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Calcium release-activated calcium channels and pain

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ABSTRACT

Calcium release-activated calcium (CRAC) channels are unique among ion channels that are activated in response to depletion of intracellular calcium stores and are highly permeable to Ca^{2+} compared to other cations. CRAC channels mediate an important calcium signal for a wide variety of cell types and are well studied in the immune system. They have been implicated in a number of disorders such as immunodeficiency, musculoskeletal disorders and cancer. There is growing evidence showing that CRAC channels are expressed in the nervous system and are involved in pathological conditions including pain. This review summarizes the expression, distribution, and function of the CRAC channel family in the dorsal root ganglion, spinal cord and some brain regions, and discusses their functional significance in neurons and glial cells and involvement in nociception and chronic pain. Although further studies are needed to understand how these channels are activated under physiological conditions, the recent findings indicate that the CRAC channel Orai1 is an important player in pain modulation and could represent a new target for pathological pain.

1. Introduction

Acute pain serves a warning or protective function and only lasts a few days or weeks. On the other hand, chronic pain serves no useful purpose and is often associated with an altered sensitivity to stimuli. Although the exact molecular and cellular mechanisms underlying chronic pain remain to be determined and may indeed vary depending on the type of pain and initiating events, evidence has accumulated for a role of intracellular Ca^{2+} in the development of persistent pain. Calcium-permeable ion channels and receptors have been implicated in pain as well as in the neuroplasticity associated with chronic pain states. Neurons express a variety of voltage-gated and ligand-gated Ca^{2+} channels [1–4]; however, recent studies have shown that newly discovered calcium release-activated calcium (CRAC) channels are also important in mediating Ca^{2+} influx in neurons and glial cells in the central nervous system (CNS) [5–8]. While CRAC channels have emerged as promising therapeutic targets for the treatment of immune disorders, thrombosis and cancer [9–12], the role of CRAC channels in pain and other CNS diseases is just beginning to be explored.

CRAC channels were firstly proposed by Putney to refill intracellular calcium stores after depletion by IP_3 receptors [13]. It took two decades to identify molecular components of CRAC channels, but recent progress has elucidated several significant features of CRAC channels. In most cell types, CRAC channels are composed of ER calcium sensors STIM1/2 and pore-forming proteins Orai1/2/3 [14–17]. When Ca^{2+} is released from the endoplasmic reticulum (ER) to the cytosol, STIM1 and STIM2 undergo oligomerization and translocate to ER–plasma membrane (PM) junctions, where they activate CRAC channels and induce Ca^{2+} entry [15,18,19]. It is well-established that CRAC channels are essential for immune cells to regulate their activation and maturation [20], cytokine production [21], and antigenic responses [22]. However, the functional significance of CRAC channels in the nervous system, especially under pathological conditions including pain, is unclear. Recently, we and others have reported that the CRAC channel family is expressed in dorsal horn neurons [23,24], glia [25–27] and dorsal root ganglion (DRG) neurons [5,28]. We have identified the CRAC channel Orai1 as a primary player in store-operated calcium entry (SOCE) in dorsal horn neurons and astrocytes [24,25], while both Orai1 and Orai3 contribute to SOCE in DRG neurons [28]. Using pharmacological and genetic approaches, we have demonstrated that inhibition or knockout of Orai1 reduces nociception and chronic pain [23,29,30]. In this review, we summarize recent work related to CRAC channels and pain, and highlight the role of the CRAC channel Orai1 in nociception and pathological pain.

2. Expression and function of CRAC channels in the nervous system

2.1. CRAC channels in DRG

DRG neurons are primary sensory neurons innervating the skin and are responsible for conveying signals for pain sensation to the spinal cord. We and others have shown that STIM1, STIM2 and Orai1/2/3 are expressed at both mRNA and protein levels in DRG tissue from adult

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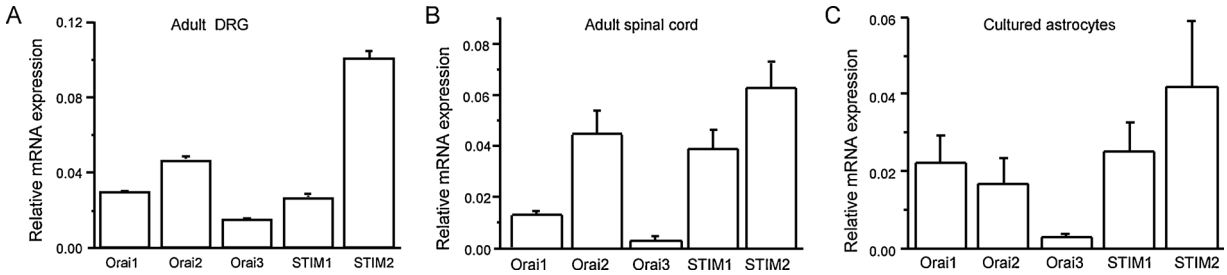

Fig. 1. Expression of CRAC channels and STIM proteins. mRNA levels of STIM1, STIM2, Orai1, Orai2 and Orai3 in adult DRGs (A), adult spinal cord tissue (B), and cultured astrocytes (C) by quantitative PCR (normalized to GAPDH), n = 4-5. Figures are modified from Wei et al. [28], Xia et al. [24], and Gao et al. [25] with permission.

mice determined by RT-qPCR and Western blotting [5,28]. Interestingly, mRNA levels of STIM2 are approximately 3-fold greater than those of STIM1 (Fig. 1A) [28]. As STIM2 is more sensitive to ER Ca^{2+} changes than STIM1, the high expression of STIM2 may endow DRGs with high sensitivity to fluctuations in $[Ca^{2+}]_{ER}$ [19].

