Biochem. J. (1990) 268, 605–610 (Printed in Great Britain)

Regulation of bombesin-stimulated inositol 1,4,5-trisphosphate generation in Swiss 3T3 fibroblasts by a guanine-nucleotide-binding protein

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The stimulation of inositol phosphate generation by bombesin and GTP analogues was studied in Swiss 3T3 cells permeabilized by electroporation. Bombesin-stimulated inositol phosphate generation is potentiated by guanosine 5'-[γ-thio]triphosphate (GTP[S]) and inhibited by guanosine 5'-[β-thio]diphosphate at all peptide concentrations tested, with no change in the EC_{50} value (concn. giving half-maximal response) for the agonist. Kinetic analysis showed that, although bombesin-stimulated [^{3}H]InsP_3 generation in [^{3}H]inositol-labelled cells was rapid (maximal by 5–10 s), the response to GTP[S] alone displayed a distinct lag time of 20–30 s. This lag time was significantly decreased by the addition of bombesin, suggesting that in this system agonist-stimulated GTP/GDP exchange occurs. In addition, bombesin-stimulated generation of Ins(1,4,5)P_3 mass at 10 s was enhanced by GTP[S] in the absence of a nucleotide response alone, a result consistent with this proposal. Pretreatment of the cells with phorbol 12-myristate 13-acetate (PMA) resulted in a dose-dependent inhibition of bombesin-, but not GTP[S]-, stimulated inositol phosphate generation. Furthermore, although PMA pretreatment did not affect the lag time for InsP_3 formation in response to GTP[S] alone, the degree of synergy between bombesin and the nucleotide was severely decreased at early time points. The results therefore demonstrate that the high-affinity bombesin receptor is coupled via a G-protein to phospholipase C in a manner consistent with a general model for receptor–G-protein interactions and that this coupling is sensitive to phosphorylation by protein kinase C.

INTRODUCTION

It is now believed that receptors linked to the hydrolysis of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] couple to the effector enzyme, phospholipase C (PLC), through interaction with a specific guanine-nucleotide regulatory binding protein (G_p) (Cockcroft & Gomperts, 1985; Gilman, 1987). In contrast with the hormonal regulation of adenylate cyclase and the rhodopsin activation of cyclic GMP phosphodiesterase, the characteristics of receptor–G-protein-coupled activation of PLC are poorly understood. Recently it has been suggested that, in common with the adenylate cyclase system, receptor-stimulated acceleration of GTP/GDP exchange is a component part of the receptor–G-protein activation cycle (Boyer *et al.*, 1989*a*,*b*). However, it is unclear whether all types of receptor linked to PtdIns(4,5)P_2 hydrolysis couple to G_p in the same manner and thus activate PLC with similar kinetics. Furthermore, it is now known that at least two distinct G-proteins may regulate inositol phospholipid hydrolysis, one of which appears to be sensitive to pertussis toxin (Paris & Pouyssegur, 1986). Also several isoforms of PLC have recently been identified. Several types of receptor–G-protein–PLC interactions may therefore be possible.

Several reports have described the inhibitory effects of protein kinase C activators on agonist-stimulated phosphoinositide hydrolysis (Rittenhouse & Sasson, 1985; Brown *et al.*, 1987; Geny *et al.*, 1988). Since in many tissues inactivation occurs in the absence of receptor down-regulation, it is possible that an effect at the G-protein level may be involved. Although phorbol 12-myristate 13-acetate (PMA)-stimulated protein kinase C is thought to phosphorylate G-proteins in other transduction systems (Katada *et al.*, 1985), the effect of protein kinase C activation on G_p is unclear.

It has previously been shown that bombesin stimulates the formation of Ins(1,4,5)P_3 in Swiss 3T3 fibroblasts, through the activation of a pertussis-toxin-insensitive G-protein (Heslop *et al.*, 1986; Taylor *et al.*, 1988). We therefore decided to assess the kinetics of both bombesin- and guanine-nucleotide-stimulated inositol phosphate formation in electropermeabilized Swiss 3T3 cells so as to determine the way in which peptide receptors interact with G_p in this system. We have also used phorbol esters to determine the site of protein-kinase-C-mediated inhibitory feedback of bombesin-stimulated inositol phosphate formation. In this study we show that the kinetics of bombesin- and GTP[S]-stimulated InsP_3 formation are consistent with the recently proposed model for receptor–G_p–PLC interactions and that the site of PMA inhibition of bombesin-stimulated inositol phosphate generation is at the level of the receptor–G-protein interaction.

