Accumulation of Lysophosphoglycerides with Arrhythmogenic Properties in Ischemic Myocardium

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ABSTRACT Lysophosphoglycerides, products of membrane phospholipid catabolism known to influence membrane function in several systems, appeared in the effluents of anoxic isolated rabbit hearts perfused at low flow and accumulated in perfused hearts and myocardium rendered ischemic in situ. Comparable concentrations of lysophosphoglycerides bound to albumin markedly and reversibly altered action potentials of isolated canine Purkinje fibers in vitro. Changes induced included diminution of the maximum diastolic potential, peak *dV/dt* of phase zero, amplitude, and action potential duration—alterations resembling those seen in ischemic myocardium in vivo. These electrophysiological alterations are compatible with changes implicated in predisposing to dysrhythmia dependent on reentry, a phenomenon potentiated by the presence of zones of decreased conduction. Thus, accumulation of lysophosphoglycerides induced by ischemia may contribute to the genesis of malignant dysrhythmia early after its onset.

INTRODUCTION

Functional alterations in myocardial sarcolemma, reflected by electrophysiological derangements, and ultrastructural discontinuity of the plasma membrane itself (1) are among early manifestations of ischemic injury. Because lysophosphoglycerides exhibit marked effects on membrane integrity in many systems (2) and because phospholipase A_2 activation appears to accompany myocardial ischemia (3), we evaluated the possibility that these compounds might accumulate in ischemic myocardium and contribute to cardiac electrical instability. Accumulation of lysophosphoglycerides is precluded under physiological conditions by both reacylation and hydrolysis (2). However, lysophosphoglycerides may accumulate in ischemic myocardium because of augmented phospholipase A_2 activity, inhibition of reacylation, and(or) decreased washout.

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METHODS

Occlusion of the left anterior descending coronary artery was produced in rabbits (1.9 = 2.3 kg), anesthetized with 30 mg/kg pentobarbital sodium, and ventilated with room air. One pair of fast-frozen myocardial samples was obtained with a suction biopsy drill as previously described (4) from normal and cyanotic zones of the same heart at selected intervals after occlusion, and fast frozen in liquid nitrogen. Lysophosphoglycerides were assayed also in effluents and myocardium of isovolumic rabbit hearts perfused retrograde without recirculation at constant heart rate (maintained by ventricular pacing) at high (20 ml/min) or low (2 ml/min) flow for 3–15 min at 37°C after equilibration for 20 min at 20 ml/min with oxygenated Krebs buffer, pH 7.4, 5 mM glucose, and equimolar defatted albumin and palmitate (0.4 mM). During equilibration, P_{O_2} in the effluent exceeded 300 mm Hg. Perfused hearts were isovolumically beating preparations with rate maintained at 180 beats/min by ventricular pacing, with left ventricular end-diastolic pressure maintained between 8 and 12 mm Hg.

Chemical procedures. Because lysophosphoglycerides are selectively lost with chloroform:methanol and other conventional lipid extraction procedures, tissues were extracted in acidified *n*-butanol containing 0.01% butylated hydroxytoluene (BHT)[1] at 0–4°C (5, 6). Under these conditions, we recovered lysophosphoglyceride standards added to the tissue extraction medium (95±1% (SE), (n = 12). Fast-frozen myocardium was weighed rapidly, placed in a Duall homogenizer (Kontes Co., Vineland, N. J.) containing 0.5 ml of 1 N HCl to which 0.5 ml of H_2O and 0.5 ml of *n*-butanol with BHT was added after thawing of the tissue at 0–4°C. Homogenization was performed at 10–15-min intervals for 2 h, and the supernatant fraction separated after centrifugation.

Thin-layer chromatography on silica gel OF plates (New England Nuclear, Boston, Mass.) was performed in two

[1] *Abbreviations used in this paper*: BHT, butylated hydroxytoluene; LPC, lysophosphatidyl choline; LPE, lysophosphatidyl ethanolamine; PhC, phospholipase C.

