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# Phospholipid and Guanine Nucleotide Sensitive Properties of the Smooth Muscle Adenylate Cyclase Catalytic Unit†

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ABSTRACT: The adenylate cyclase catalytic unit was partially purified from uterine smooth muscle by chromatography on columns of SM-2 Bio-Beads and Sepharose 6B. Stimulation of catalysis by forskolin was much greater in the presence of $Mn^{2+}$ than in the presence of $Mg^{2+}$. Neither NaF nor guanine nucleotide stimulated catalysis in the presence of $Mg^{2+}$ or $Mn^{2+}$. These properties indicated the catalytic unit was not sensitive to regulation by the $G_S$ regulatory protein. Guanine nucleotide inhibited catalysis, however, and was a competitive inhibitor of the ATP substrate ($K_i \sim 50$ $\mu$M). Since inhibition affected $K_m$ but not $V_{max}$, the catalytic unit also seemed insensitive to regulation by the $G_i$ regulatory protein, which does not act like a competitive inhibitor in other enzyme systems. The catalytic unit was also phospholipid sensitive. Only phosphatidic acid (Pho-A) had a direct effect on catalysis and was a potent inhibitor. Its effects were antagonized by the concomitant addition of phosphatidylcholine (Pho-C) but not by phosphatidylethanolamine, phosphatidylserine, or phosphatidylinositol. Acyl chain composition had a marked effect on Pho-C binding when this was determined by antagonism of Pho-A-dependent inhibition. These properties suggest the catalytic unit has both polar head group and acyl chain requirements for phospholipid binding.

The components of adenylate cyclase have been purified to homogeneity, and the enzyme reconstituted from the purified subunits synthesizes cAMP in an agonist-dependent manner (Lefkowitz et al., 1985; May et al., 1985). The subunits are frequently purified from different enzyme sources, so the absence of species- or tissue-specific differences in functional reconstitution is testimony to the accuracy of the fundamental control mechanisms that have been proposed. Despite the functional homology of subunits obtained from different sources, however, there may be subtle differences in their properties that contribute to tissue-specific differences in the regulation of cAMP production.

cAMP synthesis mediates the relaxing effects that $\beta$-adrenergic catecholamines have on smooth muscle from the uterus and other organs (Krall et al., 1983). As in other target tissues, $\beta$-adrenergic receptor-dependent cAMP production is regulated by guanine nucleotide binding by the $G_S$ regulatory protein in rat uterine smooth muscle (Krall et al., 1985). Characterization of the smooth muscle cell catalytic unit has been difficult, however, because it is not easily obtained in a state that is free of the functional influence of $G_S$ (Frolich et al., 1983).

We previously characterized some guanine nucleotide sensitive properties of adenylate cyclase solubilized from rat uterine smooth muscle with Lubrol PX (Frolich et al., 1983). We now show that chromatographic removal of the detergent from unactivated smooth muscle adenylate cyclase produces

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a form of the catalytic unit that is not affected by the guanine nucleotide binding $G_S$ unit. This partially purified catalytic unit is sensitive to the addition of phospholipid dispersions in a manner that suggests it differs in some respects from catalytic units purified from non-smooth-muscle sources.

## Experimental Procedures

*Detergent Extraction and Partial Purification of Adenylate Cyclase.* Adenylate cyclase in its unactivated form was solubilized from uterine smooth muscle strips with 1% Lubrol PX as described previously by Frolich et al. (1983). Briefly, diced muscle strips were homogenized (0.5-g wet wt./mL) with a Brinkman Polytron in ice-cold 0.01 M Tris[1]-HCl (pH 7.6) that contained 0.3 mM DTT and 0.001 M EGTA. Lubrol PX was added to a final concentration of 1%, and after stirring for 10 min, the mixture was clarified by centrifugation at 100000x*g* for 60 min at 4 °C. The soluble supernatant was quickly passed over a 1.2 × 50 cm Bio-Bead SM-2 column (Bio-Rad, Richmond, CA). SM-2 Bio-Bead chromatography has been reported to remove Lubrol PX almost completely from membrane proteins solubilized with the detergent (Levitzki, 1985).

