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Role of the actin cytoskeleton in store-mediated calcium entry in glioma C6 cells

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Abstract

The effects of actin cytoskeleton disruption by cytochalasin D and latrunculin A on Ca^{2+} signals evoked by ADP, UTP or thapsigargin were investigated in glioma C6 cells. Despite the profound alterations of the actin cytoskeleton architecture and cell morphology, ADP and UTP still produced cytosolic calcium elevation in this cell line. However, calcium mobilization from internal stores and Ca^{2+} influx through store-operated Ca^{2+} channels induced by ADP and UTP were strongly reduced. Cytochalasin D and latrunculin A also diminished extracellular Ca^{2+} influx in unstimulated glioma C6 cells previously incubated in Ca^{2+} free buffer. In contrast, the disruption of the actin cytoskeleton had no effect on thapsigargin-induced Ca^{2+} influx in this cell line. Both agonist- and thapsigargin-generated Ca^{2+} entry was significantly decreased by the blocker of store-operated Ca^{2+} channels, 2-aminooethoxydiphenylborate. The data reveal that two agonists and thapsigargin activate store-operated Ca^{2+} channels but the mechanism of activation seems to be different. While the agonists evoke a store-mediated Ca^{2+} entry that is dependent on the actin cytoskeleton, thapsigargin apparently activates an additional mechanism, which is independent of the disruption of the cytoskeleton. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Actin cytoskeleton; Store-mediated Ca^{2+} influx; ADP; UTP; Thapsigargin; Glioma C6

Nonexcitable cells, e.g., hepatocytes, blood, and epithelial cells do not express voltage operated Ca^{2+} channels. Ca^{2+} entry into these cells is regulated by the concentration of Ca^{2+} ions in the endoplasmic reticulum (ER) pool [1,2]. The depletion of this pool (the first phase) causes the opening of voltage-independent Ca^{2+} channels in the plasma membrane (PM) that permits the Ca^{2+} entry (second phase), whereas refilling of the ER store blocks this Ca^{2+} influx. The above process has been termed "capacitative" or "store-mediated" calcium entry [3]. Ca^{2+} entry coupled to the state of filling of the ER Ca^{2+} store is independent of the mechanism of its depletion. First messengers mediating inositol-1,4,5-trisphosphate (IP_3) formation liberate Ca^{2+} from the ER via the IP_3 receptors [4], whereas thapsigargin, an irreversible inhibitor of the SERCA ATPase, promotes the

leak of Ca^{2+} from the ER without IP_3 formation. Both physiological agonists and thapsigargin initiate Ca^{2+} entry into the cell as a consequence of the depletion of the ER stores. The nature of the signal linking the ER Ca^{2+} store to store-mediated Ca^{2+} channels of the PM is still debated [2,5].

One of the hypotheses that have been proposed postulates a conformational coupling in which information is transferred by the ER IP_3 receptor directly to the store-operated Ca^{2+} channel (SOC) [5]. A secretion-like-coupling model has also been proposed, which involves the reversible interaction between the ER and the PM [6]. In the secretion-like-coupling model, the actin cytoskeleton may be involved as in secretion [7,8]. It is worth adding that in excitable cells, like starfish oocytes, IP_3 -mediated Ca^{2+} release is involved in the cytoskeletal rearrangement that occurs during the maturation process [9].

We have previously shown that in glioma C6 cells the stimulation of nucleotide receptors by agonists ATP,

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UTP, and ADP, and the exposure to thapsigargin, initiate a biphasic Ca^{2+} response compatible with the typical capacitative model of Ca^{2+} influx [10–12]. The aim of the present study was to test whether the actin cytoskeleton had a role in this process in glioma C6 cells. The data have shown that in the case of ADP and UTP both the Ca^{2+} release from the ER store and the influx of Ca^{2+} are significantly reduced by the disruption of the cytoskeleton. By contrast, the thapsigargin-mediated influx of extracellular Ca^{2+} is unaffected by the cytoskeleton modification. The results thus suggest an additional cytoskeleton-independent mechanism for the thapsigargin-induced store-mediated Ca^{2+} entry.

Materials and methods

Materials. Minimum essential medium (MEM), calf serum, and antibiotics were from Gibco-BRL. ADP, UTP, 2-aminoethoxydiphenylborate (2-APB), cell dissociation solution, bovine serum albumin, and EGTA were purchased from Sigma Chemical. Latrunculin A, cytochalasin D, tetramethylrhodamine isothiocyanate (TRITC)-labelled phalloidin, and Fura-2 AM were from Molecular Probes, Eugene, OR. Thapsigargin was from LC Services.

