The Role of Membrane Phospholipids in Arrhythmogenesis: From Basic Research to Possible Clinical Application

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SUMMARY

The work from our laboratory and others presented above has helped to form the concept that at least some of the functional abnormalities of the ischemic heart are the consequence of abnormal lipid metabolism. It was postulated that drugs which circumvent such changes should have some protective effects. In fact, certain amphiphilic drugs such as AM, CQ and CZ have been shown by us and others to reduce structural and functional abnormalities both in animal experiments and in clinical treatment. This strongly suggests that lipid abnormalities contribute to myocardial malfunction. Whether the beneficial effects of these drugs lie solely in their ability to inhibit phospholipases is difficult to prove, since all these agents also affect a large number of other cellular functions. This question can best be answered by finding or by synthesizing a phospholipase-specific inhibitor and by determining whether this agent alone is capable of reducing the structural and functional abnormalities caused by ischemia. Given the calibre and scope of cardiac research today, it is expected that such an agent will become available in the not too distant future.

INTRODUCTION

Coronary artery disease is one of the major causes of death in the world. Arrhythmic deaths associated with myocardial ischemia occur mainly in the first 1 or 2 hours of acute myocardial infarction and are due to ventricular fibrillation. Functional alterations in myocardial sarcolemma, reflected by dysrhythmia and surface electrocardiographic changes, as well as ultrastructural sarcolemmal discontinuity, are among early manifestations of ischemic injury. In experimental animal models, within seconds to minutes of acute coronary artery occlusion, loss of contractility, electrophysiological (EP) and metabolic alterations and cell swelling begin to occur in subendocardial layers. These changes progress to subcellular disruption of membranes (1-4). Reperfusion of the ischemic myocardium leads to further Ca^{2+} influx, extensive necrosis and hemorrhagic infarction which are accompanied by electrocardiographic changes and arrhythmias (5-12).

Many aspects of metabolic changes associated with myocardial ischemia have been studied and extensively reviewed (13-19). These changes include: shift in carbohydrate metabolism due to anaerobic glycolysis, accumulation of lactic acid, decrease in intracellular pH, inhibition/stimulation of several enzymatic activities, decrease in cellular ATP levels, reduction in fatty acid oxidation, accumulation of free fatty acids and their derivatives and lysophospholipids (lyso-PL), etc. Despite early functional changes in the membrane properties that are responsible for the well-known EP manifestations of ischemia, little is known of phospholipid metabolism. Phospholipids and other lipids constitute a substantial portion of the plasma membranes that serve as an important barrier for the ready diffusion of substances. Phospholipids in particular exert direct physical effects on protein-lipid interactions, which in turn, affect enzymatic reactions. In recent years it has become increasingly evident that these lipids also participate in complex biological processes, serving

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both as substrates and chemical mediators (see 20, 21). Thus, altered metabolism of these membrane constituents can alter cardiac cell functions by changing the properties of cardiac cell membranes (17-19). Such functional changes could lead to the rapid loss of contractile function, arrhythmias and eventual cell death.

LIPIDS AND MEMBRANE FUNCTIONS

The biochemical, EP and ultrastructural studies described earlier are indicative of membrane damage as a key early event in ischemia. Biological membranes as proposed by Singer and Nicolson, consist of phospholipid lilayers in which both integral and peripheral membrane proteins are embedded (22). Among these proteins are phospholipases, the enzyme machinery responsible for the hydrolysis of membrane phospholipids (23). The constituent fatty acids along with the polar head groups of phospholipids are essential for the integrity of biological membranes and for various processes occurring at biological interfaces. The presence of both negatively and positively charged moieties in the polar head groups in many phospholipids allow them to interact with each other as well as with proteins; the latter interactions may also affect the conformation of membrane proteins. Because many of these interactions are dependent on the presence of ionizable groups, they are very sensitive to changes in pH and the ionic environment (24-26). Phospholipids have also been implicated in a number of vital membrane functions. They have been shown to be required for the activity of Ca^{2+} —ATPase (27, 28) and other enzymes (29), required directly or indirectly for the active transport of Na^+ and K^+ (30, 31), for certain phosphorylation enzyme activities (32, 33), chemical and electrical excitation and insulation, selective permeability properties, phagocytosis, pinocytosis and for other more general functions related to membrane stability (34, 35). The conformation of intrinsic membrane proteins and thus their functions as receptors, enzymes and channels, etc. can be influenced by the physical state and the nature of the surrounding membrane phospholipids and other lipids (36-40). Recent studies from this laboratory and others have suggested that a disturbance in lipid metabolism may be one of the critical alterations that lead to specific EP abnormalities and irreversible cell injury in myocardial ischemia, presumably due to activation of lipases and phospholipases (17, 18, 41-49, 51, 68, 69).

There are at least two possible mechanisms by which lipid abnormalities might be implicated in structural and functional damage to cardiac membranes and these can be categorized as follows: (a) accumulation of lipid metabolites (long chain fatty acid [FFA], fatty acyl (FA) CoA, FA-carnitines, β —OH FA, etc) in ischemic myocardium that may influence cardiac function; (b) hydrolysis of membrane phospholipids which produces lyso-compounds or results in their depletion, thereby influencing membrane function.

LIPID METABOLITES AND THEIR EFFECTS

Ischemia induces intracellular accumulation of lipid metabolites, such as FFA, FA CoA, and FA carnitine. β —OH FFA have been shown to have detrimental effects on cell function (for review see 17, 19, 70, 71). Elevated levels of FFA in the plasma and myocardium have been correlated with the appearance of ventricular arrhythmias (72) and extent of enzyme release from the ischemic myocardium (73). Under some conditions FFA inhibit specific subcellular enzymes and uncouple mitochondrial respiration (74). Acyl CoA inhibits adenine nucleotide translocase in vitro (75) and acyl carnitine inhibits Na^+—K^+—dependent ATPase (76, 77) as does lysophosphatidylcholine (LPC) in low concentrations (78). The ability of low oleic acid concentrations to inhibit Ca^{2+} efflux from the sarcoplasmic reticulum (SR) (79), and of palmitic acid (80, 81) and palmitoylcarnitine (76) to increase Ca^{2+} sequestration within islolated SR vesicles could contribute to the decreased Ca^{2+} release from this membrane system in cardiac ischemia. Also, increased concentrations of linoleic and arachidonic acid may result in an increased biosynthesis of prostaglandin F_2 α in ischemic myocardial tissue (82) which may have some protective effects (83). On the other hand unsturated FFA have been shown to inhibit adenylyl cyclase activity and to exert a biphasic response to Ca^{2+} permeability (79). The accumulation of FFA derivatives is the consequence of increased liploysis and of impaired β —oxidation of FFA, which in turn results from increased NADH: NAD ratios and the inability of the myocardium to reoxidize $FADH_2$. The FFA release from the

