Biochemical Pharmacology, Vol 31, No 5, pp. 775–780, 1982
Printed in Great Britain

0006-2952/82/050775-06 $03 00/0

EFFECT OF CYCLOOXYGENASE INHIBITORS AND PROTEASE INHIBITORS ON PHORBOL-INDUCED STIMULATION OF OXYGEN CONSUMPTION AND SUPEROXIDE PRODUCTION BY RAT PULMONARY MACROPHAGES*

MAUREANE HOFFMAN and ANNE P. AUTOR†
The Toxicology Center, Department of Pharmacology, The University of Iowa, Iowa City, IA 52242, U.S.A.

(*Received* 3 *April* 1981; *accepted* 6 *August* 1981)

Abstract—Oxygen consumption and superoxide anion production by pulmonary macrophages are both increased by phorbol myristate acetate (PMA), but the two processes have been separated using protease inhibitors and cyclooxygenase inhibitors. Pretreatment with the protease inhibitors (L-1-tosylamido-2-phenylethylchloromethyl ketone (TPCK) and *N*-α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK), as well as with the cyclooxygenase inhibitors acetylsalicylic acid (ASA) and ibuprofen (IBU), inhibited the stimulation of superoxide production and oxygen consumption by phorbol myristate acetate. However, whereas the order of potency for inhibition of stimulation of superoxide production was TPCK > TLCK > IBU > ASA, the order of potency for inhibition of stimulation of oxygen consumption was ASA > IBU > TPCK = TLCK. Although all four agents were effective inhibitors of PMA-stimulated superoxide production and oxygen consumption when added before PMA, in contrast to the cyclooxygenase inhibitors, TPCK was unable to inhibit oxygen consumption by more than 70–80% regardless of the concentration used, although superoxide generation could be inhibited completely. When added after PMA, ASA did not suppress either oxygen consumption or superoxide production and ibuprofen was only one-half as effective as an inhibitor. TPCK and TLCK, when added after PMA, accelerated the return to basal rates of both oxygen consumption and superoxide production. None of the four agents had any effect on basal superoxide production or oxygen consumption at the concentrations used. The data support the interpretation that both prostaglandin biosynthesis and protease activity may be associated with the activation of the superoxide-generating system of pulmonary macrophages. The consumption of molecular oxygen following stimulation of the cells with phorbol myristate acetate is not due solely to the generation of superoxide, however, since each process is inhibited with different potency by the same group of inhibitors. There appears to be a component of oxygen consumption which results from the activation of cyclooxygenase and, unlike superoxide production, cannot be completely inhibited by treatment with protease inhibitors.

Phagocytosis or membrane perturbation of phagocytic cells, including pulmonary macrophages, causes a cyanide-insensitive increase in oxygen consumption with concomitant production of superoxide anion (O_2^-)‡ [1]. Superoxide anions produced by pulmonary macrophages are generated by an NADPH-dependent oxygen reductase present in the cell membranes which is activated when the cells are stimulated [2, 3] and which catalyzes the univalent reduction of molecular oxygen. The increase in oxygen consumption by stimulated phagocytic cells has been attributed solely to the increased production of superoxide. However, no consistent stoichiometric relationship appears to exist between O_2 consumed and O_2^- produced by stimulated phagocytic cells, although a consistent stoichiometric relationship is evident between O_2 consumption and latex particle engulfment [4]. It has been suggested that O_2^- is not a major product of the stimulatory process (also known as the "respiratory burst") [5] or, alternatively, that an O_2 diffusion layer is rapidly established at the cell surface which alters the expected stoichiometry [6]. Another explanation is that O_2 consumption or O_2^- production, or both, are reflections of more than one process and, therefore, total O_2 consumption and O_2^- production do not necessarily bear a predictable stoichiometric relationship to one another. If, however, the stimulation of oxygen consumption and the formation of O_2^- in whole cells are reflections of the activity of a single enzyme or enzyme system, that is, the NADPH-dependent oxygen reductase, then inhibition of the activity of the enzyme should decrease oxygen consumption and superoxide generation with the same potency and to the same extent.

