Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 6374–6378, September 1987
Biochemistry

Dissociation of bradykinin-induced prostaglandin formation from phosphatidylinositol turnover in Swiss 3T3 fibroblasts: Evidence for G protein regulation of phospholipase A_2

(arachidonic acid/glucocorticoids/protein kinase C)

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ABSTRACT **In Swiss 3T3 fibroblasts bradykinin stimulated inositol phosphate (Ins*P*) formation and prostaglandin E_2 (PGE_2) synthesis. The EC_{50} values for stimulation of PGE_2 synthesis and Ins*P* formation by bradykinin were similar, 200 pM and 275 pM, respectively. Guanosine-5′-[γ-thio]triphosphate stimulated PGE_2 synthesis and Ins*P* formation, and guanosine-5′-[β-thio]diphosphate inhibited both PGE_2 synthesis and Ins*P* formation stimulated by bradykinin. Neither bradykinin-stimulated PGE_2 synthesis nor Ins*P* formation was sensitive to pertussis toxin. Phorbol ester, dexamethasone, and cycloheximide distinguished between bradykinin-stimulated PGE_2 synthesis and Ins*P* formation. Phorbol 12-myristate 13-acetate enhanced bradykinin-stimulated PGE_2 synthesis but inhibited bradykinin-stimulated Ins*P* formation. Pretreatment of cells with dexamethasone for 24 hr inhibited bradykinin-stimulated PGE_2 synthesis but was without effect on bradykinin-stimulated Ins*P* formation. Cycloheximide inhibited bradykinin-stimulated PGE_2 synthesis but was without effect on bradykinin-stimulated Ins*P* formation. When bradykinin was added to cells prelabeled with [^{3}H]choline, the phospholipase A_2 products lysophosphatidylcholine and glycerophosphocholine were generated. In cells pretreated with dexamethasone, lysophosphatidylcholine and glycerophosphocholine formation induced by bradykinin were inhibited. Treatment of cells with phorbol ester enhanced bradykinin-induced formation of these metabolites. The data suggest that bradykinin receptors are coupled by GTP-binding proteins to both phospholipase C and phospholipase A_2 and that phospholipase A_2 is the enzyme that catalyzes release of arachidonate for prostaglandin synthesis.**

Recent evidence suggests that GTP-binding proteins (G proteins) are involved in the receptor-mediated activation of phosphatidylinositol-specific phospholipase C (1–5). The identities of the G proteins that couple receptors to phosphatidylinositol-specific phospholipase C are unknown. In some tissues the G proteins that couple receptors to phospholipase C exhibit pertussis toxin sensitivity (1, 2), whereas in other tissues pertussis toxin is without effect (6, 7). *In vitro*, the GTP-binding proteins G_i and G_o appear to activate phospholipase C (5). More recently, it has been recognized that phospholipase A_2 is also coupled to receptors by G proteins (8–10). In the systems described thus far, the G proteins coupled to phospholipase A_2 have been found to be pertussis toxin-sensitive.

Bradykinin stimulates arachidonic acid release and prostaglandin synthesis in a variety of tissues (11). This peptide also increases inositol phosphate (Ins*P*) formation by activating a phosphatidylinositol-specific phospholipase C. It has been suggested that free arachidonate may be released by the action of phosphatidylinositol-specific phospholipase C followed by diglyceride or monoglyceride lipases (12), or that phospholipase A_2 is responsible for catalyzing the direct release of arachidonate (13). The mechanism by which bradykinin stimulates arachidonic acid release and metabolism to prostaglandin E_2 (PGE_2) is unclear.

Using Swiss 3T3 cells, the present study was designed to examine whether bradykinin stimulates arachidonate release and PGE_2 synthesis through a G protein-mediated mechanism and whether release is catalyzed by a phospholipase C–diglyceride lipase pathway or a phospholipase A_2 pathway. Our experiments provide evidence that bradykinin receptors coupled to a G protein activate phospholipase A_2 to release arachidonate for PGE_2 synthesis.

