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Review

Store-operated calcium entry in physiology and pathology of mammalian cells

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One of the numerous calcium-involving processes in mammalian cells is store-operated calcium entry (SOCE) — the process in which depletion of calcium stores in the endoplasmic reticulum (ER) induces calcium influx from the extracellular space. Previously supposed to function only in non-excitable cells, SOCE is now known to play a role also in such excitable cells as neurons, muscles and neuroendocrine cells and is found in many different cell types. SOCE participates not only in processes dependent on ER calcium level but also specifically regulates some important processes such as cAMP production, T lymphocyte activation or induction of long-term potentiation. Impairment of SOCE can be an element of numerous disorders such as acute pancreatitis, primary immunodeficiency and, since it can take part in apoptosis or cell cycle regulation, SOCE may also be partially responsible for such serious disorders as Alzheimer disease and many types of cancer. Even disturbances in the 'servant' role of maintaining ER calcium level may cause serious effects because they can lead to ER homeostasis disturbance, influencing gene expression, protein synthesis and processing, and the cell cycle.

Keywords: calcium signaling, calcium stores, store-operated calcium entry, store-operated channels

Calcium plays a crucial role in many important cellular processes such as gene transcription, cell proliferation and differentiation, apoptosis etc. Signaling pathways are often dependent on changes in free cytosolic calcium level. Calcium ions are also known to regulate enzymes and to interact with a large number of other calcium-binding proteins. There is growing evidence that small disturbances in calcium homeostasis may result in serious dysfunction of fundamental cellular processes and cause several diseases.

There are several mechanisms of calcium activity as a second messenger. The store-operated calcium entry (SOCE) is one of the less studied and described such mechanisms. A reason for such a situation could be the fact that SOCE is a complex process in which depletion of intracellular calcium stores in the endoplasmic reticulum (ER) of non-excitable cells results in calcium influx from extracellular space through store-operated channels (SOCs). Initially SOCE was thought to serve only as a way of replenishing ER calcium stores but soon it was found that the contribution of SOCE to cytoplasmic calcium rises is also important. Intensive studies have revealed that SOCE occurs in many cell types including also some excitable ones and that its disturbances can influence many important processes.

Although during the last decade SOCE has been closely investigated, many questions remain unanswered concerning the molecular identity of SOC channels and the mechanism linking ER depletion to the SOC channels' opening. The present review is an attempt to present the diversity of cellular processes involving SOCE.

Abbreviations: Aβ, amyloid β; 2-ABP, 2-aminoethyldiphenyl borate; AC, adenylyl cyclase; APC, antigen-presenting cell; $[Ca^{2+}]_i$, intracellular calcium concentration; CAI, carboxyamidotriazole; CaMK, calcium/calmodulin-dependent protein kinase; CIF, calcium influx factor; CRAC, calcium release-activated channels; CRE, cAMP-responsive element; CRF, corticotropin-releasing factor; ER, endoplasmic reticulum; FAD, familiar Alzheimer disease; IP_3, inositol 1,4,5-trisphosphate; IP_3Rs, IP_3 receptors; JNK, c-Jun-NH_2-terminal kinase; LTP, long term potentiation; MLCK, myosin light chain kinase; MMP-2, matrix metalloproteinase 2; NF, neurofibromatosis; NFAT, nuclear factor of activated T cells; PKA, Protein kinase A; PLC, phospholipase C; PM, plasma membrane; PS, presenilin; RBL, Rat basophilic leukemia; RPAECs, rat pulmonary arterial endothelial cells; RPMYECs, rat pulmonary microvascular endothelial cells; SERCA, sarcoplasmic/endoplasmic reticulum calcium transporting ATPase; SOC, store-operated channel; SOCE, store-operated calcium entry; TCR, T-cell receptor; THR, thyrotropin-releasing hormone; TMD, transmembrane domain; TRP, transient receptor protein; VOC, volatge-operated channel.

MECHANISM OF STORE-OPERATED CALCIUM ENTRY (SOCE)

In many cells stimulation of plasma membrane receptors evokes a biphasic calcium signal caused by release of calcium stored in the ER and the following calcium influx from the extracellular space, as shown on Fig. 1. Numerous studies have revealed that opening of the calcium channels in the plasma membrane is a result of ER store emptying rather than of the changes in the concentration of free cytoplasmic calcium or inositol 1,4,5-trisphosphate (IP_3) (Berridge, 1995b). Inhibition of SERCA ATP-ases in the ER by thapsigargin, blocking calcium reuptake into the ER, results in slow ER emptying and can induce SOCE (Thastrup *et al.*, 1989). Since this procedure bypasses the part of the pathway involving phospholipase C (PLC) activation, IP_3 activity and opening of the calcium channels in IP_3 receptors, thapsigargin is considered one of the best tools for studies on store-operated calcium influx. On this basis the store-operated calcium entry hypothesis was created (Putney, 1990).

SOCE has been found in many cell types but to date it has been studied mostly in non-excitable cells, where it is the main calcium response mechanism. The studies on SOCE are performed using physiological agonists (hormones, neurotransmitters, nucleotides) as well as thapsigargin. To separate the influx phase a special experimental protocol is used, where cells are stimulated in calcium-free medium which allows depleting the ER stores. Such a depletion results in a high but transient peak of cytosolic calcium. When calcium level returns to the basal value, addition of calcium ions to the medium evokes strong calcium influx by the store-operated channels in the plasma membrane (SOCs) reviewed by Parekh and Putney (2005).

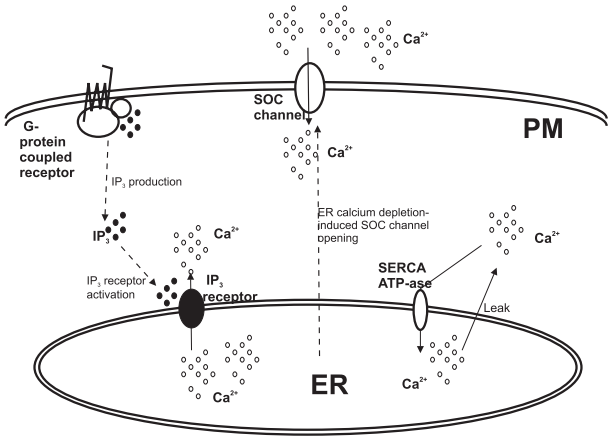

Figure 1. Scheme of store-operated calcium entry.

