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Phospholipase A_2 and phospholipase C are activated by distinct GTP-binding proteins in response to α_1-adrenergic stimulation in FRTL5 thyroid cells

(arachidonic acid/inositol phosphate/intracellular calcium/pertussis toxin/protein kinase C)

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ABSTRACT **In FRTL5 rat thyroid cells, norepinephrine, by interacting with α_1-adrenergic receptors, stimulates inositol phosphate formation, through activation of phospholipase C, and arachidonic acid release. Recent studies have shown that GTP-binding proteins couple several types of receptors to phospholipase C activation. The present study was undertaken to determine whether GTP-binding proteins couple α_1-adrenergic receptors to stimulation of phospholipase C activity and arachidonic acid release. When introduced into permeabilized FRTL5 cells, guanosine 5′-[γ-thio]triphosphate (GTP[γ-S]), which activates many GTP-binding proteins, stimulated inositol phosphate formation and arachidonic acid release. Neomycin inhibited GTP[γ-S]-stimulated inositol phosphate formation but was without effect on GTP[γ-S]-stimulated arachidonic acid release, suggesting that separate GTP-binding proteins mediate each process. In addition, pertussis toxin inhibited norepinephrine-stimulated arachidonic acid release but not norepinephrine-stimulated inositol phosphate formation. Norepinephrine-stimulated arachidonic acid release but not inositol phosphate formation was also inhibited by decreased extracellular calcium and by TMB-8, suggesting a role for a phospholipase A_2. To confirm that arachidonic acid was released by a phospholipase A_2, FRTL5 membranes were incubated with 1-acyl-2-[^{3}H]arachidonoyl-*sn*-glycero-3-phosphocholine. GTP[γ-S] slightly stimulated arachidonic acid release, whereas norepinephrine acted synergistically with GTP[γ-S] to stimulate arachidonic acid release. The results show that phospholipase C and phospholipase A_2 are activated by α_1-adrenergic agonists. Both phospholipases are coupled to the receptor by GTP-binding proteins. That coupled to phospholipase A_2 is pertussis toxin-sensitive, whereas that coupled to phospholipase C is pertussis toxin-insensitive.**

FRTL5 is a cell line derived from normal rat thyroid in which thyrotropin induces the appearance of α_1-adrenergic receptors (1). In these cells α_1-adrenergic stimulation evokes enhanced inositol phosphate formation (2) and release of arachidonic acid (3). Inositol phosphates and arachidonic acid released by stimulation of α_1-adrenergic receptors are linked to different functions. Inositol phosphates appear to enhance intracellular free calcium to mediate iodine efflux (4), while arachidonic acid via its cyclooxygenase metabolite prostaglandin E_2 enhances DNA synthesis (3). Inositol phosphate formation results from phospholipase C activity on phosphatidylinositols. The release of arachidonic acid has been ascribed either to activity of phospholipase A_2 (5) or to sequential action of phospholipase C and diacylglycerol lipase (6).

The coupling of receptors to phospholipases is unclear. However, recent evidence suggests that GTP-binding proteins [N (or G) proteins] are involved in activation of phospholipases C (7–14). Phospholipases A_2 usually are described as having a requirement for calcium, and their activation has been assumed to result from elevation of intracellular calcium induced by receptor agonists. However, the levels of calcium available after receptor activation (high nanomolar to low micromolar) are far below those usually found optimal for phospholipase A_2 activation *in vitro* (several millimolar).

The present study was undertaken to gain insight into the mechanism of activation of arachidonic acid release in FRTL5 thyroid cells. We find that activation of N proteins by the GTP analog guanosine 5′-[γ-thio]triphosphate (GTP[γ-S]) results in release of arachidonic acid and enhanced inositol phosphate formation. The N proteins mediating these effects are distinct. That mediating arachidonic acid release is pertussis toxin-sensitive, whereas that mediating inositol phosphate release is not affected by pertussis toxin.