* This work was supported by NIH Research Grant GM 12675 and a Medical Scientists Training Program Grant GM 07737 to M. H.

† Author to whom correspondence should be addressed.

‡ Abbreviations: O_2^-, superoxide anion; PMA, phorbol myristate acetate; TPCK, L-1-tosylamido-2-phenylethyl-chloromethyl ketone; TLCK, *N*-α-*p*-tosyl-L-lysine chloromethyl ketone; ASA, acetylsalicylic acid; IBU, ibuprofen; DMSO, dimethylsulfoxide; and HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

We selected two classes of agents that have been reported to be inhibitors of the stimulation of oxygen metabolism in phagocytes: cyclooxygenase inhibitors and serine protease inhibitors. Several reports in the literature document the inhibitory effects of prostaglandin synthesis inhibitors on the generation of O_2^- in polymorphonuclear leukocytes and peritoneal macrophages [7–9] and the role of prostaglandins in triggering or modulating O_2^- production. ASA, an irreversible time-dependent inhibitor of cyclooxygenase, and IBU, a competitive inhibitor of cyclooxygenase, were chosen as potential inhibitors of superoxide production and oxygen consumption in pulmonary macrophages.

Inhibition by serine protease inhibitors of the stimulation of superoxide production in monocytes and leukocytes has also been reported [10, 11]. It has been suggested that protease inhibitors exert their effects by inhibiting the activity of a serine esterase proposed to be necessary for the activation of the membrane-associated NADPH-dependent oxygen reductase. L-1-Tosylamido-2-phenylethyl-chloromethyl ketone (TPCK) and *N*-α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK), two irreversible serine protease inhibitors which were shown to be the most potent inhibitors of O_2^- production of those tested [10], were selected for use in this study. These agents are preferential inhibitors of chymotrypsin and trypsin, respectively, and both are active-site histidine alkylating agents [12].

The protease inhibitors and cyclooxygenase inhibitors exert their effects through different mechanisms and, therefore, it is unlikely that all four selected inhibitors could coincidentally inhibit both the stimulation in oxygen consumption and superoxide generation to the same degree unless both were consequences of the same process. Differential inhibition of the two processes by any or all of the agents would imply that these effects were the result of separate or overlapping reactions. This study compares the effects of ASA, IBU, TPCK and TLCK on phorbol myristate acetate-stimulated oxygen consumption and superoxide generation from pulmonary macrophages.

MATERIALS AND METHODS

Cell isolation. Pulmonary macrophages were isolated by a procedure described previously [13]. Briefly, the lungs of adult Sprague–Dawley rats were perfused through the right ventricle with 1 mM phosphate-buffered saline, removed, and minced. The tissue pieces were stirred in buffer to free the macrophages and these cells were then separated from the tissue pieces by filtration. Contaminating erythrocytes were lysed by suspension of the cells in a hypotonic salt solution for 20 sec. Macrophages were sedimented and resuspended in 1 mM HEPES-buffered saline, 1 mM $CaCl_2$, 5.6 mM glucose (pH 7.6). Cell viability was assessed by erythrosine B dye exclusion.