Fractions with the highest adenylate cyclase activity were pooled from the Bio-Bead column, and KCl was added to a final concentration of 0.4 M by stirring at 4 °C. The sample was quickly applied to a 0.9 × 90 cm Sepharose 6B column equilibrated with 0.01 M Tris-HCl, 0.4 M KCl, 0.3 mM DTT, and 0.001 M EGTA. The column was developed overnight with the equilibration buffer at 4 °C and each fraction assayed for adenylate cyclase activity. Detergent extraction and the subsequent chromatography steps took 30 h until the active (Sepharose 6B) included fractions were pooled and frozen at −90 °C. The frozen enzyme retained its properties for up to 90 days. Protein concentration was determined by using bovine albumin as standard according to the method of Lowry et al. (1951) or by using the bicinchoninic acid method described by Smith et al. (1985).

*Adenylate Cyclase Activity.* Adenylate cyclase activity was determined as the enzymatic conversion of [$\alpha$-$^{32}$P]ATP (New England Nuclear, Boston, MA) to [$^{32}$P]cAMP followed by purification of the radioactive product by Dowex and aluminum oxide chromatography as described previously (Krall et al., 1981). In some experiments, adenylate cyclase activity was determined in the presence of Gpp[NH]p, supplied by Boehringer-Mannheim (Indianapolis, IN). The analogue was free of nucleotide contamination when this was determined by thin-layer chromatography on PEI–cellulose (E. Merck, Darmstadt) developed with 0.7 M $(NH_4)_2SO_4$.

*Phospholipid Determination.* The lipid-bound phosphate content of the partially purified catalytic unit was compared with that of adenylate cyclase rich membranes from uterine smooth muscle by using the method of Bartlett (1959) as described previously (Krall et al., 1985). Briefly, membranes or the catalytic unit preparation was extracted with chloroform/methanol (2:1) at moderate ionic strength according to the method of Folch et al. (1957). The extracted lipids, hydrolyzed at 180 °C for 4 h, were used for determination of total lipid phosphorus.

[1] Abbreviations: DTT, dithiothreitol; EGTA, ethylene glycol bis($\beta$-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Gpp[NH]p, guanosine 5′-($\beta$,$\gamma$-imidotriphosphate); Pho-A, phosphatidic acid; lyso-Pho-A, lyso-[monooleoyl)phosphatidic acid; Pho-C, phosphatidylcholine; Pho-I, phosphatidylinositol; Pho-E, phosphatidylethanolamine; Pho-S, phosphatidylserine; Tris, tris(hydroxymethyl)aminomethane; PEI, poly(ethylene imine).

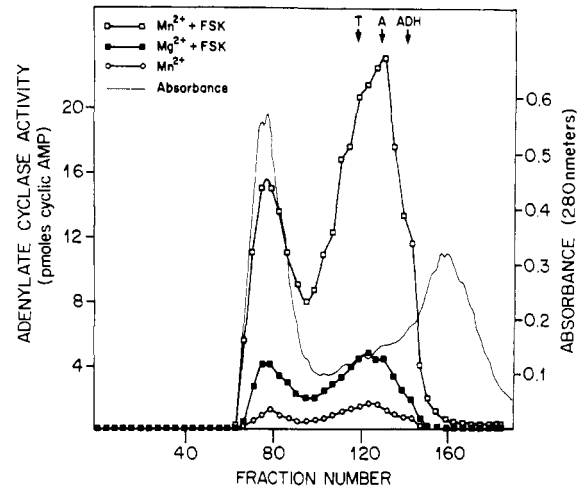


FIGURE 1: Elution profile of the solubilized adenylate cyclase from Sepharose 6B. The enzyme was extracted from uterine smooth muscle with Lubrol PX and passed over a SM-2 Bio-Bead column prior to Sepharose 6B column chromatography as described under Experimental Procedures. Column fractions were assayed in the presence of 1 mM $Mg^{2+}$ or 1 mM $Mn^{2+}$ with or without 70 $\mu$M forskolin (FSK) as indicated. T, A, and ADH mark the position at which, respectively, thyroglobulin, $\beta$-amylase, and alcohol dehydrogenase eluted from the gel exclusion column.