MATERIALS AND METHODS

Materials. RHC 80267 was a gift of Revlon Health Care Group, Tuckahoe, NY. Norepinephrine, phorbol 12-myristate 13-acetate, 1-oleoyl-2-acetyl-*rac*-glycerol, neomycin, nifedipine, TMB-8 [3,4,5-trimethoxybenzoic acid 8-(diethylamino)octyl ester], and fatty acid-free bovine serum albumin were obtained from Sigma. GTP[γ-S] was from Boehringer Mannheim. Coon's modified Ham's F-12 medium was from KC Biological (Lenexa, KS). Hanks' balanced salts solution (HBSS) was from GIBCO. [^{3}H]Arachidonic acid (84 Ci/mmol; 1 Ci = 37 GBq), *myo*-[^{3}H]inositol (15.8 Ci/mmol), and GTP[γ-^{35}S] (1106 Ci/mmol) were from New England Nuclear. Phosphatidylcholine labeled at the 2 position with [^{3}H]-arachidonic acid, was prepared by incubating FRTL5 cells with [^{3}H]arachidonic acid. The cells were extracted and 1-acyl-2-[^{3}H]arachidonoyl-*sn*-glycero-3-phosphocholine was purified as described (15). Pertussis toxin used in the reported experiments was from List Biochemical (Campbell, CA). Pilot experiments demonstrated that pertussis toxin prepared by M. Ui (Hokkaido University, Sapporo, Japan) or R. Sekura (National Institutes of Health) yielded similar results.

Cell Culture. FRTL5 cells were a gift from Leonard Kohn (National Institutes of Health) and were cultured in Coon's modified Ham's F-12 medium supplemented with thyrotropin (0.1 milliunit/ml), insulin, transferrin, hydrocortisone, somatostatin, and glycyl-L-histidyl-L-lysine acetate as previously described (3). Cells were used 2–3 days after subculturing, at which time they were 20–30% confluent.

[^{3}H]Arachidonic Acid Release. Cells grown in 6-well plates were labeled with [^{3}H]arachidonic acid (1 μCi per well) overnight and release was monitored exactly as described (3).

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Abbreviations: GTP[γ-S], guanosine 5′-[γ-thio]triphosphate; HBSS, Hanks' balanced salts solution.

In several experiments (Figs. 1 and 2) cells were labeled with [^{3}H]arachidonic acid (6 μCi/6 ml) in 100-mm plates overnight. Cells then were washed twice with Ca^{2+}- and Mg^{2+}-free HBSS containing 10 mM Hepes and fatty acid-free bovine serum albumin (1 mg/ml) and were incubated in that medium containing 4 mM EGTA for 15 min. Cells were washed from the plate with a pipet and centrifuged at 200 × *g* for 5 min. Cells were resuspended in HOST medium (see below) plus any experimental agents.

[^{3}H]Inositol Phosphate Formation. Cells grown in 6-well plates were labeled for 48–72 hr with *myo*-[^{3}H]inositol (20 μCi per well). Cells were washed twice with HBSS containing 1.5 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM Hepes, and 10 mM LiCl, then incubated for 15 min at 37°C with 1 ml of the same medium. Experimental agents were then added, and incubation was continued for an additional 15 min. The reactions were terminated and inositol phosphates were analyzed as described (2). In several experiments (Fig. 2) cells were labeled (100 μCi/6 ml) in 100-mm plates and were isolated as in the above paragraph prior to exposure to HOST medium.

