Acta Physiologica Hungarica, Volume 74 (2), pp. 195—199 (1989)

ZINC AS A POSSIBLE MEDIATOR OF SIGNAL TRANSDUCTION IN T LYMPHOCYTES*

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Received August 9, 1988
Accepted September 6, 1988

Our recent findings indicate that phorbol esters, the specific activators of protein kinase C induce the translocation of heavy metals (mostly: zinc) from the nucleus and mitochondria to the cytosol and microsomes of T lymphocytes. Phorbol ester treatment impairs the action of Ca-ionophores, this effect is mediated by intracellular heavy metal ions (most probably: by zinc). Zinc activates cytosolic protein kinase C, increases its affinity towards phorbol esters and contributes to its binding to plasma membranes. These results suggest that zinc may play a role in the "cross-talk" of second messengers and hence in signal transduction in T lymphocytes.

Keywords: zinc, heavy metals, T lymphocytes, phorbol, protein kinase C, Ca-ionophores, Ca^{2+}-dependent processes.

The pleiotropic effects of zinc on the activation of T lymphocytes were known decades ago. Zinc deprivation results in many symptoms of immune-deficiency, zinc in itself is mitogenic for T lymphocytes, while at higher concentrations it effectively blocks the action of other mitogens such as antigens or lectins [13]. However, we have very few information about the biochemical mechanisms of these processes.

On the other hand there are some recent findings which suggests that zinc may play a role in signal transduction. Zinc can substitute calcium in a number of calcium binding sites [14]. In this way changes in the intracellular zinc concentration may induce conformational and functional changes of numerous calcium binding proteins. On the other hand several DNA-binding proteins such as the cortisol, oestradiol and thyroid hormone receptors [17, 18] contain a zinc binding site, a so-called "zinc-finger" [2, 3, 11]. Parker et al. [16] defining the primary structure of protein kinase C demonstrated the presence of these "zinc-fingers" in the regulatory domain of the enzyme. However, the function of this zinc-binding site of protein kinase C is not yet known.

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* Presented at the Membrane Symposium, May, 1988, Sümeg, Hungary

Based on these results we planned some experiments to get an insight into the possible role of zinc in the regulation of Ca^{2+}- and protein kinase C-dependent signal transduction in T lymphocytes.

Methods

Subcellular fractions of T lymphocytes were prepared by differential centrifugation after the cells have been ruptured by nitrogen cavitation [12]. The amount of various heavy metals was analysed by X-ray fluorescence [5] and plasma emission spectroscopy [4]. The intracellular calcium concentration was determined by the fluorescent indicators quin2 and fura-2 [6]. Zinc was depleted from T lymphocytes and from their subcellular fractions by the intracellular heavy metal chelator tetrakis-(2-pyridyl-methyl)-ethylenediamine (TPEN, [1]). The activity of protein kinase C was determined by measuring the transfer of ^{32}P to the basic H1 histone (Type III-S, Sigma) as described earlier [9]. Binding of ^{3}H-phorbol-dibutyrate was measured by polyethylene-glycol precipitation [9].

Results and discussion

Studying the possible role of zinc in signal transduction of T lymphocytes we have selected an approach which may indicate that the concentration of heavy metals (zinc) changes during the activation of T lymphocytes. The detection of such concentration changes is a prerequisite for the postulation that zinc indeed may participate in signal transduction. Since we wanted to study the role of zinc in the "cross-talk" of known signal transduction systems we used phorbol esters, the selective activators of protein kinase C for lymphocyte stimulation.

Isolating the subcellular fractions of control and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) treated rabbit thymocytes and analysing their heavy metal content by X-ray fluorescence [5] and plasma emission spectroscopy [4] we have found that phorbol ester treatment (20 nM TPA for 90 minutes) induces the translocation of approximately 270 nmol Zn from the nucleus and mitochondria to the cytosol and microsomes of 5×10^9 cells. Smaller amounts of other heavy metals (Cu and Fe) are also translocated in a similar way. Our results suggest that the activation of protein kinase C induces a substantial increase in zinc concentration of cytosolic and microsomal fractions of T lymphocytes. Investigation of changes in *free* zinc concentration during lymphocyte stimulation is in progress in our laboratory.

As a next step we investigated the interrelationship between zinc and Ca^{2+}-induced signal transduction. It is well-known that phorbol esters attenuate the calcium signals induced by various hormones and agonists. In this systems protein kinase C has a number of possible targets to prevent the agonist-induced increase in intracellular Ca^{2+} concentration. In this way the

action of heavy metals (zinc) may be concealed. Therefore we have selected a more simple, "model" system for our studies. In our model system we induced an increase in intracellular Ca^{2+} concentration by means of Ca-ionophores such as A23187 or ionomycin and the effect of phorbol ester treatment on these calcium-signals was studied.