MATERIALS AND METHODS

Monolayers of Swiss 3T3 fibroblasts (passage 5–20) were grown in 75 cm^2 flasks in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Paisley, Scotland, U.K.), containing 10% (v/v) newborn-calf serum (Gibco), 27 mg of glutamine/ml, 250 mg of streptomycin/ml and 250 i.u. of penicillin/ml as previously described (Cook & Wakelam, 1989). For [^{3}H]inositol phospholipid experiments the cells were prelabelled with [^{3}H]-inositol (Amersham International Amersham, Bucks., U.K.) for 30–40 h in inositol-free DMEM containing penicillin, streptomycin, glutamine and 2% (v/v) dialysed newborn-calf serum. The radioactive concentration was 2μCi/ml for total inositol phosphate measurements and 8 μCi/ml where the individual

Abbreviations used: DMEM, Dulbecco's modified Eagle's medium; EC_{50}, concentration giving half-maximal response; G-protein, guanine-nucleotide-binding regulatory protein; G_p, G-protein coupling receptors to phospholipase C; GDP[S], guanosine 5'-[β-thio]diphosphate; p[NH]ppG, guanosine 5'-[βγ-imido]triphosphate; GTP[S], guanosine 5'-[γ-thio]triphosphate; PLC, phospholipase C; PMA, phorbol 12-myristate 13-acetate.

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phosphates were separated. After the labelling period, the cells were harvested mechanically with a cell scraper, incubated for a further 45 min in prelabelling medium, washed twice by centrifugation (800 *g* for 2 min) and resuspended in incubation buffer (20 mM-Hepes, pH 7.5, containing 120 mM-KCl, 6 mM-$MgCl_2$, 2 mM-KH_2PO_4, 2.5 mM-NaCl, 2.5 mM-HCl, 2 mM-Na_2ATP, 0.1 mM-EGTA and 1 mg of BSA/ml). The final cell suspension (6×10^6 cells) was then exposed to four discharges of a 3 μF capacitor with a field strength of 2 kV/cm spaced by 2 s intervals. Approx. 90–95% of the fibroblasts were found to be permeable to ethidium bromide after this procedure.

For total [^{3}H]inositol phosphate measurements, 100 μl of permeabilized cells [$(2.5–5) \times 10^4$] were incubated with agonists in incubation buffer containing 10 mM-LiCl in a final volume of 250 μl. After 30 min at 37 °C, the reaction was terminated by the addition of 0.94 ml of chloroform/methanol (1:2, v/v) and the water-soluble inositol phosphates were extracted and assayed by the method of Berridge *et al.* (1982). For measurements of the individual inositol phosphates, 50 μl of cells [$(2–3) \times 10^5$] were incubated with agonist in a final volume of 100 μl, in the absence of Li^+. The reaction was terminated by addition of an equal volume of ice-cold 10% (v/v) $HClO_4$, and the water-soluble inositol phosphates were then extracted on ice, centrifuged (14000 *g* for 5 min) and neutralized with 2 M-KOH/0.5 mM-Hepes. Anion-exchange chromatography on Dowex formate columns (1 × 8; 200–400 mesh) was used to separate the mono-, bis- and tris-phosphates as previously described by Wakelam *et al.* (1986).

Mass measurements of bombesin-stimulated Ins(1,4,5)P_3 formation were by the method of Palmer *et al.* (1989), with modification of the methods employed above. Non-radiolabelled cells, treated as previously described, were permeabilized with six pulses at 2 kV/cm in order to ensure complete permeabilization of a higher density of cells [$(20–30) \times 10^6$ cells/ml]. Batches of cells [$(1.0–1.5) \times 10^6$ in 50 μl] were incubated with agonists in a final volume of 75 μl, and the reaction was terminated by addition of 25 μl of 10% $HClO_4$. Amounts of endogenous Ins(1,4,5)P_3 produced were assayed by competitive displacement of [^{3}H]Ins(1,4,5)P_3 binding to bovine adrenal-cortex microsomes, quantified by using an Ins(1,4,5)P_3 standard curve.

Dose/response curves were fitted to a logistic equation as defined by Delean *et al.* (1980).

Materials were obtained from previously reported sources (Cook & Wakelam, 1989; Cook *et al.*, 1990).

