value was used as the threshold to differentiate labeled neurons from negative ones (Pezet et al., 2005; Baiou et al., 2007; Hong et al., 2011). The number of positively labeled neurons was normalized to the total number of neurons in the selected fields. The values from staining on 6 coverslips (n) of 3 cultures were averaged and presented as mean ± SEM.

The primary antibodies used for immunohistochemistry included rabbit anti-CB1 (1:1500, EMD Millipore, Billerica, MA) (Coutts et al., 2001; Bouchard et al., 2003), rabbit anti-trkA (1:500, Abcam, Cambridge, MA) (Manca et al., 2012; Lu et al., 2010), and goat anti-TRPV1 (1:200, Santa Cruz Biotechnology, Inc., Dallas, TX) (Ma et al., 2009; Ferrini et al., 2010). The antibodies used have been validated in previous studies and by the manufacturer.

Triple-immunostaining was performed to study co-localization of TRPV1, trkA, and CB1 in DRG neurons. The staining was performed in sequence. Neurons were first incubated with rabbit anti-trkA (1:500, Abcam) overnight at 4 °C. After rinsing, the neurons were visualized with a FITC-conjugated donkey anti-rabbit antibody (1:500, Jackson ImmunoResearch). The neurons were next incubated with goat anti-TRPV1 antibody (1:200, Santa Cruz) for 48 h at 4 °C. Staining with TRPV1 was revealed with a rhodamine red-conjugated donkey anti-goat antibody (1:500, Jackson ImmunoResearch). Staining of trkA and CB1 required using primary antibodies, both raised from rabbit hosts. The neurons were therefore blocked with unlabeled