depot triglycerides or probably from membrane PL is believed to be the consequence of the hormone sensitive lipase which is activated by circulatory catecholamines and by sympathetic stimulation (84-86). Ischemia-induced stimulation of the sympathetic tone (87) could lead to the general activation of the adrenergic receptors and adenylyl cyclase and, consequently, to the stimulation of the CAMP-dependent protein kinase (85). Protein kinase may be involved in the activation of phospholipase A_2 (PLA_2) and hormone sensitive triglyceride lipase (69, 88, 89). Also catecholamines produce an increase in intracellular Ca^{2+} levels and phospholipase activation has been shown to be Ca^{2+} – dependent.

PHOSPHOLIPID CHANGES, PHOSPHOLIPASES AND ISCHEMIC MYOCARDIAL INJURY

The enzymes responsible for the catabolism of membrane phospholipids basically fall into 4 categories and their sites of attack are schematically described in Fig. 1. PLA_1 and A_2 hydrolyse fatty acyl esters at sn-1 and sn-2 positions of diacyl-phosphoglycerides to produce equimolar amount of sn-2 and sn-1 acyl phosphoglycerides (lyso-PL) and free fatty acids, respectively. Plasmalogenase hydrolyses the vinyl ether linkage at the sn-1 position to produce lyso-PL similar to those produced by PLA_1. Phospholipase C (PLC) hydrolyses the phosphodiester bond producing sn-1, 2-diacylglycerols and phosphorylated bases. Phospholipase D liberates free base and phosphatidic acid. Lyso-PL are further hydrolysed by lysophospholipases.

The evidence which implicates increased PLA activities due to ischemia falls into two categories: Indirect and direct. The indirect evidence basically rests on the studies where phospholipid content and/or its hydrolysis products were determined in various tissues after acute experimental ischemia. These studies are summarized as follows: Using a hepatic ischemia model, Chien and co-workers reported a loss of over 50% in phospholipid content of dog liver after 1 to 2 hours of acute hepatic ischemia (90, 91). This loss was associated with marked decreases in several membrane marker enzyme activities, marked increase in Ca^{2+} content in vivo and the development of a 25 to 50 fold increase in microsomal membrane permeability to Ca^{2+}. Pretreatment with chlorpromazine (CZ) (inhibitor of phospholipases) decreased the extent of cellular injury, reduced phospholipid depletion and prevented the associated Ca^{2+} permeability defects. Liver is a highly active metabolic organ, which secretes phospholipids for lipoprotein synthesis and for biliary requirements. It could easily be anticipated that alteration in the normal function of liver by ischemia, prolonged starvation, choline deficiency, or other means (drugs) could produce substantial changes in the lipid content of liver cell membranes. Although studies on the liver model may have some relevance to ischemic myocardium, a substantial decrease in phospholipid content, at least in the early periods of myocardial ischemia, should not be expected, since in the heart phospholipids basically constitute the structural component of the membrane. On the other hand, minor changes in the structural lipid content or metabolism of the cardiac cell membranes could have severe consequences on myocardial functions. Sobel and co-workers subsequently reported a significant decrease in the phosphatidylethanolamine (PE) content of rabbit myocardium after 30-60 min of ischemia. The phosphatidylcholine (PC) content did not change significantly, but both lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) content increased by about 60% of the pre-occlusion control levels (44, 92). These quantities of lysolipids when superfused over normoxic Purkinje fibres in tissue bath experiments were sufficient to produce EP changes similar to those observed in ischemia (44, 92, 93). These studies led the authors to postulate a possible involvement of lysolipids in the genesis of malignant dysrhythmias induced by ischemia ("the lysolipid hypothesis"). The mechanism uderlying these effects appears to be related to their ability to disrupt the structure of biological membranes (94). In addition, these lipids have been shown to inhibit oxidative phosphorylation in mitochondria (95) and Na–K ATPase in sarcolemma preparations (96).

After developing highly reproducible methods for the extraction, quantification and fractionation of lipids (Table 1), we criticized the "lysolipid" hypothesis for the generation of malignant dysrhythmias (50-52). We pointed out (52)that the much higher levels of lyso-PL reported earlier by Sobel and his co-workers, as grounds for this hypothesis (44), were artifactual

TABLE 1 Recoveries of Phospholipids

Extraction scheme	No. of analyses	Lysophosphatidylcholine ng P	Lysophosphatidylcholine dpm	Lysophosphatidylethanolamine ng P	Lysophosphatidylethanolamine dpm	Total Phospholipid ug P
Folch/column	4	150 ± 1.6	7936 ± 48	229 ± 2.8	6831 ± 61	103 ± 3.8
Classical Folch	4	135 ± 2.1	7063 ± 58	200 ± 3.2	6012 ± 72	94 ± 4.3
Modified Folch	6	149 ± 0.8	7921 ± 23	228 ± 1.2	6839 ± 43	102 ± 3.1
Acid-butanol	6	2454 ± 361	7941 ± 98	3800 ± 623	6824 ± 110	101 ± 4.6

Data are expressed as mean ± SD. Control myocardial tissue was homogenized in 0.9% NaCl at 4°C and equal portions of the homogenate representing 125 mg tissue wet wt. were extracted under each protocol. Lysophospholipids were then analyzed after TLC/GLC using internal standards. Similar protocols were repeated with added ^{32}P-labelled rat liver lysophospholipids (8021 and 7004 dpm/6ug P of LPC and LPE, respectively) and, after fractionation of phospholipids by TLC, radioactivity was determined directly on silica gel scrapings (58) of appropriate fractions (for details see reference 51).

