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STIM and ORAI proteins in the nervous system

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Stromal interaction molecules (STIM) 1 and 2 are sensors of the calcium concentration in the endoplasmic reticulum. Depletion of endoplasmic reticulum calcium stores activates STIM proteins which, in turn, bind and open calcium channels in the plasma membrane formed by the proteins ORAI1, ORAI2, and ORAI3. The resulting store-operated calcium entry (SOCE), mostly controlled by the principal components STIM1 and ORAI1, has been particularly characterized in immune cells. In the nervous system, all STIM and ORAI homologs are expressed. This review summarizes current knowledge on distribution and function of STIM and ORAI proteins in central neurons and glial cells, i.e. astrocytes and microglia. STIM2 is required for SOCE in hippocampal synapses and cortical neurons, whereas STIM1 controls calcium store replenishment in cerebellar Purkinje neurons. In microglia, STIM1, STIM2, and ORAI1 regulate migration and phagocytosis. The isoforms ORAI2 and ORAI3 are candidates for SOCE channels in neurons and astrocytes, respectively. Due to the role of SOCE in neuronal and glial calcium homeostasis, dysfunction of STIM and ORAI proteins may have consequences for the development of neurodegenerative disorders, such as Alzheimer's disease.

STIM and ORAI Proteins Mediate Store-Operated Ca^{2+} Entry

Increases in the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) control diverse functions in virtually all cell types. These Ca^{2+} signals differ spatially, temporally and in magnitude and are mediated by numerous channels, transporters and Ca^{2+}-sensing proteins. Opening of Ca^{2+} channels in either the plasma membrane or the membrane of intracellular Ca^{2+} stores directly elevates $[Ca^{2+}]_i$. Ca^{2+} entry from extracellular space and Ca^{2+} release from endoplasmic reticulum (ER) operate independently or together to regulate different Ca^{2+}-dependent processes, such as exocytosis of neurotransmitters, contraction of skeletal muscle, activation of T lymphocytes or contraction of smooth muscle cells. Signaling via many cell surface receptors involves the activation of Ca^{2+} release from ER via inositol-1,4,5-triphosphate (IP_3).[1] Because a second phase of IP_3-mediated Ca^{2+} mobilization was frequently observed and identified as Ca^{2+} influx from extracellular space,[2] the hypothesis of "capacitative Ca^{2+} entry" was formulated by Putney in 1986.[3] He proposed a receptor-regulated pathway for Ca^{2+} that enters the depleted Ca^{2+} pool directly via plasma and ER membranes and is controlled by IP_3 and the Ca^{2+} content of the ER.

The capacitative or store-operated Ca^{2+} entry (SOCE), has been recognized as a an essential route for Ca^{2+} uptake in a wide variety of cell types to replenish intracellular Ca^{2+} stores and to regulate secretion, gene transcription and cell cycle progression.[4] Several candidate molecules and ion channels mediating SOCE were discussed in recent years. In particular, some members of the transient receptor potential (TRP) cation channel family were found to generate receptor-activated Ca^{2+} entry.[5] However, electrophysiological characterization of SOCE in mast cells revealed a "Ca^{2+}-release activated Ca^{2+} (CRAC) current",[6] whose properties can be clearly distinguished from those of TRP channels.[5,7] In 2005 and 2006, different groups identified stromal interaction molecules (STIM) and ORAI proteins (named after the keepers of heaven's gate in greek mythology) as essential components of SOCE.[8-15]

Stromal interaction molecule 1 (*STIM1*) was originally identified as a potential tumor suppressor gene coding for a transmembrane protein.[16,17] In vertebrates, one additional *STIM1*-related gene, *STIM2*, was described.[18] Both STIM homologues are ubiquitously expressed in different cell types with higher STIM1 levels in most tissues but a predominant expression of STIM2 in brain.[18,19] STIM1 and STIM2 each contain highly conserved domains, including a single transmembrane segment, an EF-hand Ca^{2+}-binding domain, a sterile α-motif (SAM) domain and coiled-coil regions (for review see refs. 20 and 21). STIM1 and STIM2 are primarily located to the ER; STIM1 was also detected in the plasma membrane.[22] The EF-hand Ca^{2+}-binding domains (together with the SAM domains) of STIMs are responsible for sensing $[Ca^{2+}]$ in the lumen of the ER.[8,10,23] A decrease in ER luminal Ca^{2+} concentration results in dissociation of Ca^{2+} from the EF-hand domain which, in turn, triggers oligomerization and activation of STIM1, a process that is reversed when luminal $[Ca^{2+}]$ returns to resting level (that is, 250–600 μM).[24] The luminal EF-hand domain of STIM1 binds Ca^{2+} with an apparent dissociation constant (K_d) of ~250 μM, whereas the K_d of STIM2 for Ca^{2+} is ~500 μM.[24,25] Hence, STIM2 is more sensitive to small changes in luminal $[Ca^{2+}]$ and partially active at resting ER