Measurement of oxygen consumption. Oxygen concentration in the cell suspensions was measured using a Clark oxygen electrode (Yellow Springs Instruments, Inc., Yellow Springs, OH). The suspensions contained $5–7 \times 10^5$ pulmonary macrophages/ml in 1 mM HEPES-buffered saline with 1 mM $CaCl_2$, 5.6 mM glucose (pH 7.6) with or without inhibitor. It is known that stimulation of O_2^- production and O_2 consumption by leukocytes is dependent on extracellular Ca^{2+} concentration [14]. A test of these processes in stimulated macrophages in the presence and absence of calcium indicated that this cation was required by pulmonary macrophages as well. Therefore, 1 mM $CaCl_2$ was included in the buffer solutions during stimulation. Oxygen concentration within the sealed oxygen electrode cell was monitored for an 8-min period at 25° with or without inhibitor (ASA, IBU, TPCK or TLCK). PMA was then added to the suspension and the oxygen concentration was monitored for 10 min. Similar experiments were conducted in the presence of 0.6 mM NaCN to verify that stimulated oxygen consumption was cyanide-resistant under all conditions. The ability of inhibitors to suppress oxygen uptake after stimulation by PMA was studied by adding the inhibitor 2 min after the addition of PMA. From these measurements, the basal (prior to PMA addition) and stimulated (following PMA addition) rates of oxygen consumption in the presence and absence of inhibitors were calculated. Cell viability was assessed following each set of measurements, and values were calculated on the basis of the number of viable cells at the end of each assay. Loss of cell viability during these measurements was always less than 5% of the initial cell number.

Measurement of superoxide production. Superoxide was measured spectrophotometrically by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c* in an Aminco DW2A dual wavelength spectrophotometer. Aliquots of the suspended cells were added to 1 mM HEPES-buffered saline containing 1 mM $CaCl_2$, 5.6 mM glucose, 75 μM ferricytochrome *c*, and either with or without added inhibitors. The final concentration of pulmonary macrophages was $5–7 \times 10^5$ cells/ml. The reduction of ferricytochrome *c* was monitored for 8 min at 25° at 550 nm with a reference wavelength of 540 nm. PMA was added to the cell suspension and the absorbance was monitored for an additional 10 min. The abilitity of inhibitors to suppress PMA-stimulated superoxide production was assessed by adding an inhibitor to the cuvette 2 min after the addition of PMA. Ferricytochrome *c* reduction was calculated using $E_{550\text{-}540\,nm} = 15.5 \times 10^3$ $M^{-1}\,cm^{-1}$ [15]. Reduction of ferricytochrome *c* was not linear with time, but calculations of rate were based on the most rapid and linear portion of the tracing following stimulation by PMA (see Fig. 2). Cell viability was assessed following each set of measurements, and values were calculated on the basis of the number of viable cells at the end of each assay. Loss of cell viability during the measurements was always less than 5% of the initial cell number.

Effect of inhibitors on a superoxide-generating system. To determine whether or not any of these agents either quenched superoxide or chemically generated free radicals, the effect of a 100 μM concentration of each inhibitor on a superoxide-generating system was assessed. Test mixtures contained 0.1 mM xanthine, 5 μl xanthine oxidase, 75 μM ferricytochrome *c*, and a 100 μM concentration of inhibitor in 1 mM

HEPES-buffered saline containing 1 mM $CaCl_2$, 5.6 mM glucose (pH 7.6) at 25°.

Materials. IBU (donated by Dr. J. McConnell, Upjohn, Inc., Kalamazoo, MI), ASA (Ruger, Hillside, NJ), TPCK (Sigma Chemical Co., St. Louis, MO) and TLCK (Sigma) were prepared as 0.1 M stock solutions in DMSO. When appropriate, these solutions were diluted with DMSO so that in all cases inhibitors were added in volumes of DMSO between 5 and 10 μl. Identical concentrations of DMSO added to control cells had no effect on either oxygen uptake or superoxide production. Phorbol myristate acetate (Sigma) was prepared as a 0.5 mg/ml stock solution (0.75 mM) in DMSO and diluted in DMSO as necessary with a final volume of 5–10 μl of DMSO added to the cells. In all experiments except the dose–response relationship of PMA to oxygen consumption and O_2^- production, macrophages were stimulated by the addition of 10 μl of the stock PMA solution with a final PMA concentration of 7.5 μM.