RESULTS

In permeabilized Swiss 3T3 fibroblasts, bombesin stimulated the accumulation of [^{3}H]inositol phosphates in a dose-dependent manner (Fig. 1), eliciting a 5–6-fold response over basal values at maximal peptide concentrations (30 nM). An EC_{50} value of 0.37 ± 0.14 nM ($n = 5$) for the bombesin response compared favourably with those obtained for bombesin-stimulated inositol phosphate accumulation in intact Swiss 3T3 cells (Heslop *et al.*, 1986; Hasegawa-Sasaki *et al.*, 1988). The EC_{50} value for the peptide decreased after electroporation (intact cells 1.78 ± 0.3 nM, permeabilized cells 0.54 ± 0.02 nM; $n = 3$), although the maximal response was decreased by 25–30% (results not shown). Prostaglandin $F_{2\alpha}$ and platelet-derived growth factor also stimulated inositol phosphate accumulation in electroporated cells (2–3-fold and 10–12-fold over basal respectively), with EC_{50} values in the concentration range expected for these agonists (Black & Wakelam, 1990; Brown *et al.*, 1987), suggesting that overall receptor integrity is maintained after electoporation.

Bombesin-stimulated [^{3}H]Ins$P$ generation was significantly potentiated by the addition of the non-hydrolysable guanine nucleotide analogue GTP[S] (Fig. 1): 30 μM-GTP[S], in the absence of bombesin, elicited a 2-fold increase in [^{3}H]Ins$P$ accumulation, but in the presence of bombesin an 8–10-fold increase in [^{3}H]Ins$P$ accumulation was observed. The EC_{50} value for bombesin was, however, not significantly altered by the presence of GTP[S] (control = 0.42 ± 0.13 nM, +GTP[S] = 0.31 ± 0.15 nM; $n = 3$). The potentiation was found to be specific

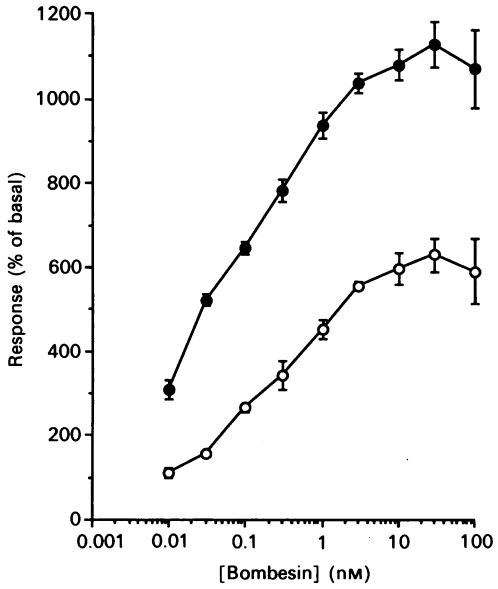

Fig. 1. Effect of GTP[S] on bombesin-stimulated accumulation of total [^{3}H]inositol phosphates in permeabilized Swiss 3T3 fibroblasts

Cells treated as outlined in the Materials and methods section were incubated with increasing concentrations of bombesin in the absence (○) or presence (●) of 30 μM-GTP[S] for 30 min at 37 °C in incubation buffer containing 10 mM-LiCl. Each point represents the mean ± S.D. of triplicate determinations from a single experiment representative of three others.

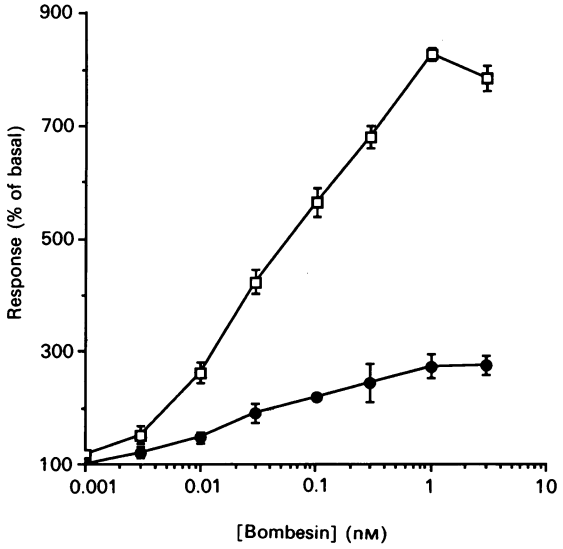

Fig. 2. GDP[S] inhibition of bombesin-stimulated accumulation of total [^{3}H]inositol phosphates in permeabilized Swiss 3T3 fibroblasts

[^{3}H]Inositol-labelled cells were incubated with increasing concentrations of bombesin in the absence (□) or presence (●) of 2 mM-GDP[S] in a buffer containing 10 mM-LiCl. Each point represents the mean ± S.D. of triplicate determinations from a single representative experiment ($n = 3$).