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Ca^{2+} levels.[26] STIM2 shows a slower oligomerization kinetics and is less effective than STIM1 in ORAI1 binding and activation, which might be critical for limiting excessive SOCE.[27,28]

ORAI1 and its homologues ORAI2 and ORAI3 were discovered by genome wide RNAi screens for SOCE inhibition and by positional cloning in patients with immune deficiency and CRAC channel dysfunction.[11,13,15] ORAI1 proteins have 4 transmembrane domains and cytosolic N- and C-termini. Co-expression with STIM1 showed that ORAI1 is the pore-forming subunit of the CRAC channel.[12,13,29] ORAI1 channels (therefore, also named CRAC modulator 1; CRACM1) are activated upon Ca^{2+} store depletion, translocation of activated STIM1 to ER-plasma membrane junctions and interaction of cytosolic STIM1 domains with the C-terminal domains of ORAI1 tetramers.[30,31] Heterologously expressed ORAI2 and ORAI3 channels (together with STIM1) conduct CRAC-like currents but exhibit distinct inactivation and permeability properties.[32,33] Store-depletion signaling via STIM2 also activates all 3 ORAI channels.[34] Over-expression of ORAI1 or ORAI2 (but not ORAI3) alone reduced endogenous SOCE, suggesting a fine stoichiometry between STIM and ORAI proteins.[14] Although TRP channels do not account for CRAC currents, a contribution of some TRP family members to STIM1-induced and store-dependent Ca^{2+} entry has been shown.[35] In this context, a SOCE-signaling complex consisting of TRPC1, ORAI1, and STIM1 has been proposed.[36]

The principal components STIM1 and ORAI1 essentially or nearly exclusively contribute to SOCE in different cell types. The function of the innate and adaptive immune system requires STIM1/ORAI1-dependent SOCE.[37,38] In mouse T lymphocytes, STIM2 partially contributes to cytokine production.[39] ORAI2 and/or ORAI3 appear to have redundant functions in mouse T cells, whereas human T cells lacking functional ORAI1 failed to proliferate in vitro and to produce cytokines.[38] STIM1 and ORAI1 regulate mast cell activation,[40,41] and STIM1 is essential for Fcγ receptor-dependent Ca^{2+} signaling and phagocytosis in macrophages and neutrophils.[42,43] Roles of both STIM1 and ORAI1 in other cell types include growth and contractility of skeletal muscle,[44,45] proliferation of vascular smooth muscle cells,[46,47] and aggregation of platelets.[48,49]

SOCE in the nervous system has been reviewed with respect to Ca^{2+} signaling and homeostasis in neurons and neuroglia.[50-53] The present review is mostly based on studies regarding expression analyses or using gene silencing or knockout of STIMs and ORAIs and gives a current overview on the putative functions of these SOCE components in both neurons and glial cells, i.e., astrocytes and microglia.

Expression of STIMs and ORAIs in Brain

STIM and ORAI isoforms are broadly expressed in murine and human tissues. At the RNA level, STIM1 was consistently detected in skeletal muscle and brain,[18,54,55] whereas STIM2 was preferentially found in brain and heart from both species.[18,19] Both STIM proteins were detected in murine and human brain.[19,55,56] Within the murine brain, STIM1 and STIM2 are distributed in the cerebral cortex and can be assigned to hippocampal and cerebellar structures. Whereas STIM1 is most prominent in the cerebellum, STIM2 dominates in hippocampus and cortex.[57] In human brain, STIM1 protein expression is high in cerebellum, medium in cerebral cortex, and low in hippocampus. STIM2 protein levels are high in hippocampus and cerebral cortex, and medium in cerebellum,[58] indicating that the differential distribution of STIM1 and STIM2 in cerebrum and cerebellum is similar in human and murine brain.

Expression of STIM mRNAs at the cellular level was analyzed using cell isolation or separation by laser capture, cell soma harvest and cell culture. In primary hippocampal and cortical neurons, STIM2 is the predominant STIM isoform.[19,55,59] In hippocampal cultures, STIM2 was detected in both neuronal soma and dendrites, whereas STIM1 protein is restricted to the soma.[60] Furthermore, the expression of STIM1 and STIM2 increases during development of hippocampal neurons *in vitro*.[61,62]

In Purkinje neurons, the principal neurons of the cerebellar cortex, STIM1 levels were higher than those of STIM2.[63] The second most important neuron within the cerebellum is the cerebellar granule cell, which is located in the nuclear layer and whose parallel fibers project to Purkinje neurons in the upper molecular layer. In cultured cerebellar granule cells, also a dominant STIM1 expression was found.[64]

