Activation of myocardial cAMP-dependent protein kinase by lysoplasmenylcholine

Scott D. Williams, David A. Ford*

Department of Biochemistry and Molecular Biology, St. Louis University Health Science Center, 1402 South Grand Boulevard, St. Louis, MO 63104, USA

Received 25 October 1997; revised version received 17 November 1997

Abstract Plasmalogen-specific, calcium-independent phospholipase A_2 ($iPLA_2$) is activated during myocardial ischemia. Accordingly, we have assessed the activation of myocardial protein kinases by the $iPLA_2$ product, lysoplasmenylcholine. Lysoplasmenylcholine-activated protein kinase activity from heart cytosol fractionated on a DE-52 column was identified as cAMP-dependent protein kinase (PKA) based on the following: (1) protein kinase activity stimulated by cAMP and lysoplasmenylcholine co-eluted on sequential chromatographic steps; (2) lysoplasmenylcholine-activated protein kinase activity was inhibited by the PKA inhibitor, PKI; and (3) the unprimed PKA form generated from the primed form of PKA was activated by cAMP and lysoplasmenylcholine. These results demonstrate a novel mechanism for PKA activation by lysoplasmenylcholine.

Key words: Plasmalogen; Lysoplasmenylcholine; cAMP-dependent protein kinase

1. Introduction

Plasmalogens are the predominant phospholipid constituents of myocardial sarcolemma [1,2]. Although plasmalogens are likely important regulators of trans-sarcolemmal proteins such as the sodium-calcium exchanger [3], the physiological role of plasmalogens is only partially understood. The presence of plasmalogens in plasma membranes suggests that their hydrolysis by phospholipases could result in the generation of plasmalogenic lipid second messengers. In fact, plasmalogen-specific $iPLA_2$ is rapidly activated in myocardial membranes over 4-fold within 2 min of global ischemia [4]. Furthermore, plasmalogen-specific $iPLA_2$ activation has been demonstrated in cardiac myocytes stimulated with IL-1 and thrombin [5,6]. Additionally, plasmalogens can be hydrolyzed by choline glycerophospholipid-specific PLC [7] and the product of this enzyme activity (i.e. 1-O-alk-1′-enyl-2-acyl-sn-glycerol) accumulates during myocardial ischemia [7,8]. Very little, however, is known about the effects of the metabolic products from the breakdown of plasmalogens in myocardial cells.

As an initial step in identifying the biological activities of the $iPLA_2$ hydrolysis product of plasmenylcholine, we have assessed the activation of myocardial protein kinases by lysoplasmenylcholine. The present study demonstrates that myocardial cAMP-dependent protein kinase (PKA) is activated by lysoplasmenylcholine as well as other choline-containing lysophospholipids. This mechanism may represent a key signal transduction mechanism for the phosphorylation of membrane-bound proteins by PKA.

2. Materials and methods

2.1. Reagents and purity of lipid species

Plasmenylcholine, plasmenylethanolamine, lysoplasmenylcholine, and lysoplasmenylethanolamine were prepared as previously described [9]. Phosphatidylcholine and lysophosphatidylcholine were purchased from Avanti Polar Lipids (Alabaster, AL). Phosphocholine, and choline were the purest available and were purchased from Sigma Chemical (St. Louis, MO). Organic solvents were purchased from Fisher Chemical. DEAE sepharose fast flow resin and Mono Q columns were purchased from Pharmacia-LKB (Piscataway, NJ). Preparations of PKA catalytic and regulatory subunits were obtained from Promega (Madison, WI). All other reagents were of the highest grade available and were purchased from Sigma Chemical (St. Louis, MO).

2.2. Preparation of rabbit heart cytosol

New Zealand White rabbits (<1 kg) were cervically dislocated and their hearts excised immediately and placed into normal saline solution (0.9% NaCl). Hearts were trimmed of fat, connective tissue, and atrial tissues, and the ventricular tissue was minced at 4°C in 5 volumes (w/v) of homogenization buffer (300 mM sucrose, 1.2 mM dithiothreitol, 1.2 mM EDTA, 24 mM Tris, pH 7.5) and then homogenized in a Potter-Elvehjem tube with a rotating Teflon pestle. The suspension was then subjected to treatment with a Polytron for 2 min at a setting of 4. The homogenate was centrifuged at $10\,000\times g_{max}$ for 10 min at 4°C. The resulting supernatant was decanted and then subjected to ultracentrifugation at $100\,000\times g_{max}$ for 1 h at 4°C. Cytosol (supernatant) was decanted and dialyzed twice against 2 l of buffer A (10 mM β-mercaptoethanol, 0.5 mM EDTA, 0.5 mM EGTA, 20 mM Tris, pH 7.8) at 4°C. Dialyzed cytosol was then filtered through glass wool prior to anion exchange chromatography.