Alternatively, extracted lipids were dissolved in hexane/2-propanol/water (40:54:6) for phospholipid analysis by isocratic HPLC using a LiChrosphere Si-100 column developed with hexane, 2-propanol, 0.025 M sodium phosphate buffer, ethanol, and acetic acid (Patton et al., 1982). Application of this method for characterizing the phospholipids of smooth muscle membranes, using UV detection at 205 nm, has been described more fully in Krall et al. (1985).

Phospholipids were obtained as chloroform solutions (dimyristoleoyl[14:1]Pho-C, dierucoyl[22:1]Pho-C, diarachidonoyl[20:4]Pho-C, diphytanoyl[20:0]Pho-C, egg Pho-C, egg Pho-A) from Avanti Polar Lipids, Inc. (Birmingham, AL) or in powdered form (soy Pho-C, egg Pho-A, lyso(monooleoyl)Pho-A, brain Pho-S, soy Pho-I, cholesterol) from Sigma (St. Louis, MO) and subsequently dissolved in chloroform. The chloroform solutions were filtered through Millex-GV filters (0.22 $\mu$m, Millipore Corp., Bedford, MA) and evaporated to dryness under a stream of dry nitrogen. The dried lipids were suspended in distilled water by sonication at 4 °C for 10 min for inclusion in the adenylate cyclase assay.

*Expression of the Experimental Results.* Except where noted, the results are the average of two to four experiments using the same catalytic unit preparation as the enzyme source. The results presented are representative, however, of those obtained with three to eight separate preparations.

## Results

The properties of the soluble myometrial adenylate cyclase, extracted from uterine smooth muscle membranes with 1% Lubrol PX, have been described previously (Frolich et al., 1983; Krall et al., 1984). In the current experiments, the Lubrol-solubilized enzyme, passed first over an SM-2 Bio-Bead column, eluted as two activity peaks on Sepharose 6B chromatography; a void volume peak and a heterodisperse peak of included activity with maxima that were coincident with the $\beta$-amylase marker (Figure 1). The properties of the two activity peaks were similar and both were stimulated slightly with $Mn^{2+}$ or with forskolin in the presence of $Mg^{2+}$, but markedly with forskolin in the presence of $Mn^{2+}$. The specific activity in the presence of $Mn^{2+}$–forskolin of the included enzyme peak was increased to 4.9 from 0.05 nmol/mg of

Table I: $M^{2+}$ Sensitivity of the Partially Purified Catalytic Unit

| | adenylate cyclase activity[a] | | | |
|---|---|---|---|---|
| $[M^{2+}]$ | control | +NaF | +Gpp[NH]p | +forskolin |
| 1 mM $Mg^{2+}$ | 0.08 | 0.07 | 0.08 | 0.15 |
| 1 mM $Mn^{2+}$ | 0.45 | 0.45 | 0.38 | 6.75 |

[a] In the presence of the indicated activators at the following concentrations: NaF, 150 mM; Gpp[NH]p, 0.3 mM; forskolin, 0.070 mM.

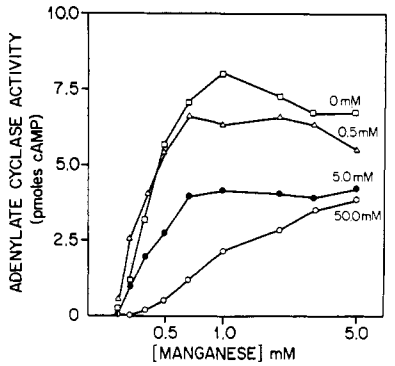


FIGURE 2: $Mg^{2+}$ and $Mn^{2+}$ sensitivity of the catalytic unit. The Sepharose 6B purified activity was assayed in the presence of forskolin (70 $\mu$M) at the indicated concentrations of $Mn^{2+}$ and in the presence of 0.0, 0.5, 5.0, or 50.0 mM $MgCl_2$ as shown.

protein in the myometrial homogenate in four separate preparations, a ~100-fold purification. It was the partially purified activity in this included peak that was used in the subsequent characterizations.