All three ORAI isoforms are detectable in murine and human brain. Interestingly, ORAI1 mRNA levels in both species appeared to be lower than those of ORAI3.[54,65,66] ORAI2 is prominently expressed in murine but not in human brain tissue.[54,57,58,65] A high abundance of ORAI2 was found in hippocampal neurons, cerebellar tissue and Purkinje neurons.[19,63] The origin of the strong ORAI3 expression, particularly in human brain, is presently unclear and might come from glial cells. In mouse cerebellum, ORAI3 overlaps with ORAI2 and was also detected in Purkinje neurons.[57,63] The role of ORAI2 in brain as well as in other tissues is still unclear.[67]

Neurons

Store-operated Ca^{2+} entry has been implicated in neuronal Ca^{2+} signaling even before the discovery of STIM and ORAI. Ca^{2+} imaging experiments revealed SOCE in cultured and freshly dissociated cortical and hippocampal neurons.[68-70] A role for SOCE in spontaneous synaptic activity and in synaptic plasticity of hippocampal neurons was suggested from studies using the common SOCE inhibitors 2-aminoethoxydiphenyl borate (2-APB), SKF96365 and La^{3+}.[71,72]

First molecular evidence for neuronal SOCE provided a study using STIM and ORAI knockout mice. Berna-Erro et al. have shown that STIM2-deficient mice were protected from cerebral damage after ischemic stroke.[19] The reduced infarct size and the improved neurological outcome of $STIM2^{-/-}$ animals were independent of functional changes within the haematopoietic system. In cultured cortical neurons, SOCE was significantly decreased in the absence of STIM2 but not of STIM1 or ORAI1.[19] Hypoxia

and hypoglycemia induced an increase in $[Ca^{2+}]_i$ which was markedly reduced in STIM2$^{-/-}$ neurons.[19] Long lasting elevation of intracellular Ca^{2+} is critical for ischemic neuronal cell death.[73] Hypoxia/hypoglycemia inhibits ATP-dependent Ca^{2+} transport into the ER and might, therefore, trigger persistent STIM2 activity and SOCE-induced accumulation of cytosolic Ca^{2+} (**Fig. 1A**). Glutamate-induced excitotoxicity is a process triggered by and contributing to neuronal Ca^{2+} accumulation during ischemia.[74] Sodero et al. reported that glutamate-mediated excitotoxicity leads to a loss of cholesterol in brain.[75] Cholesterol, a major component of the plasma membrane, plays a key role in regulating neuronal functions. Glutamate-induced cholesterol loss required high intracellular $[Ca^{2+}]$ and functional STIM2 in hippocampal neurons.[75] Apart from the role of STIM2 in ischemic stroke, behavioral tests revealed an impairment of hippocampus-dependent spatial learning in STIM2-deficient mice.[19]

Whereas no obvious abnormalities were reported for brain structures of STIM2$^{-/-}$ mice,[19] Sun et al. showed that conditional knockout of the *STIM2* gene in the hippocampus of 2–6 month old mice induced a massive neuronal loss in this brain region.[60] Deletion of STIM2 in hippocampal neurons caused a moderate reduction of somatic SOCE but a dramatic decrease of SOCE in dendritic spines.[60] The absence of STIM2 also reduced the number of spines and changed their morphology by reducing the fraction of mushroom spines which play an important role in the storage of memories.[60] A loss of mushroom spines, concurrent with the decrease in synaptic SOCE and the downregulation of STIM2 was observed in hippocampal neurons from the presenilin-1 M146V knockin mouse model of Alzheimer's disease.[60] Overexpression of STIM2 (but not of STIM1) rescued synaptic SOCE and increased the expression of phosphorylated Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) suggesting that STIM2-dependent SOCE is required for CaMKII activity and stabilization of mushroom spines in healthy neurons (**Fig. 1B**).[60] It is not clear why STIM2 but not STIM1 regulates synaptic SOCE in hippocampal neurons. STIM2 has a higher K_d for Ca^{2+} than STIM1 and induces SOCE near resting ER Ca^{2+} levels.[26] Thus, STIM2 stabilizes basal $[Ca^{2+}]_i$ even at incomplete store depletion and, in turn, maintains steady-state CaMKII activity and integrity of mushroom spines.

