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·Review·

Store-operated calcium entry in neuroglia

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Neuroglial cells are homeostatic neural cells. Generally, they are electrically non-excitable and their activation is associated with the generation of complex intracellular Ca^{2+} signals that define the "Ca^{2+} excitability" of glia. In mammalian glial cells the major source of Ca^{2+} for this excitability is the lumen of the endoplasmic reticulum (ER), which is ultimately (re)filled from the extracellular space. This occurs *via* store-operated Ca^{2+} entry (SOCE) which is supported by a specific signaling system connecting the ER with plasmalemmal Ca^{2+} entry. Here, emptying of the ER Ca^{2+} store is necessary and sufficient for the activation of SOCE, and without Ca^{2+} influx *via* SOCE the ER store cannot be refilled. The molecular arrangements underlying SOCE are relatively complex and include plasmalemmal channels, ER Ca^{2+} sensors, such as stromal interaction molecule, and possibly ER Ca^{2+} pumps (of the SERCA type). There are at least two sets of plasmalemmal channels mediating SOCE, the Ca^{2+}-release activated channels, Orai, and transient receptor potential (TRP) channels. The molecular identity of neuroglial SOCE has not been yet identified unequivocally. However, it seems that Orai is predominantly expressed in microglia, whereas astrocytes and oligodendrocytes rely more on TRP channels to produce SOCE. In physiological conditions the SOCE pathway is instrumental for the sustained phase of the Ca^{2+} signal observed following stimulation of metabotropic receptors on glial cells.

Keywords: calcium signaling; astrocyte; oligodendrocyte; microglia; store-operated calcium entry; TRP; STIM; Orai

Store-Operated Calcium Entry: The Concept

The spatio-temporal organization of intracellular Ca^{2+} signals, which regulate a wide variety of cellular functions, results from tightly coordinated fluxes of Ca^{2+} through intracellular and plasmalemmal membranes. These fluxes are mediated by several families of Ca^{2+}-permeable membrane channels, and Ca^{2+} transporters powered by ATP hydrolysis (Ca^{2+} pumps) or by transmembrane ionic concentration gradients (Ca^{2+} exchangers). The relative contribution of different types of Ca^{2+} fluxes depends on the physiological context and defines the remarkable versatility of the resulting Ca^{2+} signals.

Plasmalemmal Ca^{2+} channels can be activated by different factors. In many excitable cells, plasmalemmal Ca^{2+} influx mainly occurs through ligand-gated Ca^{2+} channels (also known as ionotropic receptors) activated by extracellular transmitters such as glutamate or acetylcholine

and through voltage-gated Ca^{2+} channels gated by changes in membrane potential. In some excitable cells, activation of voltage-gated Ca^{2+} channels triggers massive release of Ca^{2+} from intracellular stores; the trigger mechanisms rely either on direct coupling between plasmalemmal Ca^{2+} channels and type 1 ryanodine receptors localized in the endoplasmic reticulum (ER) membrane (this mechanism underlies electro-mechanical coupling in skeletal muscle) or on an initial flux of Ca^{2+} ions that is positively amplified by Ca^{2+}-dependent opening of type 2 and 3 ryanodine receptors (this pathway dominates in cardiomyocytes and is expressed in neuronal and secretory cells). In electrically non-excitable cells, the predominant mechanism for Ca^{2+} signal generation involves a specific transduction system in which activation of metabotropic receptors results in synthesis of the second messenger inositol 1,4,5 trisphosphate ($InsP_3$), which subsequently activates Ca^{2+} channels (generally known as $InsP_3$ receptors) expressed in the ER membrane that results in Ca^{2+} release. The ER Ca^{2+} source is, however, finite and can be depleted, this depletion being a trigger for opening of the distinct set of plasmalemmal Ca^{2+} channels that mediate a store-operated Ca^{2+} entry (SOCE).

