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PHYSIOLOGY

The Role of the Key Enzymes of the Phosphoinositide Signaling Pathway in the Effect of Oxidized Glutathione and Glutoxim on Intracellular Ca^{2+} Concentration in Macrophages*

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Nowadays the functioning of cellular redox systems and the influence of oxidizing and reducing agents on different cellular processes in normal and pathological conditions are of particular interest. Thus, the synthetic analogue of oxidized glutathione (GSSG), the drug glutoxim (FARMA-VAM, Moscow), is used in clinics as a wide range immunomodulator and hemostimulator, which stimulates the hematogenesis, activates the phagocytosis systems, for example in immunodeficiency states, promotes functional activity of tissue macrophages [1]. However, the mechanisms that mediate the effect of GSSG and glutoxim on cellular processes, are still unclear.

Recently, we showed that the GSSG and glutoxim increase intracellular Ca^{2+} concentration, ($[Ca^{2+}]_i$) due to mobilization of Ca^{2+} from thapsigargin-sensitive Ca^{2+} stores and subsequent Ca^{2+} influx in rat peritoneal macrophages [2, 3, 4]. Moreover, using two structurally different tyrosine kinase inhibitors (genistein and methyl-2,5-dihydroxycinnamate), as well as Na orthovanadate, a tyrosine phosphatase inhibitor, we demonstrated for the first time the involvement of tyrosine kinases and tyrosine phosphatases in the effect of GSSG and glutoxim on $[Ca^{2+}]_i$ in macrophages [3–5]. The data obtained suggested that both receptor and cytoplasmic tyrosine kinases participated in the signaling cascade triggered by GSSG and glutoxim. In addition, using two structurally distinct inhibitors of phosphatidylinositol kinases wortmannin and LY294002 we showed for the first time the involvement of phosphatidylinositol-3- and phosphatidylinositol-4-kinases in the effect of GSSG and glutoxim on $[Ca^{2+}]_i$ in rat macrophages [6].

In peritoneal macrophages, receptors possessing intrinsic tyrosine kinase activity were identified that contained cysteine-rich regions in their extracellular domains [7], which could serve as targets for GSSG and glutoxim. Activation of these receptor tyrosine kinases could promote the effect of SH2-domain-containing proteins, such as phospholipase $C\gamma$, cytoplasmic tyrosine kinases of src family and phosphatidylinositol-3-kinase, and trigger the signaling cascade, for example the phosphoinositide signaling pathway [7, 8]. It is known, that the key components of phosphoinositide system phospholipase C and protein kinase C have a high redox sensitivity and their activities are modulated by oxidizing and reducing agents [9, 10]. Therefore, the purpose of the present research was to study the possible role of the key enzymes of the phosphoinositide signaling pathway, phospholipase C, and protein kinase C, in the regulatory effect of GSSG and glutoxim on $[Ca^{2+}]_i$ in macrophages.

To determine the possible involvement of phospholipase C in the effect of GSSG and glutoxim on $[Ca^{2+}]_i$ in rat peritoneal macrophages, we studied the influence of phospholipase C inhibitor neomycin [11] on Ca^{2+}-response induced by GSSG and glutoxim. To investigate the role of protein kinase C in the effect of GSSG and glutoxim we used the specific protein kinase C inhibitors the compound H-7 [12] and calphostin C [13]. The cultivation of macrophages and the automated device for $[Ca^{2+}]_i$ measurement with the use of the fluorescent probe Fura-2AM were previously described [14]. The experiments were performed at 20–22°C on the second or third day of the cell cultivation. The figures show the results obtained with the use of glutoxim (100 μg/ml). Similar data were obtained in experiments with GSSG (100 μg/ml).

Macrophage incubation with 100 μg / ml glutoxim for 20 min led to a significant increase of $[Ca^{2+}]_i$ due to Ca^{2+} mobilization from intracellular stores. The addition of 2 mM Ca^{2+} to the external medium

