Chemiluminescence of Human Neutrophils Induced by Soluble Stimuli: Effect of Divalent Cations

LAWRENCE R. DeCHATELET* AND PAMELA S. SHIRLEY

Department of Biochemistry, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina 27103

The effect of three soluble stimuli, phorbol myristate acetate, concanavalin A, and the calcium ionophore A23187, on the luminol-enhanced chemiluminescence of human neutrophils was investigated. All three stimuli elicited a strong burst of chemiluminescence which was dose dependent. The effect of phorbol myristate acetate was independent of the presence of divalent cations in the medium and, in fact, was greater in the presence of ethylene glycol-bis(β-aminoethyl ether)-N,N-tetraacetic acid. The effect of concanavalin A was greatly stimulated by the presence of calcium in the medium, although some reaction was observed in the absence of this cation. In contrast, chemiluminescence induced by A23187 was absolutely dependent upon the presence of extracellular calcium. These results suggest that the mobilization of calcium into the cell is a sufficient, but not necessary, stimulus for initiation of the respiratory burst. Compounds such as phorbol myristate acetate, which act independently of extracellular divalent cations, may function by altering the intracellular ratio of bound/free calcium.

When human neutrophils (PMN) phagocytize particulate material, they typically undergo alterations in oxidative metabolism, collectively referred to as the "respiratory burst" (2). In addition to resulting from phagocytosis, the respiratory burst may be elicited by certain soluble stimuli. These include the plant lectin concanavalin A (ConA) (6), the tumor promoter phorbol myristate acetate (PMA) (9), and the calcium ionophore A23187 (20).

One of the most sensitive measurements of respiratory burst activity involves the generation of chemiluminescence as originally described by Allen et al. (1). This chemiluminescence was suggested to be due to relaxation of singlet oxygen to the ground state (1), but the situation appears to be far more complex, involving secondary reactions of oxygen species with particles or serum components (5, 16). We recently demonstrated that soluble stimuli such as PMA would not elicit a chemiluminescence response from PMN unless there was some type of secondary emitter in the reaction, such as protein or luminol (24). In the presence of luminol, the reaction is especially sensitive, and a marked response can easily be observed with as few as 10^5 cells (24).

The mechanism of initiation of the respiratory burst is generally conceded to be due to activation of an NADPH oxidase in the cell (2, 7), but the sequence of events leading to this activation remains obscure. An early event appears to involve membrane depolarization (12, 25), and a

number of studies have suggested that this results in alterations in Ca^{2+} flux and changes in the intracellular level of free calcium (3, 12, 15, 19). The present investigation examines the effect of divalent cations on the luminol-dependent chemiluminescence of PMN induced by three different soluble stimuli of oxidative metabolism.

MATERIALS AND METHODS

Isolation of PMN. Human PMN were isolated from heparinized venous blood by sedimentation of the erythrocytes with plasma gel as previously described (8). Because of substantial delays (greater than 9 months) in obtaining commercial shipments of plasma gel, we developed a procedure for manufacturing a similar material in the laboratory. Into a 2-liter flask was placed 990 ml of deionized water, 7.0 g of NaCl, and 1.5 g of anhydrous $CaCl_2$. After dissolution of the salts, the mixture was brought to a boil, and 30 g of gelatin (Difco Laboratories, Detroit, Mich.) was slowly added. Boiling with occasional stirring was continued until the gelatin was dissolved. The hot solution was filtered under vacuum through a Büchner funnel with the use of Whatman no. 1 paper, and the filtrate was divided into 100-ml portions in 250-ml screw-top Erlenmeyer flasks. The flasks were then autoclaved for 20 min at 250 lb/in², cooled, sealed, and stored in the cold until use. Comparison of this preparation with commercial plasma gel (HTI Corp., Buffalo, N.Y.) indicated similar yields of cells and degrees of purity. Further, no differences in either resting or stimulated hexose monophosphate shunt activity (as measured by the oxidation of [1-^{14}C]glucose) were noted between cells isolated in parallel with the two preparations.

