Amphipathic Metabolites and Membrane Dysfunction in Ischemic Myocardium

Peter B. Corr, Richard W. Gross, and Burton E. Sobel

From the Cardiovascular Division, Washington University School of Medicine, St. Louis, Missouri

Many laboratory and clinical observations have implicated altered lipid metabolism as a contributor to membrane dysfunction, electrophysiological derangements, malignant arrhythmia, and cellular injury in ischemic myocardium. Amphipathic metabolites, moieties with both hydrophobic and hydrophilic properties, are capable of exerting deleterious effects on biological membrane systems through multiple mechanisms. Because of their structure, amphiphiles can alter membrane function by inserting into the phospholipid bilayer, and, under some conditions, can act as detergents with resultant dissolution of membrane constituents including cholesterol and phospholipids. This selective review will consider the metabolism of fatty acids, fatty acid esters, and phospholipid catabolites which increase in ischemic myocardium and have been implicated as biochemical mediators of membrane dysfunction.

Two reviews dealing with the biophysics of lipid alterations in membranes and alterations of carbohydrate and fatty acid metabolism during ischemia have been published recently (Liedtke, 1981; Katz and Messineo, 1981).

Metabolism

Fatty Acids and Fatty Acid Esters

Under physiological conditions, the heart preferentially utilizes fatty acids to meet its energy requirements (Fig. 1). Unesterified free fatty acids (FFA) in plasma are bound to albumin, with small quantities free in solution in equilibrium with albumin-bound FFA. Unbound FFA can traverse the sarcolemma by passive diffusion or via a saturable fatty acid-binding process involving sarcolemmal proteins (Stein and Stein, 1968). Some cytosolic unbound FFA exists in equilibrium with a larger quantity of intracellular FFA bound to soluble proteins and membranes (Mishkin et al., 1972, 1975; Ockner et al., 1972; Fournier et al., 1978, 1983).

A high affinity intracellular binding protein (Z protein) in the heart can bind and translocate both fatty acids and acyl CoA esters, and appears to exist in at least four different molecular states reflecting self-aggregation (Fournier et al., 1983). The relative proportion of the different molecular states is modulated by both the protein and fatty acid concentration, and may regulate the membrane-bound enzyme activity because of differences in substrate delivery (Fournier et al., 1983). Thus, this binding protein may modulate the rates of the two enzymatic systems (microsomal esterification and mitochondrial β-oxidation) by regulating the cytosolic concentrations of fatty acid and coenzyme A (CoA).

Metabolism of intracellular FFA proceeds initially by thioesterification to fatty acid—CoA esters (acyl CoA) mediated by acyl CoA synthetase located on the outer mitochondrial membrane, and may be present in other subcellular compartments, as well (Fig. 1). Activity of this mitochondrial enzyme is modulated through product inhibition by AMP, inorganic phosphate, acyl CoA, and substrate availability modulated by the relative affinities of each form of the intracellular binding protein. Enzyme activity is substrate dependent due to the limited availability of CoA, most of which is contained in the mitochondrial compartment (Oram et al., 1973). Long-chain acyl CoA does not rapidly traverse the inner mitochondrial membrane. To facilitate rapid translocation of acyl CoA, sequential transesterification reactions convert acyl CoA to acyl carnitine (Haddock et al., 1970; Brosnan and Fitz, 1971), which is rapidly transferred across mitochondrial membranes prior to β-oxidation (Pande, 1975; Ramsay and Tubbs, 1975). There are two carnitine acyl transferases on either side of the mitochondrial...
membrane, as well as a carnitine-acyl carnitine translocase, which is specific for the L-isomer (Wolkowicz et al., 1982). The acyl CoA is first converted to acyl carnitine on the outside of the inner mitochondrial membrane, which is translocated across the inner mitochondrial membrane in exchange for free carnitine via carnitine-acyl carnitine translocase. This translocase is critical, not only for movement of acyl carnitine across the inner mitochondrial membrane, but, also, for regeneration of free carnitine for exchange back to the cytosolic side of the inner mitochondrial membrane (Wolkowicz et al., 1982). Once inside the mitochondrial matrix, the acyl carnitine is transesterified back to yield carnitine and acyl CoA by the inner carnitine acyl transferase (Fig. 1).

Complete \( \beta \)-oxidation results in catabolism of long-chain fatty acyl CoA to two-carbon, acetyl CoA fragments with concomitant production of reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH\(_2\)). Subsequent oxidation of these moieties and the associated trans-
port of electrons through the mitochondrial electron transport chain are tightly coupled to production of ATP. Acetyl CoA can enter the citric acid cycle with production of additional NADH, which can be oxidized to yield additional ATP.

Alternatively, free fatty acids can be stored in the myocyte by incorporation into phospholipids or neutral lipids, primarily triglycerides, which serve as a reservoir of substrate for β-oxidation (Scheuer and Brachfeld, 1966; Stein and Stein, 1968; Vasdev and Kako, 1977; Lochner et al., 1978; Klein et al., 1979). The relative fractions oxidized, incorporated into phospholipid, and incorporated into neutral lipids change in response to myocardial energy requirements and to prevailing concentrations of circulating FFA (Crass, 1972).

**Phospholipids**

Synthesis of most myocardial phospholipids (Fig. 2) is mediated by sequential enzymatic reactions involving activation of a choline or ethanolamine base prior to transfer to 1,2-diacylglycerol (Kennedy and Weiss, 1956). Choline kinase, a soluble enzyme, catalyzes the transformation of choline to phosphocholine, which can undergo condensation with cytidine triphosphate to form cytidine diphosphocholine (CDP-choline) in a reaction catalyzed by the enzyme, phosphocholine cytidyltransferase. In the hamster heart, this enzyme is the rate-limiting enzyme in the de novo pathway for phosphatidylcholine synthesis (Zelinski et al., 1980). Two forms of phosphocholine cytidyltransferase have been found in rat liver (Choy et al., 1977) with different molecular weights, enzymic activity, and intracellular loci (Vance and Pelech, 1983). Relative ratios of the two subforms are influenced by fatty acid availability which, therefore, modulate the rate of de novo phospholipid synthesis. The final step in the de novo pathway of phospholipid synthesis is the transfer of choline phosphate from CDP-choline to diacylglycerol to form phosphatidylcholine in a reaction catalyzed by CDP-choline:diacylglycerol phosphocholine transferase, an enzyme present in the heart (Zelinski et al., 1980). Ethanolamine glycerophospholipids are synthesized by a similar series of reactions.

Less abundant phospholipids, such as phosphatidyl serine, phosphatidyl inositol, and cardiolipin, are synthesized by condensation of phosphatidic acid and cytidine triphosphate to form the activated intermediate, CDP-diacylglycerol. The cytidine monophosphate moiety may be regarded as a carrier of phosphatidic acid because, in the ensuing condensation reactions, phosphatic acid is transferred to serine, inositol, or glycerol phosphate with synthesis of phosphatidylserine, phosphatidylinositol, or phosphatidylglycerophosphate, an intermediate in the synthesis of cardiolipin.

Alternate pathways for the synthesis of choline or ethanolamine glycerophospholipids involve the synthesis of phosphatidyl serine, followed by decarboxylation to form phosphatidylethanolamine, transmethylation of phosphatidylethanolamine to form phosphatidylcholine, or base exchange. In isolated hamster heart, phosphatidylcholine is synthesized from CDP-choline at a velocity of 39 nmol/min per g wet weight (Zelinski et al., 1980). The flux of phosphatidylethanolamine into phosphatidylcholine by base exchange or sequential methylations is slower than the rate of de novo synthesis from CDP-choline (Zelinski et al., 1980). Thus, the rate-limiting step for myocardial phosphatidylcholine biosynthesis appears to be the conversion of choline phosphate to CDP-choline, catalyzed by phosphocholine cytidyltransferase (Zelinski et al., 1980).

