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Effects of Adenosine and N^6-cyclopentyladenosine on Superoxide Production, Degranulation and Calcium Mobilization in Activated Neutrophils*

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ABSTRACT

The effects of adenosine and N^6-cyclopentyladenosine (CPA) on superoxide production, myeloperoxidase release and Ca^{2+} mobilization stimulated by fMLP in neutrophils were investigated. The effects were also observed on the stimulatory actions of C5a and PMA and the responses in lipopolysaccharide-primed neutrophils. In addition, the involvement of cAMP in the inhibitory action of adenosine was examined.

The fMLP-stimulated neutrophil respiratory burst, degranulation and intracellular Ca^{2+} mobilization may be regulated by activation of adenosine receptors. Adenosine may not affect the stimulated neutrophil responses due to activation of protein kinase C. fMLP-stimulated respiratory burst in lipopolysaccharide-primed neutrophils may be less sensitive to adenosine, compared with nonprimed cells. The inhibitory effect of theophylline in the presence of adenosine on neutrophil responses appears to be ascribed to accumulation of intracellular cAMP.

Key Words: Adenosine, N^6-Cyclopentyladenosine, Theophylline, Neutrophil responses

INTRODUCTION

Adenosine, an endogenous substance present in plasma and extracellular fluids, modulates cellular functions by interacting with cell surface receptors (Olsson and Pearson, 1990). Adenosine has been shown to inhibit neutrophil response to chemoattractants, including adherence to endothelium, phagocytosis and superoxide production (Cronstein, 1994). However, adenosine does not inhibit phorbol myristate acetate (PMA)-induced adherence and NaF-or A_{23187}-stimulated superoxide production (Burkey and Webster, 1993). Thus, the inhibitory mechanism of adenosine on the respiratory burst is still not clearly defined. Inhibitory or no effect of adenosine on degranulation and aggregation of neutrophils has been reported (McGarrity *et al.*, 1989; Walker *et al.*, 1989). Adenosine and adenosine analogues have little or no effect on degranulation in cytochalasin B-treated neutrophils. In contrast, in cytochalasin B-nontreated neutrophils the effective inhibitory effects of adenosine and 2-chloroadenosine on N-formyl-methionyl-leucyl-phenylalanine (fMLP)- induced degranulation are investigated (Richter, 1992). In addition, in the same assay condition tumor necrosis factor (TNF)-induced degranulation is less sensitive to them, compared with fMLP. Accordingly, effect of adenosine on the secre-

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tion of lysosomal enzymes from activated neutrophils also has not been elucidated.

Elevation of intracellular cyclic AMP (cAMP) in neutrophils is considered to inhibit neutrophil functions, including chemotaxis (Stephens and Snyderman, 1982), respiratory burst (Nielson, 1987) and lysosomal enzyme release (Lad *et al.*, 1985). In cultured neural cells, A_1 receptor occupancy diminishes accumulation of cAMP in response to β-adrenergic agents, presumably via activation of inhibitory G (Gi) signal transduction proteins (Van Calker *et al.*, 1979; Ramkumar and Stiles, 1988), whereas occupancy of A_2 receptor appears to stimulate cAMP accumulation through activation of stimulatory G protein (Gs) (Cronstein *et al.*, 1988; Laghi Pasini *et al.*, 1990). However, involvement of cAMP in the inhibition of superoxide production by adenosine is uncertain. The inhibitory action of dibutyryl cAMP on superoxide production is completeley reversed by inhibitors of the cAMP-dependent kinase (protein kinase A) (Cronstein *et al.*, 1992). On the contrary, the effect of adenosine A_2 receptor occupancy on superoxide production is not affected by protein kinase A inhibitors. Meanwhile, other neutrophil functions may be affected by change of intracellular cAMP concentrations (Cronstein, 1994).

An increase of cytosolic Ca^{2+} appears to be involved in superoxide production and degranulation (Smolen *et al.*, 1981; Painter *et al.*, 1984). Several experiments indicate that adenosine may inhibit superoxide production by inhibiting Ca^{2+} influx or mobilization (Laghi Pasini *et al.*, 1990; Tsuruta *et al.*, 1993). However, it is reported that in Ca^{2+} free media, adenosine inhibits superoxide production by stimulating agents (Cronstein *et al.*, 1988). Thus, role of Ca^{2+} in the inhibitory action of adenosine on neutrophil responses has not been clarified.

This study was done to investigate the effects of adenosine and N^6-cyclopentyladenosine (CPA) on superoxide production, myeloperoxidase release, intracellular Ca^{2+} level and adherence stimulated by fMLP in neutrophils. The effects were also observed on the stimulatory actions of C5a and PMA and the responses in lipopolysaccharide-primed neutrophils. In addition, the involvement of cAMP in the inhibitory action of adenosine was examined.

MATERIALS AND METHODS

N-Formylmethionylleucylphenylalanine (fMLP), C5a, phorbol 12-myristate 13-acetate (PMA), adenosine, N^6-cyclopentyladenosine (CPA), theophylline, dibutyryl cyclic AMP, histamine, staurosporine, genistein, cytochalasin B, lipopolysaccharide (from Escherichia coli), ferricytochrome c, o-dianisidine and fura-2/AM were purchased from Sigma Chemical Co.. Other chemicals were of analytical grade.