EXPERIMENTAL PROCEDURES

Cells. Swiss albino 3T3 cells (14) were obtained from the American Type Culture Collection (ATCC CCL 92) and maintained in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g of glucose per liter, 10% calf serum, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. For experiments, nearly confluent cells in a 10-cm dish were divided into ten 6- or 12-well plates (Costar, Cambridge, MA) and incubated 24–36 hr. When indicated, dexamethasone, [^{3}H]Ins (10 μCi per well; 1 Ci = 37 GBq), [^{3}H]arachidonic acid (1 μCi per well), or [^{3}H]choline (10 μCi per well) was included during the last 18–24 hr of incubation.

Prostaglandin Synthesis. Immediately before an experiment the media were aspirated and the wells were washed with 1 ml of DMEM without calf serum but including 20 mM Hepes, pH 7.4 (DMEM-Hepes). These media were aspirated and replaced with 0.5 ml of DMEM-Hepes containing bradykinin or phorbol 12-myristate 13-acetate (PMA) and the cells were incubated at 37°C for 5 min or the times indicated in the figure legends. The media were aspirated and frozen until assayed for prostaglandins. PGE_2 and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) were measured directly from the media by RIA using antisera from Seragen (Boston, MA). Bradykinin, dexamethasone, or PMA did not interfere with the assay. PGE_2 did not interfere with the 6-keto-$PGF_{1\alpha}$ assay; nor did 6-keto-$PGF_{1\alpha}$ interfere with the PGE_2 assay at the levels of metabolites found in the experiments.

[^{3}H]Ins*P* Formation. Before an experiment media were aspirated, and the wells were washed with 1 ml of DMEM-Hepes. This media was aspirated and replaced with 1 ml of DMEM-Hepes containing 20 mM LiCl. The cells were incubated for 15 min at 37°C; then bradykinin or PMA was

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Abbreviations: Ins, inositol; Ins*P*, Ins phosphate; PGE_2, prostaglandin E_2; 6-keto-$PGF_{1\alpha}$, 6-ketoprostaglandin $F_{1\alpha}$; GTP[γ-S], guanosine 5′-[γ-thio]triphosphate; GDP[β-S], guanosine 5′-[β-thio]diphosphate; PMA, phorbol 12-myristate 13-acetate.

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added, and the cells were incubated an additional 5 min at 37°C. Trichloroacetic acid was added to a final concentration of 5%, and the plates were incubated on ice for 30 min. Ins*P*s (InsP_{1-3}) were measured using anion-exchange chromatography as previously described (15). LiCl was found to increase the recovery of InsP_1 without affecting the recoveries of InsP_2 or InsP_3.

Permeabilization. Permeabilization of the cells to allow entry of guanine nucleotide analogues was accomplished by transient hypoosmotic shock treatment as previously described (8, 16) or by incubation of cells growing in 12-well plates with culture media minus serum and containing saponin (20 μg/ml) for 3 min. The media also contained guanosine-5′-[γ-thio]triphosphate (GTP[γ-S]) or guanosine-5′-[β-thio]diphosphate (GDP[β-S]) where indicated. The wells were then rinsed two times with culture media not containing saponin but with nucleotide analogues, and the cells were incubated at 37°C for 20 min. In some experiments, after recovery at 37°C for 20 min, the wells were rinsed, and bradykinin was added for 5 min.

Phospholipid Analysis. Lysophosphatidylcholine was extracted from the cells plus media with 1-butanol (18) and assayed by thin-layer chromatography (19). Glycerophosphocholine was extracted and assayed by thin-layer chromatography (20).

Phospholipase A_2 Activity. Cell membranes were prepared as described for ADP-ribosylation above. To 50 μl of 100 mM Tris, pH 9 (37°C)/1 mM $CaCl_2$/1 mM $MgCl_2$, 10 μl of GTP[γ-S] and/or bradykinin was added as well as 25 μl of the cell membrane preparation that had been sonicated with a probe (Kontes, setting 6) for 15 sec. To initiate the reaction, 15 μl of phosphatidyl[^{3}H]choline, 10 μM final concentration, was added. The reaction continued for 15 min. Lysophosphatidyl[^{3}H]choline was extracted with 1-butanol and assayed by thin-layer chromatography as above.

