Vol. 140, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1986 Pages 1-11

DOUBLE STIMULATION WITH FMLP AND Con A RESTORES THE ACTIVATION OF THE RESPIRATORY BURST BUT NOT OF THE PHOSPHOINOSITIDE TURNOVER IN Ca^{2+}-DEPLETED HUMAN NEUTROPHILS. A FURTHER EXAMPLE OF DISSOCIATION BETWEEN STIMULATION OF THE NADPH OXIDASE AND PHOSPHOINOSITIDE TURNOVER

Filippo ROSSI, Miroslawa GRZESKOWIAK and Vittorina DELLA BIANCA

INSTITUTE OF GENERAL PATHOLOGY, UNIVERSITY OF VERONA,
STRADA LE GRAZIE, 37134 VERONA, ITALY

Received August 4, 1986

The results reported here show that the activation of the NADPH oxidase in neutrophils by formyl-methionyl-leucyl-phenylalanine (FMLP) and concanavalin A (Con A) may occur with a stimulus response coupling sequence that bypasses the activation of phosphoinositide hydrolysis, monitored as accumulation of inositol phosphates and glycerophosphoinositol, and the increase in $[Ca^{2+}]_i$. In fact: i) in Ca^{2+}-depleted neutrophils FMLP and Con A do not induce the respiratory burst and the activation of phosphoinositide hydrolysis. The addition of Ca^{2+} restores both the respiratory and the phosphoinositide responses ; ii) the double treatment of Ca^{2+}-depleted neutrophils with FMLP and Con A in sequence, before FMLP and then Con A and viceversa, or simultaneously, restores the capacity to respond to the second stimulus with the respiratory burst but not with the activation of phosphoinositide hydrolysis. These findings suggest that, for the activation of the NADPH oxidase by FMLP and by Con A: i) the transduction pathway including the stimulation of phosphoinositide turnover, the Ca^{2+} changes and the activity of the protein kinase C is not required, or is not the unique, and ii) one stimulus may trigger more than one transduction pathway. Possible transduction pathways are discussed.

Several lines of evidence suggest that the stimulation of phosphoinositide turnover, the increase in $[Ca^{2+}]_i$ and the activation of protein kinase C play an essential role in the transduction reactions for the activation of the NADPH oxidase, the enzyme responsible for the free radicals producing respiratory burst in leukocytes: a) leukocyte response induced by chemotactic peptides, Con A and calcium-mobilizing stimuli is constantly

Abbreviations used: FMLP, formyl-methionyl-leucyl-phenylalanine; Con A, Concanavalin A; OAG, 1-oleyl-2-acetyl-glycerol; DAG, 1,2-diacylglycerol; IP_3, inositol trisphosphate; IP_2, inositol bisphosphate; IP, inositol monophosphate; IPs, inositol phosphates; PIP_2, phosphatidylinositol 4,5-bisphosphate; PMA, phorbol 12-mirystate 13-acetate; TCA, trichloroacetic acid; 5-L-HETE, 5-hydroxicosatetrenoate.

0006-291X/86 $1.50

associated with the activation of phosphodiesteric hydrolysis of phosphatidylinositol 4,5-bisphosphate (1-10) with production of diacylglycerol, a physiological activator of the protein kinase C, and of inositol trisphosphate, responsible for the increase in $[Ca^{2+}]_i$; b) activators of the protein kinase C as phorbol esters, 1-oleyl-2-acetyl-glycerol (OAG) and fatty acids (11-13) induce in leukocytes a respiratory burst (14-16). Furthermore FMLP, acts synergistically with OAG in eliciting the respiratory burst (17); c) the respiratory response is often associated with translocation of the protein kinase C from the cytosol to the plasma membrane (18-20) and with increase in protein phosphorylation (21-24).

Recent data obtained in our laboratory question the above sequence of transduction reactions showing that the activation of phosphoinositide turnover and the increase in $[Ca^{2+}]_i$ may be dissociated from the respiratory burst by FMLP (25,26). In fact, i) pretreatment of neutrophils with non stimulatory doses of PMA depresses the activation of phosphoinositide turnover and $[Ca^{2+}]_i$ change, while potentiates the respiratory burst by FMLP (25); ii) the pretreatment of Ca^{2+}-depleted neutrophils, with non stimulatory doses of PMA restores the activation of the respiratory burst but not that of the turnover of phosphoinositides by FMLP (26).

The results reported in this paper are a further and stronger evidence that the activation of the NADPH oxidase by FMLP and Con A may occur independently of the increase in phosphodiesteric hydrolysis of phosphoinositides and in $[Ca^{2+}]_i$. In fact, the synergistic activity of FMLP and Con A allows these agonists to stimulate a respiratory burst in Ca^{2+}-depleted neutrophils in the absence of any changes of phosphoinositide metabolism.