Activation of plasma membrane receptor coupled with G protein leads to IP_3 production which opens calcium channel in IP_3 receptor and releases Ca^{2+} stored in ER. Store depletion activates SOC channel and causes Ca^{2+} influx from extracellular space. Passive calcium leak from ER is counterbalanced by Ca^{2+} uptake by SERCA ATP-ases. Similarly to receptor-dependent signal, SERCA inhibition leads to ER depletion which activates SOCE. Ca^{2+}, calcium ions; ER, endoplasmic reticulum; IP_3, inositol trisphopsphate; PM, plasma membrane; SERCA, sarco-endoplasmic reticulum calcium ATP-ase; SOC channel, store-operated calcium channel.

Two main unsolved problems concerning SOCE are the molecular identity of SOC channels and the mechanism which links ER depletion with SOC opening. In general, two main models of SOC opening have been proposed — one based on a small diffusible messenger (CIF) released from depleted ER (Randriamampita & Tsien, 1993), and the second suggesting a direct or indirect physical contact between ER proteins and plasma membrane (Berridge,

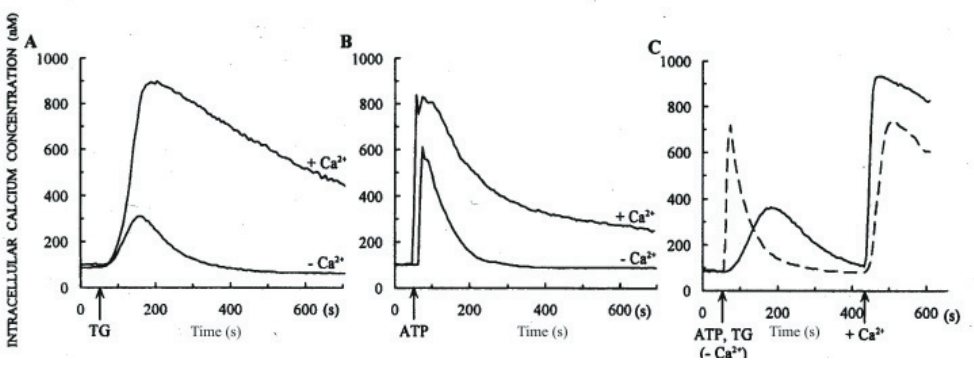

Figure 2. Effect of thapsigargin (TG) and ATP on changes in intracellular Ca^{2+} concentration in glioma C6 cells.

(**A**) Thapsigargin (100 μM) was added to cells in standard buffer containing 2 mM $CaCl_2$ (upper trace, $+Ca^{2+}$), or no $CaCl_2$ and 500 μM EGTA (lower trace; $-Ca^{2+}$); (**B**) ATP (100 μM) was added to cells in standard buffer containing 2 mM $CaCl_2$ (upper trace, $+Ca^{2+}$), or no $CaCl_2$ and 500 μM EGTA (lower trace; $-Ca^{2+}$); (**C**) ATP (100 μM) (dashed line) or thapsigargin (100 nM) (solid line) were added to cells in standard buffer containing no $CaCl_2$ and 500 μM EGTA. After 6 min medium was changed for standard buffer containing 2 mM $CaCl_2$. Each trace in A, B and C is mean value for all of cells measured in particular experiment (from: Sabala *et al.*, 1997).

Table 1. TRP proteins studied as potential candidates for SOC channel elements.

Protein	Function	Properties in different cells
TRPC1	Probably forming SOC channels with TRPC3	Store-operated in H19-7 neurons and in B lymphocytes Store-operated in CHO, COS, salivary gland cells (heterologously expressed)
TRPC2	A role in sexual behavior of mice (TRPC2 knock-outs exhibit sexual behavior deficits)	Store operated in COS-M6 cells (heterologously expressed)
TRPC3	Probably forming SOC channels with TRPC1, SOC properties only when expressed at low levels	Store-operated in H19-7 neurons Store-operated in HEK and COS cells (heterologously expressed) Not store-operated in bovine arterial cells (heterologously expressed)
TRPC4	A role in endothelial permeability and vasorelaxation	Store-operated in mouse aortic endothelial cells and in bovine adrenal cortical cells Not store-operated in proliferating H19-7 neurons and in HEK cells (heterologously expressed)
TRPC5		Store-operated when heterologously expressed in HEK cells – or not (conflicting data)
TRPC6	A role in contractility of tracheal and aortic smooth muscle	
TRPC7	A role in vasorelaxation	Not store-operated in proliferating H19-7 neurons
TRPV6 (CaT1)	Involved in prostate cancer progression	Some properties of SOC in RBL cells Store-operated in CHO cells (heterologously expressed at low levels)

Based on: Minke & Cook, 2002; Harteneck, 2003; Nilius, 2003; Putney, 2003; Freichel *et al.*, 2004; Nilius, 2004; Wu *et al.*, 2004.

1995b). Many different factors have been shown to modulate capacitative calcium influx, including actin cytoskeleton and some other cytoskeleton-associated proteins, small G proteins, tyrosine kinases and protein kinase C (Baranska *et al.*, 1999; Rosado & Sage, 2000).

The SOC channels are mostly identified by their properties revealed by patch-clamp experiments which help to discriminate some functional categories but do not give an answer about their molecular identity. Nevertheless, intensive studies on this topic have led to the conclusion that the best group of candidates for SOC channels is the TRP family.

The first TRP protein was found in the *Drosophila* photoreceptor signaling complex and many more different TRPs have been identified to date, including 28 mammalian homologues. According to their structure these mammalian proteins are classified in groups as TRPC, TRPV, TRPM, TRPP, TRPML and TRPA. Some of these proteins have been shown to be able to form heteromultimeric structures (especially ones from the very well studied TRPC group) and are supposed to form cation channels of different properties.