Preparation of human neutrophils

Neutrophils were prepared from fresh whole human blood, anticoagulated with 10% acid-citrate-dextrose, by dextran sedimentation, hypotonic lysis of erythrocytes and Ficoll-Hypague density centrifugation (Markert *et al.*, 1984). The neutrophils were suspended in Dulbecco's phosphate-buffered saline (PBS), pH 7.4 at a concentration of 1×10^7/ml. Final suspensions of neutrophils were comprised of about 97% neutrophils as judged by Wright-Giemsa stain, and viability was more than 98% as judged by trypan blue dye exclusion.

Cytochalasin B treatment: After neutrophils were pretreated with cytochalasin B (5 μg/ml for 10^7 cells) for 5 min, the assay for the respiratory burst and degranulation was done.

Lipopolysaccharide priming: Neutrophils (10^7 cells/ml) were incubated with 1 μg/ml lipopolysaccharide for 30 min at 37℃ (Guthrie *et al.*, 1984).

Assay of superoxide production

The superoxide dependent reduction of ferricytochrome c was measured by the method of Markert *et al.* (1984). The reaction mixtures in plastic microfuge tubes contained 2×10^6 neutrophils, 75 μM ferricytochrome c, stimulating agent, 20 mM HEPES-tris and Hanks' balanced salt solution (HBSS), pH 7.4 in a total volume of 1.0 ml. The reactions were performed in a 37℃ shaking water bath for 15 min. The reaction was then stopped by placing the tubes in

melting ice, and the cells were rapidly pelleted by centrifuging at 1,500 g for 5 min at 4℃. The supernatants were taken, and the amount of reduced cytochrome c was calculated by using an extinction coefficient of 2.1×10^4 M^{-1} cm^{-1} at 550 nm (Cohen and Chovaniec, 1978).

Assay of myeloperoxidase release

A 5×10^6/ml neutrophils in HBSS buffer with or without inhibitors were stimulated by adding fMLP (or PMA). After 15 min of incubation, 250 μl of 0.2 M phosphate buffer, pH 6.2 and 250 μl of an equal mixture of 3.9 mM o-dianisidine HCl and 15 mM H_2O_2 were added. After 10 min of reincubation, the reaction was stopped by the addition of 250 μl of 1% sodium azide. The absorbance was read at 450 nm (Spangrude *et al.*, 1985).

Assay of cytosolic free calcium

Fura-2 loading and fluorescence measurement were performed by the method of Luscinskas *et al.* (1990). Neutrophils (approximately 5×10^7 cells/ml) were loaded with 2 mM fura-2/AM to 1 μM/10^7 cells at 37℃ for 10 min in the reaction mixtures contained HBSS buffer without calcium and magnesium (HBSS-CMF) and 20 mM HEPES-tris, pH 7.4. The suspension was then diluted 5 fold with 0.5% bovine serum albumin containing HBSS-CMF and further incubated at 37℃ for 15 min. After loading, the suspension was centrifuged at 200 g for 10 min, and neutrophils were resuspended in 0.1% bovine serum albumin containing HBSS-CMF. This procedure was performed twice. Neutrophils were finally suspended in bovine serum albumin-free, HBSS-CMF as approximately 5×10^7 cells/ml. Fluorescence measurement was done with a Turner Spectrofluorometer (Model 430). Preloaded neutrophils (4×10^6) were suspended in 1.23 mM Ca^{2+} and 1 mM Mg^{2+} containing HBSS in a final volume of 1.0 ml. After preincubation at 37℃ for 5 min with compounds, the response was initiated by the addition of 1 μM fMLP. The fluorescence change was read at an excitation wavelength of 340 nm and emission wavelength of 505 nm.

Assay of Mn^{2+} influx

Influx of Mn^{2+} into cells was measured using the fura-2 fluorescence quenching technique (Demaurex *et al.*, 1992). Fura-2 loaded neutrophils (4×10^6/ml) were suspended in Ca^{2+}-and Mg^{2+}-containing HBSS media. After 90 sec of stimulation with fMLP, Mn^{2+} (0.5 mM) was added, and quenching of fura-2 fluorescence by Mn^{2+} influx was measured at an excitation wavelength of 360 nm and emission wavelength of 505 nm.

RESULTS

Effects of adenosine and CPA on superoxide production in activated neutrophils

The effects of adenosine and N^6-cyclopentyladenosine (CPA) on neutrophil respiratory burst stimulated by fMLP, complement C5a and

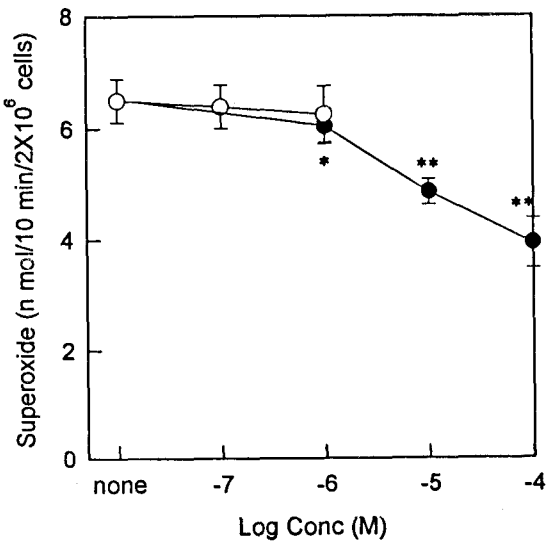

Fig. 1. Effects of adenosine and CPA on superoxide production in neutrophils activated by fMLP. Neutrophils(2×10^6 cells/ml) were stimulated with 1 μM fMLP in the presence of adenosine or CPA. Values are means±S.D., n=4. ●, adenosine; ○, CPA. **$p<0.01$,* $p<0.05$ by Student's *t*-test

