Prostaglandins E_1 and E_2 enhance the stimulation of superoxide release by 1-oleoyl-2-acetylglycerol from human neutrophils

Adrienne Penfield and M. Maureen Dale*

Department of Pharmacology, University College London, Gower Street, London WC1E 6BT, England

Received 23 December 1984

Superoxide release from human neutrophils was stimulated either by receptor activation (using fMet-Leu-Phe) or by activating, independently, each of the two pathways considered to be involved in signal transduction – calcium mobilization (using the ionophore, A23187) and protein kinase C activation (using phorbol myristate acetate or 1-oleoyl-2-acetylglycerol). Prostaglandin E_1 (3×10^{-5} M) decreased fMet-Leu-Phe-stimulated superoxide release, had no effect on superoxide release stimulated by A23187, or by phorbol myristate acetate, and markedly enhanced the superoxide release stimulated by 1-oleoyl-2-acetylglycerol. Similar enhancement was obtained with prostaglandin E_2.

Neutrophil *Phorbol ester* *Protein kinase C* *Prostaglandin E* *fMet-Leu-Phe* *Phosphatidylinositol*

1. INTRODUCTION

Neutrophils, when activated by soluble stimulants or during phagocytosis, as occurs in areas of inflammation, manifest an increased uptake of O_2 and generate substantial amounts of superoxide (O_2^-), hydrogen peroxide and other O_2 products. This process is important in oxygen-dependent microbial killing (review [1]) and may also be implicated in cell and tissue damage [2]. However, as has been emphasized in a recent review [3], the events involved in stimulus-activation coupling for the respiratory burst are not yet fully understood.

Prostaglandins E_1 and E_2, which are thought to be produced in inflammatory reactions, have been reported to inhibit superoxide release from neutrophils when the stimulus is fMet-Leu-Phe [4–6], but not when the stimulus is PMA [5,6]. The effect of these prostaglandins correlates with an increase of cyclic AMP [4,6] but the site of action of these agents on the transduction mechanism is not known.

It has recently been postulated that in many cells, two pathways may be involved in receptor-mediated signal transduction: (i) the generation of diacylglycerol which activates protein kinase C, and (ii) an increase in cytosolic calcium – both pathways being initiated, possibly, by the breakdown of polyphosphoinositides (review, [7,8]). Some evidence that the model proposed may be applicable to signal transduction by fMet-Leu-Phe in neutrophils has been reported [9–12]. Each pathway can be independently activated – the calcium pathway by a calcium ionophore, such as A23187, and the protein kinase C pathway by either PMA or OAG, which can substitute for endogenous diacylglycerol (review, [7]). Evidence that both pathways may be involved in O_2^- generation in neutrophils has recently been provided, using, as the protein kinase C activator, PMA [13–15] or OAG [16]. Here, we compared the ef-

* To whom correspondence should be addressed

Abbreviations: OAG, 1-oleoyl-2-acetylglycerol; DAG, diacylglycerol; PMA, phorbol myristate acetate; PGE_1, prostaglandin E_1

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

fect of PGE_1 on O_2^- generation produced by receptor activation (using fMet-Leu-Phe) with O_2^- generation produced by activating, independently, each of the pathways specified above. We report that PGE_2 had different effects in each case and the results might provide a clue as to its site of action in stimulus-activation coupling.

2. MATERIALS AND METHODS

Neutrophils were collected from human volunteers by venipuncture, and prepared as in [15]. The cells were equilibrated for 20 min at 37°C; those cells which were to be stimulated either by FMLP or A23187 were incubated with cytochalasin B (10 μg/ml) for a further 10 min. Following this, 2.5×10^6 cells were dispensed into 2.5 ml tubes (Sterilin, NA2S) to which had been added 1 mg ferricytochrome *c* (horse heart type III, Sigma), appropriate dilutions of the stimulant (FMLP, OAG, A23187 or PMA), 3×10^{-5} M PGE_1 (or Tyrode solution, in control tubes) and either Tyrode solution or 75 units superoxide dismutase (bovine blood, Sigma). The final calcium concentration in all samples was 3 mM. After 30 min incubation at 37°C, the reaction was stopped and the amount of O_2^- generated measured as in [15]. All reagents were obtained from Sigma, except OAG which was synthesized by Dr A. Watts, Biochemistry Department, University of Oxford, England.

3. RESULTS

In 4 experiments, PGE_1 (3×10^{-5} M) markedly reduced the O_2^- release stimulated by 10^{-7} M fMet-Leu-Phe (fig.1a). The difference between the test readings (with PGE_1) and control readings was statistically significant at the level $p = 0.01$ on a paired-sample *t*-test. PGE_2 had a similar effect (not shown).

