Abolishment of inhibitory effects of 3′-deazaadenosine on superoxide generation of guinea pig phagocytes by pre-exposure to phorbol myristate acetate

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Superoxide generation by guinea pig macrophages and polymorphonuclear leukocytes induced by wheat germ agglutinin, immune complexes or formyl-methionyl-leucyl-phenylalanine was inhibited considerably by 3′-deazaadenosine. The pre-exposure of the 3′-dezazaadenosine-treated cells to a small amount of phorbol myristate acetate abolished the inhibitory effect of 3′-deazaadenosine on the generation of superoxide.

Superoxide generation *3′-Deazaadenosine* *Phorbol myristate acetate* *Wheat germ agglutinin* *Immune complex* *Phagocyte*

1. INTRODUCTION

Surface-active agents such as lectins, chemotactic factors, immune complexes (IC) and phorbol myristate acetate (PMA) stimulate phagocytic cells for oxidative metabolism, leading to the release of superoxide anion (O_2^-), hydrogen peroxide and hydroxyl radicals [1]. An NADPH oxidase in the plasma membrane of the cells is responsible for O_2^- generation [2–4]. It has recently been shown that the activation of NADPH oxidase in phagocytic cells is closely linked to protein kinase C and Ca^{2+} mobilization in the plasma membrane [5–7]. However, it still remains unclear how the stimulation on the cells due to the binding of various stimuli is propagated to the NADPH oxidase via protein kinase C.

We observed that 3′-deazaadenosine (DZAdo) selectively inhibited the O_2^- generation of guinea pig macrophages stimulated by wheat germ agglutinin (WGA), IC or formyl-methionyl-leucyl-phenylalanine (fMLP), without affecting the PMA-induced O_2^- generation [8], hence we considered that the process of O_2^- generation of phagocytic cells induced by WGA, IC and fMLP might differ from events related to PMA. However, there were few clues to differentiate the processes of O_2^- generation induced by these stimuli. As pretreatment of phagocytic cells with a small amount of PMA activated protein kinase C [9], and abolished the inhibitory effects of DZAdo on O_2^- generation of the cells induced by WGA, IC or fMLP, the signals initiated by the binding of WGA, IC or fMLP to the receptors of the cells might be transduced to protein kinase C to activate NADPH oxidase, and DZAdo might block the transduction. From this aspect, we investigated the O_2^- generation of guinea pig macrophages and polymorphonuclear leukocytes (PMNs) induced by WGA, IC or fMLP in the presence of DZAdo.

2. MATERIALS AND METHODS

2.1. *Reagents*

Ferricytochrome *c*, cytochalasin E (Cyt-E), fMLP, superoxide dismutase and egg albumin

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were purchased from Sigma (St. Louis, MO). WGA and PMA were purchased from P-L Biochemicals (Milwaukee, WI). DZAdo was synthesized as described [10].

2.2. *Preparation of IC*

Purification of rabbit antibodies against egg albumin was performed as described [11]. The purified antibodies (1 mg/ml) were mixed with egg albumin at a molar ratio of 1 : 1 for the preparation of IC.

2.3. *Preparation of phagocytic cells*

6–8 days after the injection of 20 ml liquid paraffin into the peritoneal cavity of Hartley guinea pigs, peritoneal cells were collected. Purification of macrophages from the peritoneal cells was performed as described [11]. The PMNs obtained from the guinea pig peritoneal cavity 12 h after peritoneal injection of 20 ml of 10% polypeptone consisted of over 90% PMNs, as determined by Giemsa staining.

2.4. *Treatment of the cells with DZAdo and determination of the generated O_2^-*

Cells suspended (5×10^5) in 1 ml Hepes buffer were incubated at 37°C with or without 10^{-4} M DZAdo in a plastic cuvette for a spectrophotometer. Ferricytochrome *c* (100 μM) was added to the cell mixture. 5 min later, WGA (40 μg/ml), IC (20 μg antibodies/ml), fMLP (10^{-6} M) or PMA (10^{-9} M) was added to the mixture and the rate of superoxide dismutase-inhibitable reduction of ferricytochrome *c* was measured continuously by recording increases at 550–540 nm (molar absorption coefficient = 19×10^3), using a dual-beam spectrophotometer [12]. Cyt-E (5 μg/ml) was added to WGA, IC or fMLP to augment the O_2^- generation stimulated by each agent. To prevent sedimentation of the cells, a cell mixture was attached to the cuvette.

3. RESULTS

3.1. *Inhibition of O_2^- generation of guinea pig macrophages and PMNs by DZAdo and abolishment of the inhibitory effects by pretreatment with PMA*

We studied the O_2^- generation of DZAdo-treated or untreated macrophages of guinea pig after the addition of WGA, IC or fMLP (fig.1A). When the untreated cells (controls) were stimulated with either WGA, IC or fMLP, O_2^- was generated extensively, and then considerably suppressed by treatment of the cells with DZAdo.

