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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Sigma-1 Receptor Antagonist Haloperidol 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 sigma-1 receptor antagonist haloperidol 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 the involvement of the sigma-1 receptor in the regulation of store-dependent Ca^{2+} entry in macrophages.

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Sigma-1 receptors are unique ligand-regulated molecular chaperones located in the endoplasmic reticulum membrane at the interface with mitochondria. These receptors are expressed in cells of various types, including the immune system cells [1]. Their ligands are endogenous steroids, antidepressants, antipsychotics, anticonvulsants, and analgesic agents [2]. Sigma-1 receptors interact with target proteins (including ion channels and receptors) and modulate many cellular processes [3]. For example, it was found that, by interacting with inositol 1,4,5-trisphosphate receptors, sigma-1 receptors modulate Ca^{2+} signaling processes in cells (the mobilization of Ca^{2+} from stores and the entry of Ca^{2+} from the external medium [3]).

The store-dependent ("capacitative") entry of Ca^{2+} is a universal mechanism of regulated Ca^{2+} entry into eukaryotic cells [4]. In accordance with the model of "capacitative" Ca^{2+} entry, this process is regulated by the degree of filling of the Ca^{2+} store so that the store emptying activates the Ca^{2+} entry [4]. A functional unit of the store-dependent Ca^{2+} entry is the multimolecular protein complex (store-operated calcium influx complex, SOCIC), the components of which exhibit a high mobility and the interactions between which are strictly regulated [4, 5]. The main components of the complex, which are necessary and sufficient to activate the store-dependent Ca^{2+} entry, are the Ca^{2+} channels Orai1 in the plasma membrane and the Ca^{2+} sensor STIM1 in the Ca^{2+} store membrane. The complex also includes the regulatory proteins: calmodulin, adenylyl cyclase, and Ca^{2+} store membrane Ca^{2+}-ATPase [4, 5].

In view of above, the aim of this study was to investigate the possible role of sigma-1 receptors in the regulation of the store-dependent Ca^{2+} entry induced by endoplasmic Ca^{2+}-ATPase inhibitors thapsigargin (TG) and cyclopiazonic acid (CPA) in rat peritoneal macrophages. In the experiments we used the sigma-1 receptor antagonist, typical neuroleptic haloperidol [2, 6], which is widely used for the treatment of schizophrenia.

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 the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), based on the Leica DM 4000B fluorescent microscope (Leica Microsystems, Germany), were described in detail earlier [7]. $[Ca^{2+}]_i$ 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 [8]. Statistical analysis was performed using Student's t test. Differences were considered significant at $p \le 0.05$.

Figures 1 and 2 show the results of typical experiments. Data are represented as plots showing the changes in the ratio of Fura-2AM fluorescence intensities at excitation wavelengths 340 and 380 nm ($F_{340/380}$ ratio) over time, which reflects the dynamics of changes in $[Ca^{2+}]_i$ in cells depending on the measurement time [9].

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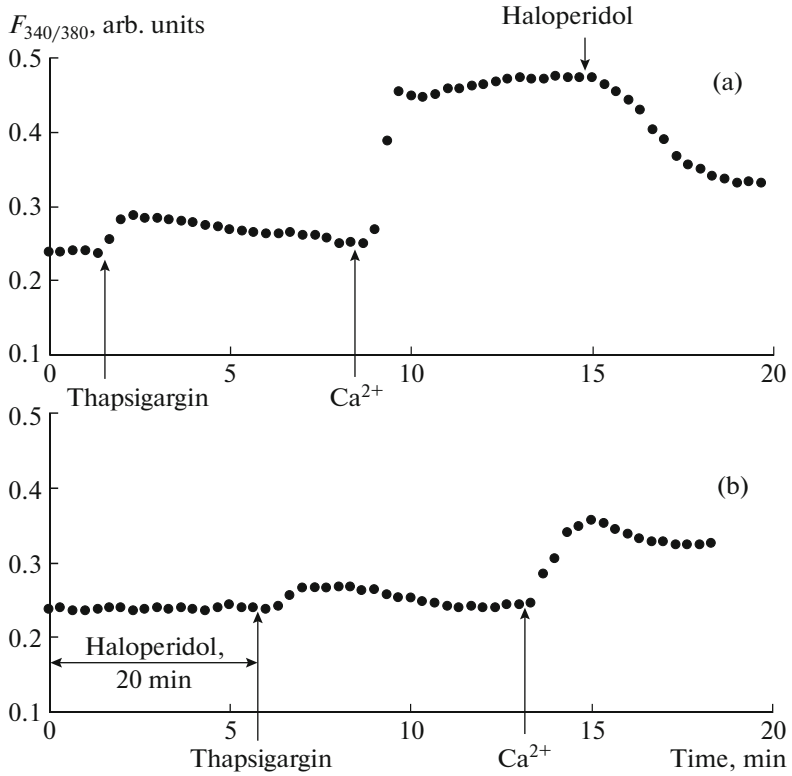