The concept of a specific signaling system connecting the ER and plasmalemmal Ca^{2+} entry pathways emerged in the late 1970s[1-3] and was formalized by Jim Putney within the next decade[4,5]. This concept postulated that: (1) the emptying of the ER Ca^{2+} store is necessary and sufficient for the activation of SOCE; (2) without Ca^{2+} influx *via* SOCE the ER store cannot be refilled; and (3) in physiological conditions the SOCE pathway is instrumental for the sustained phase of the Ca^{2+} signal observed following stimulation of metabotropic receptors. Initially, the SOCE pathway was named "capacitative Ca^{2+} entry"[5], due to certain similarities with the electrical circuitry comprising a resistor (the Ca^{2+} channel) and a capacitor (the Ca^{2+} store). Incidentally, Jim Putney also suggested that the transduction mechanism required a direct link between the ER membrane and the plasmalemma, this suggestion being confirmed in recent experiments.

Molecular Physiology of SOCE

The molecular arrangements underlying SOCE are relatively complex and include plasmalemmal channels, ER Ca^{2+} sensors, and possibly ER Ca^{2+} pumps (of the SERCA type). All this machinery is localized at ER-to-plasma membrane (PM) junctions that provide the morphological substrate for the ER-PM link (see Fig.1 and[6-10]). At least two sets of plasmalemmal channels mediate SOCE, the Ca^{2+}-release activated channels mediating the current known as I_{CRAC}[11-13] and transient receptor potential (TRP) channels[14-16]. It is now generally accepted that the I_{CRAC} channel is formed by a family of homologous proteins, named Orai (after the Greek goddesses, who were the keepers of heaven's gate[17]). Three of these proteins (Orai 1 to 3) have been identified in vertebrates, with each protein being able to form the plasmalemmal channel[17-19]. The Ca^{2+} sensor monitoring intra(luminal)-ER Ca^{2+} concentration has been associated with the stromal interaction molecule (STIM), of which two homologues, STIM1 and STIM2, are expressed in vertebrates[20-22]. Co-expression of Orai and STIM1 results in the appearance of a large I_{CRAC}[23]. The Ca^{2+}-sensing properties of STIM1 are associated with two EF-hand Ca^{2+} binding motifs localized at the N-terminus that faces the lumen of the ER. At ER Ca^{2+} levels above ~400 μmol/L, the EF motifs are assembled with the so-called sterile α-motif (SAM) domain. Decrease in the ER Ca^{2+} content results in dissociation of Ca^{2+} from this EF-hand motif and unfolds the EF-SAM complex, which triggers the oligomerization of STIM1 monomers and initiates the migration of STIM1 oligomers close to the plasmalemma where they form distinct puncta, the loci of their interaction with and activation of Orai Ca^{2+} channels (see for example[8] for details and further references). Replenishment of the ER store with Ca^{2+} triggers the opposite process, which results in dissociation of the STIM1 complex from Orai and inactivation of I_{CRAC}. The clusters of STIM1 and Orai also co-localize with SERCA pumps; this complex apparently ensures the preferential uptake of SOCE-originated Ca^{2+} into the ER lumen. The functional activity of the entire complex is also controlled by mitochondria that may regulate the amplitude of the I_{CRAC} and the degree of Ca^{2+} accumulation by the ER and mitochondria[24,25].

TRP channels, however, may also contribute to the SOCE mechanism. In particular, STIM1 can interact with several TRPC isoforms and activate TRPC1 and TRPC3 channels[7,26-28]. The TRP-mediated currents are of course very different from I_{CRAC} in their gating, conductance and permeation; TRP channels are cation-permeable and do not have strict Ca^{2+} selectivity.

