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Phosphatidate and Monooleylphosphatidate Inhibition of Fibroblast Adenylate Cyclase Is Mediated by the Inhibitory Coupling Protein, N_i

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SUMMARY

It has previously been shown that monooleylphosphatidate (MOPA) and phosphatidate inhibit cAMP accumulation in VA13 and WI-38 fibroblasts. In this study we investigated whether this inhibition might be due to a decrease in adenylate cyclase activity. Our results showed that both MOPA and phosphatidate inhibit prostaglandin E_1-stimulated adenylate cyclase in WI-38 membranes in a concentration-dependent manner with half-maximal inhibitions at 0.1 and 0.5 μM, respectively, and maximal inhibitions of 35–55%. A 5 μM concentration of structurally similar lipids caused no significant inhibition. The inhibitory effects of MOPA and phosphatidate on adenylate cyclase were (i) GTP dependent, (ii) greater at low concentrations of Mg^{2+}, (iii) eliminated following treatment of cells with islet-activating protein, (iv) nonadditive with carbachol, and (v) noncompetitive with prostaglandin E_1. Collectively these data suggested that MOPA and phosphatidate inhibitions of cAMP accumulation were due at least in part to an N_i-mediated inhibition of adenylate cyclase. Furthermore, the inhibitions showed the same characteristics normally associated with hormonal inhibition of this enzyme.

INTRODUCTION

MOPA,[1] phosphatidate, and muscarinic agonists are known to inhibit cAMP accumulation of fibroblasts (1–4). The muscarinic cholinergic inhibition is at least partially due to inhibition of adenylate cyclase activity (3), and it also may be due in part to an increase in phosphodiesterase activity (5). There is no direct evidence that phosphatidate or MOPA alters cAMP accumulation by affecting the activity of either of these enzymes.

Hormonal inhibition of adenylate cyclase activity has been shown to be GTP dependent (6, 7) and enhanced by low concentrations of Mg^{2+} (8). It also appears to be regulated by a guanine nucleotide-binding regulatory protein (N_i) that interacts with both the hormone receptor and the adenylate cyclase catalytic unit (8). One line of evidence for the existence of N_i came about through studies which utilized IAP, an exotoxin from *Bordetella pertussis* (7–15). Treating cells with IAP attenuates hormonal inhibition of cAMP accumulation and adenylate cyclase activity, and it inhibits the increase in GTPase activity and GDP release normally induced by inhibitory hormones. These effects appear to be the result of an IAP-dependent ADP-ribosylation of a membrane protein (molecular weight = 41,000) that is thought to be a subunit of N_i.

The objective of this study was to determine if MOPA and phosphatidate inhibitions of cAMP accumulation in fibroblasts might be due to an inhibition of adenylate cyclase activity. Therefore, their effects on cAMP accumulation and on adenylate cyclase in WI-38 and VA13 cells were further investigated. Both MOPA and phosphatidate inhibited adenylate cyclase in WI-38 and VA13 membranes. Furthermore, the inhibitions resembled hormonal inhibition since they were GTP dependent, enhanced by low concentrations of Mg^{2+}, and attenuated by IAP treatment.

EXPERIMENTAL PROCEDURES

Cell culture. VA13 and WI-38 cells were grown in Eagle's minimum essential medium supplemented with fetal calf serum (Flow Laboratories) with minor modifications of previously described methods (16). Kanamycin was omitted from the medium, and stock cultures were maintained in T-150 (Falcon) flasks. For intact cell studies, the cells were seeded into 35-mm dishes at a concentration of 2–3 × 10^5 cells/dish and used 4 days later when they had reached confluency. The cells were fed once after seeding at approximately 24 hr prior to the experiment. For broken cell studies, 5–7 × 10^6 cells were seeded into 150-mm dishes or flasks and used 5–6 days later at confluency. Medium was replaced 3 days after plating and 24 hr prior to the experiment.

In the pertussis toxin studies the cells were treated with IAP (10 ng/ml) or vehicle (9) for 17 hr prior to the experiment or preparation of membranes.

Intact cell studies. The effects of various compounds on cAMP accumulation were determined by the prelabeling techniques previously

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[1] The abbreviations used are: MOPA, monooleylphosphatidate; IAP, islet-activating protein (pertussis toxin); Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PGE_1, prostaglandin E_1; N_i, inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase.

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described (1). Briefly, the ATP pool was prelabeled with [2,8-^{3}H] adenine, and then the percentage of conversion of [^{3}H]ATP to [^{3}H] cAMP was calculated by the equation (cAMP)/(AXP + cAMP), where AXP was ATP + ADP.

Broken cell studies. Adenylate cyclase was measured in WI-38 and VA13 membranes prepared by the previously described method of Clark and Butcher (17). Except for the experiment where the effects of varying Mg^{2+} concentrations on adenylate cyclase were studied, all buffers used in preparing and storing the membranes contained 1 mM EDTA.