Another recent study supports the role of STIM2 in regulating dendritic spine density and morphology in hippocampal neurons. Garcia-Alvarez et al. showed that STIM2 preferentially localizes to large dendritic spines and is enriched in the postsynaptic density.[61] STIM2 is required for regular synaptic activity and mediates cAMP-dependent phosphorylation and trafficking of the AMPA receptor subunit GluA1 to plasma membrane-ER junctions.[61] However, SOCE does not appear to be involved in STIM2-dependent phosphorylation of GluA1. Analogous to STIM interaction with ORAI1, the authors suggest that STIM2 binds via its cytosolic domain to GluA1 and couples GluA1 to cAMP-dependent protein kinase (PKA).[61]

Signaling of STIM proteins through other pathways than SOCE has also been shown for the interaction of STIM1 with the voltage-gated Ca^{2+} channel (VGCC) subtype $Ca_v1.2$.[76,77]

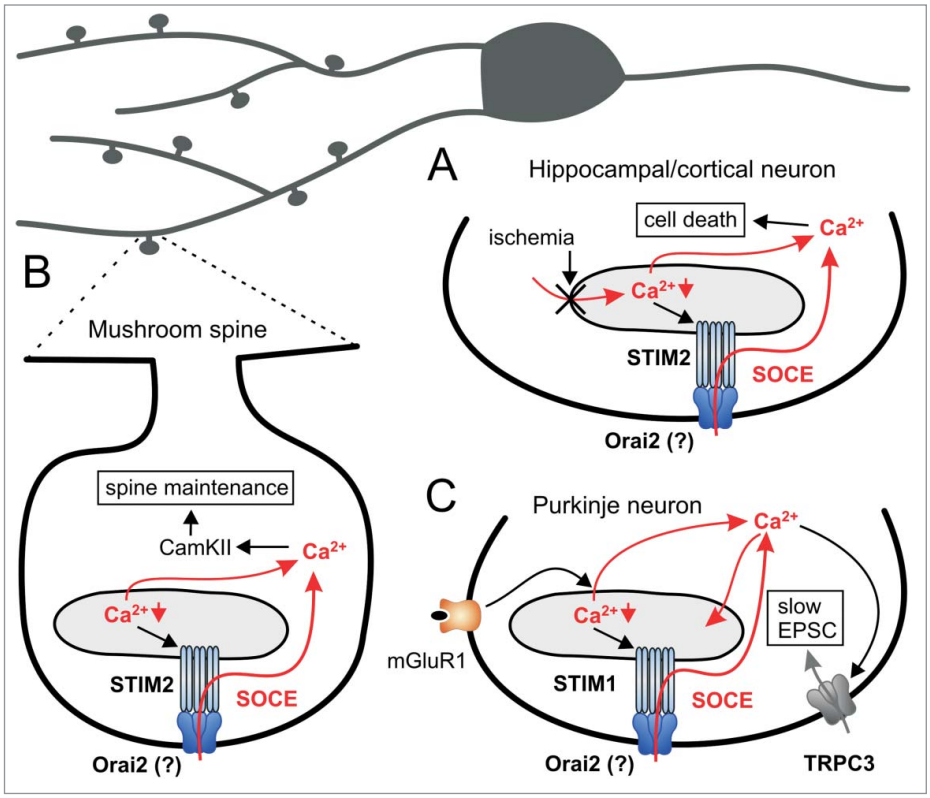

Figure 1. Roles of STIM1 and STIM2 in neuronal Ca^{2+} homeostasis and synaptic function. (**A**) Proposed model for the involvement of STIM2 in ischemic Ca^{2+} accumulation in hippocampal and cortical neurons. A disturbed refilling of intracellular Ca^{2+} stores during ischemia may be induced by inhibition of sarco-endoplasmic reticulum Ca^{2+} pumps. The reduced $[Ca^{2+}]$ in the endoplasmic reticulum (ER), $[Ca^{2+}]_{ER}$, leads to the activation of STIM2 and, possibly, of ORAI2 channels in the plasma membrane. Opening of ORAI channels results in SOCE, which contributes to deleterious Ca^{2+} accumulation in the cytosol. (**B**) Role of STIM2 in maintenance of postsynaptic mushroom spines in hippocampal neurons. Activation of STIM2 due to reduced $[Ca^{2+}]_{ER}$ induces continuous SOCE via ORAI (supposedly, ORAI2) channels. Increased cytosolic $[Ca^{2+}]$ supports constant levels of Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII) and long-term stability of mushroom spines. (**C**) Role of STIM1 in cerebellar Purkinje neurons. Activation of metabotropic glutamate receptor type 1 (mGluR1) induces Ca^{2+} release from ER, a decrease in $[Ca^{2+}]_{ER}$, and the activation of STIM1. SOCE is probably mediated by opening of ORAI2 channels through STIM1. SOCE results in Ca^{2+} store refilling and supports Ca^{2+}-dependent activation of the transient receptor potential channel TRPC3. TRPC3 mediates slow excitatory postsynaptic currents (EPSC) which are important for Purkinje neuron function and cerebellar motor behavior.