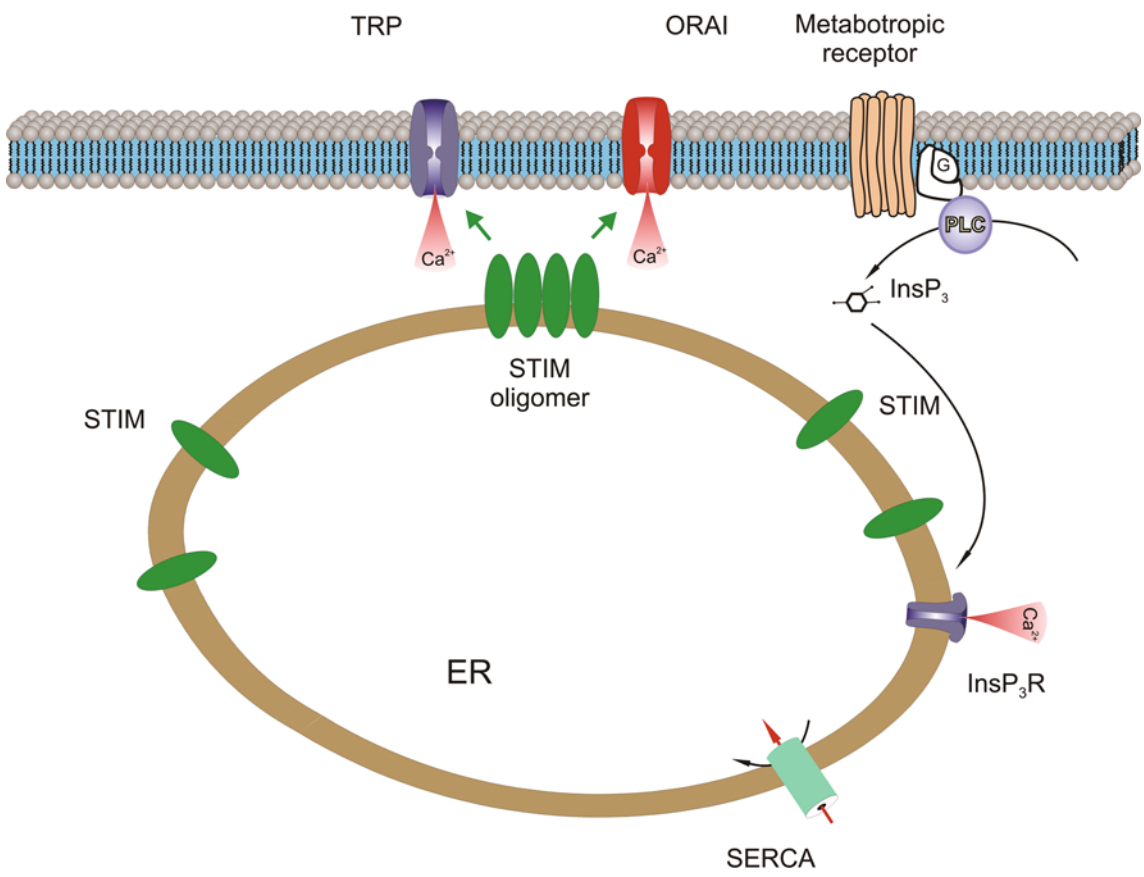

Fig. 1. Schematic representation of the store-operated calcium entry pathway. See text for explanation.

Store-Operated Ca^{2+} Entry in Neuroglia

Neuroglial cells (which include highly heterogeneous astrocytes, oligodendrocytes, NG2 cells and microglia in the central nervous system, and myelinating and non-myelinating Schwann cells, satellite glia, enteric glia and olfactory ensheathing cells in the peripheral nervous system) are homeostatic neural cells[29-31]. Generally, neuroglial cells are electrically non-excitable and their activation is associated with the generation of complex intracellular Ca^{2+} signals and propagating Ca^{2+} waves that define the "Ca^{2+} excitability" of glia[32,33]. As in other non-excitable cells, glial Ca^{2+} signaling is primarily associated with ER Ca^{2+} release[33]; consequently neuroglial cells are in full possession of the SOCE mechanism.

Glial (and particularly astroglial) glutamate receptors have generated specific interest in the last decade, mostly because of the role of glutamate as the principal excitatory neurotransmitter in the CNS. Several types of ionotropic and metabotropic receptors have been discovered in glial cells; these include AMPA and NMDA ionotropic receptors[34-36] and several types of metabotropic receptors of which mGluR5 has been considered to govern ER-originating Ca^{2+} signaling[37,38]. Recently however, it was found that the expression of glutamate receptors undergoes substantial remodeling during ageing and maturation[39,40].