Cell culture. Glioma C6 cells (40–60 passages) were cultured in MEM supplemented with 10% (v/v) calf serum, antibiotics: penicillin (50 IU/ml), streptomycin (50 $\mu\text{g}/\text{ml}$), and 2 mM L-glutamine, under the humidified atmosphere of 5% CO_2 at 37 °C. The cells were passaged when confluent by using nonenzymatic cell dissociation solution and the medium was changed twice a week. The cells used for the experiments were seeded on glass coverslips and cultured for two days under the same conditions.

Intracellular calcium measurements. Intracellular Ca^{2+} levels were measured, as described previously [13] with the following modifications to the procedure. Cells on coverslips were washed twice with a solution containing: 137 mM NaCl, 2.7 mM KCl, 1 mM Na_2HPO_4 , 25 mM glucose, 20 mM Hepes (pH 7.4), 1 mM MgCl_2 , 1% (v/v) bovine serum albumin, and 2 mM CaCl_2 (standard buffer). In the experiments in the absence of external Ca^{2+} , 500 μM EGTA replaced CaCl_2 . Glioma C6 cells were then incubated at 37 °C for 30 min in the standard buffer with 2 μM Fura-2 AM. Thereafter, the cells were washed twice with the standard buffer and the coverslips were mounted in the heated chamber of a Zeiss Axiovert 200 microscope, equipped with a Plan-NEOFLUAR 40 \times /0.75 (air) objective lens. The experiments were carried out with the use of digital imaging system with MicroMax 5 MHz Cooled CCD camera (Princeton Instruments, Trenton, NJ), LAMBDA 10-2 filter-wheel with shutter (Sutter Instruments, Novato, CA), and the MetaMorph/MetaFluor Imaging System software (Universal Imaging, West Chester, PA). The cells were alternatively illuminated with light of 340 and 380 nm wavelengths from a xenon lamp and the emitted light was passed through a 510 nm barrier filter. The 340 and 380 nm images were captured at 0.5 s intervals and the 340 and 380 nm signals were processed and examined for real changes in $[\text{Ca}^{2+}]_i$ with MetaFluor software. All data are expressed as 340 nm/380 nm Fura-2 fluorescence ratio and were estimated according to Grynkiewicz et al. [14].

Fluorescent staining of the actin cytoskeleton. The cells were fixed in experimental buffer containing 3% paraformaldehyde and 0.1% glutaraldehyde for 15 min at 23 °C, rinsed three times with PBS, and permeabilized with 1% Triton X-100 for 10 min. Then, they were rinsed three times with PBS and stained with 4 μM TRITC-labelled phalloidin for 20 min at 23 °C. The observations were made under a laser scanning confocal microscope system LSM 510 (Carl Zeiss) with Plan Apo 63 \times , 1.4 N.A. oil immersion objective lens using an excitation light of 543 nm.

Actin cytoskeleton disruption. Cells on coverslips were washed twice with standard buffer and incubated with 5 μM cytochalasin D for 3 h or with 3 μM latrunculin A for 1 h. Following the treatment, cells were loaded with Fura-2 AM, as described above (the loading time was included in the cytoskeleton disrupting treatment). All incubations were performed in the standard buffer in a humidified atmosphere of 5% CO_2 at 37 °C.

All reagents were added as solutions made in standard buffer at the following final concentrations: 10 μM ADP, 100 μM UTP, and 100 nM TG. Each experiment was repeated at least three times and data are expressed as means \pm SD. The statistical analysis was carried out using Statistica 5.77 software.

Results

Breakdown of actin cytoskeleton

Cytochalasin D binds to the barbed end of filamentous actin and latrunculin A binds to actin monomers. Both compounds are membrane-permeant inhibitors of actin polymerization [15–17]. The treatment of glioma C6 cells with latrunculin A or with cytochalasin D as described in the Materials and methods section resulted in profound changes in the actin cytoskeleton architecture. Analysis under a laser scanning confocal microscope revealed that stress fibres were not present (depolymerized). The actin cytoskeleton was deeply rearranged or disrupted in cytochalasin D pretreated cells stained with TRITC-conjugated phalloidin (Fig. 1). Similar effects on actin cytoskeleton architecture were observed when the cells were treated with latrunculin A (not shown). Additionally, morphological changes were visually checked before each experiment by differential interference contrast microscopy. Under the cytoskeleton-disrupting conditions, cells became spherical with long thin projections of cytoplasm attached to the surface of the coverslip. Control cells treated only with vehicle in the same conditions were flat and elongated, showing typical fibroblast-like phenotype (not shown).