Myocardial membrane remodeling involves the modification of the fatty acyl composition of phospholipids synthesized de novo by a decylation-reacylation cycle initiated by phospholipase A₁ or
**Phospholipases**

**FIGURE 3.** Major enzymatic reactions involved in the catabolism of phospholipids. The sites of enzymatic cleavage of each of the different phospholipases are shown in the top portion of the figure (I). In the bottom portion of the figure (II) the pathways of lysophospholipid catabolism are shown. In pathway a, acylation of lysophosphatidicholine (LPC) to phosphatidicholine is shown catalyzed by the enzyme LPC acyltransferase. In pathway b, lysophospholipase-transacylase catalyzes the disproportionation of two molecules of LPC to PC and glycerophosphorylcholine (GPC). In pathway c, lysophospholipase hydrolyzes LPC to form GPC and FFA.

A2 with generation of 1-deacyl or 2-deacyl lysophospholipids, respectively (Fig. 3). Subsequent acyl transfer from a high energy fatty acyl CoA thioester is catalyzed by the enzyme, coenzyme A:LPC acyltransferase, with generation of phosphatidylcholine with a modified fatty acid composition. Myocardial coenzyme A:LPC acyltransferase is a membrane-bound enzyme with a broad pH optimum between 6 and 8, and an apparent Michaelis constant for palmitoyl CoA of 7 μM (Gross and Sobel, 1982).

**Lysophospholipids**

Myocardial phosphoglycerides can be catabolized in reactions mediated by phospholipase A1, phospholipase A2, phospholipase C, and phospholipase D (Fig. 3). Some of these reactions lead to formation of lysophospholipids. Phospholipase A1 and A2 activities in rat liver have been described (Waite and van Deenan, 1968). Several different myocardial phospholipases have been reported with specific subcellular loci and pH optima (Weglicki et al., 1972). Acid active myocardial phospholipases A1 and A2, presumably of lysosomal origin, have been characterized (Franson et al., 1972), and a calcium-dependent neutral active phospholipase A2 from rabbit heart has been partially purified and characterized (Franson et al., 1983). The myocytic localization of this enzyme was demonstrated by identification of phospholipase activity in myocytes from chick embryos (Franson et al., 1983). Myocardial phospholipase C activity has been recently demonstrated in rat myocardial homogenates (Hosteller and Hall, 1980). A novel phospholipase D in rat cytosol has been demonstrated, also, but it is active only with N-acyl phosphatidylethanolamine as substrate (Schmid et al., 1983). Myocardial lysophospholipases, enzymes that catalyze lysophospholipids, were demonstrated indirectly in early studies of phospholipase activity by identification of the reaction product, glycerophosphorylcholine (Weglicki et al., 1972). More recently, three distinct lysophospholipases have been delineated in homogenates of rabbit myocardium. They include microsomal lysophospholipase (Gross and
Sobel, 1982), cytosolic lysophospholipase (Gross and Sobel, 1983), and cytosolic lysophospholipased-transacylase (Gross et al., 1983). Lysophospholipase-transacylase catalyzes the disproportionation of two molecules of lysophosphatidylcholine leading to formation of glycerophosphocholine plus fatty acid or phosphatidylcholine, depending upon the concentration of substrate present (Gross et al., 1983).

Unusual Phospholipids

Myocardial infarction, for 24 hours, in dogs, is associated with the accumulation of long chain N-acylethanolamine phosphoglycerides, which comprise 3.6 to 6% of total lipid phosphorous in the infarct, but not in the normal region (Epps et al., 1979, 1980). The appearance of N-acylethanolamine correlates with a decrease in ethanolamine glycerophospholipids (Epps et al., 1980), suggesting either enzymatic N-acylation of the ethanolamine glycerophospholipids, or transacylation (Matsumoto and Miwa, 1973). Calcium permeability in liver and heart mitochondria is attenuated by high concentrations N-acylthanolamines (Epps et al., 1982b). At low concentrations, N-acylthanolamine derivatives stimulate Ca**, Mg**-ATPase activity in sarcoplasmic reticulum vesicles, potentially underlying a positive inotropic effect in the heart (Epps et al., 1982a). However, little is known regarding the time course of accumulation of N-acylthanolamines after the onset of myocardial ischemia, the effects of their accumulation on membrane function, or their possible role in arrhythmogenesis.

Free Fatty Acids and Membrane Dysfunction

Regardless of the specific amphiphile being considered, it is difficult to define the operative concentration after a net change in total tissue content. The reasons for this include the lack of definition of subcellular distribution and effects of intra- and extracellular protein binding. Thus, extrapolation of effects of changes in total tissue concentrations associated with pathological conditions from effects of exogenous amphiphile in test conditions in vitro can be misleading. Studies are needed to determine the specific subcellular loci of amphiphiles in ischemic tissue with respect to total membrane phospholipid content. Subsequently, amounts incorporated into selected subcellular loci with respect to total membrane phospholipid in test systems should be determined after exposure of such systems to exogenous amphiphiles. With data from such studies, more meaningful comparisons can be drawn between amounts of amphiphiles in ischemic hearts and amounts required to alter function at specific subcellular sites in vitro.

Electrophysiological Effects of Fatty Acids

Oliver and coworkers suggested many years ago that elevated circulating FFA contribute to arrhythmogenesis in patients with myocardial ischemia and infarction (Oliver et al., 1968). Although several findings support such an association (Gupta et al., 1969; Kurien et al., 1971; Reimann and Schwandt, 1971; Prakash et al., 1972) others have failed to demonstrate close correlations between arrhythmogenesis and absolute levels of circulating FFA (Ravens and Jipp, 1972). Thus, direct cause and effect relationships have not been established unequivocally. Although circulating catecholamines increase within 30 minutes of the onset of symptoms due to ischemia (Vetter et al., 1974), accompanied by lipolysis, plasma fatty acids do not generally increase above 1200 mEq/liter until 1–2 hours later (Vetter et al., 1974). Although increases in plasma FFA will increase fatty acid uptake into myocytes, increases in intracellular fatty acids can occur independently, without an increase in plasma FFA. Thus, changes in fatty acids and their derivatives in myocardium may vary independent of plasma FFA. Intramyocardial lipolysis may lead to increased intramyocardial FFA in ischemic tissue, especially because elevated plasma catecholamines often associated with ischemia mediate intramyocardial lipolysis (Simonsen and Kjekshus, 1978; Vik-Mo et al., 1981) as well as augmenting myocardial extraction of FFA from the blood (Vik-Mo et al., 1979). Concentrations of nonesterified fatty acids, particularly arachidonic and linoleic, increase in ischemic zones of dog hearts despite decreased net uptake by the ischemic tissue, suggesting release from turnover of endogenous phospholipids as well as triglycerides (van der Vasse et al., 1982). Although ischemia stimulates myocardial lipolysis, as reflected by release of glycerol from ischemic zones (Vik-Mo et al., 1979), the increased glycerol may be due in part to inhibition of glycolysis. Thus, with hypoxia, intracellular FFA may increase because of both impaired oxidation and enhanced lipolysis.