In contrast to myometrial adenylate cyclase in its membrane-associated form or when solubilized with Lubrol PX without detergent removal, the activity of the partially purified enzyme was low in the presence of $Mg^{2+}$ concentrations up to 100 mM and could not be increased by the addition of NaF, Gpp[NH]p, or the vasoactive diterpene forskolin (Table I). NaF and Gpp[NH]p also failed to increase activity determined in the presence of $Mn^{2+}$. Unlike activity measured in the presence of $Mg^{2+}$, however, $Mn^{2+}$-dependent activity was increased 15-fold by the addition of forskolin (Table I). In contrast to its poor ability to support catalysis, $Mg^{2+}$ inhibited enzyme activation by $Mn^{2+}$ in the presence of forskolin (Figure 2).

Besides $Mg^{2+}$, the activity of the catalytic unit was also inhibited by Gpp[NH]p. The nonhydrolyzable analogue of GTP was a competitive inhibitor of the substrate and increased the $K_m$ but not the $V_{max}$ with respect to ATP ($K_m$ = 35 $\mu$M, Figure 3). As shown in Figure 4, Gpp[NH]p was a potent inhibitor with $K_i$ ~50 $\mu$M when this was analyzed by the method of Dixon. Like Gpp[NH]p, GTP was also a competitive inhibitor of ATP (data now shown).

In three separate preparations, detergent solubilization and partial purification reduced the phospholipid content associated with the catalytic unit by about 85%, from 0.030 ± 0.009 to 0.005 ± 0.002 $\mu$g of lipid phosphorus/$\mu$g of protein. When two separate preparations were analyzed by HPLC for phospholipid composition, >85% of the phospholipids detectable by UV absorption were Pho-A or Pho-E, and there were only low amounts of Pho-I and Pho-S and no Pho-C (Table II).

Membrane phospholipids, removed from the partially purified catalytic unit by detergent solubilization and SM-2 Bio-Bead and Sepharose 6B chromatography, were restored by the addition to the enzyme assay mixture of purified phospholipid dispersions. Low concentrations of Pho-A had a marked inhibitory effect (data not shown) on the activity of the catalytic unit, which was inhibited by 50% ($v/V$ = 0.5)

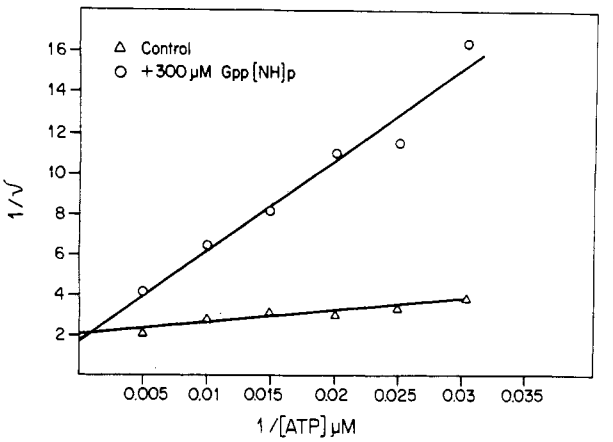


FIGURE 3: Reciprocal plot of the effect of Gpp[NH]p addition on catalytic unit activity. Catalytic activity in the presence of 1 mM $MnCl_2$ + 70 $\mu$M forskolin was determined in the absence or presence of 300 $\mu$M Gpp[NH]p at the concentrations of ATP indicated. The lines were drawn with the aid of a least-squares program.

