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Trifluoperazine Attenuates Store-Dependent Ca^{2+} Entry in Macrophages

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Abstract—Using Fura-2AM microfluorimetry, we have shown for the first time that preincubation of macrophages with the calsequestrin inhibitor neuroleptic trifluoperazine leads to a significant inhibition of the store-dependent Ca^{2+} entry induced by endoplasmic Ca^{2+}-ATPase inhibitors thapsigargin or cyclopiazonic acid in rat peritoneal macrophages. The results suggest calsequestrin involvement in the regulation of the store-dependent Ca^{2+} entry in macrophages.

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A universal mechanism of a regulated Ca^{2+} entry into eukaryotic cells is the store-dependent, or "capacitative" Ca^{2+} entry [1, 2]. In accordance with the "capacitative" Ca^{2+} entry model, this process is regulated by the degree of filling of Ca^{2+}-stores so that the store depletion activates Ca^{2+} entry [1, 2].

The functional unit of the store-dependent Ca^{2+} entry is the multimolecular protein complex SOCIC (store-operated calcium influx complex), the components of which are highly mobile, and interactions between them are strictly regulated [2, 3]. The main components of the complex that are necessary and sufficient for the activation of the store-dependent Ca^{2+} entry are the Ca^{2+} channels Orai1 in the plasmalemma and the Ca^{2+} sensor STIM1 in the Ca^{2+}-store membrane [2, 3]. When the Ca^{2+}-store is depleted, STIM1 is oligomerized, translocated into the endoplasmic reticulum regions located near the plasma membrane, and directly interacts with the Orai1 proteins, causing the store-dependent Ca^{2+} entry [2, 3].

One of the main Ca^{2+}-buffers in the sarco/endoplasmic reticulum lumen is the protein calsequestrin (CSQ). At a low Ca^{2+} concentration in the store lumen, CSQ is present in the store in the monomeric form. When the Ca^{2+} concentration in the store increases, CSQ undergoes dimerization and subsequent polymerization [4]. It is found that calsequestrin 1 (CSQ1) is expressed not only in skeletal muscles but also in nonexcitable cells and plays an important role in the regulation of Ca^{2+} mobilization from the stores in human and rat platelets [5]. In addition, it was shown recently that the monomeric CSQ1 is involved in the regulation of the store-dependent Ca^{2+} entry in skeletal muscles [6] and in a number of nonexcitable cells [5, 7].

In view of above, the aim of this study was to investigate a possible role of CSQ in the regulation of the store-dependent Ca^{2+} entry induced by the inhibitors of endoplasmic Ca^{2+}-ATPases—thapsigargin (TG) and cyclopiazonic acid (CPA)— in rat peritoneal macrophages.

In experiments we used a CSQ inhibitor trifluoperazine (TFP, triftazin), neuroleptic of the phenothiazine series, which binds to CSQ and inhibits its assembly and subsequent aggregation with increasing Ca^{2+} concentrations in the store lumen, i.e., increases the proportion of monomeric CSQ [4, 8].

Experiments were performed on cultured residential peritoneal macrophages of Wistar rats at room temperature (20–22°C) 1–2 days after the beginning of cell culturing. The macrophage culturing procedure and the automated system for measuring $[Ca^{2+}]_i$ on the basis of Leica DM 4000B fluorescence microscope (Leica Microsystems, Germany) were described in detail earlier [9]. $[Ca^{2+}]_i$ was measured using the Fura-2AM fluorescent probe (Sigma-Aldrich, United States). Fluorescence of objects was excited at wavelengths of 340 and 380 nm, and emission was recorded at 510 nm. To prevent photobleaching, measurements were performed every 20 s irradiating the object for 2 s. The $[Ca^{2+}]_i$ values were calculated using the Grynkiewicz equation [10]. The results were statistically processed using Student's *t* test. Differences were considered significant at $p \leq 0.05$.

The figure shows the results of typical experiments. Data are represented as plots showing the changes in