2.3. Chromatographic purification of lysoplasmenylcholine-activated protein kinase

Anion exchange chromatography was performed using either a 10 ml DEAE sepharose fast flow or DE-52 column. The column was first equilibrated with 50 ml of buffer A and then loaded with 25 ml of filtered cytosol. Following a 50 ml wash of the column with buffer A, myocardial protein kinases were eluted from the DEAE sepharose fast flow column with a linear gradient from 0 to 300 mM NaCl in buffer A at a flow rate of 1 ml/min. During gradient elution, 5 ml fractions were collected. For DE-52 anion exchange chromatography, myocardial protein kinases were eluted by a 100 ml gradient from buffer A to buffer A containing 300 mM NaCl. Fractions were assayed for kinase activity with cAMP or lysoplasmenylcholine using the Kemptide phosphorylation assay or the myocardial protein phosphorylation assay (*vide infra*).

Primed and unprimed PKA were separated using FPLC on a Mono Q column using a Beckman Bio Sys 500. The column was equilibrated with 25 ml buffer A and then loaded with 2 ml of pooled fractions from DEAE sepharose fast flow chromatography which contained lysoplasmenylcholine-activated protein kinase activity. The primed and unprimed forms of PKA were eluted from the Mono Q column with a 0 to 500 mM NaCl gradient in buffer A over 75 min at a flow rate of 1 ml/min. Fractions were collected at one minute intervals and

*Corresponding author. Fax: +1 (314) 577-8156.
E-mail: fordda@wpogate.slu.edu

Abbreviations: PKA, cAMP-dependent protein kinase; PKI, cAMP-dependent protein kinase-specific inhibitory peptide; PAF, platelet activating factor; $iPLA_2$, calcium-independent phospholipase A_2

PII S0014-5793(97)01482-8

assayed for kinase activation with cAMP or lysoplasmenylcholine using the Kemptide phosphorylation assay. For removal of cAMP from PKA, a 2 ml aliquot of pooled DEAE fractions containing lysoplasmenylcholine-activated protein kinase was treated with 0.125 U of phosphodiesterase (Boehringer Mannheim) at 30°C for 45 min and then immediately injected onto the Mono Q column for FPLC analysis.

2.4. Hydroxyapatite column chromatography of lysoplasmenylcholine-activated protein kinase

Anion exchange chromatography-purified, lysoplasmenylcholine-activated protein kinase was subsequently subjected to hydroxyapatite chromatography. In brief, DE-52-purified kinase was loaded onto a Koken high-performance hydroxyapatite column (0.6×13 cm) that was equilibrated with buffer B comprised of 0.5 mM EGTA, 0.5 mM EDTA, 10 mM β-mercaptoethanol and 20 mM potassium phosphate (pH 7.4). Protein kinase activity was eluted from the column by a 60 ml gradient from 20 mM to 280 mM potassium phosphate at a flow rate of 1 ml/min. One ml fractions were collected and UV absorbance was monitored at 280 nm. Protein kinase activity was detected utilizing the Kemptide phosphorylation assay.

2.5. Purification of cAMP-dependent protein kinase

Protein kinase A holoenzyme was purified from rabbit heart cytosol following a modification of the method described by Cobb et al. [8]. Briefly, rabbit heart cytosol was subjected to fractionation by DEAE sepharose fast flow anion exchange column chromatography (*vide supra*). Fractions exhibiting kinase activity stimulated by both cAMP and lysoplasmenylcholine were pooled and 160 g/l ammonium sulfate was added. After incubation for 1 h, the pooled fractions were centrifuged at $17\,000\times g_{max}$ for 30 min. An additional 270 g/l of ammonium sulfate was added to the supernatant and gently stirred for 1 h. The precipitated proteins were then pelleted by centrifugation at $17\,000\times g_{max}$ for 30 min and subsequently resuspended in buffer A. The conductivity of the enzyme solution was adjusted with additional buffer A until the conductivity equalled that of 1.2 M ammonium sulfate in buffer A. The enzyme was clarified by centrifugation at $17\,000\times g_{max}$ for 30 min and the supernatant loaded onto a 1 ml phenyl sepharose column. The column was washed by the application of 1 ml of 1 M ammonium sulfate in buffer A followed by 2 ml of 0.2 M ammonium sulfate in buffer A and the enzyme was eluted with 5 ml of 1 mM potassium phosphate, 0.1 mM EDTA, and 2 mM β-mercaptoethanol (pH 6.8). The phenyl sepharose fractions (1 ml) were assayed for kinase activity using the Kemptide phosphorylation assay.

