Effect of transmethylation-reaction and increased levels of cAMP on superoxide generation of guinea-pig macrophages induced with wheat germ agglutinin and phorbor myristate

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Superoxide (O_2^-) generation of guinea-pig macrophages induced by wheat germ agglutinin (WGA) was suppressed to a great extent by the inhibition of transmethylation with 3'-deazaadenosine. When macrophages were stimulated with phorbor myristate (PMA) instead of WGA, the suppression of O_2^- generation of macrophages was observed to be slight despite the presence of 3'-deazaadenosine. These results were confirmed under various conditions. Thus the WGA-stimulated O_2^- generation of macrophages is probably associated with transmethylation, but the PMA-stimulated O_2^- generation is not. WGA-stimulated O_2^- generation of macrophages was also inhibited in the presence of dibutyryl cAMP or prostaglandin E_2 (PGE_2), substances that increase intracellular cAMP, but PMA-stimulated O_2^- generation was only slightly affected by these compounds. These results suggest that the mechanism for O_2^- generation of macrophages caused by WGA is different from that for O_2^- generation caused by PMA.

Transmethylation of phospholipids *cAMP* *Superoxide generation* *Wheat germ agglutinin* *Phorbor myristate*

1. INTRODUCTION

Macrophages produce a large amount of O_2^- when phagocytic particles [1], immune complexes [2] and some lectins such as WGA [3] interact with the receptors on the plasma membrane of the cells, or when certain chemical substances such as PMA [4] are added. An NADPH oxidase in the plasma membrane is responsible for O_2^- generation [1-4]. However, it it still unclear how the information is transferred to NADPH oxidase after the binding of agonists to the receptors and how this enzyme is regulated in the lipid bilayers of the membrane.

Recently, it has been shown [5] that immune complexes including IgE and anti-IgE induce the methylation of phospholipids of basophils, and that histamine-release from the cells is associated with the methylation of phospholipids in the membrane, followed by an increase in membrane fluidity, Ca^{2+} influx, and activation of phospholipase A_2. Since O_2^- generation of macrophages is analogous to histamine release from basophils in terms of a receptor-mediated phenomenon, it is interesting to see whether methylation of phospholipids may affect the O_2^- generation by NADPH oxidase of the cell membrane. In spite of this, the effect of methylation of phospholipids on O_2^- generation in macrophages has not been studied in detail.

We studied the effect of transmethylation and increased levels of cAMP on O_2^- generation in macrophages stimulated by either WGA or PMA. The results suggested that WGA-mediated O_2^- generation is associated with transmethylation and inhibitable by an increase in cAMP, and that the mechanism for WGA-mediated O_2^- generation of macrophages is different from that for PMA mediated O_2^- generation.

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2. MATERIALS AND METHODS

2.1. *Stimuli and reagents*

PMA, cytochalasin E (cyt-E), superoxide dismutase (SOD), cytochrome *c* (cyt-*c*), dibutyryl cAMP and PGE_2 were purchased from Sigma (St. Louis Mo); WGA was purchased from P-L biochemicals (Milwaukee WI); 3′-deazaadenosine was prepared as in [6].

2.2. *Cell preparation*

Peritoneal exudate macrophages were collected from Hartley Guinea pigs 4–5 days after injection of 20 ml liquid paraffin as in [7]. The cells were suspended in HEPES buffer (pH 7.3) at a concentration of 5×10^5 cells/ml.

2.3. *Determination of released* O_2^-

O_2^- determination was performed as described in [7]. One ml of reaction mixture containing 100 μM ferricytochrome *c* and 5×10^5 cells in HEPES buffer as preincubated for different time intervals in shaken plastic cuvettes with or without reagents. WGA (40 μg/ml) and cyt-E (5 μg/ml) or PMA (100 ng/ml) were then added to the reaction mixtures, and the rate of SOD-inhibitable reduction of cyt-*c* was measured continuously by recording the increase in absorption at 550 to 540 nm with a Hitachi 556 double beam spectrophotometer.

3. RESULTS

Fig. 1A shows the effect of 3′-deazaadenosine, which strongly inhibits transmethylation reactions, at low concentrations [5], on O_2^- generation of macrophages stimulated with either WGA plus cyt-E or PMA alone. In the presence of 3′-deazaadenosine (100 μM), the O_2^- generation of macrophages stimulated with WGA + cyt-E was inhibited as much as 80% of the control without 3′-deazaadenosine. The O_2^- generation with WGA plus cyt-E was completely inhibited by the presence of SOD, the scavenger of O_2^-. The inhibition of O_2^- generation of macrophages stimulated with PMA alone was only 15% in the presence of 3′-deazaadenosine (not shown). This suggest that the mechanism for O_2^- generation of macrophages with WGA is quite different from that with PMA.