Data analysis. Values for "percent maximal response" for stimulation of oxygen consumption and O_2^- production by pulmonary macrophages were calculated by dividing the difference between the stimulated and basal rates in the presence of inhibitor by the maximal response (stimulated minus basal rates) of the preparation in the absence of any inhibitor. Regression analysis of the scatter plots of the "percent maximal response" values was performed to generate the concentration–response line for PMA-induced stimulation of O_2^- production and oxygen consumption in the presence of increasing concentrations of each inhibitor. For clarity, only the mean values at each concentration of inhibitor are shown in the figures, however.

RESULTS

Stimulation of the rate of oxygen consumption and O_2^- generation by PMA. Inspection of the relationship of PMA to stimulation of O_2^- generation indicates that the two are directly related up to 7.5 μM PMA (Fig. 1). Viability of the pulmonary macrophages was unchanged from control cells after treatment with PMA at concentrations up to 15 μM. At concentrations greater than 15 μM, there was a decline in cell viability; therefore, in subsequent studies, 7.5 μM PMA was used to obtain maximal stimulation of macrophages without causing loss of viability. PMA at 7.5 μM stimulated a doubling of the rate of oxygen consumption (Table 1). The stimulated rate was not inhibited by CN^- whereas the basal rate was completely inhibited by CN^- (data not shown). Superoxide anion production by unstimulated cells was essentially zero but, like oxygen consumption, was stimulated in a concentration-dependent manner by PMA. At 7.5 μM, the rate of superoxide production was slightly lower than the rate of oxygen consumption attributable to PMA stimulation (Table 1).

A typical response of macrophages treated with 7.5 μM PMA shows a short lag period followed by a steady rate of O_2^- production for approximately 2–3 min (Fig. 2). Production of O_2^- returned to the basal rate in approximately 6–10 min. O_2^- production could not be restimulated by the addition of more PMA added immediately after the return to the basal rate.

Effect of inhibitors on stimulation of oxygen consumption and O_2^- generation. PMA-induced stimulation of oxygen consumption was inhibited in a concentration-dependent manner by the protease inhibitors TPCK and TLCK and the cyclooxygenase inhibitors IBU and ASA when the inhibitors were added 8 min before PMA (Fig. 3). The order of potency was ASA > IBU > TPCK = TLCK. The stimulation of O_2^- production by PMA was also inhibited in a concentration-dependent manner by each inhibitor (Fig. 4). The order of potency differed from that seen with oxygen consumption and was TPCK > TLCK > IBU > ASA. The concentrations

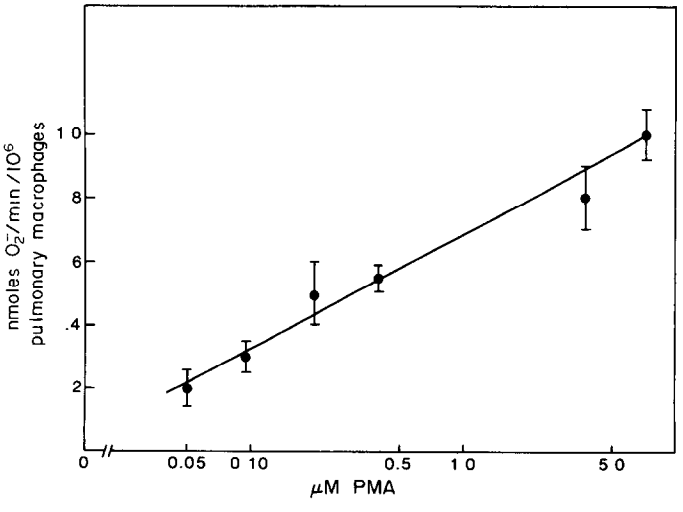

Fig. 1. Stimulation of superoxide anion production by pulmonary macrophages at increasing concentrations of PMA. Data are given as means ± S.E.M. PMA concentration is reported as the log of the concentration. Each point is the mean of four determinations. The basal level of superoxide production was 0.1 ± 0.1 nmole of O_2^- per min per 10^6 viable macrophages.

