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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Amitriptyline 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 agonist, tricyclic antidepressant amitriptyline, significantly inhibits glutoxim- and molixan-induced Ca^{2+}-responses in rat peritoneal macrophages. The results suggest possible involvement of sigma-1 receptors in the signaling cascade induced by glutoxim or molixan and leading to intracellular Ca^{2+} concentration increase in macrophages.

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Sigma-1 receptors—molecular chaperones of endoplasmic reticulum membranes—have unique structure and pharmacological properties [1, 2]. The ligands of these receptors are compounds with different chemical structure and pharmacological activity: antidepressants, neuroleptics, analgesics, antitussives, and anticonvulsants [3]. When functioning as chaperones, sigma-1 receptors interact with the target proteins (ion channels, receptors, etc.) and modulate many cellular processes, including Ca^{2+} signaling [4].

Previously [5], we have shown for the first time that the disulfide-containing immunomodulators Glutoxim® (G, disodium salt of oxidized glutathione with an additive of a *d*-metal at a nanoconcentration, PHARMA-VAM, Russia) and Molixan® (M, a complex of glutoxim and 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 entry of Ca^{2+} into rat peritoneal macrophages. In this connection, it was reasonable to study the possible involvement of sigma-1 receptors in the action of G and M on $[Ca^{2+}]_i$ in peritoneal macrophages, which was the subject of the present study.

We used the sigma-1 receptor agonist, tricyclic antidepressant amitriptyline [1, 2, 6], which is widely used to treat anxiety and depression [7].

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. After the completion of culturing, $[Ca^{2+}]_i$ was determined fluorometrically. 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 [8]. $[Ca^{2+}]_i$ 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 [9].

In the first series of experiments (results are shown in Fig. 1a), the cells were incubated for 20 min in the presence of 100 μg/mL G in a nominally calcium-free medium, after which Ca^{2+} entry into the cell was initiated by adding 2 mM Ca^{2+} into the external medium. Then, 40 μg/mL amitriptyline was added to the incubation medium. In the second series of experiments (Fig. 1b), the cells were preincubated for 20 min with 20 μg/mL a mitriptyline in a calcium-free medium and then incubated with 100 μg/mL G for 20 min, after which 2 mM Ca^{2+} was added to the external medium. The effect of amitriptyline in the presence of M (at the same concentration as G) was studied using the same experimental scheme (experimental series 3 and 4).

Data were statistically processed 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}/F_{380} ratio) over time, reflecting the dynamics of

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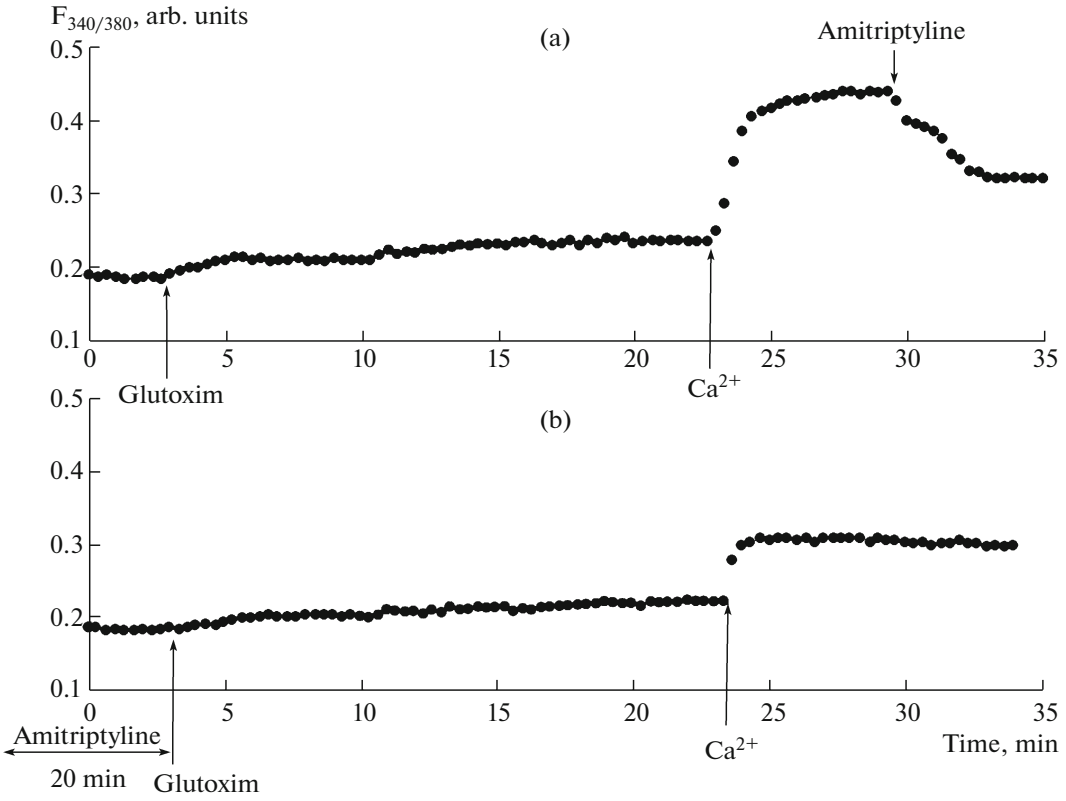