PMA were investigated. In cytochalasin B treated neutrophils, 1 μM fMLP and 20 nM C5a produced 6.50±0.39 (n=4) and 10.40±0.52 (n=5) n mol/10 min/2×10^6 cells of superoxide anion, respectively. Superoxide production in 1 μM fMLP -or 20 nM C5a- activated neutrophils was inhibited by adenosine in a dose dependent fashion, and at 100 μM adenosine 40~45% of inhibition was observed (Fig. 1 and 2). The specific A_1 agonist CPA did not affect the stimulatory effects of fMLP and C5a up to 1 μM. Effect of adenosine on stimulation of the respiratory burst by receptor-independent agonist was studied. As shown in Fig. 3, superoxide production was stimulated by PMA (0.1 μg/ml), a direct activator of protein kinase C, and 47.88±1.35 n mol/10 min/2×10^6 cells of superoxide anion (n=4) was produced. PMA-induced superoxide production was not affected by adenosine and CPA.

Role of cAMP in the inhibitory action of adenosine on the respiratory burst was examined. Theophylline (10 μM), which is known to antagonize adenosine action (Cronstein *et al.*, 1992) and to inhibit cyclic nucleotide phosphodiesterase (Wright *et al.*, 1990), did not exert an antagonizing action on the inhibitory effect of adenosine on superoxide production in fMLP-activated neutrophils and rather further inhibited it (Fig. 4). An elevation of intracellular cAMP is considered to lead to subsequent inhibition of neutrophil function. Influence of cAMP elevating agents on the respiratory burst was compared with adenosine. Fig. 5 shows that 1 mM dibutyryl cAMP and 100 μM histamine inhibited superoxide production by fMLP by 44% and 32%, respectively, whereas neither dibutyryl cAMP nor histamine inhibited the stimulatory effect of PMA. Thus, the inhibitory pattern of dibutyryl cAMP and histamine on superoxide production was similar to that of adenosine.

In lipopolysaccharide-primed neutrophils, effect of adenosine on the respiratory burst was investigated. In primed neutrophils, 1 μM fMLP produced 11.81±0.78 n mol/10 min/2×10^6 cells

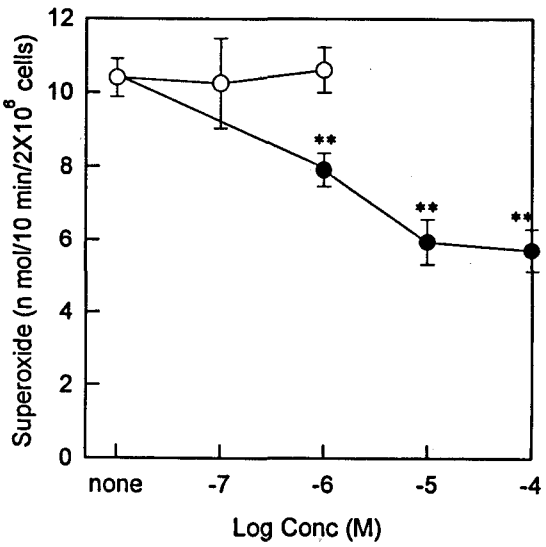

Fig. 2. Effects of adenosine and CPA on C5a-induced superoxide production. Neutrophils were stimulated with 20 nM C5a in the presence of adenosine agonists. Values are means±S.D., n=5. ●, adenosine; ○, CPA. ** $p<0.01$ by Student's *t*-test.

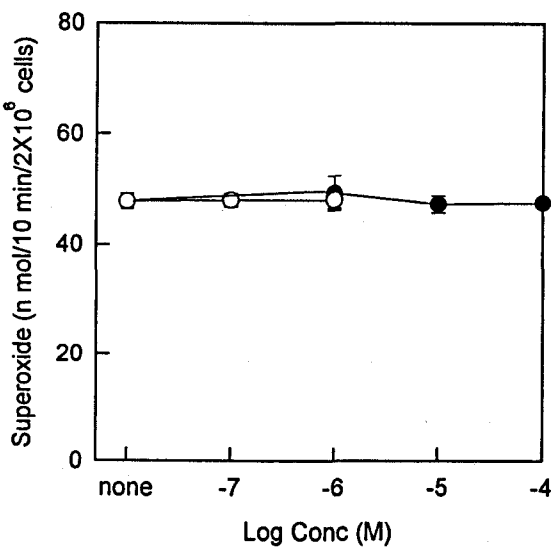

Fig. 3. Effects of adenosine and CPA on PMA-induced superoxide production. Neutrophils were stimulated with 0.1 μg/ml PMA in the presence of adenosine agonists. Values are means±S.D., n=4. ●, adenosine; ○, CPA.