Permeabilization. To permit introduction of GTP[γ-S] and neomycin, cells were transiently permeabilized by the hypoosmotic (HOST) method (16). HOST medium contained 3 mM Hepes, 5 mM MgATP, and, when appropriate, 100 μM GTP[γ-S] or 100 μM neomycin. To initiate the experiment at the end of the permeabilization period, cells were centrifuged at 200 × *g* for 5 min and resuspended in HBSS containing Ca, Mg, and Hepes at 37°C. In experiments in which [^{3}H]-arachidonic acid release was measured, the reaction was terminated by centrifugation for 15 sec in a Beckman Microfuge and the radioactivity in the supernatant was measured by liquid scintillation counting. To test whether the cells did become permeable to GTP[γ-S] by means of this procedure, GTP[γ-^{35}S] (1 μCi/2 ml) was included in the HOST medium in several experiments. At the end of the permeabilization period, the cells were centrifuged and washed three times with HBSS containing Ca^{2+}, Mg^{2+}, and Hepes at 37°C. Control cells, which were incubated with GTP[γ-^{35}S] in HBSS containing Ca^{2+}, Mg^{2+}, and Hepes instead of in HOST medium, retained 148 ± 15 cpm (n = 3). Cells incubated with HOST medium retained 656 ± 51 cpm (n = 3). In determinations of whether cells were functional after the HOST procedure, norepinephrine-stimulated [^{3}H]arachidonic acid release was found to be 3.6 ± 0.4-fold of basal after the HOST procedure and 3.7 ± 0.4-fold of basal in control cells. If the cells were returned to culture medium after HOST treatment, they continued to replicate.

Assay of Phospholipase A_2 Activity. Cells growing in 100-mm plates were rinsed with 10 ml of Ca^{2+}- and Mg^{2+}-free HBSS. Next, 1 ml of ice-cold 10 mM Tris·HCl (pH 8) was added and the cells were scraped from the plate. Cells were placed into a Dounce homogenizer and homogenized by hand with three strokes. The homogenate was centrifuged at 25,000 × *g* for 20 min. The pellet was resuspended in 10 ml of ice-cold 50 mM Tris·HCl, pH 7.5/5 mM $MgCl_2$/1 mM EGTA, rehomogenized, and centrifuged at 600 × *g* for 20 min. The supernatant was centrifuged at 25,000 × *g* for 20 min and the crude membrane pellet was resuspended in 100 mM Tris·HCl (pH 8.5). Assays were conducted in 100 μl of 100 mM Tris·HCl (pH 8.5) containing 1 mM Ca^{2+}, 1.5 nmol of substrate (in dimethyl sulfoxide), and 100 μg of membrane protein. The reaction mixture minus membrane protein was sonicated with a Kontes probe sonicator for 15 sec, and then the membrane protein was added. The membrane preparation was sonicated for 15 sec before aliquots were removed for assay. After 5 min at 37°C, the reaction was stopped with the addition of 300 μl of ethanol. The extract was separated into released fatty acid and the unreacted substrate by thin-layer chromatography using chloroform/methanol/water (63:27:4) on Whatman LK6D plates.

RESULTS

GTP[γ-S] Stimulates Arachidonic Acid Release and Inositol Phosphate Formation. GTP[γ-S] is a hydrolysis-resistant analog of GTP that, in many systems, binds to and activates N proteins (13, 14). GTP[γ-S], however, is not readily able to cross the plasma membranes of most cells (14). In the present experiments GTP[γ-S], when added to intact FRTL5 cells, had no effect on [^{3}H]arachidonic acid release (data not shown). However, after cells were transiently permeabilized in the presence of GTP[γ-S], [^{3}H]arachidonic acid release was enhanced 2-fold over release in the absence of GTP[γ-S] (Fig. 1). In several tissues GTP[γ-S] has also been shown to enhance inositol phosphate formation by activation of phospholipase C (13). GTP[γ-S], when incorporated into FRTL5 cells, stimulated [^{3}H]inositol phosphate formation 4-fold (Fig. 2).

GTP[γ-S] Stimulates Release of Arachidonic Acid After Inhibition of Phospholipase C. To determine whether arachidonic acid release was an ultimate consequence of phospholipase C activation, the effect of GTP[γ-S] on arachidonic acid release was assessed after inhibition of phospholipase C. Neomycin, which "inhibits" phospholipase C by binding phosphatidylinositols (14, 17), reduced GTP[γ-S]-stimulated [^{3}H]inositol phosphate formation to near the control level but did not block GTP[γ-S]-stimulated [^{3}H]arachidonic acid release (Fig. 2).