2.6. Kemptide phosphorylation assay

Lysoplasmenylcholine-activated myocardial protein kinase activity from column fractions was determined by modifications of the method described by Roskoski [10]. Briefly, 25 μl of the fraction containing lysoplasmenylcholine-activated protein kinase was incubated in assay buffer (50 mM MOPS, pH 7.0, 10 mM $MgCl_2$, 0.25 mg/ml BSA, 100 μM [^{32}P]ATP (77 cpm/pmol), 100 μM Kemptide) and either selected concentrations of lipid activators, 10 μM cAMP, or no activators in a final volume of 200 μl at 30°C for 15 min. Reactions were terminated by spotting 80 μl of each assay sample onto phosphocellulose filters (2.4 cm, Whatman) and washed 3 times in 75 mM phosphoric acid for 2 min each. Protein kinase activity was quantitated by counting the phosphocellulose filters by Cerenkov counting. For experiments involving the inhibition of PKA, PKI was added at concentrations of either 0.5, 5 or 50 μM to the assay buffer before the addition of the stimulant.

2.7. Assay of myocardial protein phosphorylation stimulated by lysoplasmenylcholine

To identify myocardial proteins that are phosphorylated by lysoplasmenylcholine-activated protein kinase in column chromatography fractions, 10 μl of individual or pooled DE-52 fractions were incubated in reaction buffer (20 mM Tris, pH 7.4, 10 mM $MgCl_2$, and 10 μM [^{32}P]ATP (1000 cpm/pmol) in a volume of 100 μl at 30°C for 5 min. Endogenous kinase activity was stimulated by the addition of 50 μM lysoplasmenylcholine. Phosphorylation reactions were stopped by the addition of 100 μl of SDS-PAGE sample buffer followed by boiling. Samples were separated on 10% SDS-PAGE gels and the phosphoproteins visualized by autoradiography following 24 h of exposure.

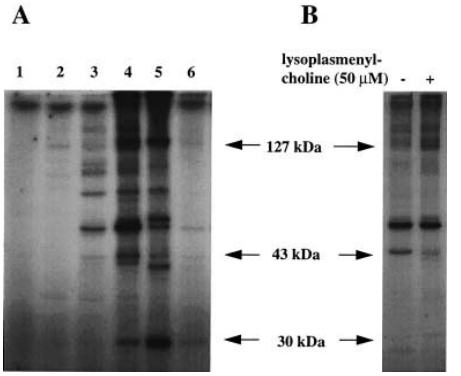

Fig. 1. Protein phosphorylation stimulated by lysoplasmenylcholine in DE-52 fractionated rabbit heart cytosol. Cytosol was prepared from rabbit myocardium and was fractionated by DE-52 column chromatography as described in Section 2. To expedite the identification of protein kinases activated by lysoplasmenylcholine, aliquots of fractions were pooled and assayed for kinase activity (panel A; lane 1, fractions 3 and 4; lane 2, fractions 5 and 6; lane 3, fractions 7 and 8; lane 4, fractions 9 and 10; lane 5, fractions 11 and 12; and lane 6, fractions 13 and 14). Protein kinase activity was identified in each pooled fraction by the addition of [^{32}P]ATP, 50 μM lysoplasmenylcholine, and other cofactors with analysis of phosphoproteins by autoradiography of SDS-PAGE gels as described in Section 2. Kinase activity stimulated by lysoplasmenylcholine in fraction 9 was assessed in the presence or absence (+ or −) of lysoplasmenylcholine (50 μM) (panel B).