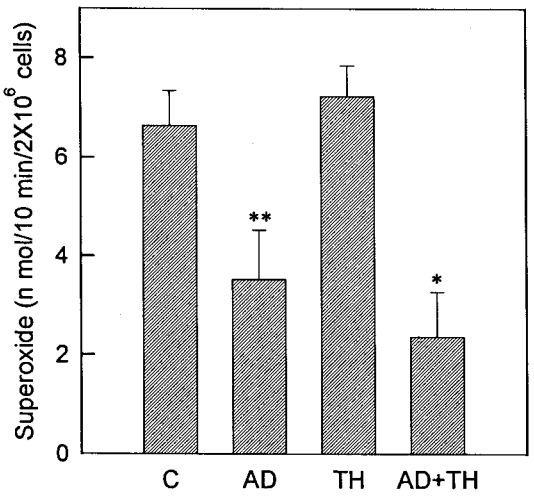

Fig. 4. Effect of theophylline on inhibition of fMLP-induced superoxide production by adenosine. Neutrophils were stimulated with 1 μM fMLP in the presence of 100 μM adenosine and 10 μM theophyllines. Values are means $\pm$ S.D., n=4. C, no addition; AD, adenosine; TH, theophylline; AD+TH, adenosine + theophylline. **p $<$ 0.01, *p $<$ 0.05 by Student's *t*-test.

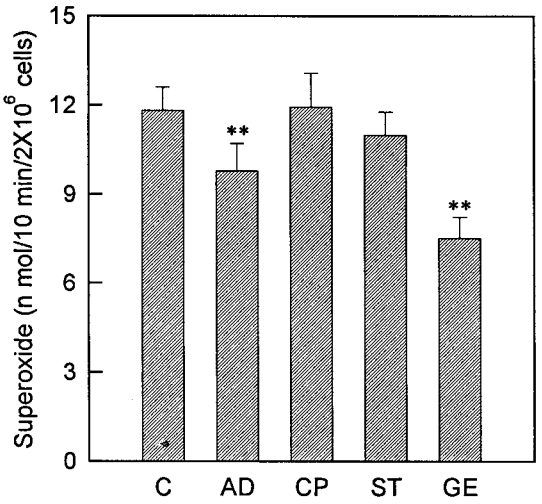

Fig. 6. Effects of adenosine agonists on fMLP-stimulated superoxide production in lipopolysaccharide-primed neutrophils. Primed neutrophils were incubated with 1 μM fMLP (C) and other agents. Values are means $\pm$ S.D., n=5-9. AD, 100 μM adenosine; CP, 0.1 μM CPA; ST, 100 nM staurosporine; GE, 10 μM genistein.** p $<$ 0.01 by Student's *t*-test.

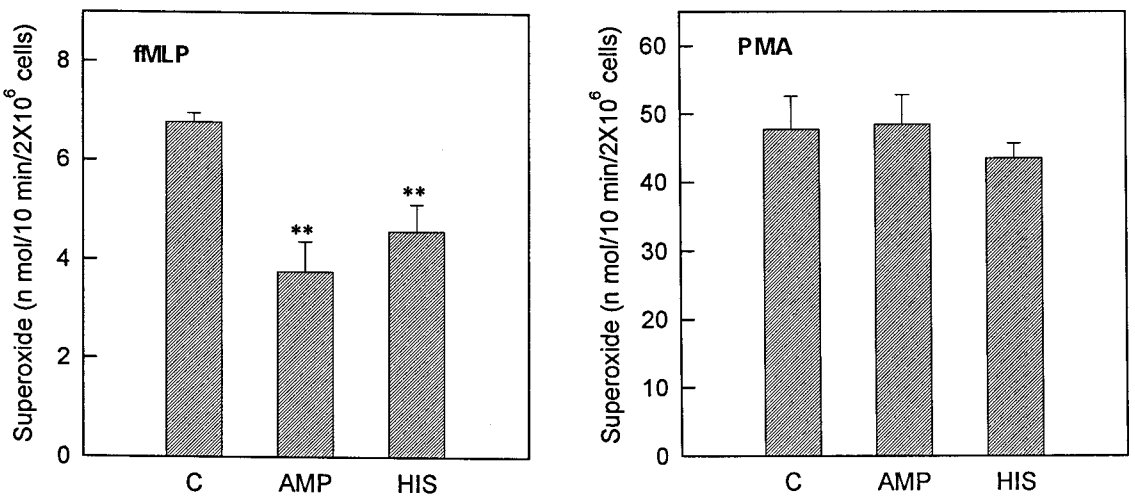

Fig. 5. Effects of cAMP elevating agents on stimulated superoxide production by fMLP and PMA. Neutrophils were incubated with 1 μM fMLP (or 0.1 μg/ml PMA) and cAMP elevating agents. Values are means $\pm$ S.D., n=4. C, no addition; AMP, 1 mM dibutyryl cAMP; HIS, 100 μM histamine.
** p $<$ 0.01 by Student's *t*-test.