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dimensions (6): first with chloroform, methanol, ammonium hydroxide (65:25:5) as solvent. Samples containing ≅200 nmol of phosphorous in a total volume of 50 μl were spotted 3 cm from each edge near the bottom right corner of the plate. After each 10-μl addition, the spot was dried with a hair dryer to minimize its total size. Each plate was developed until the solvent front came within 0.5 cm of the edge, and was then removed from the chamber, dried in air for 15 min, over-dried for 5 min at 80°C, and cooled in air for 10 min. The intervals involved during each step were critical for obtaining reproducible results. Subsequently, the plate was rotated 90° and run in a saturated chamber containing chloroform, acetone, methanol, acetic acid, and water (6:8:2:2:1), and developed until the solvent front was 3 cm from the edge. It was then air dried and placed in an iodine vapor chamber. Identified spots were outlined with pencil before removal of the emulsion for chemical assay. Phosphate assays were performed without prior elution.

Recovery of total phosphorous in the lipid extractable fraction of myocardium was evaluated by comparing the amount of phosphorous recovered from all identified phospholipid spots on the chromatogram and to total lipid phosphorous in the extract. Recovery of total lipid phosphorous from the thin-layer plates averaged 95±2% (SE) of the amount in the initial *n*-butanol ischemic myocardium and 98±2% in extracts from controls. A representative chromatogram is shown in Fig. 1. Standards (≅1–3 μg of phosphorous) of compounds of interest were run concomitantly. In some experiments, radioactively labeled phospholipids (including L-[1-*palmitoyl*-^{14}C]lysophosphatidyl choline, with the label on the carboxyl carbon of the acyl group, from New England Nuclear) were added to the tissue extract to provide an internal standard for verification of recovery.

Plasmalogen content was estimated assuming one vinyl ether linkage per molecule and the ether linkages were measured in extracts and eluates of selected regions of the thin-layer chromatograms by the Gottfried and Rapport method (7). Plasmalogens in mammalian tissues may be neutral lipid derivatives, which do not comigrate with lysophosphoglycerides or other phospholipid moieties, and phospholipid compounds (8). The phospholipid plasmalogens, phosphatidals, contain an alkenyl group that can be hydrolyzed to an aldehyde, rather than a fatty acid, in vinyl ether linkage in the 1-position (9). Because they could comigrate with phosphatidyl compounds, their identification was important. In these experiments, potential effects of BHT in the extraction medium on the detection system were accounted for by running blanks with iodine standards in media with and without BHT.

Fatty acid constituents of lysophosphatidyl choline (LPC) and lysophosphatidyl ethanolamine (LPE) were assayed by gas chromatography of the methyl ester derivatives (10) on a Varian aerograph (Varian Aerograph, Walnut Creek, Calif.) with N_2 as carrier on 10% diethylene glycol succinate at 150°C, and fatty acid position determined by thin-layer chromatography of the monoacylglycerol derivatives on borate-impregnated silica gel after enzymatic hydrolysis of isolated lysophosphoglycerides (11). Monoacylglyceride derivatives were formed from the lysophosphoglycerides and identified by thin-layer chromatography with boric acid-impregnated plates. The presumed LPC (≅6 μg of phosphorous with material combined from spots from six thin-layer chromatograms) isolated from ischemic myocardium was converted to a monoacylglyceride that comigrates with glyceryl-1-monopalmitin standard obtained from Supelco, Inc., Bellefonte, Pa. The procedure used involved elution of the samples in chloroform: methanol, evaporation to dryness, addition of 0.2 ml of medium containing 0.05 M Hepes-HCl, pH 7.4; 0.002 M $CaCl_2$; and 0.15 M NaCl with 10 μl of phospholipase C (Sigma Chemical Co., St. Louis, Mo.) comprising 5 U of activity, sonica-