Studies using numerous molecular methods have revealed that some TRP proteins can form cation channels *in vivo* and since TRP proteins may form heteromultimers, their properties depend on the pattern and level of expression. It seems clear now that SOC channels formed by TRP proteins do not have the same structure in all cell types but their elements, construction and regulation mechanisms can differ between different cells (Table 1). The function of SOC channels can depend not only on their multimeric construction but also on many additional modulators. It seems that, for example, calmodulin, caveolin, ankyrin, annexin 2, IP_3 receptors, PLCγ and some other proteins can bind to TRPC proteins and probably modify their function, making the whole regulation mechanism more complicated.

SOCE IN DIFFERENT CELL TYPES

Glioma C6 — a model non-excitable cell

Among the many types of non-excitable cells glioma C6 seems to be one of the best models for studies on SOCE. It has been shown that these cell belong to the non-excitable cells class, i.e. they do not contain voltage-dependent calcium channels (Baranska *et al.*, 1995). These cells also lack active purinergic P2X ionotropic receptors (Sabala *et al.*, 2001) which makes them a good object for SOCE studies with a clear and undisturbed image of capacitative calcium influx (Baranska *et al.*, 1999).

The shape of the calcium response to ATP-stimulation of purinergic metabotropic receptors in glioma C6 cells is characteristically biphasic, with a peak due to calcium release from the ER and a sustained, more prolonged elevation in $[Ca^{2+}]_i$ as a result of store-operated influx (Fig. 2). Stimulation with thapsigargin evokes a similar but more prolonged signal (Sabala *et al.*, 1997). In experimental conditions it is possible to separate calcium release

from the capacitative influx by cell incubation in a calcium-free medium followed by subsequent addition of Ca^{2+} to the extracellular medium, as described above. Thapsigargin action proves that the calcium influx following ER depletion is store-operated and not activated by the previous rise in cytosolic $[Ca^{2+}]_i$. Glioma C6 cells proved also to be the perfect model for experimental study of numerous factors influencing SOCE. Figure 3 shows the influence of disorganization of the cell cytoskeleton on calcium transient resulting from the activity of SERCA pump inhibitors (Suplat *et al.*, 2004).

SOCE in lymphocytes

In contrast to glioma C6 cells, the intracellular calcium stores in lymphocytes — also non-excitable cells — are relatively small and cannot produce a prolonged elevation in cytosolic calcium level (Donnadieu *et al.*, 1994). Hence, the main source for prolongation and amplification of calcium signal is calcium influx from the extracellular space. In lymphocytes it occurs mainly through SOC channels (Lewis, 2001). These thoroughly described channels have been named CRAC and found in many lymphocytic lines (Lewis, 2001). The calcium current conducted by them, called I_{CRAC}, is one of the best described SOCE currents.

As a main calcium influx pathway, SOCE in lymphocytes can control such cellular events as regulation of transcription factors, organization of the cytoskeleton, cell motility, cytokine production and release as well as cell proliferation (Partiseti *et al.*, 1994; Emptage *et al.*, 2001). SOCE is also necessary for lymphocyte activation (Partiseti *et al.*, 1994; Lewis, 2001). In activated T cells the expression of SOC channels is enhanced and SOCE is significantly augmented. It has been shown that activation of TCR on the cell surface triggers a signaling cascade leading to calcium influx through CRACs (Lewis, 2001). This current provides calcium for sustained cytosolic calcium elevation that is absolutely necessary for interleukin-2 production (Lewis, 2001). Calcium release from intracellular stores cannot induce T cell activation if not followed by calcium influx. In addition, a role of SOCE in cytotoxicity of T lymphocytes has been suggested (Zweifach, 2000).

Apart from proliferation, activation and motility of lymphocytes there is one more process somehow connected with lymphocytic SOCE-regulated processes: tumor cell defence against the T cell. It has been shown that stimulation of acid sphingomy-

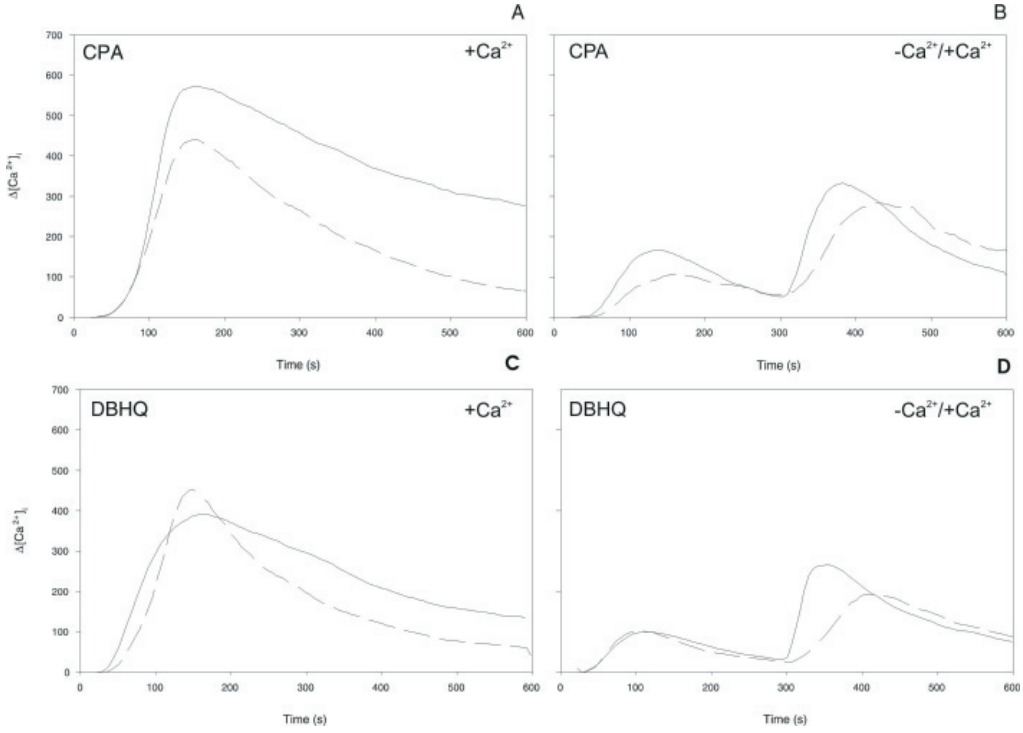

Figure 3. Effect of disorganization of cytoskeleton by cytochalasin D on SERCA ATPase inhibitor induced SOCE.