Astrocytes

The SOCE mechanism is ubiquitously expressed in astroglia (Fig. 2). Stimulation of astrocytes *in vitro* or *in situ* with metabotropic agonists (such as ATP, noradrenalin, histamine or endothelin) triggers a biphasic $[Ca^{2+}]_i$ response with a plateau phase almost entirely dependent on extracellular Ca^{2+} (see e.g.[41-45]); this dependency reflects functional expression of the SOCE pathway. Similarly, depletion of the ER Ca^{2+} store in astrocytes with SERCA blockers triggers Ca^{2+} entry through SOCE[46,47]. Astroglial

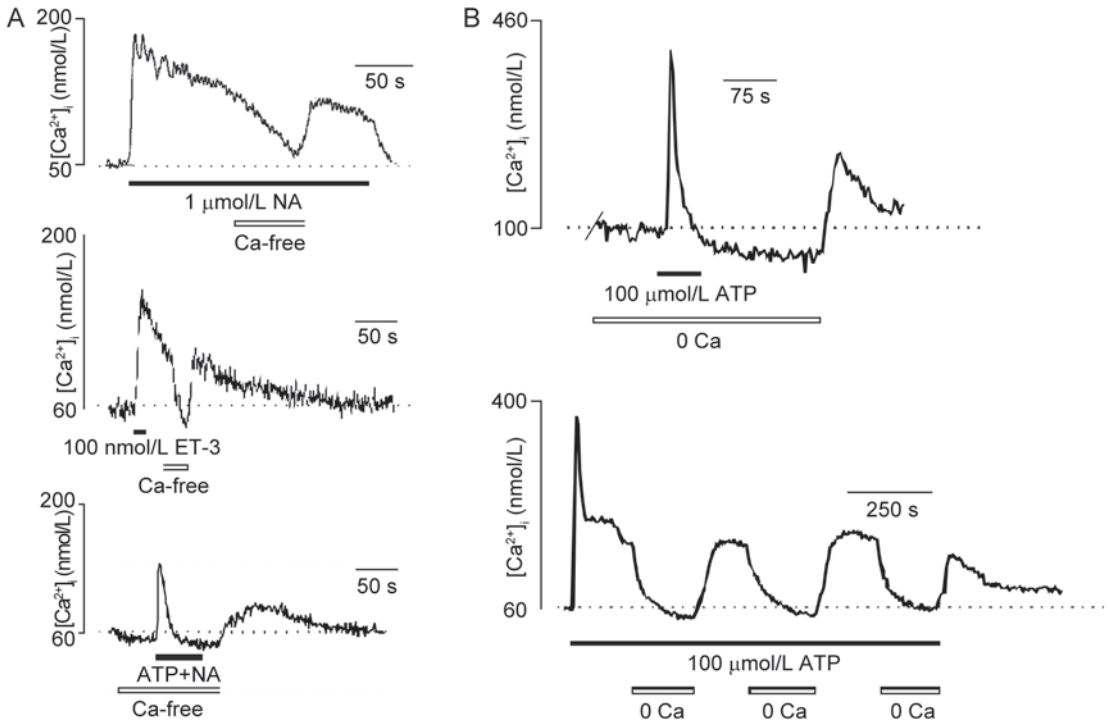

Fig. 2. Examples of SOCE in different types of neuroglial cells. A: SOCE in cerebellar Bergmann glia. The traces show $[Ca^{2+}]_i$ responses to metabotropic stimulation with noradrenalin (NA), endothelin-3 (ET3) and a mixture of ATP and NA. The plateau phase of the response is entirely dependent on extracellular Ca^{2+}, indicating the primary role of SOCE. Reproduced with permission from[74, 75]. B: SOCE in glioblastoma cells. Upper panel shows the $[Ca^{2+}]_i$ response of a single cell stimulated by ATP in Ca^{2+}-free extracellular medium. Restoration of extracellular Ca^{2+} causes a $[Ca^{2+}]_i$ transient which slowly returns to baseline. The lower panel shows the mean response of 51 cells to a long-lasting ATP application. ATP triggers an initial peak which is followed by a plateau phase. Removal of extracellular Ca^{2+} eliminates the plateau phase; after restoration of Ca^{2+} into the extracellular medium, complete recovery of the plateau occurs. Reproduced with permission from[48].

SOCE is highly localized with the sites of Ca^{2+} entry close to the ER that arguably ascertains effective replenishments of the stores[43,45]. The SOCE mechanism has also been identified in pathologically remodeled glia, in particular in glioblastoma cells[48].