In contrast, when the macrophages were pretreated with a small amount of PMA (2.5×10^{-10} M) for only 2 min and then stimulated with WGA, IC or fMLP, the DZAdo-treated cells recovered from the DZAdo-induced inhibition of O_2^- generation to the level seen in the untreated controls (fig.1B). Similar results were obtained for the O_2^- generation of guinea pig PMNs. Table 1 is a summary of effects of DZAdo on the O_2^- generation of guinea pig PMNs induced by WGA, IC or fMLP and the effects of pretreatment of the cells

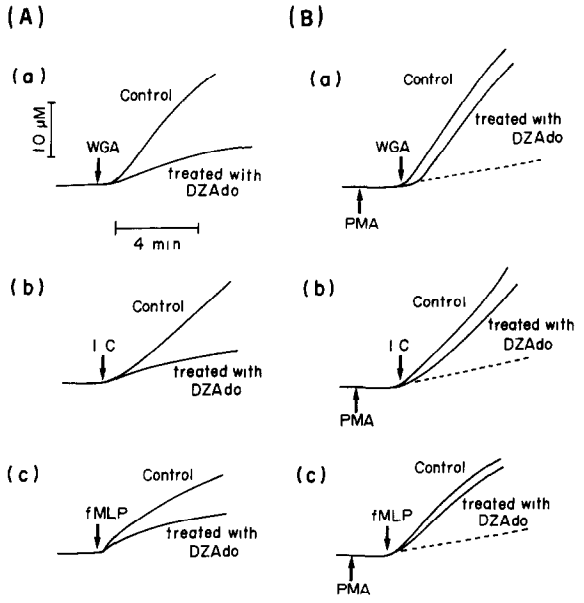

Fig.1. O_2^- generation of untreated guinea pig macrophages induced by WGA, IC, or fMLP and the effect of PMA pretreatment on O_2^- generation of the cells. (A) Guinea pig macrophages were pretreated with DZAdo (10^{-4} M) or medium only (control) for 10 min, following which the O_2^- generation of the cells stimulated by WGA (a), IC (b) or fMLP (c) was assayed. (B) Guinea pig macrophages were pretreated with DZAdo or medium only (control) for 10 min. The cells were then pre-exposed to PMA (2.5×10^{-10} M) for 2 min before the challenge with each stimulus for O_2^- generation. (a–c) Addition of WGA, IC and fMLP, respectively. The broken lines indicate the O_2^- generation of the cells stimulated by PMA alone (2.5×10^{-10} M).

Table 1

O_2^- generation of guinea pig PMN (nmol/min per 5×10^5)

Pre-exposure to PMA[a]	Stimuli for O_2^- generation	Control	DZAdo treated[b]	% inhibition of O_2^- generation
(−)	WGA (40 μg/ml)	4.6 ± 1.1	1.3 ± 0.4	71.7
	IC (20 μg Abs/ml)	3.6 ± 0.9	1.6 ± 0.4	55.6
	fMLP (10^{-6} M)	3.4 ± 0.5	1.7 ± 0.6	50.6
	PMA (10^{-8} M)	8.9 ± 1.4	8.0 ± 0.9	10.1
	PMA (10^{-9} M)	3.1 ± 0.4	2.9 ± 0.2	6.5
PMA (2.5×10^{-10} M)	WGA (40 μg/ml)	5.7 ± 0.9	5.5 ± 0.4	3.5
	IC (20 μg Abs/ml)	5.5 ± 1.2	4.7 ± 0.6	14.5
	fMLP (10^{-6} M)	4.6 ± 0.6	3.9 ± 0.4	15.2
	(−)PMA	1.1 ± 0.2	0.9 ± 0.3	

[a] The cells were pre-exposed to PMA (2.5×10^{-10} M) for 2 min before challenges with each stimulus for O_2^- generation

[b] Guinea pig PMN (5×10^5 cells/ml) were pretreated with DZAdo (10^{-4} M) for 10 min. The control shows the experiments without DZAdo. Data represent mean ± SD (n = 3)

with a small amount of PMA. The O_2^- generation by guinea pig PMNs was suppressed considerably in the presence of DZAdo when the cells were stimulated with either WGA, IC or fMLP. However, generation of O_2^- was greatly recovered when the DZAdo-treated cells were pretreated with a small amount of PMA (2.5×10^{-10} M) and stimulated with WGA, IC or fMLP.