Intracellular calcium signals after actin cytoskeleton disruption

The addition of ADP or UTP to glioma C6 cells treated with cytochalasin D in the presence of extracellular Ca^{2+} (Figs. 2A and C) or latrunculin A (Figs. 2B and D) generated a cytosolic calcium rise, which was significantly lower than that in control. Statistical analysis (mean value \pm SD from 4 to 10 separate experiments) of the data revealed that in control cells the initial Ca^{2+} peak evoked by ADP, which was associated with the depletion of intracellular stores, amounted to 0.98 ± 0.14 arbitrary units ($n = 403$) whereas that evoked by UTP amounted to 0.91 ± 0.17 ($n = 198$). After cytochalasin D treatment, the initial Ca^{2+} transient amounted to 0.71 ± 0.14 ($n = 230$) for ADP and to 0.77 ± 0.16 ($n = 137$) for UTP. After latrunculin A treatment, the initial peak reached 0.77 ± 0.18

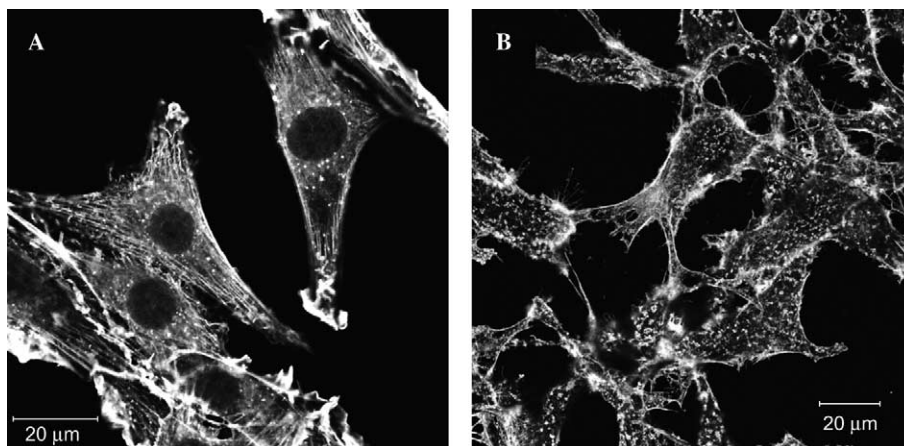


Fig. 1. Actin cytoskeleton disassembly in glioma C6 cells after cytochalasin D treatment. Laser scanning confocal microscopy of glioma C6 cells before (A) and after (B) the incubation with 5 μM cytochalasin D for 3 h. The cells were stained with TRITC-phalloidin to detect filamentous actin.

($n = 174$) for ADP and 0.71 ± 0.19 ($n = 78$) for UTP. The initial peak triggered by ADP and UTP was followed by a longer, sustained Ca^{2+} elevation, which was associated with the Ca^{2+} entry. This second phase was also significantly diminished with respect to the controls in cytochalasin D and latrunculin A treated cells. However, the disruption of the actin cytoskeleton had no effect on the Ca^{2+} increase induced by thapsigargin in glioma C6 cells, where the maximal values of Ca^{2+} elevation amounted to 0.79 ± 0.1 ($n = 143$) for the controls and to 0.85 ± 0.11 ($n = 179$) or 0.80 ± 0.14 ($n = 108$) in cells treated with cytochalasin D or latrunculin A, respectively (Figs. 2E and F).

The lack of effect of actin cytoskeleton disruption on the thapsigargin-induced intracellular Ca^{2+} elevation was analysed more closely by expressing the relative Ca^{2+} rise as the increase in $[\text{Ca}^{2+}]_i$ above the resting level (Fig. 3). When the actin cytoskeleton was modified with cytochalasin D, the calcium signal evoked by ADP was clearly inhibited (Figs. 3A and C). The treatment resulted in a 30% decrease in the values for the initial peak (I) and for the sustained phase (II) while no changes occurred in the action of thapsigargin (Figs. 3B and D) in glioma C6 cells tested in standard buffer containing 2 mM CaCl_2 ($p < 0.001$).

The values above bars correspond to the increase of intracellular calcium measured in experiments on cells after cytoskeleton disrupting treatment and are expressed as a percentage of the calcium increase in control experiments when cells not treated with cytochalasin D were used (Figs. 3C and D).