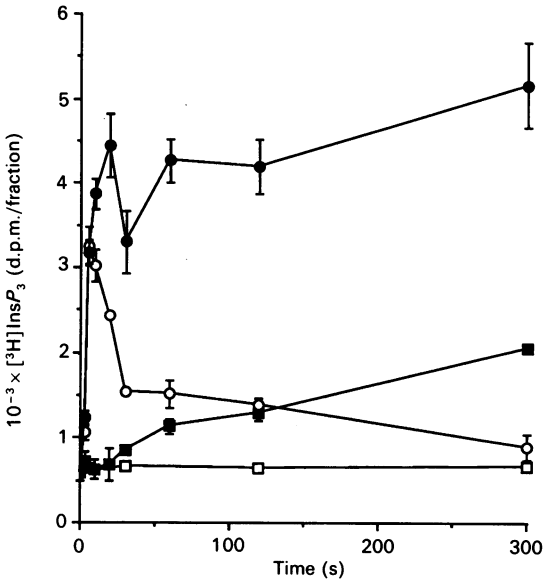

Fig. 3. Kinetics of bombesin- and GTP[S]-stimulated [^{3}H]InsP_3 formation in permeabilized Swiss 3T3 fibroblasts

Cells treated as outlined in the Materials and methods section were incubated with vehicle (□), 30 nM-bombesin (○), 100 μM-GTP[S] (■), or both (●), for the times indicated. Each point represents the mean ± S.D. of triplicate determinations from a single experiment representative of two others.

Table 1. Effect of GTP[S] on bombesin-stimulated Ins(1,4,5)P_3 formation

Cells were treated as outlined in the Materials and methods sections. Each value represents the mean ± S.D. of triplicate determinations from a single representative experiment ($n = 4$). The degree of bombesin stimulation varied between preparations. Stimulation of Ins(1,4,5)P_3 formation in response to GTP[S] alone at 5 min was used to confirm the efficacy of the nucleotide.

Treatment	Ins(1,4,5)P_3 (pmol/fraction)	Time of stimulation
Control	0.60 ± 0.11	10 s
GTP[S] (100 μM)	0.32 ± 0.10	10 s
Bombesin (30 nM)	3.36 ± 0.31	10 s
Bombesin + GTP[S]	6.66 ± 0.98	10 s
Control	0.50 ± 0.26	5 min
GTP[S]	1.33 ± 0.54	5 min

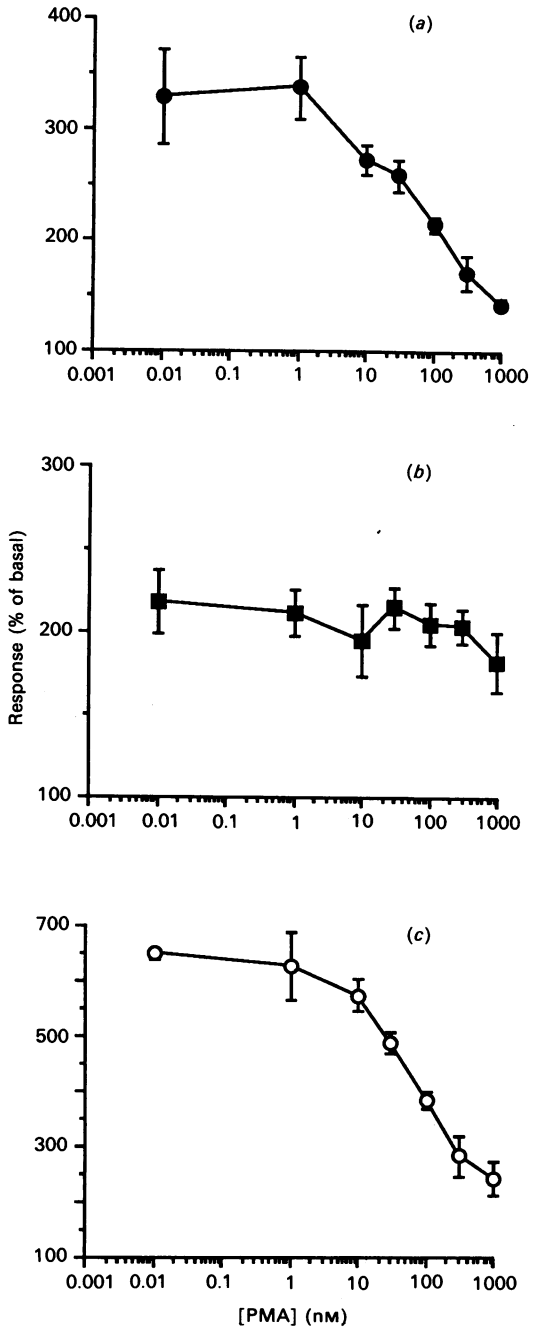

Fig. 4. Dose-dependency of inhibition by PMA of bombesin- and GTP[S]-stimulated accumulation of total [^{3}H]inositol phosphates in permeabilized Swiss 3T3 fibroblasts