Fig. 1. The effect of amitriptyline on the glutoxim-induced increase in $[Ca^{2+}]_i$ in rat macrophages. Here and in Fig. 2, the ordinate axis shows the Fura-2AM fluorescence intensity ratio F_{340}/F_{380} at excitation wavelengths 340 and 380 nm, respectively (expressed in arbitrary units (arb. units)). The abscissa axis shows time. The results of experimental series (a) 1 and (b) 2 are shown. Each record was obtained for a group of 40–50 cells and represents a typical variant of six to eight independent experiments.

changes in $[Ca^{2+}]_i$ in cells depending on the measurement time [10].

In the control experiments in series 1 and 3, we found that the incubation of macrophages for 20 min with 100 μg/mL G (Fig. 1a) or 100 μg/mL M (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 sample), 20 min after the addition of the agents, $[Ca^{2+}]_i$ increased from the basal level (90 ± 18 nM) to 150 ± 19 nM for G and 158 ± 20 nM for M. When 2 mM Ca^{2+} was added to the external medium, we observed a further increase in $[Ca^{2+}]_i$, reflecting the entry of Ca^{2+} into the cytosol (Figs. 1a, 2a). On average (according to the results of six experiments for each sample), during the entry of Ca^{2+}, $[Ca^{2+}]_i$ increased to 382 ± 32 and 394 ± 34 nM for G and M, respectively.

In the experiments of series 2, we found that the preincubation of macrophages with 20 μg/mL amitriptyline for 20 min before the addition of 100 μg/mL G significantly attenuated the glutoxim-induced Ca^{2+} mobilization from the stores (on average by 39.6 ± 9.2%, according to the results of seven experiments) and subsequent Ca^{2+} entry into the cell (on average by 46.3 ± 10.1%, according to the results of seven experiments) (Fig. 1b). Similar data were obtained when studying the effect of 20 μg/mL amitriptyline on the Ca^{2+}-responses induced by 100 mg/mL M (Fig. 2b, experiments of series 4). On average (according to the results of seven experiments), amitriptyline attenuated the molixan-induced Ca^{2+} mobilization from the stores and the Ca^{2+} entry into the cell by 46.8 ± 8.2 and 55.4 ± 9.0%, respectively.

The addition of 40 μg/mL amitriptyline on the background of developed Ca^{2+} entry induced by G (Fig. 1a) or M (Fig. 2a) caused a significant (on average, 67.8 ± 15.0%, according to the results of 12 experiments) inhibition of the store-dependent Ca^{2+} entry into the macrophages.