TABLE 2 Lysophospholipid Content of Pig and Rabbit Myocardium

Time of Ligation (min)	Shaikh and Downar (51) LPC ug P/g wet wt.	Shaikh and Downar (51) LPE ug P/g wet wt.	Sobel et al. (92) LPC ug P/g wet wt.	Sobel et al. (92) LPE ug P/g wet wt.
.Control	.13 ± 0.04 (22)	1.9 ± 0.12 (22)	43.4 ± 3.7 (13)	37.2 ± 6.2 (13)
Ischemic zone				
2	1.5 ± 0.09 (9)	2.6 ± 0.31 (9)	—	—
8	2.0 ± 0.16 (10)	3.0 ± 0.26 (10)	—	—
5 – 15	—	—	74.4 ± 12.4 (7)	55.8 ± 6.2 (7)
20	1.7 ± 0.11 (7)	3.2 ± 0.34 (7)	—	—
40	1.7 ± 0.08 (8)	3.1 ± 0.31 (8)	—	—
30 – 60	—	—	68.2 ± 12.4 (6)	68.2 ± 6.2 (6)

Data expressed as mean ± SD of (n) analyses. All values are significantly different from respective controls ($P<0.1$)

Data modified from Shaikh and Downar (51) and Sobel et al. (92)

The data from ref. 92 is recalculated by assuming 80% water content of the tissue and is expressed as mean ± SE of (n) analyses.

in nature and must have arisen from the use of unsuitable extraction and analysis techniques. This was subsequently acknowledged by the authors (97). We evaluated the time course of ultrastructural changes of ischemic injury due to coronary artery occlusion in the in situ model of porcine heart (53) and correlated this with the time course of changes in phospholipid (PL) levels (Tables 2 & 3). We showed that, although tissue levels of lyso-PL increased by 60% in in situ ischemic porcine heart after 8-12 min of acute myocardial ischemia, the absolute quantities of these lipids remained very low (0.6% of the total PL) and about 25 fold lower (Table 3) than reported for rabbit heart (44, 92). We were the first to report that the loss of membrane PL due to prolonged ischemia is not as severe in heart (52) as was observed for liver, understandably so, since the liver synthesizes PL for assembly and transport (lipoprotein,

TABLE 3 Phospholipid Composition of Normal and Ischemic Myocardium

	Normal Zone		Ischemic Zone		
Time Ligation	(o min)	(20-40 min)	(2-20 min)	(40 min)	(24 hr)
	ug P/g wet wt. (mean ± S.D. of (n) observations)				
Total Phospholipids	825.3 ±43.1 (5)	831.6 ±52.6 (5)	818.3 ±49.2 (5)	801.7 ±62.8 (5)	715.6 ±38.2 (3)
Phospholipid Fractions*	% of total lipid-P				
CPG	37.85	37.10	38.16	37.22	38.40
Diacyl	24.22	23.68	24.36	23.86	24.56
Alkenyl-acyl	13.63	13.42	13.80	13.36	13.84
EPG	25.34	25.68	25.37	26.69	28.87
Diacyl	12.16	12.26	12.07	12.81	13.98
Alkenyl-acyl	13.18	13.42	13.30	13.88	14.89
CL	19.24	18.73	18.70	17.50	14.47
PI	6.29	7.12	6.09	6.35	6.39
PS	4.34	4.12	4.51	4.21	4.52
Sph	6.04	6.18	6.02	6.81	6.65
PA	0.18	<0.60	<0.60	<0.60	<0.60
PG	0.38				
LPC	0.15	0.19	0.21	0.22	0.25
LPE	0.23	0.28	0.34	0.40	0.45

* Mean of duplicate analyses of 3 independent samples.

Abbreviations used: CPG, choline phosphoglycerides: EPG, ethanolamine phosphoglycerides; CL, cardiolipin; PI, phosphatidylinositol; PS phosphatidylserine; Sph, sphingomyelin; PA, phosphatidic acid; PG, phosphatidylglycerol. Total phospholipid content or individual phospholipid fractions were determined by phosphate analysis directly on silica gel scrapings (57, 59). For details see reference 51.

biliary secretion, etc.), while in the hart, PL constitute membrane components. In our studies, no change in the total PL content or the individual PL classes could be demonstrated for up to 45 min of in situ ischemia. However, the total PL content fell from 6%and 14% after 8 and 24 hours of ischemia. Subsequently, Chien and co-workers supported our observation and reported a much smaller decrease (3-10%) in the total PL content of the subendocardium (42,43). This decrease was also associated with Ca^{2+} –permeability defects. In our other studies (NA Shaikh, unpublished), levels of FFA and diacylglycerols increased significantly at as early as 5-10 min of left anterior descending coronary artery (LAD) occlusion (Table 4), while triacylglycerol, cholesterol and cholesterol esters remained unchanged (Table 5). Although absolute quantities of lyso-PL were subsequently reported to be much lower and were comparable to ours, Corr et al (98) found a similar per cent increase in their content after 10 minutes of ischemia in cat hearts. Man et al (99) reported an increase of 107 and 137% in LPC and LPE content after 24 hours of ischemia in dog hearts. A substantial increase in the lyso–PL content of whole dog hearts, after 24 hours incubation in a 37° water bath, was also reported by Steenbergen and Jennings (100). Other workers have found increased levels of LPC in the venous blood draining ischemic regions (101) and progressive increase in non-esterified arachidonic

TABLE 4 Time Course of Changes in the Free Fatty Acid and Diacylglycerol Levels of Porcine Myocardium after LAD Occlusion

Lipid Component	Time after LAD Occlusion (min.) 0	5	10	30	90	180
Free Fatty Acids (umoles/g wet wt.)	32.6 (26.6-42.3)	38.4 (29.8-44.6)	45.3 (36.6-54.8)	64.8 (41.9-73.1)	86.8 (68.4-100.1)	92.6 (71.3-113.4)
Diacylglycerols (nmoles/g wet wt.)	0.51 (0.43-0.56)	0.91 (0.82-1.31)	1.12 (0.91-1.34)	0.88 (0.78-1.14)	0.96 (0.78-1.36)	1.20 (0.81-1.41)

Data expressed as mean of 3-6 determinations. Numbers in parentheses designate range of values. Experimental protocol similar to that described elsewhere (51). Free fatty acid levels were determined by GLC on polar columns using heptadecanoic acid as an internal standard. Diacylglycerols were quantitated by GLC on non-polar columns using tridecanoin as an internal standard as described previously (60-62). N. A. Shaikh (1986) unpublished data.