Adenylate cyclase assay. Adenylate cyclase was measured by the method of Salomon *et al.* (18). Unless noted otherwise, the assay mixture contained 2 mM $MgCl_2$, 40 mM Hepes, 1 mM EDTA, 8 mM creatine phosphate, creatine phosphokinase (16 units/ml), 0.2 mM ATP, 10 μM GTP, 0.1 mM methylisobutylxanthine, approximately 2 μCi of [α-^{32}P]ATP, and 60 mM NaCl. The assay was initiated by the addition of 10–30 μg of protein, and a 10-min incubation at 30° was routinely used.

ADP-ribosylation. Membranes of vehicle- or IAP-treated cells (approximately 100 μg/tube) were incubated with a 10 μM NAD, 1 mM ATP, 0.2 mM GTP, 5 mM $MgCl_2$, 10 mM thymidine, 10 mM arginine, 5 mM creatine phosphate, creatine phosphokinase (16 units/ml), 1 mM EDTA, 20 mM Tris-HCl (pH 7.4), 1–5 μCi of [^{32}P]NAD, and IAP (5μg) or its vehicle for 30 min at 30°. The incubation was terminated by the addition of cold Tris-HCl buffer. The membranes were then centrifuged at 18,000 rpm for 15 min and washed once with Tris buffer. The pellet was suspended in 50 μl of gel sample buffer (10% glycerol, 5% mercaptoethanol, 3% sodium dodecyl sulfate, and 62.5 mM Tris-HCl, pH 6.8, and heated for 3 min at 90°. Electrophoresis of the radiolabeled membranes was performed according to the method of Laemmli (19). The ADP-ribosylated proteins were localized by autoradiography and quantitated by cutting and counting the dried gel.

Materials. Carbachol and atropine sulfate from Sigma were dissolved in water prior to use. PGE_1 and epinephrine, also from Sigma, were dissolved in 70% ethanol and 100 mM thiourea plus 10 mM ascorbate, respectively. L-α-Phosphatidic acid from egg lecithin and phosphatidylinositol were purchased from Sigma. All other lipids were purchased from Serdary Research Laboratories. The lipids were dispersed in water by sonication. Forskolin from Calbiochem was dissolved in 95% ethanol. IAP was a generous gift from Dr. Michio Ui and Dr. Toshiaki Katada, and [^{32}P]NAD was a generous gift from Dr. Ravi Iyengar and Dr. Lutz Birnbaumer.

RESULTS

Phosphatidate inhibition of cAMP accumulation. Changes in cAMP accumulation in VA13[2] cells treated with either PGE_1 of PGE_1 plus phosphatidate for 2–40 min are shown in Fig. 1. Phosphatidate inhibited PGE_1-stimulated cAMP accumulation, and the level of inhibition (approximately 50%) was constant over the 40-min period. The decrease in cAMP accumulation following phosphatidate treatment was not due to an increase in the amount of cAMP extruded from the cell, because there were comparable decreases in intracellular cAMP accumulation and in cAMP escape into the medium when cells were treated with phosphatidate. It also seemed unlikely that phosphatidate inhibition was due to an increase in phosphodiesterase activity, since VA13 cells have low phosphodiesterase activity, and cAMP levels were essentially unaffected by the phosphodiesterase inhibitor, 1-methyl-3-isobutylxanthine, in the presence or absence of phosphatidate (data not shown). Additionally, the constant inhibition measured during the 40-min time course suggested that the fractional turnover constant, which is primarily a function of phosphodiesterase activity, was unchanged (20).

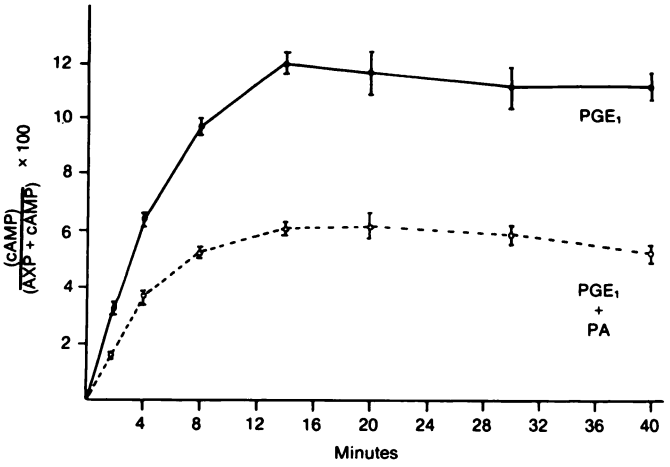

FIG. 1. *Time course of cAMP accumulation in VA13 cells treated with PGE_1 or PGE_1 plus phosphatidate*

The % conversion of [^{3}H]ATP to [^{3}H]cAMP was measured in VA13 cells treated with 1 μM PGE_1 or 1 μM PGE_1 plus 10 μM phosphatidate (*PA*) for the indicated times. Basal per cent conversion was 0.04 ± 0.001 without and 0.03 ± 0.001 with phosphatidate. Values shown are the mean ± S.E. of triplicate determinations.

