Guanine nucleotide regulation of phospholipase C activity in permeabilized rabbit neutrophils

Inhibition by pertussis toxin and sensitization to submicromolar calcium concentrations

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Rabbit neutrophils labelled with [^3H]inositol and permeabilized with saponin produced [^3H]inositol trisphosphate (InsP_3) when incubated with stable analogues of GTP or millimolar concentrations of Ca^{2+} . [^3H] InsP_3 production elicited by guanosine 5'-[γ -thio]triphosphate was enhanced by the chemoattractant formylmethionyl-leucyl-phenylalanine and inhibited by pertussis-toxin pretreatment. A pertussis-toxin-sensitive stimulation of [^3H] InsP_3 concentration was also observed with guanosine 5'-[$\beta\gamma$ -imido]triphosphate, but not with guanosine 5'-[β -thio]diphosphate or GTP. Millimolar Ca^{2+} alone was sufficient to stimulate [^3H] InsP_3 production; however, in the presence of guanosine 5'-[γ -thio]triphosphate, the Ca^{2+} dose-response curve was shifted to submicromolar concentrations. These findings directly confirm the role of a pertussis-toxin-sensitive guanine nucleotide regulatory protein (G protein) in chemoattractant-stimulated phospholipase C activity in rabbit neutrophils. Moreover, the ability of guanine nucleotides to sensitize phospholipase C to physiologically relevant Ca^{2+} concentrations suggests that the role of the activated G protein may be to enhance the apparent affinity of phospholipase C for Ca^{2+} and thus to activate the enzyme without an increase in the Ca^{2+} concentration.

INTRODUCTION

The accelerated turnover of inositol phospholipids is an essential reaction which precedes Ca^{2+} mobilization during stimulus-secretion coupling (Michell, 1975). Activation of a wide variety of Ca^{2+} -mobilizing receptors causes the breakdown of polyphosphoinositides, with the resultant formation of the cellular second messengers diacylglycerol and InsP_3 (Downes & Michell, 1982; Nishizuka, 1984; Berridge, 1984; Berridge & Irvine, 1984).

In neutrophils, an increased metabolic turnover of inositol lipids was first demonstrated by using cells which had been stimulated to undergo phagocytosis by presentation of starch particles (Karnovsky & Wallach, 1961; Sastry & Hokin, 1966). Subsequent studies of neutrophils which utilized the soluble chemoattractant peptide fMet-Leu-Phe as a stimulus showed increased incorporation of ^{32}P into phosphatidylinositol, which accompanied Ca^{2+} fluxes, superoxide generation and lysosomal enzyme secretion (Bennett *et al.*, 1980). The idea that phospholipase C-mediated hydrolysis of polyphosphoinositides is an early step in stimulus-secretion coupling in the neutrophil was substantiated by several laboratories (Volpi *et al.*, 1983; Yano *et al.*, 1983; Dougherty *et al.*, 1984; Bradford & Rubin, 1985a).

The transduction mechanism of this receptor-mediated response appears to be controlled by a guanine nucleotide regulatory protein, even though the target enzyme is not adenylate cyclase (Bokoch & Gilman, 1984; Okajima & Ui, 1984). This conclusion is based on the finding that the coupling of receptor activation by

fMet-Leu-Phe to phospholipase C stimulation in neutrophils can be inhibited by pertussis toxin (Bradford & Rubin, 1985b; Brandt *et al.*, 1985; Verghese *et al.*, 1985; Ohta *et al.*, 1985). This toxin specifically ADP-ribosylates a 41000-Da protein in neutrophil membranes that co-migrates with the α subunit of G_i of the adenylate cyclase system (Bokoch & Gilman, 1984; Okajima & Ui, 1984). Moreover, GTP analogues modulate the affinity of fMet-Leu-Phe for its receptor (Koo *et al.*, 1983; Okajima *et al.*, 1985a), and fMet-Leu-Phe stimulates guanine nucleotide exchange in a neutrophil membrane fraction (Lad *et al.*, 1985). Pertussis toxin inhibits both of these effects (Lad *et al.*, 1985), as well as the extent of guanine-nucleotide-sensitive high-affinity binding of radiolabelled fMet-Leu-Phe (Lad *et al.*, 1985; Okajima *et al.*, 1985b). These findings, taken together with the demonstrated stimulation by guanine nucleotides of polyphosphoinositide hydrolysis and inositol phosphate production in neutrophil plasma membranes (Cockcroft & Gomperts, 1985; Smith *et al.*, 1985), suggest the involvement in fMet-Leu-Phe-induced phospholipase C activation of a guanine nucleotide regulatory protein similar to G_i of the adenylate cyclase system.