Total counts on the isolated preparations were per-

formed in a hemacytometer, and the cells were sus-
pended in Dulbecco's phosphate-buffered saline (PBS)
containing glucose (100 mg/100 ml). Cells were sus-
pended in PBS containing no added cations or contain-
ing 1.4 mM CaCl$_2$ and 0.7 mM MgCl$_2$ as dictated by
the individual experiments. The cells were suspended
to a concentration of 5 × 10^6/ml and stored in plastic
tubes on ice; immediately before use, the stock solu-
tion of cells was diluted to 5 × 10^5/ml with the
appropriate buffer at room temperature. The prepara-
tion typically contained 85 to 90% PMN as determined
on Wright-stained smears.

Reagents. PMA (Sigma Chemical Co., St. Louis,
Mo.) was dissolved in dimethyl sulfoxide to give a
stock solution of 2.0 mg/ml. The stock solution was
stored frozen; a portion was diluted to the appropriate
concentration with PBS (with or without divalent
cations) immediately before use. ConA (Sigma Chemi-
cal Co.) was prepared fresh daily as a stock solution of
10 mg/ml in the appropriate buffer; a portion was
added to each incubation vessel to yield the designated
final concentration. The calcium ionophore A23187
(Calbiochem, La Jolla, Calif.) was stored in a refriger-
ator as a stock solution of 0.04 M in dimethyl sulfox-
ide. Dilutions of this were made in the appropriate
buffer immediately before use to yield the designated
final concentrations. Luminol (Sigma) was dissolved
in dimethyl sulfoxide to a concentration of 10 mg/ml,
stored in a dark bottle at room temperature, and
diluted with PBS immediately before use to give a final
concentration in the assay of 10^{-6} M. CaCl$_2$ and MgCl$_2$
(Fisher Scientific Co., Fairlawn, N.J.) were stored at
room temperature as stock solutions of 1 M and were
diluted with deionized water just prior to use. These
could not be diluted in buffer because of precipitation
of the metal phosphates. EDTA and ethylene glycol-
bis(β-aminoethyl ether)-N,N-tetraacetic acid (EGTA;
Fisher) were stored at room temperature as stock
solutions of 0.10 M, pH 7.4, and were diluted to the
desired concentration just prior to use with cation-free
PBS.

Measurement of chemiluminescence. Chemilumines-
cence was measured at ambient temperature in a
Beckman LS 100C liquid scintillation spectrometer set
in the out-of-coincidence mode with a ^{14}C-^{3}H window
and a gain of 2.5. The use of the luminol-enhanced
system permitted performance of the assay in a lighted
room without the inconvenience of dark-adapting re-
agents or working under red (actinic) light (18). The
reactions were performed in polyethylene minivials
with a capacity of 4.0 ml (Fisher Scientific Co.). Each
vial contained a final volume of 2.0 ml, including
buffer, luminol (10^{-6} M), the indicated concentration
of stimulus, and, where indicated, the appropriate
concentrations of Ca^{2+}, Mg^{2+}, or EGTA. Reaction
was initiated in a timed sequence by the addition of
0.20 ml of isolated PMN (10^5 cells), and the vials were
mixed by inversion and counted for 0.20 min. When
the last vial was in place, the scintillation counter was
closed and repetitive counts (every 3 to 5 min depend-
ing upon the experiment) were recorded. The initial
(zero time) values were spurious as a result of the
luminescence of the vials; however, later time points
were entirely reproducible. Since the resting values
(no stimulus) did not vary significantly over the course
of 2 h, it was a simple matter to extrapolate to the zero-
time value.

In several experiments, the generation of chemilu-
minescence in a model system was examined by use of
the xanthine oxidase-acetaldehyde system (14). These
experiments were performed in the same fashion as
those described except that the substrate was 0.10 M
freshly distilled acetaldehyde and the reaction was
initiated by the addition of 0.024 U of xanthine oxi-
dase. It was necessary to reduce the luminol concen-
tration to 5 × 10^{-8} M in these experiments to keep the
activity on scale. Other additions to the reaction are
described in the text.

RESULTS

Figure 1 illustrates the effect of various doses
of PMA on the luminol-dependent chemilumi-
nescence. With a PMA concentration of 10 ng/
ml, there was a rapid and intense burst of
chemiluminescence which reached a peak within
15 to 20 min and then decayed. As the dose was
reduced, the peak response was more delayed
and lower in intensity. At much higher concen-
trations of PMA (e.g., 1 to 2 μg/ml), the measur-
able chemiluminescence was actually less than
that observed with 10 ng/ml (data not shown). In
the absence of PMA, there was a low back-
ground level of chemiluminescence (10,000 to
20,000 cpm) which did not vary significantly
over the course of 2 h.