TABLE 5 Triacylglycerol, Free and Esterified Cholesterol Levels in in situ Porcine Heart after LAD Occlusion

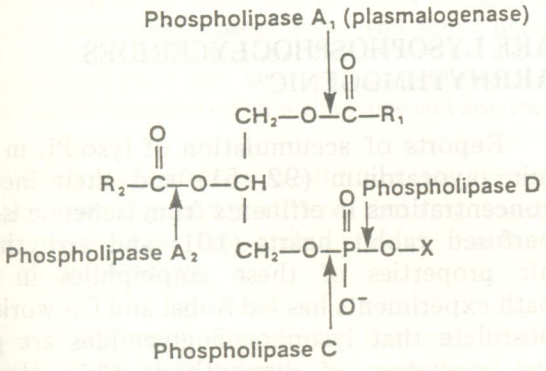
Time of Ligation (min)	Triacylglycerol	Cholesterol (mg/g wet wt.)	Cholesterol ester
0	2.9 (2.1-3.8)	1.8 (1.4-2.1)	0.42 (0.31-0.46)
90	2.3 (1.7-2.9)	1.6 (1.3-1.9)	0.40 (0.29-0.48)
180	3.1 (1.7-3.8)	1.9 (1.4-2.3)	0.38 (0.32-0.41)

Data expressed as mean of 3-6 determinations. Numbers in parentheses designate range of values. Experimental protocol similar to that described elsewhere (51). The determinations were made by GLC on non-polar columns using tridecanoin as an internal standard as described previously (61). N. A. Shaikh (1986) unpublished data.

acid and other free fatty acids during ischemia in dog heart (68, 102, 103). The increase in tissue free fatty acids could arise from the sequential degradation of phospholipids by phospholipases and lysophospholipases; the triglyceride levels in ischemia are believed to remain unchanged. Subcellular fractions prepared from hypoxic or ischemic hearts also showed varying degrees of increases in lyso-PL content and decrease in total phospholipid levels after 3 hours of ischemia (104-106). Despite the differences in experimental protocols and animal models, the studies outlined above are suggestive of membrane phospholipase activation due to myocardial ischemia.

The studies describing the direct evidence for the activation of phospholipases in ischemia are few and apparently reflect inherent technical difficulties. However, PLA activities have been described in a number of normal cardiac membranes as well as cardiac lysosomes (107 - 110) and lysosomal enzyme activity increases in the ischemic heart (41). Studies in vitro have shown activation of endogenous phospholipase(s) of myocardial sarcolemma and of rat liver endoplasmic reticulum by exogenous Ca^{2+} (108, 111). Since intracellular Ca^{2+} levels increase in myocardial ischemia with a decrease in phospholipid content, it can be deduced that phospholipase(s) are activated. A more direct line

of evidence comes from Needleman and co-workers who described synthesis and release of prostaglandins by isolated perfused rebbit hearts upon bradykinin stimulation or by the onset of global ischemia. They suggested stimulation of lipases which specifically liberate arachidonic acid for prostaglandin synthesis (bradykinin) and non-specifically release (global ischemia) oleic as well as arachidonic acid (112, 113). In these experiments, ischemia was produced by stopping the perfusion of the heart for 5 minutes and the effluent was then collected for 4 minutes after re-establishing the cardiac perfusion with albumin-containing buffer. The release of raiolabelled fatty acids and prostaglandin in the effluent was described to be the consequences of a non-specific activation of lipases by ischemia. In these studies, however, it is not clear whether increased lipase activities are due to ischemia or are in fact due to reperfusion of the ischemic heart. Reperfusion injury is accompanied by a massive influx of Ca^{2+} and membrane phospholipase activation has been shown to be Ca^{2+}—dependent (108, 111, 1140.



Phospholipase A_1	=	1-Lyso-PL (2 monoacyl PL) + FA
Phospholipase A_2	=	2-Lyso-PL (1 monoacyl PL) + FA
Phospholipase C	=	Diglyceride + Phosphoryl-base
Phospholipase D	=	Phosphotidic acid + base

Figure 1. Schematic diagram showing the sites of attack of different phospholipases.

Using isolated perfused cat hearts we provided the first direct evidence for phospholipase activation in ischemia.Cat hearts after dissection were perfused retrogradely with Krebs-Henseleit bicarbonate buffer (pH 7.35), containing radiolabelled stearic and arachidonic acids, at 37°C in a closed circuit apparatus (Fig. 2). The LAD was ligated proximal to its first diagonal branch for 40 min with a silk thread; and samples from ischemic and non-ischemic zones of the same heart were dissected and analyzed (for details, see 49). Radioactivity in fatty acids occupying position 1 and 2 of the glycerol backbone of phospholipids in ischemic and non-ischemic zones of cat heart after 40 minutes of LAD occlusion was determined (Table 6). A significant decrease in the ratio of the radioactivities of arachidonic acid and stearic acid in ischemic zone as compared to non-ischemic zone of the heart after 40 minutes of LAD occlusion strongly suggests that PLA_2 activity is stimulated by ischemic insult (Table 7). This change in the rate of deacylation-reacylation of membrane phospholipids could constitute a major event in at least early phases of ischemia which could be responsible for the EP abnormalities since lipids maintain a membrane architecture crucial for a hormonally responsive state of membrane-bound enzymes (115-117). This finding is also supported by the simultaneous work of Chien (118) who showed a 70% increase in arachidonic acid content of ischemic canine myocardium after 60 minutes of LAD occlusion. Since arachidonic acid is found entirely in membrane phos-

