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PHYSIOLOGY

Cyclooxygenase and Lipoxygenase Inhibitors Modulate the Glutoxim and Molixan Effects on the Intracellular Ca^{2+} Concentration in Macrophages

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Glutoxim (disodium salt of GSSG with cisplatin at nano-concentration, FARMA-VAM, Moscow), a pharmacological analogue of oxidized glutathione (GSSG), is used as an immunomodulator and hemostimulator in integrated treatment of bacterial and viral diseases and psoriasis, as well as in radiation therapy and chemotherapy of cancer [1]. Another disulfide-containing drug, Molixan (a complex of Glu-

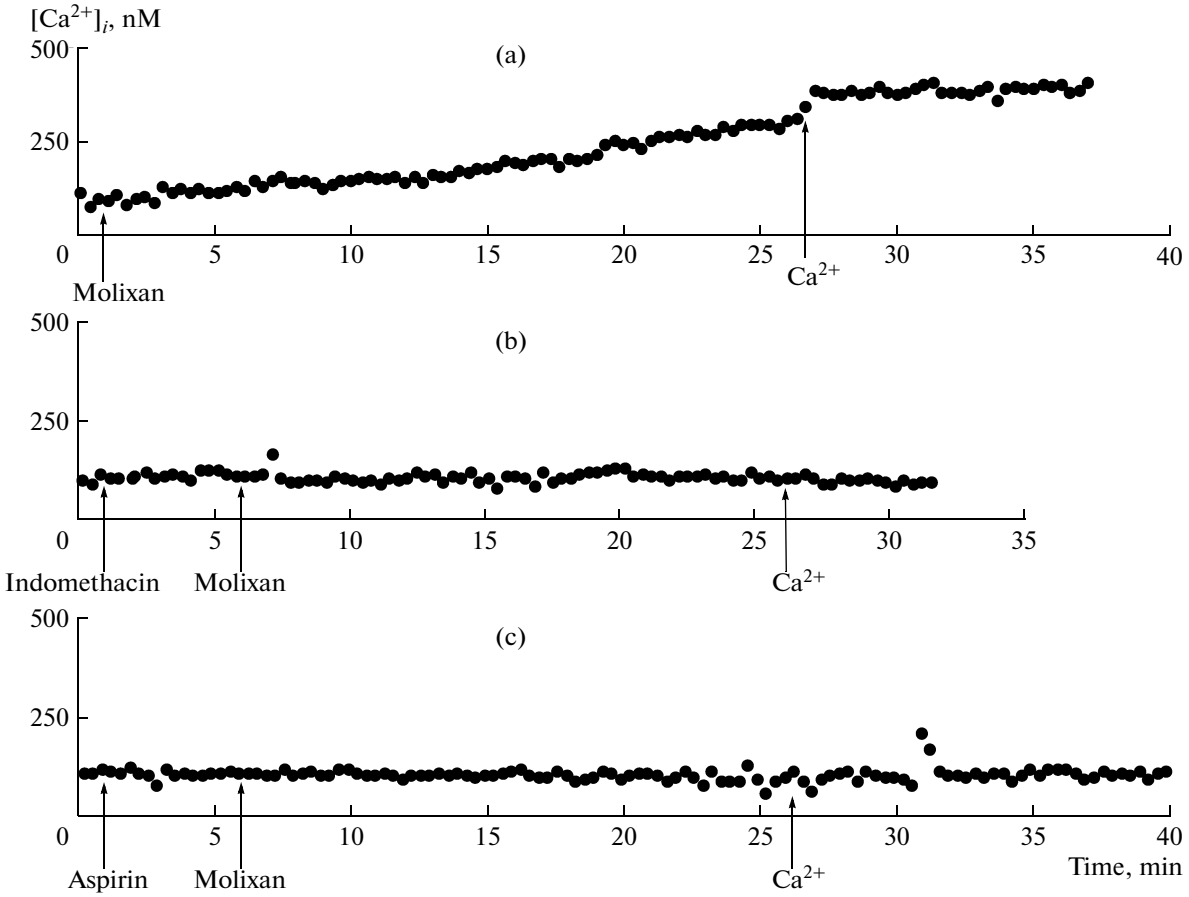

Fig. 1. Influence of (b) indomethacin and (c) aspirin on the (a) Molixan effect on $[Ca^{2+}]_i$ in peritoneal macrophages. Here and in Figs. 2 and 3, each recording has been obtained for a group of 40–50 cells and represents a typical variant for three to seven experiments.