It has been suggested that FFA contributes to depressed contractility, inhibition of several sarcoplasmic reticular, and mitochondrial enzymes, and electrophysiological derangements in ischemic hearts. Since FFA bind avidly to plasma proteins, primarily albumin, in a molar ratio of approximately 1:1 in normal plasma, uptake of FFA into the myocyte and the effects elicited in test systems are critically dependent on the ratio of FFA to protein. In addition, the intracellular fatty acid-binding protein plays a critical role in fatty acid uptake due to high affinity binding and the fact that only unbound FFA would be translocated across the sarcolemma. In general, the threshold value of increased FFA for induction of mechanical dysfunction (molar ratio = 4.5:1) is lower than the threshold required for induction of electrophysiological alterations (7:1), although species-dependent differences may exist (Opie and Lubbe, 1975). In guinea pig heart, a mixture of as little as 1:1 palmitate to albumin elicits electrophysiological derangements.
Including ventricular fibrillation (Opie and Lubbe, 1975), in contrast to the case in rat heart in which fibrillation does not occur despite exposure to mixtures with molar ratios as high as 12:1 (Evans et al., 1963). It is essential in the assessment of the effects of any amphiphile, including FFA, fatty acid esters, and phospholipid products, that the extent of protein binding be considered, because the degree of protein binding markedly influences the free concentration available.

The role of FFA in arrhythmogenesis has been supported by observations with nicotinic acid and sodium salicylate. These moieties depress free fatty acid extraction and inhibit myocardial lipolysis. Nicotinic acid inhibits, at least to some extent, changes in conduction and refractory periods otherwise induced early after the onset of ischemia in the canine heart (Russell and Oliver, in press). Interventions that suppress myocardial fatty acid uptake, such as a 2-fold augmentation of plasma glucose, improve conduction and blunt disparities in refractory periods with mild but not severe ischemia (Russell and Oliver, in press). The decrease in action potential duration in ischemic myocardium induced by FFA can be prevented by high concentrations of glucose (Russell et al., 1978a). Some reports indicate that infusion of neutral lipid and heparin in dogs with myocardial ischemia exacerbates arrhythmia (Kurien et al., 1969, 1971), but results have been inconsistent (Opie et al., 1971). In patients, anti-polytic agents decrease ST alterations induced by exercise or ischemia (Luxton et al., 1976; Russell and Oliver, 1978b) with a concomitant reduction of plasma FFA (Russell and Oliver, 1978b). Antiarrhythmic effects are particularly marked when the induced fall in plasma free fatty acids is rapid (Rowe et al., 1975). A marked decrease in the incidence of ventricular tachycardia (i.e., 50%) in patients with myocardial infarction results from infusion of glucose-insulin-potassium, but only in those patients who manifest marked reduction of plasma FFA (Rogers et al., 1976). Specific, circulating fatty acids may be particularly arrhythmogenic. For example, elevations of circulating linoleic acid appear to correlate well with arrhythmogenesis (Ravens and Jipp, 1972).

Despite these associations, no differences in the frequency of arrhythmias or incidence of ventricular fibrillation were observed in dogs with ischemia, despite marked increases in the circulating FFA:albumin ratio to 13:1 (Opie et al., 1971); no change in ventricular fibrillation threshold was elicited by elevated FFA in dogs (Kostis et al., 1973); and no increase in ventricular arrhythmia in pigs with coronary occlusion resulted from augmentation of circulating FFA (Most et al., 1976). Furthermore, marked increases of plasma oleic acid did not alter the incidence of ventricular fibrillation associated with ischemia (Riemersma et al., in press). In our studies (Corr et al., 1979), as well as in those by others (Cowan and Vaughan Williams, 1977), elevation of FFA by 7-fold failed to alter significantly the transmembrane action potentials in isolated ventricular muscle, Purkinje fibers, or isolated hearts when glucose was available as substrate. In contrast, with moderate hypoxia or ischemia in the isolated heart, palmitate exacerbated the shortening of action potential duration (Cowan and Vaughan Williams, 1977).

Conflicting conclusions regarding FFA and arrhythmogenesis after experimentally induced myocardial ischemia may be related to a number of factors including: (1) diversity of tissue preparations used (Opie et al., 1979), (2) variation in the severity of the ischemic insult, (3) direct membrane-depressant effects, independent of effects on lipolysis of anti-lipolytic agents such as nicotinic acid and β-Pyridyl carbinol (Opie and Lubbe, 1975), (4) species differences, (5) alterations in the molecular structure and the affinity of the intracellular binding protein thereby influencing the intracellular concentration of unbound fatty acid (Fournier et al., 1983), and (6) possible dependence of observed effects on secondary metabolites including acyl CoA and acyl carnitine (see below). The accumulation of such metabolites is, of course, influenced by numerous factors besides the absolute concentrations of circulating free fatty acid, including availability of glucose and the prevailing level of neurohumoral stimulation of the heart.

**Non-Electrophysiological Effects of Fatty Acids**

FFA increases myocardial mitochondrial O2 consumption of effects on cardiac performance (Challoner and Steinberg, 1966; Mjos, 1971). Although high concentrations of free fatty acids can lead to formation of micelles which can exert detergent effects on membranes (Pande and Mead, 1968), the micelles can also act as a pool releasing free monomers of fatty acid into the membrane, thereby perturbing membrane function. Under physiological conditions, FFA in myocardium comprise less than 0.1% of total cellular fatty acid (van der Vusse, 1982). Most long chain fatty acid is present in an esterified form in phospholipids (86%) or neutral lipids (13%), primarily triglycerides. Thus, mean concentrations of FFA are as low as 23 μM, with most bound to intracellular protein. Interstitial fluid FFA is approximately 40 μM, and intracellular fluid values are as low as 10 μM (van der Vusse, 1982). Despite the fact that most fatty acid in tissue is bound to specific proteins (Mishkin et al., 1972, 1975; Ockner et al., 1972; Fournier et al., 1978, 1983), in severely ischemic myocardium, FFA values can increase 6- to 7-fold (van der Vusse, 1982). However, the proximity of the increased FFA to cellular membranes and enzyme systems and the extent of protein binding in ischemic tissue have not yet been elucidated definitively.

Increased endogenous FFA may exert several del-
Fatty Acid Esters and Membrane Dysfunction

Under physiological conditions, O₂ availability is sufficient for oxidation of the reduced adenine nucleotides (NADH and FADH₂) formed during the course of β-oxidation of FFA. However, when oxygen is limited, FADH₂ and NADH accumulate, inhibiting β-oxidation and diminishing the rate of formation of acetyl CoA. Mitochondrial β-oxidation of FFA involves at least four enzymatic steps (Stanley and Tubbs, 1975). Ischemia results in an imbalance among them. Oxidation of β-hydroxy acyl CoA is inhibited due primarily to an increase in the NADH:NAD⁺ ratio (Moore et al., 1980). Accordingly, acyl CoA and acyl carnitine can increase in the ischemic heart, although in some experimental preparations changes in long-chain acyl carnitine have not been detected. This lack of an increase in long-chain acyl carnitine with ischemia may be secondary to inhibition of acyl transferase (McMillin et al., 1973), possibly due to the increase in acyl CoA which competitively inhibits this enzyme (Bremer and Norum, 1967). Thus, increases in acyl CoA may inhibit the formation of acyl carnitine and limit its accumulation in the ischemic heart. Increases of long-chain acyl CoA (Shug et al., 1978; Hochachka et al., 1977; Whitmer et al., 1978; Idell-Wenger et al., 1978; Liedtke et al., 1978) and acyl carnitine (Hochachka et al., 1977; Whitmer et al., 1978; Idell-Wenger et al., 1978; Liedtke et al., 1978) with ischemia have been observed in isolated hearts and experimental animals in vivo. The increase of acyl CoA occurs almost exclusively in the mitochondrial fraction, whereas long-chain acyl carnitine accumulates predominantly in the cytosol (89% of the total in control and 73% in ischemic tissue). The small increase of long-chain acyl carnitine in mitochondria in ischemic tissue and the decreased percentage of total long-chain carnitine in the cytosol appear to reflect a small reverse flux of the amphi-ple into mitochondria (Idell-Wenger et al., 1978). Thus, accumulating cytosolic acyl carnitine is likely to have access to sarcolemma in ischemic tissue (Idell-Wenger et al., 1978; Liedtke et al., 1978).

