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PHYSIOLOGY

Possible Involvement of Phosphatidylinositol Kinases in the Effect of the Oxidized Glutathione and Glutoxim on the Intracellular Ca^{2+} Concentration in Macrophages

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Redox regulation of signal transduction and gene expression is one of fundamental regulatory mechanisms of cell physiology. The system glutathione/oxidized glutathione (GSH/GSSG) is an oxidation–reduction cell system of special importance. We showed previously that GSSG and its synthetic analog Glutoxim® (FARMA-VAM, Russia), which is used in clinic as immunomodulator and hemostimulator [1], augmented the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) because they mobilized Ca^{2+} from the thapsigargin-sensitive Ca^{2+} stores and promoted Ca^{2+} influx into rat peritoneal macrophages [2]. Using two structurally different tyrosine kinase inhibitors (genistein and methyl-2,5-dihydroxycinnamate), as well as Na orthovanadate, a tyrosine phosphatase inhibitor, we demonstrated that tyrosine kinases and tyrosine phosphatases were involved in the effect of GSSG and Glutoxim on $[Ca^{2+}]_i$ in macrophages [3]. The evidence obtained suggested that both receptor and cytoplasm tyrosine kinases participated in the signaling cascade triggered by GSSG and Glutoxim. At the same time, the mechanisms mediating GSSG and Glutoxim regulatory effect on $[Ca^{2+}]_i$ should be studied additionally.

In peritoneal macrophages, receptors possessing intrinsic tyrosine kinase activity were identified that contained cysteine-rich regions in their extracellular domains [4]; these regions could serve as targets for GSSG and Glutoxim. Activation of the receptor tyrosine kinases promoted the effect of SH2-domain-containing proteins, such as phospholipase Cγ, cytoplasmic tyrosine kinases of the src family, and phosphatidylinositol-3 kinase [4, 5]. We showed previously that phosphatidylinositol-3 and phosphatidylinositol-4 kinases participated in the regulation of Ca^{2+} signals in macrophages [6]. Now, we studied the role of phosphatidylinositol kinases in the effect of GSSG and Glutoxim on $[Ca^{2+}]_i$ in macrophages.

To this end, we studied the effects of two structurally different inhibitors of phosphatidylinositol-3 and phosphatilylinositol-4 kinases, wortmannin [7] and LY294002 [8], on the Ca^{2+} response induced by GSSG or Glutoxim.

Macrophage cultivation and the device for $[Ca^{2+}]_i$ recording by means of a Fura-2AM probe were described in detail earlier [9]. The experiments were performed at the room temperature, 20–22°C, on the second or third days of cell cultivation. Similar results were obtained with Glutoxim and GSSG used at doses of 100 μg/ml (Fig. 1).

Macrophage incubation with Glutoxim, 100 μg/ml, for 20 min led to a significant increase of $[Ca^{2+}]_i$ because of Ca^{2+} mobilization from intracellular stores. Ca^{2+} (2 mM) added to the medium induced Ca^{2+} influx, apparently, because of Ca^{2+} store depletion (Fig. 1a). After the addition of 1 μM wortmannin against the background of Ca^{2+} influx, the Glutoxim-induced $[Ca^{2+}]_i$ increase was completely inhibited, and this parameter returned to the basal level (Fig. 1a). This was in agreement with previous data demonstrating that wortmannin inhibited thapsigargin-induced Ca^{2+} influx into rat peritoneal macrophages [6].

Preliminary cell incubation for 10 min with 1 μM wortmannin before Glutoxim administration almost completely inhibited the Glutoxim-induced $[Ca^{2+}]_i$ increase and Ca^{2+} influx (Fig. 1b). Similar results were obtained using LY294002, another inhibitor of phosphatidylinositol-3 and phosphatidylinositol-4 kinases. Preliminary incubation of macrophages with 100 μM LY294002 for 10 min before Glutoxim administration also almost completely prevented the Glutoxim-induced $[Ca^{2+}]_i$ increase and Ca^{2+} influx (Fig. 1c).