Table 1. Effect of inhibitors and order of addition on PMA-stimulated oxygen consumption and O_2^- production by pulmonary macrophages

Addition(s)	O_2 consumption* (nmoles · min^{-1} · 10^6 cells^{-1})	O_2^- production* (nmoles · min^{-1} · 10^6 cells^{-1})
None	1.7 ± 0.1	0.1 ± 0.1
PMA	3.0 ± 0.2†	1.0 ± 0.1†
ASA	1.7 ± 0.2‡	0.1 ± 0.1‡
PMA + ASA§	2.9 ± 0.2‖	1.1 ± 0.1‖
ASA + PMA¶	2.2 ± 0.2	0.8 ± 0.1
IBU	1.6 ± 0.2‡	0.1 ± 0.1‡
PMA + IBU§	2.4 ± 0.1**	0.8 ± 0.2‖
IBU + PMA¶	2.0 ± 0.1	0.4 ± 0.1

* Each value in the mean ± S.D. of separate determinations from at least four different macrophage preparations.

† Significantly different from untreated cells ($P \leq 0.05$).

‡ Not significantly different from untreated cells.

§ Cells were incubated in 7.5 μM PMA for 2 min before the addition of 20 μM ASA or 100 μM IBU.

‖ Not significantly different from the PMA-treated preparation.

¶ Cells were incubated in 20 μM ASA or 100μM IBU for 8 min before the addition of 7.5 μM PMA.

** Significantly different from the PMA-treated preparation ($P \leq 0.05$).

of each inhibitor resulting in 50% inhibition of PMA-induced stimulation of oxygen consumption and superoxide generation (IC_{50}) were compared in Table 2. These data showed not only that the inhibitors of cyclooxygenase were more effective inhibitors of PMA-stimulated oxygen consumption and that the protease inhibitors were more effective against superoxide generation but also that except in the case of IBU there was nearly a 10-fold difference in the IC_{50} of the inhibitors for each process.

If each of the four agents was added to macrophages after stimulation with PMA, inhibition was either abolished, diminished, or altered. Addition of inhibitory concentrations of ASA 2 min after PMA had no effect on the rate or time course of either O_2^- production or oxygen consumption by the pulmonary macrophages (Table 1). IBU addition 2 min following PMA caused an immediate decrease in the cellular oxygen consumption but the extent of the inhibition was less than if IBU was added prior to PMA stimulation. When either TPCK or TLCK was added to cell suspensions 2 min after stimulation with PMA, there was a time-dependent decrease in oxygen consumption and O_2^- production such that values returned to basal levels more rapidly than in the absence of a protease inhibitor (data not shown). Thus, total O_2^- production or O_2 consumption was decreased. None of the agents studied had any effect on basal oxygen consumption or O_2^- production at the concentrations used (see Table 1 for ASA and IBU basal values).

TPCK, the more potent of the two protease inhibitors in terms of inhibition of O_2^- production, was able to inhibit totally the stimulation of superoxide production when added before PMA at a concentration of 70 μM. However, TPCK only partially inhibited PMA-stimulated oxygen consumption. The extent of TPCK inhibition of stimulated oxygen con-

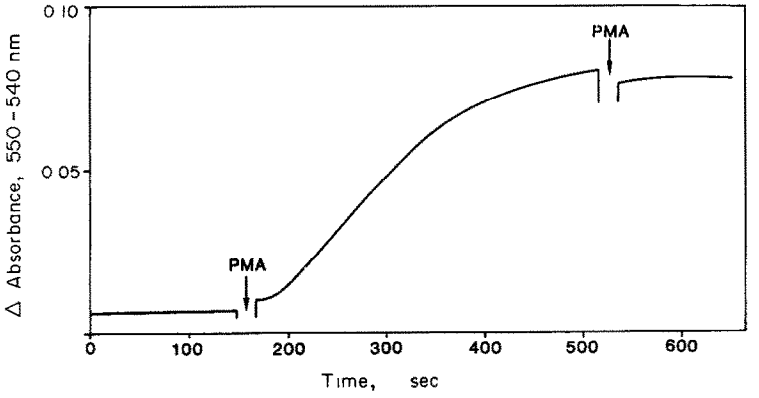

Fig. 2. Effect of PMA on superoxide production by pulmonary macrophages. This figure is a representative spectrophotometric tracing showing the rate of reduction of ferricytochrome *c* by superoxide anion produced by stimulated macrophages. The arrows indicate the addition of 7.5 μM PMA to the suspended cells.