Since mitochondrial β-oxidation appears to involve four enzymatic steps (Stanley and Tubbs, 1975), involving the formation of α,β-unsaturated acyl CoA, β-hydroxy acyl CoA, and β-keto acyl CoA, these intermediates may accumulate in the ischemic heart. This was initially suggested by findings obtained in isolated mitochondria wherein increases in the NADH:NAD⁺ ratio or inhibition by rotenone increased accumulation of β-hydroxy palmitate (Bremer and Wojtczak, 1972; Hull et al., 1975). More recent studies by Hull and coworkers indicate that, in ischemic isolated perfused rabbit hearts, β-hydroxy palmitate and stearate accumulate (Hull et al., 1976; Moore et al., 1980, 1982). Small increases in β-hydroxy palmitate are found in effluents from ischemic isolated perfused hearts (Moore et al., 1980). The accumulation of β-hydroxy palmitate and stearate with ischemia is seen within 2 minutes. It occurs first in the mitochondrial fraction, followed by progressive increases in the cytosolic fraction during the first 10 minutes. Nearly 60% is in the form of β-hydroxy palmitoyl carnitine in the cytosol, with only 15% as the CoA ester (Moore et al., 1982). Although this represents only 8% of the total acyl carnitine, the results suggest that rapid inhibition of β-oxidation can lead to accumulation of not only precursors (i.e., acyl CoA), but also intermediates of β-oxidation. It appears that these intermediates can react with carnitine acyl transferase as well as carnitine translocase for exchange out of the mitochondria to the cytosol (Moore et al., 1982). This possibility is supported by observations indicating that β-hydroxy palmitoyl carnitine is an avid substrate for carnitine acyl transferase (Al-Arif and Blecher, 1971). Although it is not clear whether the α,β-unsaturated or β-keto fatty acid intermediates accumulate in the ischemic heart, the unsaturated intermediates may induce profound effects on membranes and associated enzyme systems, since the degree of saturation of acyl CoA appears to markedly influence enzyme inhibition (Owens et al., 1982).

Liedtke et al. (1978) have reported that concentrations of long-chain acyl carnitine increase 18-fold in ischemic tissue in vivo to approximately 75 μM when coronary perfusion is reduced to 57% of control flow. The metabolic response is accentuated when circulating FFA levels are elevated by pretreatment of animals with neutral lipid and heparin, and is associated, under these conditions, with deterioration of ventricular performance (Liedtke et al., 1978). Thus, total interruption of coronary flow precluding...
delivery of FFA attenuates the increase of either acyl CoA or acyl carnitine (Idell-Wenger et al., 1978).

Both long-chain acyl CoA and acyl carnitine are amphiphatic metabolites with hydrophobic and hydrophilic constituents capable of interacting with and disrupting membranes. Accumulating acyl carnitines appear to inhibit carnitine-acyl carnitine translocase, and might thereby decrease the movement of acyl carnitine into the mitochondrial matrix and deprive exchange for free carnitine to the outer mitochondrial membrane (Wołkowicz et al., 1982). Long-chain acyl CoA can inhibit several enzyme systems non-competitively (Morel et al., 1974) and mitochondrial oxidative phosphorylation competitively (Pande and Blanchaer, 1971; McMillin-Wood et al., 1977). The mechanism appears to involve inhibition of transport of adenine nucleotides into the mitochondria, thereby compromising mitochondrial production of ATP. Adenine nucleotide translocase, which exchanges intramitochondrial ATP for cytosolic ADP, is inhibited by acyl CoA (Shrago, 1976; LaNoue and Schoolwerth, 1979). The apparent inhibitory constant ($K_i$) of 0.3 $\mu$M is compatible with regulation of this critical step in energy production in vivo (Ho and Pande, 1974; Shug et al., 1975; Shrago, 1976; Vignais, 1976; Shrago et al., 1976; Woldegiorgis and Shrago, 1979; Shrago and Woldegiorgis, 1981; Woldegiorgis et al., 1981, 1982). Acyl CoA can inhibit translocase on both sides of the inner mitochondrial membrane (Woldegiorgis and Shrago, 1979; Shrago and Woldegiorgis, 1981), apparently through interaction with two different receptors (Woldegiorgis et al., 1982) and can inhibit purified translocase incorporated into liposomes, regardless of its orientation in the liposome (Woldegiorgis et al., 1981). Only those acyl CoA esters which are carnitine dependent for mitochondrial membrane transport, such as long- rather than short-chain esters, inhibit the translocase (Woldegiorgis et al., 1981). Long-chain acyl carnitine does not inhibit adenine nucleotide translocase (Shrago et al., 1976). Others have reported divergent findings with acyl CoA within the matrix of the mitochondria appearing not to alter adenine nucleotide translocase activity (LaNoue et al., 1981). Since the increase in acyl CoA with ischemia is confined largely to the mitochondrial compartment because of the lack of permeation of this amphiphile across the mitochondrial membrane, its influence on adenine nucleotide translocase remains controversial. However, mitochondrial membrane function may be altered within minutes of ischemia in vivo (McMillin-Wood et al., 1979). Accumulating acyl CoA may have access to the outer mitochondrial membrane because of early changes in permeability, and might thereby inhibit adenine nucleotide translocase. This possibility is consistent with the ability of acyl CoA to increase mitochondrial membrane permeability at higher concentrations (Wołtczak, 1974).

Long-chain acyl CoA can inhibit Na+,K+-ATPase (McMillin-Wood et al., 1977a). However, the effect is dependent not only on overall concentration but also on the particular fatty acid esterified. Na+,K+-ATPase of an enriched sarcolemmal fraction was activated by arachidonyl CoA (100-250 $\mu$M) (Owens et al., 1982), an effect blocked by propranolol (Kramer et al., 1983). In contrast, palmitoyl CoA inhibited Na+,K+-ATPase only in concentrations above 100 $\mu$M (Owens et al., 1982). Since most acyl CoA in ischemic tissue is in the mitochondrial compartment, it appears unlikely that the increased acyl CoA would have access to sarcolemmal Na+,K+-ATPase. In contrast, increased long-chain acyl carnitine is primarily in the cytosolic compartment, and has access to sarcolemma. Presently, little is known regarding the subcellular localization of these amphiphiles in the ischemic heart under conditions proven to avoid intrapreparative conversion and exchange between subcellular sites.

Palmitoyl carnitine alters activity of the calcium pump of the sarcoplasmic reticulum (Pitts et al., 1978), enhances release of calcium from sarcoplasmic reticular vesicles (Pitts et al., 1978; Messineo et al., 1982; Lamers et al., in press), inhibits Na+, Ca++-antiporter activity in sarcolemmal vesicles (Lamers et al., 1983), and inhibits sarcolemmal Na+,K+-ATPase (McMillin-Wood et al., 1977a; Lamers and Hulsmann, 1977; Adams et al., 1979a, 1979b). Biochemical effects of free fatty acids, acyl carnitines, and related compounds may be markedly dependent on chain length or degree of saturation of the acyl group, judging from the striking differences in effects on Na+,K+-ATPase activity induced by arachidonyl and palmitoyl CoA (Owens et al., 1982). Low concentrations of palmitoyl carnitine can stimulate Ca++-ATPase and binding of calcium by the sarcoplasmic reticulum (Adams et al., 1979b). Higher concentrations can inhibit the enzyme and inhibit binding of calcium, as well (Pitts et al., 1978; Adams et al., 1979b). Palmitoyl carnitine may increase contractile force by increasing membrane permeability to calcium (Inoue and Pappano, 1983) and may contribute to the K+ loss and intracellular Na+ accumulation with ischemia (Osumo-Vargas et al., 1981) by inhibiting Na+,K+-ATPase. Recent studies with purified sarclemma suggest that palmitoyl carnitine, even at high concentrations (400 $\mu$M), does not alter Na+,K+-ATPase activity with or without concomitant reduction of pH (Owens et al., 1982). This observation might be explained by the fact that the sarcolemma preparation used by Owens and colleagues was primarily right-sided out vesicles with low initial Na+,K+-ATPase activity. Addition of palmitoyl carnitine, through its detergent action, may allow expression of latent Na+,K+-ATPase activity and, in a sense, both exposure and inhibition would occur with no net change in enzymatic activity. Thus, it is likely that acyl carnitines inhibit Na+,K+-ATPase in vivo.
Although increased acyl carnitine and CoA could contribute to cellular injury in the ischemic heart, their role might not be obligatory. Such an interpretation is consistent with findings in rat hearts incubated at zero flow at 37°C. Autolysis and cellular damage occurred without any change in either fatty acid ester observed over the 60-minute incubation period (Neely et al., 1979). Thus, accumulation of fatty acid esters does not appear to be a sine qua non for irreversible damage.

