Biochimica et Biophysica Acta 1864 (2017) 1071–1087

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Overexpression of STIM1 in neurons in mouse brain improves contextual learning and impairs long-term depression☆

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ARTICLE INFO

Article history:
Received 28 September 2016
Received in revised form 25 November 2016
Accepted 26 November 2016
Available online 29 November 2016

Keywords:
STIMs
ORAIs
SOCE
LTD
mGluR
Behavior

ABSTRACT

STIM1 is an endoplasmic reticulum calcium sensor that is involved in several processes in neurons, including store-operated calcium entry. STIM1 also inhibits voltage-gated calcium channels, such as $Ca_v1.2$ and $Ca_v3.1$, and is thus considered a multifunctional protein. The aim of this work was to investigate the ways in which transgenic neuronal overexpression of STIM1 in FVB/NJ mice affects animal behavior and the electrophysiological properties of neurons in acute hippocampal slices. We overexpressed STIM1 from the Thy1.2 promoter and verified neuronal expression by quantitative reverse-transcription polymerase chain reaction, Western blot, and immunohistochemistry. Mature primary hippocampal cultures expressed STIM1 but exhibited no changes in calcium homeostasis. Basal synaptic transmission efficiency and short-term plasticity were comparable in slices that were isolated from transgenic mice, similarly as the magnitude of long-term potentiation. However, long-term depression that was induced by the glutamate receptor 1/5 agonist (*S*)-3,5-dihydroxyphenylglycine was impaired in STIM1 slices. Interestingly, transgenic mice exhibited a decrease in anxiety-like behavior and improvements in contextual learning. In summary, our data indicate that STIM1 overexpression in neurons in the brain perturbs metabotropic glutamate receptor signaling, leading to impairments in long-term depression and alterations in animal behavior. This article is part of a Special Issue entitled: ECS Meeting edited by Claus Heizmann, Joachim Krebs and Jacques Haiech.

1. Introduction

Stromal interaction molecule 1 (STIM1) was originally discovered as a stromal cell surface protein [1] and potential tumor suppression gene [2,3]. STIM1 is a highly conserved sensor of calcium concentration in the endoplasmic reticulum (ER) and ubiquitously expressed in different tissues [4]. Together with the closely related STIM2 protein and a family of ORAI calcium channel proteins, it has been identified as a key component of the process called store-operated calcium entry (SOCE; alternatively referred to as capacitative calcium entry [CCE]); [5–9]. In SOCE (for review, see [10–12]), the depletion of Ca^{2+} from the ER is sensed by STIM molecules, which then cause an influx of Ca^{2+} ions from the extracellular space through calcium release-activated channels (CRACs), which are composed of ORAI protein subunits. SOCE was first found to regulate a number of functions in non-excitable cells [13,14], but several studies have recently demonstrated its importance in excitable cells, including neurons [15–20]. STIMs and ORAIs are ubiquitously expressed throughout the human and murine brain [20–24]. STIM2, a more Ca^{2+}-sensitive homologue [25], is expressed at levels comparable to STIM1 in the human brain and is the predominant isoform in the murine central nervous system. Our group demonstrated that both STIM isoforms interact with ORAI and form complexes in neurons [26,27]. Although neurons possess multiple mechanisms of Ca^{2+} entry (e.g., via voltage-gated calcium channels [VGCCs] and receptor-operated channels [ROCs]), SOCE in neurons orchestrates a variety of processes, such as gene expression, spine morphology, neuronal excitation, stem and progenitor cell proliferation, and axonal growth (for comprehensive reviews, see [28–31]).

