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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Phospholipase A_2 Inhibitors Modulate the Effect of Trifluoperazine on the Intracellular Ca^{2+} Concentration in Macrophages

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Abstract—Using Fura-2AM microfluorimetry, it was shown for the first time that phospholipase A_2 inhibitors 4-bromophenacyl bromide and glucocorticosteroids prednisolone and dexamethasone attenuate Ca^{2+} responses induced by neuroleptic trifluoperazine in macrophages. The results suggest the involvement of phospholipase A_2 and arachidonic acid metabolism cascade in the effect of trifluoperazine on intracellular Ca^{2+} concentration in macrophages.

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Trifluoperazine (TFP, triftazin) belongs to the first generation of typical neuroleptics of the phenothiazine series, which are widely used in the treatment of schizophrenia and other mental disorders [1]. The multifactorial effect of TFP on cellular processes was established [2]. For example, it was shown [3] that phenothiazines (TFP, chlorpromazine) affect the Ca^{2+}-dependent activation of macrophages and have an immunomodulatory effect on rat peritoneal macrophages.

Earlier [4], we for the first time showed that, in a Ca^{2+}-containing medium, TFP causes a dose-dependent increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in rat peritoneal macrophages. However, the mechanisms underlying the TFP-induced increase in $[Ca^{2+}]_i$ in macrophages are not fully understood.

The plurality of TFP effects may be due to its amphiphilic nature, which enables its penetration through cell membranes. It was found [5, 6] that TFP interacts mostly with the inner monolayer of the plasmalemma, in which the anionic phospholipids (primarily phosphoinositides) are located. Due to this fact, TFP can regulate intracellular processes such as signaling and intracellular transport. In addition, experiments on human platelets showed that psychotropic compounds (e.g., TFP) can modulate the activity of phospholipase A_2 (PLA_2), the key enzyme of the arachidonic acid (AA) metabolism cascade [5, 7].

In view of above, it was reasonable to study the possible involvement of PLA_2 and arachidonic acid (AA) metabolic cascade in the effect of TFP on $[Ca^{2+}]_i$ in macrophages, which was the subject of this communication.

In experiments, we used three PLA_2 inhibitors: 4-bromophenacyl bromide (4-BPB) [8] and synthetic glucocorticoids dexamethasone and prednisolone [9].

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 [10]. $[Ca^{2+}]_i$ 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 [11]. Statistical analysis was performed using Student's t test. Differences were considered significant at $p \leqslant 0.05$.

The figure shows 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 [12].

In the control experiments, we found that the addition of TFP (4 μg/mL) to macrophages in normal saline containing Ca^{2+} ions caused a rapid increase in $[Ca^{2+}]_i$ compared to the basal level (92 ± 17 nM), after which a long-term plateau phase of Ca^{2+}-response was observed (Fig. 1a), in which $[Ca^{2+}]_i$ was 176 ± 26 nM.