Two SERCA inhibitors: cyclopiazonic acid (CPA) and 2,5-di-(t-butyl)-1,4-benzohydroquinone (DBHQ) were used, each at 100 μM. Note that even if disorganization of cytoskeleton has different effect on calcium release from ER, in both cases it weakens SOCE resulting from SERCA pump inhibition (second transient on the right plot) (**A**) CPA 2 mM Ca^{2+} environment (**B**) CPA in calcium free environment (500 μM EGTA), and then exchange of medium into 2 mM Ca^{2+} environment (**C**) DBHQ in 2 mM Ca^{2+} environment (**D**) DBHQ in calcium free environment (500 μM EGTA), and then exchange of medium into 2 mM Ca^{2+} environment. Solid line: response of control cells, dashed line: response of cells incubated for 1 hour with cytochalasin D (from: Suplat *et al.*, 2004).

elinase pathway leads to inhibition of SOCE in RBL and T cells. Thus, it was suggested that sphingolipids produced by cancer cells could play a role of immmunosupressors due to their SOCE-inhibiting action (Lewis, 2001).

Considering the mechanism of SOCE effects, it was suggested that the calcium signal evoked by T cell activation could act by calcineurin stimulation since some immunosupressors, such as cyclosporine A, are also calcineurin inhibitors (Berridge, 1995a). However, the SOCE-mediated calcium signal in lymphocytes can influence numerous processes depending on the antigen or cell type and maturity (Lewis, 2001). The same stimulus that causes activation of mature T cells can also cause apoptosis in immature lymphocytes (Blackshaw *et al.*, 2000). The duration and amplitude of calcium signal can precisely regulate different subsets of transcription factors. In HEL-specific naive lymphocytes, where calcium response includes a high peak and a following lower plateau, the first calcium elevation selectively regulates NFκB and JNK while activation of NFAT is plateau-dependent (Lewis, 2001). Moreover, each transcription factor depends on a different calcium oscillation frequency (Dolmetsch *et al.*, 1998), hence a single universal second messenger can precisely modulate many different and often opposite processes.

SOCE in excitable cells

SOCE has also been found in many types of excitable cells and this fact by itself suggests that it should be an important element of calcium homeostasis in these cells and might play a significant role in many processes in the nervous system.

For a long time cells of the nervous system had been thought not to have a store-operated calcium influx pathway. It was obvious that in excitable cells, containing voltage-dependent calcium channels and ionotropic receptors, there is no place for such a pathway of calcium influx. But during the last five years many studies have proved that SOCE occurs also in numerous types of excitable cells (Akbari *et al.*, 2004), including neurons (Emptage *et al.*, 2001). Calcium release from the ER triggering calcium influx through neuronal SOCs may also occur *via* ryanodine receptors (Emptage *et al.*, 2001). Nevertheless, it seems that the SOCE pathway in excitable cells is an additional but important possibility of regulation of cellular calcium-dependent processes.

Calcium stores' depletion with thapsigargin in dorsal root ganglion neurons is known to inhibit neurite initiation and elongation (Mattson *et al.*, 2000). Since thapsigargin-induced depletion activates also store-dependent Ca^{2+} influx it cannot be ruled out that also this process is involved in neurite growth regulation. A recent paper reports an involvement of SOCE in a taste receptor function (Perez *et al.*, 2003). The involvement of SOCE in long-term potentiation and its role in neurodegenerative disorders will be described in the next parts of this review.

SOCE has also been found in different smooth and skeletal muscle lines (Hopf *et al.*, 1996), where it is supposed to have a real but still unclear physiological role (Kurebayashi & Ogawa, 2001).

THE ROLE OF SOCE IN A CELL'S LIFE

Proliferation

Among the processes in which calcium plays a significant role two opposite determinants of cell life are found: proliferation and apoptosis. Cellular calcium homeostasis is an important factor regulating the switch points of the cell cycle (Whitaker & Patel, 1990). Particularly the ER calcium homeostasis is crucial for cell growth (Waldron *et al.*, 1997). The abnormal proliferation of some cancer cell lines is connected with strongly elevated IP_3 levels and overexpression of PLC which must influence calcium signaling, especially calcium release from the ER stores (Berridge, 1995a). Being connected with the nuclear envelope, ER can relay the changes of calcium balance to the nucleus. As a result, changes of calcium level in the ER and cytosol are closely tied to nuclear calcium homeostasis (Berridge, 1995a). Calcium signal is needed to induce expression of many immediate-early response genes (Roche & Prentki, 1994), main examples being c-*Jun*, c-*Fos* and *CRE*. On entry into mitosis one of the main effectors of the calcium signal is calmodulin and CaMK. Inhibition of these proteins can disturb the cell cycle (Whitaker & Patel, 1990).

Strong evidence can be found for SOCE importance in calcium signaling regulating the cell division (Golovina *et al.*, 2001). SOCE could mediate cell proliferation by influencing calcium homeostasis in the ER. Inhibition of SOCE could prevent store replenishment which in turn would inhibit cell growth (Short *et al.*, 1993) and proliferation (Gill *et al.*, 1996) probably by influencing gene expression and protein processing. Additionally, disturbances of SOCE could change the pattern of calcium oscillations that is important for gene expression (Enfissi *et al.*, 2004).

The best known examples of SOCE-dependent regulation of proliferation have been found in lymphocytes and several types of cancer cells. Lymphocyte proliferation depends almost exclusively on SOCE, as was shown mostly in the Jurkat T cell line (Lewis, 2001). Proliferation and activation of T cells correlates with enhanced expression of proteins from the transient receptor proteins family (TRP), namely TRPC and TRPV (Lewis, 2001). This suggests that

these proteins may be involved in SOC channel formation.

In hepatoma cells the proliferation was correlated with the level of SOCE (Enfissi *et al.*, 2004). LNCaP cancer cells stimulated with EGF present enhanced SOCE and proliferation. Both these processes are attenuated in serum-deprived cells (Vanden Abeele *et al.*, 2003). Noteworthy is that the level of expression of different TRP proteins seems to correlate with cell proliferation, especially for TRPC4 in pulmonary artery endothelial cells (Fantozzi *et al.*, 2003) and TRPC6 in pulmonary artery smooth muscle cells (Yu *et al.*, 2003), where both TRPC types are thought to mediate SOCE. Some SOC blockers, such as CAI and 2-APB, have been shown to inhibit proliferation in hepatoma cells (Enfissi *et al.*, 2004) which raises the possibility that SOCE inhibitors could be used as antiproliferative drugs in cancer therapy.

