BIOPHYSICS AND BIOCHEMISTRY

Effect of Ricin and Its B-Subunit on Calcium Responses in Human Lymphocytes

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The effect of ricin and its B-subunit on cytoplasmic Ca^{2+} concentration in human lymphocytes was studied using a fluorescence probe. Both agents dose-dependently increased cytosolic free Ca^{2+} by stimulating its release from of thapsigargin-sensitive store. It is suggested that this effect is associated with the increase in cytosolic content of inositol-1,4,5-trisphosphate.

Key Words: *lymphocytes; ricin; ricin B-subunit; Ca^{2+} response; inositol-1,4,5-trisphosphate*

Ricin (RT) is a plant toxin isolated by Stillmark from castor beans (*Ricinus communis*) more than 100 years ago [6]; its molecule consists of two polypeptide chains, A and B [9]. A-subunit is responsible for toxic effect of RT based on irreversible inhibition of ribosomal protein synthesis [4], while B-subunit (RTB) binds to receptors expressed on the plasma membrane of target cells [1]. RT represents a classic plant lectin which selectively recognizes certain cell surface sugars. Galactose and N-galactosamine in the binding site of the receptor and responsible for toxin-receptor binding efficiently block RT binding to cells [11,13]. RT induces agglutination of different cell types [4]. Reportedly, it increases the concentration of cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) [5,7], but the mechanism of this effect remains unknown.

In view of wide application of RT in the past years, its biological and cytotoxic activities are now intensely studied. It is used not only as a molecular probe in cell biology, but also as a basis for the development of immunotoxins representing the covalent antibody-RT A-subunit complexes [13]. Immunotoxins are applied to eliminate undesirable cells including tumor cells [5].

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The aim of this study was to investigate the effect of RT and RTB on $[Ca^{2+}]_i$ and calcium responses to other substances and to elucidate the mechanisms of these effects.

MATERIALS AND METHODS

Lymphocytes were isolated from the peripheral blood of healthy donors by differential centrifugation in a Ficoll-Histopaque density gradient [1]. The final suspension contained no less than 92-95% lymphocytes, viability assessed by trypan blue exclusion was no less than 95%.

$[Ca^{2+}]_i$ was determined by measuring Fura-2AM fluorescence on a Shimadzu RF-15-01 spectrofluorimeter equipped with a thermostated cuvette and magnetic stirrer [2].

The following compounds were used: Fura-2AM, EGTA, thapsigargin, digitonin, and Ficoll-Histopaque gradient (1.077 g/ml, Sigma). RT and RTB were kindly provided by I. I. Agapov (Institute of Genetics and Selection of Industrial Microorganisms).

RESULTS

In lymphocytes incubated in a medium with physiological Ca^{2+} concentration, RTB (2.5×10^{-7} M) increa-

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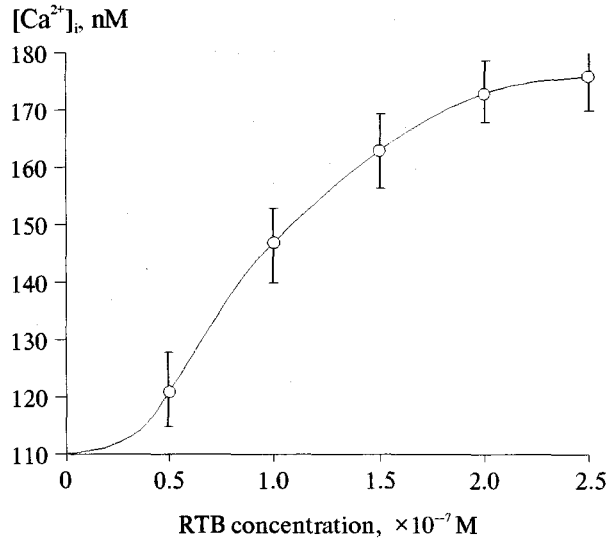

Fig. 1. Effect of ricin B-subunit (RTB) on cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in human lymphocytes in a Ca^{2+}-containing medium.