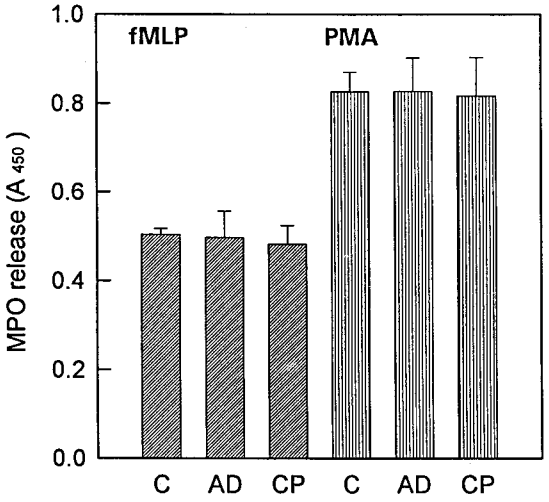

Fig. 7. Effects of adenosine agonists on myeloperoxidase release from activated neutrophils. After 5 min of preincubation with adenosine agonists, neutrophils were stimulated with 1 μM fMLP or 0.1 μg/ml PMA. Values are expressed as absorbance change and are means±S.D., n=4. C, no addition; AD, 100 μM adenosine; CP, 0.1 μM CPA.

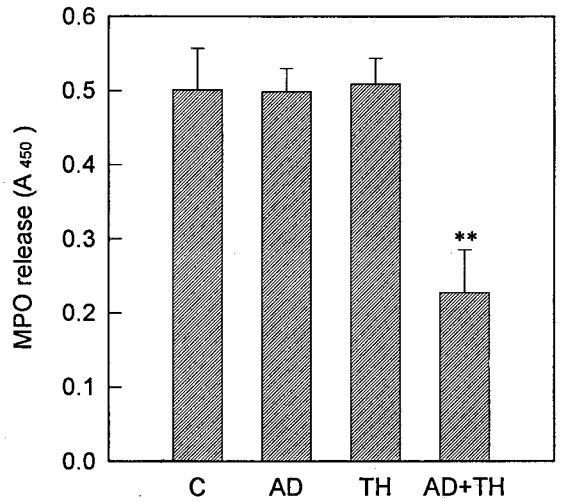

Fig. 8. Inhibitory effect of theophylline in the presence of adenosine on myeloperoxidase release. Neutrophils were incubated with 1 μM fMLP, 100 μM adenosine and 10 μM theophylline. Values are expressed as absorbance change are means±S.D., n=4. C, no addition; AD, adenosine; TH, theophylline, AD+TH, adenosine+theophylline. **$p<0.01$ by Student's t-test.

of superoxide anion (n=9). A 100 μM adenosine and 10 μM protein tyrosine kinase inhibitor, genistein inhibited fMLP-induced superoxide production (Fig. 6). The designated concentration of adenosine showed a 17% of inhibition. The inhibitory effect of adenosine on superoxide production in lipopolysaccharide-primed neutrophils was apparently smaller than that in nonprimed neutrophils. In these cells, 0.1 μM CPA and 100 nM protein kinase C inhibitor, stauosporine did not have inhibitory effect on it.

Effects of adenosine and CPA on myeloperoxidase release

The secretion of lysosomal enzymes was assayed by measuring the release of myeloperoxidase. Fig. 7 shows that in cytochalasin B treated neutrophils, release of myeloperoxidase in response to 1 μM fMLP (or 0.1 μg/ml PMA) was not inhibited by both 100 μM adenosine and 0.1 μM CPA.

Influence of cAMP on lysosomal enzyme release was examined. Theophylline (10 μM) itself did not affect fMLP- induced myeloperoxidase release. However, as shown in Fig. 8, in the presence of 100 μM adenosine and 10 μM theophylline, fMLP-induced myeloperoxidase release was inhibited by 55%.

Effects of intracelluar cAMP elevating agents on lysosomal enzyme release was studied. Release of myeloperoxidase in response to 1 μM fMLP was inhibited by 1 mM dibutyryl cAMP and 100 μM histamine by 68% and 21%, respectively (Fig. 9). In contrast to fMLP, dibutyryl cAMP and histamine at the same concentration did not affect myeloperoxidase release by PMA.

In lipopolysaccharide-primed neutrophils, fMLP-induced myeloperoxidase release was not inhibited by 100 μM adenosine, 0.1 μM CPA and 100 nM staurosporine but was inhibited by 10

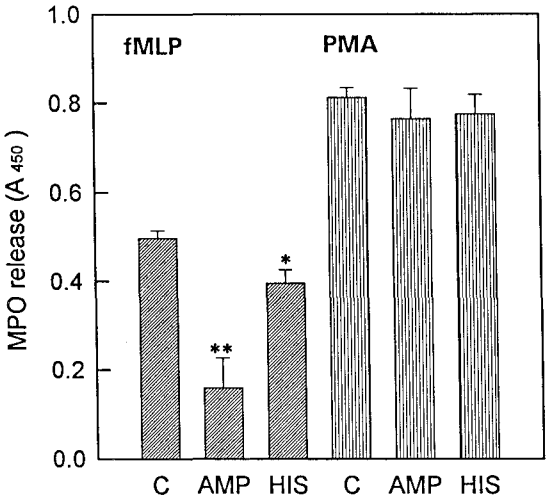

Fig. 9. Effects of cAMP elevating agents on myeloperoxidase release. Neutrophils were stimulated with 1 μM fMLP(or 0.1 μg/ml PMA) in the presence of 1 mM dibutyryl cAMP and 100 μM histamine. Values are expressed as absorbance change and are means±S.D., n=4. C, no addition; AMP, dibutyryl cAMP; HIS, histamine. $^{**}p<0.01$, $^{*}p<0.05$ by Student's *t*-test.