Electrophysiological Effects of Fatty Acid Esters

At concentrations comparable with those found in ischemic tissue (Liedtke et al., 1978), palmitoyl carnitine superfused over isolated normoxic canine Purkinje fibers induces concentration-dependent decreases in maximum diastolic potential, amplitude, V_max of phase 0, and action potential duration—effects that are completely reversible by superfusion with medium devoid of palmitoyl carnitine (Corr et al., 1981). Catabolites of palmitoyl carnitine, including palmitic acid and carnitine, fail to induce significant electrophysiological changes (Corr et al., 1981). As stated previously, with ischemia in vivo, the absolute concentrations of long-chain acyl carnitines and their specific subcellular loci have not yet been well delineated. Furthermore, influences of intracellular protein binding have, for the most part, not been elucidated. Useful insights could be gained from assessment of the intracellular loci of the accumulated fatty acid ester with respect to amounts incorporated in each in terms of subcellular membrane phospholipid content. Subsequent studies in vitro could then be interpreted in terms of comparable amounts of exogenous fatty acid ester incorporated. Palmitoyl carnitine can induce a decrease in excitability, postrepolarization refractoriness, and electrical alternans, phenomena, which are all characteristic of ischemic tissue in vivo (Downar et al., 1977; Russell et al., 1977). These electrophysiological effects are seen with 3-fold lower concentrations of palmitoyl carnitine in the presence of reduced pH (Corr et al., 1981) comparable to that seen in ischemic tissue in vivo (e.g., 6.7). Palmitoyl carnitine decreases the rate of rise of phase 0 depolarization in chick ventricular muscle and increases the duration of the action potential without changing diastolic membrane potential (Inoue and Pappano, 1983). In tissue depolarized to −60 mV, palmitoyl carnitine increases the rate of phase 0 depolarization, presumably because of an increase in calcium influx (Inoue and Pappano, 1983). The magnitude of the electrophysiological effects elicited by palmitoyl carnitine depends not only on the type of tissue (Purkinje tissue or ventricular muscle), but possibly also on the species of origin (Corr et al., 1981; Inoue and Pappano, 1983). Although, with prolonged ischemia, some palmitoyl carnitine may leak from the cytosol to the extracellular space (Shug et al., 1978), most is probably intracellular early after the onset of ischemia at the time when electrophysiological changes are first prominent. It is likely that gating mechanisms for the currents responsible for depolarization and repolarization differ on the two sides of the membrane. Thus, effects induced by intracellular compared with extracellular palmitoyl carnitine may differ with respect to arrhythmogenesis early after the onset of ischemia. However, the issue is complex, since fatty acid carnitine esters may readily exchange from one side of the membrane bilayer to the other (Levitsky and Skulachev, 1972).

Long-chain acyl carnitines circulate in plasma. Their concentrations increase more than 2-fold to 29 µM after 36 hours of fasting, despite increased renal clearance (Frohlich et al., 1978). It is possible that alterations in the concentration of acyl carnitine in the circulation may contribute to electrophysiological derangements under some conditions, particularly when changes in regional myocardial pH are pronounced. A concentration of 29 µM can induce electrophysiological alterations in vitro in the presence of reduced pH (Corr et al., 1981).

Protective Influences of Free Carnitine

Although free carnitine is not synthesized by myocardium, levels in rat heart are high (1–4 mM) with respect to those in plasma (approximately 50 µM) (Idell-Wenger et al., 1978; Vary et al., 1981). Transport of carnitine into the heart utilizes both a carrier component which is saturable (Bohmer et al., 1977; Mølstad et al., 1977) as well as noncarrier, diffusion process which is not saturable (Vary and Neely, 1982). The transport appears to be energy independent and little affected by anoxia (Vary and Neely, 1982). Several findings suggest that increased free carnitine may benefit the ischemic heart. Adverse effects of free fatty acids may be attenuated (Oram et al., 1975; Shug et al., 1975; Shrago et al., 1976; Folts et al., 1978; Liedtke and Nellis, 1979), effects of acyl CoA on adenine nucleotide translocation blunted (Shrager et al., 1976; Shug et al., 1978), and antiarrhythmic effects evident (Folts et al., 1978), possibly mediated by a reduction of amphiopathic effects of nonesterified FFA on the cell membrane (Liedtke and Nellis, 1979). The protective effects of free carnitine in ischemic hearts may be secondary to reduction of acyl CoA (Neely et al., 1979), although conflicting results have been reported (Opie, 1979). L-Carnitine inhibits accumulation of triglycerides in isolated, normoxic rat hearts exposed to neutral lipid (Hulsmann et al., 1982) independent of altered fatty acid oxidation but associated with increased synthesis of acyl carnitine (Hulsmann et al., 1982).

Phospho- and Lysophospholipids and Membrane Dysfunction

Degradation of myocardial phospholipids with prolonged ischemia has been implicated in mem-
brane dysfunction and cell death in liver (Chien et al., 1978) and in the heart subjected to ischemia (Sobel et al., 1978; Chien et al., 1981). After 3 hours of myocardial ischemia in dogs, mitochondrial phospholipids decrease, although lysosomal phospholipid levels expressed with respect to lysosomal protein increased (Vasdev et al., 1979), probably because of net loss of lysosomal protein. Thus, irreversible damage appears to be accompanied by depletion of cellular phospholipids secondary to activation of phospholipases. In contrast, ischemia in vitro results in irreversible membrane damage before gross degradation of phospholipid occurs (Steenbergen and Jennings, in press). However, delivery of substrate via collateral flow, coupled with neural and hormonal influences known to stimulate phospholipases, may accelerate phospholipid degradation in vivo.

Even modest degradation of phospholipids may be deleterious if catabolites are pathogenetically active. One catabolite, lysophosphatidicholine, is increased 2-fold in mitochondria isolated from liver subjected to 2 hours of ischemia (Boime et al., 1970). In the heart, lysophospholipids increase within the first few minutes of ischemia (Snyder et al., 1981; Shaikh and Downar, 1981; Corr et al., 1982), as do they in the ischemic kidney (Matthys et al., 1982). Relative increases of lysophospholipids in lysosomes after prolonged ischemia (Vasdev et al., 1979) may not reflect actual increases in tissue content because of concomitant loss of lysosomal protein (Wildenthal et al., 1978). Although overall tissue content of lysosphospholipid is elevated after ischemia of 5 hours in vitro, the increase is not likely to be a cause of the irreversible injury (Steenbergen and Jennings, in press).