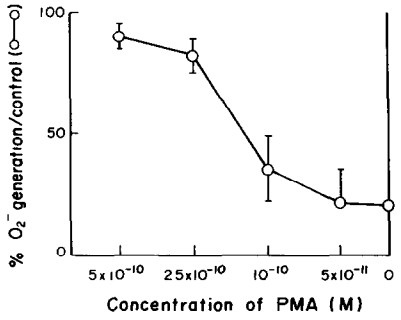

Fig.2. The dose-dependent effect of PMA on abolishment of the inhibitory effect of DZAdo on O_2^- generation. Guinea pig macrophages were pretreated with DZAdo (10^{-4} M) for 10 min, then the cells were pre-exposed to various doses of PMA for 2 min before the challenge with WGA for O_2^- generation. The control shows the O_2^- generation of untreated cells. The values were expressed by [(O_2^- generation of DZAdo-treated cells)/(O_2^- generation of untreated cells)] × 100 (= %).

3.2. *The dose-dependent effect of PMA on abolishment of inhibitory effects of DZAdo on* O_2^- generation of macrophages

Fig.2 shows the dose-dependent effect of PMA on the abolishment of the inhibitory effect of DZAdo on the O_2^- generation stimulated by WGA. Inhibitory effects of DZAdo on the O_2^- generation induced by WGA were abolished in accord with increases with concentration of PMA. The inhibition of O_2^- generation by DZAdo decreased to less than 20% when the DZAdo-treated cells were pre-exposed to 2.5×10^{-10} M PMA. At this concentration, PMA itself led to O_2^- generation, albeit the production being scanty. However, at the lower concentrations of PMA (less than 2.5×10^{-10} M), PMA failed to induce the O_2^- generation (not shown) and also to recover O_2^- generation of DZAdo-treated cells.

4. DISCUSSION

The present results plus data obtained previously show that the generation of O_2^- in guinea pig macrophages and PMNs induced by WGA, IC or fMLP is suppressed when the cells are treated with DZAdo. Since DZAdo is an inhibitor of the transmethylation reaction of the phospholipids in the plasma membrane [12,13], DZAdo may possibly exert inhibitory effects on the WGA-, IC-

or fMLP-induced O_2^- generation through inhibition of transmethylation of plasma membrane phospholipids of the cells [8,13]. However, here we found that the inhibitory effects of DZAdo on the O_2^- generation of the phagocytic cells induced by WGA, IC or fMLP were abolished by pretreatment of the cells with a small amount of PMA (2.5×10^{-10} M: a minimal effective concentration to activate protein kinase C in terms of O_2^- generation of the cells by PMA itself). From these results, the process of signal transduction from cell receptors to NADPH oxidase, which is responsible for the generation of O_2^- in the plasma membrane of the phagocytic cells, can be deduced. Protein kinase C is a crucial enzyme for transduction to final targets of the signals caused by binding of stimuli to the receptors in various cells [9]. This enzyme is activated by diacylglycerol, a product of phosphatidylinositol metabolism in the plasma membrane, or directly by PMA and functions with Ca^{2+} synergistically [14,15]. According to Nishizuka [9], PMA at low concentrations induces the activation of protein kinase C alone and the cells are thus made ready to function once Ca^{2+} becomes available.

It has also been shown that protein kinase C plays an important role in the activation of NADPH oxidase and generation of O_2^- in phagocytic cells [5–7]. Changes in the plasma membrane of phagocytic cells caused by binding of stimuli such as WGA, IC or fMLP may lead to activation of protein kinase C and increased mobilization of Ca^{2+} accompanied by the activation of NADPH oxidase to produce O_2^-. Therefore, it is conceivable that the signals evoked by the binding of the stimuli to the plasma membrane of phagocytic cells are propagated to NADPH oxidase in the presence of PMA, even if the supply of diacylglycerol is inhibited. As shown in fig.1A, DZAdo inhibited O_2^- generation in guinea pig macrophages and PMNs induced by WGA, IC or fMLP. This finding indicates that DZAdo selectively inhibits the production of diacylglycerol without affecting the mobilization of Ca^{2+}, resulting in decreased signal transduction from receptors to protein kinase C and NADPH oxidase. The results in fig.1B and table 1 support this view, because the addition of a low concentration of PMA, which in itself activates protein kinase C, abolished the inhibitory effects of DZAdo on O_2^- generation of phagocytic cells.

Mori et al. [16] showed that phospholipid-interacting drugs such as chlorpromazine, dibucaine, imipramine and tetracaine inhibit the action of protein kinase C in the plasma membrane without affecting the mobilization of Ca^{2+}. Thus, the inhibitory effects of DZAdo may be similar to these drugs. The action of DZAdo on the metabolism of phosphatidylinositol in phagocytic cells remains to be clarified.

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