Apoptosis

The participation of calcium in apoptotic pathways is undisputed but many of its aspects remain elusive. It is generally accepted that changes in intracellular calcium compartmentalization can induce and regulate apoptosis (Lam *et al.*, 1994; He *et al.*, 1997). There are two general hypotheses about calcium role in apoptosis: the first implies that the main trigger of apoptotic signaling cascades is the depletion of intracellular calcium stores in the endoplasmic reticulum (Lam *et al.*, 1994; He *et al.*, 1997; Pinton *et al.*, 2000; Vanden Abeele *et al.*, 2002). The second hypothesis is based on the role of the prolonged rise of cytosolic calcium level (Berridge, 1995a; Distelhorst & Dubyak, 1998). In both cases SOCE may be an important part of calcium-involving apoptotic pathways.

In models based on ER depletion SOCE could play a role as one of the sources for the replenishment of calcium stores and have an anti-apoptotic activity. In prostate cancer cells inhibition of SOC channels strongly augmented cell death (Vanden Abeele *et al.*, 2003). Also in osteoclasts inhibition of SOCE resulted in great enhancement of ER depletion-induced apoptosis (Mentaverri *et al.*, 2003).

In the second hypothesis the main trigger for calcium-modulated apoptosis would be sustained elevation of cytosolic calcium level (Wang *et al.*, 1999). Some studies have proved that chelation of cytoplasmatic calcium inhibits apoptosis in prostate cancer cells and thymocytes (He *et al.*, 1997; Wei *et al.*, 1998). Apoptosis of cancer cells caused by irradiation, TGF-β, doxorubicin or 5-fluorouracil requires calcium influx by an unknown pathway (Prevarskaya *et al.*, 2004). It has been suggested that some threshold level of cellular calcium must be reached to induce cell death (He *et al.*, 1997).

In CHO cells incubated without extracellular calcium (which prevents calcium influx) apoptosis was significantly attenuated and authors linked it with a delayed expression of the GADD153 transcription factor (Pigozzi *et al.*, 2004). In hamster embryonic cells apoptosis is preceded by a drop in ER calcium level (Preston *et al.*, 1997) and the authors suggest that the lower calcium pool in the ER may be a result of attenuated SOCE.

A strong correlation between SOCE and apoptosis was found by Jayadev *et al.* (1999) who described a correlation between SOCE disturbances and impaired secretion. Since it is known that apoptosis is accompanied by secretion disturbances (Wyllie, 1997) these results support the thesis on a significant role of SOCE in apoptosis.

Thus, the data about the role of calcium and SOCE in apoptosis are somehow vague. One of the reasons may be the cellular specificity of some apoptotic pathways and of the calcium involvement in this process. Besides, the anti- or proapoptotic action of the calcium signal may depend on its amplitude and duration (Yu *et al.*, 2001). SOCE itself, acting as the source of calcium for store replenishment, might have a protective role in the normal state of the ER but promote apoptosis in case of any disturbances in ER calcium homeostasis (Pigozzi *et al.*, 2004) or prolonged activity of SOC channels.

Little is known in general about the mechanisms of SOCE-dependent apoptosis, however, some cases have been studied in detail. Among the many effects which may be evoked by a SOCE-related calcium signal during apoptosis is activation of calcineurin which is dependent on sustained cytosolic calcium signal (Jayaraman & Marks, 2000). Calcineurin causes dephosphorylation of the NFAT transcription factor, its translocation to the nucleus (Timmerman *et al.*, 1996) and activation of NFAT-dependent genes. There is evidence that in lymphocytes dephosphorylation of NFAT is SOCE-dependent and that this process is mediated by calcineurin (Jayaraman & Marks, 2000). It has been also shown that induction of NFAT-regulated transcription depends on the duration and amplitude of cytosolic calcium rise (Lewis, 2001).

It is also worth to mention that a correlation between high concentration of IP_3 receptors (IP_3Rs) and apoptosis was found in lymphocytes and prostate cancer cells (Blackshaw *et al.*, 2000; Jayaraman & Marks, 2000). In this context especially interesting are type 3 IP_3Rs, associated with the plasma membrane (Putney, 1990) and participating in regulation of SOC channels (Kiselyov *et al.*, 1999; Blackshaw *et al.*, 2000). Taken together these data suggest that both IP_3Rs and SOCE are involved in apoptosis reg-

ulation and that their role may be somehow correlated.

Differentiation

Besides an involvement in proliferation and apoptosis, there is growing evidence that SOCE can also be linked to cell differentiation. The correlation between proliferation and differentiation of H19-7 neuronal cells and TRPC1 and TRPC3 expression proves that SOCE in neurons may modulate also these important processes. TRPC1 and 3 are known to form at least part of SOC channels not only in H19-7 cells, but also in HEK-293 (Wu *et al.*, 2004). Thus their role in SOCE seems to be well proved. Supression of these' proteins expression with antisense RNA results in diminished SOCE and causes apoptosis instead of differentiation (Wu *et al.*, 2004).

Cell differentiation has been shown to result in changed properties of SOCE and expression of SOC channels. Such a relationship was described for muscle cells (Broad *et al.*, 1996), monocytic line U937 (Floto *et al.*, 1996) and HL60 cells (Gardner *et al.*, 1997). In dendritic cells, where SOCE has been shown to be the main pathway of calcium influx, it promotes cell maturation (Hsu *et al.*, 2001). Similar data were obtained for monocytes and bone marrow cells (Koski *et al.*, 1999).

THE ROLE OF SOCE IN CELL FUNCTION

Cell shape and motility

Store-operated calcium influx has been found in many endothelial cells (Luckhoff & Clapham, 1994; Fasolato & Nilius, 1998), including vascular and pulmonary endothelium (Chetham *et al.*, 1999; Norwood *et al.*, 2000; Freichel *et al.*, 2004). It was shown that in endothelial cells SOCE was responsible for nitric oxide production and prostaglandin synthesis (Freichel *et al.*, 2004). There is also strong evidence for a crucial role of SOCE in regulation of pulmonary endothelium permeability (Chetham *et al.*, 1999; Norwood *et al.*, 2000). It has been shown that thrombin-induced permeability is connected with ER depletion-activated calcium influx SOCE (Freichel *et al.*, 2004).