Ca^{2+} ions play a central role in the processes of synaptic plasticity, the key phenomenon underlying learning and memory formation [32]. As an ER Ca^{2+} sensor, STIM1 can potentially play a role in modulation of synaptic strength. Indeed, Garcia-Alvarez and colleagues have shown that *Stim1/Stim2* double knockout mice demonstrate an increased long-term potentiation of excitatory synaptic transmission (LTP) [33]. Importantly, an enhanced LTP was reported upon the suppression of constraints in cyclic adenosine monophosphate (cAMP)

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http://dx.doi.org/10.1016/j.bbamcr.2016.11.025

signaling [34,35], while STIM1 was shown to influence cytosolic cAMP levels [36]. Interestingly, this process occurred independently of changes in cytosolic Ca^{2+}, which is characteristic of SOCE. Similarly, cAMP treatment induced STIM1 translocation to the plasma membrane without associations with ORAI and independently of ER Ca^{2+} depletion [37]. The latest findings points to interactions between STIM proteins and ionotropic receptors [38,39]. STIM1 was recently shown to be necessary for metabotropic glutamate receptor 1 (mGluR1)-dependent synaptic transmission that is mediated by transient receptor potential channel 3 [40]. Moreover, in *Stim1* knockout mice, inositol triphosphate (IP_3)- and caffeine-evoked Ca^{2+} release from the ER was abolished, suggesting a role for STIM1 in the IP_3- and ryanodine receptor (RyR)-dependent filling of internal Ca^{2+} stores. However, upon membrane depolarization, store refilling in *Stim1* knockout mice was rescued by Ca^{2+} influx via VGCCs. STIM1 was shown to associate with L-type VGCCs, causing their internalization and inhibition of Ca^{2+} currents [41,42]. It has been speculated that this interaction might play a role in the differentiation of embryonic stem cells into neurons [43].

The association between STIM1 and VGCCs is mediated by Homer [44], a family of postsynaptic scaffolding proteins that are crucial for mGluR-dependent synaptic long-term depression of excitatory transmission (LTD; [45]). Long-term depression can be broadly divided into *N*-methyl-D-aspartate (NMDA)- or mGluR-dependent forms and occurs either presynaptically via a decrease in neurotransmitter release probability or postsynaptically through the internalization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs). In NMDA receptor (NMDAR)-LTD, which can be evoked by low-frequency stimulation, slow Ca^{2+} flux through NMDARs activates phosphatases that subsequently dephosphorylate AMPARs, thus promoting their endocytosis [46]. The nature of mGluR-LTD remains more elusive. The signaling cascades that are responsible for mGluR-LTD involve activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK)/(ERK1/2) and PI3K-mechanistic target of rapamycin (mTOR) pathways [47,48]. Depending on the induction protocol, however, different mechanisms lead to the expression of mGluR-LTD [49,50]. In particular, the pre- and postsynaptic components of mGluR-LTD remain a matter of debate [51]. Notably, LTD that is induced by application of the mGluR1/5 agonist (*S*)-3,5-dihydroxyphenylglycine (DHPG) is considered to occur independently of the canonical mGluR pathway, which involves phospholipase C (PLC), protein kinase C (PKC), and intracellular Ca^{2+} [52–54]. mGluR-LTD that is evoked by 5 Hz stimulation is blocked by chelating intracellular Ca^{2+} and has been shown to involve L-type and T-type VGCCs [55,56].

The aim of the present study was to elucidate the role of STIM1 in synaptic transmission and animal behavior. Using transgenic mice that overexpressed neuronal STIM1, we observed a significant reduction of DHPG-LTD but no changes in LTP or basal synaptic transmission. Behavioral analyses revealed a decrease in anxiety-like behavior in parallel with improvements in contextual learning and no changes in locomotor performance. These data provide further evidence for the involvement of SOCE proteins in shaping animal behavior [33,40]. Altogether, our results point to novel functions of STIM1 in the regulation of synaptic plasticity in the mouse hippocampus.