Statistical Analysis. All experiments were repeated at least three times. When 12-well plates were used (PGE_2 synthesis), each experimental manipulation was done in triplicate wells; the values obtained were averaged and counted as a single observation. When 6-well plates were used (Ins*P* formation), each experimental manipulation was done in duplicate wells; the values obtained were averaged and counted as a single observation. Data are presented as mean ± SEM. Statistical comparisons were made using Student's *t* test for paired observations. EC_{50} concentrations for bradykinin stimulation of PGE_2 synthesis and Ins*P* formation were calculated using linear regression analysis of logarithm-logit transformations of the data in Fig. 2.

Materials. Bradykinin was from Peninsula (San Carlos, CA), PMA was from Calbiochem, and GTP[γ-S] and GDP[β-S] were from Boehringer Mannheim. [5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid (84 Ci/mmol), *myo*-[^{3}H]Ins (16 Ci/mmol), [^{3}H]choline, and [5,6,8,11,12,14,15-^{3}H]PGE_2 (165 Ci/mmol) were obtained from New England Nuclear. Other chemicals were from Sigma.

RESULTS

Bradykinin Stimulates Arachidonic Acid Release, Prostaglandin Synthesis, and Ins*P* Formation in Swiss 3T3 Cells. When bradykinin (1 μM) was added to prelabeled cultures of Swiss 3T3 cells, [^{3}H]arachidonic acid release was rapidly stimulated (Fig. 1). Half-maximal stimulation occurred in <1 min and had reached an apparent plateau by 5 min. Bradykinin also stimulated PGE_2 synthesis (Fig. 1). Half-maximum stimulation occurred within 2 min and had reached an apparent plateau by 10 min. Thus, bradykinin stimulation of PGE_2 synthesis occurred somewhat more slowly than arachidonate release, consistent with a precursor–product relationship. Swiss 3T3 cells also synthesize prostacyclin, as

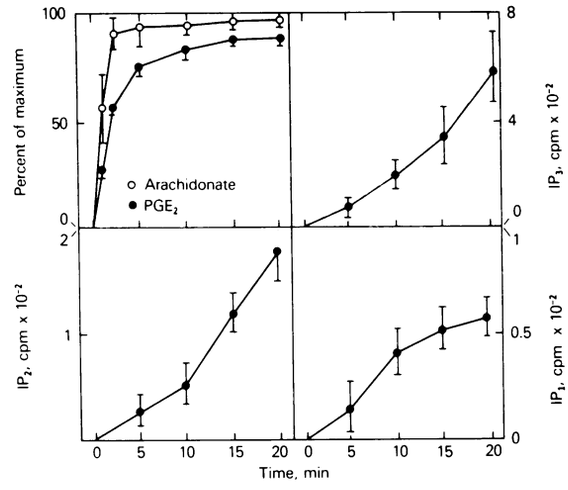

FIG. 1. Bradykinin stimulates arachidonic acid release and PGE_2 synthesis and Ins*P* (IP) formation in Swiss 3T3 fibroblasts. (*Upper left*) ○, [^{3}H]Arachidonic acid release; ●, PGE_2 synthesis. Reference time for maximum release or synthesis was 30 min. The data are from three experiments. The three other panels indicate the formation of InsP_1, InsP_2, and InsP_3. Data are from six experiments.

indicated by the detection of the stable hydrolysis product of prostacyclin, 6-keto-$PGF_{1\alpha}$, in the media, but the amount of this product was only ≈20–30% the amount of PGE_2. Bradykinin also stimulated the release of 6-keto-$PGF_{1\alpha}$ into the media (data not shown), but because the quantity of this metabolite was difficult to detect in the size of culture wells used, the present study will consider PGE_2 as an index of prostaglandin synthesis in the cells. The dose–response for bradykinin-stimulated PGE_2 synthesis revealed an EC_{50} of 200 nM (Fig. 2).