The effect of divalent cations on this response
is illustrated in Fig. 2. In this experiment the
cells were suspended in PBS without divalent
cations or containing 1.4 mM Ca^{2+} and 0.7 mM
Mg^{2+}. No difference was observed in either the
magnitude or the time course of the response
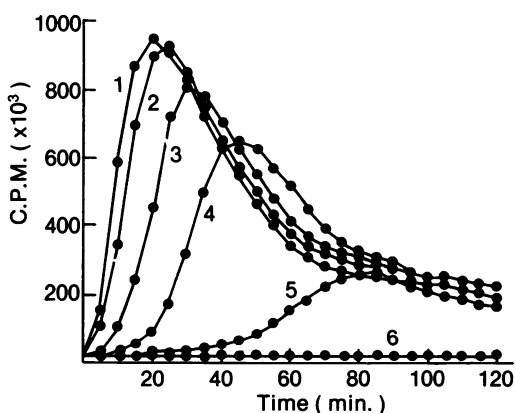with the different buffers.

FIG. 1. Effect of PMA concentration on the lu-
minol-dependent chemiluminescence. Each vial con-
tained 10^5 PMN in 2.0 ml of PBS containing 1.4 mM
Ca^{2+} and 0.7 mM Mg^{2+}. Luminol concentration was
10^{-6} M. Curve 1, 10 ng of PMA/ml; curve 2, 5 ng of
PMA/ml; curve 3, 2.5 ng of PMA/ml; curve 4, 1.0 ng of
PMA/ml; curve 5, 0.50 ng of PMA/ml; curve 6, con-
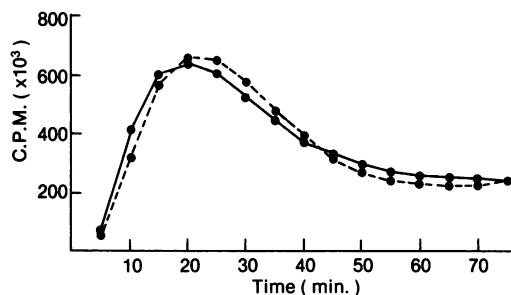trol, no PMA added.

FIG. 2. Effect of divalent cations on chemilumines-
cence. Conditions were the same as described for Fig.
1, with a final PMA concentration of 10 ng/ml. Solid
line, PBS containing 1.4 mM Ca^{2+} and 0.7 mM Mg^{2+};
dotted line, PBS in the absence of added divalent
cations. The data are from a single experiment which
is representative of four separate experiments.

In an attempt to demonstrate conclusively
that chemiluminescence in this system was inde-
pendent of extracellular calcium, the experiment
was performed in the presence of 1 mM EGTA.
Results of a typical experiment are presented in
Fig. 3. As observed in Fig. 2, the PMA-induced
chemiluminescence was not significantly affect-
ed by the addition of 1.4 mM Ca^{2+} in the
medium. Surprisingly, the response was mark-
edly stimulated by the addition of 1 mM EGTA
in the absence of Ca^{2+}. Although the magnitude
of stimulation by EGTA varied from day to day
(130 to 160% of control), the effect was observed
in each of five separate experiments. This stimu-
lation was completely abolished when Ca^{2+} and
EGTA were present simultaneously. Similar re-
sults were obtained when EDTA was substituted
for EGTA, although the degree of stimulation
was usually not as marked (data not shown).

We next examined the effect of EGTA on
chemiluminescence generated in a model system
to determine whether the stimulation represent-
ed a direct effect on the cell. As shown in Fig. 4,
1 mM EGTA markedly stimulated the chemilu-
minescence response observed in the xanthine
oxidase-acetaldehyde system. This stimulation
was partially inhibited by the inclusion of Mg^{2+},
but was dramatically inhibited (below the con-
trol level) when EGTA and Ca^{2+} were present
simultaneously. Similar results were obtained
with EDTA in a single experiment (data not
shown).