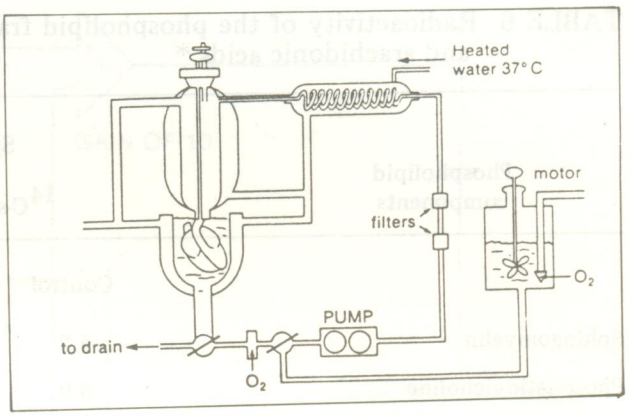


Figure 2. Schematic diagram of the isolated heart perfusion apparatus.
After dissection, the heart is immediately flushed with Krebs-Henseleit bicarbonate buffer, pH 7.35 (KHB) containing heparin and mounted on the apparatus through the aorta with a cannula. The heart is then reperfused retrogradely with oxygenated KHB at 37°C. When labelling is required, radiolabelled fatty acids are introduced directly to the heart through a small tube passing through the reservoir chamber and the effluent buffer is redirected to close the perfusion circuit.

TABLE 6 Radioactivity of the phospholipid fractions of isolated perfused cat hearts labelled with stearic and arachidonic acids.*

Phospholipid components	Specific activity (dpm/ug P)			
	^{14}C-stearate		^{3}H-arachidonate	
	Control	Ischemic	Control	Ischemic
Sphingomyelin	5.2	5.3	18	23
Phosphatidylcholine	6.0	6.2	440	401
Phosphatidylethanolamine	3.1	3.4	1075	1075
Phosphatidylinositol	30.0	28.1	3989	3715
Phosphatidylserine	19.4	19.7	788	707
Cardiolipin	1.6	3.2	135	108
Lysophosphatidylcholine	18.7	20.6	—	—
Lysophosphatidylethanolamine	4.2	6.3	—	—

*Results are expressed as radioactivity per ug phosphate of individual phospholipid components. Cat hearts were perfused with 3 uCi of ^{14}C-stearic acid or 5 uCi of ^{3}H-arachidonic acid. After 30-40 min of LAD occlusion, both ischemic and non-ischemic zones of the hearts were analyzed for phosphate content and radioactivity of the individual phospholipid fractions. Other experimental details are described elsewhere (49).

pholipids, he suggested that the increase in unesterified arachidonic acid is due to increased phospholipid degradation.

TABLE 7 Ratio of the radioactivity of arachidonic acid and stearic acid of certain phospholipid fractions in isolated perfused cat hearts.*

PHOSPHOLIPIDS	ARACHIDONIC ACID/ STEARIC ACID	
	Control	Ischemic
Phosphatidylcholine	16	8
Phosphatidylethanolamine	43	35
Phosphatidylinositol	9	7.5

*Results are expressed as ratio of the radioactivity of arachidonic acid occupying position 2 and stearic acid occupying position 1 of the glycerol backbone of phospholipids.
Cat hearts were perfused with 5 uCi each of ^{14}C-stearic and ^{3}H-arachidonic acids. After 40 min of LAD occlusion, samples from ischemic and non-ischemic zones of the hearts were analyzed. Other experimental details are described elsewhere (49).

ARE LYSOPHOSPHOGLYCERIDES ARRHYTHMOGENIC?

Reports of accumulation of lyso-PL in ischemic myocardium (92, 51) and their increased concentrations in effluents from ischemic isolated perfused rabbit hearts (101) and arrhythmogenic properties of these amphiphiles in tissue bath experiments has led Sobel and Co-workers to postulate that lysophosphoglycerides are potential mediators of dysrhythmia (44). However, lyso-PL at their maximum levels in ischemic heart account for only 160 nmoles/g wet wt. or 0.6% of total PL-phosphorus (51). A much higher concentration of lyso-PL (approx. 10-fold) bound with albumin is required to induce EP alterations in normoxic Purkinje fibres in a tissue bath that are analogous to changes in ischemic tissue in vivo (119). Unbound lyso-PL in quantities similar to those found in ischemic myocardium or lower amounts have recently been shown to produce EP derangements (119-121) and to induce arrhythmia in isolated perfused hearts analogous to other detergents (122). However, free concentrations of lyso-PL equivalent to those employed in the fore-