3. Results

3.1. Identification of a myocardial protein kinase that is activated by lysoplasmenylcholine

As an initial step in the delineation of the biological targets of lysoplasmenylcholine produced by $iPLA_2$, the activation of myocardial protein kinases by lysoplasmenylcholine was determined in column fractions from DE-52 anion exchange chromatography of rabbit heart cytosol. Fractions were assayed for kinase activity in the presence and absence of 50 μM lysoplasmenylcholine utilizing proteins present in each fraction for both kinase activity and protein kinase substrates with activity determined by autoradiography of ^{32}P-labeled proteins as described in Section 2. Protein kinase activity that was activated by lysoplasmenylcholine eluted in fractions 9 through 13 corresponding with a sodium chloride concentration of ∼150 mM (Fig. 1A). Fig. 1B demonstrates the dependence of kinase activity in fraction 9 on the presence of 50 μM lysoplasmenylcholine. Subsequently, it was determined that this kinase activity could phosphorylate Kemptide and further assays employed this phosphorylation assay system. Fig. 2A depicts a typical plot of the myocardial protein kinase activity from DEAE sepharose fast flow fractions of rabbit heart cytosol utilizing the Kemptide phosphorylation assay. Under these conditions, kinase activity stimulated by lysoplasmenylcholine is identified in a peak centered around fraction 48 at a NaCl concentration of ∼150 mM. Lysoplasmenylcholine did not activate any other myocardial protein kinases including protein kinase C which elutes prior to lysoplasmenylcholine-activated protein kinase. When the same fractions were stimulated with cAMP, kinase activity coeluted with that activated by lysoplasmenylcholine (Fig. 2A). The anion exchange-purified lysoplasmenylcholine-activated protein kinase was further purified by hydroxyapatite chromatography (Fig. 2B) and lysoplasmenylcholine-activated

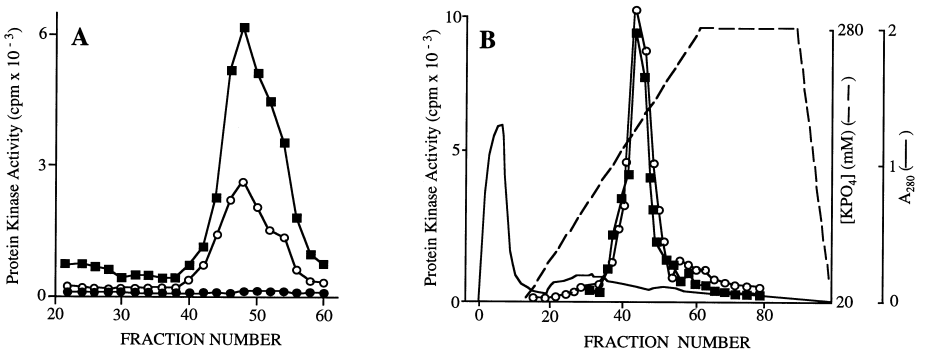

Fig. 2. Chromatographic separation of lysoplasmenylcholine-activated protein kinase. Proteins from rabbit heart cytosol were fractionated by DEAE sepharose fast flow chromatography as described in Section 2. The even numbered fractions from 22–60 were assayed independently for myocardial protein kinase activity stimulated by no stimulant (●), 10 μM cAMP (■), or 100 μM lysoplasmenylcholine (○) in the Kemptide phosphorylation assay (Section 2) (panel A). Anion exchange chromatography-purified lysoplasmenylcholine-activated protein kinase was subsequently subjected to FPLC hydroxyapatite chromatography as described in Section 2. Protein kinase activity was detected in the fractions using the Kemptide phosphorylation assay as described in Section 2 using cAMP (■) or lysoplasmenycholine (○) as the stimulants (panel B).

protein kinase activity again co-eluted with cAMP-stimulated kinase activity. The results from these experiments clearly demonstrate the presence of a myocardial kinase (lysoplasmenylcholine-activated protein kinase) that can be activated by one of the products of $iPLA_2$ in rabbit myocardium (i.e. lysoplasmenylcholine).