We next attempted to employ other soluble
stimuli of neutrophils to determine whether the
independence of extracellular calcium was a
general phenomenon. The results in Fig. 5 dem-
onstrate that 100 µg of ConA per ml, in the
presence of Ca^{2+} and Mg^{2+}, stimulated a signifi-
cant burst of chemiluminescence in the present
system; this is in agreement with previously
published dose-response curves (24). In contrast

to the stimulation by PMA, there was a marked
diminution of the response to ConA when Ca^{2+}
and Mg^{2+} were omitted from the reaction; this
response was further attenuated when 1 mM
EGTA was added. These observations are con-
firmed and extended in Fig. 6. Some chemilumi-
nescence was observed with ConA in the ab-
sence of divalent cations; this was marginally
stimulated when Mg^{2+} was included in the reac-
tion, but was markedly enhanced by the addition
of extracellular Ca^{2+}. The simultaneous addition
of both Mg^{2+} and Ca^{2+} did not yield significantly
greater chemiluminescence than the presence of
Ca^{2+} alone.

Finally, we investigated the effect of the calci-
um ionophore A23187 on the luminescent re-
sponse. The effect of various concentrations of
ionophore is illustrated in Fig. 7. At high con-
centrations of ionophore (i.e., 1.0 µM), there
was a very pronounced initial response which
declined rapidly with time. As the concentration
of ionophore was decreased, there was a de-
crease in peak intensity with a corresponding
shift in the time of maximal response, so that a
concentration of 0.01 µM gave a very usable
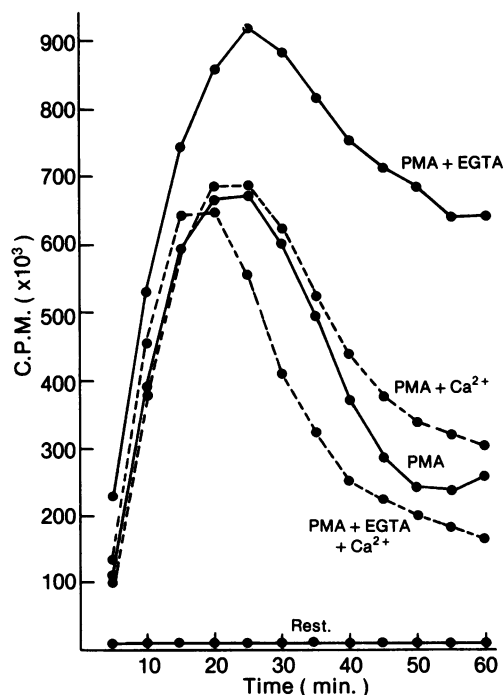response. That this response was dependent

FIG. 3. Effect of EGTA and Ca^{2+} on the PMA-
induced chemiluminescence of PMN. Where indicat-
ed, PMA was added to a final concentration of 10 ng/
ml, and EGTA and Ca^{2+} were added to a final concen-
tration of 1.0 mM. The data are from a single experi-
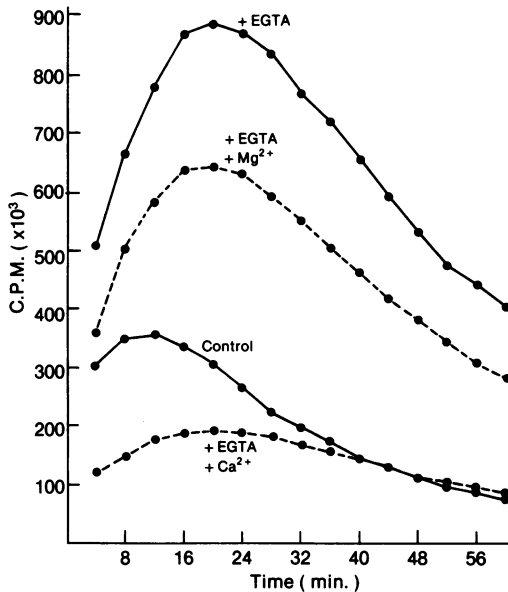ment which is representative of three separate experi-
ments.