In order for arachidonic acid to be released as a result of phospholipase C activity, a diacylglycerol lipase is postulated as the enzyme catalyzing release of free arachidonic acid (6). To determine whether diacylglycerol lipase is involved in arachidonic acid release, an inhibitor of this enzyme, RHC 80267, was used (18). At no concentration tested did RHC 80267 inhibit [^{3}H]arachidonic acid release (Table 1). At a high concentration RHC 80267 enhanced arachidonic acid release (Table 1).

Pertussis Toxin Inhibits Norepinephrine-Stimulated Arachidonic Acid Release but Not Inositol Phosphate Formation. Since the above experiments suggested the involvement of an N protein in arachidonic acid release, the effect of pertussis toxin was investigated. Pertussis toxin catalyzes the ADP-ribosylation of certain N proteins, reducing their ability to couple receptors to effector proteins (19). Pertussis toxin (100 ng/ml), when incubated with FRTL5 cells for 4 hr prior to HOST treatment, blocked GTP[γ-S]-stimulated [^{3}H]ara-

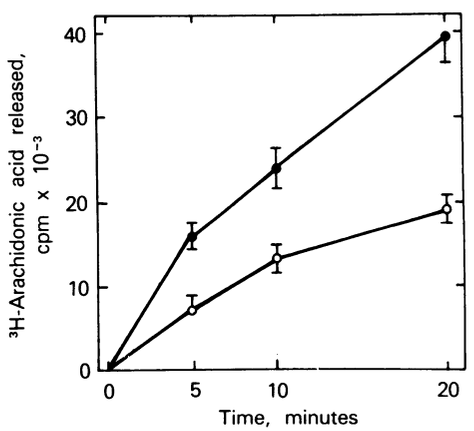

FIG. 1. GTP[γ-S] stimulates arachidonic acid release. Cells prelabeled with [^{3}H]arachidonic acid were permeabilized in the presence or absence of 100 μM GTP[γ-S] as described in *Materials and Methods*. After resuspension in HBSS, the cells were incubated in a shaking water bath at 37°C, and aliquots were taken at various times to determine arachidonic acid release from control cells (○) and from cells incubated with GTP[γ-S] (●). Data are shown for four preparations measured in duplicate.

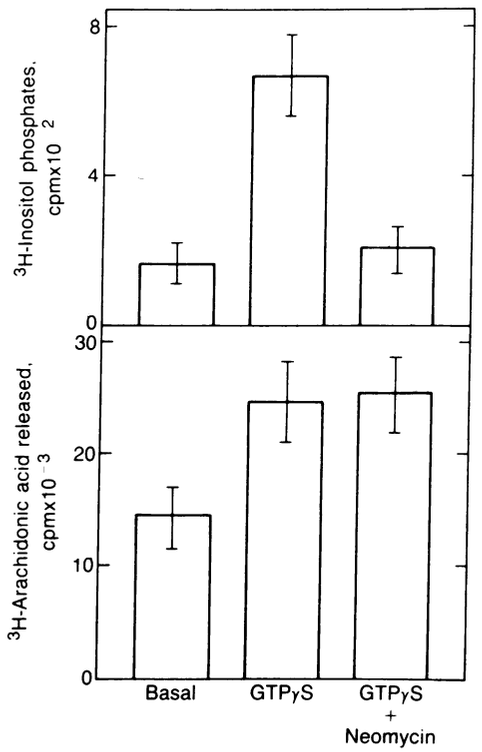

FIG. 2. Neomycin inhibits GTP[γ-S]-stimulated inositol phosphate formation but not arachidonic acid release. Cells were prelabeled with [^{3}H]arachidonic acid or *myo*-[^{3}H]inositol and then permeabilized in the presence of GTP[γ-S] (100 μM) or neomycin (100 μM). Cells were incubated in a shaking water bath at 37°C for 10 min before the reaction was terminated. (n = 4 preparations, measured in duplicate.)

chidonic acid release but was without effect on GTP[γ-S]-stimulated inositol phosphate formation (data not shown). Of greater interest was the observation that when cells were incubated with pertussis toxin (0.1–500 ng/ml) for 4 hr, subsequent stimulation of [^{3}H]arachidonic acid release by norepinephrine was inhibited in a dose-dependent manner (Fig. 3). The effect of pertussis toxin was dependent on the length of time it was incubated with the cells, indicating that uptake into the cells is rate-limiting (19). No effect of pertussis toxin was apparent with incubation <2 hr.