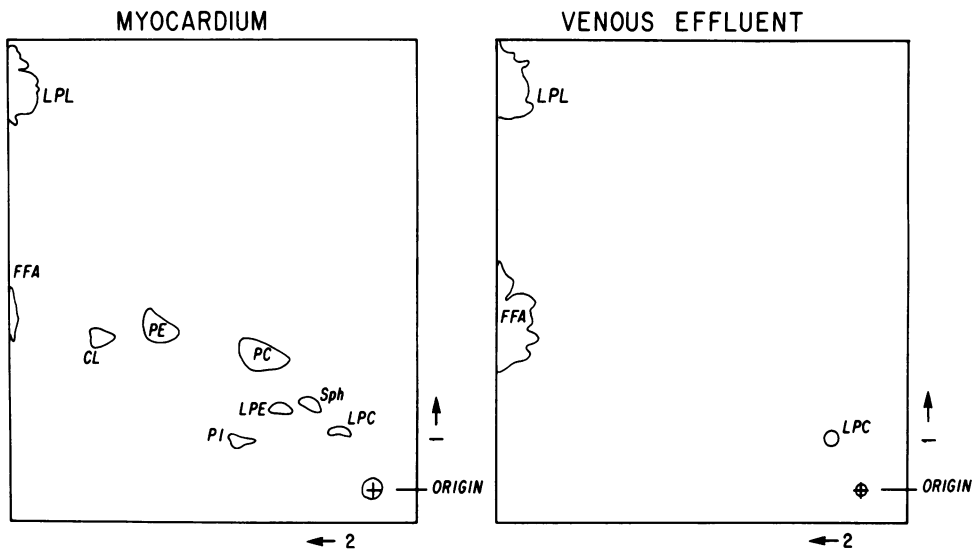

FIGURE 1 Tracings of thin-layer chromatograms of lipid extracts of 25 mg (wet wt) of ischemic myocardium (left) and 5 ml of coronary venous effluent from an isolated heart perfused at low flow (2 ml/min) (right) developed in two dimensions. Separated compounds were visualized by exposure to iodine vapor and identified by comparing mobilities with those of authentic standards. Solvent systems were (1) chloroform:methanol:28% ammonium hydroxide (65:25:5) and (2) chloroform:acetone:methanol:acetic acid:water (6:8:2:2:1). The abbreviations are CL, cardiolipin; FFA, free fatty acid; LPL, less polar lipids; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PI, phosphatidyl inositol; and Sph, sphingomyelin.

tion for 20 min at 37°C, rapid cooling, acidification with a drop of 1 N HCl, extraction in 1 ml of chloroform:methanol (1:2) containing 0.01% BHT, followed by addition of 0.33 ml of cold chloroform and 0.33 ml H_2O with mixing of the sample for 30 s between additions. After centrifugation, the lower phase was evaporated to dryness, the sample resuspended in chloroform:methanol with BHT, and applied to a New England Nuclear silica gel OF thin-layer-chromatogram plate with glyceryl-1- and glyceryl-2-monopalmitin standards applied in alternate rows. Each plate was sprayed twice with 0.4 M boric acid and activated for 1 h at 110°C immediately before use; developed in chloroform, acetone, acetic acid, and methanol (145:50:1:4) until the solvent front had advanced a total of 14 cm; air-dried; and sprayed with a 1:10 dilution of 0.12% Rhodamine 6G (Analabs, Inc., North Haven, Conn.) and visualized with long-range (375 nm) ultraviolet light. Because the 1- and 2-monoacylglyceride derivatives are well separated (Fig. 2), results obtained reflect the parent lysophosphoglycerides present in the sample.

Chemical verification of the composition of lysophosphoglycerides in myocardial tissue extracts to determine molar ratios of phosphate and fatty acid glycerol was performed after separation of the phospholipids by two-dimensional, thin-layer chromatography. Although more sensitive procedures are available, results in preliminary experiments indicated that quantities available were adequate for assay of phosphate by a modification of the Bartlett procedure in which lipid extracts were evaporated to dryness, extracted in 0.4 ml of 70% perchloric acid heated at 132°C for 3 h, and reacted with ammonium molybdate under reducing conditions at 100°C for