FIG. 4. Effect of EGTA and divalent cations on the chemiluminescence emitted by xanthine oxidase and acetaldehyde. The experimental conditions are described in Materials and Methods. EGTA, Mg^{2+}, and Ca^{2+} were added, where indicated, to a final concentration of 1.0 mM. The data are from a single experiment which is representative of three separate experiments.

upon the presence of divalent cations is illustrated by the data in Fig. 8. A good chemiluminescent response was elicited by 0.01 μM ionophore in the presence of 1.0 mM Ca^{2+} and Mg^{2+}; essentially the same response was observed in the presence of 1.0 mM Ca^{2+} alone. In contrast, the omission of Ca^{2+} resulted in no significant activity above background regardless of whether Mg^{2+} was present. Similarly, the inclusion of 0.10 mM EGTA or EDTA with the ionophore resulted in no chemiluminescence response.

DISCUSSION

The activation of the respiratory burst as monitored by luminol-dependent chemiluminescence with PMA as a stimulus was not dependent upon the presence of extracellular Ca^{2+} or Mg^{2+} (Fig. 2 and 3). This is in agreement with other studies in which the PMA-induced stimulation of O_2^- production was independent of extracellular calcium ion (13, 17). In the presence of EGTA, a metal ion chelator, the chemiluminescence was markedly enhanced; this was completely reversed by the presence of an equimolar amount of extracellular calcium (Fig. 3). This observation suggested the possibility that initiation of the respiratory burst involves the efflux of Ca^{2+} from the cell, as suggested by the data of Barthélemy et al. (3); the presence of

EGTA in the medium might facilitate this efflux. This turned out not to be the case, however, since EGTA also stimulated chemiluminescence in a cell-free system in which xanthine oxidase and acetaldehyde were used (Fig. 4). These results suggest that EGTA was somehow acting as an additional amplification signal, perhaps in a manner analogous to the role of luminol itself. This amplification by EGTA apparently required EGTA in the free (unchelated) form. The stimulation was only moderately reduced by equimolar quantities of Mg^{2+} but was completely obliterated by the same concentration of Ca^{2+}. This correlates with the fact that EGTA has a much greater affinity for Ca^{2+} than for Mg^{2+} (4).

In contrast to PMA, the stimulation of chemiluminescence by ConA was heavily dependent upon the presence of extracellular cations. As shown in Fig. 6, the most significant cation for the ConA response was Ca^{2+}.

The addition of the ionophore A23187 in the presence of divalent cations resulted in a marked generation of chemiluminescence which was dose dependent (Fig. 7). To observe a reproducible peak response, we found it necessary to employ a concentration of 0.01 μM, an amount substantially lower than that generally employed in other systems (10, 22, 27). Wilson et al. (26) had previously reported a very modest stimulation of chemiluminescence by 1 μM A23187 (less than 3% of the value obtained with opsonized zymosan). However, their system did not em-
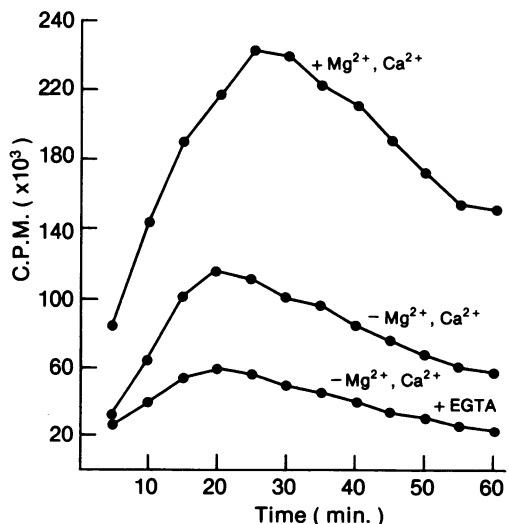