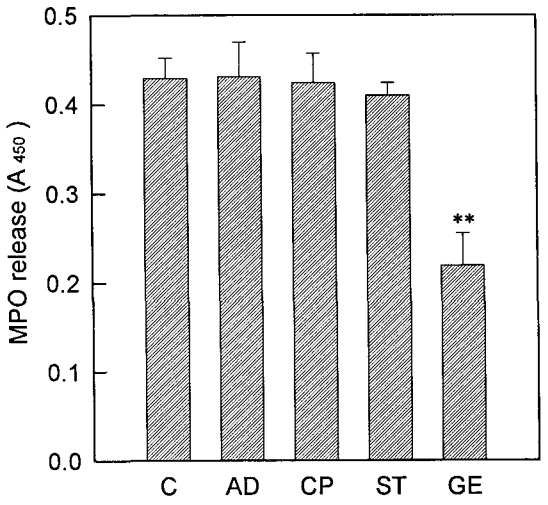

Fig. 10. Effects of adenosine agonists on myeloperoxidase release from lipopolysaccharide-primed neutrophils. Primed neutrophils were incubated with 1 μM fMLP (C) and other agents. Values are expressed as absorbance change and are means±S.D., n=3. AD, 100 μM adenosine; CP, 0.1 μM CPA; ST, 100 nM staurosporine; GE, 10 μM genistein. $^{**}p<0.01$ by Student *st*-test.

μM genistein (Fig. 10).

Effects of adenosine and CPA on change in intracellular calcium

One μM fMLP elicited an increase of intracellular Ca^{2+} level ($[Ca^{2+}]_i$) in neutrophils. The $[Ca^{2+}]_i$ rose to a maximum within 30 sec post addition, and then the $[Ca^{2+}]_i$ was gradually decreased to the resting level over the subsequent several minutes (Fig. 11). Influence of adenosine on fMLP-induced Ca^{2+} mobilization was investigated. Fig. 11 shows that the initial peak of $[Ca^{2+}]_i$ in response to fMLP was slightly decreased by 10 μM adenosine and 1 mM dibutyryl cAMP. However, adenosine and dibutyryl cAMP inhibited the sustained elevation of $[Ca^{2+}]_i$ after the stimulation. fMLP-induced elevation of $[Ca^{2+}]_i$ was not affected by 0.1 μM CPA.

The rise in $[Ca^{2+}]_i$ is attained by both re-

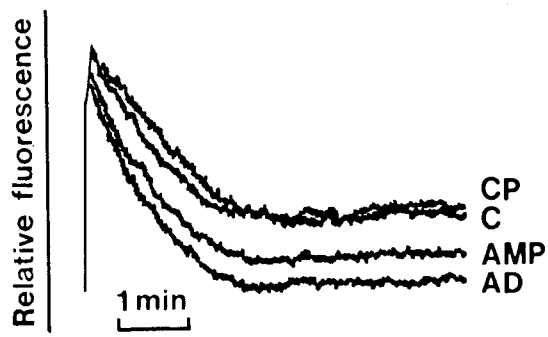

Fig. 11. Effects of adenosine agonists on fMLP-induced elevation of $[Ca^{2+}]_i$. Fura-2 loaded neutrophils (4×10^6 cells/ml) were preincubated with 10 μM adenosine (AD), 0.1 μM CPA (CP) and 1 mM dibutyryl cAMP (AMP) or not (C) for 5 min, and then the response was initiated by 1 μM fMLP. The traces are representative of three experiments.

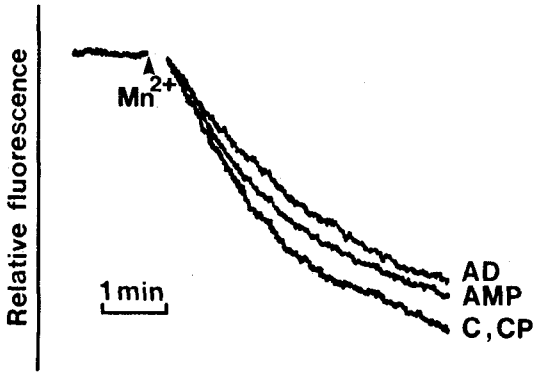

Fig. 12. Effects of adenosine agonists on Mn^{2+} influx induced by fMLP. Mn^{2+} influx into the cytoplasm of neutrophils were initiated by adding 0.5 mM Mn^{2+} after 90 sec of stimulation with 1 μM fMLP. Fura-2 loaded neutrophils were preincubated with 10 μM adenosine(AD), 0.1 μM CPA (CP) and 1 mM dibutyryl cAMP (AMP) or not (C). The traces are representative of three experiments.