MATERIALS AND METHODS

Materials. Quin-2 AM was purchased from Calbiochem-Behringer (La Jolla, CA); [myo-2-^{3}H]inositol (10-20 Ci/mmol) from Amersham; AG 1-X8 resin from Biorad; FMLP, Con A, cytochalasin B, were purchased from Sigma. Pertussis toxin was kindly provided by dr. R. Rappuoli from Centro Ricerche Sclavo (Siena-Italy).
Neutrophil preparation. Human neutrophils were prepared from venous blood of healthy donors as in (10). The cells were resuspended in a Ca^{2+}-free Hank's balanced salt solution containing 20 mM Hepes and 5.6 mM glucose (pH 7.4).
Preparation of labelled and Ca^{2+}-depleted human neutrophils. Human neutrophils (20×10^7/ml) were labelled for 2 h at 37°C with 30µCi/ml [^{3}H]inositol in the presence of 0.025% bovine serum albumin. During the second hour 1mM EGTA and 60µM quin-2 were

added to the incubation medium according to (27) in order to deplete the cells of Ca^{2+}. At the end of incubation the cells were washed twice and resuspended in Hank's balanced salt solution containing 1 mM EGTA and 10mM LiCl to a final concentration of $3x10^7$/ml. This treatment results in a decrease of $[Ca^{2+}]_i$ to < 10nM, monitored according to (28).

Metabolics studies. O_2 consumption was measured at 37°C with a Clark oxygen electrode using $3x10^7$ neutrophils/ml in Hank's balanced salt solution containing 1mM KCN and 5µg/ml cytochalasin b, and when required, 2 mM $CaCl_2$ added 5 min. before the stimulants. In some experiments neutrophils were pretreated with pertussis toxin (1 µg/ml) for 2 h at 37°C, then depleted of calcium with quin-2 and EGTA as described before.

The phosphoinositide turnover was investigated by measuring the accumulation of inositol phosphates according to (29), in the same conditions of incubation and treatment used for O_2 consumption. Briefly aliquots of cells suspensions were drawn and quenched with TCA, final 7.5%, and kept on ice. The sample were extracted four times with diethyl eter, neutralized with sodium-tetraborate (final pH 8.0) and resuspended in 2 ml of distilled water. The radioactive phosphate-esters were separated on AG resin (1-X8, 200-400 mesh formate form) exchange column. The column was eluted sequentially with water (for free [^{3}H]inositol); 5 mM Na-tetraborate/60 mM sodium formate (for glycerophospho[^{3}H]-inositol); ; 0.1-M formic acid/0.2-M ammonium formate (for [^{3}H]IP); 0.1-M formic acid/0.5-M ammonium formate (for [^{3}H]IP_2); 0.1-M formic acid/1-M ammonium formate (for [^{3}H]IP_3). Samples of 1 ml of each eluate were taken for liquid-scintillation counting.

RESULTS

Double treatment with FMLP and Con A restores the respiratory responsiveness of Ca^{2+}-depleted neutrophils

Fig. 1 and table I show that in human neutrophils, depleted of Ca^{2+} by Quin-2 loading in Ca^{2+}-free medium containing EGTA , FMLP and Con A do not induce the activation of the respiratory burst. The addition of 2 mM Ca^{2+} restores the respiratory responsiveness to FMLP and Con A. The Ca^{2+}-restored respiratory burst by FMLP is inhibited by pertussis toxin, while that by Con A is only slightly sensitive as previously reported (6).

Fig. 1 and table I show also that Ca^{2+}-depleted neutrophils, firstly exposed to (primed with) FMLP or Con A, recover the capacity to respond with a respiratory burst to the subsequent addition of Con A and FMLP respectively. The characteristics of these respiratory bursts, in terms of maximal velocity and of onset (lag time) of oxygen consumption, are similar to those induced by the single treatment in presence of Ca^{2+}. Furthermore, the duration of the respiratory response by FMLP in Con A pretreated neutrophils is longer than that by FMLP alone in presence of Ca^{2+}. The respiratory responsiveness of Ca^{2+}-depleted neutrophils is restored also when the two stimuli are added