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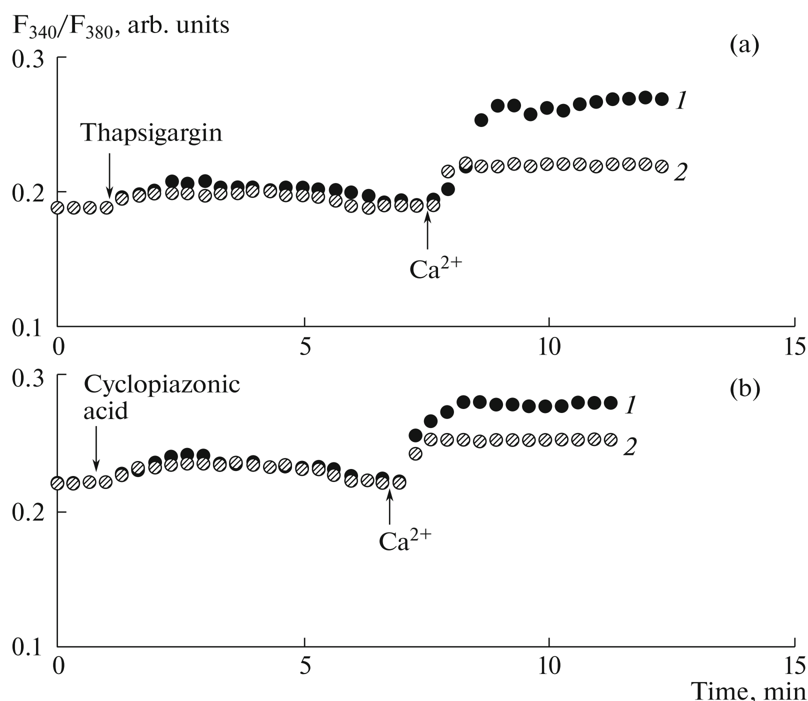

Fig. 1. Effect of trifluoperazine on the Ca^{2+}-responses induced by (a) thapsigargin or (b) cyclopiazonic acid in rat peritoneal macrophages. The ordinate axis shows the Fura-2AM fluorescence intensity ratio F_{340}/F_{380} at excitation wavelengths 340 and 380 nm, respectively (expressed in arbitrary units (arb. units)). The horizontal axis shows time. Panel (a), curve *1:* cells were stimulated with 0.5 μM thapsigargin in a nominally calcium-free medium, after which Ca^{2+} entry was initiated by the addition of 2 mM Ca^{2+} to the external medium. Panel (a), curve *2*: macrophages were preincubated for 10 min with 2 μg/mL trifluoperazine in a calcium-free medium and then stimulated with 0.5 μM thapsigargin, after which Ca^{2+} entry was initiated by the addition of 2 mM Ca^{2+} to the external medium. Panel (b), curve *1*: macrophages were stimulated with 10 μM cyclopiazonic acid in a calcium-free medium, after which Ca^{2+} entry was initiated by the addition of 2 mM Ca^{2+} to the external medium. Panel (b), curve *2*: cells were preincubated for 10 min with 2 μg/mL trifluoperazine in a calcium-free medium and then stimulated with 10 μM cyclopiazonic acid, after which Ca^{2+} entry was initiated by the addition of 2 mM Ca^{2+} to the external medium. Each record was obtained for a group of 40–50 cells and represents a typical variant of seven independent experiments.

the ratio of Fura-2AM fluorescence intensities at excitation wavelengths 340 and 380 nm (F_{340}/F_{380} ratio) over time, reflecting the dynamics of changes in $[Ca^{2+}]_i$ in cells depending on the measurement time [11].

In preliminary control experiments, we found that the addition of 0.5 μM TG to macrophages incubated in a calcium-free medium caused a slight increase in $[Ca^{2+}]_i$, reflecting the Ca^{2+} mobilization from the intracellular Ca^{2+}-stores (Fig. 1a, curve *1*). On average (according to the results of seven experiments), the increase in $[Ca^{2+}]_i$ in the phase of Ca^{2+} mobilization from the stores was 25 ± 9 nM. Subsequent addition of 2 mM Ca^{2+} to the external medium caused a store-dependent Ca^{2+} entry into the cytosol (Fig. 1a, curve *1*). On average (according to the results of seven experiments), the $[Ca^{2+}]_i$ value during the Ca^{2+} entry increased to 80 ± 20 nM. Similar results were obtained when using 10 μM CPA (Fig. 1b, curve *1*). On average (according to the results of seven experiments), the increase in $[Ca^{2+}]_i$ during the CPA-induced Ca^{2+} mobilization from the stores was 22 ± 8 nM and amounted to 52 ± 10 nM during the Ca^{2+} entry (Fig. 1b, curve *1*).

We for the first time showed that the preincubation of macrophages with 2 μg/mL TFP for 10 min before the addition of 0.5 μM TG almost had no effect on the phase of Ca^{2+} mobilization from the stores but considerably (according to the results of seven experiments, by 66.7 ± 11.2%) inhibited subsequent store-dependent Ca^{2+} entry into macrophages (Fig. 1a, curve *2*). Similar results were obtained in the experiments with the addition of 10 μM CPA (Fig. 1b, curve *2*). On average (according to the results of seven experiments), the inhibition of the CPA-induced store-dependent Ca^{2+} entry by TFP was 57.3 ± 13.4%.

Thus, in this study we for the first time showed on rat peritoneal macrophages that the calsequestrin inhibitor TFP suppresses the store-dependent Ca^{2+} entry induced by TG or CPA in macrophages. These data are consistent with results of other authors, who found that TFP suppressed the store-dependent Ca^{2+} entry induced by ATP in HL-60 promyelocytic leukemia cells [12, 13] and directly blocked the voltage-