[^{3}H]Inositol-labelled cells were incubated with increasing concentrations of PMA 20 min before permeabilization, and then incubated with 30 nM-bombesin (*a*), 100 μM-GTP[S] (*b*) or both (*c*) for 30 min in the presence of 10 mM-LiCl. Each point represents the mean ± S.D. of triplicate determinations from one experiment similar to two others.

for non-hydrolysable analogues of GTP. GTP itself, GDP or GMP were inactive at millimolar concentrations. Only GTP[S] and p[NH]ppG stimulated [^{3}H]Ins$P$ accumulation when added alone, with EC_{50} values in the low-micromolar range (GTP[S] = 19.55 ± 8.5 μM, p[NH]ppG = 57.76 ± 19.5 μM). The bombesin response was also significantly decreased by preincubation of the permeabilized cells with millimolar concentrations of GDP[S] (Fig. 2).

The kinetics of the interaction between bombesin and GTP[S] was examined after separation of the individual inositol phosphates (Fig. 3). In response to maximal concentrations of bombesin, [^{3}H]InsP_3 formation increased rapidly, reaching a peak after 5–10 s before decreasing to near-basal values within 1 min. GTP[S] alone stimulated [^{3}H]InsP_3 formation in a linear manner over 5 min, although a clear lag of 20–30 s was observed before the onset of the response. However, in the presence of GTP[S] bombesin-stimulated [^{3}H]InsP_3 formation was markedly enhanced. By 10–20 s a clear potentiation of the initial signal was observed, although no GTP[S] response was apparent at this time. In addition, the response was maintained at potentiated levels for up to 5 min by the presence of the nucleotide. A similar pattern of response was observed in [^{3}H]InsP_2 formation, but in the [^{3}H]InsP_1 fraction potentiation was only manifest after 2 min (results not shown). Further confirmation of this phenomenon is shown in Table 1. Bombesin-stimulated formation of Ins(1,4,5)P_3 mass at 10 s is potentiated by GTP[S], although alone GTP[S] is