Airways endothelial permeability is caused by changes of cell shape and intercellular gap formation (Chetham *et al.*, 1999). Since the shape changes are caused by reorganization of the cytoskeleton and its contractility, the correlation between the cytoskeleton and SOCE was investigated. The hypothesis about an involvement of the cytoskeleton in SOCE regulation (Rosado & Sage, 2000) was confirmed also for endothelial cells (Norwood *et al.*, 2000). Cytoskeleton tension is mediated by myosin light chain kinase (MLCK) and it was shown that inhibition of MLCK not only promoted reorganization of the cytoskeleton and cell shape changes but also inhibited SOCE. It is possible that cytoskeleton participates in maintaining contact between SOC channels in the plasma membrane and ER (Chetham *et al.*, 1999; Norwood *et al.*, 2000). Nevertheless, there is also the possibility that calcium release from the ER can induce cytoskeleton contractility and cause opening of stretch-activated membrane calcium chanels (Pletjushkina *et al.*, 2001; Pomorski *et al.*, 2004). Such mechanism could co-exist with SOCE and therefore ought to be taken into account.

As described earlier, TRPC4 protein was postulated to be either a part of SOC channel or a SOCE modulator in vascular endothelial cells (Freichel *et al.*, 2004). In mouse microvascular endothelial cells induction of SOCE should stimulate production of nitric oxide and cause vasorelaxation. It was shown that in cells with a knock-out of TRPC4, SOCE was almost completely inhibited and the vasorelaxation induced by agonists was attenuated (Freichel *et al.*, 2004). This leads to the conclusion that TRPC4-mediated SOCE is an important part of the microvascular vasorelaxation mechanism. Moreover, monolayers of knock-out TRPC4 endothelial cells show significantly lower resistance to permeability which again links SOCE impairment with endothelial barrier function (Freichel *et al.*, 2004).

Interestingly, the involvement of SOCE in permeability regulation in endothelial cells seems to be cell type-dependent. Although both in rat pulmonary arterial endothelial cells (RPAECs) and in rat pulmonary microvascular endothelial cells (RPMVECs) the strength of SOCE related calcium transients does not differ, induction of SOCE does not cause a shape change or influence endothelial permeability in RPMVECs (Chetham *et al.*, 1999) while it does so in RPAECs (Chetham *et al.*, 1999; Norwood *et al.*, 2000). These differences may result from different cytoskeleton organization in these cell lines (Chetham *et al.*, 1999).

In the pulmonary circulation SOCE is involved not only in the endothelial function but also regulates agonist- and hypoxia-induced contraction of vascular smooth muscle cells. The main argument is that inhibition of SOCE can block contraction induced by both stimuli in pulmonary aorta cells (Ng & Gurney, 2001). Potential SOC channels in aorta smooth muscle cells have been described (Trepakova *et al.*, 2001). Since supression of TRP1 was shown to block SOCE (Xu & Beech, 2001), a role for TRP proteins, i.e. TRPC1 and TRPC3, in SOC channel construction in these cells has been suggested. SOCE involvement in the regulation of vascular smooth muscle contraction makes SOC channels a potentially good target for pulmonary hypertension therapy.

The cellular shape change induced by SOCE has also been suggested to play a role in angiogenesis (Beck & D'Amore, 1997). Inhibition of plasma membrane calcium channels in HUVEC endothelial cells blocks cell proliferation, migration and tube formation during angiogenesis (Kohn *et al.*, 1996). Short inhibition of calcium influx by CAI (a nonspecific voltage-independent calcium channel blocker) inhibits cell flattening that is a prerequisite for normal and pathological vase formation. CAI also weakens cell adhesion to the substrate, extracellular matrix-stimulated migration and collagenase IV production. A lack of SOCE can also inhibit production of metalloproteinase MMP-2 and attenuate collagenolysis, an important part of angiogenesis and cancer cell invasion (Kohn *et al.*, 1996).

The mechanism of vascular formation is very similar to that of cancer invasion. SOCE inhibition by CAI can stop not only angiogenesis but also cancer cell proliferation and invasion (Kohn *et al.*, 1996). Calcium influx is one of processes involved in cell adhesion to the extracellular matrix induced by integrin crosslinking. Some extracellular matrix proteins, i.e. fibronectin and vitronectin, can induce calcium influx in endothelial cells (Alessandro *et al.*, 1996).

Store-operated calcium influx also takes part in the regulation of T cell motility. Shortly after contact with the antigen-presenting cell (APC) cloned T cells present elevation of cytosolic calcium level followed by cell rounding and inhibition of motility (Donnadieu *et al.*, 1994). The same processes can be induced by use of thapsigargin or ionophores. It has been shown that the calcium signal is not needed for the formation of the lymphocyte-APC contact but is necessary to maintain this connection (Delon *et al.*, 1998). Cytosolic calcium elevation induces reorientation of the cortical actin cytoskeleton to the contact region (Wulfing & Davis, 1998).

An important role of SOCE in the regulation of cell function has also been shown in platelets, where calcium mobilization controls platelet aggregation. It is suggested that impairment of SOCE may be responsible for hyperreactivity of platelets from patients with non-insulin-dependent diabetes mellitus, possibly being a cause of micro- and macroangiopathy in these patients (Saavedra *et al.*, 2004).

Secretion and neurotransmission

As mentioned above, capacitative calcium influx can also be found in excitable cells. One of its roles in these cells seems to be regulation of neurotransmitter secretion. Recently, it was proved that SOCE regulates spontanic release of neurotransmitters in neurons (Emptage *et al.*, 2001). SOCE is known to take part in regulation of exocytosis in non-excitable cells (Parekh & Penner, 1997), interneurons of hippocampus (Savic & Sciancalepore, 1998). In bovine chromaffin cells, SOCE is an element of the angiotensin-induced signaling cascade that mediates secretion (Robinson *et al.*, 1992). It is possible that in synaptic boutons in the hippocampus SOCE is also involved in exocytosis regulation (Emptage *et al.*, 2001). Since store-operated calcium influx has been found in hippocampal neurons (Bouron, 2000) also in these cells it may be involved in neurotransmitter release. Moreover, participation of SOCE in synaptic transmission makes it one of the regulators of synaptic plasticity (Emptage *et al.*, 2001).