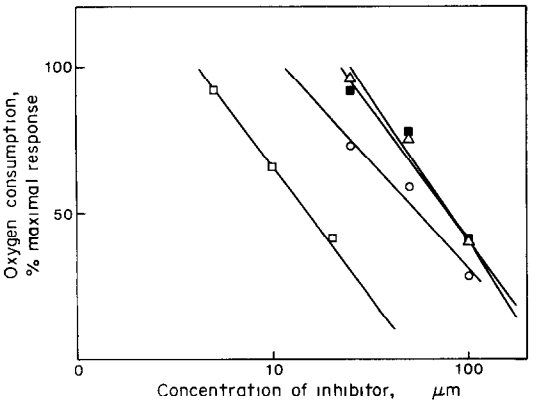

Fig. 3. Inhibition of PMA-induced stimulation of oxygen consumption following an 8-min preincubation with increasing concentrations of inhibitors. Each point is the mean of at least three determinations, each on a different macrophage preparation. The lines were fitted to the data by linear regression of the scatter plot. The range of values at each concentration of inhibitor was between 2 and 15% of the maximal response. Key: TPCK (■), TLCK (△), ASA (□), and IBU (○).

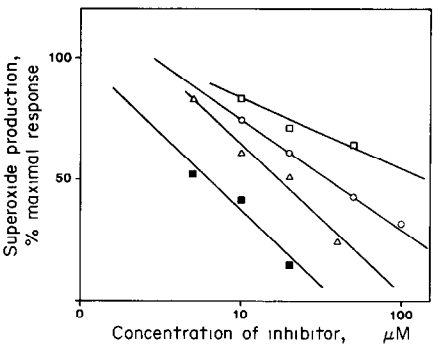

Fig. 4. Inhibition of the stimulation of PMA-induced superoxide production following an 8-min preincubation with increasing concentrations of inhibitors. Each point is the mean of at least three different determinations, each on a different macrophage preparation. The lines were fitted to the data by linear regression of the scatter plot of all determinations. The range of values at each concentration of inhibitor was between 5 and 18% of the maximal response. Key: TPCK (■), TLCK (△), ASA (□), and IBU (○).

sumption was 70–80% at 70 μM, and increasing concentrations of TPCK (between 150 μM and 1 mM) did not result in further inhibition.

None of the four inhibitors had any effect on the reduction of ferricytochrome *c* when tested at a concentration of 100 μM in a system where xanthine–xanthine oxidase system was used to generate superoxide radicals at rates of 0.5 to 2.0 nmoles · min^{-1} · ml^{-1} which are comparable to the rate of superoxide production by the stimulated pulmonary macrophages in these experiments.