FIG. 5. Effect of divalent cations on the ConA-induced chemiluminescence of PMN. ConA was added to a final concentration of 100 μg/ml. Ca^{2+} and Mg^{2+} concentrations were 1.4 and 0.7 mM, respectively. EGTA was added to a final concentration of 1 mM. These data are from a single experiment which is representative of three separate experiments.
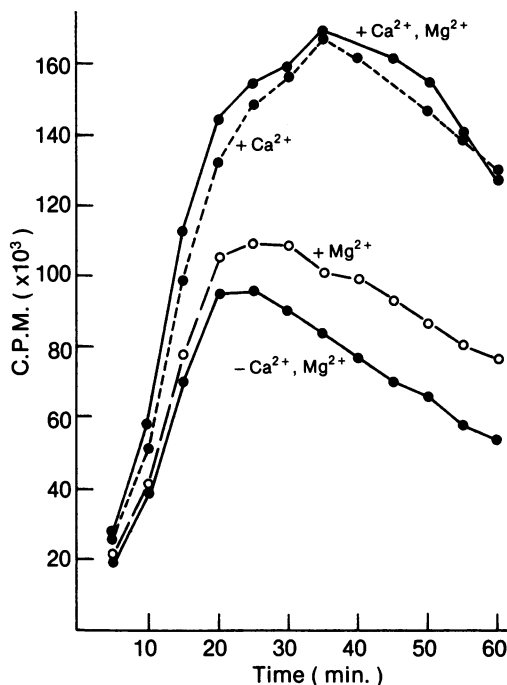
FIG. 6. Effect of addition of divalent cations on the ConA-induced chemiluminescence of PMN. ConA was added in all cases to a final concentration of 100 μg/ml. Ca^{2+} and Mg^{2+} were added, where indicated, to give a final concentration of 1.0 mM each. Similar results were obtained with a Ca^{2+} concentration of 1.4 mM and an Mg^{2+} concentration of 0.7 mM. The data are from a single experiment which is representative of five separate experiments.

ploy luminol, and Westrick et al. (24) had previously demonstrated the need for a secondary emitter when using soluble stimuli. Thus, the chemiluminescent response to A23187 is much greater than was previously appreciated. The stimulation of chemiluminescence by A23187 was entirely dependent upon the presence of extracellular Ca^{2+}. No chemiluminescence above background was noted in the absence of Ca^{2+} even if Mg^{2+} was present (Fig. 8). Thus, this phenomenon appeared to be related to the Ca^{2+}-transporting properties of the ionophore and not simply to alterations secondary to the insertion of the organic molecule into the membrane.

The three soluble stimuli employed gave three distinct profiles with regard to Ca^{2+} ion requirement for the luminol-dependent chemiluminescence. PMA generated a chemiluminescence response which was completely independent of extracellular divalent cations and, in fact, occurred in the presence of a potent chelator of Ca^{2+}. This observation suggests that initiation of the respiratory burst does not require the influx of extracellular calcium into the cell. On the

other hand, the results with the calcium ionophore demonstrated that an influx of Ca^{2+} would, indeed, cause initiation of the respiratory burst. Thus, calcium influx appears to be a sufficient, but not necessary, condition for activation of the oxidative response of the cell. Results with ConA were intermediate between these two extremes. Some chemiluminescence was observed in the absence of divalent cations, but this was markedly stimulated when Ca^{2+} was present. The stimulation by calcium in this case is probably related to the need for this cation for binding of ConA to the cell membrane (12) and not to transport of calcium into the cell.

It seems likely that stimuli such as PMA which act independently of extracellular calcium still work through this ion by increasing the free intracellular Ca^{2+} concentration, perhaps by liberating the ion from some binding protein (11); the possible involvement of calmodulin in the regulation of oxidative metabolism has also been suggested (23). This involvement of intracellular Ca^{2+} concentrations in initiation of the respiratory burst is also suggested by recent studies
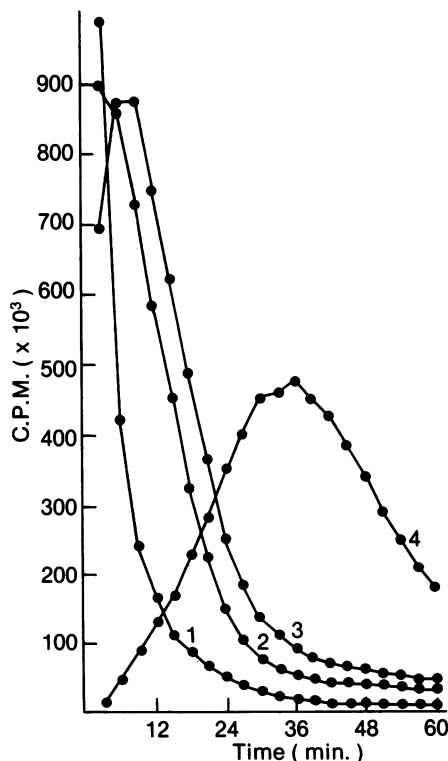