The molecular identity and biophysical properties of SOCE in astroglia remain to be fully characterized. The classical I_{CRAC} has not been hitherto recorded from astrocytes. The expression of Orai/STIM1 in astroglial cells has only been found *in vitro*. In the glioblastoma cell line U373 MG mRNAs for Orai1 and STIM1 have been detected; however their contribution to the SOCE has not been directly confirmed[49]. The STIM1 and Orai1 proteins have been identified in primary cultured rat cortical astrocytes. Their role in SOCE following activation of protease activated receptor (PAR) with thrombin has been substantiated based on (1) detection of STIM1 puncta, (2) decrease of SOCE following *in vitro* PAR knockdown with siRNA, and (3) increase of SOCE following Orai1/STIM1 over-expression[50].

There is also substantial evidence for a role of TRP channels in the generation of astroglial SOCE. Various types of TRP channels including several TRPC isoforms have been found in astrocytes[43,44,51,52]. Manipulations with the TRPC1 isoform (that is known to be obligatory for channel formation) substantially affects SOCE in cultured astrocytes. For example siRNA knockdown of the TRPC1 gene reduces SOCE following depletion of the ER stores in astrocytes[43]. Similarly, inhibition of TRPC1 function with a specific antibody inhibits $[Ca^{2+}]_i$ transients following stimulation with ATP affecting primarily the plateau phase of the response (Fig. 3A). Hence, immunological blockade

of TRPC1 results in ~30% reduction of the ATP-induced transient Ca^{2+} elevation, along with full abolishment of the $[Ca^{2+}]_i$ transient plateau. Similarly, the mechanically-induced Ca^{2+} response in astrocytes is greatly reduced by acute immunological inhibition of TRPC1 protein, which also inhibits Ca^{2+}-dependent glutamate release from astrocytes[44] (Fig. 3B, C). Importantly, store-operated activation of TRP channels results in a substantial Na^+ influx which may link the activation of metabotropic receptors and ER Ca^{2+} release with astroglial Na^+ signaling[53].

Oligodendroglia and NG2 cells

Very little experimental evidence on SOCE in cells of the oligodendroglial lineage has been accumulated. Store-operated Ca^{2+} influx is detectable in oligodendrocyte