Ischemia results in rapid, nonselctive release of fatty acids from myocardial phospholipids (Hsueh et al., 1977), potentially leading to accumulation of lysophospholipids, particularly if reacylation or catabolism is inhibited. Studies from our laboratory showed that lysophospholipids increased in myocardium rendered ischemic for as little as 5 minutes, with values exceeding control by more than 60% (Sobel et al., 1978). Substantial concentrations were found in normal tissue as well (Sobel et al., 1978). In these studies, tissues were extracted with acidified media because others had shown that recovery of lysophospholipid was enhanced under these conditions (Bierve et al., 1974). Subsequently, with the use of 31P-NMR, we found that acidified extraction media led to substantial intrapreparative conversion of plasmalogens to lysophospholipids (Mogelson et al., 1980), resulting in overestimation of endogenous levels in both normal and ischemic myocardium. Because plasmalogens comprise approximately 40% of myocardial phospholipid, artifact due to this phenomenon is likely to be particularly serious in studies of cardiac muscle compared with those of other tissues, such as liver. Accordingly, we reevaluated concentrations of lysophospholipids in normal and ischemic myocardium under conditions in which acidified media were avoided (Corr et al., 1982). Comparable percentage increases were observed in our laboratory in cats (Corr et al., 1982) and by others in studies of ischemic porcine myocardium extracted with nonacidified extraction media (Shaikh and Downar, 1981). In tissue from ischemic cat heart extracted with chloroform:methanol and assayed after separation of phospholipids by isocratic high performance liquid chromatography (Corr et al., 1982), lysophospholipids increased by 53% compared with values in control tissue within 10 minutes after the onset of ischemia (the sum of lysophosphatidylcholine plus lysophosphatidylethanolamine from 4.9 ± 0.27 to 7.5 ± 0.33 nmol/mg protein, P < 0.001). Thus, lysophospholipids increase in myocardium rendered ischemic within a few minutes after the onset of the insult.

Two recent studies (Shaikh and Downar, 1981; Steenbergen and Jennings, in press) reported values of lysophosphatidylcholine in control tissue much lower than those reported by many others (Gloster et al., 1969, 1970; Goshima, 1971; Singh and Swartwout, 1972; Chauhan and Singh, 1978; Broekman et al., 1980; Corr et al., 1982) who found lysophospholipid to comprise consistently between 1 and 3% of total phospholipid phosphorus. The very low levels of lysophospholipids reported by Shaikh and Downar (1981) may be related to differential recovery of internal standard and endogenous lysosphospholipid. It is likely that some lysophospholipids in the tissue are less stable than l-heptadecanly-sn-glycero-3-phosphocholine, the standard used by these workers. Of possibly even more importance, 2-deacetyl lysolipids derived from plasmalogens in vivo (lysoplasmalogens) would not be detected by the analysis used (Shaikh and Downar, 1981). Plasmalogen-derived lysophospholipids are likely to be prominent in myocardium, as they are in platelets (Broekman et al., 1980), particularly since plasmalogens comprise approximately 40% of myocardial sarcolemmal phospholipids (Gross, 1984). An alternative explanation for the relatively low levels observed by Shaikh and Downar may be species' differences between the pig and cat (Shaikh and Downar, 1981; Corr et al., 1982).

**Mechanisms Potentially Responsible for Accumulation of Lysophospholipids**

Myocardial lysophospholipids may accumulate as a result of (Fig. 3): increased production mediated by phospholipase A1 or A2, decreased catabolism mediated by attenuation of lysophospholipase activity, increased production due to reversal of the disproportionation reaction catalyzed by lysophospholipase-transacylase, or combinations of these phenomena. The liberation of unsaturated free fatty acid accompanying ischemia probably reflects increased phospholipase A2 activity (Hsueh et al.,...
Corr et al. / Metabolites in Ischemic Myocardium

1977; van der Vusse et al., 1982). Accumulation of intracellular calcium and increased free calcium resulting from displacement of bound calcium by increased concentrations of hydrogen ion are likely to stimulate phospholipase $A_2$ and $A_2$ activity in ischemic regions. Intense local adrenergic stimulation in such regions may augment the activity of phospholipase $A_2$ (Franson et al., 1979). On the other hand, assay of phospholipase activity in homogenates and subcellular fractions isolated from ischemic and control tissue have not demonstrated consistent alterations in phospholipase activity (Gross and Sobel, 1979), suggesting that observed alterations of activity in situ may reflect effects of the altered intracellular milieu.

Rabbit myocardium has abundant lysophospholipase activity. The activity exceeds phospholipase $A_2$ activity in vitro by 16-fold (Gross and Sobel, 1982). Attenuation of lysophospholipase activity may therefore be a critical determinant of accumulation of lysophospholipids in ischemic tissue. Because of the structural similarity between long-chain acyl carnitines and lysophospholipids, we considered the possibility that long-chain acyl carnitines competitively inhibit cytosolic lysophospholipase. Myocardial cytosolic lysophospholipase was purified to homogeneity. Three metabolites that accumulate with ischemia, long-chain acyl carnitine, free fatty acid, and acyl CoA, inhibited lysophospholipase activity (Gross and Sobel, 1983; Gross, 1983). Another lysophospholipid catabolic enzyme, lysophospholipase-transacylase, was purified to homogeneity and was inhibited by low concentrations of palmitoyl carnitine (Gross et al., 1983). Furthermore, membrane-bound lysophospholipase activity was attenuated markedly at pH 6 to 6.5 (Gross and Sobel, 1982). Thus, several metabolic determinants may result in inhibition of catabolism of lysophospholipids in ischemic tissue with consequent accumulation of these amphipathic moieties.

**Electrophysiological Effects of Phospho- and Lysophospholipids**

Since the mean sum of the two most prominent myocardial lysophosphoglycerides, lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE), averages 7.5 nmol/mg protein in ischemic myocardium (Corr et al., 1982), the overall cellular concentration is in the range of 1200 nmol/ml or 1.20 mM based on an average value of 160 mg protein/g wet weight. This estimate is conservative with respect to the concentration of lysophospholipids in cellular or subcellular membranes, because amphiphiles are not distributed homogeneously throughout the cell. Thus, the local concentrations of lysophospholipids near and within the membrane may be substantially higher. As pointed out previously, the amount incorporated with respect to total membrane phospholipid content is likely to be critical (see below). In solutions containing 0.4 mM albumin at physiological pH, 1.20 mM lysophospholipid induces marked electrophysiological derangements closely resembling changes seen in ischemic tissue in vivo (Corr et al., 1979). These electrophysiological alterations are reversible with superfusion with media devoid of exogenous lysophospholipid. Even lower concentrations (10–50 µM) of protein-free exogenous lysophospholipids induce marked electrophysiological derangements in vitro (Arnsdorf and Sawicki, 1981; Corr et al., 1981; Clarkson and Ten Eick, 1983). Arnsdorf and Sawicki (1981) demonstrated increased membrane resistance, altered length and time constants, and biphasic effects on excitability due to modification of passive and active membrane properties induced by lysophosphatidylcholine. Clarkson and Ten Eick (1983) observed decreased membrane potential induced by lysophosphatidylcholine in isolated ventricular muscle, analogous to that seen in ischemic tissue secondary to a reduction of permeability to $K^+$.

Several observations support the view that lysophospholipids are likely to contribute to arrhythmogenesis because of their specific biochemical properties. Neither glycerophosphorylcholine nor free fatty, each a catabolite of lysophosphatidylcholine (LPC), induces significant alterations of the transmembrane action potential resembling those elicited by LPC (Corr et al., 1979). On the other hand, lysophosphatidylethanolamine (LPE), an amphiphile resembling LPC, induces comparable effects (Corr et al., 1979). The effects of LPC and LPE do not appear to be due to removal of sarcolemmal cholesterol, based on several lines of evidence. For example, superfusion for even brief intervals induces electrophysiological effects, the effects are reversible by superfusion with media without cholesterol, and superfusion with phosphatidylcholine-rich liposomes (which also removes membrane cholesterol) does not elicit comparable electrophysiological changes (Corr et al., 1981). In contrast to effects seen in the presence of exogenous albumin, in its absence, 10-fold lower concentrations of exogenous LPC are sufficient to induce electrophysiological effects in normoxic Purkinje fibers resembling those elicited by ischemia (Corr et al., 1981).