STIM1 is mainly expressed in IB4- and CGRP-positive C-fibers, which are primarily responsible for nociception, and to lesser extent, in NF-200-positive A-fibers [28]. SOCE was observed in cultured DRG neurons, especially in small-diameter sensory neurons after Ca^{2+} depletion by thapsigargin (TG), a non-competitive inhibitor of endoplasmic reticulum Ca^{2+} ATPase [28]. Interestingly, SOCE is robust in nociceptors including TRPV1-, TRPA1-, TRPM8-, and IB4-positive DRG neurons [28], indicating that CRAC channels are functional predominantly in nociceptors. Knockdown of Orai1 and Orai3, but not Orai2, by specific siRNA significantly attenuates SOCE in cultured DRG neurons [28]. Despite previous reports showing that Orai1 and Orai3 can form heteromers mediating SOCE [31,32], it seems that they can independently contribute to SOCE in DRG neurons [28].

2.2. CRAC channels in the spinal cord

The spinal cord dorsal horn is a relay center for sensory information. Dorsal horn neurons process sensory input received from DRG neurons and transmit it to several brain regions. While voltage-gated sodium, potassium and calcium channels, as well as ionotropic glutamate receptors, are key players, G-protein coupled receptors and other ion channels also play modulatory roles in this process [33]. We have found that Orai1/2/3 and STIM1/2 are expressed in the spinal cord dorsal horn (tissue) and acutely isolated dorsal horn neurons [24]. Similar to that found in DRGs, STIM2 mRNA levels are higher than STIM1 in the dorsal horn (Fig. 1B) [24]. Interestingly, Orai1 mRNA level is greater in neurons than in the tissue while Orai2 mRNA level is higher in the tissue than that in neurons (Fig. 1B), suggesting that Orai2 mRNA expression is higher in non-neuronal cells including cells in the white matter. CRAC channels are functional in majority of dorsal horn neurons [24]. Different from DRG neurons, only Orai1 is responsible for SOCE in dorsal horn neurons and both STIM1 and STIM2 contribute to SOCE [24], indicating Orai1 and STIM1/2 are the main functional components in spinal cord dorsal horn neurons.

2.3. CRAC channels in supraspinal brain regions

Sensory information is further processed and terminated in a number of brain regions. SOCE has been reported in several supraspinal regions [34–36], however, the molecular components that mediate SOCE in these regions have remained unclear until recently. Several studies have demonstrated that STIMs and Orais are expressed in the cerebral cortex, hippocampus, amygdala [7], thalamus, and cerebellum [37,38]. Whereas, STIM2 is predominantly expressed in most brain regions [7,39], STIM1 is highly expressed in the cerebellum [38,40]. All three Orai isoforms are detectable in the brain, but their mRNA levels vary in different brain regions [41]. The Orai1 expression pattern in the brain and spinal cord of rodents is similar to that of humans [42]. In the cortex and hippocampus, Orai2 expression levels are much greater than Orai1 and Orai3 [39,43]. While STIM1 and Orai1 are major components mediating SOCE in many cell types including neurons, this is not the case in cortical neurons [39], suggesting that expression levels of STIM1/2 and CRAC channels are tissue-dependent. However, expression, distribution and function of CRAC channels in pain-processing brain regions have not been reported.

2.4. CRAC channels in glia

Glial cells play essential roles in brain homeostasis. Microglia, astrocytes and oligodendrocytes are the main types of glia in CNS. Normal activities of astrocytes and microglia are essential for maintaining many CNS functions. However, excessive activation of these cells is a hallmark of many acute and chronic neuropathologies including pain [44–46]. Reactive astrocytes and microglia release excessive proinflammatory cytokines and chemokines, which are involved in the development, maintenance and exaggeration of chronic pain [35,47]. Cytokine production is a Ca^{2+}-dependent process [48]. It is well-established that CRAC channels play an important role in cytokine production in immune cells [49,50].

Previous studies have shown that STIM1/2 and Orai1/2/3 are expressed and functional in microglia [26,27,51]. Inhibition of CRAC channels by 2-APB, La^{3+} and N(p-amylcinnamoyl) anthranilic acid (ACA) dramatically blocks SOCE and CRAC currents in microglia [30–32]. Using global knockout mouse lines of STIM1, STIM2 and Orai1, Michaelis et al. have demonstrated that STIM1/2 and Orai1 are primary components mediating SOCE in cultured cerebral microglia [26].

Like microglia, astrocytes in the cortex and spinal cord also express STIM1/2 and Orai1/2/3 [25,52]. However, in hippocampal astrocytes, Orai1 is undetectable while Orai3 is the predominant isoform [53]. Calcium imaging data reveal that CRAC channels are functional and mediate a large calcium influx in cultured spinal astrocytes [25]. Using the siRNA knockdown approach, we have found that Orai1 is responsible for SOCE in spinal cord astrocytes. Although STIM2 expression levels are greater than STIM1, both almost equally contribute to SOCE in spinal cord astrocytes (Fig. 1C) [25]. Interestingly, an independent study has reported that both Orai1 and Orai3 contribute to the major portion of SOCE in cortical astrocytes [52], indicating that the functional components of CRAC channels in astrocytes are also tissue-dependent.

3. Functional significance of CRAC channels in the nervous system

3.1. Downstream events of CRAC channels activation in the CNS

Ca^{2+} serves as a second messenger and regulates diverse aspects of cellular function. Ca^{2+} influx through CRAC channels is essential for