3.2. *Identification of lysoplasmenylcholine-activated protein kinase as PKA*

The observations that lysoplasmenylcholine-activated protein kinase activity co-eluted with PKA activity in fractions obtained from the anion exchange chromatography and hydroxyapatite chromatography suggested that the protein kinase stimulated by lysoplasmenylcholine was PKA. As an initial approach to testing this hypothesis, the cAMP-dependent protein kinase inhibitor was utilized in Kemptide phosphorylation assays using lysoplasmenylcholine or cAMP as stimulants. The addition of PKI to the Kemptide assays completely inhibited the myocardial protein kinase activity stimulated by both cAMP and lysoplasmenylcholine (Fig. 3). These results were predicted since PKI inhibits kinase activity by binding to the substrate binding site of the protein kinase and since both PKA and lysoplasmenylcholine-activated protein kinase phosphorylate the synthetic Kemptide substrate used in the assay. It should also be noted that Kemptide is not a specific substrate of PKA, other protein kinases such as S6 kinase can phosphorylate Kemptide [11]. Accordingly, alternative strategies were employed to identify lysoplasmenylcholine-activated protein kinase as PKA. The PKA holoenzyme was partially purified utilizing multiple chromatographic and fractionation steps by the procedure described by Cobb and co-workers [12]. Rabbit heart cytosol was fractionated by anion exchange chromatography and the fractions with lysoplasmenylcholine-stimulated protein kinase activity were pooled, the enzyme precipitated by the addition of ammonium sulfate, and subjected to phenyl sepharose chromatography. After each step in the purification process, the lysoplasmenylcholine-stimulated kinase activity was observed to coincide with cAMP-stimulated kinase activity (data not shown).

Further support for the conclusion that lysoplasmenylcholine-activated protein kinase is PKA was accrued from experiments designed to interconvert the primed and unprimed forms of PKA which are readily purified by anion exchange chromatography [12]. The unprimed form of PKA is characterized as being cAMP free and the primed form of PKA is characterized as having half of the cAMP binding sites containing bound cAMP [12]. An interesting feature of this system is that upon removal of cAMP by phosphodiesterase from the primed PKA fractions, rechromatography yields the unprimed form of PKA. Therefore, PKA from anion exchange chromatography of rabbit heart cytosol was separated by FPLC on a Mono Q column. PKA activity was measured

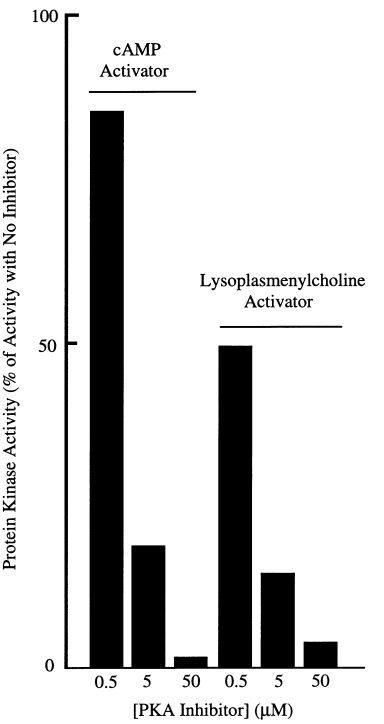

Fig. 3. Inhibition of lysoplasmenylcholine-activated protein kinase activity by the PKA inhibitory peptide. Mono Q-purified lysoplasmenycholine-activated protein kinase from DE-52 fractionated rabbit heart cytosol was assayed for kinase activity utilizing the Kemptide phosphorylation assays as described in Section 2. Assays were performed in the presence of either 50 μM lysoplasmenylcholine or 10 μM cAMP as well as in the presence or absence of either 0.5, 5 or 50 μM PKI. Each data bar represents the mean of three independent assays.

using cAMP and lysoplasmenylcholine as activators and the results indicated that all of the PKA activity is present in the primed form as peak 2 which also exhibited activity stimulated by lysoplasmenylcholine (Fig. 4A). When the primed fractions of PKA were treated with phosphodiesterase and rechromatographed, the formation of the unprimed peak 1 of PKA activity was observed along with a residual peak of PKA in the primed state resulting from incomplete removal of cAMP (Fig. 4B). Additionally, this unprimed peak of PKA now is activated by lysoplasmenylcholine (Fig. 4B). To confirm that the primed and unprimed peaks had been successfully separated, primed PKA was treated with phosphodiesterase and then loaded onto the Mono Q column in equal volumes with untreated primed PKA. The results obtained indicate that the added untreated primed PKA increases peak 2 with the unprimed peak still evident (Fig. 4C). Both the primed and unprimed forms of PKA are effectively separated and are both activated by lysoplasmenylcholine. Thus it is concluded that lysoplasmenylcholine stimulates PKA in both the primed and unprimed forms and that cAMP is not required for the stimulation of the unprimed form.