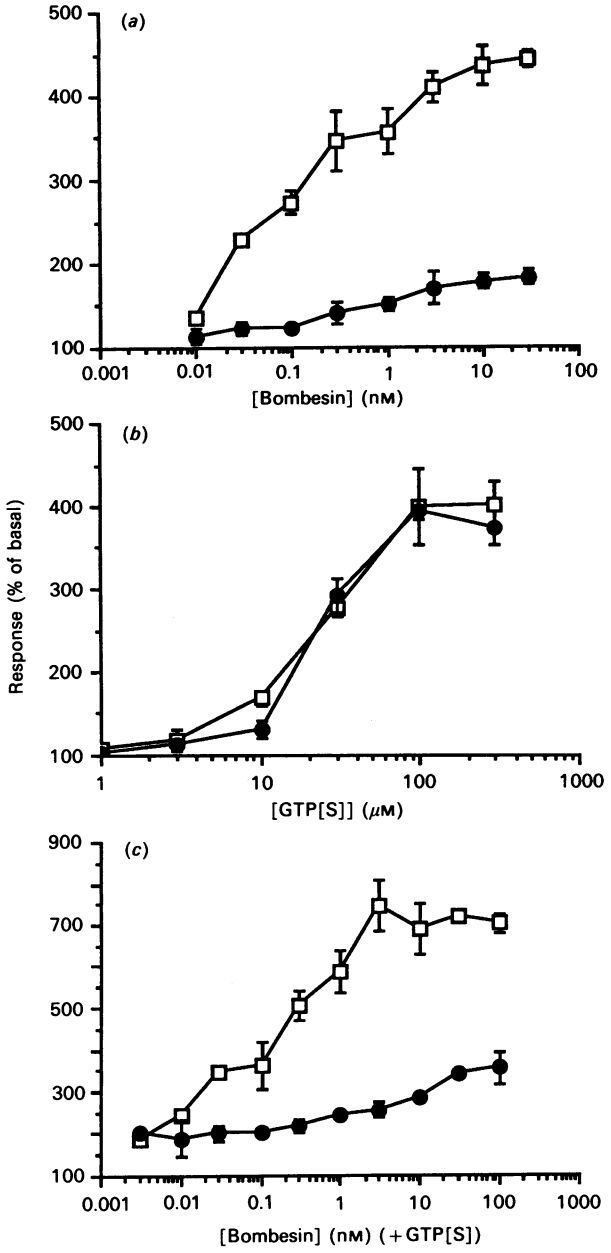

Fig. 5. Dose/response curves for bombesin- and GTP[S]-stimulated [^{3}H]inositol phosphate accumulation in control and PMA-pretreated permeabilized Swiss 3T3 fibroblasts

Cells labelled as outlined in the Materials and methods section were pretreated with vehicle (□) or 500 nM-PMA (●) for 20 min before permeabilization, and then incubated with bombesin (*a*), GTP[S] (*b*) or both (*c*) for 30 min in the presence of 10 mM-LiCl. Each point is the mean ± S.D. of triplicate determinations from a single experiment ($n = 3$). In (*c*) the GTP[S] concn. was 30 μM.

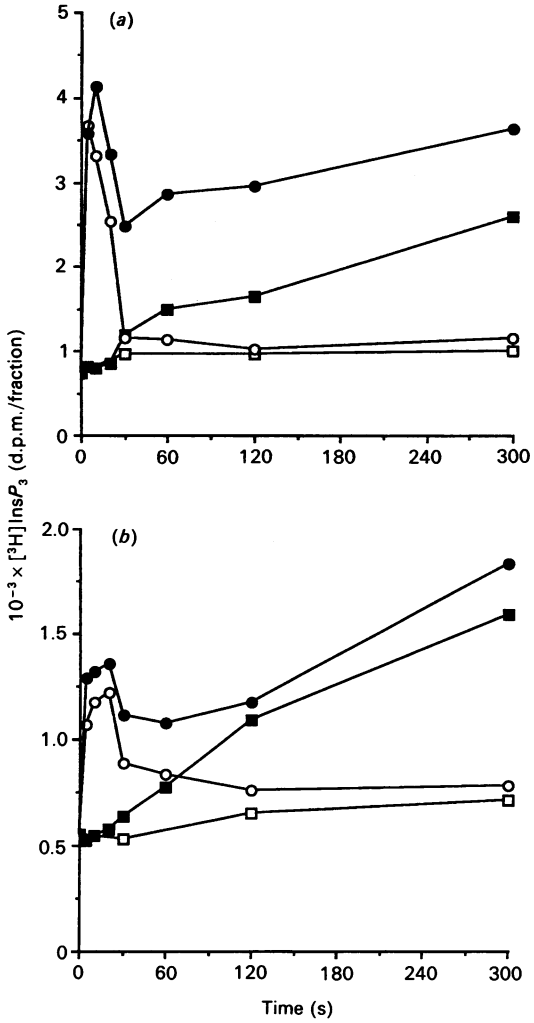

Fig. 6. Effect of PMA pretreatment on the kinetics of bombesin- and GTP[S]-stimulated [^{3}H]InsP_3 formation in permeabilized Swiss 3T3 fibroblasts

Labelled cells were pretreated with 1 μM-β-phorbol (*a*) or 500 nM-PMA (*b*) for 20 min before pulsing, and then incubated with vehicle (□), 30 nM-bombesin (○), 100 μM-GTP[S] (■) or both (●) for the times indicated. Each point represents the mean ± S.D. of a single experiment repeated on two other occasions.

without effect at this early time point. GTP[S] also potentiated InsP_3 formation in response to sub-maximal doses of bombesin, although in this instance the lag in the onset of the InsP_3 response to bombesin itself was not decreased by GTP[S] (results not shown).