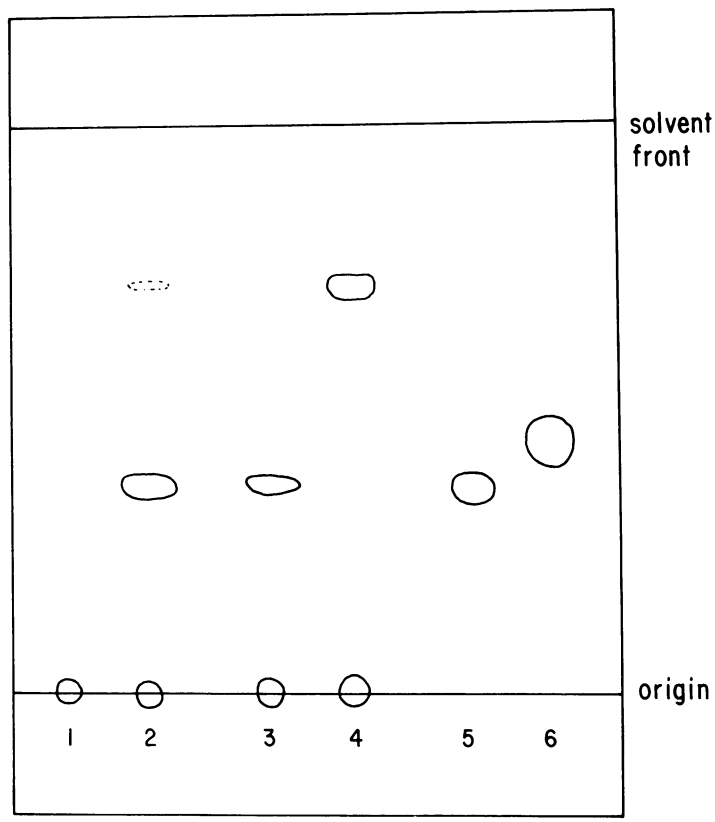

FIGURE 2 Tracing of a thin-layer-chromatogram plate showing products after incubation of isolated lipids and standards with 0.25 U of phospholipase C (PhC) at 37°C for 1 h to produce monoacylglycerol from phospholipids. Phosphoglycerides were assayed by analysis of lipid phosphorous detected by the Bartlett procedure. Spots were visualized with Rhodamine 6G spray, 0.012%: (1), Standard 1-palmitoylglycerol-3-phosphoryl choline (LPC) 50 μg, after incubation without PhC; (2), Standard LPC 50 μg after incubation with PhC; (3), Rabbit heart LPC (pooled extracts from six preparative thin-layer chromatogram plates equivalent to 100 μg of LPC assuming all the LPC is in the 1-palmitoyl compound) after incubation with PhC; (4), Standard dipalmitoylphosphatidyl choline 50 μg after incubation with PhC; (5), rac-glyceryl-1-monopalmitin; (6), rac-glyceryl-2-monopalmitin. As can be seen, the compound derived from standard dipalmitoylphosphatidyl choline is a diacylglycerol. A trace of this compound was visible in the standard LPC channel, presumably resulting from either reacylation during the incubation procedure or a contaminant in the PhC. However, the incubation product of LPC extracted from the tissue was exclusively the monoacylglycerol derivative, and its mobility corresponded to that of glyceryl-1-monopalmitin. Because the reaction was not run to completion some of the LPC is visualized at the origin. It is possible that some of this material was 1-deacyl LPC and that it would have given rise to 1-deacyl monoacylgylcerol under more prolonged incubation conditions.

10 min according to a modification of the Fiske and SubbaRow procedure (12). Absorbance at 830 nm was compared to results obtained with phosphate standards. Spots from thin-layer chromatography plates were assayed directly for phosphorus (without prior elution) with particulate material precipitated by centrifugation before spectrophotometry.

Glycerol content was assayed fluorometrically with a NADH coupled enzyme system permitting detection of as little as 0.1 nmol of glycerol after elution of lysophosphoglycerides with chloroform:methanol (4:1) with two washes of the silica gel, and conversion to glycerol by reflux in 2 N HCl for 96 h (cleaving acyl and phosphate ester bonds) (12, 13). The completeness of hydrolysis was verified with phosphatidyl choline standards. Observed fluorescence was calibrated with the use of quinine sulfate standard in 0.1 N H_2SO_4. Fluorescence as a result of contamination with glycerol-3-phosphate in the sample was excluded by initial assay after additon of glycerol-3-phosphate dehydrogenase to the system but before addition of glycerol kinase, and results were corrected for fluorescence obtained with blank regions of thin-layer chromatograms without detectable phospholipid spots.

Total fatty acid content in separated phospholipid fractions was assayed after elution of the spots in chloroform:methanol overnight at 0–4°C with two washes. Fatty acids were hydrolyzed (12) in 2 ml of 0.2 N KOH in 50% ethanol, at 100°C for 2 h, washed with petroleum ether, neutralized with H_2SO_4, extracted in petroleum ether, washed with H_2O, and total fatty acid content assayed colorimetrically after formation of cobalt soaps and reaction with α-nitroso-β-napthol (14). This procedure detected as little as 2 nmol of fatty acid reproducibly (SD = 8%). Results were calibrated with palmitate as standard, recrystallized three times from absolute ethanol, dried in air, stored for 2 wk under vacuum, and dissolved in redistilled heptane at a concentration of 50 mM and diluted before use.