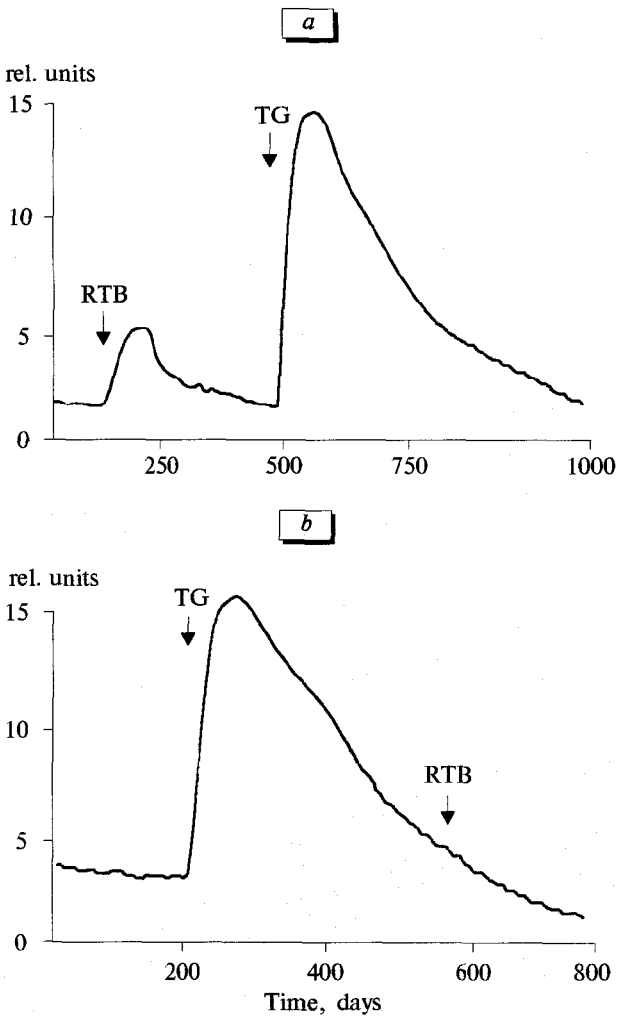

Fig. 2. Effect of ricin B-subunit (RTB) and thapsigargin (TG) on Ca^{2+} release from intracellular Ca^{2+} stores in human lymphocytes in a Ca^{2+}-free medium. Ordinate: fluorescence intensity.

sed $[Ca^{2+}]_i$ from 110±12 nM (basal level) to a steady-state level of 180±15 nM for 55±10 sec. The time course of lymphocyte Ca^{2+}-response corresponded to a saturation curve. The effect of RTB was dose-dependent (Fig. 1) with saturation at 1.8×10^{-7} M. RT exerted similar effects.

In lymphocytes incubated in a Ca^{2+}-free medium, RTB (2.5×10^{-7} M) induced a transient calcium response (Fig. 2, *a*): fluorescence intensity peaked after 45±8 sec and then gradually decreased to the basal level. Subsequent treatment with thapsigargin (400 nM) induced a typical Ca^{2+} response. It was assumed that the effect of RTB in a Ca^{2+}-free medium is determined by Ca^{2+} release from intracellular thapsigargin-sensitive stores (TSS).

To test this assumption, the agents were added in inverse order (Fig. 2, *b*). Thapsigargin (400 nM) in a Ca^{2+}-free medium induced a specific transient Ca^{2+} response. Thapsigargin is known to produce irreversible inhibition of microsomal Ca^{2+}-ATPase [12]. It does not affect the formation of intracellular inositol-1,4,5-trisphosphate [9] and the observed increase in $[Ca^{2+}]_i$ results from passive Ca^{2+} efflux from Ca^{2+} stores to the cytoplasm through leakage channels. Being added after thapsigargin, RTB (2.5×10^{-7} M) produced no Ca^{2+}-response. Thus, complete depletion of TSS with thapsigargin prevented Ca^{2+}-response to RTB. These data confirm the assumption that RTB stimulates Ca^{2+} release from TSS. Minor decrease in the amplitude of Ca^{2+}-response to thapsigargin after RTB application can result from its reversible effect on TSS.

Since the treatment of lymphocytes with RTB in Ca^{2+}-containing medium produced a pronounced Ca^{2+}-response, it can be assumed that the absence of Ca^{2+}-response to RTB after thapsigargin (Fig. 2, *b*) was due to RTB binding to receptors involved in phosphoinositide metabolism followed by the formation of inositol-1,4,5-trisphosphate stimulating Ca^{2+} efflux from TSS [3]. Similar results were obtained in experiments with RT.

Thus, both RTB and the whole RT molecule dose-dependently increased $[Ca^{2+}]_i$ in human lymphocytes. These effects could be associated with activated metabolism of membrane phosphoinositides and the formation of inositol-1,4,5-trisphosphate which depleted intracellular Ca^{2+} stores. It is likely that RT-induced increase in $[Ca^{2+}]_i$ is necessary for A-subunit permeation into the cytoplasm.

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