The effect of the protein-kinase-C-activating phorbol ester PMA on bombesin- and GTP[S]-stimulated [^{3}H]inositol phosphate accumulation is shown in Figs. 4 and 5. In permeabilized cells preincubation with PMA decreased the bombesin response in a dose-dependent manner, over the nanomolar range (Fig. 4*a*). In contrast, there was no significant effect of PMA on GTP[S]-stimulated Ins$P$ accumulation over the concentration range tested (Fig. 4*b*). The potentiation by GTP[S] of the bombesin response was also inhibited by PMA, this effect mirroring the loss in the bombesin response (Fig. 4*c*). Preincubation with a maximal concentration of PMA (500 nM) was without effect on the GTP[S] dose/response curve (Fig. 5*b*), but clearly decreased the magnitude of the bombesin response (Fig. 5*a*) and the synergy between the two agents (Fig. 5*c*). In HL60 and WRK cells PMA inhibition of the GTP[S]- or hormone-stimulated inositol phosphate generation was shown to be time-dependent (Geny *et al.*, 1989; Monaco & Mufson, 1986). We therefore examined the effect of the preincubation period on both bombesin- and GTP[S]-stimulated inositol phosphate formation. Maximum inhibition of the bombesin response was obtained after 15 min of PMA pretreatment (results not shown), after which no further decrease occurred. The GTP[S] response was not significantly affected over the 60 min incubation period (results not shown). Again the decrease in the synergy between bombesin and GTP[S] was a function of the inhibition of the peptide response (results not shown).

The effects of PMA on the kinetics of [^{3}H]InsP_3 formation are shown in Fig. 6. In the presence of 1 μM-β-phorbol, an inactive analogue of PMA, both bombesin- and GTP[S]-stimulated [^{3}H]InsP_3 generation was unaffected (Fig. 6*a*). In cells pretreated with PMA (Fig. 6*b*), bombesin-stimulated [^{3}H]InsP_3 formation was markedly decreased, from 300–400% to 150% of basal values at 5–10 s. In addition, the agonist-induced enhancement of the onset of the GTP[S] effect was less pronounced. However, neither the lag time nor the magnitude of the [^{3}H]InsP_3 response to GTP[S] was affected in these cells.

DISCUSSION

The results in the present paper show that in permeabilized Swiss 3T3 fibroblasts bombesin and GTP[S] act in a synergistic manner to stimulate the accumulation of [^{3}H]inositol phosphates. GTP[S] and p[NH]ppG both potentiate the response, with GTP, GDP, GMP and GDP[S] being ineffective. These results are consistent with those in other systems (Uhing *et al.*, 1986; Claro *et al.*, 1989), and support previous proposals that a GTP-binding regulatory protein is involved in the coupling of bombesin receptors to PLC (Hasegawa-Sasaki *et al.*, 1988). This is further supported by the results in Table 1. Stimulation of Ins(1,4,5)P_3 formation by bombesin is significantly enhanced by addition of GTP[S]. Since Ins(1,4,5)P_3 is derived directly from the hydrolysis of PtdIns(4,5)P_2, this indicates that coupling of bombesin receptors to a PtdIns(4,5)P_2-specific PLC is involved. The stimulation by bombesin, in the absence of GTP[S], of both [^{3}H]inositol phosphates and Ins(1,4,5)P_3 mass in the electropermeabilized cells is probably due to the presence of residual cellular GTP. This interpretation is supported by the response to bombesin alone being short-lived, whereas that to bombesin and GTP[S] is sustained (Fig. 3). Other additional evidence is obtained by studies on the kinetics of GTP[S]-stimulated [^{3}H]InsP_3 formation. The lag time observed in the onset of the stimulated GTP[S] response (20–30 s) is clearly decreased by bombesin-receptor occupation (Fig. 3). Furthermore, bombesin-stimulated formation of Ins(1,4,5)P_3 at 10 s is enhanced by GTP[S], in the absence of a nucleotide response alone (Table 1).

By analogy with previous studies examining the kinetics of hormone-dependent activation of adenylate cyclase (Ferguson *et al.*, 1986; Casey & Gilman, 1988), these observations suggest that the dissociation of bound GDP is the rate-limiting step in the activation of the G-protein and also that agonist receptor stimulation significantly enhances the exchange of GTP for GDP. A similar phenomenon has also been observed in turkey erythrocyte membranes (Harden *et al.*, 1988; Boyer *et al.*, 1989*a*), where ATP, acting via a P_{2y} purinergic receptor, markedly decreased the lag time for the onset of GTP[S]-stimulated InsP_3 formation. However, the work presented here is the first study assessing the kinetics of high-affinity peptide-receptor-stimulated inositol phosphate formation.