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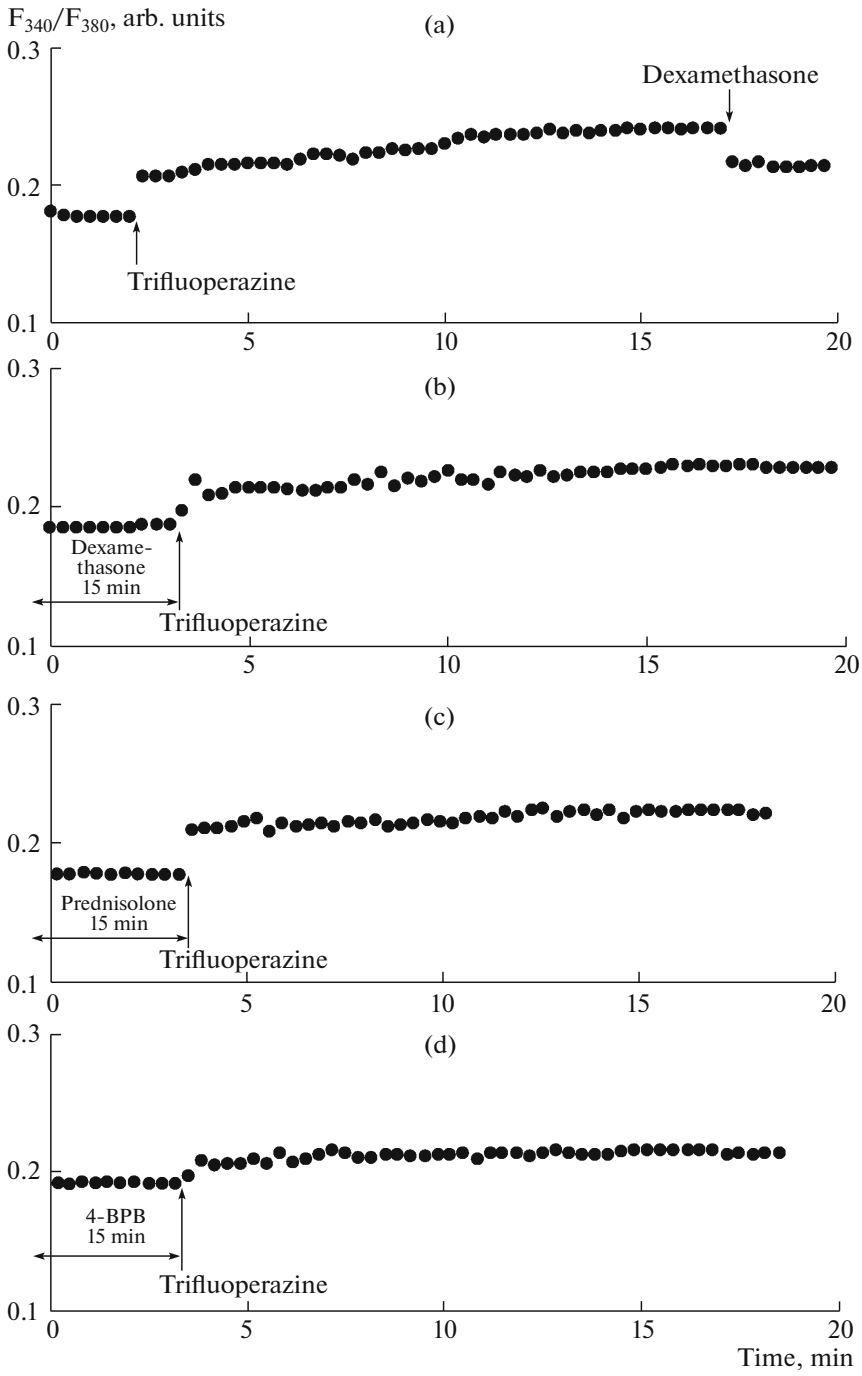

Fig. 1. Effect of phospholipase A_2 inhibitors on the trifluoperazine-induced Ca^{2+}-responses in rat peritoneal macrophages. The ordinate axis shows the Fura-2AM fluorescence intensity ratio F_{340}/F_{380} at excitation wavelengths 340 and 380 nm, respectively (expressed in arbitrary units (arb. units)). The abscissa axis shows time. (a) Normal Ca^{2+}-containing saline with macrophages was supplemented with 4 μg/mL trifluoperazine, after which 16 μg/mL dexamethasone was added against the background of developed Ca^{2+}-response. (b) Normal saline with macrophages was incubated for 15 min with 8 μg/mL dexamethasone. (c) Cells were incubated with 25 μg/mL prednisolone. (d) Cells were first incubated with 20 μM 4-bromophenacyl bromide (4-BPB). Then (b–d) 4 μg/mL trifluoperazine was added. Each record was obtained for a group of 40–50 cells and represents a typical variant of seven independent experiments.

In this study, we for the first time showed that a 15-min preincubation of macrophages with dexamethasone (8 μg/mL) before the addition of TFP (4 μg/mL) resulted in a significant inhibition of TFP-induced Ca^{2+}-responses (according to the results of seven experiments, by 37.6 ± 9.3% (Fig. 1b)). Similar results were obtained when using 25 μg/mL prednisolone (Ca^{2+}-responses were inhibited by 54.3 ± 13.5%