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PHYSIOLOGY

Involvement of Microtubules in the Effects of Glutoxim and Molixan on the Intracellular Concentration of Ca^{2+} in Macrophages

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Currently, a great number of disulfide-containing drugs altering the redox status and having a physiologically significant effect on cells have been developed and introduced into clinical practice. The pharmaceutical agent Glutoxim®, disodium salt of oxidized glutathione (GSSG) with a platinum nanoadditive (PHARMA-VAM, Moscow, Russia) is used as an immunomodulator and a hemostimulant in the integrated therapy of bacterial and viral diseases, psoriasis, as well as radio- and chemotherapies of cancer [1]. Another disulfide-containing agent, molixan (a complex of glutoxim and inosine nucleoside), has a similar application. However, the mechanisms of the cellular and molecular effects of these drugs are not completely understood.

We were the first to find out earlier that GSSG, glutoxim, and molixan enhance the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) due to Ca^{2+} mobilization from the thapsigargin-sensitive Ca^{2+} stores and subsequent entry of Ca^{2+} into rat peritoneal macrophages [2–4]. Using a wide range of agents affecting the components of signaling systems in cells, we have demonstrated that tyrosine kinases, tyrosine phosphatases [3, 5], phosphatidylinositol kinases [6], small G proteins of the Ras superfamily, and the most important enzymes of the phosphoinositide system of signal transduction (phospholipase C and protein kinase C) [7] are the key players in the signaling cascade triggered by GSSG and glutoxim and leading to an increase in $[Ca^{2+}]_i$ in macrophages. It has also been found that elements of the actin cytoskeleton are involved in the effects of glutoxim and molixan on $[Ca^{2+}]_i$ in macrophages [8, 9].

It is known that the microtubule protein tubulin has a high redox sensitivity and can be easily S-glutathionylated [10]. Correspondingly, it was reasonable to study the possible role of microtubules in the regulatory effect of glutoxim or molixan on $[Ca^{2+}]_i$ in the rat peritoneal macrophages.

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The procedure of macrophage culturing and an automated system for $[Ca^{2+}]_i$ recording with the use of the fluorescent probe Fura-2AM were described earlier in detail [11]. The experiments were performed at a room temperature of 20–22°C on the second or third day of cell culturing. The involvement of microtubules in the effects of glutoxim and molixan on $[Ca^{2+}]_i$ in the rat peritoneal macrophages was studied using two structurally different agents capable of inducing depolymerization of microtubules, colcemid and nocodazole, as well as the microtubule stabilizer taxol [12].

It has been demonstrated that incubation of macrophages in the presence of 100 μg/mL molixan (Fig. 1a) or 100 μg/mL glutoxim (Fig. 2a) for 20 min in a medium without calcium causes a significant increase in $[Ca^{2+}]_i$, which reflects mobilization of Ca^{2+} from intracellular Ca^{2+} stores. Addition of 2 mM Ca^{2+} to the external medium induces entry of Ca^{2+} into the cytosol, which is apparently mediated by depletion of the Ca^{2+} store (Figs. 1a, 2a).

It has been found that nocodazole, colcemid, or taxol almost completely prevent the increase in $[Ca^{2+}]_i$ when glutoxim or molixan are applied (Figs. 1, 2). For example, preincubation of macrophages with 10 μM nocodazole (Fig. 1b) for 25 min before application of 100 μg/mL molixan causes an almost complete suppression of both phases of the Ca^{2+} response induced by molixan. Preincubation of cells in the presence of 50 μM colcemid (Fig. 1c) for 20 min prior to introduction of 100 μg/mL molixan also leads to a full inhibition of the $[Ca^{2+}]_i$ increase induced by molixan. Similar results have been obtained with 100 μg/mL glutoxim. The results suggest that depolymerization of the tubulin cytoskeleton prevents the regulatory effects of glutoxim and molixan on the processes of Ca^{2+} signaling in macrophages. Moreover, it has been shown that preincubation of macrophages with 40 μM taxol for 30 min before application of 100 μg/mL glutoxim also leads to an almost complete suppression of the Ca^{2+} responses induced by glutoxim (Fig. 2b). Similar