We have found that phorbol ester treatment impairs the action of Ca-ionophores. This effect is concentration- and time-dependent being maximal at TPA concentrations higher than 10 nM (this is the range where protein kinase C is fully activated) and after an incubation time of 90 minutes. Our studies indicate that the effect of phorbol esters can not be explained by the activation of Ca^{2+}-extrusion systems, "freezing" of Ca-ionophores to the lymphocyte plasma membrane or by other means of ionophore immobilization. On the other hand the intracellular heavy metal chelator TPEN restores the Ca-ionophore-induced Ca^{2+} translocation to the control level in TPA, or diacyl glycerol treated lymphocytes. This suggests that intracellular heavy metals (most probably the increased amount of zinc in the cytosol and microsomes — see above —) may be responsible for the attenuation of Ca-ionophore-induced Ca-signals in T lymphocytes [7].

As a third step we investigated whether the protein kinase C-induced excess of zinc in the cytosol and microsomes had any regulatory effect on protein kinase C itself. This question is interesting all the more because Parker et al. [16] suggested the existence of putative zinc binding site(s) on protein kinase C.

We have found that zinc activates cytosolic protein kinase C at nanomolar concentrations while it does not affect the membrane-bound form of the enzyme [8, 9]. Our results are in agreement with that of Murakami et al [15] who also demonstrated a zinc-induced activation of protein kinase C. Zinc increases the phorbol ester binding affinity of cytosolic protein kinase C also at nanomolar concentrations while it has a much smaller effect on the membrane-bound form of the enzyme [10].

The presence of the intracellular heavy metal chelator TPEN prevents the phorbol ester- and antigen-induced translocation of protein kinase C from the cytosol to the microsomes of T lymphocytes [9]. This suggests that heavy metals (most probably: zinc) may contribute to the membrane binding of protein kinase C. Taken our data together we suggest that cytosolic protein kinase C is a mixture of enzymes which are differently saturated with zinc. Zinc saturation may be a prerequisite of membrane binding of the enzyme. This may explain the relative insensitivity of membrane-bound protein kinase C to the addition of zinc. However, to strengthen this hypothesis further experiments are needed.

Figure 1 summarizes our present view on the possible role of zinc in signal transduction of T lymphocytes. The activation of protein kinase C

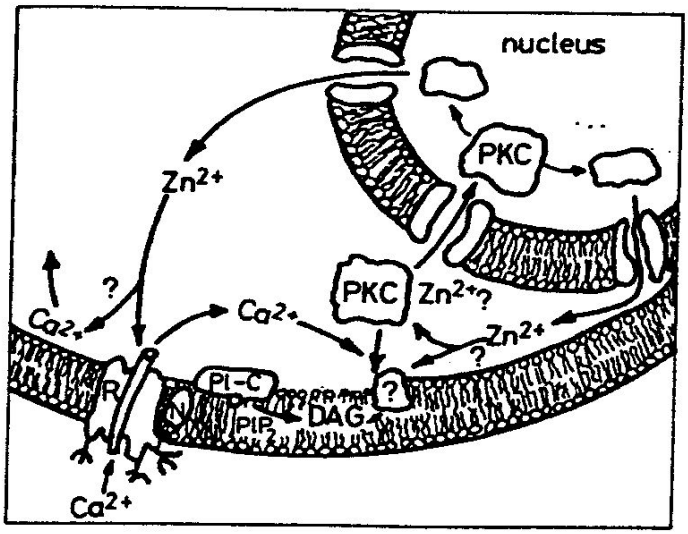

Fig. 1. The possible role of zinc in signal transduction of T lymphocytes
Abbreviations: DAG, diacyl glycerol; N, GTP-binding protein; PIP_2, phosphatidyl inositol bis-phosphate; PKC, protein kinase C; PI-C, phosphatidyl inositol specific phospholipase C; R, T cell receptor

(PKC) releases zinc from the nucleus. The mechanism of this action is unknown. In Figure 1 we illustrated one possibility which supposes that after prolonged activation of the enzyme protein kinase C translocates to the nucleus, phosphorylates some of the zinc-containing nuclear proteins inducing a decrease in their zinc-binding affinity. As a consequence these proteins release zinc which appears in the cytosol and microsomes. However, we would like to stress that this mechanism is merely speculative (at the moment).

The excess of zinc in the cytosol may disturb the calcium-signalling mechanisms and causes the activation and membrane binding of protein kinase C. This "secondary" activation of protein kinase C may represent a new mechanism of the autoregulation of the enzyme since it occurs at a later phase of lymphocyte activation (after 1.5 hours) when the initial activators of the enzyme (e.g.: diacyl glycerols) have been already metabolized.

Our results present pieces of evidence that zinc should be considered as an active constituent of the sophisticated signalling mechanism of T lymphocytes. These observations might help to clarify the heretofore mainly unknown mechanisms of both the action of zinc and tumor promoter phorbol esters in these cells.

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