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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Sigma-1 Receptor Antagonist Haloperidol Attenuates Ca^{2+} Responses Induced by Glutoxim and Molixan in Macrophages

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Abstract—Using Fura-2AM microfluorimetry, we have shown for the first time that sigma-1 receptor antagonist, antipsychotic haloperidol, significantly inhibits glutoxim- and molixan-induced Ca^{2+}-response in peritoneal macrophages. These results indicate possible involvement of sigma-1 receptors in the signal cascade induced by glutoxim or molixan and leading to intracellular Ca^{2+} concentration increase in macrophages.

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Sigma-1 receptors are unique ligand-regulated molecular chaperones localized in endoplasmic reticulum membranes at the interface with mitochondria. These receptors are expressed in cells of different types, including the immune system cells [1]. Their ligands are endogenous steroids, antidepressants, antipsychotics, anticonvulsants, and analgesics [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, when interacting with inositol 1,4,5-trisphosphate receptors, sigma-1 receptors modulate Ca^{2+} signaling processes in cells: Ca^{2+} mobilization from the stores and Ca^{2+} entry into the cell from the extracellular environment [4, 5].

Earlier [6, 7], we have shown for the first time that disulfide-containing immunomodulators glutoxim® (G, disodium salt of oxidized glutathione with d-metal at a nanoconcentration, PHARMA VAM, Russia) and molixan® (M, complex of glutoxim with nucleoside inosine, PHARMA VAM) increase the intracellular concentration of Ca^{2+}, $[Ca^{2+}]_i$, causing Ca^{2+} mobilization from the thapsigargin-sensitive Ca^{2+} stores and subsequent store-dependent Ca^{2+} entry into rat peritoneal macrophages.

In this regard, it was reasonable to study the possible involvement of sigma-1 receptors in the effect of G and M on $[Ca^{2+}]_i$ in peritoneal macrophages, which was the subject of this communication.

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In experiments, we used the sigma-1 receptor antagonist neuroleptic haloperidol [2, 8], which is widely used for the treatment of schizophrenia. Experiments were performed on cultured resident peritoneal macrophages of Wistar rats at room temperature (20–22°C). The drugs were added to the culture 1–2 days after the beginning of cell culturing. The macrophage cultivation procedure and the automated system for measuring $[Ca^{2+}]_i$ based on a Leica DM 4000B fluorescent microscope (Leica Microsystems, Germany) were described in detail in [6]. $[Ca^{2+}]_i$ was measured using the fluorescent probe Fura-2AM (Sigma-Aldrich, United States). Fluorescence of the object was excited at wavelengths 340 and 380 nm, and emission was detected at 510 nm. To prevent photobleaching, measurements were performed every 20 s, irradiating the object for 2 s. $[Ca^{2+}]_i$ was calculated using the Grynkiewicz equation [9]. Statistical analysis was performed using Student's *t* test.

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}/F_{380} ratio) over time, reflecting the dynamics of changes in $[Ca^{2+}]_i$ in cells depending on the measurement time.

The control experiments showed that the incubation of macrophages for 20 min with 100 μg/mL of M (Fig. 1a) or 100 μg/mL G (Fig. 2a) in a calcium-free medium caused a slowly developing increase in $[Ca^{2+}]_i$, reflecting the mobilization of Ca^{2+} from the intracellular stores. On average (according to the results of six experiments for each drug), 20 min after the addition of the agents, $[Ca^{2+}]_i$ increased from the basal level (90 ± 18 nM) to 134 ± 20 nM for M and