Characteristics of phospholipid inhibition of adenylate cyclase. Since the phosphatidate inhibition did not appear to be due to an increase in phosphodiesterase activity or to an increase in cAMP escape, we examined whether the inhibition might be due at least in part to a decrease in adenylate cyclase activity. In the majority of the studies we used MOPA rather than phosphatidate, because it has previously been shown that MOPA was about 3-fold more potent as an inhibitor than phosphatidate in intact cell studies (3). Also, since the inhibitory effects of hormones are greater in WI-38 than in VA13 cells (1–3), WI-38 membranes were primarily used. Both phosphatidate and MOPA caused a concentration-dependent inhibition of PGE_1-stimulated adenylate cyclase in WI-38 membranes (Fig. 2). In this experiment, the concentration required to produce half-maximal inhibition by MOPA was approximately 0.1 μM. It can be seen that phosphatidate was less potent than MOPA. The maximal inhibitions varied from 35–55% in different membrane preparations, but they were typically equivalent for the two drugs at concentrations of 10 μM or greater. Maximal concentrations of MOPA also caused a 20–55% inhibition of basal adenylate cyclase activity in WI-38 membranes (*e.g.*, see Fig. 5). Additionally, there was no detectable lag in MOPA inhibition of adenylate cyclase (data not shown).

To determine the specificity of the MOPA inhibition, other lipids with similar structures were tested as inhibitors of adenylate cyclase in WI-38 membranes (Table

[2] The responses of VA13 and WI-38 cells to active phospholipids were qualitatively identical but varied quantitatively. The VA13 cells are less sensitive than WI-38 cells to hormonal and phospholipid inhibition of cAMP accumulation, but they grow more readily in culture. Since the magnitude of inhibition is less in broken cell studies than in the intact cell studies, the more sensitive WI-38 cells were used for most broken cell studies. For intact cell studies, we used primarily the VA13 line.

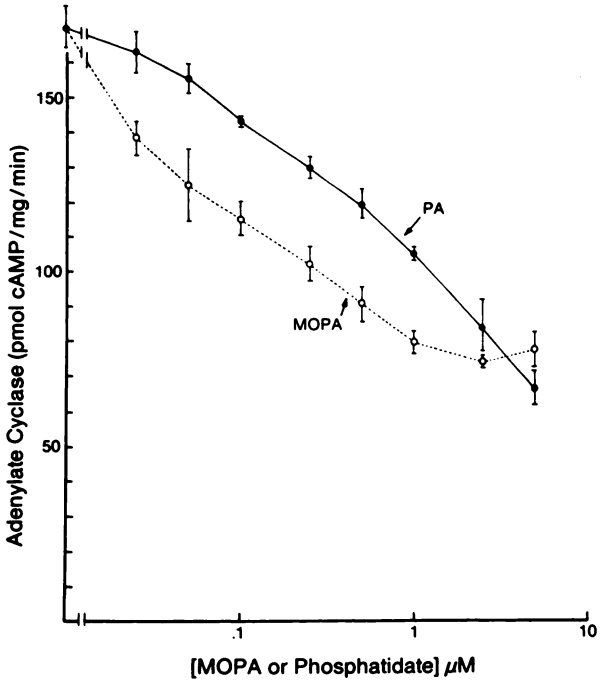

FIG. 2. *Effect of varying concentrations of MOPA or phosphatidate (PA) on PGE_1-stimulated adenylate cyclase in WI-38 membranes*

Adenylate cyclase was measured in WI-38 membranes exposed to 5.7 μM PGE_1 or 5.7 μM PGE_1 plus various concentrations of MOPA or PA. Values shown are the mean ± S.E. of triplicate determinations.

TABLE 1

Specificity of the lipid effect on WI-38 adenylate cyclase

Adenylate cyclase was measured in WI-38 membranes exposed to 5.7 μM PGE_1 or 5.7 μM PGE_1 and a 5 μM concentration of the indicated compounds. Values shown are the mean ± S.E. of triplicate determinations.

Additions	cAMP	Inhibition
	pmol/mg/min	*%*
Basal	31 ± 1	
PGE_1	142 ± 4	
PGE_1 + monooleylphosphatidate	85 ± 1	40
PGE_1 + monooleylphosphatidylcholine	127 ± 4	10
PGE_1 + dioleylphosphatidylcholine	130 ± 6	8
PGE_1 + phosphatidylinositol	130 ± 2	8
PGE_1 + 1,2-diolein	133 ± 7	6
PGE_1 + platelet-activating factor	123 ± 7	13

1). A 5 μM concentration of MOPA inhibited PGE_1-stimulated adenylate cyclase by 40%, but the same concentration of the other lipids tested caused, at most, an inhibition of 13%. These results agreed with earlier intact cell studies which showed that the concentration of these and other phospholipids required to produce significant inhibition of cAMP accumulation was about 20–100-fold greater than the IC_{50} values of MOPA or phosphatidate (2). The inhibition produced by high concentrations of the phospholipids might be due to the presence of MOPA or phosphatidate in the commercially available lipids or to a nonspecific effect of lipids in general.