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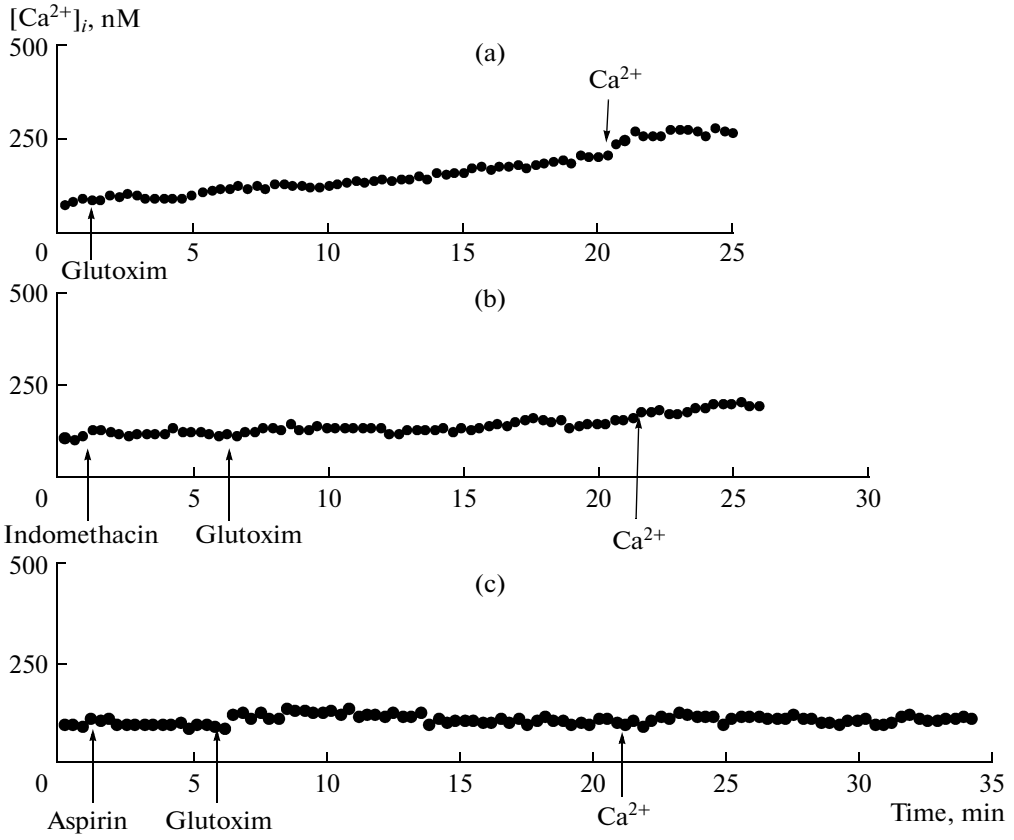

Fig. 2. Influence of (b) indomethacin and (c) aspirin on the (a) Glutoxim effect on $[Ca^{2+}]_i$ in peritoneal macrophages.