In cells prelabeled with *myo*-[^{3}H]inositol, pertussis toxin (10 ng/ml) had no effect on norepinephrine-stimulated [^{3}H]inositol phosphate formation (Fig. 4).

Role of Calcium in Norepinephrine Stimulation of Arachidonic Acid Release. It has been demonstrated in numerous tissues that arachidonic acid release catalyzed by phospho-

Table 1. Diacylglycerol lipase inhibitor RHC 80267 does not block norepinephrine-stimulated release of [^{3}H]arachidonic acid

Treatment		[^{3}H]Arachidonic acid released, cpm
Norepinephrine	RHC 80267, μM	
−	0	264 ± 21
+	0	923 ± 103*
+	1	897 ± 114*
+	10	906 ± 83*
+	100	1012 ± 96*
+	1000	3617 ± 312*

RHC 80267 in dimethyl sulfoxide was added (1:30 to 1:1000) to wells 15 min before addition of norepinephrine (10 μM). Controls also received dimethyl sulfoxide. Release was measured for 90 sec. *P < 0.01 compared to control (n = 3 preparations, assayed in duplicate).

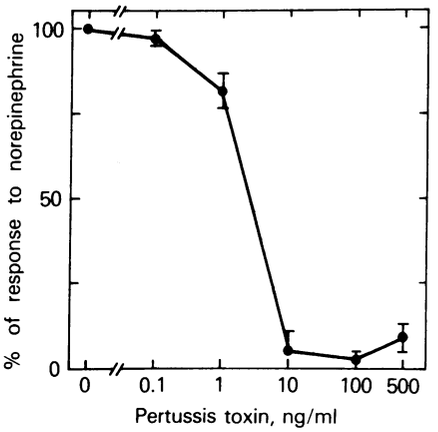

FIG. 3. Pertussis toxin inhibits norepinephrine-stimulated arachidonic acid release. Cells prelabeled with [^{3}H]arachidonic acid were incubated with pertussis toxin for 4 hr. Norepinephrine (10 μM)-stimulated arachidonic acid release was measured for 90 sec. (n = 3 preparations, measured in duplicate.)

lipase A_2 is dependent on the presence of millimolar calcium, whereas activation of phospholipase C generally requires micromolar or less calcium (20, 21). In FRTL5 cells, addition of EGTA to the incubation medium to chelate extracellular calcium and reduce intracellular free calcium blocked the norepinephrine-stimulated [^{3}H]arachidonic acid release (Fig. 5). Similarly, TMB-8, which "antagonizes" intracellular calcium, blocked norepinephrine-stimulated [^{3}H]arachidonic acid release. However, nifedipine, which inhibits calcium uptake into cells through voltage-dependent calcium channels, had no effect on norepinephrine-stimulated [^{3}H]-arachidonic acid release (Fig. 5). This finding is in agreement with previous evidence that these cells lack voltage-dependent calcium channels (4). In contrast, addition of EGTA or TMB-8 had no effect on norepinephrine-stimulated [^{3}H]inositol phosphate formation (data not shown).