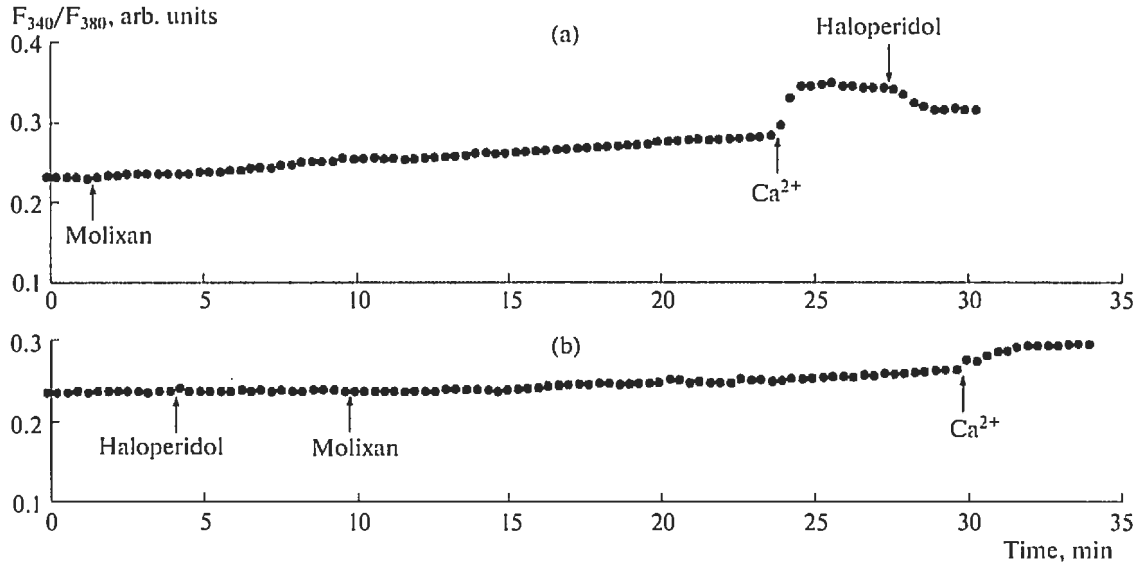

Fig. 1. Effect of haloperidol on the molixan-induced $[Ca^{2+}]_i$ increase in rat macrophages. Here and in Fig. 2, the ordinate axis shows the ratio of Fura-2AM fluorescence intensities F_{340}/F_{380} at excitation wavelengths 340 and 380 nm, respectively (arb. units). The abscissa axis shows time. (a) Cells were incubated with 100 μg/mL molixan for 20 min in a nominally calcium-free medium, after which Ca^{2+} entry was initiated by adding 2 mM Ca^{2+} to the external medium. Haloperidol (50 μg/mL) was added against the background of developing Ca^{2+} entry. (b) Macrophages were preincubated for 6 min with 30 μg/mL haloperidol in a calcium-free medium, followed by the addition of 100 μg/mL molixan and incubation for another 20 min, 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 is a typical variant of six or seven independent experiments.