STIM1 inhibits VGCC activation by binding to $Ca_v1.2$ via the cytosolic STIM-ORAI activating domain.[76,77] It has been suggested that STIM1-mediated suppression of VGCCs is involved in the differentiation of neurons from embryonic stem cells.[78,79] Knockdown of STIM1 or STIM2 reduced SOCE and inhibited entry of mouse embryonic stem cells into neural lineage.[78] ORAI1 contributed to SOCE but was not required for neuronal differentiation. STIM1 knockdown induced an increase in voltage-dependent Ca^{2+} entry and treatment of cells with the VGCC blocker nifedipine facilitated neural differentiation.[78] Another study corroborates the role for STIM1 in neurogenesis. Somasundaram et al. found a markedly reduced SOCE after knockdown/knockout of STIM1 or ORAI1 in embryonic and neonatal neural precursor cells.[80] Suppression of STIM1 or ORAI1 diminished proliferation of neural precursors and inhibited activation of the transcription factor NFAT (nuclear factor of activated T cells).[80]

Whereas STIM2 regulates SOCE in hippocampal and cortical neurons, STIM1 appears to be the primary STIM isoform in cerebellar granule cells and Purkinje neurons.[79] Lalonde et al. have shown that changes in the extracellular K^+ concentration rapidly induce redistribution of overexpressed STIM1 together with overexpressed ORAI1 and ORAI2 in cerebellar granule neurons.[64] Because a switch of extracellular [K^+] from a high (25–65 mM) to a low level (5 mM; near resting extracellular [K^+]) induces plasma membrane hyperpolarization (repolarization), the authors suggest that changes in the membrane potential induce SOCE in granule neurons. Furthermore, ryanodine receptor-dependent Ca^{2+} stores were depleted by K^+-induced hyperpolarization and a sustained SOCE signal was observed at resting extracellular [K^+].[64] Knockdown of STIM1 in a neuroblastoma cell line and pharmacological inhibition of SOCE in granule neurons inhibited hyperpolarization-induced degradation of the neuron-specific transcription factor Sp4.[64] Sp4 has been implicated in dendrite patterning in cerebellar and hippocampal neurons as well as in memory and synaptic plasticity.[64,79] The activation of SOCE at resting (hyperpolarized) membrane potential suggests a homeostatic function of STIMs in neurons, i.e. the regulation of Ca^{2+}-dependent gene transcription by controlling resting intracellular Ca^{2+} levels.

Hartmann et al. reported that STIM1 controls glutamate receptor-dependent synaptic transmission in Purkinje neurons and motor learning in mice.[63] The metabotropic glutamate receptor type 1 (mGluR1), that is highly expressed in Purkinje neurons, plays an important role in cerebellar functions like motor coordination.[81] Signal transduction downstream of mGluR1 involves the IP_3-dependent Ca^{2+} release from ER and the activation of a slow excitatory postsynaptic current which is mediated by the TRP channel TRPC3.[82] In the absence of STIM1, the IP_3-dependent Ca^{2+} release from dendritic ER Ca^{2+} stores and the TRPC3-mediated currents were abolished.[63] Interestingly, STIM1 was only required for Ca^{2+} store refilling and slow synaptic currents when Purkinje neurons were held at resting membrane potential. Depolarization-induced Ca^{2+} entry via VGCCs, however, induced a STIM1-independent recovery of ER Ca^{2+} release and TRPC3 activity.[63] The latter observation leads to the conclusion that activation of TRPC3 channels depends on intracellular [Ca^{2+}] but not on interaction with STIM1 or the mGluR1-induced activation of phospholipase C (**Fig. 1C**). The essential role of STIM1 in resting Purkinje neurons suggests that neuronal SOCE replenishes intracellular and ER Ca^{2+} levels when VGCC activity is low.

Astrocytes

Astrocytes are glial cells which are derived, like neurons and oligodendrocytes, from neuroepithelial progenitors. They perform a variety of homeostatic functions within the nervous system and communicate with neighboring glial cells and neurons by generating (intercellular) Ca^{2+} signals and by releasing and binding of transmitter molecules.[83] Cultured astrocytes and tumor cells of astroglial origin, i.e., glioblastoma cells, exhibit SOCE in response to metabotropic receptor agonists like ATP, histamine, and glutamate.[84-86]

STIM1 and STIM2 proteins are expressed in cultured astrocytes while STIM1 appeared to be the dominant isoform.[55] STIM1 and ORAI1 have been implicated in Ca^{2+} signaling of astrocytes and glioblastoma cells. Moreno et al. reported that knockdown of STIM1 and ORAI1 or of ORAI1 alone diminishes thrombin-induced Ca^{2+} signals and SOCE in cultured rat astrocytes.[87] Motiani et al. showed a reduction of SOCE and of CRAC currents by silencing of STIM1 or ORAI1 in human glioblastoma cells.[88] Knockdown of STIM1 and ORAI1 slightly affected proliferation but clearly inhibited invasive glioblastoma cell migration.[88]