Thus, we have demonstrated that phosphatidylinositol-3 and phosphatidylinositol-4 kinases participate in GSSG and Glutoxim regulatory influence on $[Ca^{2+}]_i$ in rat peritoneal macrophages. This is in agreement with

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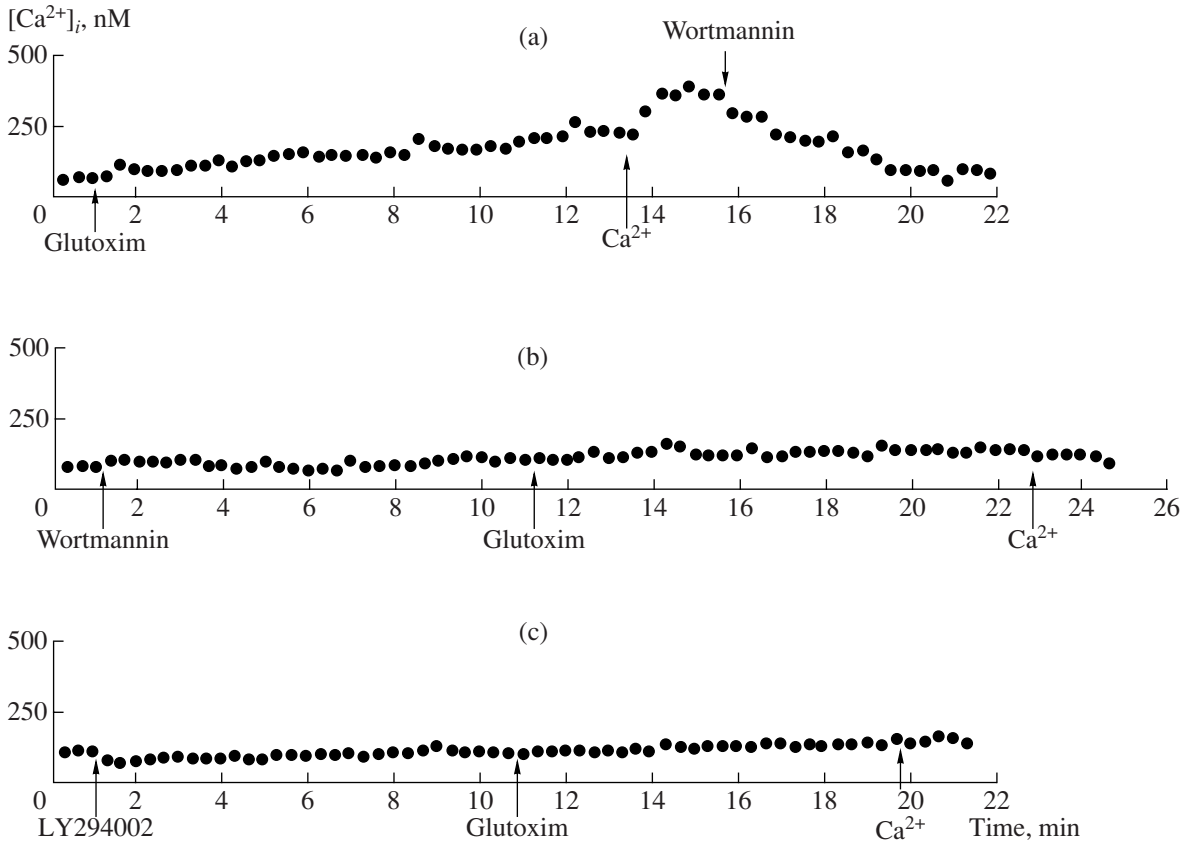

Fig. 1. The influence of wortmannin and LY294002 on the effect of Glutoxim on $[Ca^{2+}]_i$ in the peritoneal macrophages. Each recording was obtained for a group of 40–50 cells to demonstrate a typical pattern obtained in three to seven experiments. See the text for more detail on panels (a–c).

our previous data on an important role of phosphatidylinositol-3 and phosphatidylinositol-4 kinases in the regulation of Ca^{2+} signaling in peritoneal macrophages [6].

The results obtained in this study, as well as our earlier data [2, 3], suggest that GSSG and Glutoxim are capable of transactivating receptors with intrinsic tyrosine kinase activity and of triggering a complex signaling cascade, including tyrosine kinases, tyrosine phosphatases, and phosphatidylinositol kinases; this leads to increase of $[Ca^{2+}]_i$ in macrophages.

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