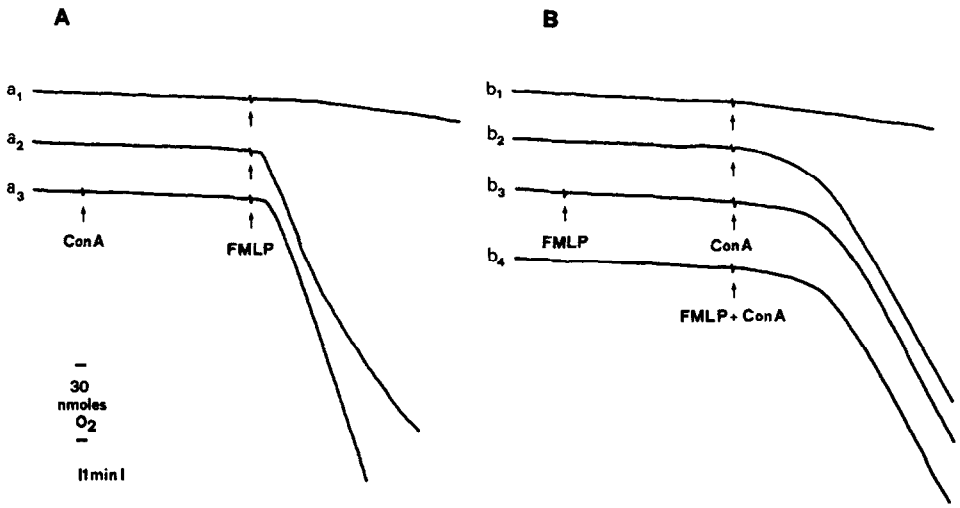

Fig.1 Poloragraphic traces of the O_2 consumption by $3x10^7$ human neutrophils.
A) Stimulation with 50 nM FMLP: a_1) in the absence of Ca^{2+}; a_2) in the presence of 2 mM Ca^{2+}; a_3) in the absence of Ca^{2+} after pretreatment with 100 µg/ml Con A.
B) Stimulation with 100 µg/ml Con A: b_1) in the absence of Ca^{2+}; b_2) in the presence of 2 mM Ca^{2+}; b_3 in the absence of Ca^{2+} after pretreatment with 50 nM FMLP; b_4) in the absence of Ca^{2+} simultaneously with 50 nM FMLP.

simultaneously. In this case the characteristics of the respiratory burst are similar to those of the burst by Con A alone in presence of Ca^{2+}, indicating that the priming agonist is FMLP.

Table I

Respiratory burst by FMLP and Con A in Ca^{2+}-depleted human neutrophils

Stimulus	Second stimulus	O_2 Consumption (nmoles/min/$3x10^7$ cells) Control	Pertussis Toxin
FMLP	--	2.8 ± 1.1 (14)	---
FMLP (Ca^{2+})	--	52.5 ± 10.3 (14)	3.4 ± 1.2 (3)
Con A	--	2.7 ± 1.4 (14)	---
Con A (Ca^{2+})	--	37.3 ± 9.4 (14)	32.8 ± 6.0 (3)
FMLP *	Con A	33.7 ± 5.0 (14)	6.7 ± 1.3 (3)
Con A *	FMLP	48.2 ± 8.0 (14)	7.2 ± 1.8 (3)
FMLP + Con A simultaneously		29.0 ± 4.2 (5)	---

Where indicated 2 mM Ca^{2+} was added 5 min before the stimulants.
* Cells were exposed to the first stimulus for 2-4 min.
Neutrophils were treated with pertussis toxin as described in materials and methods. For other conditions see fig. 1. Data are means ± S.D. of the experiments indicated in the brackets.