Electrophysiological studies. Experiments were performed with isolated canine Purkinje fibers exposed to selected concentrations of albumin-bound lysophosphoglycerides (0.75–3.0 mM) equivalent to the range found in ischemic myocardium in vivo (see Results). The canine Purkinje fiber preparation was used because of the relative ease of isolation of fibers in this preparation, and the extensive experience and literature pertaining to it. Technical considerations limit the suitability of rabbit Purkinje fibers for this purpose. Adult mongrel dogs were anesthetized with thiopental sodium (10 mg/kg), the heart removed quickly and placed in oxygenated Krebs solution, and distal portions of the right or left bundle branches removed and impaled for recordings of the transmembrane action potential at sites several millimeters proximal to the insertion of the Purkinje fibers into the ventricular muscle. The dissected tissue was pinned through the attached endocardial muscle to the bottom of a 7.5-ml wax-lined bath and continuously superfused at a rate of 6–8 ml/min, with flow calibrated, and held constant throughout each experiment with a modified Krebs solution at 37.5°C, pH 7.4, aerated with 95% O_2-5% CO_2 containing 5 mM glucose and the following ions (in meq/liter): Na^+ = 150, K^+ = 4.0, Mg^{++} = 2.0, Ca^{++} = 2.4, Cl^- = 136, PO_4^{--} = 0.9, HCO_3^- = 22, and 0.4 mM palmitate bound to equimolar defatted albumin, dialyzed against Krebs-Henseleit buffer for 10 h. Concentrations of all ionic constituents, albumin, and palmitate in the perfusate were verified by direct assay before utilization of the perfusate. Solutions with selected constituents were introduced simply by turning a stopcock and providing injectate with total flow maintained constant. Oxygenation was provided by equilibrating the perfusate through silastic tubing thereby avoiding foaming and providing a measured oxygen tension consistently exceeding 350 mm Hg.

Synthetic 1-palmitoylglycerol-3-phosphoryl choline (Sigma Chemical Co.) LPC was bound to albumin by dissolving the material in ethanol, slowly adding the solution to the albumin-palmitate-Krebs perfusate with constant stirring at 40°C under nitrogen until the ethanol had been completely evaporated. Controls were run with albumin treated identically without added lysophosphoglycerides. Identical results were obtained when lysophosphoglycerides were bound to defatted albumin that was not first bound to palmitate. However, because albumin in the systemic blood circulation in vivo is virtually always exposed to substantial concentrations of fatty acids, we elected to perform most experiments with protein with some binding sites occupied. Purity of lysophosphoglycerides (and other constituents used in control experiments) bound to albumin was monitored by two-dimensional, thin-layer chromatography as described under chemical procedures. Binding and solubility were verified spectrophotometrically.

Purkinje fibers were stimulated with the use of a four-channel programmable, photo-optically isolated stimulator through bipolar Teflon-coated stainless steel electrodes with square wave pulses of 2.0 ms duration at ×2 voltage threshold at a basic cycle length of 800 ms. After calibration signals of 0 and −100 mV and a linear generated ramp of 0 and 1,000 V/s were stored on separate channels of analog tape, intracellular potentials were recorded with glass microelectrodes filled with 3 M KCl (6–20 M Ω DC resistance). Signals were visualized on an oscilloscope and processed sequentially through a high impedance unity gained electrometer, model M4A (W-P Instruments, Inc., New Haven, Conn.), a constant gain operational amplifier (×50), and a variable gain amplifier before storage on FM analog tape (15 inch/s, frequency response 1.6 KHz at 3 decibels). Adjustments were made for leakage currents, DC voltage offsets, and inherent capacitance of the microelectrodes. An analyzer designed in our laboratory, employing a peak-hold circuit with delay to compensate for the stimulus artifact, was used for electronic differentiation of the maximum rate of rise of phase 0 of each action potential.

Maximum diastolic potential, overshoot of phase 0, action potential duration at 50, 70, and 95% of full recovery, and the maximum rate of rise of voltage of phase 0 of action potentials stored on FM analog tape were analyzed statistically with the use of a PDP-12 computer and disc with analog-to-digital conversion of each action potential at a sampling frequency of 10 KHz for the first 100 ms of each potential and at 2.5 KHz for the next 300 ms. A threshold criterion, placed by the operator with the use of a joystick on the computer, allows capture of each phase 0 depolarization at the 20 ms mark in the analyzation window. Up to 200 action potentials can be analyzed in any one sequence, with subsequent incremental plotting of any selected action potential with a Houston Instruments DPI-5H plotter (Houston Instrument Div., Bausch & Lomb, Inc., Austin, Tex.). At least 25 consecutive action potentials were used for statistical analysis of data under each set of experimental conditions, such as during selected intervals after administration of exogenous lysophosphoglycerides.