In the present paper, we provide data directly linking a guanine nucleotide regulatory protein to fMet-Leu-Phe-mediated phospholipase C activation by demonstrating in permeabilized [^3H]inositol-labelled neutrophils that stable analogues of GTP stimulate [^3H] InsP_3 production through a process which is potentiated by fMet-Leu-Phe and Ca^{2+} and inhibited by pertussis toxin. We also show that GTP[S] shifts the dose-response of the Ca^{2+} -stimulated [^3H] InsP_3 production, suggesting an

Abbreviations used: fMet-Leu-Phe, formylmethionyl-leucyl-phenylalanine; InsP_3 , inositol trisphosphate; InsP_2 , inositol bisphosphate; GTP[S], guanosine 5'-[γ -thio]triphosphate; p[NH]ppG, guanosine 5'-[$\beta\gamma$ -imido]triphosphate; GDP[S], guanosine 5'-[β -thio]diphosphate.

action of the guanine nucleotide regulatory protein in facilitating Ca^{2+} activation of phospholipase C.

MATERIALS AND METHODS

Materials

[^3H]Inositol (15 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO, U.S.A.). [$\alpha\text{-}^{32}\text{P}$]NAD $^{+}$ was obtained from ICN Radiochemicals. [^3H]Ins(1,4,5) P_3 was obtained from Amersham Corp. (Arlington Heights, IL, U.S.A.). Pertussis toxin was obtained from List Biological Laboratories (Campbell, CA, U.S.A.). Dowex-1 anion-exchange resin and electrophoresis reagents were purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.). All other reagents were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN, U.S.A.) or Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Preparation of cells

Peritoneal neutrophils from New Zealand White rabbits were collected by heparinized-saline lavage 10–12 h after intraperitoneal injection of molluscan glycogen as described by Bradford & Rubin (1985a). Cells (> 95% neutrophils) were washed and resuspended (2×10^8 /ml) in a modified Hanks' balanced salts solution (pH 7.4) containing (mM): NaCl 124, KCl 4, Na_2HPO_4 0.64, KH_2PO_4 0.66, NaHCO_3 15, Hepes 10, MgCl_2 0.2, CaCl_2 0.5, glucose 5.6, and 0.025% (w/v) bovine serum albumin. Neutrophils (6×10^8 – 8×10^8) were labelled with [^3H]inositol (200–400 μCi) for 3 h in an agitator water bath under O_2/CO_2 (19:1). Where indicated, pertussis toxin was included during the final 90–120 min of incubation. The toxin treatment had no effect on [^3H]inositol incorporation into lipid.

Permeabilization of cells

After labelling with [^3H]inositol, the neutrophils were washed once in Hanks' buffer and then resuspended in a cytosolic-type medium of the following composition (mM): NaCl 20, KCl 100, MgSO_4 5, NaHCO_3 25, NaH_2PO_4 0.96, EGTA 1, CaCl_2 0.38; at pH 7.2 and 37 °C. The medium also contained albumin (0.2%), and the pH was maintained by equilibration with O_2/CO_2 (19:1). Saponin (80 $\mu\text{g}/\text{ml}$) was added to the cell suspension (6×10^7 cells/ml) and incubation was continued for 8–10 min until the cells (> 90%) were permeable to Trypan Blue. The cells were centrifuged (200 g for 4 min) and then resuspended (5×10^7 cells/ml) in the same cytosolic-type medium, but without added saponin. The calculated free [Ca^{2+}] of this cytosolic-type medium was 120 nM, which was equivalent to the cytosolic free [Ca^{2+}] of unstimulated rabbit neutrophils, as measured by quin2 fluorescence. The free [Ca^{2+}] was modified in the reaction buffers as required by varying the amount of CaCl_2 added (Burgess *et al.*, 1983). The permeabilized cells were also pretreated for 5 min with the mitochondrial inhibitors antimycin (10 μM), oligomycin (10 μM) and dinitrophenol (0.5 mM), followed by the addition of ATP (1.5 mM) and an ATP-regenerating system consisting of phosphocreatine (5 mM) and creatine kinase (10 units/ml). Initial studies showed that added ATP was necessary to maintain polyphosphoinositide concentrations and optimize Ins P_3 formation in permeabilized cells,

and so ATP and a regenerating system were included in all subsequent studies. The presence of mitochondrial inhibitors ensures that the observed responses are entirely those of permeabilized cells and not of remnant intact neutrophils (< 10%).