SOCE may also be involved in regulation of secretion in neuroendocrine cells (Rohacs *et al.*, 1994), but this seems to be species- and cell population-dependent. It is possible that in excitable cells SOCE may be used as an alternative pathway of calcium influx.

SOCE involvement in secretion has also been described in non-excitable cells. A role of SOC channels formed by TRPC4 in calcium regulation of pancreatic β cells and in insulin secretion has also been suggested but not proven (Freichel *et al.*, 2004). SOCE is part of calcium influx stimulated by thyrotropin-releasing hormone (TRH) in GH_3 pituitary cells and regulates hypophyseal secretion of prolactin (Villalobos & Garcia-Sancho, 1995). Capacitative calcium influx is also one of the calcium influx pathways in mature placenta and probably is involved in neuropeptide-4-mediated secretion of CRF during pregnancy (Belkacemi *et al.*, 2005).

Crosstalk with adenylyl cyclases

One of the most interesting examples of SOCE-regulated processes is the very close relation between store-dependent calcium influx and some types of adenylyl cyclase (AC) dependent signaling. Among the many adenylyl cyclases some are negatively regulated by calcium. These calcium-inhibited ones are localized in the plasma membrane and seem to be regulated mostly by calcium influx from the extracellular space (Fagan *et al.*, 2000). AC I, VI and VIII have been shown to be dependent on calcium influx but not on its release from intracellular stores (Cooper *et al.*, 1994; Chiono *et al.*, 1995; Fagan *et al.*, 2000). Moreover, calcium influx stimulated by ionomycin was ineffective in AC regulation (Fagan *et al.*, 2000), which led the authors to a conclusion that SOCE-dependent ACs and SOC channels have to be localized together in PM microdomains. Calcium influx through voltage-gated channels could also regulate ACs but to a lesser extent. This suggests very close colocalization of ACs and SOCs, in contrast to VOCs (Fagan *et al.*, 2000). The lack of an effect of cytoskeleton disruption with cytochalasin D on SOCE-dependent AC regulation seems to prove that colocalization and coupling of ACs and SOCs

depends rather on lipid segregation or protein–protein interaction than on cytoskeleton structure (Fagan *et al.*, 2000). This relationship is to date the only published example of such an intimate dependence of regulation of a particular protein on SOCE.

SOCE-RELATED DISEASES

Acute pancreatitis

An important role has been suggested for SOCE in acute pancreatitis in which premature activation of trypsin precursor stored in secretory granules leads to autodigestion of the pancreas. The enzyme activation has been shown to require prolonged cytosolic calcium elevation while calcium oscillations or short calcium peaks with a high amplitude seem to be insufficient (Parekh, 2000). SOCE was shown to be the source for calcium signal prolongation (Raraty *et al.*, 2000) and SOC channels are considered a good target for acute pancreatitis therapy.

Primary immunodeficiency

In a disease called primary immunodeficiency, stimulation of TCR receptor cannot cause T cell activation, the well-documented reason being a lack of functional SOCE (Partiseti *et al.*, 1994). Lymphocytes obtained from patients with primary immunodeficiency have normal calcium stores and present calcium release comparable to the control, but depletion-induced calcium influx is almost absent, probably because of dysfunctional SOC channels or an impaired link between store depletion and SOC opening.

Duchenne's dystrophy

There is some evidence for SOCE involvement in Duchenne's dystrophy which is connected with changes of calcium homeostasis. Cells obtained from patients with Duchenne's dystrophy present enhanced calcium influx that has been shown to be SOCE (Vandebrouck *et al.*, 2002). In the cell line mdx from a mouse model of dystrophy, suppression of TRPC1 and 4 attenuated SOCE. Having a dystrophin-homology domain (Lockwich *et al.*, 2000), TRPC1 could be dependent on proper cytoskeleton organization and a lack of dystrophin might result in enhanced SOCE that in turn could activate calcium-dependent proteases that have been shown to be overactivated in dystrophic muscle fibers (Vandebrouck *et al.*, 2002). Those findings are also an argument for a physiological role of SOCE in such excitable cells as skeletal muscles.

Neurofibromatosis

In neurofibromatosis NF1 — a disease which causes bone neoplasms, learning deficits, mental handicap and predispositions to some malignant tumors — significant deficits of SOCE have been described without any apparent changes in ER calcium storage and release (Korkiamaki *et al.*, 2002). Since SOCE has been shown to play an important role in osteoclast survival and apoptosis (Mentaverri *et al.*, 2003) and in LTP induction (Emptage *et al.*, 2001; Ris *et al.*, 2003), it is possible that deficits of SOCE could be responsible for some of the many disorders observed in neurofibromatosis (Korkiamaki *et al.*, 2002).

Neurodegenerative disorders

Even though Alzheimer disease cannot be considered a calcium homeostasis disorder, SOCE in affected cells usually changes during this illness. Some of familial forms of Alzheimer disease are evidently caused by particular missense mutations in genes coding for presenilins 1 and 2 (PS-1, PS-2), known to be responsible for the processing of amyloid precursor protein. Mutations found in familiar type of Alzheimer disease (FAD) have been shown to cause not only the well described changes in amyloid β (Aβ) production (Akbari *et al.*, 2004) but also to disturb calcium homeostasis (Mattson *et al.*, 2000; Yoo *et al.*, 2000). These specific mutations causing FAD are not equivalent to a lack of PS function — knock-outs of PS do not present Alzheimer disease phenotype (Putney, 2000). The question of the potential role of PS-1 in the regulation of calcium homeostasis is a widely studied one but this mechanism has not been established yet.

Since PS1 is localized mostly in the ER and its mutations may lead to abnormalities of intraluminal calcium homeostasis it has been postulated that the SOCE changes observed in Alzheimer disease are a simple result of disturbed ER calcium storage and release (Smith *et al.*, 2002). Numerous groups have reported enhanced calcium release from the ER (Mattson *et al.*, 2000; Yoo *et al.*, 2000) and lower SOCE in cells with FAD PS1 mutations (Waldron *et al.*, 1997). Taken together these two processes may lead to ER depletion and induce apoptosis (He *et al.*, 1997) as well as influence many other processes dependent on ER calcium level (Meldolesi & Pozzan, 1998).