FIG. 7. Effect of concentration of A23187 on the luminol-dependent chemiluminescence of PMN. Ca^{2+} and Mg^{2+} were present in all vials to a final concentration of 1.0 mM. Curve 1, 1 μM A23187; curve 2, 0.10 μM; curve 3, 0.05 μM; curve 4, 0.10 μM. The data are from a single experiment which is representative of two separate experiments.
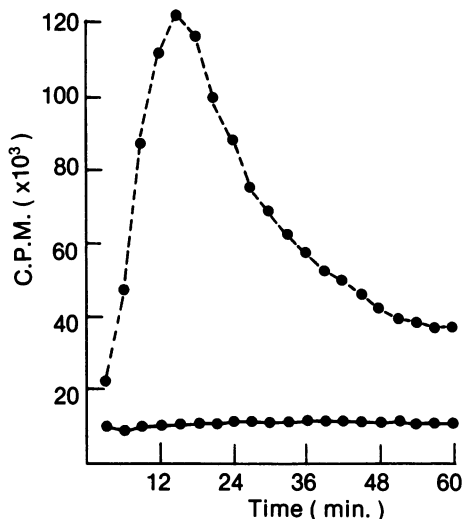
FIG. 8. Effect of divalent cations on the chemilu-minescence of neutrophils stimulated with 0.01 µM A23187. Dotted line, Ca^{2+} and Mg^{2+} present to a final concentration of 1.0 mM each; solid line, Ca^{2+} and Mg^{2+} omitted. The addition of Mg^{2+} alone gave results which were superimposable on those obtained in the absence of both cations. Results are from a single experiment which is representative of two separate experiments.

employing TMB-8, a compound purported to chelate intracellular Ca^{2+} (21). In the presence of TMB-8, but not of EGTA, the PMA-dependent generation of superoxide anion was found to be markedly inhibited. Caution must be exercised in interpreting such data based on inhibitor studies, however, since there is no assurance that the compound is acting only via the intracellular calcium pool.