In another set of experiments, glioma C6 cells were treated with cytochalasin D as described in the Materials and methods section, but the intracellular calcium was monitored in Ca^{2+} free buffer containing 500 μM EGTA (Fig. 4). Under such conditions, the ADP-evoked rise in the cytosolic Ca^{2+} , which was exclusively caused by the depletion of the intracellular stores, reached a value of

0.53 ± 0.11 ($n = 190$) while in control cells it amounted to 0.84 ± 0.13 ($n = 150$). The Ca^{2+} rise (I phase) was thus inhibited by 67% ($p < 0.001$) after actin cytoskeleton disruption (Fig. 4C). If the Ca^{2+} free buffer was replaced by standard buffer containing 2 mM CaCl_2 when the intracellular calcium concentration had declined to the basal level, the calcium influx was triggered through the store-operated channels (SOC). Again, it was lower in cytochalasin D treated cells: The $[\text{Ca}^{2+}]_i$ rise amounted to 0.49 ± 0.12 ($n = 190$) as compared to 0.67 ± 0.14 ($n = 150$) in control cells. The relative calcium rise during the Ca^{2+} entry (II phase) was inhibited by 45% ($p < 0.001$) (Figs. 4A and C). The intracellular Ca^{2+} rise induced by the depletion of the internal Ca^{2+} stores with thapsigargin (100 nM) under the same experimental conditions was diminished only by 25% ($p < 0.001$), most probably due to a cytochalasin D-induced rearrangement of the endoplasmic reticulum membranes. However, the calcium influx following the replacement of Ca^{2+} -free buffer with that containing 2 mM Ca^{2+} was unchanged in the I and II phases (Figs. 4B and D).

Effect of actin cytoskeleton disruption on the calcium influx

The differences between nucleotide agonists and thapsigargin-induced calcium entry were analysed further in glioma C6 cells pretreated with cytochalasin D or latrunculin A as described in the Materials and methods section. Fura-2 loaded cells were placed in the standard buffer with 2 mM CaCl_2 for 1 min after which time the buffer was replaced by a Ca^{2+} free buffer containing 500 μM EGTA. The cells were kept in this medium for additional 5 min and then the buffer was replaced by that containing 2 mM CaCl_2 . The decrease of intracellular calcium was not monitored by the fluorescence ratio of Fura-2 up to the second buffer change, since it was low and slow. However, when cells were placed in