With OAG maximum O_2^- release was obtained at a concentration of either 50 or 100 μg/ml. In 6 experiments, PGE_1 (3×10^{-5} M) markedly enhanced submaximal superoxide release stimulated by a submaximal concentration of OAG, either 30 or 50 μg/ml (fig.1a), the difference between test and control readings being significant at $p = 0.001$ on a paired-sample *t*-test. In 3 out of the 5 experiments in which the full dose-response curve

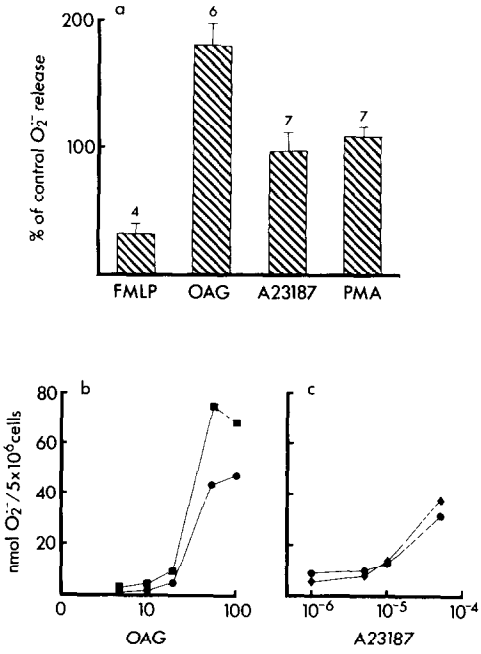

Fig.1. (a) The effect of PGE_1, 3×10^{-5} M, on the stimulation of superoxide release by submaximal concentrations of stimulants: fMet-Leu-Phe (10^{-7} M), OAG (30 or 50 μg/ml), A23187 (5×10^{-5} M) and PMA (10 ng/ml). The data are expressed as the percentage of control values (i.e., in the absence of PGE_1). The number of experiments with each stimulus is given above the columns. The mean control O_2^- release at the concentrations specified was 36.2 ± 2.6 nmol per 5×10^6 cells with fMet-Leu-Phe, 28.4 ± 3.7 with OAG, 22.0 ± 9.7 with A23187 and 59.6 ± 40 with PMA. (b) The dose-response curve of O_2^- release with OAG (μg/ml), in the absence (●) and presence (■) of PGE_2 (3×10^{-5} M). (c) The dose-response curve of O_2^- release with A23187 (M), in the absence (●) and presence (♦) of PGE_1 (3×10^{-5} M).

was investigated, the curve was not only shifted to the left in the presence of PGE_1, but the maximum response was increased. A similar result was obtained with PGE_2 (shown in fig.1b).

Seven experiments were carried out with PMA over a concentration range 1–50 ng/ml, maximum responses being obtained at 20–50 ng/ml in different experiments. In several experiments PGE_1 (3×10^{-5} M) caused an increase, sometimes marked, in O_2^- release with low concentrations of PMA, but the differences between test and control readings were not statistically significant at the level $p = 0.05$ in a paired-sample *t*-test. The results

with a submaximal concentration of PMA, 10 ng/ml, are shown in fig.1a.

In 7 experiments, PGE_1 (3×10^{-5} M) had no significant effect on the stimulation of superoxide release by A23187 (5×10^{-5} M) (fig.1a,c).

4. DISCUSSION

It is usually considered that the inhibitory action of prostaglandins such as PGE_1, PGE_2 and PGI_2 on O_2^- generation by neutrophils is produced mainly by increasing cyclic AMP [4,6]. It has been pointed out that these prostaglandins inhibit fMet-Leu-Phe-stimulated O_2^- release but not PMA-stimulated O_2^- release [5,6] and it has been suggested that the inhibition is due to modulation of calcium-dependent events [6]. There is also evidence for a mechanism of action involving a decrease in affinity of the formyl peptide receptor [17]. In this study, PGE_1 did not have any significant effect on A23187-stimulated O_2^- release, which could imply that it does not have much effect on calcium-dependent events.

A tentative hypothesis, based on the model of signal-transduction outlined in [7], which could explain the results obtained in this study with all four agents, would be that PGE_1 and PGE_2 modify the turnover of polyphosphoinositides by decreasing the conversion of DAG to phosphatidic acid through an action on DAG kinase. OAG is metabolized rapidly in situ, to its corresponding phosphatidic acid [7], presumably by DAG kinase. An inhibitory action on this enzyme would result in high, maintained concentrations of OAG and thus enhanced OAG responses. It would also decrease the effect of fMet-Leu-Phe by decreasing the availability of phosphatidylinositol biphosphate. The calcium pathway would be affected at a point prior to the rise in cytosolic calcium (i.e., by inhibition of production of inositol triphosphate) and thus there would be no effect on the response to ionophore-induced increase in cytosolic calcium.

PMA is metabolised very slowly [18]; neither its metabolism nor its effect should be affected by agents which modify inositol lipid turnover.