ACKNOWLEDGMENTS

LITERATURE CITED

1. **Allen, R. C., R. L. Stjernholm, and R. H. Steele.** 1972. Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bactericidal activity. Biochem. Biophys. Res. Commun. **47:**679–684.
2. **Babior, B. M.** 1978. Oxygen-dependent microbial killing by phagocytes. N. Engl. J. Med. **298:**659–668 and 721–725.
3. **Barthélemy, A., R. Paridaens, and E. Schell-Frederick.** 1977. Phagocytosis-induced ^{45}calcium efflux in polymorphonuclear leukocytes. FEBS Lett. **82:**283–287.
4. **Blaedel, W. J., and V. W. Meloche.** 1963. Elementary quantitative analysis, 2nd ed., p. 576. Harper & Row, Publishers, New York.
5. **Cheson, B. B., R. L. Christensen, R. Sperling, B. E. Kohler, and B. M. Babior.** 1976. The origin of the chemiluminescence of phagocytizing granulocytes. J. Clin. Invest. **58:**789–796.
6. **Cohen, M. S., J. A. Metcalf, and R. K. Root.** 1980. Regulation of oxygen metabolism in human granulocytes: relationship between stimulus binding and oxidative response using plant lectins as probes. Blood **55:**1003–1010.
7. **DeChatelet, L. R.** 1978. Initiation of the respiratory burst in human polymorphonuclear neutrophils: a critical review. RES J. Reticuloendothel. Soc. **24:**73–91.
8. **DeChatelet, L. R., and M. R. Cooper.** 1970. A modified procedure for the determination of leukocyte alkaline phosphatase. Biochem. Med. **4:**61–68.
9. **DeChatelet, L. R., P. Shirley, and R. B. Johnston, Jr.** 1976. Effect of phorbol myristate acetate on the oxidative metabolism of human polymorphonuclear leukocytes. Blood **47:**545–554.
10. **Goldstein, I. M., J. K. Horn, H. B. Kaplan, and G. Weissmann.** 1974. Calcium-induced lysozyme secretion from human polymorphonuclear leukocytes. Biochem. Biophys. Res. Commun. **60:**807–812.
11. **Hoffstein, S.** 1979. Ultrastructural demonstration of calcium loss from local regions of the plasma membrane of surface-stimulated human granulocytes. J. Immunol. **123:**1395–1402.
12. **Korchak, H. M., and G. Weissmann.** 1978. Changes in membrane potential of human granulocytes antecede the metabolic responses to surface stimulation. Proc. Natl. Acad. Sci. U.S.A. **75:**3818–3822.
13. **Lehmeyer, J. E., R. Snyderman, and R. B. Johnston, Jr.** 1979. Stimulation of neutrophil oxidative metabolism by chemotactic peptides: influence of calcium ion concentration and cytochalasin B and comparison with stimulation by phorbol myristate acetate. Blood **54:**35–45.
14. **McPhail, L. C., L. R. DeChatelet, and R. B. Johnston, Jr.** 1979. Generation of chemiluminescence by a particulate fraction isolated from human neutrophils. Analysis of molecular events. J. Clin. Invest. **63:**648–655.
15. **Naccache, P. H., M. Volpi, H. J. Showell, E. L. Becker, and R. I. Sha'afi.** 1979. Chemotactic factor-induced release of membrane calcium in rabbit neutrophils. Science **203:**461–463.
16. **Nelson, R. D., M. J. Herron, J. R. Schmidtke, and R. L. Simmons.** 1977. Chemiluminescence response of human leukocytes: influence of medium components on light production. Infect. Immun. **17:**513–520.
17. **Newburger, P. E., M. E. Chovaniec, and H. J. Cohen.** 1980. Activity and activation of the granulocyte superoxide-generating system. Blood **55:**85–92.
18. **Prendergast, E., and R. A. Proctor.** 1981. Simple procedure for measuring neutrophil chemiluminescence. J. Clin. Microbiol. **13:**390–392.
19. **Romeo, D., G. Zabucchi, N. Miani, and F. Rossi.** 1975. Ion movement across leukocyte plasma membrane and excitation of their metabolism. Nature (London) **253:**542–543.
20. **Schell-Frederick, E.** 1974. Stimulation of the oxidative metabolism of polymorphonuclear leukocytes by the calcium ionophore A23187. FEBS Lett. **48:**37–40.
21. **Smith, R. J., and S. S. Iden.** 1981. Modulation of human neutrophil superoxide anion generation by the calcium antagonist 8-(N,N-diethylamino)-octyl-(3,4,5-trimethoxy) benzoate hydrochloride. RES J. Reticuloendothel. Soc. **29:**215–225.
22. **Smith, R. J., and L. J. Ignarro.** 1975. Bioregulation of lysosomal enzyme secretion from human neutrophils: roles of guanosine-3':5'-monophosphate and calcium in stimulus-secretion coupling. Proc. Natl. Acad. Sci. U.S.A. **72:**108–112.
23. **Taheshige, K., and S. Minakami.** 1981. Involvement of calmodulin in phagocytotic respiratory burst of leukocytes. Biochem. Biophys. Res. Commun. **99:**484–490.
24. **Westrick, M. A., P. S. Shirley, and L. R. DeChatelet.** 1980. Generation of chemiluminescence by human neutrophils exposed to soluble stimuli of oxidative metabolism. Infect. Immun. **30:**385–390.
25. **Whitin, J. C., C. E. Chapman, E. R. Simons, M. E. Chovaniec, and H. J. Cohen.** 1980. Correlation between

membrane potential changes and superoxide production in human granulocytes stimulated by phorbol myristate acetate. J. Biol. Chem. 225:1874–1878.

26. Wilson, M. E., M. A. Trush, K. Van Dyke, and W. Neal. 1978. Induction of chemiluminescence in human polymor-

phonuclear leukocytes by the calcium ionophore A23187. FEBS Lett. 94:387–390.

27. Zabucchi, G., and D. Romeo. 1976. The dissociation of exocytosis and respiratory stimulation in leukocytes by ionophores. Biochem. J. 156:209–213.