GTP[γ-S] Stimulates Membrane-Bound Phospholipase A_2 Activity. To further establish that the phospholipase responsible for arachidonic acid release in FRTL5 cells is a phospholipase A_2, experiments were performed with cell-free membrane preparations. When exogenous phosphatidylcholine labeled with [^{3}H]arachidonic acid in the 2 position

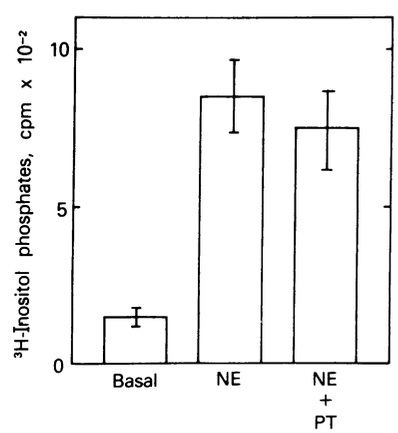

FIG. 4. Pertussis toxin is without effect on norepinephrine-stimulated inositol phosphate accumulation in FRTL5 thyroid cells. Cells were prelabeled with *myo*-[^{3}H]inositol. Norepinephrine (NE)-stimulated inositol phosphate formation was measured for 15 min. Pertussis toxin (PT) at 10 ng/ml was incubated with the cells for 4 hr prior to norepinephrine (10 μM) addition. (n = 3 preparations, measured in duplicate.)

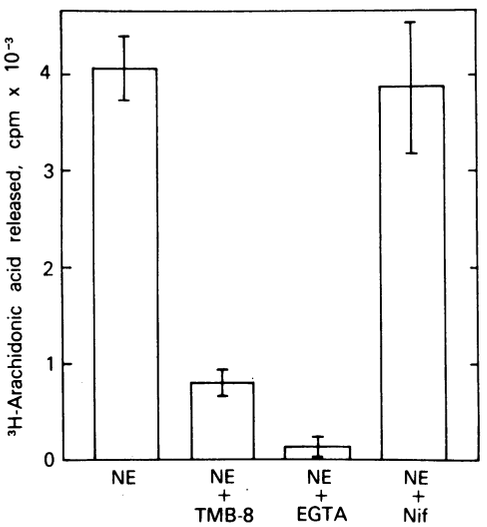

FIG. 5. Calcium is necessary for norepinephrine (NE)-stimulated arachidonic acid release in FRTL5 thyroid cells. TMB-8 (100 μM), EGTA (2 mM), or nifedipine (Nif, 100 μM) were incubated with the cells for 5 min prior to the addition of norepinephrine (10 μM) for 90 sec. (n = 3 preparations, measured in duplicate.)

was used to assay membrane-bound phospholipase A_2 activity, GTP[γ-S] was found to stimulate activity. In control membranes, 638 ± 76 cpm of [^{3}H]arachidonic acid was released. When GTP[γ-S] (100 μM) was added, 1086 ± 260 cpm was released ($P < 0.05$ compared to control, n = 4 preparations). Membranes to which norepinephrine (10 μM) alone was added released 686 ± 54 cpm. However, when membranes were incubated with norepinephrine plus GTP[γ-S], arachidonic acid release was enhanced in a synergistic manner to 2042 ± 320 cpm.

Diacylglycerol Analogs Inhibit Adrenergic Receptor-Stimulated Arachidonic Acid Release. Our laboratory has previously shown that phorbol esters, which activate protein kinase C, desensitize β-adrenergic receptors (22). Stimulation of α_1-adrenergic receptors in other tissues has also been shown to activate protein kinase C, which catalyzes phosphorylation of the receptor to inhibit generation of second messengers (23). In FRTL5 cells, treatment with phorbol 12-myristate 13-acetate blocked norepinephrine-stimulated arachidonic acid release (Table 2). Another activator of protein kinase C is 1-oleoyl-2-acetyl-*rac*-glycerol (24). This agent also blocked norepinephrine-stimulated [^{3}H]arachidonic acid release (Table 2).

Table 2. Inhibitory effect of activators of protein kinase C on norepinephrine-stimulated [^{3}H]arachidonic acid release

Pretreatment	[^{3}H]Arachidonic acid released, cpm
None	624 ± 43
Norepinephrine	2176 ± 262*
Phorbol myristate acetate	584 ± 56
Norepinephrine + phorbol myristate acetate	865 ± 98†
1-Oleoyl-2-acetyl-*rac*-glycerol	646 ± 48
Norepinephrine + 1-oleoyl-2-acetyl-*rac*-glycerol	724 ± 43†

Phorbol 12-myristate 13-acetate (10 ng/ml) or 1-oleoyl-2-acetyl-*rac*-glycerol (50 μg/ml) was added 5 min prior to norepinephrine (10 μM). Release was measured for 90 sec. (n = 3 preparations, assayed in duplicate).