Thus, in this study, we have shown for the first time that the sigma-1 receptor agonist tricyclic antidepressant amitriptyline inhibits both phases of the Ca^{2+}-response induced by G or M in rat peritoneal macrophages. These results are consistent with the published data obtained under different experimental conditions. For example, it was shown earlier [11] that amitriptyline suppresses the Ca^{2+} mobilization from the stores and subsequent store-dependent Ca^{2+} entry induced by ATP or thapsigargin in human leukemia cells (HL-60 line). It was also shown that the sigma-1

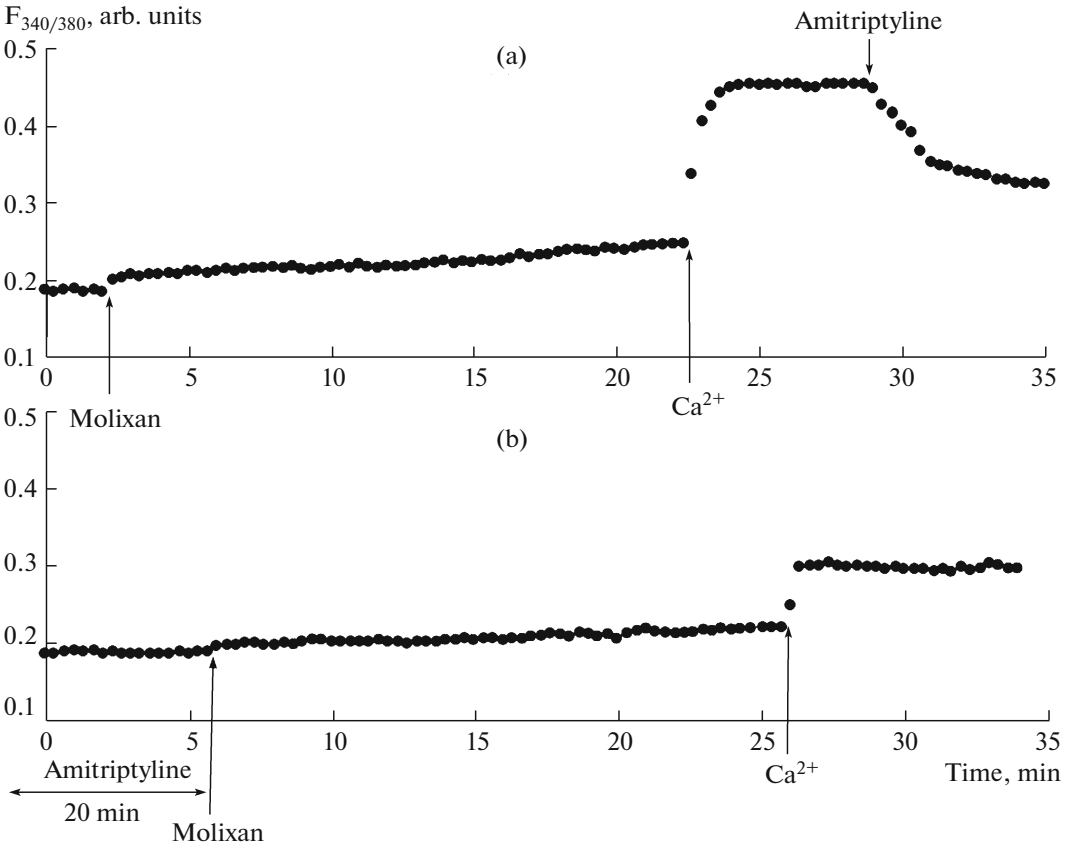

Fig. 2. The effect of amitriptyline on the molixan-induced increase in $[Ca^{2+}]_i$ in rat macrophages. The results of experimental series (a) 3 and (b) 4 are shown.

receptor agonist cocaine inhibits the store-dependent Ca^{2+} entry induced by thapsigargin in the endothelial cells of rat brain vessels [12], and the sigma-1 receptor agonist (+)-SKF-10047 inhibits the Ca^{2+} mobilization from the stores and the store-dependent Ca^{2+} entry induced by thapsigargin in Chinese hamster oocytes and human embryonic kidney cells (HEK 293 line) [13]. In addition, it is known that amitriptyline blocks the voltage-gated L-type Ca^{2+} channels (Cav1.2) in rat heart ventricular cardiomyocytes [14, 15].

The results obtained in the present study suggest a possible involvement of sigma-1 receptors in the complex signaling cascade triggered by G and M and leading to an increase in $[Ca^{2+}]_i$ in rat peritoneal macrophages. The results also indicate that a combined use of drugs G or M and the antidepressant amitriptyline in clinical practice is undesirable.

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