Since it previously has been shown that hormonal inhibition of adenylate cyclase is GTP-dependent (6), the effects of varying concentrations of GTP on MOPA inhibition of forskolin-stimulated adenylate cyclase were measured (Fig. 3). In the absence of GTP, there was no measurable MOPA inhibition. Increasing concentrations of GTP caused a progressive increase in the percentage of inhibition due to MOPA. The concentration of GTP required for half-maximal MOPA inhibition was approximately 50 nM, and for maximal inhibition it was 0.25–1.0 μM GTP. Phosphatidate inhibition of forskolin-stimulated WI-38 adenylate cyclase also required GTP.

Another characteristic of hormonal inhibition of adenylate cyclase is that it is generally more prominent at concentrations of Mg^{2+} that are less than optimal for hormonal stimulation (8). We found similar results when we measured MOPA inhibition of PGE_1-stimulated adenylate cyclase in the presence of varying concentrations of Mg^{2+} (Fig. 4). MOPA and carbachol caused 40–60% inhibition when the concentration of free Mg^{2+} was less than 2.5 mM, but the percentage of inhibition decreased to 12–13% when 9.3 mM free Mg^{2+} was present. Phosphatidate inhibition was also more prominent at low concentrations of Mg^{2+} (data not shown).

Carbachol and MOPA inhibitions of cAMP accumulation in intact VA13 cells are nonadditive (3), and we found that carbachol and MOPA inhibitions of PGE_1-stimulated adenylate cyclase were also nonadditive when present either at concentrations around the IC_{50} or at maximally effective concentrations (Table 2). These findings are consistent with the mediation of MOPA and carbachol inhibitions by a common intermediate.

Hormonal inhibition of adenylate cyclase is usually noncompetitive (22) in nature. To determine whether MOPA inhibition of PGE_1-stimulated adenylate cyclase resembled the kinetics of hormonal inhibition, MOPA inhibition in the presence of varying concentrations of PGE_1 was measured (Fig. 5). At concentrations of PGE_1

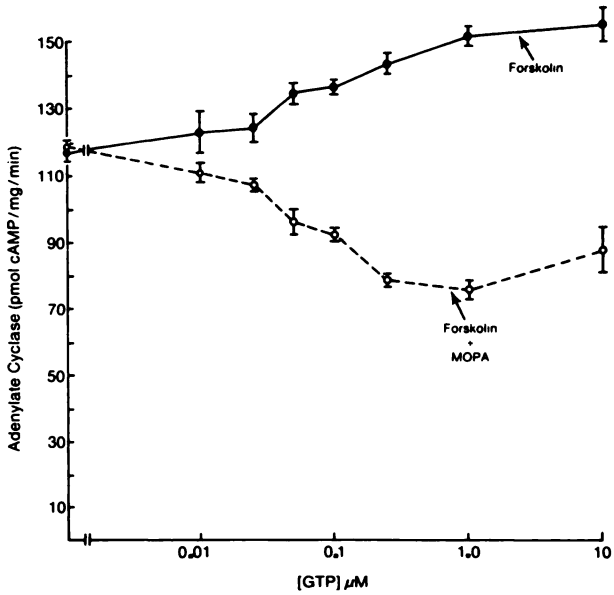

FIG. 3. *Effect of varying concentrations of GTP on MOPA inhibition of forskolin-stimulated adenylate cyclase in WI-38 membranes*

Adenylate cyclase was measured in WI-38 membranes exposed to 25 μM forskolin or 25 μM forskolin and 5 μM MOPA in the presence of varying concentrations of GTP. The concentration of NaCl in the incubation mixture was 120 μM. Values shown are the mean ± S.E. of triplicate determinations.