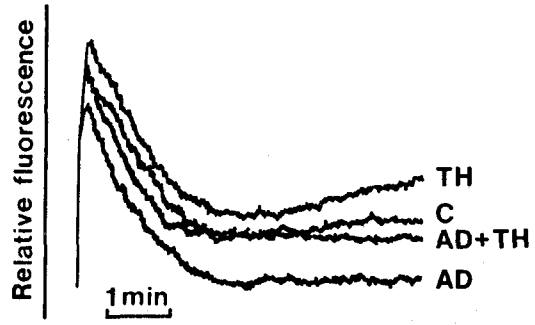

Fig. 13. Inhibitory effect of theophylline on the effect of adenosine on fMLP-induced elevation of $[Ca^{2+}]_i$. Fura-2 loaded neutrophils were stimulated with 1 μM fMLP (C) in the presence of 10 μM adenosine (AD) and 10 μM theophylline (TH). The traces are representative of three experiments.

lease of Ca^{2+} from intracellular stores and Ca^{2+} influx across the plasma membrane (Pozzan *et al.*, 1983; Westwick and Poll, 1986). The sustained elevation of $[Ca^{2+}]_i$ following the initial peak is thought to be regulated by Ca^{2+} influx across the plasma membrane. Mn^{2+} has been shown to permeate through Ca^{2+} influx pathway in neutrophils activated by chemoattractants (Demaurex *et al.*, 1992; Jaconi *et al.*, 1993). fMLP-stimulated Mn^{2+} entry into neutrophils was inhibited by 10 μM adenosine and 1 mM dibutyryl cAMP, whereas the effect of 0.1 μM CPA was not detected (Fig. 12).

In Fig. 13, the antagonistic effect of theophylline on the adenosine action in intracellular Ca^{2+} mobilization was observed. A 10 μM theophylline itself did not affect elevation of $[Ca^{2+}]_i$ induced by fMLP. However, theophylline antagonized the inhibitory action of adenosine on elevation of $[Ca^{2+}]_i$ by fMLP.

DISCUSSION

Endogenous adenosine has been shown to modulate the inflammatory response. Adenosine affects cellular function by binding to adenosine receptors, A_1 and A_2, on the plasma membrane (Stiles, 1992; Cronstein, 1994). Activation of adenosine A_1 receptor augments stimulated neutrophil response, chemotaxis and phagocytosis, mediated by agonists. However, its effect on superoxide production is uncertain (Rose *et al.*, 1988; Salmon and Cronstein, 1990). Occupancy of adenosine A_2 receptor inhibits superoxide production by neutrophil in response to chemoattractant but does not affect chemotaxis. On the other hand, adenosine does not inhibit stimulation of neutrophil responses by receptor-independent agonists (Burkey and Webster, 1993). Thus, the modulating actions of adenosine on neutrophil responses to stimulating agents are complex and unclear. In addition, the action mechanism of adenosine has still not been elucidated.

The neutrophil superoxide production in response to fMLP and C5a was suppressed by adenosine but was not affected by specific A_1 agonist, CPA. While the effects of adenosine

and CPA on superoxide production by PMA were not detected. The inhibitory action of adenosine on stimulation of the respiratory burst by the activation of cell surface receptors appears to be mediated by occupancy of adenosine A_2 receptor but not by A_1 receptor. It seems unlikely that adenosine affects the protein kinase C-dependent activation process. Influence of adenosine on stimulated neutrophil degranulation is not clarified. Adenosine and adenosine analogues have been reported to have little or no effect on stimulated degranulation (McGarrity *et al.*, 1989; Walker *et al.*, 1989). However, it is found that they effectively inhibit lysosomal enzyme release by fMLP in the absence of cytochalasin B (Richter, 1992). The effect of adenosine on neutrophil responses is considered to be abrogated by cytochalasin B (de la Harpe and Nathan, 1989). In cytochalasin B-nontreated neutrophils, stimulating effect of fMLP on cellular responses is weak. Thus, it would be predicted that the interpretation on the certain effect of compounds may be somewhat difficult. In cytochalasin B-treated neutrophils, adenosine and CPA did not affect myeloperoxidase release by fMLP and PMA. The present data partly support that the activation process of degranulation may be different from the respiratory burst.

Adenosine receptors on neutrophils mediate the intracellular accumulation of cAMP (Cronstein *et al.*, 1988; Richter, 1992). Occupancy of adenosine receptors does not cause a detectable change in neutrophil cAMP content, whereas in the presence of phosphodiesterase inhibitor it markedly provokes accumulation of intracellular cAMP (Cronstein *et al.*, 1988). However, since the inhibitory effect of the specific A_2 agonist, 5' N-ethylcarboxamidoadenosine (NECA), on fMLP-stimulated superoxide production are not inhibited by inhibitor of the cAMP-dependent protein kinase (Cronstein *et al.*, 1992), role of cAMP as an intracellular messenger for inhibition of superoxide production by adenosine is uncertain. In the present study theophylline, known as adenosine antagonist and cyclic nucleotide phosphodiesterase inhibitor, in the presence of adenosine significantly inhibited neutrophil superoxide production and myeloperoxidase release by fMLP, while theophylline alone did not show any significant effect on the responses. Above views and this finding indicate that an increased intracellular cAMP appears to be involved in the inhibitory effect of adenosine and theophylline on neutrophil responses. The increased intracellular cAMP level in neutrophils is associated with a decreased neutrophil responses, including superoxide production and degranulation (Wright *et al.*, 1990; Tyagi *et al.*, 1991). This finding was also investigated in this study. In addition, the effects of cAMP elevating agents, dibutyryl cAMP and histamine, on the stimulated superoxide production and myeloperoxidase release by fMLP and PMA were similar to that of adenosine. The result probably suggest role of cAMP in inhibition of neutrophil responses caused by adenosine.