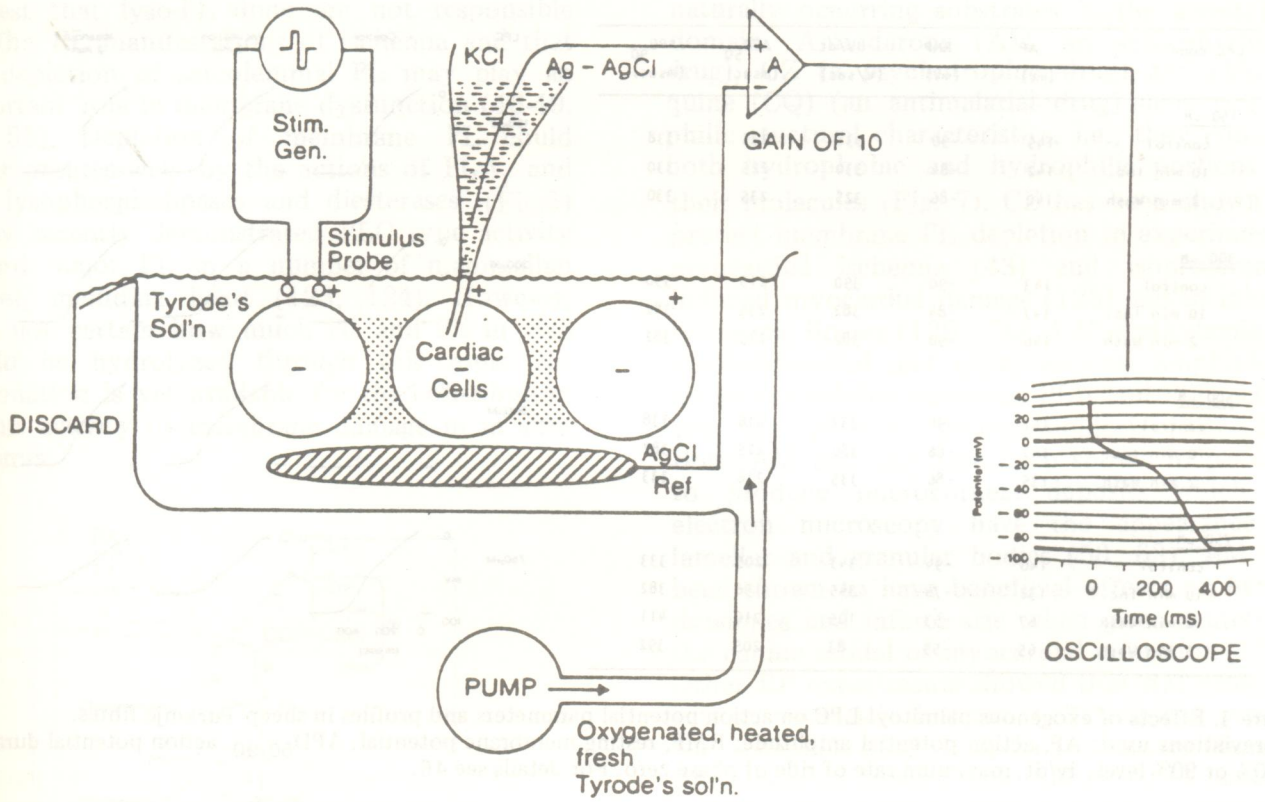


Figure 3. Schematic diagram of a tissue bath set up. The myocardial tissue containing muscle and Purkinje fibres is secured at the bottom of the tissue bath by means of stainless steel pins. The tissue is superfused with Tyrode's buffer (pH 7.35) at 37°C and is electrically paced at one end with a bipolar probe. The stimulus is maintained at 2 X threshold with a biphasic pulse width of 1 msec duration. Transmembrane action potentials are recorded using glass microelectrodes (10-30 megaOhm tip impedance) filled with 3 M KCl and connected via driven-shield cables to high input impedance buffer amplifiers. Action potentials are visualized on an oscilloscope and also recorded simultaneously.

mentioned experiments would not be expected in the plasma compartment bathing cardiac tissue where these amphiphiles would be bound with plasma albumin.

We investigated, in a tissue bath set up (Fig. 3), the possibility of whether the accumulation of lyso-PL or depletion of PL molecules in the sarcolemma is responsible for the EP abnormalities of ischemic hearts by producing in vitro phospholipase-induced abnormalities in muscle and Purkinje fibres of the sheep heart. Under control conditions, exogenous PLA_2 was employed to produce lyso-PL in the membrane matrix, and PLC was used to achieve depletion of membrane PL (1.0%) without lyso-PL production. For comparative purposes, we also investigated the EP effects of exogenous free lyso-PL on sheep cardiac fibres (46-49, 54, 55). In contrast to previous reports, our results clearly showed that incubation with exogenous lyso-PL in quantities smimilar to (016 mM) or 5 times the amount did not alter the EP characteristics of either muscle or Purkinje fibres. Resting membrane potentials, upstroke velocity, action potential duration and amplitude remained similar to controls (Fig. 4). Incubation with PLA_2 which produced endogenous lyso-PL (up to 0.4 mM) in membranes also showed no effects. At higher endogenous lyso-PL concentrations (0.6-1. mM), action potentials deteriorated but normal EP properties returned after 6-10 min of washing with oxygenated normal buffer (Fig. 5A). At this stage of complete recovery, lyso-PL levels in fibres remained abnormally high (0.4 mM). On the other hand, incubations with PLC resulted in less than 0.1% loss of membrane PL with no lyso-PL production, but completely abolished the action potential in 6 min with the fibre remaining unresponsive to stimulus, even after prolonged washing with oxygenated normal buffer (Fig. 5B). Within the limitations imposed by tissue bath experiments, these results nevertheless

LPC conc.	AP (mV)	RMP (mV)	dV/dt (V/sec)	APD_{50} (msec)	APD_{90} (msec)
150 μM					
control	145	-90	338	235	338
10 min Inc.	142	-84	330	225	330
2 min Wash	140	-86	325	235	330
300 μM					
control	143	-90	390	215	390
10 min Inc.	147	-84	382	235	382
2 min Wash	146	-90	382	215	382
500 μM					
control	142	-90	337	216	338
10 min Inc.	147	-86	380	225	382
2 min Wash	135	-86	335	216	333
750 μM					
control	140	-94	343	205	333
10 min Inc.	132	-74	345	196	382
2 min Wash	67	-53	105	216	411
7 min Wash	65	-55	83	205	392