When cells were prelabeled with [^{3}H]Ins, bradykinin elicited an increase in [^{3}H]Ins*P* formation (Fig. 1). Bradykinin significantly stimulated InsP_1 formation by 5 min, but InsP_1 continued to accumulate for the entire 20-min stimulation. Formation of InsP_2 and InsP_3 was also stimulated by bradykinin (Fig. 1); both of these metabolites continued to accumulate for the entire 20-min incubation with bradykinin. Thus, although bradykinin-stimulated arachidonate release and PGE_2 synthesis were transient, bradykinin stimulation of Ins*P* formation was sustained over the entire incubation with bradykinin. The EC_{50} for bradykinin-stimulated Ins*P* formation was 275 nM (Fig. 2), similar to its EC_{50} for stimulation of PGE_2.

G Proteins Mediate Bradykinin-Stimulated PGE_2 Synthesis and Ins*P* Formation. Our previous studies have demonstrated that a G protein mediates α_1-adrenergic-stimulated arachi-

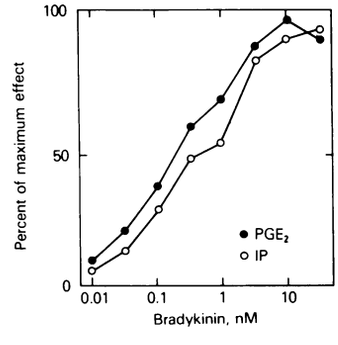

FIG. 2. Dose–response for bradykinin stimulation of PGE_2 synthesis and Ins*P* formation in Swiss 3T3 cells. InsP_{1-3} was measured. Data are from three experiments for PGE_2 synthesis and from five experiments for Ins*P* formation, IP, Ins*P*.

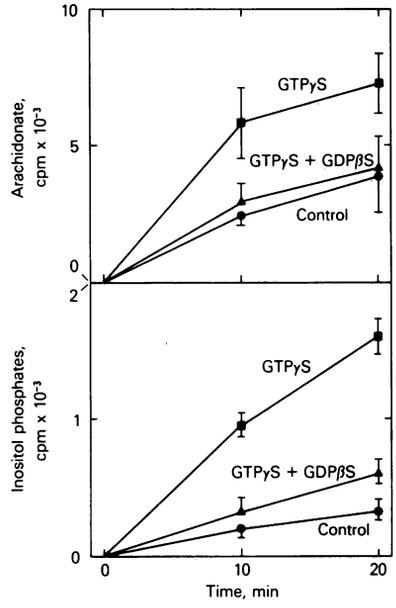

FIG. 3. GTP[γ-S]stimulates arachidonic acid release and Ins*P* formation in Swiss 3T3 cells. Cells were labeled with [^{3}H]arachidonic acid or [^{3}H]inositol, and then transiently permeabilized in the presence of GTP[γ-S] (100 μM) or GTP[γ-S] (100 μM) plus GDP[β-S] (2 mM). Data are from three experiments.

donic acid release and prostaglandin synthesis in FRTL5 thyroid cells (8). To determine whether a G protein regulates arachidonic acid release in Swiss 3T3 cells, the cells were prelabeled with [^{3}H]arachidonic acid overnight, then permeabilized by hypoosmotic shock to incorporate GTP[γ-S]. GTP[γ-S] is a poorly hydrolyzed GTP analogue that activates G proteins (21). GTP[γ-S] stimulated [^{3}H]arachidonate plus metabolite release into the culture media (Fig. 3). When the cells were osmotically permeabilized in the presence of GDP[β-S], which irreversibly inactivates G proteins, GTP[γ-S]-stimulated [^{3}H]arachidonate release was inhibited (Fig. 3). In similar experiments done with cells prelabeled with [^{3}H]Ins, GTP[γ-S] stimulated [^{3}H]Ins*P* release, which was blocked by GDP[β-S] (Fig. 3).

To determine whether the bradykinin receptor inducing arachidonate release and Ins*P* formation was coupled via a G protein, GDP[β-S] was incorporated into the cells using saponin to permeabilize the plasma membranes, followed by addition of bradykinin. GDP[β-S] inhibited bradykinin-stimulated PGE_2 synthesis and Ins*P* formation (Table 1).