DISCUSSION

The results reported demonstrate that the protease inhibitors TPCK and TLCK inhibit PMA-stimulated O_2^- production by pulmonary macrophages in proportion to the concentration of each inhibitor. The potency of both inhibitors is in the same range as that reported for inhibition of O_2^- production of neutrophils and monocytes stimulated with cytochalasin E and concanavalin A [10]. Cyclooxygenase inhibitors also inhibited PMA-stimulated O_2^- production by pulmonary macrophages in a concentration-dependent manner. The effective concentrations of these inhibitors were within a range appropriate for inhibition of cyclooxygenase [16]. All four agents inhibited PMA-stimulated O_2 consumption, as might be expected. However, the order of potency for inhibition of PMA-stimulated O_2 consumption and O_2^- production was not the same. The cyclooxygenase inhibitors were more potent inhibitors of stimulated O_2 consumption than O_2^- production, whereas the protease inhibitors were more potent inhibitors of the stimulation of O_2^- production than O_2 consumption. Furthermore, while ASA abolished the stimulation of O_2 consumption, TPCK was unable to inhibit stimulated O_2 consumption by more than 70–80%. Thus, a portion of O_2 consumption stimulated by PMA cannot be accounted for by the generation of O_2^- by stimulated macrophages, but appears to originate through the action of cyclooxygenase. Oxygen consumption resulting from PMA stimulation of pulmonary macrophages is not the consequence solely of the activity of the stimulated NADPH-dependent oxygen reductase, therefore, but reflects the activity of both the oxygen reductase and cyclooxygenase. It is concluded that the measurement of stimulated oxygen consumption is an inadequate means of estimating the activity of the superoxide-generating enzyme. The contribution of both cyclooxygenase and oxygen reductase to PMA-stimulated oxygen consumption also explains the lack of a stoichiometric relationship between phagocyte O_2^- production and O_2 consumption.

Table 2. Inhibition of PMA stimulation of the rate of oxygen consumption and superoxide anion production: IC_{50}*

Inhibitor	IC_{50} for O_2 consumption (μM)	IC_{50} for O_2^- production (μM)
ASA	15	148
IBU	50	51
TPCK	80	7
TLCK	80	19

* Concentration of inhibitor causing a 50% inhibition of PMA-induced stimulation.

The greater effectiveness of the inhibitors in preventing PMA stimulation of oxygen consumption and superoxide production than in suppressing these processes once stimulation had been initiated could be attributed to irreversible, time-dependent inhibition in the cases of ASA [17], TPCK and TLCK. All three of these inhibitors act via enzyme modification. IBU, however, is a competitive inhibitor of the cyclooxygenase but does not have the property of time-dependent inhibition as does ASA [18], yet it is also a less effective inhibitor when added after the macrophages have been stimulated with PMA. One possible explanation of this effect is that the concentration of free arachidonate which will influence the competitive effectiveness of IBU for cyclooxygenase is probably greater when macrophages are initially stimulated by PMA. Thus, one might expect that the same concentration of IBU would be less effective after stimulation than when added before stimulation. TPCK and TLCK decrease O_2^- generation and O_2 consumption with time following their addition to stimulated macrophages and, thus, diminish the stimulated rate of both processes. It seems likely, therefore, that both act to inhibit PMA-stimulated oxygen consumption and superoxide generation by inhibition of events subsequent to the initial interaction of PMA with the plasma membrane. TPCK and TLCK, when added after PMA stimulation, may cause partial inhibition of the rate of superoxide generation by preventing the activation of further superoxide-producing sites, while inactivation of sites already activated proceeds at an unaltered rate. This interpretation would also account for the observation that the extent as well as the rate of O_2^- production is less in inhibitor-pretreated preparations than in control preparations.

The occurrence of inactivation of some type is necessary to explain the time course of O_2^- generation upon PMA stimulation. The rate of O_2^- generation accelerates following PMA addition with only a short lag period. The rate of O_2^- production eventually returns to the basal level and cannot be immediately restimulated by the addition of more PMA. It seems unlikely that NADPH has been exhausted, since the hexose monophosphate shunt which provides the reduced nucleotide is activated by macrophage stimulation [1]. A restoration of those components either associated with the initial membrane effects or with activity of the NADPH-dependent enzyme itself must occur if the pulmonary macrophages will again be responsive to stimulation.

An explanation of the initial lag period is not yet available. However, since participation of the cyclooxygenase system is necessary, the short lag may reflect the time necessary to accumulate an adequate concentration of peroxides in order to activate cyclooxygenase [19]. Because the stimulated macrophage continuously produces oxygen radicals within a prescribed time, maintenance of an adequate peroxide level would be expected after stimulation.