The percent inhibition of O_2^- generation of

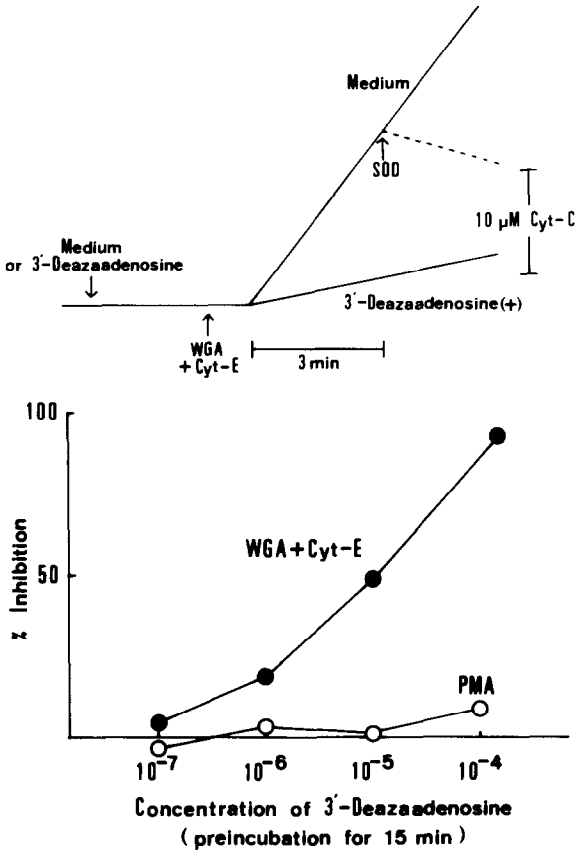

Fig. 1. Percent inhibition of O_2^- generation in guinea-pig macrophages by 3′-deazaadenosine: (A) Inhibition of O_2^- generation in macrophages stimulated with WGA + cyt-E in the presence or absence (medium) of 3′-deazaadenosine; (B) Effect of various concentrations of 3′-deazaadanosine on O_2^- generation in macrophages stimulated with WGA + cyt-E or PMA alone.

macrophages stimumated with WGA plus cyt-E or PMA was studied at various concentrations of 3′-deazaadenosine (fig. 1B) The results showed that O_2^- generation with WGA was maximally inhibited by 10^{-4} M 3′-deazaadenosione. This means that transmethylation is critical to the O_2^- generation by NADPH oxidase in macrophages. We also observed that methylation of phospholipids occurs within a few minutes when macrophages are exposed to immune-complexes (not shown). Therefore, it is likely that O_2^- generation which is finally elicited by NADPH oxidase is coupled with methylation of phospholipids induced by the interaction of WGA and WGA receptors

of macrophages. The inhibition of PMA-stimulated O_2^- generation, however, was small at various concentrations of 3'-deazaadenosine.

In order to further investigate the different mechanism for O_2^- generation in WGA- or PMA-stimulated macrophages, we studied the effect of increased levels of cAMP on O_2^- generation by macrophages. Fig. 2 shows the effect the addition of dibutyryl cAMP on the O_2^- generation of macrophages stimulated with WGA + cyt-E or PMA alone. The O_2^- generation of macrophages stimulated with WGA + cyt-E was inhibited to a great extent (about 40%) by the addition of dibutyryl cAMP which permeates the cell membrane. When macrophages were stimulated with PMA, the inhibition of O_2^- generation by dibutyryl cAMP was far less than that with WGA.

It is well documented that PGE_2 increases the level of intracellular cAMP by stimulating the activity of adenylate cyclase [8]. We observed that the adenylate cyclase of macrophages was 20-times more activated than that of the cells without PGE_2 (not shown). We therefore studied the effect of PGE_2 on O_2^- generation of macrophages stimulated with WGA or PMA (fig. 3A). The O_2^- generation of macrophages with WGA + cyt-E was suppressed by PGE_2 maximally (about 60%) at a concentration of 1 μg/ml. On the other hand,

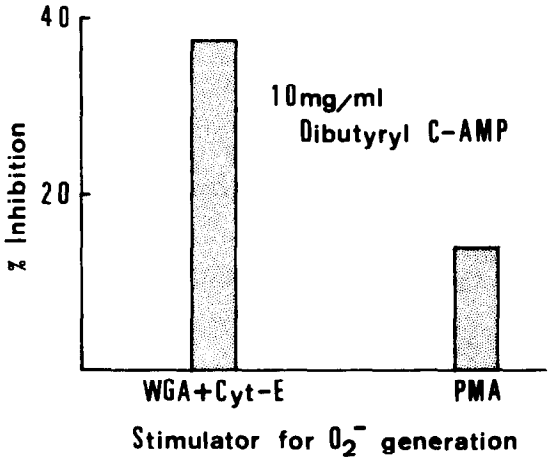

Fig. 2. Effect of dibutyryl cAMP on O_2^- generation in macrophages stimulated with WGA + cyt-E or PMA alone. Percent inhibition of O_2^- generation in macrophages stimulated with WGA + cyt-E or PMA alone was studied in the presence of dibutyryl cAMP (10 mg/ml).