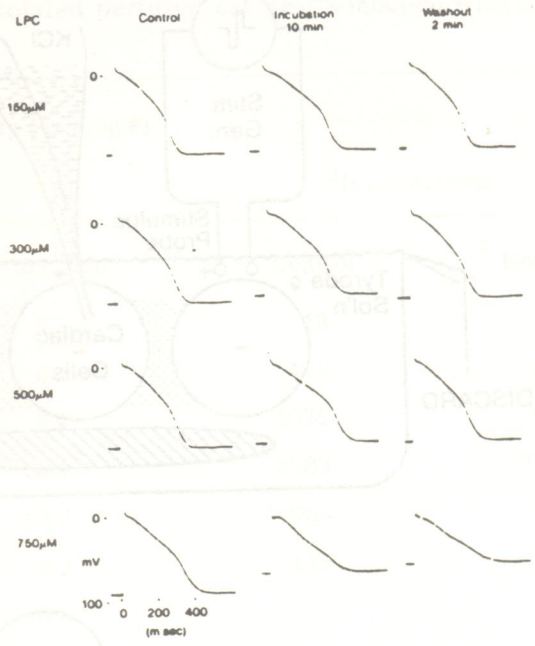
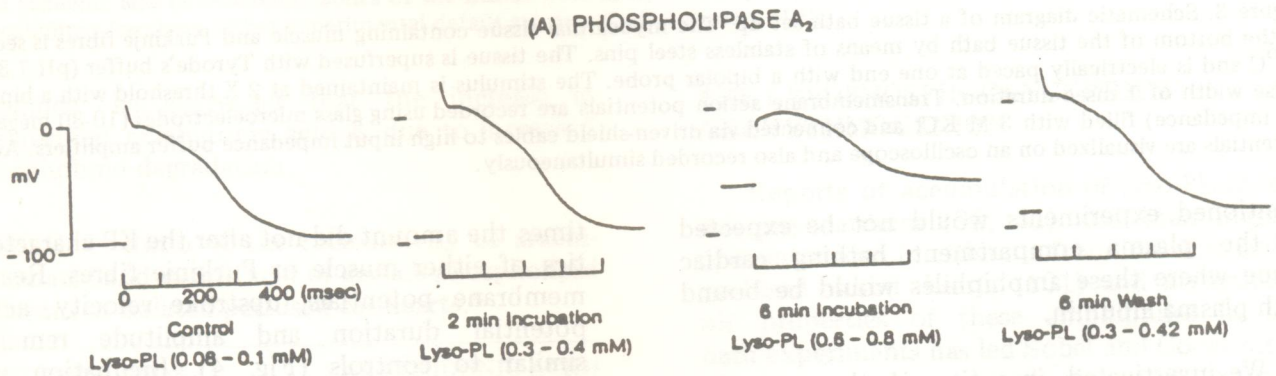


Figure 4. Effects of exogenous palmitoyl-LPC on action potential parameters and profiles in sheep Purkinje fibres. Abbreviations used: AP, action potential amplitude; RMP, resting membrane potential; APD_{50-90}, action potential duration at 50% or 90% level; dv/dt, maximum rate of ride of phase zero. For details see 46.



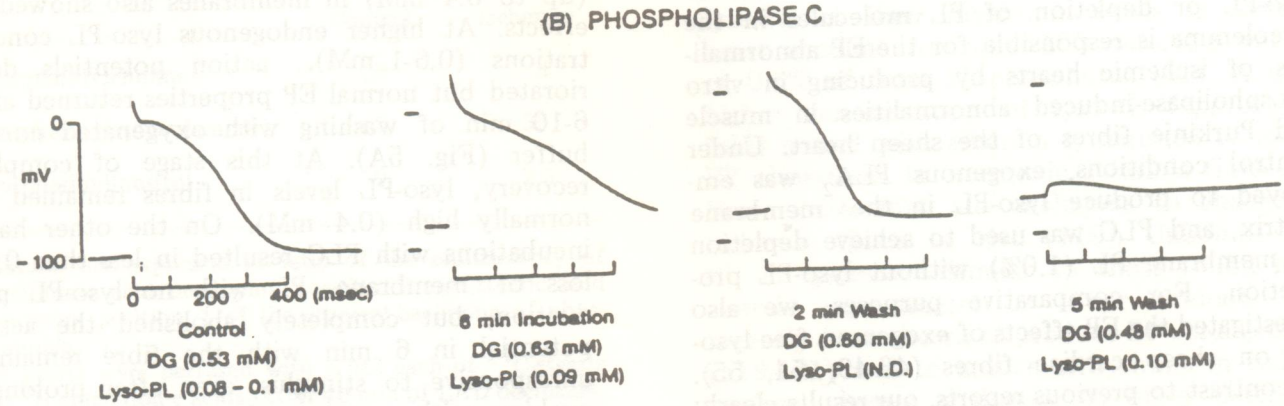
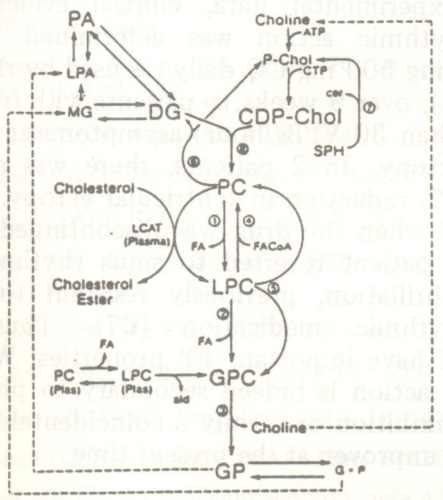


Figure 5. Effects of endogenously produced lyso-PL (A) or membrane PL depletion (B) on EP profiles of sheep Purkinje fibres (for details see 49).

suggest that lyso-PL alone are not responsible for the EP manifestations of ischemia and that the depletion of sarcolemmal PL may play an important role in membrane dysfunction (46-49, 54, 55). Depletion of membrane PL could occur progressively by the actions of PLA_1 and A_2, lysophospholipases and diesterases (Fig.6) or by recently demonstrated PLC type activity toward major PL in a number of mammalian tissues, including heart (123, 124). However, it is not certain how much PC and PE in vivo would be hydrolyzed through this route. No information is yet available for the contribution of this activity to membrane damage in in situ-ischemia.



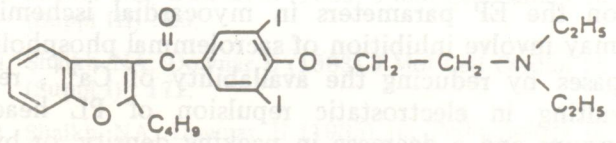
1. Phospholipase A_1 & A_2
2. Lysophospholipase
3. Glycerophosphorylcholine diesterase
4. Lysophospholipid acyltransferase
5. Lysophospholipase-transacylase
6. Phospholipase C
7. Sphingomyelinase
8. Choline phosphotransferase

Abbreviations:
PC, phosphatidylcholine; LPC, lyso-phosphatidylcholine; GPC, glycero-phosphorylcholine; GP, glycerophos-phate; SPH, sphingomyeline; FA, fatty acid; FACoA, fatty acyl coenzyme A; cer, ceremide; Plas, plasmalogen; ald, fatty aldehyde; DG, diglyceride.