In several cell types, receptor-mediated arachidonate release (2, 8–10) or Ins*P* formation (1, 2) was inhibited by pertussis toxin, an agent that inactivates certain G proteins by catalyzing ADP-ribosylation of their α subunits. In FRTL5 cells, for example, α_1-adrenergic-stimulated arachidonate release was pertussis toxin-sensitive, whereas Ins*P* formation was not (8). When Swiss 3T3 cells were incubated with pertussis toxin (1 ng–1 μg per ml) for 4 hr or overnight, neither bradykinin-stimulated PGE_2 synthesis nor Ins*P* formation was significantly affected (Table 2). To demonstrate that Swiss 3T3 cells have a pertussis toxin substrate, membranes were prepared and incubated with activated pertussis toxin and [^{32}P]NAD. Gel electrophoresis demonstrated that Swiss 3T3 cells do contain a pertussis toxin substrate at M_r about 40,000 (data not shown), consistent with either G_i or G_0 (22, 23). To demonstrate that pertussis toxin could cross the cell membrane to reach its substrate, cells were incubated with pertussis toxin for 4 hr. Then membranes were prepared and incubated with activated pertussis toxin and [^{32}P]NAD. In these membranes, pertussis toxin-catalyzed incorporation of ^{32}P into the M_r 40,000 substrate was markedly reduced (data not shown), suggesting that pertussis toxin had catalyzed ADP-ribosylation of its substrate in the intact cells.

Table 1. GDP[β-S] inhibits bradykinin-induced PGE_2 synthesis and Ins*P* formation

	PGE_2, pg/well	[^{3}H]Ins*P*, cpm/well
Control	212 ± 44	640 ± 72
GDP[β-S] (2 mM)	226 ± 34	576 ± 64
Bradykinin (1 μM)	564 ± 68*	1466 ± 118*
+ GDP[β-S] (100 μM)	518 ± 52	1116 ± 126†
+ GDP[β-S] (1 mM)	376 ± 46†	864 ± 88†
+ GDP[β-S] (2 mM)	322 ± 40†	782 ± 92†

Data are from three experiments. $Ins P_{1-3}$ was measured.
*$P < 0.05$ compared with control.
†$P < 0.05$ compared with bradykinin alone.

Table 2. Bradykinin-stimulated PGE_2 synthesis and Ins*P* formation are pertussis toxin-insensitive in Swiss 3T3 cells

	PGE_2, pg/well	[^{3}H]Ins*P*, cpm/well
Control	154 ± 18	582 ± 74
Pertussis toxin (100 ng/ml)	160 ± 24	610 ± 82
Bradykinin (1 μM)	742 ± 88*	973 ± 112*
+ pertussis toxin	686 ± 56*†	965 ± 96*†

Data are from three experiments.
*$P < 0.05$ compared with control.
†Not significantly different from bradykinin alone.

Dexamethasone Blocks Bradykinin-Stimulated PGE_2 Synthesis but Is Without Effect on Ins*P* Formation. Glucocorticoids inhibit receptor-mediated arachidonic acid release and metabolism in many tissues (24). To determine whether a similar effect occurs in Swiss 3T3 cells, these cells were incubated with dexamethasone for 18–24 hr. With this treatment, bradykinin-stimulated PGE_2 synthesis was inhibited. The IC_{50} for dexamethasone inhibition of bradykinin-stimulated PGE_2 synthesis was 0.9 nM (Fig. 4). In contrast, in cells that had been prelabeled with [^{3}H]Ins, dexamethasone had no effect on [^{3}H]Ins*P* formation (Fig. 4).