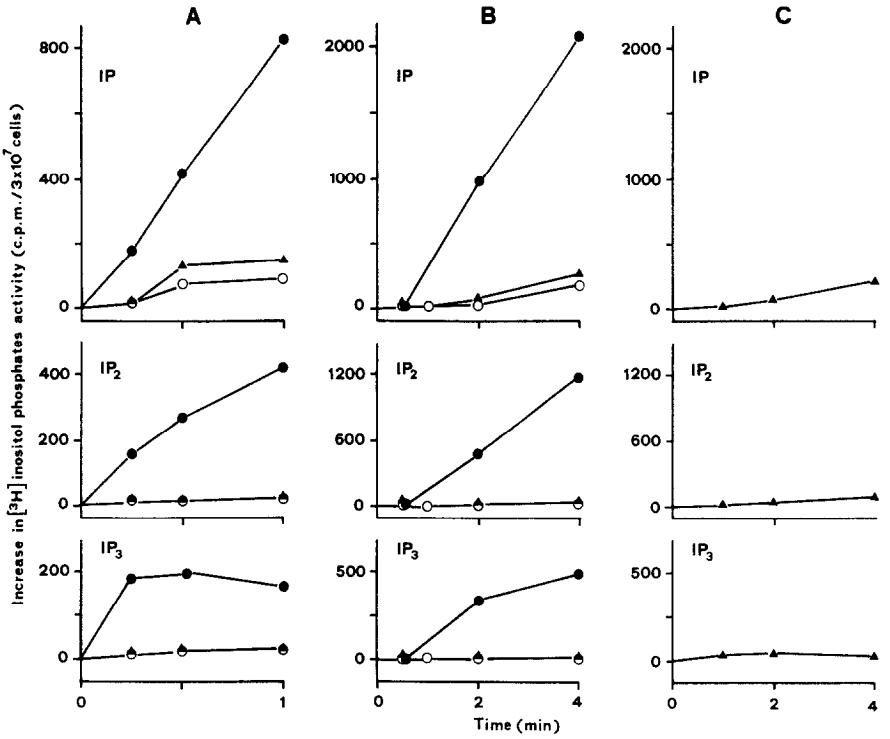

Fig.2 Time course of [^{3}H]inositol phosphates formation in human neutrophils.
Panel A. Stimulation with 50 nM FMLP: (●—●) in the presence of 2 mM Ca^{2+}; (○—○) in the absence of Ca^{2+}; (▲—▲) in the absence of Ca^{2+} after pretreatment with 100 µg/ml Con A for 4 min.
Panel B. Stimulation with 100 µg/ml Con A: (●—●) in the presence of 2 mM Ca^{2+}; (○—○) in the absence of Ca^{2+}; (▲—▲) in the absence of Ca^{2+} after pretreatment with 50 nM FMLP for 4 min.
Panel C. Stimulation with FMLP and Con A added simultaneously in the absence of Ca^{2+}. Basal values of [^{3}H]IP, [^{3}H]IP_2, [^{3}H]IP_3 were 2100, 550, 216 and 1800, 272, 118 cpm in the presence and absence of Ca^{2+} respectively. Data are of one experiment representative of four.

In all the conditions, when the first stimulus is FMLP and the second is Con A and viceversa, the respiratory burst requires cytochalasin B and is inhibited by pertussis toxin.

These results i) confirm that the sequence of transduction reactions for the activation of NADPH oxidase by FMLP and by Con A requires Ca^{2+}; ii) show that this requirement may be bypassed; iii) demonstrate that the Ca^{2+}-bypassing sequence involves a GTP binding protein also when the final stimulus is Con A, which usually does not require this step.

Double treatment of Ca^{2+}-depleted neutrophils with FMLP and Con A does not restore the phosphoinositide response

With the aim to understand the stimulus-response coupling sequence of this Ca^{2+}-independent respiratory burst we have

