Molecular and Cellular Neuroscience 52 (2013) 152–160

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Neuronal IL-17 receptor upregulates TRPV4 but not TRPV1 receptors in DRG neurons and mediates mechanical but not thermal hyperalgesia

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ARTICLE INFO

Article history:
Received 15 May 2012
Revised 10 October 2012
Accepted 2 November 2012
Available online 10 November 2012

Keywords:
TRPV1
TRPV4
IL-17A
IL-17RA
ERK
NFκB

ABSTRACT

In addition to the proinflammatory cytokines tumor necrosis factor-α, interleukin-6 and interleukin-1ß, the cytokine interleukin-17 (IL-17) is considered an important mediator of autoimmune diseases such as rheumatoid arthritis. Because tumor necrosis factor-α and interleukin-1ß have the potential to influence the expression of transduction molecules such as transient receptor potential vanilloid 1 (TRPV1) in dorsal root ganglion (DRG) neurons and thus to contribute to pain we explored in the present study whether IL-17A activates DRG neurons and influences the expression of TRPV1. The IL-17A receptor was visualized in most neurons in dorsal root ganglion (DRG) sections as well as in cultured DRG neurons. Upon long-term exposure to IL-17A, isolated and cultured rat DRG neurons showed a significant upregulation of extracellular-regulated kinase (ERK) and nuclear factor κB (NFκB). Long-term exposure of neurons to IL-17A did not upregulate the expression of TRPV1. However, we found a pronounced upregulation of transient receptor potential vanilloid 4 (TRPV4) which is considered a candidate transduction molecule for mechanical hyperalgesia. Upon the injection of zymosan into the paw, IL-17A-deficient mice showed less mechanical hyperalgesia than wild type mice but thermal hyperalgesia was not attenuated in IL-17A-deficient mice. These data show, therefore, a particular role of IL-17 in mechanical hyperalgesia, and they suggest that this effect is linked to an activation and upregulation of TRPV4.

Introduction

The proinflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-6, and interleukin-1ß not only evoke and/or maintain inflammatory reactions. By acting on cytokine receptors they also contribute to the generation of inflammatory pain. Interleukin-17 (IL-17) is another proinflammatory cytokine which received recently strong attention in immunology and rheumatology but which was not in the focus of pain research so far. IL-17A, the prototype member of the IL-17 family (Pappu et al., 2010), is secreted from Th17, $CD8^+$ T, γδT, natural killer cells, and from activated monocytes and neutrophils (Pappu et al., 2010) and plays a role in both innate and adaptive immunity (Alber and Kamradt, 2007; Gaffen, 2009; Lubberts, 2008). Because the intraarticular injection of IL-17 in mice causes hyperalgesia (Pinto et al., 2010) we began to address the putative role of IL-17 in nociception. We found that IL-17A sensitizes unmyelinated joint nociceptors to mechanical stimuli at a time course of 2–3 h (Richter et al., 2012). This effect is likely to be caused by the activation of neuronal IL-17 receptors because the majority of DRG neurons express the interleukin-17A receptor (IL-17RA). In isolated and cultured rat DRG neurons IL-17A caused rapid phosphorylation of protein kinase B (PKB/Akt) and extracellular-regulated kinase (ERK), and in patch clamp studies bath application of IL-17A enhanced the excitability of DRG neurons within several minutes (Richter et al., 2012). In addition, an antibody against IL-17A reduced mechanical hyperalgesia in mice with unilateral antigen-induced arthritis in the knee (Richter et al., 2012).

Because TRP ion channels are major transduction molecules of nociception (Basbaum et al., 2009; Levine and Alessandri-Haber, 2007), and because the expression of TRPV1 in DRG neurons can be regulated by inflammation in the long term (Amaya et al., 2003; Ji et al., 2002) we investigated in the present study whether IL-17A

Abbreviations: 4αPDD, 4α-phorbol 12,13-didecanoate; COX, cyclooxygenase; DRG, dorsal root ganglion; ERK, extracellular-regulated kinase; IL-17A, interleukin-17A; IL-17RA, interleukin-17A receptor; IL-17RA-like IR, interleukin-17A receptor-like immunoreactivity; NFκB, nuclear factor κB; PBS, phosphate buffered saline; Th17 cell, T helper 17 cell; TNF-α, tumor necrosis factor-α; TRPV1, transient receptor potential vanilloid 1; TRPV4, transient receptor potential vanilloid 4; TTX, tetrodotoxin; WT, wild type.

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1044-7431/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.mcn.2012.11.006

has long-term effects on the expression of TRPV ion channels in DRG neurons. We focused on TRPV1 and TRPV4. TRPV1 is expressed in a proportion of nociceptive neurons, contributes to the transduction of noxious heat stimuli and is essential for the development of inflammation-evoked thermal hyperalgesia (Basbaum et al., 2009; Stein et al., 2009). Incubation of cultured DRG neurons with either TNF-α (Hensellek et al., 2007) or IL-1ß (Ebbinghaus et al., 2012) for 24–48 h significantly increased the proportion of DRG neurons which express TRPV1. We asked, therefore, whether IL-17A has a similar effect. TRPV4 is gated by temperature in the innocuous range with a maximum at 37 °C, but TRPV4 is also considered a candidate molecule for the transduction of noxious mechanical stimuli, at least under inflammatory conditions (Levine and Alessandri-Haber, 2007; Liedtke, 2008). Because IL-17A sensitizes joint nociceptors for mechanical stimuli it was of interest whether it regulates the expression of TRPV4. In order to further explore the pattern of hyperexcitability of IL-17A we also performed behavioral experiments in mice which were deficient for IL-17A. In these experiments we compared the withdrawal threshold for mechanical and thermal stimuli applied to the hindpaws, then we induced an inflammation in the paw by the injection of zymosan and tested whether WT and IL-17$^{-/-}$ mice develop similar mechanical and thermal hyperalgesia.