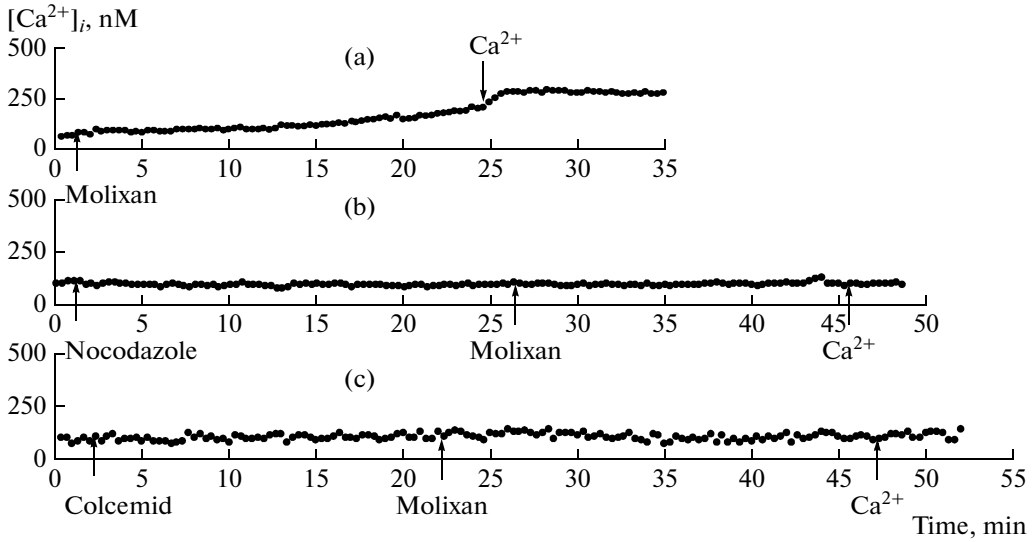

Fig. 1. (a) The effect of molixan on $[Ca^{2+}]_i$ and the influences of (b) nocodazole and (c) colcemid on the effect of molixan on $[Ca^{2+}]_i$ in peritoneal macrophages. Each recording is obtained for 40–50 cells and is typical of three to seven experiments. Panels (a–c), see the text.

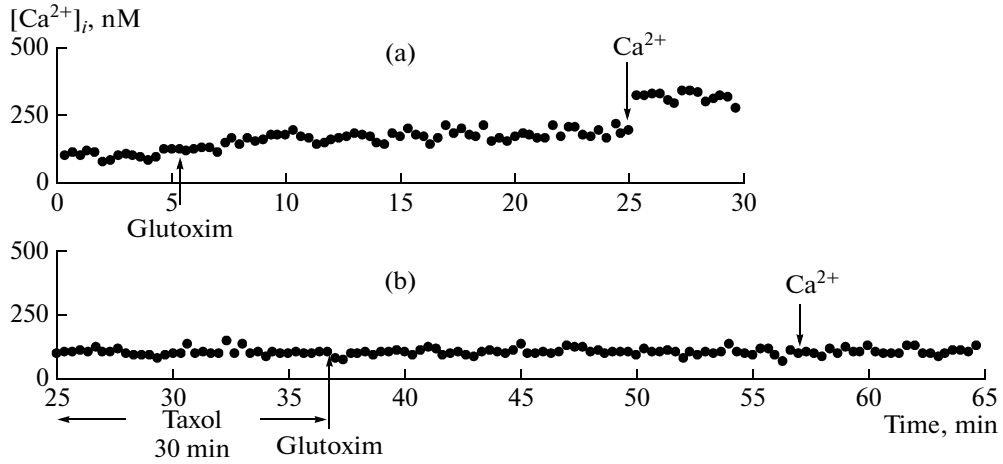

Fig. 2. (a) The effect of glutoxim on $[Ca^{2+}]_i$ and (b) the influence of taxol on the effect of glutoxim on $[Ca^{2+}]_i$ in peritoneal macrophages. Each recording is obtained for 40–50 cells and is typical of three to seven experiments. Panels (a, b), see the text.

results have been obtained when using 100 μg/mL molixan. This suggests that stabilization of microtubules, as well as their disassembling, can prevent the effects of glutoxim and molixan on $[Ca^{2+}]_i$ in macrophages.

It has been found that application of 100 μM taxol during the store-operated Ca^{2+} entry induced by molixan causes a complete inhibition of Ca^{2+} entry and return of $[Ca^{2+}]_i$ to the basal level (data not shown). This proves our earlier data about inhibition by taxol of store-operated Ca^{2+} entry, induced by the purinergic agonist ATP or the inhibitor of endoplasmic Ca^{2+} ATPases thapsigargin [13] and suggests the involvement of microtubules not only in the generation, but also in the maintenance of store-operated Ca^{2+} entry in macrophages.

Thus, we have demonstrated that any changes in the tubulin cytoskeleton structure (depolymerization or stabilization) modulate the effects of glutoxim and molixan on $[Ca^{2+}]_i$ in macrophages. Moreover, these data suggest that application of glutoxim or molixan together with nocodazole, colcemid, or taxol, which are used in the therapy of cancer as cytostatic drugs, is undesirable.

It is also known that microtubules are involved in the regulation of the intracellular transport of secretory vesicles regulating the efficacy of the transport and, like "roads" or "highways," determine the direc-

tion of the vesicle traffic. Agents causing microtubule depolymerization inhibit secretion in cells of different types [14]. Hence, the nocodazole or colcemid suppression of Ca^{2+} responses to glutoxim or molixan suggests that the regulation of $[Ca^{2+}]_i$ by these drugs is mediated by a mechanism similar to the process of secretion.

Thus, we have demonstrated the involvement of microtubules in the regulatory effects of glutoxim and molixan on $[Ca^{2+}]_i$ in the rat peritoneal macrophages. We can conclude that the tubulin cytoskeleton is a direct participant in the signaling cascade triggered by glutoxim or molixan and leading to an increase in $[Ca^{2+}]_i$ in rat peritoneal macrophages.

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