Effects of these amphipathic metabolites in ischemic tissue may be enhanced by concomitant metabolic derangements. Thus, reduction of pH to 6.7, comparable with that seen early after the onset of ischemia in vivo, results in a 3-fold increase in sensitivity of Purkinje fibers to the electrophysiological effects of exogenous lysophosphatidylcholine (Corr et al., 1981). The increased concentration of lysophosphatidylcholine trapped by albumin in venous effluent from ischemic but reperfused myocardium in vivo is sufficient to induce marked electrophysiological effects in vivo when pH is reduced to 6.7 (Snyder et al., 1981). In contrast, when venous effluent is evaluated only under conditions of continuous low flow, a reflection primarily of perfusion...
from normal regions, it is not surprising that lysophospholipids do not increase in the overall ventricular venular effluent (Reimersma and Michorowski, in press). Studies employing quantitative electron microscopic autoradiography indicate that electrophysiological alterations are induced in normoxic Purkinje fibers and ventricular myocardium when as little as 1% of myocardial phospholipid is replaced by exogenous lysophospholipid. This amount is at the lower limit of detection of augmented concentrations of lysophospholipids in ischemic myocardium (Corr et al., 1982, and Fig. 4).

Membrane effects of low concentrations of lysophospholipids have been demonstrated in several systems including: alterations of erythrocyte potassium content (Lawrence et al., 1974), inhibition of oxidative phosphorylation in mitochondria (Honjo et al., 1968; Boime et al., 1970), contracture of isolated perfused hearts (Hajdu et al., 1957; Bergmann et al., 1981), increased contractile force in isolated tissue (Clarkson and Ten Eick, 1983), inhibition of activity of membrane-bound enzymes such as Na⁺,K⁺-ATPase (Karli et al., 1979; Owens et al., 1982), and simulation of adenylate cyclase in broken myocardial cell preparations and isolated hearts independent of catecholamines (Ahumada et al., 1979). Accumulation of lysophospholipids may therefore augment cAMP in ischemic myocardium, a phenomenon associated with arrhythmogenesis (Corr et al., 1978; Podzuweit et al., 1978). Thus, diverse observations from several laboratories with tissue preparations from several species support the view that the increased concentrations of endogenous lysophospholipids observed in ischemic myocardium are sufficient to elicit membrane dysfunction. The concentrations observed in vivo early after the onset of ischemia exceed those required to induce comparable membrane dysfunction with exogenous lysophospholipids. In addition, their electrophysiological effects may be exacerbated by con-

![Figure 4](http://circres.ahajournals.org/content/55/1/146/fig/4)

**Figure 4.** Effect of reduction in pH on electrophysiological parameters (left) and incorporation and metabolism of [14C]lysophosphatidylcholine ([14C]LPC-100 μM) (right) in canine ventricular muscle strips (n = 10). For electrophysiological parameters, results are given for control, treatment of 10 minutes, and recovery at normal (○) and reduced (□) pH, and * indicated a significant (P < 0.01) difference from control at each pH. Simultaneous measurements of the labeled fraction are given at the end of the treatment interval and at recovery for lysophosphatidylcholine (LPC), phosphatidyl choline (PC), and free fatty acid (FFA); * = P < 0.05, ** = P < 0.01, *** = P < 0.001 significance level for recovery vs. treated for each of the biochemical indexes. Values are means ± SEM. Reproduced from Gross et al., 1982.
comitants of ischemia such as accumulation of hydrogen ion (Corr et al., 1981; Snyder et al., 1981). Although the site of production of lysophospholipids in ischemic tissue is not yet clear, increased trapping of material in effluents from ischemic zones suggests that much of the endogenously generated lysophospholipid may be extracellular (Snyder et al., 1981) with resultant direct access to the external surface of the sarcolemma. Accumulation in this locus may reflect catecholamine stimulation of phospholipase A$_2$ (Stam and Hulsmann, 1981). Results of electron microscopic autoradiography with [$^{14}$C]- and [$^3$H]lysophosphatidylcholine indicate that selective sarcolemmal incorporation comprising less than 2% of sarcolemmal phospholipid (as opposed to 1% of overall tissue phospholipid) is sufficient to induce marked electrophysiologic derangements (Saffitz et al., 1984). Using [$^{14}$C]LPC in isolated canine ventricular muscle, we have recently demonstrated that incorporation of approximately 0.4 nmol/mg protein of additional LPC was associated with electrophysiologic alterations. The change represented less than 1% of cellular phospholipid, judging from measured values of 160 nmol/mg protein (Fig. 4). Metabolism of 50% of this exogenous labeled LPC to PC and FFA was associated with reversal of the electrophysiologic alterations (Gross et al., 1982, and Fig. 4). Thus, the “threshold” value for induction of the electrophysiologic effects appeared to be of the order of 0.2 nmol/mg protein. This conclusion is supported by results of experiments in which the first exposure to [$^{14}$C]LPC and subsequent recovery was followed by a second exposure. The latter required less incorporation prior to induction of electrophysiologic effects because of the persistent contributions from residual [$^{14}$C]LPC (Gross et al., 1982). Because the increase of lysophospholipids in ischemic tissue is in excess of 1 nmol/mg protein, it is likely to be sufficient to cause marked electrophysiologic derangements if accumulation occurs at or near the sarcolemma. Additional studies are required to delineate the subcellular localization manifest throughout the time course of ischemia, not only for lysophospholipids, but also for other amphiphiles such as acyl CoA and acyl carnitine.

Lysophospholipids are moieties with both hydrophobic and hydrophilic constituents. Because of their hydrophobic properties, they are incorporated readily into membranes. Thus, it is not surprising that exposure of sarcolemma to exogenous or endogenous lysophospholipid impairs membrane function. Effects of lysophospholipid do not appear to require incorporation of micelles; monomers appear to be sufficient (Weltzien, 1979; Arnsdorf and Sawicki, 1981; Bergmann et al., 1981; Clarkson and Ten Eick, 1983). Incorporation of monomers may result from increased endogenous synthesis or decreased catabolism of lysophospholipids within cell membranes. Alternatively, it may be a consequence of exposure of membranes to lysophospholipids known to be elevated in extracellular fluid with ischemia (Snyder et al., 1981).

The electrophysiologic effects of lysophospholipids are complex. Their dependence on specific ionic currents has been resolved only partially. Depression of the rapid inward current carried by Na$^+$ appears likely, since $V_{\text{max}}$ of phase 0 is reduced in association with a downward and rightward shift of the membrane response curve (Corr et al., 1979; Arnsdorf and Sawicki, 1981). Thus, although $V_{\text{max}}$ may be reduced in part as a consequence of the reduction of resting membrane potential (RMP), LPC appears to exert direct depressant effects on gNa$^+$ independent of alterations in RMP (Corr et al., 1979, 1981; Arnsdorf and Sawicki, 1981; Clarkson and Ten Eick, 1983). Low concentrations of LPC shorten the refractory period. High concentrations lengthen refractoriness while inducing postpolarization refractoriness (Corr et al., 1979). Inhomogeneity of refractoriness is a characteristic of ischemia in vivo (Russell and Oliver, 1978). It may occur because of differences in the accumulation of amphiphatic metabolites across an ischemic region. Disparity of refractoriness may be of considerable importance in the induction of unidirectional block. Together with slowed conduction inducible by amphiphiles such as LPC (Corr et al., 1979, 1981; Arnsdorf and Sawicki, 1981), it would predispose to maintenance of reentrant pathways within ischemic myocardium. The occurrence of electrical alternans in action potentials in subepicardial ischemic zones (Downar et al., 1977; Russell et al., 1977), a phenomenon that can be elicited by LPC (Corr et al., 1979), may be a reflection of regional variation of refractoriness.