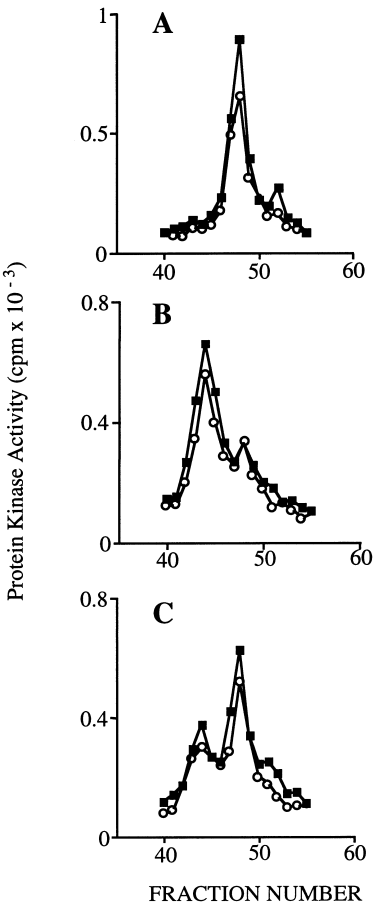

Fig. 4. Separation of unprimed and primed PKA conformations by FPLC and activation by lysoplasmenylcholine. Partially purified rabbit myocardial PKA from anion exchange chromatography was subjected to FPLC through a Mono Q column (Section 2) and then assayed for protein kinase activity using the Kemptide phosphorylation assay and 10 μM cAMP (■) or 100 μM lysoplasmenylcholine (○) as stimulants as described in Section 2 (panel A). Mono Q-purified PKA in the primed conformation was pooled and treated with 0.125 U of phosphodiesterase and rechromatographed by FPLC through a Mono Q column (Section 2) and then assayed for protein kinase activity as described in panel A (panel B). Mono Q-purified PKA in the primed conformation and primed PKA treated with phosphodiesterase were simultaneously subjected to FPLC through a Mono Q column and then assayed for protein kinase activity as described in panel A (panel C).

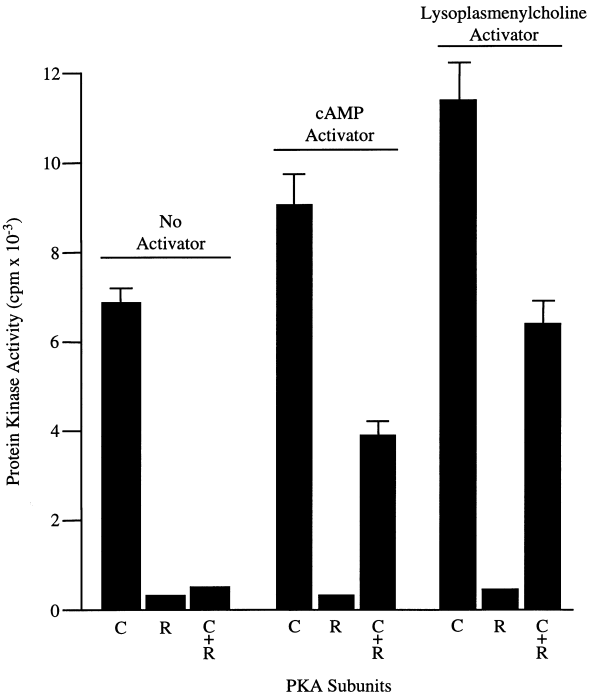

Fig. 5. Reversal of regulatory subunit inhibition of catalytic subunit activity by cAMP or lysoplasmenylcholine. PKA catalytic (1 unit) (C) and regulatory (1.5 units) (R) subunits were assayed for protein kinase activity using the Kemptide phosphorylation assay as described in Section 2. For conditions utilizing both the catalytic and regulatory subunits (C+R), 0.5 units of catalytic and 0.75 units of regulatory subunits were combined and incubated on ice for 5 min prior to Kemptide phosphorylation assays. Assays were performed in the presence of no activator, 10 μM cAMP, or 50 μM lysoplasmenylcholine as indicated. Each value represents the mean+standard deviation of three independent assays.

To further demonstrate the activation of PKA by lysoplasmenylcholine, commercially available, preparations of both the catalytic and regulatory subunits of PKA purified from bovine heart were assayed for protein kinase activity in the presence and absence of lysoplasmenylcholine using the Kemptide phosphorylation assay. Fig. 5 demonstrates that the activity of the catalytic subunit alone is constitutive and exhibits maximal protein kinase activity independent of the activator present which is due to the absence of the regulatory subunit. Protein kinase assays performed on the regulatory subunit alone exhibited no protein kinase activity in the presence of the activators due to the fact that no catalytic subunit was present (Fig. 5). The addition of the regulatory subunit to the catalytic subunits prior to measurement of protein kinase activity prevented catalytic activity in the absence of cAMP or lysoplasmenylcholine. The addition of cAMP as an activator releases the inhibitory effects of the regulatory subunit and activates the protein kinase. Similar to cAMP, the addition of lysoplasmenylcholine to the combined catalytic and regulatory subunits releases the inhibitory effects of the regulatory subunits and activates protein kinase activity. Thus it is concluded that lysoplasmenylcholine stimulates commercial preparations of PKA in a cAMP-independent manner and further