Although the kinetics of bombesin- and GTP[S]-induced InsP_3 formation are in keeping with a general model for describing receptor–G-protein–PLC interactions, the dose/response relationship for bombesin- and GTP[S]-stimulated [^{3}H]Ins$P$ accumulation is at variance with other systems. In both ATP-stimulated turkey erythrocyte membranes and carbachol-stimulated SH-SY5Y human neuroblastoma cells (Boyer *et al.*, 1989*a*; Wojcikiewicz *et al.*, 1990), a marked shift to the left in the dose/response curve for agonist-stimulated inositol phosphate generation is observed in the presence of GTP[S]. In the present study, addition of GTP[S] did not significantly affect the EC_{50} value for bombesin-stimulated [^{3}H]inositol phosphate accumulation, although the maximum response is greatly enhanced (Fig. 1). A similar result has recently been obtained by Cattaneo & Vicentini (1989). This may suggest that peptide and non-peptide agonist receptors couple to PLC in different ways. In support of this hypothesis we have found that in Swiss 3T3 fibroblasts the EC_{50} value for prostaglandin-$F_{2\alpha}$-stimulated [^{3}H]inositol phosphate accumulation decreases in the presence of GTP[S] (control = 0.21 ± 0.08 μM, +GTP[S] = 0.073 ± 0.006 μM; $n = 3$). Alternatively, these results could suggest that no receptor reserve exists for bombesin on Swiss 3T3 cells, a proposal supported by the identity between the EC_{50} for inositol phosphate generation and the K_d value for bombesin-receptor binding to Swiss 3T3 cells (Zachary & Rozengurt, 1985).

In intact Swiss 3T3 fibroblasts, phorbol esters inhibit bombesin-stimulated InsP_3 formation through protein-kinase-C-mediated negative feedback (Brown *et al.*, 1987) (also results not shown). We therefore sought to determine the site at which bombesin-receptor coupling to PLC was impaired. Several possible sites of action can be identified: (1) coupling of the bombesin receptor to the G-protein; (2) the interaction between the G-protein and PLC; (3) a direct effect on PLC. In permeabilized Swiss 3T3 fibroblasts we find that preincubation with PMA inhibits bombesin-stimulated [^{3}H]inositol phosphate accumulation and also the synergy between bombesin and GTP[S], but does not significantly affect the GTP[S] response (Fig. 4). This is confirmed by assessing the kinetics of InsP_3 formation (Fig. 6), and suggests that PMA impairs the linkage between the bombesin receptor and the G-protein. In rat renal glomerulosa cell membranes angiotensin-II-stimulated inositol phosphate formation was similarly affected by PMA (Pfeilschifter & Bauer, 1987). The authors of that study have proposed that protein-kinase-C-mediated phosphorylation may uncouple the receptor from the G-protein. In DDT_1 MF2 smooth-muscle cells, PMA-stimulated phosphorylation of α_1 adrenoceptors resulted in decreased agonist–receptor binding (Leeb-Lundberg *et al.*, 1985). Although that possibility has not been examined in the present study, PMA has previously been shown to be without effect on the characteristics of ^{125}I-bombesin binding to intact Swiss 3T3 fibroblasts (Brown *et al.*, 1987). It is of course possible that phosphorylation of the receptor may not affect ligand-binding characteristics, but could still impair the coupling between the receptor and the G-protein. Alternatively, the G-protein itself may be phosphorylated by protein kinase C activation, as has been previously observed for G_i in Cyc$^-$ S49 lymphoma cells (Katada *et al.*, 1985). Once again, the coupling of the receptor to the G-protein may be impaired without affecting the function of the G-protein itself.

The results shown in the present study contrast with the effect of phorbol esters on inositol phosphate responses in human neutrophils (Smith *et al.*, 1987), HL60 cells (Geny *et al.*, 1989) and 1321N1 astrocytoma-cell membranes (Orellena *et al.*, 1987). In these systems, PMA inhibits GTP[S]-stimulated inositol phosphate formation, indicating that the coupling between the G-protein and PLC is impaired, rather than that between the receptor and G-protein. Furthermore, in HL60 cells Ca^{2+}-stimulated inositol phosphate formation is also inhibited by PMA (Geny *et al.*, 1989), suggesting that phosphorylation of PLC may itself be a mechanism by which phorbol esters act. The difference in the sensitivity of HL-60 and Swiss 3T3 cells to PMA may be due to the presence of different isoenzymes of PLC coupled to receptor-stimulated PtdIns(4,5)P_2 hydrolysis, or the involvement of two distinct G-proteins in the transduction mechanism. It is, however, likely that PMA has multiple sites of action on transduction pathways in different tissues, only one of which may involve an effect on the G-protein.

This work was supported by grants from the Cancer Research Campaign and the Medical Research Council (U.K.).

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Received 19 December 1989/26 February 1990; accepted 28 February 1990