Results

Expression of the IL-17A receptor (IL-17RA) in rat DRG neurons

The staining of lumbar DRG sections for IL-17RA-like immunoreactivity (IR) revealed that the vast majority of DRG neurons and satellite cells express IL-17RA-like IR. Fig. 1A shows a DRG section in which numerous neurons and satellite were labeled with an antibody

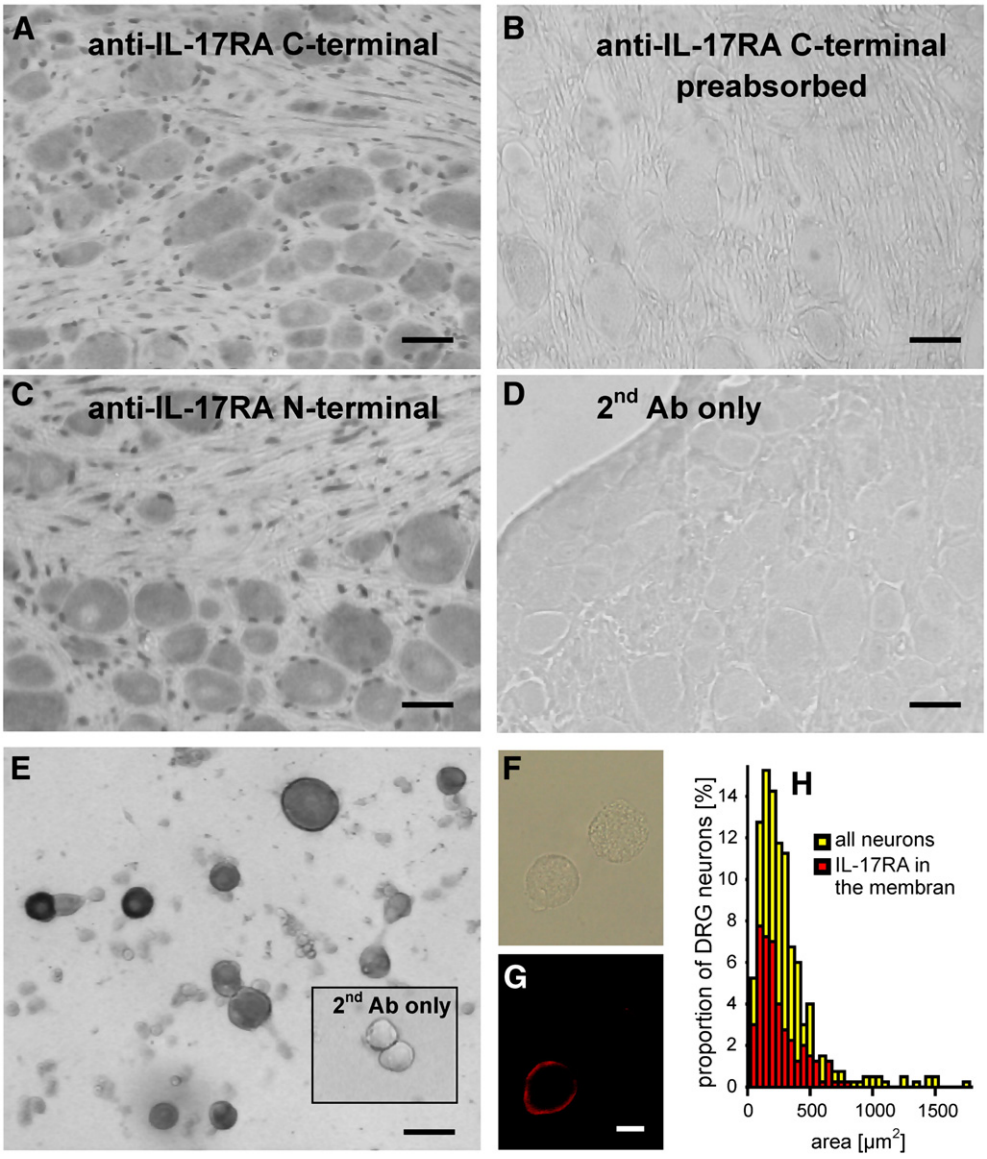

Fig. 1. Localization of IL-17A receptors (IL-17RA) in rat DRG neurons. (A) IL-17RA-like IR in a DRG section using an antibody against a cytoplasmatic peptide. (B) Control section after preabsorption of the antibody with the peptide. (C) IL-17RA-like IR in a DRG section using an antibody against the N-terminal extracellular domain of the IL-17RA. (D) Control section without adding the first antibody. Bars in A, B, C and D 15 µm. (E) IL-17RA-like IR in DRG neurons cultured over night. The inset shows cells from the control incubation cells were not treated with the primary antibody. Bar: 10 µm. (F) Bright field image of two neurons. (G) Image of the some neurons as in F with IL-17RA labeling (N-terminal antibody). One neuron shows IL-17RA-like IR in the membrane (labeling without permeabilization of the cells). Illumination in G was 40 ms. (H) Size distribution of all analyzed neurons without permeabilization of the cells (yellow) and of the neurons with IL-17RA-like IR within the membrane (red).