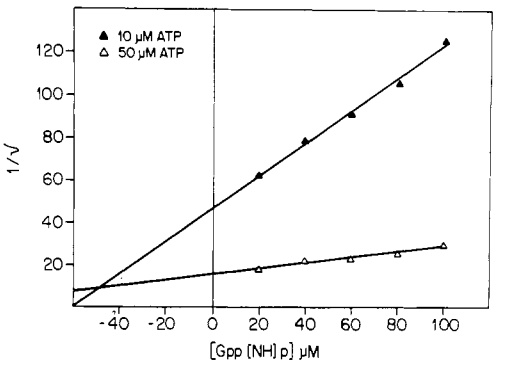


FIGURE 4: Analysis by the method of Dixon of the competitive inhibition of catalysis by Gpp[NH]p. The effect of Gpp[NH]p at the indicated concentrations on the activity of the catalytic unit was determined in the presence of 1 mM $MnCl_2$ + 70 $\mu$M forskolin at ATP concentrations of 10 and 50 $\mu$M as shown. The intercept determined with the aid of a least-squres program of the two lines at $V_{max}^{-1}$ was 50 $\mu$M. Graphic determination of the $K_i$ at each of the ATP concentrations separately differed from this value ($K_i$ = 50 $\mu$M) by <7%.

Table II: Phospholipid Composition of the Catalytic Unit Preparation

| phospholipid[a] | relative abundance[b] |
|---|---|
| Pho-A | 57 |
| Pho-E | 30 |
| Pho-S | 8 |
| Pho-I | 7 |
| Pho-C | ND[c] |

[a] Determined by Si-100 HPLC with UV detection at 205 nm. [b] The results are the average of those obtained from the analysis of two separate preparations. They are the percent of the integrated area of each specific peak relative to the sum of the area of all of the detectable peaks. [c] ND = none detected.

by 100 $\mu$M phospholipid. In contrast to Pho-A, lyso(monooleoyl)Pho-A was about 20-fold less potent as an inhibitor of catalytic unit activity. Other purified phospholipids including Pho-C, Pho-E, Pho-I, and Pho-S had little effect, even at concentrations at which Pho-A inhibited activity >85% (Table III).

These results suggested that either Pho-A was the only phospholipid which was bound by the catalytic unit or that other phospholipids were bound in a manner that failed to effect catalysis. We distinguished between these two possibilities by investigating the effects that the phospholipids had on the catalytic unit activity when it was partially inhibited

Table III: Phospholipid Sensitivity of the Catalytic Unit

| phospholipids[b] | adenylate cyclase activity[a] | |
|---|---|---|
| | no additions | +0.4 mM Pho-A |
| 3 mM Pho-A | 0.13 | 0.11 |
| 3 mM Pho-C | 1.12 | 0.97 |
| 3 mM Pho-S | 0.77 | 0.21 |
| 3 mM Pho-E | 0.78 | 0.23 |
| 3 mM Pho-I | 0.92 | 0.10 |

[a] Activity in the presence of $Mn^{2+}$ and forskolin expressed as $v/V_{max}$, the ratio of the activity in the presence of the indicated combinations of phospholipids to the activity in the absence of any phospholipid. [b] Phospholipids added to the assay at the concentration indicated in the absence (no additions) or presence or an inhibiting concentration of Pho-A (0.4 mM).

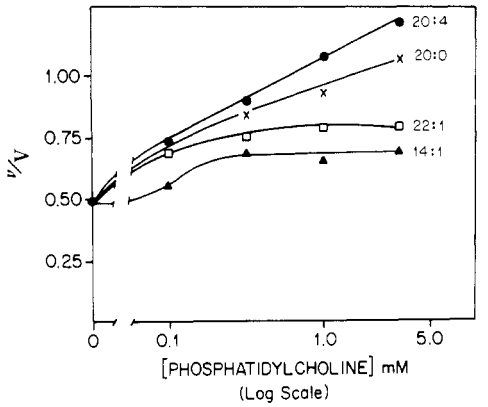


FIGURE 5: Effect of acyl chain composition on the capacity of PC to antagonize Pho-A-dependent inhibition of catalysis. The catalytic unit was assayed in the presence of 1 mM $MgCl_2$ + 70 $\mu$M forskolin in the absence ($V$) or presence ($v$) of enough Pho-A to inhibit activity ~50% ($v/V = 0.5$, 0.1–0.4 mM Pho-A). Pho-C of the defined acyl chain composition indicated here and described under Experimental Procedures was added at the concentrations shown. The results are the average of those obtained with three to five separate catalytic unit preparations.

by Pho-A. Table II shows that Pho-C but not Pho-S, Pho-E, or Pho-I was capable of antagonizing the inhibitory effect of Pho-A. Pho-C had to be added concomitantly with Pho-A in the enzyme assay. Inhibition by Pho-A could not be reversed if the enzyme was incubated with Pho-A alone for as little as 5 min before the addition of Pho-C (data not shown).