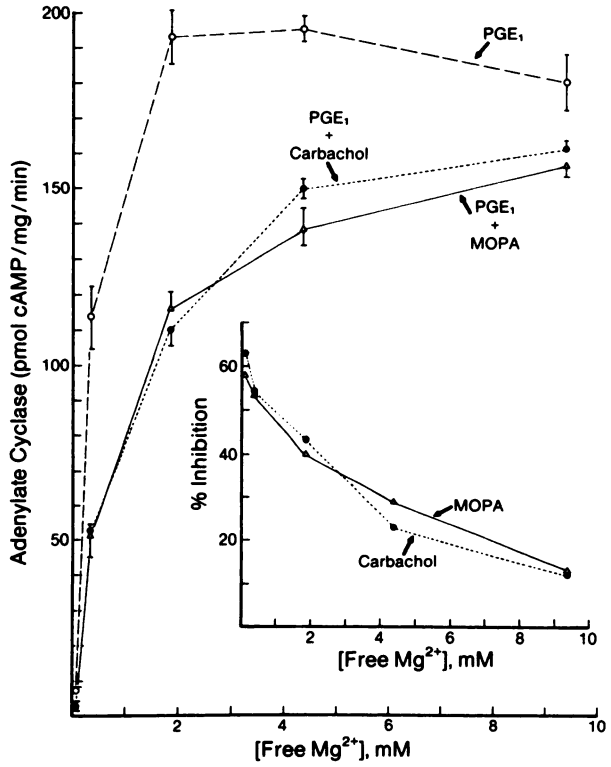

FIG. 4. *Effect of varying concentrations of free Mg^{2+} on MOPA and carbachol inhibitions of PGE_1-stimulated adenylate cyclase in WI-38 membranes*

Adenylate cyclase was measured in WI-38 membranes exposed to 5.7 μM PGE_1 alone, 5.7 μM PGE_1 plus 5 μM MOPA, or 5.7 μM PGE_1 plus 10 μM carbachol. All incubations contained 0.5 mM EDTA and various concentrations of Mg^{2+}. The concentration of free Mg^{2+} was calculated according to the method of Bockaert *et al.* (21). Values shown are the mean ± S.E. of triplicate determinations. *Inset*, shown is the percentage of inhibition calculated for data in Fig. 4.

TABLE 2

Lack of additivity of MOPA and carbachol inhibition of WI-38 adenylate cyclase

Adenylate cyclase was measured in WI-38 membranes exposed to 5.7 μM PGE_1 and when indicated carbachol and MOPA. Values shown are the mean ± S.E. of triplicate determinations.

Additions	cAMP	Inhibition
	pmol/mg/min	*%*
PGE_1	138 ± 1	
PGE_1 + 1 μM carbachol	104 ± 2	24
PGE_1 + 0.1 μM MOPA	110 ± 2	20
PGE_1 + μM carbachol + 0.1 μM MOPA	94 ± 2	31
PGE_1 + 10 μM carbachol	84 ± 3	39
PGE_1 + 5 μM MOPA	82 ± 2	41
PGE_1 + 10 μM carbachol + 5 μM MOPA	76 ± 2	45

ranging from 0.25–5.7 μM, 0.1 and 5 μM MOPA caused relatively constant inhibitions of approximately 20 and 35%, respectively. An Eadie-Hofstee transformation of the data revealed that there was a decrease in the V_{max} from 179 pmol/mg/min to 144 and 105 pmol/mg/min in the presence of 0.1 and 5 μM MOPA, respectively. Neither concentration of MOPA had any effect on the K_{act} for PGE_1 which is consistent with noncompetitive inhibition.

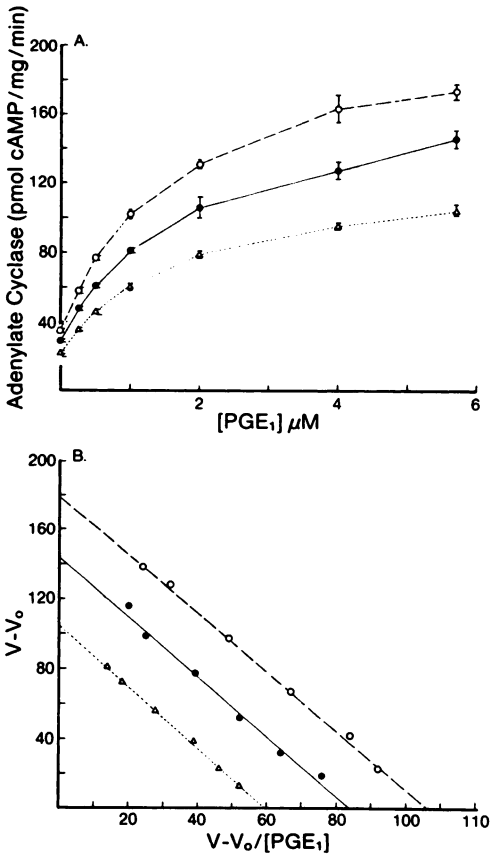

FIG. 5. *Effect of PGE_1 concentration on MOPA inhibition of PGE_1-stimulated adenylate cyclase in WI-38 membranes*

A, adenylate cyclase was measured in WI-38 membranes exposed to varying concentrations of PGE_1 in the absence (○) or presence of 0.1 μM MOPA (●) or 5 μM MOPA (△). Values shown are the mean ± S.E. of triplicate determinations. *B*, data in *A* transformed by the Eadie-Hofstee method.