RESULTS

Accumulation of LPE and LPC was evident in extracts from ischemic zones of hearts sampled in situ (Table I). As can be seen, both increased compared to control values by more than 50%. Similarly, in isolated hearts perfused at low flow (0–2 ml/min), LPE and LPC increased significantly compared to values in hearts perfused at 20 ml/min (Table I). Under these conditions, left ventricular pressure development and *dP/dt* de-

TABLE I
Lysophosphoglycerides in Rabbit Myocardium

	n	LPE	LPC	LPE + LPC	Increase in LPE + LPC compared to control
			μmol/g dry wt		%
Normal myocardium in situ	13	6±1	7±1	13±2	
Ischemic myocardium in situ (5–15 min after occlusion)	7	9±1*	12±2*	21±3*	62*
Ischemic myocardium in situ (30–60 min after occlusion)	6	11±1*	11±2*	22±3*	69*
Myocardium from hearts perfused at:					
20 ml/min (control)	13	7±0.5	10±1	17±2	
Low flow for 5–15 min	6	9±1	13±2	22±2*	29*
Low flow for 3–5 min	7	10±1*	16±1*	26±2*	53*

Results expressed represent mean±SE of LPE and LPC. Before assay of myocardium, isolated hearts were perfused at 20 ml/min (control), or 0–2 ml/min (low flow) after equilibration. Recovery of total lipid phosphorous in the extracts from thin-layer chromatograms averaged 95±2% for ischemic hearts and 98±2% for controls. Recovery of LPE and LPC standards assayed concomitantly averaged 97±1 (n = 13) and 98±1% (n = 12).
* $P < 0.01$.

clined promptly by more than 50% because of impaired oxygenation. The increase in myocardial lysophosphoglycerides was most striking within the first 5 min of low flow, averaging 53%. In hearts perfused at 2 ml/min for 3–15 min, LPC appeared in the effluent in overall average concentrations of ≅2 μM, in contrast to the case of effluents of hearts perfused at 20 ml/min despite corrections for dilution. Thus, during prolonged low flow, some washout of lysophosphoglycerides appeared to occur under these conditions in isolated perfused hearts. It should be recognized that bulk flow (2 ml/min) sufficient to produce hypoxia in vitro with blood-free media exceeds physiological coronary flow in vivo. Thus, it is not surprising that the increases of lysophosphoglycerides with ischemia in situ were greater than those seen in isolated perfused hearts.

No differences were evident between normal and ischemic myocardium in situ or control and hypoperfused isolated hearts with respect to total phospholipids recovered averaging 115±2 (SE) μM/g dry wt for all samples analyzed—values similar to those obtained by others (15–17). The percentage of phospholipids accounted for by phosphatidyl choline and phosphatidyl ethanolamine in control hearts averaged 64±1.6, also comparable to values reported by others (67%) (15).

Based on gas chromatography, both the LPE and LPC contained predominantly saturated long-chain fatty acids, usually found in the 1-position of phosphoglycerides and their derivatives (major constituents included: 12% C14:0, 43% 16:0, and 40% 18:0 in LPE and 2% 14:0, 33% 16:0, 28% 18:0, and 10% 18:1 in LPC). No palmitoleic acid, usually found in the 2-position and hence indicative of 1-deacyl lysophosphoglycerides, was liberated from either LPC or LPE. Additional evidence in support of this conclusion came from the experiments in which monoacylglycerol derivatives were formed. As shown in Fig. 2, when LPC separated chromatographically from extracts of ischemic myocardium was used as the starting material, the resulting monoacylglyceride derivative was the 1-palmitin compound, indicating that the predominant lysophosphoglyceride in the tissue was the 2-deacyl isomer. However, some 1-deacyl LPC may have been undetected because the enzymatic reaction was not run to completion.

Choline plasmalogens (8) appear to be the predominant phospholipid plasmalogens in heart muscle and could give rise to LPC intrapreparatively. However, because the vinyl ether linkage is in the 1-position, the product would be a 2-acyl lysophosphoglyceride (9) rather than the 2-deacyl compound found, unless intrapreparative transacylation was extensive. If lysoplasmalogens co-migrated with lysophosphoglycerides, they would not give rise to the monoacylglycerol derivative we observed after treatment of the presumed lysophosphoglycerides with phospholipase C.