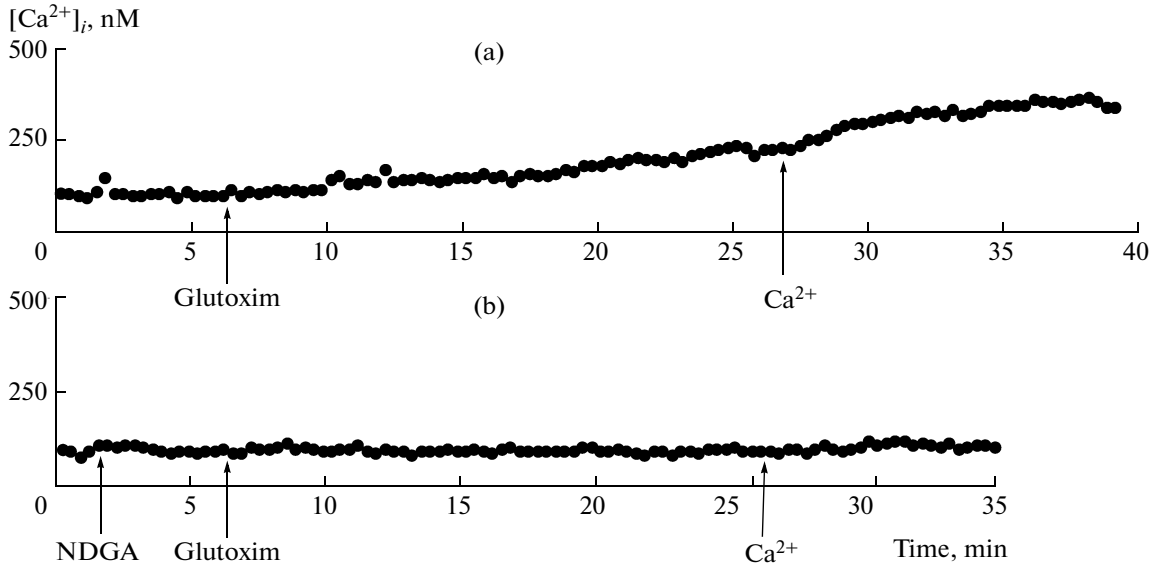

Fig. 3. Influence of (b) nordihydroguaiaretic acid (NDGA) on the (a) Glutoxim effect on $[Ca^{2+}]_i$ in peritoneal macrophages.

toxim and the nucleoside inosine) has similar applications. At the same time, the cellular and molecular mechanisms of action of both Glutoxim and Molixan are not fully understood.

Earlier, we discovered that GSSG and Glutoxim increased the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$) causing the mobilization of Ca^{2+} from the thapsigargin-sensitive Ca^{2+} store and the subsequent

entry of Ca^{2+} into rat peritoneal macrophages [2, 3]. Later, it was shown that Molixan had a similar effect on $[Ca^{2+}]_i$, in macrophages [4]. In addition, we demonstrated that the key components of the signaling cascade that is triggered by GSSG and Glutoxim and causes an increase in $[Ca^{2+}]_i$, in macrophages are tyrosine kinases, tyrosine phosphatases [3, 5], phosphatidylinositol kinases [6], small G-proteins of the Ras superfamily, phospholipase C and protein kinase C (important enzymes of the phosphoinositide signaling system) [7], and actin cytoskeleton elements [8, 9].

Polyunsaturated arachidonic acid (AA) and its oxidation products constitute one of the major signaling systems in cells [10, 11]. Enzymes of AA metabolism have a high redox sensitivity, and they are targets for oxidizing and reducing agents. One of the key events in macrophage activation is the production of a large number of eicosanoids, products of AA metabolism [12]. In peritoneal macrophages, AA, released from membrane phospholipids by phospholipase A_2 is easily oxidized in the cyclooxygenase and lipoxygenase pathways [10–12]. Thus, it seemed appropriate to investigate the possible involvement of cyclooxygenase and lipoxygenase pathways of AA oxidation in the action of Glutoxim and Molixan on $[Ca^{2+}]_i$, in rat peritoneal macrophages.

The detailed procedure for the cultivation of macrophages and the automated system for determining $[Ca^{2+}]_i$, using the fluorescent probe Fura-2AM were described previously [13]. Experiments were performed at room temperature (20–22°C) on the second or third day of cell culturing. In order to identify the possible role of the cyclooxygenase pathway of AA oxidation in the action of Glutoxim and Molixan on $[Ca^{2+}]_i$,, two structurally different inhibitors of cyclooxygenases, nonsteroid anti-inflammatory agents, indomethacin and acetylsalicylic acid (aspirin) were used. A lipoxygenase inhibitor (nordihydroguaiaretic acid, NDGA) was used in order to identify the possible involvement of the lipoxygenase pathway of the AA metabolism [10,11].

In control experiments, it was shown that the addition of 100 μg/mL Molixan (Fig. 1a) or 100 μg/mL Glutoxim (Figs. 2a, 3a) to the macrophages on the medium without calcium caused a significant increase in the $[Ca^{2+}]_i$, reflecting the mobilization of Ca^{2+} from the intracellular Ca^{2+} store. The subsequent introduction of 2 mM Ca^{2+} into the external medium induced Ca^{2+} entry into the cytosol which was apparently related to the depletion of intracellular Ca^{2+} stores (Figs. 1a, 2a, 3a).