Although Pho-C failed to have dramatic effects by itself on the catalytic unit, it was probably bound by a site that could effect catalysis. We further characterized the nature of this putative site by testing the effect that the acyl chain composition of Pho-C had on its potency as an antagonist of Pho-A. As shown in Figure 5, antagonism was highly specific, and Pho-C with 20-carbon acyl chain length was more potent as an antagonist than Pho-C with either shorter or longer chains. In contrast to chain length, acyl chain saturation seemed to have little effect on potency. Cholesterol, present in concentrations up to 4 mM, had no effect on either the sensitivity of the catalytic unit to inhibition by Pho-A or the antagonism of Pho-A's effects by Pho-C (data not shown).

## Discussion

Even extensive purification of the catalytic unit of adenylate cyclase does not necessarily lead to its separation from some units of the regulatory protein $G_S$ (Homcy et al., 1978; Smigel, 1986). Several properties of adenylate cyclase we isolated by Sepharose 6B chromatography suggest the catalytic unit was free of the influence of $G_S$, although probably not from the $G_S$ subunits. First, activity was stimulated by forskolin, which stimulates the catalytic unit isolated from many sources (Bender & Neer, 1983; Pfeuffer & Metzger, 1982; Smigel, 1986), but not by guanine nucleotide or by NaF, activators known to require the $G_S$ regulatory protein (Lefkowitz et al., 1985).

Next, catalysis was stimulated more by $Mn^{2+}$ than by $Mg^{2+}$, a widely acknowledged property of the catalytic unit in its unactivated state (Bender & Neer, 1983; Ross, 1981; Somkuti et al., 1982). Finally, forskolin, which seems to both activate the catalytic unit and to facilitate its interaction with the $Mg^{2+}$-requiring $G_S$ units when these are present (Bender & Neer, 1983; Bouhelal et al., 1985), stimulated the partially purified uterine enzyme in the presence of $Mn^{2+}$ but not in the presence of $Mg^{2+}$. Since the diterpene failed to confer $Mg^{2+}$ sensitivity upon the catalytic unit under these conditions, interation with $G_S$ probably failed to occur in our partially purified preparations.

In fact, $Mg^{2+}$ had an inhibitory effect on $Mn^{2+}$-dependent activity when this was determined in the presence of forskolin. This suggests, but does not prove, that although it could not support catalysis, $Mg^{2+}$ could compete with $Mn^{2+}$ for a divalent cation site ($M^{2+}$ site) common to adenylate cyclase catalytic units from numerous sources (Cech & Maguire, 1982; Somkuti et al., 1982). We showed previously that the activation of adenylate cyclase by guanine nucleotide is associated with an increase in $Mg^{2+}$ sensitivity in uterine smooth muscle as it is in other mammalian tissues (Krall & Korenman, 1979). The unactivated catalytic unit from uterine smooth muscle is characterized, therefore, by its $Mg^{2+}$ refractoriness. Although speculative, these properties suggest that activation, mediated by guanine nucleotide binding in the intact tissue, may be associated with the transition of the catalytic unit $M^{2+}$ site from one that binds $Mg^{2+}$ unproductively to one that binds $Mg^{2+}$ in a manner that promotes catalysis.