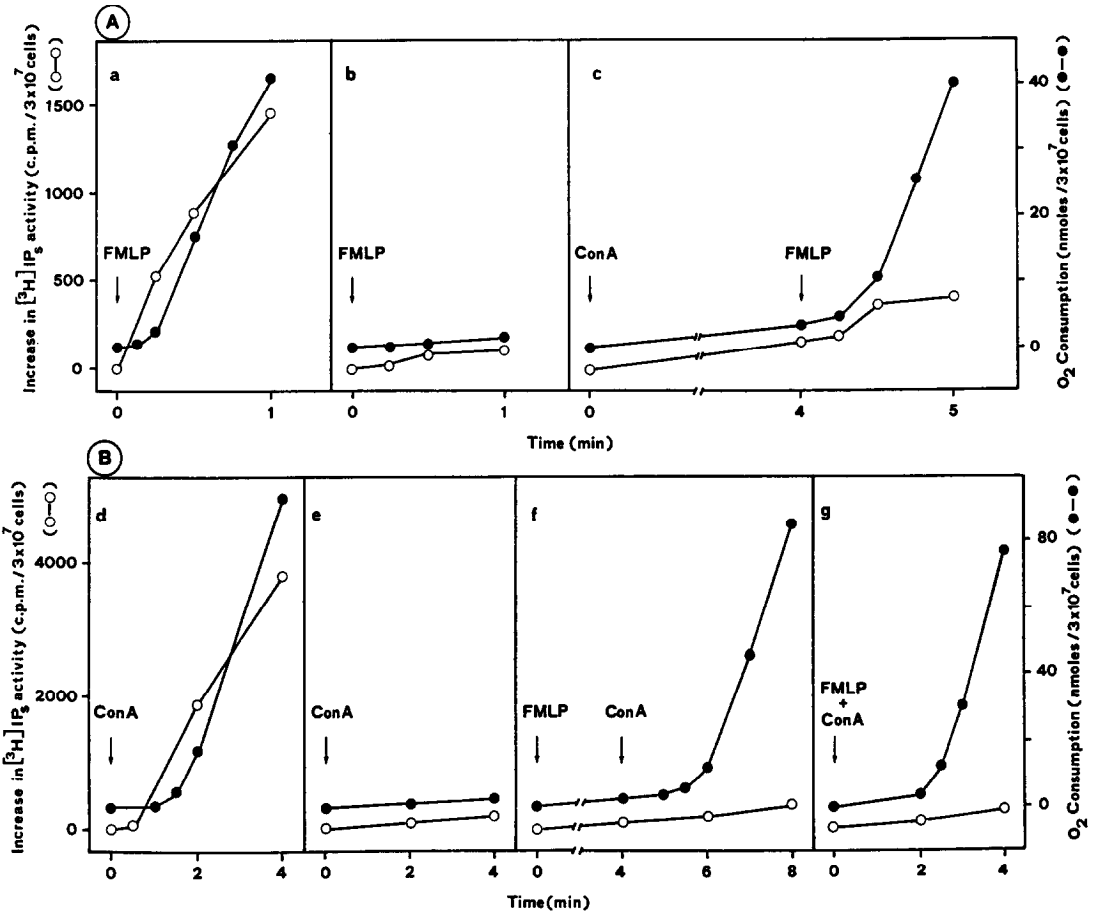

Fig.3 Relationship between the respiratory burst (●—●) and stimulation of [³H]IPs formation (○—○).
A) Neutrophils stimulated with 50 nM FMLP: a) in the presence of 2 mM Ca^{2+}; b) in the absence of Ca^{2+}; c) in the absence of Ca^{2+} after pretreatment with 100 µg/ml Con A.
B) Neutrophils stimulated with 100 µg/ml Con A: d) in the presence of 2 mM Ca^{2+}; e) in the absence of Ca^{2+}; f) in the absence of Ca^{2+} after pretreatment with 50 nM FMLP; g) in the absence of Ca^{2+} simultaneously with 50 nM FMLP. Basal values of [³H]IPs were 2800 and 2100 cpm in the presence and absence of Ca^{2+} respectively. Data are of one experiment representative of four.

investigated the behaviour of the phosphoinositide response by measuring the formation of IPs, the product of the activation of a phospholipase C, and of glycerophosphoinositol the product of phosholipases A. Fig. 2 reports the formation of the single classes of IPs, fig. 3 the correlation between formation of IPs and O_2 consumption, and fig. 4 the formation of glycerophosphoinositol. The data show that: i) The treatment of Ca^{2+}-depleted neutrophils with FMLP or Con A do not stimulate the formation of IPs and glycerophosphoinositol ; ii) when Ca^{2+} is

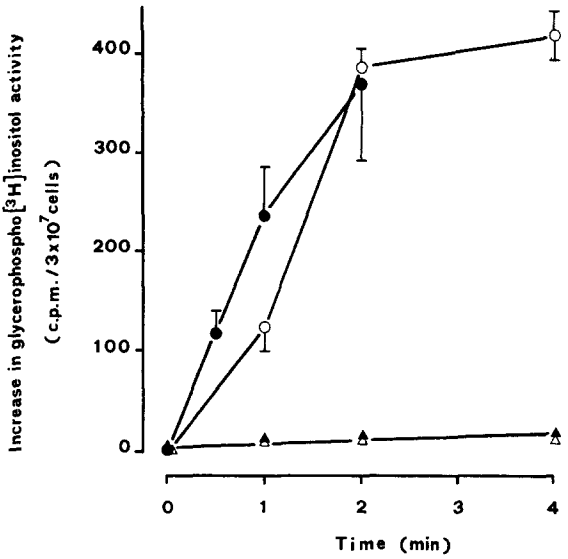

Fig.4 Time course of glycerolphospho[^{3}H]inositol formation in human neutrophils stimulated with 50 nM FMLP (●—●) and 100 µg/ml Con A (○—○) in the presence of 2 mM Ca^{2+}; with FMLP (△—△) after pretreatment with Con A; with Con A (▲—▲) after pretreatment with FMLP for 4 min. in absence of Ca^{2+}. Basal values of glycerophospho[^{3}H]inositol were 550 and 320 cpm in the presence and absence of Ca^{2+} respectively. Data are means ± S.D. of four experiments.

added to Ca^{2+}-depleted neutrophils, FMLP and Con A stimulate, beside the respiratory burst, the formation of IPs and glycerophosphoinositol; iii) the double treatment of Ca^{2+}-depleted neutrophils with FMLP and Con A, in sequence or simultaneously, while restores the activation of the respiratory burst does not restore the activation of the formation of IPs and glycerophosphoinositol.

These results demonstrate that the activation of respiratory burst by FMLP and Con A may occur with a sequence of transduction reactions that bypasses the activation of the hydrolysis of phosphoinositides by phospholipase C and by phospholipase A.

DISCUSSION

The first finding reported here is that the activation of the phosphoinositide response and of the respiratory burst by FMLP and by Con A requires Ca^{2+}, and that in the presence of Ca^{2+} the activation of the NADPH oxidase and the phosphoinositide turnover are strictly associated. These data agree with the sequence of transduction reactions involving the activation of a phospholipase

C with hydrolysis of PIP_2; the generation of IP_3 and DAG; and the activation of the protein kinase C (30,31).