Fig. 1. Effect of haloperidol on the thapsigargin-induced Ca^{2+}-response in rat peritoneal macrophages. The ordinate axis shows the Fura-2AM fluorescence intensity ratio $F_{340/380}$ at excitation wavelengths 340 and 380 nm, respectively (expressed in arbitrary units (arb. units)). The abscissa axis shows time. (a) 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, and 30 μg/mL haloperidol was added against the background of developed Ca^{2+} entry. (b) Macrophages were preincubated for 20 min with 30 μg/mL haloperidol in calcium-free medium, after which 0.5 μM thapsigargin was added; then, Ca^{2+} entry was initiated by adding 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.

In the control experiments, we found that the addition of 0.5 μM TG to macrophages placed in a calcium-free medium caused a slight increase in $[Ca^{2+}]_i$, reflecting the mobilization of Ca^{2+} from the intracellular Ca^{2+} stores (Fig. 1a). The average (according to the results of seven experiments) increase in $[Ca^{2+}]_i$ during the mobilization phase was 31 ± 9 nM (hereinafter, $M \pm m$, $n = 7$). After subsequent addition of 2 mM Ca^{2+} to the external medium, a store-dependent entry of Ca^{2+} into the cytosol was observed (Fig. 1a). The increase in $[Ca^{2+}]_i$ during the Ca^{2+} entry was 152 ± 20 nM. Similar results were obtained when using 10 μM CPA (Fig. 2a). The increase in $[Ca^{2+}]_i$ during the CPA-induced Ca^{2+} mobilization from the store was 26 ± 9 nM, and during the Ca^{2+} entry it was 141 ± 22 nM (Fig. 2a).

We for the first time showed that the preincubation of macrophages with 30 μg/mL haloperidol for 20 min before the addition of 0.5 μM TG led to the suppression of both phases of the TG-induced Ca^{2+} response (Fig. 1b). Haloperidol inhibited the Ca^{2+} mobilization from the stores by 23.2 ± 7.9% and subsequent store-dependent Ca^{2+} entry into macrophages by 42.3 ± 13.6%. Similar results were obtained in the experiments with the use of 10 μM CPA (Fig. 2b). Haloperidol suppressed the CPA-induced Ca^{2+} mobilization from the stores by 25.9 ± 8.0% and the CPA-induced store-dependent Ca^{2+} entry by 43.8 ± 12.5%. This fact indicates the involvement of sigma-1 receptors in the activation of the store-dependent Ca^{2+} entry induced by TG or CPA in macrophages.

The addition of 30 μg/mL haloperidol against the background of developed store-dependent Ca^{2+} entry induced by TG (Fig. 1a) or CPA (Fig. 2a) led to significant inhibition of the store-dependent Ca^{2+} entry into macrophages. For endoplasmic Ca^{2+}-ATPase inhibitors, the inhibition of the store-dependent Ca^{2+} entry was 48.5 ± 17.1% for TG and 48.1 ± 16.9% for CPA. This finding indicated a possible involvement of sigma-1 receptors not only in the activation but also in maintaining the store-dependent Ca^{2+} entry into macrophages.

Thus, in this paper, we for the first time showed on rat peritoneal macrophages that the sigma-1 receptor