Measurement of [^3H]inositol phosphates

Saponin-treated [^3H]inositol-labelled neutrophils (200 μl) were added to test tubes containing cytosolic-type medium (300 μl) and guanine nucleotide, formyl-peptide or vehicle to yield the indicated concentrations. The reaction mixture also contained 2 mM-2,3-bisphosphoglycerate, an inhibitor of Ins P_3 5-phosphomonoesterase (Downes *et al.*, 1982). For time-course studies, appropriately larger volumes were used and samples were removed at the indicated times. Incubations were terminated by the addition of 2 vol. of ice-cold 4.5% (w/v) HClO_4 and, after the precipitation of protein, the supernatant was neutralized with a sufficient volume of 0.5 M-KOH/9 mM- $\text{Na}_2\text{B}_4\text{O}_7$ /1.9 mM-EDTA to give pH 8–9. Soluble [^3H]inositol phosphates were separated by anion-exchange chromatography, on Dowex-1 (formate form) resin (Berridge *et al.*, 1983). The separation methods were validated by using standard [^3H]Ins(1,4,5) P_3 . Amounts of [^3H]inositol phosphates were determined by liquid-scintillation spectrometry, and radioactivity was counted for sufficient time to ensure > 90% reliability in the measurements. Amounts of [^3H]Ins P_3 are expressed as percentage of total radioactivity in the lipid fraction.

[^{32}P]ADP-ribosylation

Membranes prepared from pertussis-toxin-treated and untreated neutrophils were incubated with [$\alpha\text{-}^{32}\text{P}$]NAD $^{+}$ and activated pertussis toxin, by the procedure described by Okajima *et al.* (1985b). Proteins were solubilized in SDS and separated by polyacrylamide-gel electrophoresis (Laemmli, 1970). After autoradiography, the 41000-Da region was cut from the gel and its radioactivity content determined by liquid-scintillation spectrometry.

RESULTS AND DISCUSSION

Effect of guanine nucleotides and fMet-Leu-Phe on [^3H]Ins P_3 accumulation

GTP[S] promoted a time- and dose-dependent increase in [^3H]Ins P_3 production and hence in phospholipase C activity, in saponin-permeabilized [^3H]inositol-labelled neutrophils (Fig. 1; Table 1). No effect of GTP[S] was observed with intact cells (results not shown). With permeabilized cells, a significant increase in [^3H]Ins P_3 was observed within 1 min in response to 100 μM -GTP[S]. [^3H]Ins P_3 continued to increase for 5–10 min, resulting in a 3-fold rise over basal values (Fig. 1). The time course of [^3H]Ins P_3 accumulation observed with permeabilized cells in response to GTP[S] contrasts with that observed after fMet-Leu-Phe stimulation of intact neutrophils, where a 3-fold increase in [^3H]Ins P_3 was observed within 10–20 s, and thereafter declined to basal values by 5–15 min (Bradford & Rubin, 1985a). The maintained concentrations of [^3H]Ins P_3 observed with permeabilized neutrophils may be ascribed to the diffusion of inositol phosphates and soluble metabolizing enzymes out of the leaky cells, thus decreasing the effective concentrations of

Fig. 1. Time course of stimulated $[^3\text{H}]\text{InsP}_3$ production by permeabilized neutrophils

Rabbit peritoneal neutrophils were prelabelled with $[^3\text{H}]\text{inositol}$ and permeabilized with saponin, as described in the Materials and methods section. The cells were then treated with buffer ($\blacktriangle$), 10 nM-fMet-Leu-Phe ($\circ$), 100 μM -GTP γS ($\square$), fMet-Leu-Phe plus GTP[S] ($\blacksquare$), or 500 μM -Ca $^{2+}$ ($\bullet$). Values are means $\pm$ S.E.M. for four independent experiments, except values for Ca $^{2+}$ -stimulated samples, which are means of two experiments. $[^3\text{H}]\text{Phosphoinositide}$ radioactivity averaged 116000 c.p.m./sample.