2. Materials and methods

2.1. Chemicals and antibodies

DHPG was purchased from Sigma (catalog no. D3689). Tetrodotoxin (TTX) was purchased from Alomone Labs (catalog no. T-550). Morbital was purchased from Biowet. 3,3′-diaminobenzidine tetrahydrochloride (DAB) was purchased from Sigma (catalog no. D3939). Fura-4 AM was purchased from Thermo Fisher Scientific (catalog no. F14175). Rabbit polyclonal STIM1 antibody was purchased from Sigma (vendor 1; catalog no. HPA012123). Mouse antibody against STIM1 was purchased from BD Transduction (vendor 2; catalog no. 610954). Polyclonal antibodies against total AKT (catalog no. 2972), phospho-AKT (autophosphorylated at carboxy terminal Ser473 residue; catalog no. 4060), total ERK1/2 (catalog no. 9102) and phospho-ERK1/2 (catalog no. 9101) were purchased from Cell Signaling. Polyclonal rabbit monoclonal antibody against phospo-GluR1 Ser845 was purchased from Millipore (catalog no. EPR2148). Rabbit polyclonal antibody against GluR1 was purchased from Abcam (catalog no. ab109450). The housekeeping protein monoclonal mouse β-actin antibody was purchased from Sigma (catalog no. A2228). The housekeeping protein rabbit polyclonal GAPDH antibody was purchased from Santa Cruz Biotechnology (catalog no. FL-335). The housekeeping protein mouse monoclonal tubulin antibody was purchased from Sigma (catalog no. T5168). Secondary anti-rabbit and anti-mouse antibodies were conjugated with horseradish peroxidase (HRP; catalog no. A0545 and A9044, Sigma) or fluorophores IRDye 800CW and 680LT (catalog no. 92632212 and 92668023, LI-COR).

2.2. Animal care

The mice were housed under standard conditions on a 12 h/12 h light/dark cycle with food and water available *ad libitum*. All of the animal experiments were approved by the Local Commission for the Ethics of Animal Experimentation no. 1 in Warsaw (approval no. 756/2015) and performed in accordance with the European Communities Council Directive (63/2010/EEC).

2.3. Generation of FVB/NJ–Tg(STIM1)Ibd transgenic mice

A 2.1-kb coding sequence of human STIM1 based on cDNA clone MGC:29566 IMAGE:4899542 (Dharmacon) was amplified by polymerase chain reaction (PCR) with XhoI-containing primers: forward 5′-ACTTCACTCGAGACCATGGATGTATGCGTCCGTCTTGCC-3′, reverse 5′-ACTTCACTCGAGCTACTTCTTAAGAGGCTTCTTAAAGATTTTGAGC-3′. Because the measurements of basal Ca^{2+} levels were planned, fusion with green fluorescent protein (GFP) was intentionally omitted. The PCR product was digested with *XhoI* and subcloned into the *Xho*I site of the modified mouse Thy-1.2 expression cassette [57,58]. The STIM1 coding region and flanking Thy1.2 sequence were sequenced to confirm that no point mutations were introduced during the cloning steps. Next, the Thy1.2-STIM1 construct was digested with *PvuI* and *NotI* to excise plasmid sequences, and the linearized fragment was gel-purified and used for standard pronuclear microinjection [59] and injected into pronuclei of fertilized eggs that were derived from FVB/NJ. The procedure was performed in the Laboratory of Animal Models at the Nencki Institute of Experimental Biology (Warsaw, Poland). A total of 496 zygotes were injected, 260 of which were transferred to recipient foster mothers, resulting in 26 live pups. Genomic DNA was isolated from the tail and digested with *HindIII* restriction enzyme. The PCR analysis revealed that two *Tg* founder pups carried the *Thy1.2-STIM1* transgene. The *Tg* rate was 8% (2/26), based on the number of *Tg* pups born/number of embryos transferred. Reverse transcription PCR (RT-PCR) and Western blot confirmed specific expression of the transgene in the brain within the established FVB/NJ-*Tg*(STIM1)Ibd lines.

2.4. Maintenance of Tg(STIM1)Ibd mice and characterization of transgene expression

Founders and F1 animals were identified by Southern blot analysis of *HindIII*-digested genomic DNA with a ^{32}P-labeled STIM1 probe. Offspring of the F2 generation and onward were genotyped by PCR using the following primers: Thy_Forw_genotype 5′-TCTGAGTGGCAAAGGACCTTAGG-3′ and Stim1_Rev_genotype 5′-TGGAGTCTGTTTCCTCGCCAATAG-3′. Genomic DNA was isolated from tail fragments by phenol extraction, and standard PCR conditions were applied. All of the experiments were performed with heterozygous animals. The profile of