The effects of IAP treatment on phosphatidate, MOPA, and carbachol inhibitions of cAMP accumulation. IAP treatment blocked phosphatidate and carbachol inhibitions of cAMP accumulation following PGE_1 or epinephrine stimulation of VA13 cells (Fig. 6). The inhibitory effects of phosphatidate on VA13 cells were concentration-dependent (Fig. 7), and pretreatment with IAP completely blocked the inhibition produced by even the relatively high concentration of 30 μM phosphatidate. IAP also blocked MOPA inhibition of PGE_1-stimulated cAMP accumulation in these cells (data not shown). Similar results were obtained with the nontransformed WI-38 fibroblasts.

IAP treatment attenuates carbachol and phospholipid inhibitions of adenylate cyclase and IAP-dependent [^{32}P] NAD incorporation in membranes. Carbachol and MOPA inhibitions of PGE_1-stimulated adenylate cyclase in VA13 cells were also blocked by IAP treatment, and, as has previously been shown (9–11), the effect of IAP was time dependent (Table 3). With increasing length of IAP treatment, there was a progressive decline in MOPA and carbachol inhibitions and in the IAP-dependent incorporation of [^{32}P]NAD in membranes. The molecular

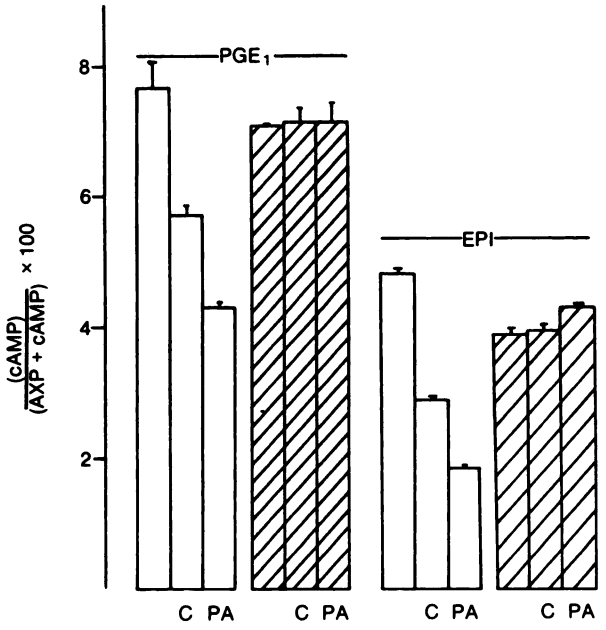

FIG. 6. *Effect of IAP on phosphatidate and carbachol inhibition of cAMP accumulation in VA13 cells*

The percentage of conversion of [^{3}H]ATP to [^{3}H]cAMP was measured in vehicle (*open bars*) or IAP pretreated (*striped bars*) VA13 cells stimulated with the indicated combinations of 1 μM epinephrine (EPI), 1 μM PGE_1, 100 μM carbachol (*C*), and 10 μM phosphatidate (*PA*) for 4 min. Values shown are the mean ± S.E. of triplicate determinations.

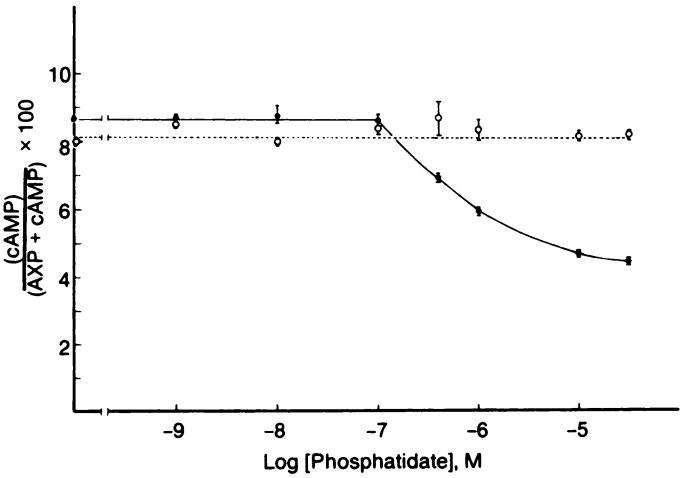

FIG. 7. *Concentration response to phosphatidate in VA13 cells pretreated with vehicle or IAP*

The percentage of conversion of [^{3}H]ATP to [^{3}H]cAMP was measured in vehicle (●) or IAP (○)-pretreated VA13 cells that were stimulated with 1 μM PGE_1 or 1 μM PGE_1 in combination with various concentrations of phosphatidate for 4 min. Values shown are the mean ± S.E. of triplicate determinations.

weight of the radiolabeled protein, as determined by sodium dodecyl sulfate-gel electrophoresis, was approximately 41 kDa, and this is in the range of molecular weights that have previously been reported for N_i. The data at the early time points are not extensive, but they suggested an increasing proportionality between loss of MOPA and carbachol inhibitions of adenylate cyclase and ADP-ribosylation with increasing time of IAP treatment. In any event, the results of the 18-h IAP treatment