Table 1. Effect of pertussis toxin on $[^3\text{H}]\text{InsP}_3$ accumulation induced by guanine nucleotides or Ca $^{2+}$

$[^3\text{H}]\text{Inositol}$ -labelled neutrophils incubated for 90 min without or with pertussis toxin (200 ng/ml) were permeabilized and then treated for 5 min with the indicated stimulus. Values are means $\pm$ S.E.M. from four experiments, each done in duplicate. $[^3\text{H}]\text{Phosphoinositide}$ radioactivity averaged 226000 c.p.m./sample. * $P < 0.10$, ** $P < 0.05$, *** $P < 0.025$, **** $P < 0.005$, for toxin treatment versus control (Student's t test).

Treatment (μM)	$[^3\text{H}]\text{InsP}_3$ (% of $[^3\text{H}]\text{lipid}$)	
	Control	Pertussis toxin
Control	0.091 $\pm$ 0.010	0.093 $\pm$ 0.011
GTP[S] (1)	0.182 $\pm$ 0.009	0.139 $\pm$ 0.021*
GTP[S] (10)	0.246 $\pm$ 0.016	0.185 $\pm$ 0.028**
GTP[S] (100)	0.299 $\pm$ 0.013	0.220 $\pm$ 0.023****
p[NH]ppG (100)	0.165 $\pm$ 0.017	0.123 $\pm$ 0.017***
GTP (100)	0.117 $\pm$ 0.027	0.109 $\pm$ 0.025
GDP[S] (100)	0.115 $\pm$ 0.021	0.104 $\pm$ 0.020
Ca $^{2+}$ (180)	0.302 $\pm$ 0.043	0.262 $\pm$ 0.038
Ca $^{2+}$ (500)	0.559 $\pm$ 0.076	0.555 $\pm$ 0.053

both. Indeed, more than 90% of the $[^3\text{H}]\text{InsP}_3$ produced by permeabilized cells is found in the supernatant, whereas with intact cells it is found entirely in the pellet (results not shown).

Fig. 1 shows that the effect of GTP[S] in stimulating $[^3\text{H}]\text{InsP}_3$ production by permeabilized cells was potentiated by fMet-Leu-Phe ($P < 0.005$; paired t test), thus demonstrating effective coupling among receptor, G protein (guanine nucleotide regulatory protein) and

Fig. 2. Effect of pertussis toxin on GTP[S]-stimulated $[^3\text{H}]\text{InsP}_3$ accumulation

Pertussis-toxin-treated cells were prelabelled with $[^3\text{H}]\text{inositol}$, permeabilized, and then treated for 5 min with buffer ($\blacktriangle$), or 100 μM -GTP[S] ($\square$), or 500 μM -Ca $^{2+}$ ($\bullet$). Values are means $\pm$ S.E.M. for four experiments each done in duplicate, or, for 2000 ng of toxin/ml, the values are means of two independent experiments done in duplicate. $[^3\text{H}]\text{Phosphoinositide}$ radioactivity averaged 197000 c.p.m./sample. * $P < 0.025$, ** $P < 0.01$, for toxin-treated versus control (t test).

phospholipase C. This potentiating effect of fMet-Leu-Phe, which was complete by 30 s, is presumably due to a receptor-mediated increase in the rate and extent of guanine nucleotide binding to the G protein. Indeed, fMet-Leu-Phe stimulates guanine nucleotide exchange in neutrophil plasma membranes (Lad *et al.*, 1985).

Without added GTP[S], fMet-Leu-Phe did not stimulate $[^3\text{H}]\text{InsP}_3$ accumulation (Fig. 1). This is consistent with the reported inability of fMet-Leu-Phe to stimulate polyphosphoinositide hydrolysis in human neutrophil membranes (Smith *et al.*, 1985). The lack of stimulation by the chemoattractant alone points to the critical role of guanine nucleotide in receptor-effector coupling. However, even in the absence of fMet-Leu-Phe and GTP[S], permeabilized cells produced $[^3\text{H}]\text{InsP}_3$ in response to millimolar concentrations of Ca $^{2+}$ (Fig. 1), which has been shown to activate directly a polyphosphoinositide phosphodiesterase of erythrocyte and neutrophil membranes (Downes & Michell, 1981; Cockcroft *et al.*, 1984).

The ability of GTP[S] to raise $[^3\text{H}]\text{InsP}_3$ concentrations was shared by p[NH]ppG, but not by GDP[S] or GTP (Table 1). Thus the specificity exhibited by GTP analogues to activate polyphosphoinositide phosphodiesterase in permeabilized cells coincided with their differential effects on stimulating the phosphodiesterase of neutrophil membranes in that GTP[S] and p[NH]ppG were active; whereas GTP and GDP[S] were inactive (Cockcroft & Gomperts, 1985). The ineffectiveness of GTP may relate to the presence in neutrophil membranes of a high-affinity GTPase (Hyslop *et al.*, 1984).