An average of 24 μmol/g dry wt of plasmalogens were detected in chloroform:methanol extracts of control and ischemic myocardium with lower values in other extraction media such as acidified butanol. The plasmalogen content in chloroform:methanol extracts of ischemic myocardium was indistinguishable from that in control tissue (with directionally inconsistent differences ≤8%, n = 4). Thus, it is unlikely that differences in intrapreparative conversion of plasmalogens to lysophosphoglycerides would account for the differences in lysophosphoglycerides observed. Plasmalogens were

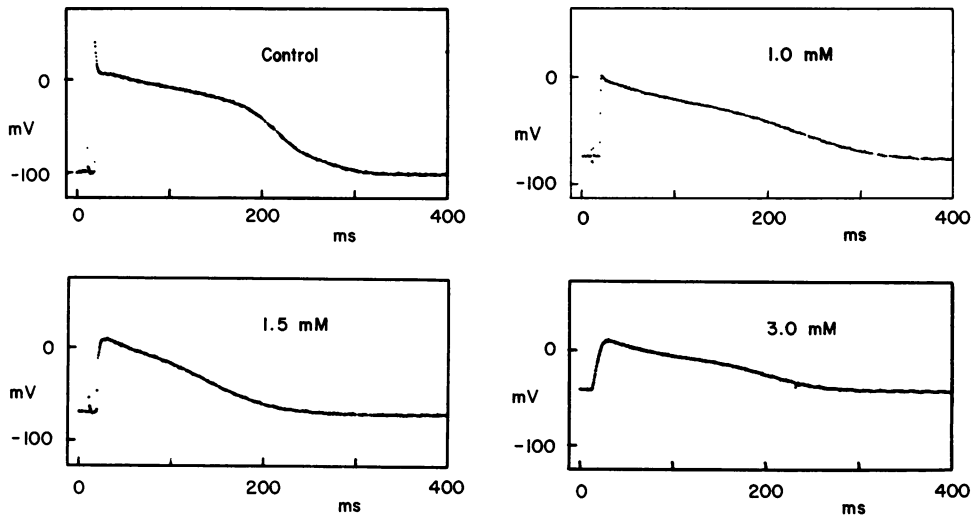

FIGURE 3 Effects of lysophosphoglycerides (1-palmitoylglycerol-3-phosphoryl choline,LPC) on canine Purkinje fibers. The concentration of LPC bound to albumin is indicated. Each panel depicts a representative computer-reconstructed action potential (one of at least 25 consecutive action potentials from the same Purkinje fiber).

not detected in the eluates of lysophosphoglyceride spots identified by two-dimensional, thin-layer chromatography from extracts of ischemic hearts in acidified butanol.

The ratio of glycerol to phosphate after acid hydrolysis of eluates from the combined loci of LPC and LPE from six chromatographic plates, run with extracts from ischemic myocardium averaged 0.87, and the corresponding ratio of fatty acid to phosphate averaged 0.91, close to the theoretical values of these ratios for pure lysophosphoglycerides (1.0).

Electrophysiological effects of lysophosphoglycerides. Albumin (0.4 mM) with or without equimolar palmitate did not affect Purkinje fiber action potentials. LPC (but not 4 mM phosphatidyl choline or 3 mM glycerophosphoryl choline) bound to albumin exerted marked effects (Fig. 3). Obviously, concentrations of endogenous, membrane constituents may differ from those of exogenous, protein-bound material exerting effects in vitro. However, the concentrations of LPC employed in our studies in vitro (0.75 to 3.0 mM) were similar to the sum of overall concentrations of LPE and LPC in ischemic myocardium in situ based on analysis of the extracts (21 μmol/g dry wt (Table I) equivalent to 4.2 μmol/g wet wt or $\cong$4 mM). LPC decreased, in a dose-dependent fashion, maximum diastolic potential, peak *dV/dt*, amplitude, overshoot of phase 0, and action potential duration (Table II).