*$P < 0.02$ compared to control.

†Not significantly different from phorbol ester or 1-oleoyl-2-acetyl-*rac*-glycerol alone.

DISCUSSION

In FRTL5 rat thyroid cells, norepinephrine, through interaction with α_1-adrenergic receptors, stimulates both arachidonic acid release (2) and inositol phosphate formation (3). The experiments described in this report demonstrate that the phospholipases responsible for both arachidonic acid release and inositol phosphate formation are GTP-dependent, suggesting that both processes are mediated by N proteins. In contrast to the phospholipase A_2-dependent release of arachidonic acid, phospholipase C-mediated inositol phosphate formation was not dependent on a high concentration of calcium, and the N protein coupled to phospholipase C was not pertussis toxin-sensitive.

In FRTL5 cells a protein of ≈40 kDa is ADP-ribosylated by pertussis toxin (25). This finding is consistent with an N_i- or N_o-like protein being present in these cells. However, α_2-adrenergic rather than α_1-adrenergic receptors have been associated with the adenylate cyclase-inhibiting N protein N_i (26). The function of N_o remains undescribed (27). Recent work from another laboratory (10) and the present results demonstrate that the α_1-adrenergic receptor is coupled to an N protein to activate phospholipase C. The present work also shows that α_1-adrenergic receptors are coupled via an N protein to a phospholipase A_2 that releases arachidonic acid.

Pertussis toxin sensitivity of receptor-mediated arachidonic acid release has been described in neutrophils (8), mast cells (7), and fibroblasts (9). In those reports the identity of the phospholipases mediating arachidonic acid release was not assessed directly. The evidence presented here indicates that the α_1-adrenergic-mediated release of arachidonic acid in FRTL5 cells is catalyzed by a phospholipase A_2 coupled to the receptor by a pertussis toxin-sensitive N protein. In several but not all of the previous reports, pertussis toxin inhibited both inositol phosphate formation and arachidonic acid release, whereas in the present study, the activity responsible for release of arachidonic acid was readily differentiated from the phospholipase C that catalyzed inositol phosphate formation, by several criteria: differential pertussis toxin sensitivity, different calcium requirements, and the inability of neomycin or a diacylglycerol lipase inhibitor to block arachidonic acid release.

The substrate from which phospholipase A_2 catalyzes release of arachidonic acid in FRTL5 cells remains unknown. Phosphatidylcholine arising from the receptor-mediated methylation of phosphatidylethanolamine is rich in arachidonic acid (28, 29). In unstimulated FRTL5 cells, arachidonic acid is preferentially incorporated into phosphatidylethanolamine (R.M.B., unpublished data). Thus, the methylation pathway may generate the substrate for the phospholipase A_2.

The interactions between calcium and N proteins in the activation of phospholipase A_2 are unknown. Recently, it has been shown that activation of an N protein dramatically lowers the calcium requirement of a phospholipase C to a level similar to the resting level of free calcium (30). Whether such a mechanism exists in FRTL5 cells for activation of phospholipase A_2 is unknown. Arachidonic acid itself may release calcium from intracellular stores without affecting inositol phosphate formation (31). It is possible that activation of the N protein coupled to phospholipase A_2 by an α_1-adrenergic receptor reduces the calcium requirement of the phospholipase. The lowered requirement for calcium by phospholipase A_2 would induce release of arachidonic acid, and the subsequent release of arachidonic acid might generate additional calcium to further activate the phospholipase A_2.

In the adenylate cyclase system there is an N protein, N_s, that activates the cyclase and another, N_i, that inhibits the cyclase (32). In the present work we have demonstrated a role

for an N protein in the activation of phospholipase A_2. As in the case of adenylate cyclase, it is possible that a dual regulation of phospholipase A_2 exists. A likely candidate for such an inhibitory protein is lipomodulin, a 40-kDa member of the lipocortin family, which inhibits phospholipase A_2 (33).