Phorbol Ester Blocks Bradykinin-Stimulated Ins*P* Formation but Enhances Bradykinin-Stimulated PGE_2 Synthesis. Phorbol esters block phosphatidylinositol turnover in response to agonists in several tissues (25, 26). In Swiss 3T3 cells prelabeled with [^{3}H]Ins, PMA had no effect on basal [^{3}H]Ins*P* formation (Table 3). However, PMA inhibited bradykinin-stimulated Ins*P* formation (Table 3). PMA slightly stimulated basal PGE_2 synthesis (Table 3). In contrast to

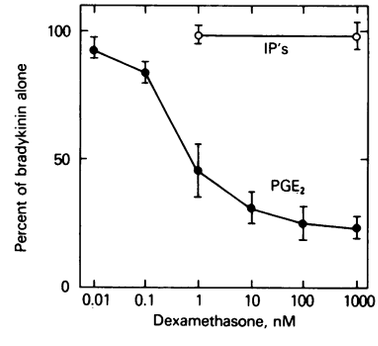

FIG. 4. Dexamethasone inhibits bradykinin-stimulated PGE_2 synthesis in Swiss 3T3 cells. Cells were pretreated with dexamethasone or dexamethasone plus [^{3}H]inositol for 24 hr. Data are from three experiments for PGE_2 synthesis and from four experiments for Ins*P* (IP) formation.

Table 3. Phorbol ester inhibits bradykinin-stimulated Ins*P* formation but enhances bradykinin-stimulated PGE_2 synthesis

	PGE_2, pg/well	[^{3}H]Ins*P*, cpm/well
Control	86 ± 19	854 ± 112
PMA (10 nM)	134 ± 24*	882 ± 85
Bradykinin (1 μM)	645 ± 72*	1762 ± 94*
+PMA	1120 ± 148†	1128 ± 86†

Data are from four experiments.
*$P < 0.05$ compared to control.
†$P < 0.05$ compared to bradykinin alone.

its effect on bradykinin-stimulated Ins*P* formation, PMA enhanced bradykinin-stimulated PGE_2 synthesis (Table 3).

Bradykinin Stimulates Phospholipase A_2 to Release Arachidonic Acid from Phosphatidylcholine. When Swiss 3T3 cells were prelabeled with [^{3}H]choline for 48 hr, bradykinin stimulated the formation of the phospholipase A_2 products lysophosphatidyl[^{3}H]choline and glycerophospho[^{3}H]choline (Table 4). When cells were pretreated with dexamethasone for 24 hr, bradykinin-stimulated formation of lysophosphatidylcholine and glycerophosphocholine were inhibited (Table 4), similar to its effect on PGE_2 synthesis (Fig. 4). When cells were incubated with PMA, bradykinin-stimulated lysophosphatidylcholine and glycerophosphocholine formation were enhanced (Table 4).

Cycloheximide Inhibits Bradykinin-Stimulated PGE_2 Synthesis. Previous studies have shown that bradykinin stimulation of PGE_2 synthesis is inhibited by the protein synthesis inhibitor cycloheximide (27). In Swiss 3T3 cells, also, cycloheximide blocked bradykinin stimulation of PGE_2 synthesis (Table 5). The RNA synthesis inhibitor actinomycin D was without effect on bradykinin-stimulated PGE_2 synthesis (Table 5). Cycloheximide had no effect on bradykinin-stimulated Ins*P* formation (Table 5).

GTP[γ-S] Stimulates Membrane-Bound Phospholipase A_2 in Swiss 3T3 Cells. The experiment in Table 4 demonstrated that bradykinin stimulated phospholipase A_2 in intact cells. However, the observation that cycloheximide inhibited bradykinin-stimulated PGE_2 synthesis raised the possibility that occupation of the bradykinin receptor was not directly coupled to phospholipase A_2, but instead occupation of the receptor stimulated synthesis of a protein and activation of phospholipase A_2 might be a secondary event. Thus, experiments were done using cell-free membrane preparations from 3T3 cells in an effort to distinguish between these

Table 4. Bradykinin stimulates phospholipase A_2 activity in Swiss 3T3 cells

	Lysophosphatidylcholine: Lysophosphatidyl[^{3}H]choline, cpm/well
Control	94 ± 12
Bradykinin (1 μM)	136 ± 22*
+ dexamethasone (100 nM)	98 ± 16†
+ PMA (10 nM)	204 ± 38†
Glycerophosphocholine	**Glycerophospho[^{3}H]choline, cpm/well**
Control	256 ± 30
Bradykinin	512 ± 75*
+ dexamethasone	318 ± 42†
+ PMA	820 ± 144†

Data are from five experiments.
*$P < 0.05$ compared to control.
†$P < 0.05$ compared to bradykinin alone.