It has been shown, for platelets (see [7]) and lymphocytes [19] that PGE_1 inhibits signal-induction of both cell-activation and turnover of inositol phospholipids, the mechanism being thought to involve an increase in cyclic AMP. Furthermore, it has been reported that, in neutrophils, dibutyryl cyclic AMP selectively inhibits $^{32}P_i$ incorporation into phosphatidic acid [20]. There is evidence that, in platelets, the effect of increased cyclic AMP is, in large part, due to an action on phospholipase C [21,22]. However, the effect in platelets is clearly different from that in neutrophils in that a signal-induced increase in cyclic AMP in platelets is also associated with a marked reduction in the concomitant increase in Ca^{2+} [23] whereas in neutrophils this is not the case [24].

Stimulation of neutrophils with fMet-Leu-Phe has been reported to result in an increase of DAG in one study [25] but in another [26] it has been suggested that this may not occur. If this latter statement is correct, an explanation other than the one proposed here would be required for the PGE_1 effect on O_2^- generation by neutrophils stimulated with this peptide.

ACKNOWLEDGEMENT

This work was supported by the Emily le Rossignol Fund, UCL and The Arthritis and Rheumatism Fund. We wish to thank Dr A. Watts of the Biochemistry Department, Oxford University for the supply of OAG.

REFERENCES

[1] Badwey, J.A. and Karnovsky, M.L. (1980) Annu. Rev. Biochem. 49, 695–726.
[2] Johnston, R.B. and Lehmeyer, J. (1976) J. Clin. Invest. 57, 836–841.
[3] Babior, B.M. (1984) J. Clin. Invest. 73, 599–601.
[4] Simchowitz, L., Fischbein, L.C., Spilberg, I. and Atkinson, J.P. (1980) J. Immunol. 124, 1482–1491.
[5] Fantone, J.C. and Kinnes, D.A. (1983) Biochem. Biophys. Res. Commun. 113, 506–512.
[6] Fantone, J.C., Marasco, W.A., Elgas, L.J. and Ward, P.A. (1984) Am. J. Pathol. 115, 9–16.
[7] Nishizuka, Y. (1984) Science 225, 1365–1370.
[8] Berridge, M.J. and Irvine, R.F. (1984) Nature 312, 315–321.
[9] Serhan, C.N., Broekman, M.J., Korchak, H.M., Smolen, J.E., Marcus, A.J. and Weissman, G. (1983) Biochim. Biophys. Acta 762, 420–428.

[10] Volpi, M., Yassin, R., Naccache, P.H. and Shaafi, R.I. (1983) Biochem. Biophys. Res. Commun. 112, 957–964.
[11] Dougherty, R.W., Godfrey, P.P., Hoyle, P.C., Putney, J.W. and Freer, R.J. (1984) Biochem. J. 222, 307–314.
[12] Burgess, G.M., McKinney, J.S., Irvine, R.F., Berridge, M.J., Hoyle, P.C. and Putney, J.W. (1984) FEBS Lett. 176, 193–196.
[13] Robinson, J.M., Badwey, J.A., Karnovsky, M.L. and Karnovsky, M.J. (1984) Biochem. Biophys. Res. Commun. 122, 734–739.
[14] Di Virgilio, F., Lew, D.P. and Pozzan, T. (1984) Nature 310, 691–693.
[15] Dale, M.M. and Penfield, A. (1984) FEBS Lett. 175, 170–172.
[16] Penfield, A. and Dale, M.M. (1984) Biochem. Biophys. Res. Commun., in press.
[17] Fantone, J.C., Marasco, W.A., Elgas, L.J. and Ward, P.A. (1983) J. Immunol. 130, 1495–1497.
[18] Nishizuka, Y. (1984) Nature 308, 693–698.
[19] Kaibuchi, K., Takai, Y., Ogawa, Y., Kimura, S., Nishizuka, Y., Nakamura, T., Tomomura, A. and Ichihara, A. (1982) Biochem. Biophys. Res. Commun. 104, 105–112.
[20] Tou, J. and Maier, C. (1976) Biochim. Biophys. Acta 451, 353–362.
[21] Imai, A., Hattori, H., Takahashi, M. and Nozawa, Y. (1983) Biochem. Biophys. Res. Commun. 112, 693–700.
[22] Knight, D.E. and Scrutton, M.C. (1984) Nature 309, 66–68.
[23] Zavoico, G.B. and Feinstein, M.B. (1984) Biochem. Biophys. Res. Commun. 120, 579–585.
[24] De Togni, P., Cabrini, G. and Di Virgilio, F. (1984) Biochem. J. 224, 629–635.
[25] Volpi, M., Yassin, R., Tao, W., Molski, T.F.P., Naccache, P.H. and Shaafi, R.I. (1984) Proc. Natl. Acad. Sci. USA 81, in press.
[26] Cockcroft, S. (1984) Biochim. Biophys. Acta 795, 37–46.