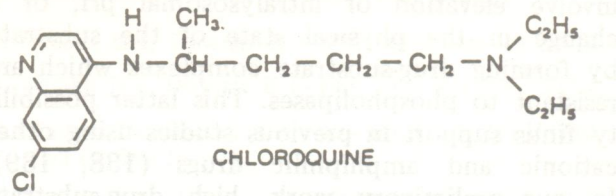
Figure 6. Schematic diagram of the metabolism of choline phosphoglycerides.

REDUCTION IN PHOSPHOLIPASE ACTIVITIES BY DRUGS

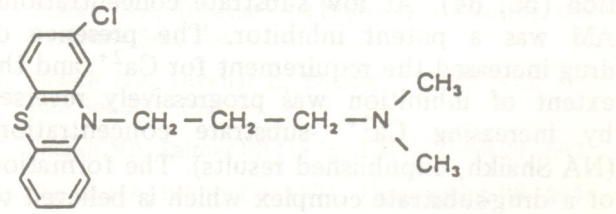
If some of the ischemia-induced abnormalities of cardiac PL metabolism are the consequence of the activation of lipases, then the agents which inhibit such enzymatic activities would have some protective effects. Ca^{2+} blocking agents and other drugs which partition between aqueous and lipid phases would inhibit non-specific lipases (including phospholipases) either by blocking Ca^{2+} or by reducing the accessibility of the intrinsic enzymatic proteins to their naturally occurring substrates in the membrane domain. Amiodarone (AM; an antiarrhythmic drug), CZ (a psychotrophic drug) and chloroquine (CQ) (an antimalarial drug) share amphiphilic stuctural characteristics, i.e., they contain both hydrophobic and hydrophilic portions in their molecules (Fig. 7). CZ has been shown to protect membrane PL depletion in experimental myocardial ischemia (43) and isoproterenol-induced myocardial damage (125) and to inhibit lysosomal lipases (126). CQ, 4,4' -diethylaminoe-thoxy-hexestrol and other cationic amphiphilic drugs also inhibit lysosomal PLA and PLC activities and produce phospholipidosis (127-129). Both AM and CQ have been shown in patients to produce microcorneal deposits which on electron microscopy have the appearance of lamellar and granular bodies (56, 63). AM has been shown to have beneficial effects on hemodynamics and infarct size when given acutely in the canine model of myocardial infarction (130). Other EP experiments showed that AM prolongs action potential duration (131) and depresses Na^+ -channels (132, 133). In preliminary observations on a rat treated with AM in which lamellar and granular bodies were present in skin and cornea, we found an increase of 20-30% in total PL (on the basis of wet wt. and protein) in both



AMIODARONE



CHLOROQUINE



CHLORPROMAZINE

Figure 7. Structures of some amphiphilic drugs.

heart and liver. We also observed widespread lysosomal inclusion bodies in all cell types, including polymorph leukocytes, alveolar macrophages, hepatocytes and cardiac myocytes, in patients that had been chronically treated with AM. These inclusions are lamellar/granular in appearance and similar to those observed with treatment of CQ and CZ (63, 64).

It is conceivable that AM, like CQ, has phospholipase inhibitory effects which are responsible for its potent and beneficial effects in reducing ischemic injury and stabilizing EP responses. In fact, we were the first to show that AM, like CQ and CZ, has potent dose response inhibitory effects on in vitro activities of PLC and PLA_2 (56, 64, 65). More recently, Hostetler and co-workers have reported inhibition of purified lysosomal PLA by exogenous AM and an increase in PL content of lysosomes prepared from animals treated with this drug (134). The delayed adverse effects of AM (neuromyopathy (135), hepatic fibrosis (136) and pulmonary toxicity (137) may involve alteration in the lysosomal functions due to massive inclusion of PL bodies (probably drug-PL complexes) not susceptible to normal hydrolysis by phospholipases. How AM effects its inhibition remains to be determined. The beneficial effects of AM on the EP parameters in myocardial ischemia may involve inhibition of sacrolemmal phospholipases by reducing the availability of Ca^{2+}, resulting in electrostatic repulsion of PL head groups and a decrease in packing density, or by stabilizing lysosomes. The inhibition of lysosomal phospholipases (A & C) by the drug may involve elevation of intralysosomal pH, or a change in the physical state of the substrate by forming drug-substrate complexes which are resistant to phospholipases. This latter possibility finds support in previous studies using other cationic and amphiphilic drugs (138, 139). In our preliminary work, high drug-substrate ratios were required to produce marked inhibition (56, 64). At low substrate concentrations, AM was a potent inhibitor. The presence of drug increased the requirement for Ca^{2+} and the extent of inhibition was progressively reversed by increasing Ca^{2+} -substrate concentrations (NA Shaikh, unpublished results). The formation of a drug-substrate complex which is believed to be resistant to hydrolysis is formed readily at lower pH, and this is reflected by almost complete inhibition of PLA_2 activity (NA Shaikh, unpublished results).

Thus, from these data it is suggested that the inhibition of phospholipase activities may be the cause of phospholipidosis and this may also be responsible for the beneficial effects of AM. If that is the case, then useful antiarrhythmic properties might be expected from other drugs that inhibit phospholipase activities.In fact, our recent tissue bath experiments show that CQ ($1x10^{-5}$, $1x10^{-6}$ M) produced a significant reduction in the maximum velocity of the upstroke of the action potential, a prolongation of the action potential duration and refractory period in Purkinje fibres (66). In addition to these experimental data, clinical evidence of antiarrhythmic action was determined by administering 500 mg CQ daily (as used by rheumatologists), over 9 weeks, to patients with frequent (more than 30 VPBs/hour) asymptomatic ventricular ectopy. In 2 patients, there was a more than 75% reduction in ventricular ectopy, which recurred when the drug was discontinued, while a third patient reverted to sinus rhythm from atrial fibrillation, previously resistant to other antiarrhythmic medication (67). Thus, CQ and AM have important EP properties. Whether this EP action is indeed secondary to phospholipase inhibition or mainly a coincidental finding remains unproven at the present time.

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