We demonstrated that preincubation of macrophages for 5 min with 20 μM indomethacin (Fig. 1b) or 100 μM aspirin (Fig. 1c) caused a practically complete inhibition of both phases of Ca^{2+} response induced by 100 μg/mL Molixan. Similar results were obtained when 100 μg/mL Glutoxim was used (Figs. 2b, 2c).

Furthermore, it was found that preincubation of macrophages for 5 min with 10 μM NDGA also leads to an almost complete inhibition of the Ca^{2+} responses induced by 100 μg/mL Glutoxim (Fig. 3b) and 100 μg/mL Molixan (data not shown).

These data indicate the involvement of products and/or enzymes of the cyclooxygenase and lipoxygenase pathways of AA oxidation in the action of Glutoxim and Molixan on $[Ca^{2+}]_i$, in macrophages. In addition, the results indicate undesirability of a combined use of the Glutoxim and Molixan drugs and nonsteroid anti-inflammatory drugs on the basis of indomethacin or acetylsalicylic acid.

On the basis of the results obtained in the present study and earlier [2–9], we can assume that Glutoxim and Molixan transactivate receptors with intrinsic tyrosine kinase activity and induce a complex signaling cascade that involves tyrosine kinases, tyrosine phosphatases, phospholipase C, protein kinase C, small G-proteins, actin cytoskeletal elements, as well as enzymes and/or products of the cyclooxygenase and lipoxygenase pathways of arachidonic acid oxidation. This leads to an increase in the $[Ca^{2+}]_i$, in macrophages.

REFERENCES

1. Zhukov, O.B., Zubarev, A.R., Mezentseva, M.V., et al., *Vracheb. Soslovie*, 2004, vols. 5–6, pp. 51–56.
2. Krutetskaya, Z.I., Lebedev, O.E., Kurilova, L.S., et al., *Dokl. Biol. Sci.*, 2007, vol. 412, no. 5, pp. 11–14.
3. Kurilova, L.S., Krutetskaya, Z.I., Lebedev, O.E., et al., *Cell Tissue Biol.*, 2008, vol. 2, pp. 322–332.
4. Krutetskaya, Z.I., Kurilova, L.S., Lebedev, O.E., et al., *Tsitologiya,* 2011, vol. 53, no. 9, p. 708.
5. Krutetskaya, Z.I., Lebedev, O.E., Kurilova, L.S., et al., *Dokl. Biol. Sci.,* 2007, vol. 417, no. 2, pp. 417–419.
6. Krutetskaya, Z.I., Lebedev, O.E., Kurilova, L.S., et al., *Dokl. Biol. Sci.,* 2008, vol. 422, no. 4, pp. 296–297.
7. Krutetskaya, Z.I., Lebedev, O.E., Kurilova, L.S., et al., *Dokl. Biol. Sci.*, 2009, vol. 428, no. 2, pp. 407–409.
8. Krutetskaya, Z.I., Lebedev, O.E., Kurilova, L.S., et al., *Dokl. Biol. Sci.*, 2011, vol. 436, no. 5, pp. 16–19.
9. Kurilova, L.S., Krutetskaya, Z.I., Lebedev, O.E., et al., *Tsitologiya,* 2012, vol. 54, no. 2, pp. 135–142.
10. Krutetskaya, Z.I., Lebedev, O.E., and Kurilova, L.S., *Mekhanizmy vnutrikletochnoi signalizatsii* (Intracellular Signaling Mechanisms), St. Petersburg: St. Petersb. Univ., 2003, 208 p.
11. Krutetskaya, Z.I. and Lebedev, O.E., *Tsitologiya*, 1993, vol. 35, nos. 11–12, pp. 3–35.
12. Scott, W.A., Pawlowski, N.A., Murry, H.W., et al., *J. Exp. Med.,* 1982, vol. 155, pp. 1148–1161.
13. Krutetskaya, Z.I., Lebedev, O.E., Tyushev, V.E., et al., *Tsitologiya*, 1997, vol. 39, nos. 2–3, pp. 164–176.

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