Ronco et al. investigated the relative mRNA expression of ORAI homologs in cultured rat astrocytes.[89] Surprisingly, the authors were unable to detect ORAI1 but found a dominant expression of ORAI3 which was 6-fold more abundant than ORAI2.[89] Furthermore, previous studies suggest a role of TRP channels in astrocytic SOCE and, therefore, challenge a sole contribution of ORAI channels. Golovina reported that antisense oligonucleotides targeted to the TRP channel gene *TRPC1* inhibited SOCE in murine astrocytes.[90] However, in a later study the same group showed a profound inhibition of astrocytic SOCE by knockdown of ORAI1.[91] Malarkey et al. reported that an antibody against TRPC1 reduces SOCE and abolishes ATP-mediated Ca^{2+} entry in cultured rat astrocytes.[92] Astrocytes also express further TRP channel subtypes, including TRPC4 and TRPC5,[90,92,93] which can form heteromeric channels with TRPC1.[94] A concordant regulation of TRPC1, TRPC4, ORAI3, and of receptor-induced Ca^{2+} entry has been observed in astrocytes treated with different pro-inflammatory agents and amyloid-β protein.[89] Thrombin treatment of astrocytes induced the up-regulation of a further TRP homolog, TRPC3, and an increase in SOCE.[95] The augmentation of SOCE was suppressed after knockdown of TRPC3.[95] In contrast to CRAC channels, which are highly selective for Ca^{2+}, TRPC3 is permeable for some other divalent cations, including Sr^{2+}.[4,96] Grimaldi et al. have shown that store depletion induces only a negligible Sr^{2+} entry in astrocytes suggesting a minor role of TRPC3 in astrocytic SOCE.[97]

Microglia

Microglia are derived from myeloid precursors and constitute up to 20% of the total glia population. Microglia sense disturbances or loss of brain homeostasis and undergo morphological and functional changes in response to brain injury and infection.[98] This "microglial activation" includes shape changes toward an ameboid appearance, directed movement, proliferation, phagocytosis, and release of cytokines. Microglia are equipped with a variety of surface receptors for neurotransmitters, signaling molecules and pathogens. Extracellular nucleotides, i.e. ATP, ADP and UDP, accumulate during brain injury, activate purinergic P2Y receptors and stimulate migration and phagocytosis in microglia.[99-101]

Receptor-induced Ca^{2+} signals play a central role in microglial function and activation.[98] SOCE and CRAC currents have been described in microglia.[102-104] Ohana et al. showed that CRAC/ORAI channels rather than TRP channels mediate SOCE in cultured rat microglia.[105] SOCE was inhibited by a high concentration of 2-APB (50 μM) and by exchange of extracellular Ca^{2+} by Sr^{2+} or Ba^{2+}. From the high expression of ORAI1 and ORAI3 in microglia and the agonistic action of 50 μM 2-APB on ORAI3,[106] the authors suggested a mayor role of ORAI1 in microglial SOCE.[105] The same group detected high levels of ORAI1 and STIM1 in microglial podosomes, actin-rich structures involved in cell motility and invasion.[107] Treatment of cultured rat microglia with SOCE inhibitors strongly reduced migration, i.e., transmigration across filters with defined pores, and invasion, i.e. transmigration through pores coated with extracellular matrix molecules.[107]

Heo et al. confirmed the expression of all ORAI isoforms and of STIM1 in purified cultured mouse microglia and showed that downregulation of ORAI1 and STIM1 reduces SOCE and UDP-induced Ca^{2+} signals.[108] Silencing of STIM1 inhibited lipopolysaccharide-induced activation of NFAT1 and production of interleukin 6 (IL-6) but did not affect activation of NF-κB. STIM1 knockdown slightly decreased release of TNF-α and reduced the UDP-stimulated phagocytosis of bacterial particles.[108] The $P2Y_6$ receptor agonist UDP was previously shown to induce activation of NFAT1 and NFAT2 as well as expression of the chemokines CCL2 and CCL3 in microglia.[109]