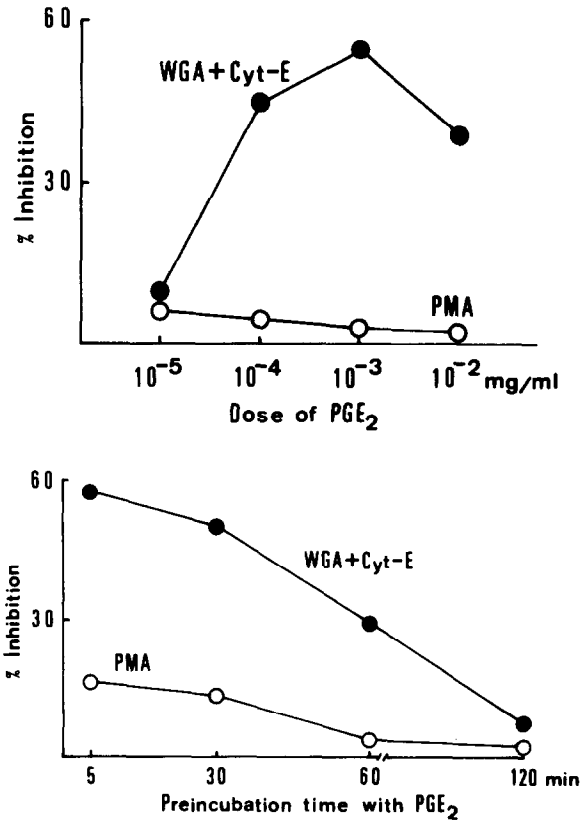

Fig. 3. Effect of PGE_2 on O_2^- generation in macrophages stimulated with WGA + cyt-E or PMA alone: (A) Percent inhibition of O_2^- generation at various concentrations of PGE_2; (B) Time-dependent inhibition of O_2^- generation of macrophages by PGE_2.

O_2^- generation of macrophages with PMA was not affected by the changes in PGE_2 concentrations, and the extent of the inhibition was small.

The inhibition of O_2^- generation of macrophages stimulated with WGA and PMA in the presence of PGE_2 was maximally observed within 5 min after exposure of macrophages to PGE_2, and was then gradually reversed (fig. 3B). After 2 h, the effect of PGE_2 almost disappeared with both WGA and PMA.

4. DISCUSSION

Although it is well known that O_2^- is generated by the activation of NADPH oxidase in the macrophage plasma membrane through the interaction of membrane receptors with lectins and

immune complexes [2,3], the mechanism of how the information on the cell surface is transferred to the enzyme is still not clear. The present results show that O_2^- generation of macrophages stimulated with WGA is associated with transmethylation reactions, while O_2^- generation with PMA is not (fig. 1A,B). The methylation of phospholipids is shown to be a trigger of histamine release of basophils causing an increase in membrane fluidity, Ca^{2+} influx and activation of phospholipase A_2 [5]. WGA stimulates the O_2^- generation of macrophages by way of WGA receptors [9], while PMA probably interacts with membrane proteins directly to stimulate O_2^- generation. These results, therefore, suggest that when macrophages were exposed to WGA, the activation of NADPH oxidase was coupled to methylation of phospholipids, which is induceable by the interaction of WGA and WGA receptors. This mechanism is analogous to that for histamine release from basophils, induced by the binding of immune complexes to the receptors [5].

On the other hand, the reason why PMA-stimulated O_2^- generation of macrophages is independent of transmethylation reaction may be explained by the nature of PMA. PMA is shown to activate protein kinase C in thrombocytes [10] and phospholipase A_2 involved in the plasma membranes of C_6 astrocytoma [11]. Therefore, it is conceivable that PMA activates an NADPH oxidase or some proteins linked to NADPH oxidase in the plasma membranes of macrophages, though the detailed mechanism is not clear at present.

A different manner of O_2^- generation caused by WGA and PMA was also observed in the case of cAMP inhibition of O_2^- generation by these compounds (fig. 2,3). WGA-stimulated O_2^- generation of macrophages was considerably suppressed by the increase in intracellular cAMP levels provoked by PGE_2 or by the addition of dibutyryl cAMP. However, PMA-stimulated O_2^- generation was not affected by the presence of PGE_2 or dibutyryl cAMP as much as WGA-stimulated O_2^- generation. These results support the view that the mechanism for the activation of NADPH oxidase through WGA is different from that through PMA. It is shown that an increase in cAMP inhibits O_2^- generation of neutrophils when the cells are stimulated with some lectins and heat-treated *Escherichia coli* which bind membrane receptors [12,13]. The significance of cAMP inhibition of O_2^- generation has not been clear. These results however, may support the view that O_2^- generation mediated by membrane receptors is regulated by cAMP, because WGA-stimulated O_2^- generation was considerably inhibited by an increase in intracellular cAMP, while PMA-stimulated O_2^- generation was not.

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