Table 5. Cycloheximide inhibits bradykinin-stimulated PGE_2 synthesis in Swiss 3T3 cells

	PGE_2, pg/well	[^{3}H]Ins*P*, cpm/well
Control	124 ± 18	1244 ± 79
Cycloheximide (100 μg/ml)	116 ± 21	1175 ± 85
Actinomycin D (10 μM)	120 ± 12	
Bradykinin (1 μM)	462 ± 34*	1820 ± 118*
+ cycloheximide	156 ± 25†	1902 ± 106‡

Data are from three experiments.
*$P < 0.01$ compared with control.
†$P < 0.01$ compared with bradykinin alone.
‡Not significantly different from bradykinin alone.

alternative mechanisms. GTP[γ-S] stimulated phospholipase A_2 activity in the cell-free preparation, whereas bradykinin in the absence of GTP[γ-S] did not fully stimulate the enzyme (Table 6). This finding is similar to our earlier results with α_1-adrenergic stimulation of phospholipase A_2 in FRTL5 cells (8), is consistent with a requirement for GTP to allow a receptor to transduce its message through G protein coupling, and suggests that protein synthesis is not an absolute prerequisite for receptor stimulation of phospholipase A_2.

DISCUSSION

In Swiss 3T3 fibroblasts, bradykinin stimulated phospholipase C-mediated Ins*P* formation and PGE_2 synthesis. Using GTP analogues, G proteins were implicated in the mediation of these effects of bradykinin. Bradykinin-stimulated Ins*P* formation, and PGE_2 synthesis appeared to be mediated by two separate GTP-dependent pathways that could be dissociated with PMA, dexamethasone, or cycloheximide. PMA inhibited Ins*P* turnover and stimulated PGE_2 synthesis, whereas dexamethasone and cycloheximide inhibited PGE_2 synthesis but not Ins*P* formation. Bradykinin also stimulated phospholipase A_2-catalyzed degradation of phosphatidylcholine. Bradykinin-stimulated phosphatidylcholine degradation was affected by PMA and dexamethasone similarly to their effects on bradykinin-stimulated PGE_2 synthesis, leading to the conclusion that arachidonate for PGE_2 synthesis is liberated by a phospholipase A_2.

G proteins have recently been implicated in receptor-mediated activation of phospholipase A_2 in several systems: α_1-adrenergic-stimulated PGE_2 synthesis in FRTL5 thyroid cells (8), leukotriene C_4- and leukotriene D_4-stimulated PGI_2 synthesis in the bovine endothelial cell line CPAE cells (9), and in light-activated arachidonate release from bovine rod outer segments (10). In all those systems the G proteins that coupled the receptors to phospholipase A_2 were pertussis toxin-sensitive. In the present study bradykinin stimulated arachidonate release and metabolism to PGE_2 in Swiss 3T3 fibroblasts by activating a phospholipase A_2 coupled to the receptor by a G protein not sensitive to pertussis toxin. Thus, more than one G protein appears to couple receptors to phospholipase A_2 activation. A similar situation exists for the G proteins that couple receptors to phosphatidylinositol-

Table 6. GTP[γ-S] and bradykinin stimulate phospholipase A_2 in cell-free Swiss 3T3 membranes

	Lysophosphatidyl[^{3}H]choline, cpm
Control	1018 ± 55
Bradykinin (1 μM)	1114 ± 81
GTP[γ-S] (100 μM)	1290 ± 70*
+ bradykinin	1324 ± 64*

Data are from three experiments.
*$P < 0.05$ compared with control.

specific phospholipase C. Some of the G proteins are pertussis toxin-sensitive (1, 2), whereas others are not (6, 7).

PMA inhibited bradykinin-induced Ins*P* formation. In several other systems phorbol esters have been found to inhibit receptor agonist-stimulated phosphatidylinositol-specific phospholipase C (25, 26). Protein kinase C-mediated phosphorylation of the phospholipase C or the G protein coupling the receptor to the phospholipase C has been proposed as potential mechanisms for the inhibitory effects of phorbol esters on receptor-mediated Ins*P* formation (26). The exact mechanism involved in Swiss 3T3 cells remains to be established.