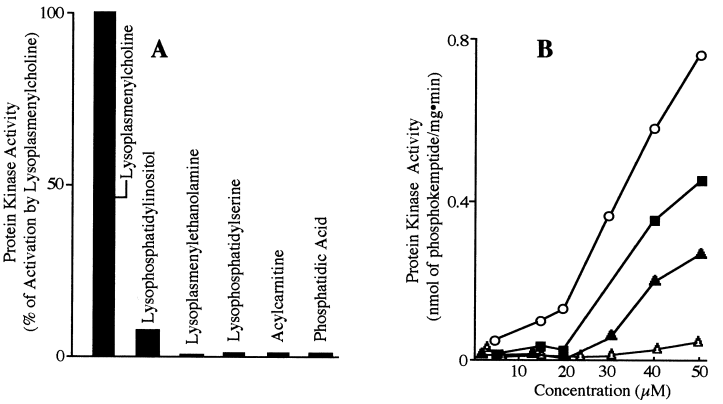

Fig. 6. Specificity and concentration dependence of lipidic activators of lysoplasmenylcholine-activated protein kinase. Mono Q-purified PKA from DE-52 fractionated rabbit heart cytosol was assayed for kinase activity utilizing the Kemptide phosphorylation assay as described in Section 2. Kinase activity was measured in the presence of either 50 μM lysoplasmenylcholine, 50 μM lysophosphatidylinositol, 50 μM lysoplasmenylethanolamine, 50 μM lysophosphatidylserine, 50 μM palmitoyl carnitine, or 50 μM phosphatidic acid (panel A). Mono Q-purified PKA from DE-52 fractionated rabbit heart cytosol was also assayed for kinase activity utilizing the Kemptide phosphorylation assay in the presence of indicated concentrations of lysoplasmenylcholine (○), lysophosphatidylcholine (■), platelet activating factor (△), or lysoplatelet activating factor (▲) as described in Section 2 (panel B).

supports our observation of myocardial PKA activation by lysoplasmenylcholine.

3.3. *Demonstration of specificity and concentration dependence of lipidic activators of PKA*

To explore the specificity of the activation of PKA by lysoplasmenylcholine, structurally similar lipids were tested at selected concentrations for activation of PKA. PKA is specifically activated by choline lysophospholipids (Fig. 6A). Other lysophospholipid classes such as lysoplasmenylethanolamine and lysophosphatidylserine did not stimulate kinase activity (Fig. 6A). Additionally, activation by choline-containing lysophospholipids possesses a subclass specificity with an order for activation of lysoplasmenylcholine > lysophosphatidylcholine > lyso-PAF (lyso-platelet activating factor, 1-O-alkylglycero-3-phosphocholine) with PAF not activating PKA (Fig. 6B). The specificity of lysoplasmenylcholine-activated protein kinase activation by choline-containing lysophospholipids is underscored by the demonstration that substitution of the hydroxyl group at the *sn-2* position with an acetate results in a lipid molecule (PAF) which does not activate PKA (Fig. 6B).

To pursue the choline-containing lysophospholipid specificity for PKA activation further, we examined the ability of phosphatidylcholine, phosphocholine, and choline to activate the PKA. At concentrations of 1, 10, and 100 mM, none of the choline derivatives stimulated PKA (data not shown). This suggests that choline or phosphocholine alone is not sufficient to activate the myocardial protein kinase. These observations underscore the specificity of PKA for lysophospholipids containing choline polar head groups.

4. Discussion

The results described herein provide evidence that lysoplasmenylcholine activates PKA in vitro. A peak of lysoplasmenylcholine-activated protein kinase activity was initially identified in rabbit myocardial cytosol fractionated by DE-52 anion exchange chromatography. The identity of the lysoplasmenylcholine-activated protein kinase activity was determined to be PKA based on four salient observations: First, protein kinase activity stimulated by both cAMP and lysoplasmenylcholine co-eluted on multiple chromatographic steps including anion exchange, high-performance hydroxyapatite, phenyl sepharose, and Mono Q column chromatographies. Second, protein kinase activity stimulated by lysoplasmenylcholine was inhibited through the use of PKI. Third, lysoplasmenylcholine-activated protein kinase was able to phosphorylate the synthetic PKA peptide substrate, Kemptide. Finally, cAMP and lysoplasmenylcholine stimulated protein kinase activity in Mono Q FPLC-resolved primed and unprimed forms of PKA. Additionally, the activation of PKA by lysophospholipids was specific for lysophospholipids containing choline polar head groups.