The poorly hydrolyzed GTP analogue Gpp[NH]p inhibited the activity of the catalytic unit, a property generally ascribed to the effects of the liganded $G_i$ unit (Jakobs et al., 1981; Katada et al., 1986). Gpp[NH]p was a competitive inhibitor of ATP, however, and increased $K_m$ without concomitant effects on $V_{max}$. Several well-characterized properties of $G_i$ suggest that Gpp[NH]p-dependent inhibition was not mediated by the $G_i$ unit in our experiments. First, the inhibitory effects of guanine nucleotide liganded $G_i$ are reduced in the presence of $Mn^{2+}$, the cation that best supported catalysis in our experiments (Jakobs et al., 1981; Katada et al., 1984). Next, $G_i$ probably binds to the same site on the catalytic unit as $G_S$, which is well characterized with respect to its capacity to increase the $V_{max}$ without effects on the $K_m$ of catalysis (Katada et al., 1986). Finally, in $cyc^-$ membranes, which lack functional $G_S$ but not $G_i$, Gpp[NH]p inhibits by lowering $V_{max}$ without an effect on $K_m$ (Hildebrandt & Birnbaumer, 1983).

These results suggest that the inhibitory effects of guanine nucleotides cannot always be ascribed to $G_i$ activity. This would be particularly true under conditions analogous to those in our experiments. Under these conditions, the activity of the catalytic unit was determined in the absence of functional $G_S$ activity that might ordinarily mediate stimulation and mask competitive inhibition.

The smooth muscle catalytic unit was also phospholipid sensitive. The importance of plasma membrane lipids to the control of cAMP synthesis by mammalian cells is widely acknowledged. The role proposed for the phospholipids in adenylate cyclase regulation is frequently attributed to the properties of the hydrophobic acyl chains that affect bulk membrane fluidity rather than the nature of the charged head groups (Atlas et al., 1980; Houslay & Gordon, 1983; Shinitzky & Inbar, 1976; Sinensky et al., 1979). Such a model is sup-

ported by reports of relatively large changes in the polar head group composition of membrane phospholipids that occur without marked effects on bulk membrane properties (McKenzie & Brophy, 1984; Schroeder et al., 1976a,b). There remain, however, many adenylate cyclase systems with properties seemingly not affected by membrane viscosity (Helmreich & Elson, 1984; Henis et al., 1982a,b; McOsker et al., 1983; Poon et al., 1981; Puchwein et al., 1974). Besides the role proposed for the acyl chains in the regulation of adenylate cyclase, there is also evidence for polar head group specificity, although these requirements appear to be diverse.

Thus, Pho-C and Pho-S in liver (Ross, 1982; Weidenbach & Massmann, 1978), Pho-S in heart (Levey & Klein, 1972), and the acidic phospholipids in liver but not in the avian erythrocyte, except in the presence cholesterol (Kirilovsky & Schramm, 1983; Rubalcava & Rodbell, 1973), have all been implicated in coupling receptors for hormones and neurotransmitters with adenylate cyclase. Pho-E is required for maximal $\beta$-adrenergic receptor binding in the avian erythrocyte (Kirilovsky & Schramm, 1983), and the conversion of Pho-E to Pho-C has been proposed as a general mechanism of receptor coupling (Hirata et al., 1979). Evidence for the role of this particular methylation pathway has proved elusive in some hormone-sensitive enzyme systems, however (Colard & Breton, 1981; Padel et al., 1982).

Like the membrane phospholipids, components of the adenylate cyclase systems from a variety of sources are also sensitive to treatment with phospholipase (Legan et al., 1985; Limbird & Lefkowitz, 1976; Rubalcava & Rodbell, 1973; Weidenbach & Massmann, 1978). When we characterized the effects that treatment with phospholipase $A_2$ had on adenylate cyclase activity and phospholipids in membranes from the uterine smooth muscle cell, the results indicated a correlation between the loss of guanine nucleotide dependent catalysis and the hydrolysis of Pho-A and Pho-E but not Pho-C, Pho-I, or Pho-S (Krall et al., 1985). As might be anticipated from these previous studies, the partially purified catalytic unit was associated principally with Pho-A and Pho-E.

This property further suggests that Pho-E and Pho-A are required to support catalysis in uterine smooth muscle, not suppress it. The physiologic importance of the phospholipid specificity that was displayed by the partially purified myometrial catalytic unit is, therefore, uncertain. Moreover, the partially purified catalytic unit failed to bind Pho-E in any manner that affected catalysis. We suggested one role of Pho-A and Pho-E in myometrial adenylate cyclase might be to promote the interaction between the $G_S$ and catalytic units during guanine nucleotide dependent activation (Krall et al., 1985). Differences in the phospholipid requirements for activation by $G_S$ and for catalysis might explain, therefore, these discrepant properties of Pho-C, Pho-A, and Pho-E in the myometrial adenylate cyclase holoenzyme compared to the properties of the partially purified catalytic unit.