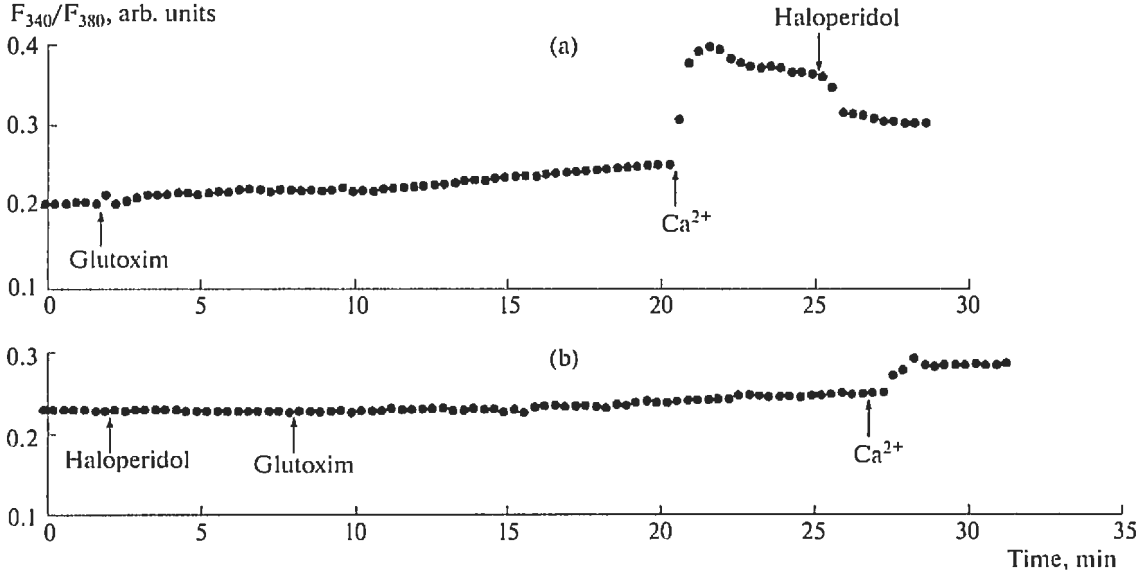

Fig. 2. Effect of haloperidol on the glutoxim-induced $[Ca^{2+}]_i$ increase in rat macrophages. (a) Cells were incubated with ug/mL glutoxim for 20 min in a nominally calcium-free medium, after which Ca^{2+} entry was initiated by adding 2 mM Ca^{2+} to the external medium. Haloperidol (50 μg/mL) was added against the background of developing Ca^{2+} entry. (b) Macrophages were preincubated for 6 min with 30 μg/mL haloperidol in a calcium-free medium, followed by the addition of 100 μg/mL glutoxin and incubation for another 20 min, after which Ca^{2+} entry was initiated by the addition of 2 mM Ca^{2+} to the external medium.

135 ± 18 nM for G. The addition of 2 mM Ca^{2+} to the external medium caused a further increase in $[Ca^{2+}]_i$, reflecting the Ca^{2+} entry into the cytosol (Figs. 1a, 2a). On average (according to the results of six experiments for each drug), the increase in $[Ca^{2+}]_i$ during the Ca^{2+} entry was 202 ± 20 and 223 ± 22 nM for M and G, respectively.

Our experiments demonstrated for the first time that the preincubation of macrophages with 30 μg/mL haloperidol for 6 min before the addition of 100 μg/mL M resulted in a significant suppression of both Ca^{2+} mobilization from the stores (on average, by 49.3 ± 8.1% according to the results of seven experiments) and subsequent Ca^{2+} entry into the cell (on average, by 47.6 ± 9.7% according to the results of seven experiments), induced by M (Fig. 1b). Similar results were obtained in the experiments on the effect of 30 μg/mL haloperidol on the Ca^{2+}-responses induced by 100 pg/mL G (Fig. 2b). On average, according to the results of seven experiments, haloperidol suppressed the Ca^{2+} mobilization from the stores by 50.3% and the Ca^{2+} entry into the cell by to 54.5%, induced by G.

In addition, we have also shown that the addition of 50 μg/mL haloperidol against the background of developing Ca^{2+} entry induced by M (Fig. 1a) or G (Fig. 2a) caused a significant (on average, by 51.4 ± 9.0% according to the results of 12 experiments) inhibition of the store-dependent Ca^{2+} entry into macrophages. This is consistent with the published data on the suppression of Ca^{2+} entry by sigma-1 receptor ligands. For example, it was shown [11] that the sigma-1 receptor antagonist antipsychotic chlorpromazine inhibits the store-dependent Ca^{2+} entry induced by thapsigargin in pheochromocytoma cells (PC12 line). It was also found [12] that the sigma-1 receptor antagonists BD1063 and BD1047 inhibit the Ca^{2+} entry induced by histamine in the endothelial cells of human saphenous vein. In addition, it is known [13] that haloperidol inhibits the voltage-gated Ca^{2+} channels in cardiomyocytes, sympathetic and parasympathetic neurons of rats [14], and in human embryonic kidney cells (HEK293 line) [15].

Thus, we have demonstrated for the first time that the sigma-1 receptor antagonist haloperidol inhibits both phases of the Ca^{2+}-response induced by G or M, which indicates the possible involvement of sigma-1 receptor in the signaling cascade triggered by these immunomodulators in rat peritoneal macrophages. Our results also indicate that it is inadvisable to use drugs G or M together with the antipsychotic haloperidol in clinical practice.

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