The second finding reported in this paper is that with FMLP and Con A the activation of NADPH oxidase may occur independently of Ca^{2+} and of the activation of phosphoinositide response which generates IP_3 and DAG, provided that the neutrophils are primed by challenging with both the stimuli, either in sequence or simultaneously. We have previously reported that in neutrophils primed with the protein kinase C activator PMA the activation of the NADPH oxidase is dissociated from the Ca^{2+} changes and from the activation of phosphoinositide turnover (26). These data do not agree with the above sequence of transduction reactions for the activation of the NADPH oxidase.

The explanation of these contradictory results is that with FMLP and Con A the transduction pathway involving Ca^{2+} changes and the protein kinase C activated by products of phosphoinositide hydrolysis (1 of fig.5) either, is not required or is not unique. Some hypotheses may be advanced on the nature of these alternative pathways for the oxidative responses of Ca^{2+}-depleted neutrophils presented in this paper (2,3,4,5, of fig.5). a) The protein kinase C is activated by messengers (arachidonic acid and its derivatives, phosphatidic acid, DAG) produced independently of the phosphodiesteric hydrolysis of PIP_2 (2 of fig. 5) (32-38), or by protease (39). The fact that in our conditions the burst by double stimulation in Ca^{2+}-depleted neutrophils is not associated with an increase in the formation of glycerophosphoinositol excludes that fatty acids are formed by deacylation of phosphoinositides by phospholipases A_1 and A_2. Some of the above mentioned messengers (arachidonic acid, phosphatidic acid) are able to activate the protein kinase C (13,39), while others (endoperoxide and thromboxane A_2) are activators of phospholipase C (40), and others (5-L-HETE and leukotriene B_4) act sinergically with the protein kinase C stimulator DAG in the activation of leukocytes (41). It is worthy pointing out that the formation of the above messengers requires Ca^{2+}-dependent reactions, while the activation of the respiratory burst in the conditions presented here, occurs in the absence of Ca^{2+}. b) Alternatively, the recruitment of the protein kinase C could be due to mechanisms different from those which require DAG, fatty acids, phosphatidic acid (3 of Fig.5). c) Another hyphothesis is that the transduction pathway independent of Ca^{2+} and phosphoinositide turnover does not involve the protein

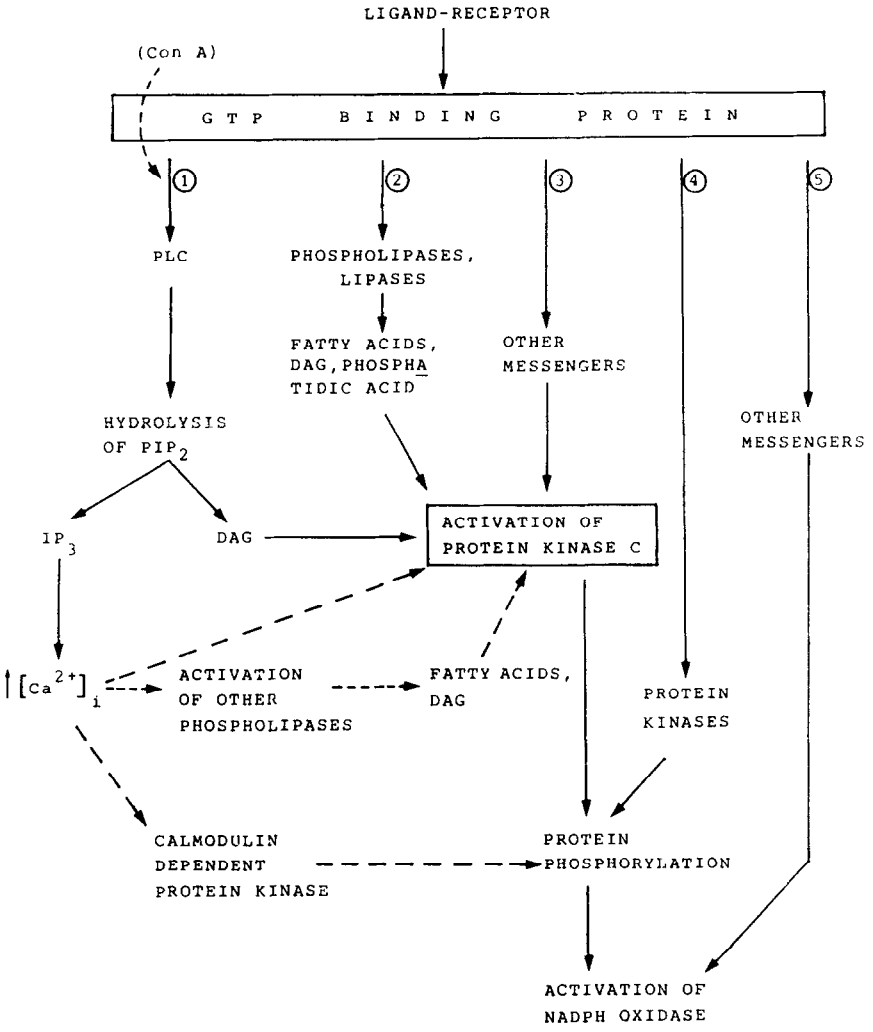

Fig.5 Possible transduction pathways for the activation of the NADPH oxidase by FMLP and Con A.
1) Pathway including GTP binding protein, phosphoinositide turnover, Ca^{2+} changes and protein kinase C. With Con A, the GTP binding protein is bypassed (dotted line).
2) 3) 4) 5) Pathways proposed on the basis of the findings presented in this paper, that the oxidase may be activated independently of Ca^{2+} and phosphoinositide turnover. For explanations see the text.