We investigated the role of STIM1, STIM2, and ORAI1 in microglia.[110] To estimate the relative contribution of STIM1 and STIM2 to microglial responses and to evaluate the role of ORAI1, cultured microglia were obtained from corresponding knockout mice.[19,48,49] Purity of microglial cultures was tested by staining with isolectin B4 and by using transgenic $CX_3CR_1^{GFP}$ mice,[111] where GFP is exclusively expressed in microglia. In our primary cultures, which showed a purity of ~99.5%, we analyzed the mRNA levels of STIM and ORAI isoforms by quantitative RT-PCR. STIM1, STIM2, and ORAI1 were more abundant than ORAI2 and ORAI3.[110] Calcium imaging revealed a graded suppression of SOCE in the absence of STIM1, STIM2, or ORAI1. SOCE evoked by blockage of sarco-endoplasmic reticulum ATPase (SERCA) was nearly absent in $STIM1^{-/-}$ microglia (91% inhibition), whereas $STIM2^{-/-}$ cells showed a less pronounced but significant inhibition (by 30%). CRAC currents were suppressed in the absence of STIM1. SOCE was substantially decreased in $ORAI1^{-/-}$ microglia (by 70%). Purinergic stimulation with the $P2Y_6$ receptor agonist UDP or the $P2Y_{12}$ receptor agonist 2-methylthio-ADP (2-MeSADP) caused SOCE amplitudes which were significantly smaller than those evoked by SERCA inhibition. The $P2Y_{12}$ receptor-mediated SOCE was blocked by 40% in $STIM2^{-/-}$ microglia and reduced by 77% in $STIM1^{-/-}$ cells, suggesting a decreased effect of STIM1 and an increased impact of STIM2 on SOCE of lower magnitude.[110]

A role for STIM2 in modulating the threshold for Ca^{2+} entry has been reported previously. Kar et al. showed that STIM2 exclusively contributed to Ca^{2+} signals evoked by tyrosine kinase receptors but not by G-protein coupled receptors in leukemia cells.[112] Thiel et al. found a STIM2-dependent SOCE signal after mild store depletion which was completely replaced by a STIM1-dependent SOCE upon strong store depletion.[113] Ong et al. proposed that STIM2 triggers SOCE by recruiting STIM1 at low stimulus intensities when ER Ca^{2+} stores are mildly depleted.[114] Together, these data suggest that STIM2 increases the sensitivity of intracellular Ca^{2+} signals to extracellular agonists.

To evaluate the role of SOCE in microglial migration and phagocytosis we used STIM and ORAI knockout mice, the SOCE blockers 2-APB, La^{3+}, and the TRP channel blocker *N*-(p-amylcinnamoyl)anthranilic acid (ACA).[115] We found that ACA effectively blocks SOCE in microglia with an IC_{50} of 0.4 μM.[110] Transmigration of cultured microglia stimulated with ATP was largely reduced by ACA and SOCE inhibitors. Uptake of zymosan particles by UDP-stimulated microglia was nearly abolished in the presence of 2-APB. P2Y receptor-induced migration (induced by 2-MeSADP, ATP, and UDP) was inhibited in $STIM1^{-/-}$, $STIM2^{-/-}$, and $ORAI1^{-/-}$ microglia. $P2Y_6$ receptor-dependent phagocytosis was nearly abolished in $STIM1^{-/-}$ cells and largely decreased in $STIM2^{-/-}$ and $ORAI1^{-/-}$ microglia.[110] Basal migration and phagocytosis in the absence of purinergic stimuli were not affected by STIM or ORAI knockout.

From these data we concluded that STIM1 and ORAI1 are the mayor constituents of microglial SOCE and that STIM2 is an important component of this signaling pathway. Consequently, SOCE is essential for P2Y receptor-dependent Ca^{2+} entry, migration, and phagocytosis in microglia (**Fig. 2**). Possible downstream effectors of microglial SOCE involve protein kinase C (PKC) and the Ca^{2+}/calmodulin-activated myosin light chain kinase (MLCK) which both activate phagocytosis in microglia.[116,117] The Ca^{2+}-dependent phosphorylation of protein kinase B (Akt) is required for ADP-induced chemotaxis of microglia.[118]

Conclusions and Implications for Neurological Disorders

STIM and ORAI proteins have been implicated in normal brain function but also in neurological disorders. STIM1, preferentially expressed in cerebellar neurons, and STIM2, more abundant in hippocampal and cortical neurons, trigger neuronal SOCE. The versatile role of STIMs and, consequently, of SOCE