N proteins have been found to mediate many processes in addition to the originally described roles of N_s and N_i in mediating activation and inhibition, respectively, of adenylate cyclase (32). Transducin, like N_s and N_i, also mediates regulation of an enzyme, the cGMP phosphodiesterase in retinal rod outer segments (34). The present report shows that a pertussis toxin-sensitive N protein mediates arachidonic acid release via phospholipase A_2 activation. Similar pertussis toxin-sensitive arachidonic acid release has been reported in neutrophils (8) and in 3T3 fibroblasts (9). The N protein mediating activation of phospholipase C in the present study was pertussis toxin-insensitive, similar to that mediating activation of phospholipase C in hepatocytes (10). However, other phospholipases C present in neutrophils (8) and mast cells (7) are pertussis toxin-sensitive and thus appear to be coupled to different N proteins. Pertussis toxin-sensitive N proteins also mediate somatostatin-induced (35), as well as γ-aminobutyric acid- and norepinephrine-induced (36), inhibition of calcium channels in neural tissues. Muscarinic receptors are coupled to potassium channels in cardiac myocytes by a pertussis toxin-sensitive N protein (37). Inositol trisphosphate-induced calcium release from endoplasmic reticulum also appears to be N-protein-mediated (38). Thus, there appear to be several GTP-dependent N proteins, some of which are pertussis toxin-sensitive, linked to many effector proteins (adenylate cyclase, phospholipases A_2 and C, phosphodiesterases, ion channels) in a stimulatory or inhibitory manner.

A model of our present understanding of α_1-adrenergic-mediated events in the FRTL5 cell is depicted in Fig. 6. Norepinephrine (NE) binds to α_1-adrenergic receptors ($R\alpha_1$) that are coupled to two distinct N proteins, N_A and N_c, which activate phospholipase A_2 [sensitive to pertussis toxin (PT)] and phospholipase C, respectively. Whether a single receptor is coupled to both N proteins or whether subpopulations of receptors exist that are coupled to only one or the other N protein is unknown. Phospholipase A_2 (PLA_2) generates free arachidonic acid, which is metabolized to prostaglandin E_2 (PGE_2), which, together with norepinephrine, mediates DNA synthesis. Indomethacin, by inhibiting cyclooxygenase, reduces PGE_2 synthesis and inhibits DNA synthesis [shown as thymidine (TdR) incorporation in the figure] (3). Activation of a phospholipase C (PLC) increases inositol trisphosphate (IP_3) formation, which, in turn, increases intracellular free calcium (2). An increase in intracellular free calcium is necessary for iodine efflux (39). Activation of phospholipase C also increases diacylglycerol (DAG) formation, which activates protein kinase C (PKC) and feeds back to inhibit the α_1-adrenergic receptor linked to phospholipases A_2 and C, probably by phosphorylation (23).

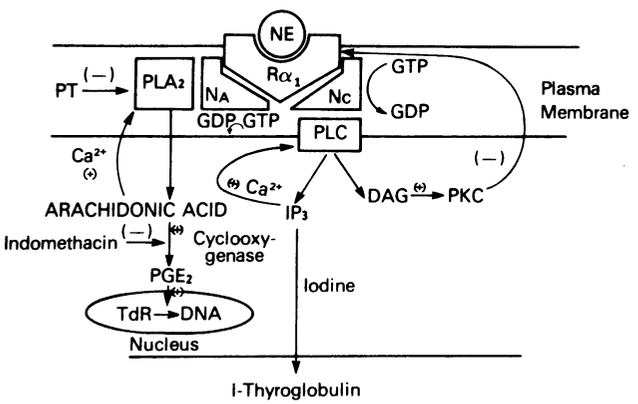

FIG. 6. Biochemical events following norepinephrine binding to α_1-adrenergic receptors in FRTL5 cells. See text for details.

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