Phospholipids markedly enhance catalytic unit activation by receptors and $G_S$ in systems reconstituted from the purified units (Lefkowitz et al., 1985; May et al., 1985; Ross, 1982). Although Pho-C stimulates catalytic units prepared from both liver and brain, phospholipid restoration is not, in general, a requirement for purified or partially purified catalytic unit activity (Bender & Neer, 1983; Bitonti et al., 1982; Pfeuffer et al., 1985; Ross, 1982; Smigel, 1986). The myometrial catalytic unit displayed complex phospholipid specificity but very modest sensitivity, since only Pho-A had a direct effect. The observed specificity indicates that the effects of Pho-A and Pho-C could not be attributed to minor contaminants of the phospholipid preparations.

Likewise, it seems improbable that the inhibitory action of Pho-A can be attributed merely to the displacement of native phospholipids that are tightly bound and required for catalysis. First, the phospholipid content of the partially purified catalytic unit was low compared to that of the enzyme in its membrane-associated state, suggesting that most phospholipids had, indeed, been dissociated. Second, of the remaining phospholipids, Pho-A was present in the highest concentration, yet the enzyme was active and Pho-C had no effect in the absence of exogenously added Pho-A.

Pho-A has been shown previously to be a potent inhibitor of cAMP production in some but not all types of cells and to stimulate smooth muscle cell contraction (Clark et al., 1980; Salmon & Honeyman, 1980). The effect Pho-A has on adenylate cyclase is suppressed by treatment with the islet-activating protein in VA-13 fibroblasts, which indicates that Pho-A-inhibition is mediated by the $G_i$ regulatory protein in these cells (Proll et al., 1985). Our studies show that Pho-A can also inhibit the adenylate cyclase catalytic unit in a direct manner and differs from the inhibitory mechanism in fibroblasts where the phospholipid's effects are guanine nucleotide dependent and more pronounced with lyso(monooleyl)Pho-A than with Pho-A (Proll et al., 1985).

Although Pho-C failed to have a direct effect on catalysis, the myometrial catalytic unit could specifically bind both zwitterionic Pho-C and anionic Pho-A. The distinct properties of these two phospholipids is additional evidence that the polar head groups contributed to the specificity of phospholipid binding. Besides head-group specificity, there appeared to be specific acyl chain requirements as well, although we cannot exclude the possibility that Pho-A-binding conferred the acyl chain specificity associated with Pho-C-binding. This seems unlikely, though, since Pho-A-dependent inhibition could be blocked by the concomitant addition of Pho-C but was not reversed when Pho-A treatment was followed a few minutes later by the addition of Pho-C.

These apparent competitive properties of Pho-C-binding suggest, therefore, that the acyl chain specificity demonstrated by the Pho-C analogues reflected similar acyl chain requirements of Pho-A binding. These acyl chain requirements were, in turn, complex. Pho-C with short (C-14) hydrocarbon chains bound poorly whether these were saturated as shown in Figure 5 or unsaturated (data not shown). Pho-C with saturated or unsaturated chains of intermediate length (C-20) bound the best in our experiments. Pho-C with longer chains (C-22) was also poorly bound. Although speculative, these results suggest a highly specific binding mechanism rather than one that reflects generic acyl chain requirements. This property, in turn, would not seem to reflect an important role for steric configuration in binding. In support of this notion, cholesterol, which perturbs acyl chain ordering in phospholipid membranes (Rothman & Engelman, 1972; Smith & Oldfield, 1984), had no effect on concentration-dependent inhibition by Pho-A or its antagonism by Pho-C.

In conclusion, the catalytic unit demonstrated phospholipid specificity that was independent of its interaction with other subunits of the adenylate cyclase system. This specificity might also play a role in regulating cAMP production when the catalytic unit is in its native (membrane) state in the intact cell.

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