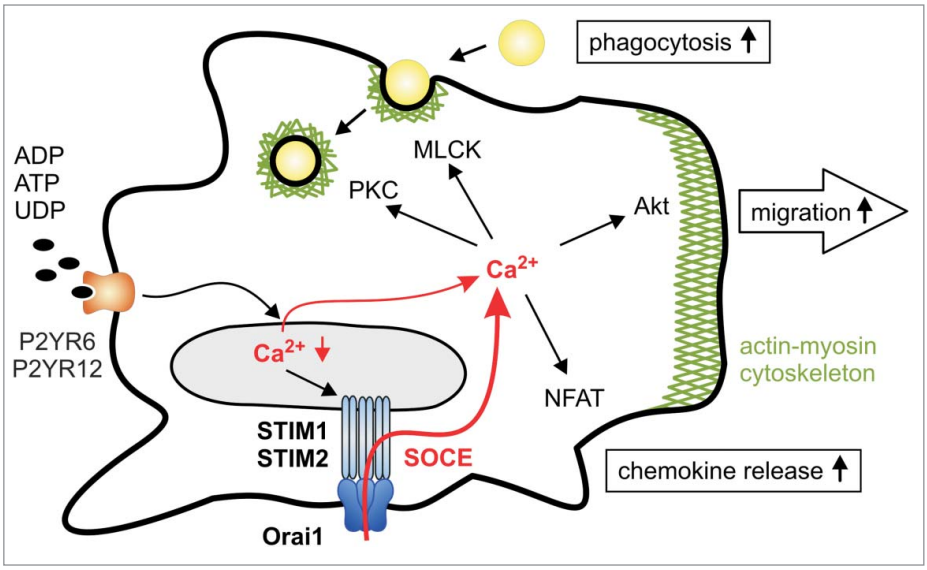

Figure 2. STIM1, STIM2, and ORAI1 regulate microglia functions. The G protein-coupled receptors $P2Y_6$ and $P2Y_{12}$ are activated by UDP and ADP/ATP, respectively. Activation of P2Y receptors induces Ca^{2+} release from endoplasmic reticulum (ER) and reduces $[Ca^{2+}]_{ER}$. In turn, STIM1 and STIM2 proteins activate ORAI1 channels. The resulting SOCE promotes activation of different Ca^{2+}-dependent signaling molecules, including Ca^{2+}/calmodulin-activated myosin light chain kinase (MLCK), protein kinase C (PKC), the serin/threonine specific kinase Akt, and the transcription factor NFAT. Remodeling of actin-myosin skeleton is probably involved in MLCK/PKC-dependent phagocytosis and in Akt-dependent cell migration. NFAT mediates UDP-induced expression of chemokines in microglia.

in neuronal signaling was rather unanticipated because synaptic activity was thought to provide a sufficient supply for intracellular Ca^{2+}. However, recent studies suggest that SOCE is a mayor source for intracellular Ca^{2+} in resting neurons. STIM1 is required for slow glutamate receptor signals in Purkinje neurons and for Ca^{2+}-dependent gene transcription in cerebellar granule neurons.[63,64] STIM2 controls steady-state activity of CAMKII and thereby stabilizes mushroom spines in hippocampal neurons.[60] Decreased STIM2 activity and loss of mushroom spines may be responsible for memory loss in aging neurons and in Alzheimer's disease.[60] STIM2 is also a potential target for the treatment of glutamate-induced excitotoxicity during epilepsy, brain trauma and cerebral infarction.[19,75] The role of ORAI proteins in neuronal Ca^{2+} signaling is still unresolved. In particular, consequences of the dominant ORAI2 expression should be clarified. In comparison to ORAI1, ORAI2-mediated currents show a smaller amplitude but undergo a decreased inactivation at higher $[Ca^{2+}]_i$.[32] In analogy to functional differences between STIM1 and STIM2, ORAI2 might, therefore, support a persistent and moderate "steady-state SOCE" in neurons.

Less is known about the role of STIMs and ORAIs in astrocytes. Here, a further ORAI isoform, ORAI3, might play a central role. ORAI3 is, in contrast to ORAI1 and ORAI2, inhibited by reactive oxygen species (ROS),[119] and is required for store-independent CRAC channels activated by arachidonic acid,[120,121] a phospholipase A2 (PLA2) metabolite. Both, PLA2 activity and ROS production were implicated in neurodegenerative diseases, such as cerebral ischemia and Alzheimer's disease,[122] while astrocytes play diverse supportive roles in maintaining neuronal health.[123] In a mouse model of Alzheimer's disease, a deregulation of astrocytic Ca^{2+} homeostasis and signaling, including increased basal $[Ca^{2+}]_i$ and spontaneous network activity, was observed.[124] Since SOCE is upregulated in astrocytes treated with amyloid-β,[89] future work on the role of STIM and ORAI proteins might help to understand the pathophysiological regulation of astrocytic Ca^{2+} signaling and function.

In microglia, STIM1, STIM2, and ORAI1 represent the mayor SOCE components. While STIM1 is the primary ER Ca^{2+} sensor, STIM2 contributes to SOCE, particularly upon weak Ca^{2+} store depletion. Several microglial functions, i.e., release of cytokines, chemotaxis, and stimulated phagocytosis, are impaired in the absence or after downregulation of these SOCE components. Dysfunction and dystrophy of microglia has been recognized as a potential cause of neurodegeneration and impaired neuronal development.[125-128] For example, engulfment of dysfunctional synapses during development and phagocytosis of cellular debris (such as amyloid-β protein) are possibly regulated by intracellular Ca^{2+} release and SOCE in microglia.[102,129,130] Therefore, STIM1, STIM2, and Orai1 represent possible targets for the treatment of microglial dysfunction that may underlie neurodegenerative and neurodevelopmental disorders.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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