From the results reported here as well as from the published literature, some conclusions can be drawn about the sequence of events following macrophage stimulation. Stimulation of phagocytes results in an influx of extracellular Ca^{2+} and mobilization of intracellular Ca^{2+} [20], causing the activation of a calcium-dependent phospholipase A_2 [21]. The phospholipase catalytically releases arachidonic acid from membrane phospholipids which results in the biosynthesis of prostanoids in macrophages and leukocytes [22, 23]. Products of the lipoxygenase pathway increase hexose transport, which is another concomitant of stimulation in leukocytes [24]. The results reported here show that products of the cyclooxygenase pathway play a role in the activation of the superoxide-generating system in pulmonary macrophages. A protease may also be involved in this process. The precise sequence of events connecting the initial interaction between the membrane and the perturbant to the activation of the superoxide-generating enzyme remains to be elucidated. The significance of ASA and IBU inhibition of superoxide generation by pulmonary macrophages in light of the potential of macrophage-generated superoxide for neutrophil recruitment to the lung should be examined as well.

REFERENCES

1. B. M. Babior, *New Engl. J. Med.* **298**, 659 (1978).
2. M. Hoffman and A. P. Autor, *Fedn Eur. Biochem. Soc. Lett.* **121**, 352 (1980).
3. A. P. Autor and M. Hoffman, *Clin. Resp. Physiol.* **17**, (Suppl.), 153 (1981).
4. A. W. Segal and S. B. Coade, *Biochem. biophys. Res. Commun.* **84**, 611 (1978).
5. A. W. Segal and T. Meshulam, *Fedn Eur. Biochem. Soc. Lett.* **100**, 27 (1979).
6. T. R. Green, R. E. Schaefer and M. T. Makler, *Biochem. biophys. Res. Commun.* **94**, 1213 (1980).
7. J. E. Smolen and G. Weissmann, *Biochem. Pharmac.* **29**, 533 (1980).
8. Y. Oyanagui, *Biochem. Pharmac.* **27**, 777 (1978).
9. R. J. Smith and S. S. Iden, *Biochem. Pharmac.* **29**, 2389 (1980).
10. B. D. Goldstein, G. Witz, M. Amoruso and W. Troll, *Biochem. biophys. Res. Commun.* **88**, 854 (1979).
11. S. Kitagawa, F. Takaku and S. Sakamato, *J. clin. Invest.* **65**, 74 (1980).
12. E. Shaw, in *The Enzymes* (Ed P. D. Boyer), p. 91. Academic Press, New York (1970).
13. J. B. Stevens and A. P. Autor, *Lab. Invest.* **37**, 470 (1977).
14. H. J. Cohen and M. E. Chovaniec, *J. clin. Invest.* **61**, 1088 (1978).
15. A. Nakagawara and S. Minakami, *Biochem. biophys. Res. Commun.* **64**, 760 (1975).
16. R. J. Flower, *Pharmac. Rev.* **26**, 33 (1974).
17. W. L. Smith and W. E. M. Lands, *J. biol. Chem.* **246**, 6700 (1971).
18. L. H. Rome and W. E. M. Lands, *Proc. natn. Acad. Sci. U.S.A.* **72**, 4863 (1975).
19. M. E. Hemler and W. E. M. Lands, *J. biol. Chem.* **255**, 6253 (1980).
20. P. H. Naccache, M. Volpi, H. J. Showell, E. L. Becker and R. I. Sha'afi, *Science* **203**, 461 (1979).
21. R. Franson and M. Waite, *Biochemistry* **17**, 4029 (1978).
22. I. M. Goldstein, C. L. Malmsten, B. Samuelsson and G. Weissman, *Inflammation* **2**, 309 (1977).
23. K. Brune, M. Glatt, H. Kälin and B. A. Peskar, *Nature, Lond.* **274**, 261 (1978).
24. D. A. Bass, J. T. O'Flaherty, P. Szejda, L. R. DeChatelet and C. E. McCall, *Proc. natn. Acad. Sci. U.S.A.* **77**, 5125 (1980).