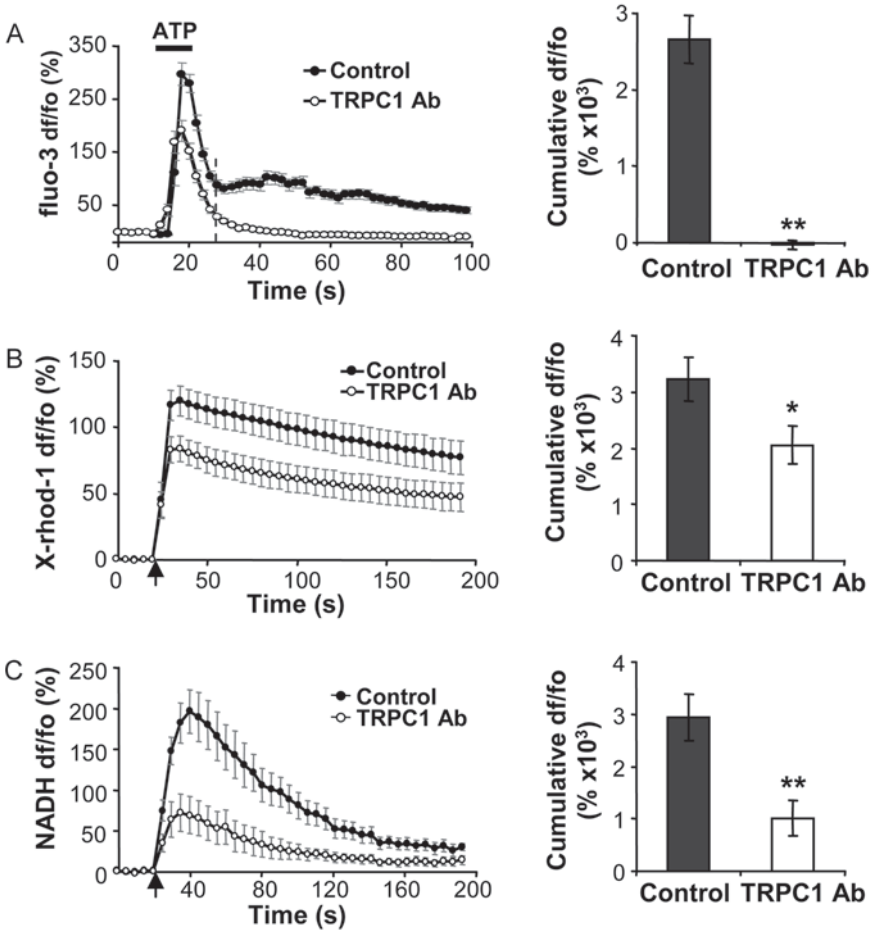

Fig. 3. The role of TRPC1 in SOCE generation in cultured astroglia. A: TRPC1 plays a role in the intracellular Ca^{2+} elevation elicited by receptor activation in astrocytes. Application of ATP (100 μmol/L) to astrocytes from rat visual cortex results in a biphasic intracellular Ca^{2+} response: an initial transient elevation and a sustained (plateau) elevation. Intracellular Ca^{2+} measurements were obtained using the Ca^{2+} indicator fluo-3. If TRPC1-containing channels are blocked by incubating cells with an antibody against TRPC1, the sustained (plateau) Ca^{2+} elevation, reporting on SOCE, is abolished. Vertical dashed line indicates the initial point of a sustained plateau Ca^{2+} response, of which the cumulative values are shown in the bar graph. B–C: TRPC1 plays a role in mechanically-elicited intracellular Ca^{2+} responses in astrocytes and the resulting Ca^{2+}-dependent glutamate release from these cells. Mechanical stimulation causes cytoplasmic Ca^{2+} elevation in astrocytes, as recorded using the Ca^{2+} indicator X-rhod-1 (B). Glutamate release from astrocytes, reported by an increase in extracellular NADH fluorescence, is induced by mechanical stimulation (C). Both responses (Ca^{2+} and glutamate) are reduced when astrocytes are incubated with TRPC1 antibody. Arrows indicate the time of mechanical stimulation. Points and bars in (A–C) indicate mean ± SEM. Asterisks indicate a significant change compared with the control group (Student's *t*-test; **P* <0.05, ***P* <0.01). Modified from[44].

precursor cells (OPCs) in culture where it is involved in the regulation of proliferation. It should be noted that TRPC1 is also important for astrocytic proliferation in cell culture[43]. Nonetheless, the SOCE in these OPCs is positively modulated by golli protein, which belongs to the basic myelin protein family[54]. This golli protein has been reported to interact with STIM1 proteins[55] as well as with TRPC1 channels, the latter being also shown to mediate SOCE in OPCs[56].

Microglia

The SOCE mechanism is widespread in microglia; stimulation of various types of metabotropic receptors (such as P2Y purinoceptors, PARs, platelet-activating factor receptors (PAFRs), complement fragment receptors, endothelin receptors, and lysophosphatidic acid receptors) triggers transient ER Ca^{2+} release, which is followed by a long-lasting plateau of elevated Ca^{2+} that is almost entirely mediated by SOCE (see for example[41,57-59] and[60] for further references). SOCE in microglia demonstrates a peculiar phenomenon of long-lasting activation following strong stimulation of metabotropic receptors. This phenomenon was initially found in cultured microglia stimulated with supramaximal doses of ATP or UTP that activate $P2Y_{2/4}$ purinoceptors and result in full depletion of the ER Ca^{2+} store. This induces a persistent activation of SOCE that lasts for tens of minutes (Fig. 4). During this period, the ER signaling is inhibited because neither metabotropic stimulation nor exposure to thapsigargin, a SERCA blocker, is able to produce an additional $[Ca^{2+}]_i$ signal[61]. Long-lasting SOCE activation also occurs in microglial cells stimulated with phorbol ester, and with brain-derived neurotrophic factor[62,63]. Similar mechanism may also account for the long-lasting elevation of $[Ca^{2+}]_i$ in lipopolysaccharide (endotoxin)-treated cells; this $[Ca^{2+}]_i$ elevation is accompanied by suppressed Ca^{2+} release from the ER[64]. The long-lasting elevation in $[Ca^{2+}]_i$ that can be mediated by chronically activated SOCE has been suggested to be fundamentally important for regulating various aspects of microglial activation[64]. The long-lasting $[Ca^{2+}]_i$ increase is found in microglia treated with another activating agent, interferon-γ[65], in microglia treated with toxic β-amyloid fragment(25-35)[66] and also in microglial cells isolated and cultured from the human post-mortem brains from Alzheimer's disease patients[67].