In contrast to its effect on Ins*P* formation, PMA potentiated bradykinin-stimulated PGE_2 synthesis. A phosphatidylcholine-specific phospholipase C that is activated by phorbol esters has been described in several tissues (28, 29). Such a phospholipase might be responsible for the enhanced release of arachidonate by increasing cellular diglyceride, from which arachidonate could be deacylated by diglyceride lipase. However, when added to cells in the absence of bradykinin, PMA had little effect on PGE_2 synthesis. That protein kinase C is not involved directly in bradykinin-stimulated PGE_2 synthesis is the observation that H-7, a protein kinase C inhibitor (30), is without effect on bradykinin-stimulated PGE_2 synthesis in 3T3 cells (R.M.B., unpublished observation).

Dexamethasone has been proposed to induce synthesis of a phospholipase A_2 inhibitory protein, lipocortin (31). It has been suggested that lipocortin may be inactivated by phosphorylation catalyzed by protein kinase C (32). Whether dexamethasone induced such a protein in Swiss 3T3 cells to inhibit bradykinin-stimulated PGE_2 synthesis remains to be determined. Phosphorylation and inactivation of lipocortin provides a second potential mechanism for the enhancement in bradykinin-stimulated PGE_2 synthesis by PMA.

Bradykinin stimulated formation of lysophosphatidylcholine and glycerophosphocholine. Lysophosphatidylcholine is the direct product of phospholipase A_2, whereas glycerophosphocholine is the product of subsequent lysophospholipase (phospholipase B) on lysophosphatidylcholine (18). These observations provide additional evidence that phospholipase A_2 was stimulated in response to bradykinin in Swiss 3T3 cells. Dexamethasone and PMA affected bradykinin-stimulated lysophosphatidylcholine and glycerophosphocholine similarly to their effects on bradykinin-stimulated PGE_2 synthesis. All of these findings again indicate that phospholipase A_2-catalyzed release of arachidonate from phospholipids is the source of substrate for PGE_2 synthesis.

In platelets, α_2-adrenergic agonists stimulate phospholipase A_2 (33). However, in those cells the phospholipase A_2 may be activated indirectly because the receptor may couple to a Na^+/H^+ antiporter that alkalinizes the cytosol to stimulate phospholipase A_2 secondarily (33). Such a mechanism for phospholipase A_2 activation is unlikely in Swiss 3T3 cells because the Na^+/H^+ antiporter inhibitor amiloride has no effect on bradykinin-stimulated PGE_2 synthesis. Further, acidifying the media to pH 6.9 or substituting choline or N-acetylglucosamine for sodium had no effect on bradykinin-stimulated PGE_2 synthesis (R.M.B., unpublished observations).

In CPAE endothelial cells bradykinin and leukotrienes stimulate prostaglandin synthesis that is inhibited by the protein synthesis inhibitor cycloheximide (34). It has been suggested that receptor activation stimulates rapid induction of a phospholipase A_2 stimulatory protein (17). In CPAE cells leukotriene-stimulated prostaglandin synthesis is pertussis toxin-sensitive (9), suggesting the involvement of a G protein in phospholipase A_2 activation by leukotriene. A similar phenomenon may occur in Swiss 3T3 cells because cycloheximide blocks bradykinin stimulation of PGE_2 synthesis. Whether the G protein couples receptor activation to phospholipase activation indirectly by controlling synthesis of a phospholipase stimulatory protein or whether the G protein couples the receptor directly to the phospholipase is unknown at present. However, in cell-free preparations from Swiss 3T3 cells, we found that GTP[γ-S] and bradykinin stimulated phospholipase A_2. Thus, there are at least two mechanisms for stimulation of phospholipase A_2. Further work will be required to clarify the interrelationships among the pathways.

R.M.B. was supported by the Pharmacology Research Associate program of the National Institute of General Medical Sciences.

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