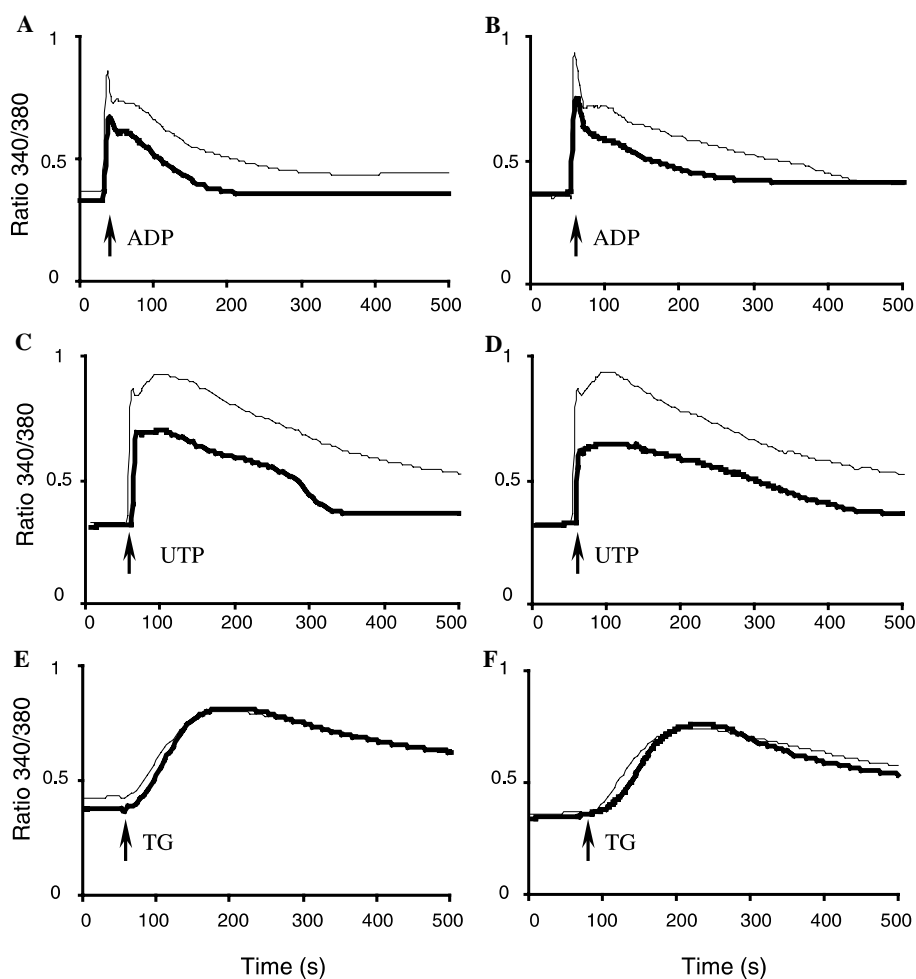


Fig. 2. Effect of actin cytoskeleton disruption on calcium signals evoked by ADP, UTP, and thapsigargin in glioma C6 cells. The cells were incubated with 5 μM cytochalasin D for 3 h (A, C, and E) or with 3 μM latrunculin A for 1 h (B, D, and F), loaded with Fura-2 and treated with either 10 μM ADP, 100 μM UTP or 100 nM thapsigargin (TG), as indicated by the arrows (thick lines). The thin lines represent the responses of the control cells (unmodified actin cytoskeleton). All experiments were performed in a buffer containing 2 mM CaCl_2 . Each trace represents the mean ratio value of the responses of 25–43 cells recorded in single experiments. Each experiment was repeated 4–10 times.

the standard buffer (2 mM CaCl_2) the Ca^{2+} influx took place which in control, untreated cells $[\text{Ca}^{2+}]_i$ reached a value of 0.64 ± 0.12 ($n = 23$), whereas in cytochalasin D or latrunculin A treated cells it reached only a value of 0.43 ± 0.12 ($n = 10$) and 0.34 ± 0.13 ($n = 12$), respectively (Fig. 5). Clearly, the disruption of the actin cytoskeleton inhibited the capacitative calcium entry.

The effect of blockade of the calcium in glioma C6 cells treated with ADP, UTP, and with thapsigargin

Calcium influx in control glioma C6 cells was blocked with 2-APB. This compound is not specific but at higher concentrations is considered a blocker of plasma membrane store-operated Ca^{2+} entry channels (SOCs). Glioma C6 cells were placed in the standard buffer and treated with 75 μM of 2-APB 30 s, prior to the addition of ADP, UTP or TG. The effect of SOC blocking was evident in all cases. This was especially evident after the addition

of ADP, which evoked only the initial peak, regardless of the presence of the Ca^{2+} in the extracellular buffer. Similarly, the Ca^{2+} response induced by thapsigargin under these conditions (Fig. 6) resembled that seen when cells were placed in the Ca^{2+} -free buffer containing 500 μM EGTA, i.e., only the first increase, corresponding to the intracellular calcium release, was observed.

Discussion

Previous work from this laboratory has provided evidence that in glioma C6 cells the stimulation of P2Y_1 receptor by ADP and the P2Y_2 receptor by UTP initiated an increase in $[\text{Ca}^{2+}]_i$ that involved both the release of Ca^{2+} from IP_3 sensitive store (the first phase) and store-mediated Ca^{2+} entry (the second phase) [11,12]. Thapsigargin produced a similar, biphasic Ca^{2+} response, but without IP_3 [10,18]. The present study has