kinase C but other protein kinases (4 of Fig.5). In this case the phosphorylation remains the final reaction responsible, in some way, for the activation of the NADPH oxidase. d) The last hypothesis is that the pathway indepedent of Ca^{2+} and phosphoinositide turnover involves neither protein kinase C nor other kinases (5 or Fig.5). In this case the activation of the NADPH oxidase is due to other reactions different from the phosphorylation, while the stimulation of phosphoinositide

hydrolysis, the protein kinase C and Ca^{2+} changes are coupled with other responses and functions. Evidence has been presented that the protein kinase C is not required for the respiratory burst by chemotactic peptides (42-44), and it does not play a critical role in the initiation of other responses (45,46).

Whatever is the case, the results presented here and those previously reported by our group (25,26) suggest that one stimulus may trigger more than one transduction pathway for the activation of the NADPH oxidase, and agree with the view that an essential role of the protein kinase C in the responses of leukocytes should be reconsidered (42-46). For example, it has been proposed that this kinase has a negative role in the stimulation of phosphoinositide hydrolysis by phospholipase C in leukocytes (25,26) as in other cells (47,48).

Investigations are in progress in our laboratory on the validity of the above hypotheses and on the mechanisms by which the state of the cells and the conditions of stimulation may regulate the choice and the activity of the transduction pathway.

ACKNOWLEDGEMENTS

This work was supported by grants from Ministero Pubblica Istruzione (Fondo 40% al Gruppo Difese Biologiche, Metabolismo, Funzioni e Patologia dei Fagociti, Coordinatore Prof. F.Rossi) and CNR (Contributo n° 85.00720.04) - Gruppo Immunologia.

REFERENCES

1. Volpi,M., Yassin.R., Naccache,P.H. and Sha'afi,R.I. (1983) Biochem. Biophys. Res. Commun. 112, 957-964.
2. Dougherty,R.W., Gofrey,P.P., Hoyle,P.C., Putney,J.W.Jr. and Freer, R.J. (1984) Biochem. J. 222, 307-314.
3. Cockcroft,S., Barrowman,M.M. and Gomperts,B.D. (1985) Febs Lett. 181, 259-263.
4. Bradford,P.G. and Rubin,R.P. (1985) Molecular Pharm. 27, 74-78.
5. Di Virgilio,F., Vicentini,L.M., Treves,S., Riz,G. and Pozzan,T. (1985) Biochem. J. 229, 361-367.
6. Verghese,M.W., Smith,C.D. and Snyderman,R. (1985) Biochem. Biophys. Res. Commun. 127, 450-457.
7. Otha,H., Okajima,F., and Ui,M. (1985) J. Biol. Chem. 260,15771-15780
8. Krauze,K.-H., Schlegel,W., Wollheim,C.B., Andersson,T., Waldvogel,F.A. and Lew,P.D. (1985) J. Clin. Invest. 76, 1348-1354.
9. Naccache,P.H., Molski,M.M., Volpi, M., Becker,E.L. and Sha'afi,R.I. (1985) Biochim. Biophys. Res. Commun. 130, 677-684.
10. Grzeskowiak,M., Della Bianca,V., De Togni,P., Papini,E. and Rossi,F. (1985) Biochim. Biophys. Acta 844, 81-90.
11. Castagna,M., Takai,Y., Kaibuchi,K., Sano,K., Kikkawa,U. and Nishizuka,Y. (1982) J. Biol. Chem. 257, 7847-7851.
12. Mori,T., Takai,Y., Yu,B., Takahashi,J., Nishizuka,Y. and Fujikura,T. (1982) J. Biochem. 91, 427-431.
13. McPhail,L.C., Clayton,C., Snyderman,R. (1984) Science 224,622-624.
14. De Chatelet,L.R., Shirley,P.S. and Johnston,R.B.Jr. (1976) Blood 47, 545-554.