TABLE 3

Effect of IAP treatment on ADP-ribosylation and carbachol or MOPA inhibitions of adenylate cyclase in VA13 membranes

IAP-dependent [^{32}P]NAD incorporation and adenylate cyclase were measured in membranes prepared from VA13 cells that had been treated with vehicle (18 hr) or IAP (10 ng/ml) for 2, 4, or 18 hr. [^{32}P]NAD incorporation was measured as described under "Experimental Procedures," and the results are expressed as (NAD incorporation in IAP-treated group/NAD incorporation in vehicle group) × 100. Values shown for the 0- and 18-hr IAP pretreatment are the means ± S.E. of three experiments, and for the 2- and 4-hr treatment values are the average of two experiments. The mean ± S.E. NAD incorporation for the vehicle-treated groups was 3.3 ± 0.6 nmol/mg (n = 3). The mean (±S.E.) PGE_1-stimulated adenylate cyclase activity was 298 ± 13 pmol/mg/min in the vehicle group and 242, 341, and 304 ± 37 pmol/mg/min following, respectively, 2, 4, or 18 hr of IAP treatment.

	IAP treatment (hr)			
	0	2	4	18
Percentage of control [^{32}P]NAD incorporation	100	60	38	10 ± 10
Percentage of inhibition of PGE_1-stimulated adenylate cyclase				
MOPA (5 μM)	41 ± 4	43	25	5 ± 3
Carbachol (100 μM)	19 ± 5	20	10	3 ± 1

were consistent with the idea that the ADP-ribosylation of N_i blocks phospholipid and carbachol inhibition.

IAP treatment also attenuated MOPA and carbachol inhibitions of WI-38 adenylate cyclase (Fig. 8). MOPA and carbachol caused concentration-dependent decreases in PGE_1-stimulated adenylate cyclase, but pretreating cells with IAP completely eliminated the inhibition produced by both drugs. IAP treatment also blocked phosphatidate inhibition of adenylate cyclase (data not shown) and attenuated the IAP-dependent [^{32}P]NAD labeling of the 40-kDa membrane protein.

Reversibility of the MOPA effect on cAMP accumulation. Reversibility is characteristic of hormone action at plasma membrane receptors, and it has previously been shown that carbachol inhibition of cAMP accumulation in WI-38 cells is reversible (1). MOPA inhibition of cAMP accumulation in WI-38 cells was also reversible (Table 4). When MOPA-containing medium was rapidly (≅30 sec) replaced with fresh medium, PGE_1 stimulation of cAMP accumulation was restored.

DISCUSSION

We have shown that MOPA and phosphatidate inhibit adenylate cyclase in WI-38 and VA13 membranes. The inhibition did not appear to be a nonspecific effect of lipids in general because other lipids with similar structures had little effect on adenylate cyclase. Furthermore, MOPA and phosphatidate inhibitions showed the same properties that have previously been associated with hormonal inhibition of this enzyme: (i) the inhibition was GTP-dependent, and the concentration of GTP required was similar to what has previously been reported for hormones (6); (ii) the inhibition appeared to be mediated at least in part by N_i because IAP treatment blocked MOPA and phosphatidate inhibitions of adenylate cyclase and cAMP accumulation, and it attenuated the IAP-dependent ADP-ribosylation of a 40-kDa pro-

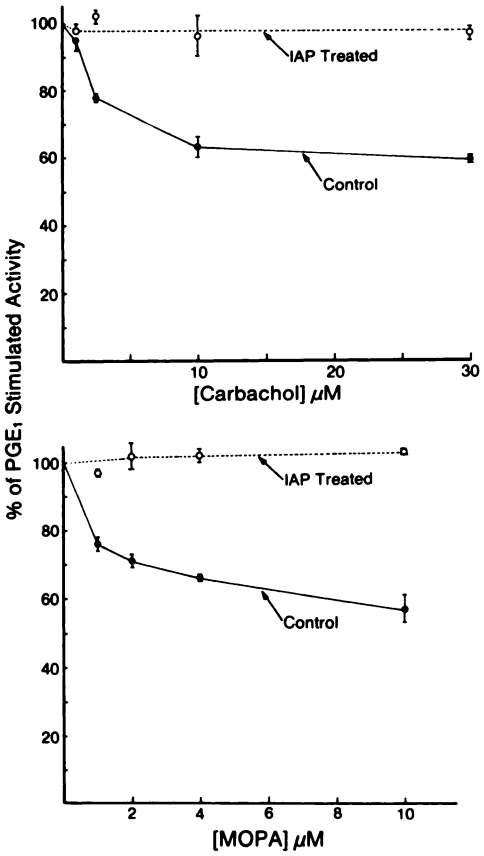

FIG. 8. *Effect of IAP treatment on carbachol and MOPA inhibitions of PGE_1-stimulated adenylate cyclase in WI-38 membranes*