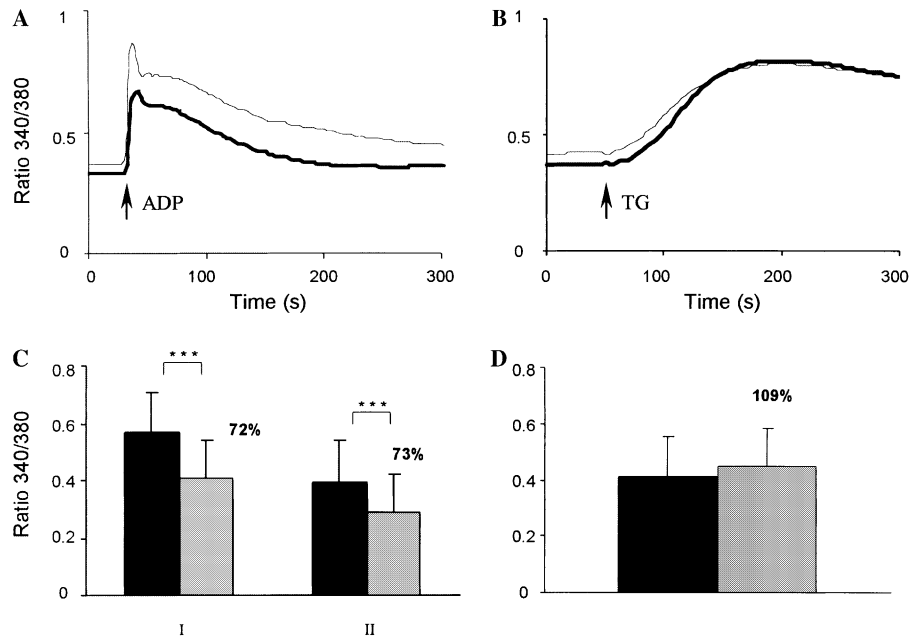


Fig. 3. Effect of cytochalasin D treatment on intracellular calcium changes evoked by ADP or by thapsigargin in glioma C6 cells. Glioma C6 cells were incubated with cytochalasin D, loaded with Fura-2, and treated with ADP or thapsigargin (TG) in a buffer containing 2 mM CaCl_2 , as described in Fig. 2 (thick lines). The thin lines represent the responses of the control cells with unmodified actin cytoskeleton. The addition of ADP or TG is indicated by the arrows. Each trace represents the mean ratio value of the responses of 25–41 cells recorded in single experiment. Each experiment was repeated 4–6 times. The histograms (C and D) show the relative changes in intracellular calcium rise as the ratio values above the resting level. The values above the bars correspond to the increase of intracellular calcium measured in experiments on cells after cytoskeleton disrupting treatment (grey bars) and are expressed as a percentage of the calcium increase in control experiments when cells not treated with cytochalasin D were used (black bars). “I”, maximal values from the initial peak corresponding to the emptying of the intracellular stores, “II”, maximal values from the sustained phase corresponding to the influx of extracellular Ca^{2+} . The histogram data are means \pm SD for 118–230 individual cells collected from 4 to 6 separate experiments. The significance values indicate the differences with respect to control cells: *** $P < 0.001$.

shown that the disruption (depolymerization) of actin microfilaments with cytochalasin D and lantrunculin A significantly decreased the agonist-induced initial release of Ca^{2+} from the ER store (the first phase) and the extracellular Ca^{2+} influx (the second phase). By contrast, disruption of the actin cytoskeleton had a much smaller effect on the thapsigargin-induced Ca^{2+} release phase and no effect on the extracellular Ca^{2+} entry.

The decrease in the initial Ca^{2+} rise in cells treated with cytochalasin D or lantrunculin A and exposed to ADP or to UTP may have resulted from a direct effect of IP_3 on the ER store. Damage of the cytoskeleton could disrupt a link between the plasma membrane nucleotide receptors, protein G, and phospholipase C, and thus diminish PIP_2 breakdown and IP_3 production. The diffusion of IP_3 through the cytosol to the ER and the binding of IP_3 into IP_3 receptor could also be reduced under such conditions. In the case of thapsigargin which acts without IP_3 production, the small decrease of the initial Ca^{2+} rise could have resulted from the rearrangement of the ER membranes.

Perhaps, the most significant result of this study is the observation that cytoskeleton disruption affects agonist-induced, store-mediated Ca^{2+} entry but not that promoted by thapsigargin. The difference cannot be traced back to the fact that agonists produce IP_3 and thapsi-

gargin does not. Leak of Ca^{2+} from the ER store can also be achieved by bathing cells in the absence of CaCl_2 and with 500 μM EGTA. We have previously shown that under such conditions the replacement of the medium with a buffer containing 2 mM CaCl_2 generated a strong elevation of the cytosolic Ca^{2+} level in glioma C6 cells, which was due to the depletion of the ER store and due to the extracellular Ca^{2+} influx [10]. The present study shows that influx of external Ca^{2+} could also be achieved by adding the buffer with 2 mM CaCl_2 only after a short time of bathing in Ca^{2+} -free medium, in a process that was blocked by cytochalasin D and lantrunculin A. These data indicate that SOCs were activated by the depletion of internal Ca^{2+} stores and that the actin cytoskeleton plays a regulatory role in this activation. The binding of IP_3 molecules to IP_3 receptors does not seem to be necessary.

Our observation of the differential response of cells treated with agonists and with thapsigargin is in agreement with results in NIH 3T3 and smooth muscle cell lines by Pedrosa Ribeiro et al. [19] and Patterson et al. [6], respectively. In NIH 3T3 cells, Ca^{2+} mobilization by ATP or platelet-derived growth factor was abolished by the treatment with cytochalasin D while the ability of thapsigargin to activate capacitative Ca^{2+} influx was unaffected. The failure of the cytoskeletal disruption to