15. Fujita,I., Irita,K., Takeshige,K. and Minakami,S. (1984) Biochem. Biophys. Res. Commun. 114, 638-645.
16. Badway,J.A., Curnutte,J.T., Robinson,J.M., Berde,C.B., Karnovsky,M.J. and Karnovsky,M.L. (1984) J. Biol. Chem. 259, 7870-7877.
17. Dewald,B., Payne,T.G. and Baggiolini,M. (1984) Biochem. Biophys. Res. Commun. 125, 367-373.
18. Wolfson,M., McPhail,L.C. and Snyderman,R. (1985) J. Immunol. 135, 2057-2062.
19. Gennaro,R., Florio,C. and Romeo,D. (1986) Biochem. Biophys. Res. Commun. 134, 305-312.
20. Melloni,E., Pontremoli,S., Michetti,M., Sacco,O., Sparatore,B. and Horecker, B.L. (1986) J. Biol. Chem. 261, 4101-4105.
21. Schneider,C., Zanetti,M. and Romeo,D. (1981) Febs Lett. 127,4-8.
22. Andrews,P.C. and Babior,B.M. (1983) Blood 61, 333-340.
23. White,J.R., Huang,C.-K., Hill,J.M.Jr., Naccache,P.H., Becker,E.L. and Sha'afi, R.I. (1984) J. Biol. Chem. 259, 8605-8611.
24. Irita,K., Takeshige,K. and Minakami,S. (1984) Biochim. Biophys. Acta 805,44-52.
25. Della Bianca,V., Grzeskowiak,M., Cassatella,M.A., Zeni,L. and Rossi,F. (1986) Biochem. Biophys. Res. Commun. 135, 556-565.
26. Grzeskowiak,M., Della Bianca,V., Cassatella,M.A. and Rossi.F. (1986) Biochem. Biophys. Res. Commun. 135, 785-794.
27. Di Virgilio,F., Lew,D.P. and Pozzan,T. (1984) Nature 310, 691-693.
28. Tsien.R.Y., Pozzan,T. and Rink, T.J. (1982) J. Cell. Biol. 94,325-33
29. Berridge,M.J., Dawson,R.M.C., Downes,C.P., Heslop,J.P. and Irvine,R.F. (1983) Biochem. J. 212, 473-482.
30. Nishizuka,Y. (1984) Nature 308, 693-698.
31. Berridge,M.J. and Irvine,R.F. (1984) Nature 312, 315-321.
32. Hirata,F., Corcoran,B.A., Venkatasubramanian,K., Schiffmann,E. and Axebrod,J. (1979) Proc. Natl. Acad. Sci. USA 76, 2640-2643.
33. Walsh,C.E., Waite,B.M., Thomas,M.J., DeChatelet,L.R. (1981) J. Biol. Chem. 256, 7228-7234.
34. Pike,M. and Snyderman,R. (1981) J. Immunol. 127, 1444-1449.
35. Borgeat,P. and Samuelsson,B. (1979) Proc. Natl. Acad. Sci. USA 76, 2148-2152.
36. Maclouf,J., DeLaclos,B.F., Borgeat,P. (1982) Proc. Natl. Acad. Sci. USA 79, 6042-6046.
37. Lindgren,J.A., Hansson,G., Samuelsson,B. (1981) Febs Lett. 128, 329-335.
38. Goldstein,J.M., Malmsten,C.L., Kindahl,H., Kaplan,H.B.,Radmark,O., Samuelsson,B. and Weissmann,G. (1978) J. Exp. Med 148, 787-792.
39. Takai,Y., Kishimoto,A., Iwasa,Y., Kawahara,Y., Mori,T. and Nishizuka,Y. (1979) J. Biol. Chem. 254, 3692-3695.
40. Siess,W., Siegel,F.L. and Lapetina,E.G. (1983) J. Biol. Chem. 258, 11236-11242.
41. O'Flaherty,J., Schmitt,J.D. and Wykle,R.L. (1985) Biochem. Biophys. Res. Commun. 127, 916-923.
42. Cooke,E. and Hallett,H.B. (1985) Biochem. J. 232, 323-327.
43. Gerard,C., McPhail,L.C., Marfat,A., Stimler-Gerard,N.P., Bass,D.A. and McCall,C.E. (1986) J. Clin. Invest. 77, 61-65.
44. Wright,C.D. and Hoffman,M.D. (1986) Biochem. Biophys. Res. Commun. 135, 749-755.
45. Naccache,P.H., Molski,M.M. and Sha'afi,R.I. (1985) Febs Lett. 193, 227-230.
46. Sha'afi,R.I., Molski,T.F.P., Huang,C.-K. and Naccache,P.H. (1986) Biochem. Biophys. Res. Commun. 137, 50-60.
47. Vicentini,L.M., Di Virgilio,F., Ambrosini,A., Pozzan,T. and Meldolesi.J. (1985) Biochem. Biophys. Res. Commun. 127, 310-317.
48. Watson,S.P. and Lapetina,E.G. (1985) Proc. Natl. Acad. Sci. USA 82, 2623-2626.