TABLE II

Concentration-Dependent Alterations in Action Potentials Induced by Lysophospholipids

Parameter	Control*	Experimental*			
	No lysophospholipid (17)	0.75 mM (4)	1.0 mM (5)	2.0 mM (4)	3.0 mM (4)
MDP, *mV*‡	−91±2.5	−82±0.6§	−65±8.7§	−56±6.2§	−50±7.9§
Overshoot of phase 0, *mV*	33±2.6	28±5.2	7±5.0§	7±9.7§	6±5.7§
Peak *dV/dt, V/s*	667±83	265±56§	258±106§	155±47§	210±24§
APD_{50}, *ms*‡	220±13	185±3.6	144±17§	137±22§	123±22§
APD_{70}, *ms*‡	242±13	206±4.2	186±27§	173±26§	180±17§
APD_{95}, *ms*‡	285±14	249±6.9	240±44§	237±35§	255±24

* All experiments were performed with equimolar albumin and palmitate (0.4 mM) in an oxygenated superfusion medium. Control = no lysophospholipid in the medium. Experimental = concentrations of LPC bound to albumin indicated for each group of preparations studied with the number of preparations in parentheses.

‡ MDP = maximum diastolic potential; $APD_{50,70,95}$ = action potential duration at 50, 70, and 95% recovery (repolarization). Numbers in parentheses = the number of preparations studied. Results expressed are means±SE based on analysis of at least 25 consecutive computer-reconstructed action potentials in each case.

§ Significantly less than corresponding control values ($P < 0.05$).

DISCUSSION

These observations indicate that LPC and LPE accumulate in myocardium early after the onset of ischemia in situ and in vivo. The accumulated lysophosphoglycerides are likely to be catabolites of membrane constituents, although the present data do not define their specific precursor pool (5). It is possible that they are derived from cardiolipin, prominant in myocardial tissue, but the paucity of linoleic acid in the isolated lysophosphoglycerides argues against this possibility, because linoleic acid constitutes as much as 85% of the nonpolar moieties in cardiolipin (8). In ischemic myocardium in situ, the sum of phosphatidyl choline and phosphatidyl ethanolamine decreased only slightly compared to controls (by an average of 4.4 μmol/g dry wt) suggesting that phosphatidyl choline and phosphatidyl ethanolamine may have been among the immediate precursors. However, the possibility that plasmalogens accumulate and serve as intermediates or precursors in vivo cannot be excluded.

In extracts of ischemic myocardium, the overall concentrations of lysophosphoglycerides are similar to those found to exert deleterious effects on the isolated Purkinje fibers in vitro. Because early malignant dysrhythmia induced by ischemia appears to be a result of reentrant mechanisms, and because several of the effects elicited by LPC have been identified as factors predisposing to reentry, it appears likely that the increased concentrations observed in ischemic myocardium may contribute to dysrhythmia induced by ischemia (18). Furthermore, the action potential alterations induced by LPC in the present study resemble those seen in subepicardial action potentials from ischemic myocardium in situ (19). Thus, accumulation of lysophosphoglycerides could explain in part the recently observed correlations between malignant dysrhythmia and enzymatically estimated infarct size in patients (20) or electrophysiological effects elicited in normal tissue by blood from ischemic zones in experimental animals (21).

The present results do not identify the cellular or subcellular locus of lysophosphoglycerides accumulating in ischemic hearts. Phospholipids from erythrocytes in vivo could be one precursor pool, but not an obligatory one based on the results with hearts perfused with blood-free media. Judging from recently recognized relationships between depletion of high energy phosphates and accumulation of lysophosphoglycerides in erythrocytes (22), depletion of specific pools of ATP in myocardial cells subjected to ischemia could lead to activation of phospholipase A_2. It is not yet clear whether the accumulated lysophosphoglycerides damage or become incorporated in membranes of viable cells surrounding severely ischemic zones. However, because they exert detergent-like effects in several other systems (2), and arrhythmogenic effects on Purkinje fibers in vitro as shown in this study, they may be important mediators of sequelae of myocardial ischemia. Definitive assessment of their role is complex because of difficulties in isolating, purifying, and characterizing myocardial sarcolemma under conditions minimizing transfer of lipid constituents between organelles and between different classes of lipids. In addition, net concentrations may change rapidly because of reacylation and lysophosphoglyceride catabolism mediated by lysophospholipases. However, clarification of factors affecting activation and inhibition of myocardial phospholipases and lysophospholipases, lysophospholipid acylases, and acyl transferases should be useful in further elucidation of the role of lysophosphoglycerides in the genesis of malignant arrhythmias induced by ischemia.

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