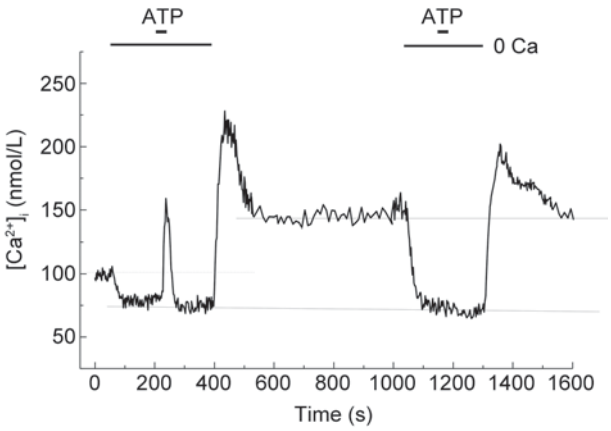

Fig. 4. Long-lasting activation of SOCE in microglial cells. Following depletion of the intracellular Ca^{2+} stores by metabotropic stimulation of glial cells in the absence of extracellular Ca^{2+}, the readmission of external Ca^{2+} induces a large rebound increase in $[Ca^{2+}]_i$. This rebound is followed by a return of $[Ca^{2+}]_i$ to a new steady-state level, higher than the initial resting level. This chronic activation of SOCE renders the ER store non-responsive, and subsequent stimulation by ATP in Ca^{2+}-free conditions fails to elicit Ca^{2+} release (observations by Toescu & Verkhratsky, and[61]).

Whether such a chronic increase in $[Ca^{2+}]_i$ is mediated by activation of SOCE, remains, however, unclear. Furthermore, in microglial cells isolated from Alzheimer's post-mortem brains, the SOCE following PAFR stimulation is substantially reduced, although the duration of the ATP-induced response is much increased[67]. The sustained, chronic SOCE activation is also speculated to be related to chronic activation of microglia in neuropsychiatric disorders[68]. Moreover, microglial SOCE has been reported to be regulated by mitochondria because inhibition of mitochondrial peripheral benzodiazepine receptors with the agent PK11195 reduces Ca^{2+} entry following the activation of PAFRs[69].

The molecular and biophysical identity of microglial SOCE has been studied in cultured cells. A current very much resembling $I_{CRAC,}$ with an amplitude of ~1.3 pA/pF, has been recorded in mouse microglia *in vitro*. The amplitude of this current is reduced to 0.5 pA/pF 48 h after activation of microglia by treatment with lipopolysaccharide[70]. Similarly, I_{CRAC} (with very high Ca^{2+}-selectivity of P_{Ca}/P_{Na} > 1 000) has been detected in cultured rat neonatal microglia. These cells also express mRNA for all three Orais and it has been

suggested that the Orai1/STIM complex is responsible for I_{CRAC} generation[71].

Microglial cells also express a wide variety of TRP channels. RT-PCR of cultured rat microglia reveals the following rank of mRNA expression TRPM7 > TRPC6 > TRPM2 > TRPC1 > TRPC3 ≥ TRPC4 > TRPC7 > TRPC5 > TRPC2[71]. High levels of expression of mRNA for TRPM2 have been detected in rat microglial cells *in vitro* and these channels are supposed to be activated by hydrogen peroxide and intracellular injection of ADP-ribose[72]. Primary cultured rat microglial cells have also been found to express TRPV1 channels as demonstrated by RT-PCR, Western blot analysis and immunocytochemistry[73]. Nonetheless, there are no clear indications that TRP channels mediate SOCE in microglia.

Conclusions

The SOCE mechanism is functionally expressed in all types of CNS neuroglial cells. The molecular identity of the neuroglial SOCE has not yet been identified unequivocally, although it seems that the STIM/Orai-composed I_{CRAC} is predominantly expressed in microglia, whereas astrocytes and oligodendrocytes rely more on TRP channels to produce SOCE.

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