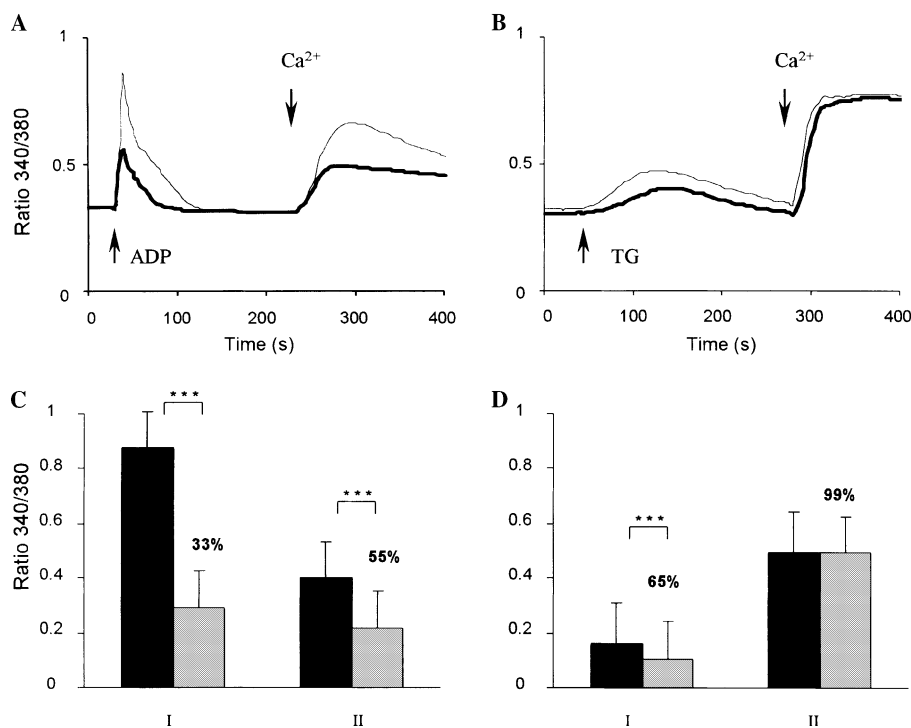


Fig. 4. Effect of intracellular store depletion evoked by ADP (A) or by thapsigargin (B) on calcium influx in cytochalasin D treated glioma C6 cells. Glioma C6 cells were incubated with cytochalasin D (5 μ M, 3 h) loaded with Fura-2 and treated with ADP or thapsigargin (TG) in a calcium-free buffer containing 500 μ M EGTA. Five minutes after the addition of ADP or TG, the calcium-free buffer was replaced with the standard buffer containing 2 mM CaCl_2 as indicated by the arrows (thick lines). The thin lines represent the responses of the control cells under the same experimental conditions. Each trace represents the mean value of the responses of 27–45 cells recorded in single experiment. Each experiment was repeated 3–4 times. The histograms (C and D) show the relative changes in intracellular calcium rise as the ratio values above the resting level. The values above the bars correspond to the increase of intracellular calcium measured in experiments on cells after cytoskeleton disrupting treatment (grey bars) and are expressed as a percentage of the calcium increase from control experiments when cells not treated with cytochalasin D were used (black bars). “I”, maximal values of the initial peak corresponding to the emptying of the intracellular stores, “II”, maximal values of Ca^{2+} influx into the cells after the exchange of the buffer. The histogram data are means \pm SD of 135–190 individual cells collected from 3 to 4 separate experiments. The significance values indicate the differences with respect to control cells: *** $P < 0.001$.

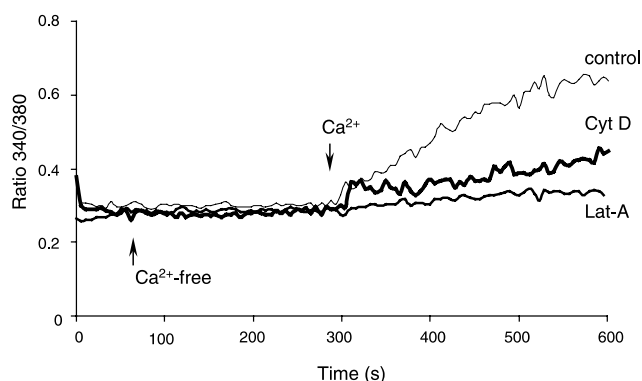


Fig. 5. Inhibitory effect of actin cytoskeleton disruption on extracellular calcium entry in glioma C6 cells. Ca^{2+} response of glioma C6 cells incubated with cytochalasin D (Cyt D; 5 μ M, 3 h) and latrunculin A (Lat A; 3 μ M, 1 h). In both cases, the cells were loaded with Fura-2 and 1 min after the start of experiment the standard buffer was replaced with a calcium-free buffer containing 500 μ M EGTA. Five minutes later, the calcium-free buffer was replaced again by the standard buffer with 2 mM CaCl_2 . The thin line represents the response of the control cells recorded under the same experimental conditions. The exchange of the buffer is indicated by the arrows. Each trace represents the mean ratio value of the responses of 10–23 cells recorded in single experiment. Each experiment was repeated three times.