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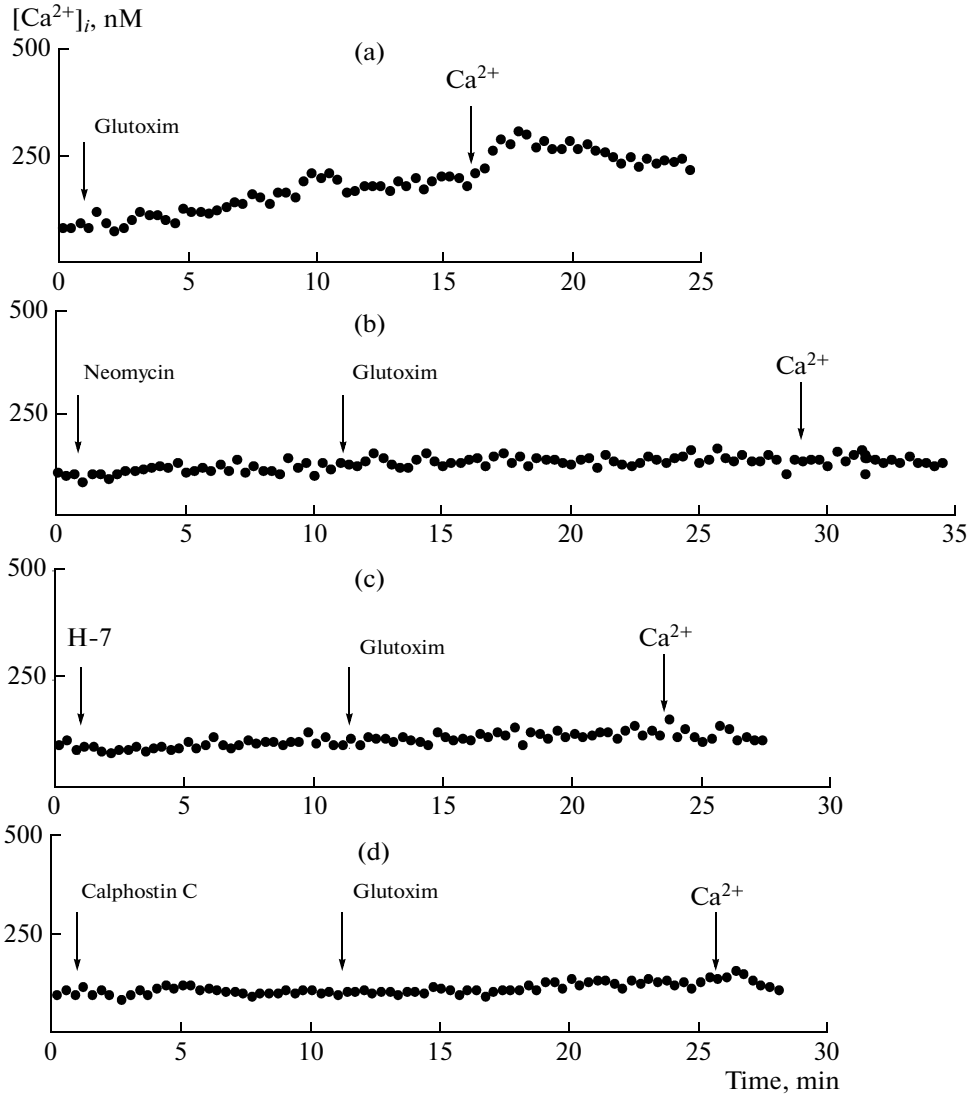

Fig. 1. The influence of neomycin, compound H-7 and calphostin C on the effect of glutoxim on $[Ca^{2+}]_i$ in the peritoneal macrophages. Each recording was obtained for a group of 40–50 cells to demonstrate a typical pattern obtained in three to seven experiments. See the text for more details on panels (a-d).

induced Ca^{2+} influx caused, apparently, by Ca^{2+} store depletion (Fig. 1a). Preliminary incubation of the cells for 10 min with 50 μM neomycin completely prevents glutoxim-induced $[Ca^{2+}]_i$ increase and Ca^{2+} influx (Fig. 1b). Preincubation of the macrophages for 10 min with 100 μM H-7 for 10 min before 100 μg/ml glutoxim addition also leads to the almost complete inhibition of glutoxim-induced $[Ca^{2+}]_i$ increase and prevents Ca^{2+} influx from the external medium (Fig. 1c). Similar results were obtained with the use of another protein kinase C inhibitor calphostin C in the concentration of 1 μM (Fig. 1d).

Thus, we showed for the first time the involvement of key enzymes of phosphoinositide signaling pathway phospholipase C and protein kinase C in the regulatory effect of GSSG and glutoxim on $[Ca^{2+}]_i$ in rat peritoneal macrophages.

The results obtained in this study, as well as our earlier data [2–6], suggest that GSSG and glutoxim transactivate receptors with intrinsic tyrosine kinase activity and trigger a complex signaling cascade, including tyrosine kinases, tyrosine phosphatases, phosphatidylinositol kinases, and key enzymes of phosphoinositide system phospholipase C and protein kinase C; this leads to increase of $[Ca^{2+}]_i$ in macrophages.

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