After exposure to low concentrations of lipopolysaccharide and platelet-activating factor (PAF), neutrophils become primed to promote greatly the stimulated superoxide production by chemoattractants (Guthrie *et al.*, 1984; Ingraham *et al.*, 1982). Adenosine is reported to inhibit PAF-mediated priming of neutrophils (Stewart and Harris, 1993). However, effect of adenosine on the responses in lipopolysaccharide-primed neutrophils has not been elucidated. In lipopolysaccharide-primed neutrophils, adenosine inhibited fMLP-stimulated superoxide production, which is responsible for genistein, but it showed an apparently diminished effect compared with the effect in nonprimed cells. fMLP-stimulated respiratory burst in lipopolysaccharide-primed neutrophils may be less sensitive to the inhibitory action of adenosine. On the other hand, as in nonprimed neutrophils, adenosine and CPA did not exert a effect on stimulated myeloperoxidase release in lipopolysaccharide-primed cells.

Receptor activation by stimulating agents leads to the elevation of $[Ca^{2+}]_i$ in neutrophils (Goldstein *et al.*, 1975; Smolen *et al.*, 1981). The rise in intracellular Ca^{2+} is thought to play an important role in the stimulation of neutrophil responses. The rise in $[Ca^{2+}]_i$ is accomplished by both release of Ca^{2+} from intracellular stores and Ca^{2+} influx across the plasma membrane (Westwick and Poll, 1986). The release of Ca^{2+} is mediated by inositol 1,4,5-trisphosphate

(Berridge, 1993). On the contrary, the mechanism implicating receptor-mediated Ca^{2+} influx is uncertain (Jaconi *et al.*, 1993). Adenosine has been reported to have little inhibitory or no effect on the early increase in $[Ca^{2+}]_i$ and inhibit the sustained rise in $[Ca^{2+}]_i$ (Ward *et al.*, 1988; Thiel and Bardenheuer, 1992). Adenosine exerted little effect on the initial peak in $[Ca^{2+}]_i$ and inhibited the sustained rise in fMLP-stimulated neutrophils. The effect of dibutyryl cAMP on Ca^{2+} mobilization was similar to adenosine. The divalent cation Mn^{2+} has been shown to permeate through the neutrophil Ca^{2+} influx pathway activated by chemoattractants (Demaurex *et al.*, 1992; Jaconi *et al.*, 1993). The inhibition of Mn^{2+} influx by adenosine and dibutyryl cAMP may contribute their inhibitory actions on the sustained rise after stimulation. Meanwhile in this response the effect of CPA was not detected. The effect of adenosine on fMLP-induced elevation of $[Ca^{2+}]_i$ was inhibited by theophylline, while theophylline alone showed minimal enhancing or no effect on Ca^{2+} mobilization. The effect of theophylline in the presence of adenosine on Ca^{2+} mobilization does not coincide with its effect on superoxide production and myeloperoxidase release. In addition, the elevated intracellular cAMP is found to decrease a $[Ca^{2+}]_i$ in platelets (Roevens and De Chaffoy de Courcelles, 1993). Thus, reversing action of theophylline on adenosine effect on Ca^{2+} mobilization appears to be attributed to its antagonizing action on adenosine receptors but not a change of intracellular cAMP level.

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=국문초록=

Adenosine과 N^6-cyclopentyladenosine이 활성화된 중성호성 백혈구에서 Superoxide 생성, 탈과립과 칼슘동원에 나타내는 영향

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fMLP에 의하여 자극된 중성호성 백혈구에서의 superoxide 생성, myeloperoxidase 유리, 칼슘 동원과 백혈구 부착에 나타내는 adenosine과 N^6-cyclopentyladenosine의 효과를 관찰하였다. 또한 이들의 효과를 C5a와 PMA의 자극효과에 대하여 그리고 lipopolysaccharide-primed 중성호성 백혈구의 반응에 대하여 관찰하였다. 이와 함께 adenosine의 억제작용에 있어 cAMP의 관여 여부를 조사하였다.

연구 결과로 부터 fMLP에 의해 자극된 중성호성 백혈구에서의 superoxide 생성, 탈과립과 세포내 칼슘 동원과 백혈구 부착은 adenosine 수용체에 의하여 조절된다고 추정된다. Adenosine은 protein kinase C의 활성화에 따른 백혈구 반응의 자극에 영향을 나타내지 않을 것으로 시사된다. Nonprimed 세포에 비하여, lipopolysaccharide-primed 중성호성 백혈구에서 fMLP에 의한 superoxide 생성은 adenosine의 영향을 적게 받을 것으로 여겨진다. Adenosine 존재하에서 백혈구 반응에 나타내는 theophylline의 억제효과는 세포내 cAMP 축적에 기인할 것으로 추정된다.