$[^3\text{H}]\text{InsP}_2$ concentrations were also increased by

Table 2. Effect of pertussis-toxin pretreatment on toxin-catalysed [32 P]ADP-ribosylation of rabbit neutrophil membranes

Neutrophils were incubated for 90 min without or with the indicated concentrations of pertussis toxin. Membranes were prepared and treated for 60 min with [α - 32 P]NAD $^{+}$ and activated pertussis toxin (25 μ g/ml). Values are means of duplicate determinations from two separate cell preparations.

Pertussis-toxin pretreatment (ng/ml)	32 P incorporation into 41 kDa fraction (% of control)
0	100
2	69
20	44
200	37

Fig. 3. Effect of Ca^{2+} and fMet-Leu-Phe alone and in combination on GTP[S]-stimulated [^3H]Ins P_3 concentrations

[^3H]Inositol-prelabelled permeabilized neutrophils were incubated for 5 min in the presence of various concentrations of GTP[S] ($\blacktriangle$), GTP[S] plus 500 nM- Ca^{2+} ($\bullet$), GTP[S] plus 10 nM-fMet-Leu-Phe ($\triangle$), or GTP[S] plus Ca^{2+} and fMet-Leu-Phe ($\square$). Results are means $\pm$ S.E.M. for four separate experiments.

GTP[S], GTP[S] plus fMet-Leu-Phe, and increased [Ca^{2+}] (results not shown). [^3H]Ins P_2 accumulation proceeded with a similar time course and reached values comparable with those observed for [^3H]Ins P_3 , except for the effect of elevated [Ca^{2+}], where [^3H]Ins P_2 concentrations were approximately twice those of [^3H]Ins P_3 .

Effect of pertussis-toxin pretreatment on stimulated [^3H]Ins P_3 concentrations and ADP-ribosylation

Pretreatment of neutrophils with pertussis toxin produced a dose-dependent inhibition of GTP[S]-stimulated [^3H]Ins P_3 concentrations in permeabilized cells (Fig. 2), as well as a dose-dependent inhibition of [32 P]ADP-ribosylation of a 41 000-Da protein in neutrophil membranes (Table 2); 50% inhibition of both responses was observed between 2 and 20 ng of

Fig. 4. Enhancement by Ca^{2+} of GTP[S]-stimulated [^3H]Ins P_3 concentrations

[^3H]Inositol-prelabelled neutrophils were permeabilized and then exposed for 5 min to increasing concentrations of Ca^{2+} in the absence ($\bullet$) or presence ($\circ$) of 100 μM -GTP[S]. Results are means $\pm$ S.E.M. for four separate experiments.

toxin/ml. Pertussis toxin also depressed the stimulatory effect of p[NH]ppG on [^3H]Ins P_3 production, but failed to inhibit that of millimolar concentrations of Ca^{2+} (Fig. 2; Table 1). These findings support the contention that the action of guanine nucleotides is at the level of the pertussis-toxin-sensitive guanine nucleotide regulatory protein (see also Smith *et al.*, 1985).

Effect of Ca^{2+} on GTP[S]-stimulated [^3H]Ins P_3 accumulation

The ability of GTP[S] to stimulate [^3H]Ins P_3 concentrations was dose-responsive and enhanced by either fMet-Leu-Phe or Ca^{2+} , even though the concentrations of fMet-Leu-Phe (10 nM) and Ca^{2+} (500 nM) used did not stimulate [^3H]Ins P_3 production on their own (Figs. 1 and 4). Moreover, in the presence of submaximal concentrations of GTP[S], the combination of fMet-Leu-Phe plus Ca^{2+} elicited greater than additive effects, relative to that elicited by each agent alone with GTP[S] ($P < 0.05$) (Fig. 3). These findings support the contention that separate sites of action exist in the transduction mechanism for chemoattractant, guanine nucleotide and Ca^{2+} .