Membranes were prepared from WI-38 cells treated with vehicle or IAP (10 ng/ml) for 17 hr. Then adenylate cyclase activity was measured in membranes incubated with either PGE_1 (5.7 μM) or PGE_1 and various concentrations of carbachol (*A*) or MOPA (*B*). Values shown are the percentage of PGE_1-stimulated adenylate cyclase activity in vehicle (117 ± 3 pmol/mg/min) or IAP-treated (174 ± 4 pmol/mg/min) membranes and represent the mean ± S.E. of triplicate determinations.

tein; (iii) the inhibitions due to phosphatidate and MOPA were greater at lower concentrations of Mg^{2+}; and (iv) MOPA inhibition was noncompetitive with PGE_1. These similarities among MOPA, phosphatidate, and the hormonal inhibitions of adenylate cyclase suggest that they might have a similar mechanism of action.

Hormonal inhibition of adenylate cyclase appears to be mediated by N_i (8–15). N_i has been purified from rabbit liver (23) and human erythrocytes (24, 25), and in these tissues it contained an α_i, β, and γ subunit with molecular weights of 39–41, 35, and 5–10 kDa, respectively. IAP catalyzes the NAD-dependent ADP-ribosylation of the α_i subunit, and it also attenuates hormonal inhibition. Since MOPA and phosphatidate inhibitions also were attenuated by IAP treatment, it is likely that they inhibit adenylate cyclase by acting either on a receptor or on N_i rather than the catalytic unit (C) directly.

Phospholipid regulation of adenylate cyclase has previously been reported. Platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine), which is a phospholipid that is released *in vitro* and *in vivo* when cells such as neutrophils are appropriately challenged (26), inhibits cAMP accumulation and adenylate cyclase in platelets (27, 28). The inhibitory effect of PAF on human platelet adenylate cyclase is GTP dependent (28). Thus, there is evidence that a phospholipid may be released and that it may also produce biochemical changes including inhibition of adenylate cyclase in adjacent cells.

It is well documented that hormones alter phospholipid metabolism (29–31). Therefore, it is possible that a hormone might cause MOPA or phosphatidate levels to increase, and these phospholipids or a metabolite could then activate N_i. Since carbachol inhibits adenylate cyclase (3) and cAMP accumulation (1) in fibroblasts and stimulates phospholipid metabolism in other tissues (31), it was originally proposed that phosphatidate might be an intermediate for carbachol inhibition of adenylate cyclase (2). A number of findings, however, have since led to the abandonment of this hypothesis (3). However, it is possible that in these or other cells phospholipids produced in response to cell regulators could activate N_i indirectly or directly. A direct effect on N_i seems unlikely because, if the structure of N_i is highly conserved, as is currently believed, one might predict that MOPA or phosphatidate would inhibit adenylate cyclase in all cells. This is not the case, because we have found that these phospholipids have no significant effects on cAMP accumulation or adenylate cyclase in S49 lymphoma cells, NG108-15 cells, turkey erythrocytes, or Chinese hamster ovary cells.

It is possible that MOPA and phosphatidate inhibition of adenylate cyclase is a phenomenon that will prove to

TABLE 4

Reversibility of MOPA effect on cAMP accumulation in WI-38 cells

The accumulation of [^{3}H]cAMP was measured following the 4-min incubation with 1 μM PGE_1 or PGE_1 + MOPA (0.1 μM). When indicated, the cells were washed rapidly 3 times with serum-free medium prior to the addition of PGE_1. The time lapse between the beginning of the wash procedure and the addition of PGE_1 was 30 sec.

Preincubation (2 min)	Wash	Additions (4 min)	$\frac{cAMP}{AXP + cAMP} \times 100$	Inhibition
				%
Vehicle	–	PGE_1	3.34 ± 0.03	
MOPA (0.1 μM)	–	PGE_1	2.28 ± 0.02	32
Vehicle	+	PGE_1	3.15 ± 0.04	
MOPA (0.1 μM)	+	PGE_1	3.36 ± 0.04	0
Vehicle	+	PGE_1 + MOPA	2.19 ± 0.08	30

be unique to *in vitro* systems and that no correlate will be found *in vivo*. To data it has been shown that MOPA or phosphatidate inhibits cAMP accumulation in hamster adipocytes *in vitro* (32) and in cultured fibroblasts (WI-38, VA13, MRC-5, 3T3) (2–4) and C6 glioma cells.[3] In this paper it has been shown that the MOPA- and phosphatidate-induced decrease in cAMP accumulation in WI-38 and VA13 cells is due at least in part to a decrease in cAMP synthesis. The inhibition of adenylate cyclase by MOPA and phosphatidate showed the characteristics normally associated with hormonal inhibition of this enzyme; hence, both may prove to be useful probes for studying the role of N_i and lipids in the regulation of adenylate cyclase.

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