affect thapsigargin-induced Ca^{2+} entry led the authors to the conclusion that the coupling between Ca^{2+} pool depletion and store-mediated Ca^{2+} entry does not depend on the cytoskeleton [19]. Our interpretation of these results is at variance with this. We suggest that thapsigargin might operate different mechanisms for store-mediated Ca^{2+} entry. It has been recently demonstrated that 2-APB blocks store-mediated Ca^{2+} entry channels in nonexcitable cells [20]. In glioma C6 cells, agonist- and thapsigargin-induced capacitative Ca^{2+} entry was both significantly diminished by 2-APB, showing that the agonists and thapsigargin activated the same PM Ca^{2+} channels, although the mechanism of their activation may be different.

Two general mechanisms have been proposed to explain how information is transferred from the ER to the PM to produce store-mediated Ca^{2+} influx. The first suggests the existence of a diffusible messenger [21] and the second the direct coupling between the plasma membrane Ca^{2+} channels and proteins resident in the membranes of intracellular stores [6]. In the latter model, the actin cytoskeleton would play a key modulatory role

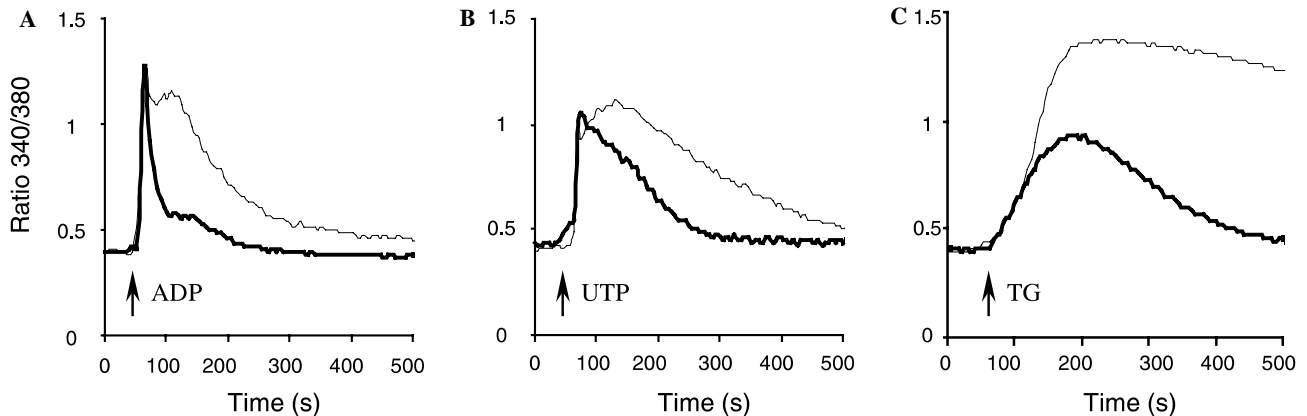


Fig. 6. Effect of 2-APB on calcium signals evoked by ADP, UTP, and thapsigargin in glioma C6 cells. The cells were treated with 2-APB (75 μ M) 30 s prior to the addition of ADP (10 μ M), UTP (100 μ M) or thapsigargin (TG, 100 nM), as indicated by the arrows (thick lines). The thin lines represent the responses of the control, untreated with 2-APB cells. All experiments were performed in a buffer containing 2 mM CaCl_2 . Each trace represents the mean ratio value of the responses of 16–24 cells recorded in single experiment. Each experiment was repeated three times.

[7], in which the reversible trafficking of the ER towards the PM has been proposed [8]. The depletion of intracellular stores may also induce a translocation and association of small GTP-binding proteins of the Ras superfamily with the PM [22] and activation of tyrosine kinases [23]. The results presented here have provided evidence for the secretion-like-coupling model based on reversible trafficking and coupling of the ER with the PM of glioma C6 cells. The depletion of the Ca^{2+} pool with agonists and that produced by bathing the cells in Ca^{2+} -free medium induced store-operated Ca^{2+} entry, which was strongly dependent on the cytoskeleton. Thapsigargin could instead affect other processes, e.g., it might generate conformational changes of some proteins, like in the case of the ER Ca^{2+} -ATPase, which is irreversibly blocked by thapsigargin binding [24]. Another possibility would be a direct effect of thapsigargin on SOC channels. It is worth adding that in human platelets the disruption of the actin cytoskeleton reduced thapsigargin-evoked Ca^{2+} entry [7]. Thus, in different cell types, the thapsigargin-induced Ca^{2+} entry may or may not be modified by the cytoskeleton. At the moment, the reasons for these discrepancies remain obscure.

Acknowledgments

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