This apparently clear picture is somewhat disturbed by the fact that other groups have reported, conversely, enhanced SOCE in cells with FAD PS1 mutations (Yoo *et al.*, 2000). Together with the evidence that in knock-outs of PS1 SOCE is strongly augmented, these findings have led the authors to conclude that the physiological role of PS1 in cal-

cium regulation is inhibition of SOCE that protects the ER from calcium overload.

Recent results of studies using different PS1 mutants helped to understand the mechanism of PS-1 mutations-related SOCE changes. Akbari *et al.* (2004) showed that mutations of PS-1 could diminish ER capacity but while point mutation enhanced SOCE, deletion of transmembrane domains caused opposite changes. These findings prove that FAD mutations-related changes in γ-secretase activity and ER calcium balance are not related to the changes observed in SOCE (Akbari *et al.*, 2004). It also looks like PS-1 regulates SOCE by its transmembrane domains 1 and 2 (TMD1 and TMD2).

Calcium influx is also connected with Aβ production and its toxicity (Nicotera & Orrenius, 1998). These connections seem to be somehow related to the cytoskeleton since it has been shown that calcium influx may induce cytoskeleton changes typical for Alzheimer disease. Moreover, depolymerization of the cytoskeleton can inhibit calcium influx and prevent Aβ toxicity (Mattson *et al.*, 2000). Thus, even if Alzheimer disease is not caused by Ca^{2+} homeostasis impairment, the latter may be responsible for many disease symptoms, e.g. cell death.

At the end it is worth noting that PS-1 mutations result in SOCE changes not only in neurons but also in other cell types, i.e. fibroblasts from patients with Alzheimer disease. This could be useful in early diagnosis of Alzheimer disease (Ito *et al.*, 1994). Given that in cells with FAD PS1 mutations many changes seem to be related to attenuated SOCE, there is a chance that substances stimulating SOC channels could be useful in Alzheimer disease prevention and/or therapy (Yoo *et al.*, 2000).

The role of SOCE in LTP, considered to be one of the mechanisms of memory formation, seems to be linked to the action of presenilin 1 (PS-1). It is worth to mention that in cells without PS-1 calcium influx through SOC channels is sufficient for LTP induction (Ris *et al.*, 2003). The second phase of LTP induction that is dependent on *de novo* protein synthesis involves a signaling cascade triggered by cAMP-dependent PKA which is activated by strong calcium influx. Probably in cells lacking PS-1 enhanced SOCE is sufficient for this PKA activation. Currently, intensive research concerns the reasons of neurodegenerative disorders and it seems that devoting some attention to SOCE involvement would be worthwhile. Participation of SOCE in LTP regulation may explain many of the memory defects observed in different neurodegenenative disorders. Based on data from *Drosophila* mutants a theory has been put forward that alterations in expression of TRP forming a SOC channel could be the basis of some neurodegenerative brain diseases (Missiaen *et al.*, 2000).

Cancer

The role of SOCE in apoptosis and proliferation was discussed above. Both processes are especially important in cell transformation and malignancy. The apoptotic resistance of cancer cells is linked to changes in calcium homeostasis, including calcium influx (Jayadev *et al.*, 1999; Pinton *et al.*, 2000).

Many studies about the role of SOCE in cancer cells were performed on prostate cancer cells, especially on a line from a lymph node metastasis of prostate cancer, LNCaP (Vanden Abeele *et al.*, 2003). This line is androgen independent mainly as a result of its enhanced apoptosis resistance. In androgen-independent cells all the elements involved in apoptosis are present but the triggering mechanism seems not to work (Prevarskaya *et al.*, 2004). It has been suggested that in androgen-dependent cells apoptosis triggered by androgen deprivation is connected with prolonged elevation of cytosolic calcium (Prevarskaya *et al.*, 2004) but the contribution of SOCE in this elevation is not known.

Cancer grade correlates with expression of TRPV6 (Fixemer *et al.*, 2003; Vanden Abeele *et al.*, 2003) that according to many studies may be a component of a SOC channel (Xu & Beech, 2001). Pharmacological inhibition of androgen receptor enhances expression of TRPV6 and augments SOCE (Vanden Abeele *et al.*, 2003). Antisense suppression of TRPV6 in LNCaP cells attenuates SOCE by about 50%. Also TRPC1, TRPC4 and TRPC6 are investigated as potential SOC forming proteins in prostate cancer cells (Vanden Abeele *et al.*, 2003).

The role of SOCE in cancer cells is controversial. It has been shown that cells that become apoptosis-resistant show enhanced Bcl-2 expression which correlates with attenuated SOCE (Prevarskaya *et al.*, 2004). In such apoptosis-resistant cells depletion of ER calcium stores is not sufficient to trigger apoptosis and the role of SOCE-dependent calcium signal in apoptosis is clearly visible (Vanden Abeele *et al.*, 2002; Vanoverberghe *et al.*, 2004). A lower number of SOC channels has been reported in such cells (Vanden Abeele *et al.*, 2002; Vanoverberghe *et al.*, 2004), consistent with attenuated SOCE but in opposition to some reports about enhanced expression of the channel-forming protein TRPV6 (Prevarskaya *et al.*, 2004).

Despite these discrepancies, the obvious coincidence between TRPV6 expression, its participation in SOCE and the important role of SOCE in proliferation and apoptosis of cancer cells encourages a search for anticancer therapeutics among SOC channels modulators (Vanden Abeele *et al.*, 2003). For example, it seems that the calcium influx inhibitor CAI, a potent inhibitor of cell adhesion, migration, vascular tube formation and multiple minor proc-

esses involved in angiogenesis, could be one of the best candidates for use in cancer therapy, being able to stop not only proliferation of cancer cells but also invasion and tumor angiogenesis (Kohn *et al*., 1996).

In conclusion, involvement of SOCE in such crucial processes as those described above makes this phenomenon an important subject of scientific studies, especially in search for the molecular identity of SOC channels. There is hope that these studies will not only solve many problems of SOCE regulation but also may have a practical use in drug targeting and therapy for some diseases.

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