The variety of phospholipids which comprise biological membranes provides a depot of diverse precursors for the generation of numerous possible second messengers (cf. [13]). Many signal transduction pathways are initiated by the activation of phospholipases located at biological membranes which hydrolyze phospholipids to produce lipid second messengers. Similarly, it is possible that the production of lysoplasmenylcholine by $iPLA_2$ at the level of the plasma membrane may represent an important process in signal transduction. Although lysoplasmenylcholine content in subcellular membrane domains of myocardium remains to be determined, it is possible that lysoplasmenylcholine production in specific membrane domains may represent a biochemical mechanism designed to target PKA activation and subsequent protein phosphorylation to membranes where phospholipolysis is occurring. It is possible that increased levels of cAMP and lysoplasmenylcholine in the cell may act synergistically to both activate and target PKA to specific subcellular membrane compartments. Regulation of this mechanism would likely be controlled by the activation of $iPLA_2$ and lysophospholipid acyltransferase activities.

The relationship between phospholipases and the activation of kinases by phospholipid intermediates is well appreciated. For example, the family of protein kinase C isozymes represents the major downstream targets for lipid second messengers produced by phospholipases (cf. [13]). Protein kinase C isozymes are differentially activated by the products of phospholipase A_2 (free fatty acids and lysophospholipids), phos-

pholipase C (diacylglycerol and the plasmalogenic diglyceride, 1-O-alk-1′-enyl-2-acyl-sn-glycerol), and phospholipase D (phosphatidic acid) [13,14]. Other kinases recently shown to be activated by phospholipids include phosphatidic acid-activated protein kinase and Raf-1 by phosphatidic acid [15,16]. The present results demonstrating the activation of PKA by lysoplasmenylcholine represent another mechanism through which accelerated phospholipolysis may be coupled to protein kinases.

Acknowledgements: This research was supported by HL42665 (D.A.F.) and Research Career Development Award, HL03316 (D.A.F.) from NIH.

References

[1] Gross, R.W. (1984) Biochemistry 23, 158–165.
[2] Post, J.A., Verklej, A.J., Roelofsen, B. and Op de Kamp, J.A.F. (1988) FEBS Lett. 240, 78–82.
[3] Ford, D.A. and Hale, C.C. (1996) FEBS Lett. 394, 99–102.
[4] Ford, D.A., Hazen, S.L., Saffitz, J.E. and Gross, R.W. (1991) J. Clin. Invest. 88, 331–335.
[5] McHowat, J. and Liu, S. (1997) Am. J. Physiol. 272, ((Cell Physiol. 41)) C450–C456.
[6] McHowat, J. and Creer, M.H. (1997) Am. J. Physiol. 272, ((Heart Circ. Physiol. 41)) H1972–H1980.
[7] Wolf, R.A. and Gross, R.W. (1985) J. Biol. Chem. 260, 7295–7303.
[8] Ford, D.A. and Gross, R.W. (1989) Circ. Res. 64, 173–177.
[9] Han, X., Zupan, L.A., Hazen, S.L. and Gross, R.W. (1992) Anal. Biochem. 200, 119–124.
[10] Roskoski, R. (1983) Methods Enzymol. 99, 3–6.
[11] Erikson, E. and Maller, J.L. (1988) Second Messengers Phosphoproteins 12, 135–143.
[12] Cobb, C.E., Beth, A.H. and Corbin, J.D. (1987) J. Biol. Chem. 262, 16566–16574.
[13] Nishizuka, Y. (1992) Science 258, 607–614.
[14] Ford, D.A., Miyake, R., Glaser, P.E. and Gross, R.W. (1989) J. Biol. Chem. 264, 13818–13824.
[15] Waite, K.A., Wallin, R., Qualliotine-Mann, D. and McPhail, L.C. (1997) J. Biol. Chem. 272, 15569–15578.
[16] Ghosh, S., Strum, J.C., Sciorra, V.A., Daniel, L. and Bell, R.M. (1996) J. Biol. Chem. 271, 8472–8480.