Although low concentrations of Ca^{2+} (0.1–10 μM) only marginally stimulated [^3H]Ins P_3 accumulation, higher concentrations ($> 100 \mu\text{M}$) produced a marked enhancement (Fig. 4). In the same permeabilized cell preparation that produced a 10–20-fold increase in [^3H]Ins P_3 in response to 500 μM - Ca^{2+} , no changes in total [^{14}C]choline- or [^{14}C]ethanolamine-labelled lipids were detected (results not shown). Likewise, there were no Ca^{2+} -evoked changes in soluble phosphoryl [^{14}C]choline and phosphoryl [^{14}C]ethanolamine concentrations. Thus the stimulant effects of millimolar concentrations of Ca^{2+} on [^3H]Ins P_3 concentrations reflect activation of a rabbit neutrophil enzyme that is specific for phosphoinositides. The stimulation of phospholipase C activity in human neutrophil membranes by high [Ca^{2+}] has also been reported to be specific for polyphosphoinositides (Cockcroft *et al.*, 1984).

In the presence of 100 μM -GTP[S], the Ca^{2+} dose-response curve was shifted markedly to the left, with significant Ca^{2+} stimulation observed with submicromolar Ca^{2+} (Fig. 4). With Ca^{2+} concentrations of 300 μM or higher, [^3H]Ins P_3 concentrations were similar in the presence and absence of GTP[S] (Fig. 4). Physiologically relevant Ca^{2+} concentrations are thus unable to augment [^3H]Ins P_3 accumulation markedly in permeabilized cells in the absence of stable guanine nucleotides, or to stimulate phosphatidylinositol 4,5-bisphosphate hydrolysis in human neutrophil membranes in the presence of GTP (Smith *et al.*, 1985). These results are consistent with our previous finding in intact neutrophils that use of ionomycin to increase cytosolic [Ca^{2+}] fails to increase [^3H]Ins P_3 (Bradford & Rubin, 1985a). Thus in intact neutrophils increased cytosolic [Ca^{2+}] in the absence of receptor stimulation appears inadequate to stimulate phospholipase C directly.

General conclusions

Previous studies have demonstrated that binding of fMet-Leu-Phe to its receptor leads to GDP-GTP exchange and activation of a guanine nucleotide regulatory protein (Bokoch & Gilman, 1984; Okajima & Ui, 1984). Pertussis toxin, by ADP-ribosylating the G protein, uncouples the regulatory interactions between the chemoattractant receptor and the guanine nucleotide regulatory protein, thus inhibiting neutrophil responses, including polyphosphoinositide breakdown and enzyme secretion (Bradford & Rubin, 1985b; Brandt *et al.*, 1985; Ohta *et al.*, 1985; Verghese *et al.*, 1985). The present finding, which demonstrates an inhibitory effect of pertussis toxin on guanine nucleotide-stimulated [^3H]Ins P_3 accumulation, supports the contention that pertussis toxin exerts its action at the level of the G protein. Perhaps of even greater significance is the demonstrated potentiating effect of fMet-Leu-Phe on guanine nucleotide-stimulated [^3H]Ins P_3 accumulation, for these findings provide direct evidence that a guanine nucleotide regulatory protein mediates coupling between the fMet-Leu-Phe receptor and phospholipase C-catalysed reactions in rabbit neutrophils.

The actions of guanine nucleotides on stimulus-secretion coupling in the neutrophil appear to be complex. In addition to an action at the level of the fMet-Leu-Phe receptor, guanine nucleotides may exert a direct effect on the exocytotic process (Barrowman *et al.*, 1986), and even regulate the interaction of Ins P_3 with its intracellular receptor in effecting Ca^{2+} release (Dawson, 1985; Gill *et al.*, 1986). The present study has uncovered what appears to be yet another possible role for the guanine nucleotides. Thus the ability of GTP[S] markedly to augment the action of physiologically relevant concentrations of Ca^{2+} to promote [^3H]Ins P_3 accumulation suggests that the activated G protein serves to sensitize the catalytic unit of phospholipase C to the action of resting Ca^{2+} concentrations. Viewed from this perspective, phospholipase C of the neutrophil is an enzyme whose activity is regulated by the G protein, but is dependent on Ca^{2+} . Catalysis can thus occur in response to receptor stimulation without a change in Ca^{2+} concentration. Such a mechanism would be analogous to the role of diacylglycerol in sensitizing protein kinase C to the stimulant action of basal concentrations of Ca^{2+} (Nishizuka, 1984). However, the system described in the present study may be more

complex in that, during cell stimulation, Ca^{2+} mobilized by phospholipase C-mediated Ins P_3 accumulation may feed-back to enhance the action of the activated G protein on the catalytic subunit.

After the present paper had been submitted for review, a study by Smith *et al.* (1986) on human neutrophil plasma membranes appeared which supports our conclusion that the G protein decreases the Ca^{2+} requirement for expressing its action on phospholipase C.

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