Changes in excitability with ischemia in vivo include a transient increase for 1–3 minutes, followed by a marked decrease (Elharrar et al., 1977). These changes are compatible with known effects of lysophospholipids. LPC elicits a biphasic effect on excitability. An initial increase reflecting alteration of passive membrane properties is followed by a subsequent decrease of excitability because of changes in active membrane properties (Arnsdorf and Sawicki, 1981). Alterations of excitability appear to vary across ischemic regions. Such variation, as well as inhomogeneity of regional refractoriness and conduction time, may reflect inhomogeneity of accumulation of amphiphiles such as lysophospholipids.

Lysophospholipids depolarize both ventricular muscle and Purkinje fibers, inducing changes similar to those seen in ischemic tissue in vivo. The mechanism responsible appears to be a reduction of outward K$^+$ conductance in the resting cell (Clarkson and Ten Eick, 1983). LPC appears to depress the slow inward current ($I_s$) carried by calcium (Clarkson and Ten Eick, 1983). Since it decreases the rapid inward current carried by sodium, the outward current carried by potassium, the slow inward current carried by calcium, and the time-independent back-
Potential Role of Free Radicals and Lipid Peroxidation

A large body of information is available which suggests that ischemia in several organ systems may result in the production of free radicals secondary to the univalent reduction of oxygen (see Lewis and Del Maestro, 1980, for review). Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids which leads to the formation of free radical intermediates and subsequent autocatalysis (Plaa and Witschi, 1976). The potential free radicals include superoxide anion (O\(_2^-\)), hydrogen peroxide (H\(_2\)O\(_2\)), and hydroxide radical (-OH). The hydroxide radical, in particular, is known to cause a variety of alterations in cell membranes, including peroxidation of lipids and lysosomal membranes (Fong et al., 1973; Sachs et al., 1978). In the central nervous system, regional ischemia appears to result in a selective loss of polyunsaturated fatty acids from membrane phospholipids because of their susceptibility to free radical damage (Demopoulos et al., 1980). In addition, lipid peroxides may selectively inhibit the synthesis of PGI\(_2\), a vasodilator, and thereby result in platelet-induced occlusions in the microcirculation exacerbating ischemia and injury (Demopoulos et al., 1980). In mitochondria isolated from brain subjected to 30 seconds to 5 minutes of ischemia, rapid hydrolysis of up to 30% of diacyl and plasmalogen phospholipids is seen with corresponding increases in unsaturated fatty acids (Majewska et al., 1978). The phospholipid hydrolysis is associated with an increase in production of malondialdehyde (MDA) indicative of lipid free radical oxidation, which is stimulated by excess calcium and inhibited by pentobarbital (Majewska et al., 1978). Thus, ischemia may result in phospholipid hydrolysis, particularly at the sn2 carbon, with release of unsaturated fatty acids secondary to excess free radical production. Although not confirmed, this sequence would be expected to result in the production of lysophospholipids, particularly in the presence of ischemia when normal catabolic pathways mediated by lysophospholipases are inhibited (Gross et al., 1983).

With respect to the heart, several indirect lines of evidence suggest that free radical production may play an important role in membrane injury induced by ischemia, as well as by subsequent reperfusion. For example, generation of free radicals with a xanthine-xanthine oxidase system inhibits Ca\(^{2+}\) uptake by isolated sarcoplasmic reticulum even at pH = 6.4, changes analogous to that seen in ischemic tissue in vivo (Hess et al., 1981). Free radicals may be particularly important with reoxygenation of previously ischemic tissue, often manifest by exacerbation of ischemic injury (Ganote et al., 1976; Hearse et al., 1975). In the normal heart, free radicals can be generated by a number of reactions in tissue, including those associated with mitochondrial electron transfer (Bors et al., 1978; Loschen et al., 1974). Under physiological conditions, the superoxide anion generated is neutralized to H\(_2\)O by superoxide dismutase (Nohl and Hegner, 1978) and then converted to H\(_2\)O by glutathione peroxidase (Little and O'Brien, 1968), an enzyme present in relatively high levels in heart (Lawrence and Burk, 1978). Indirect evidence obtained with antioxidants used to decrease lipid peroxidation during reperfusion in isolated perfused hearts confirms an associated reduction of cellular damage assessed by morphological or enzyme analysis (Guarnieri et al., 1978; Caldarera et al., 1978). Hypoxia appears to decrease the activity of superoxide dismutase and, thereby, the capacity of the cell to convert superoxide anion to hydrogen peroxide (Liu et al., 1977). Subsequent reperfusion may activate mitochondrial electron transport in cells rendered susceptible to toxic effects, reflecting accumulation of the generated superoxide anion resulting in lipid peroxidation (Zimmerman et al., 1973). Reperfusion of hypoxic perfused rat hearts does result in an increase in malondialdehyde production associated with a decrease of superoxide dismutase and glutathione peroxidase activity (Guarnieri et al., 1980). Peroxidation of unsaturated fatty acids in membranes can result in increased membrane permeability (Hicks and Gebicki, 1978), altered transport of Ca\(^{2+}\) in sarcoplasmic reticulum (Hess et al., 1981), and depressed mitochondrial respiratory function (Nohl et al., 1978). Inclusion of superoxide dismutase plus catalase in cardioplegia solutions has been shown to enhance recovery and
improve left ventricular function after prolonged ischemia (Shlafer et al., 1982a, 1982b). Thus, several lines of indirect evidence implicate free radical generation and peroxidation of membrane lipids in the genesis of membrane injury associated with myocardial ischemia. Future studies should help to define the time course of phospholipid hydrolysis associated with lipid peroxidation, particularly with regard to the accumulation and subcellular loci of phospholipid products such as lysophospholipids.

**Potential Mechanisms Accounting for Altered Membrane Function Induced by Amphiphiles**

Free fatty acids, fatty acid esters, including acyl CoA and acyl carnitine and lysophospholipid—amphiphiles—may alter membrane function through a variety of mechanisms. At relatively low concentrations, below the critical micelle concentration (CMC), these compounds exist as monomers. In this form they can insert in the inner portion of the lipid bilayer, thereby altering the physical properties of the membrane and membrane function. Alternatively, at concentrations above the CMC, these compounds aggregate into micelles with a portion in equilibrium with monomers in a concentration up to the CMC. The micelles, can insert into the lipid bilayer and leech lipids out of the membrane via detergent effects, with ultimate destruction of the membrane. Although incorporation of monomers may stabilize the membrane because of an increase in membrane volume (see Katz and Messineo, 1981, for review), an alteration in motion within the membrane may result, and conformation of membrane proteins (enzymes, receptors or ion channel constituents) may be modified. The function of such membrane proteins may be altered substantially as a result of the change in conformation reflecting disturbances in the lipid bilayer (Baumann and Mueller, 1974; Sandermann, 1978). In addition to alterations in membrane volume and alterations in the conformation of membrane proteins, insertion of amphiphiles into membranes may displace Ca²⁺ from negatively charged sites on membrane phospholipids, because of a change in the orientation of the polar head group (Hauser and Dowson, 1968). Lysophospholipids and, possibly, structurally related compounds such as long-chain acyl carnitines can alter membrane function by "wedging" into the membrane (Weltzien, 1979). Their lytic activity is determined by the length of the hydrophobic acyl chain. It is maximal with chain lengths of 16 to 18 carbons. Membrane hemolysis appears to result from monomer insertion into the membrane, rather than effects of micelles. However, the micelles may act as a reservoir—continually providing monomer for insertion (Weltzien, 1979). The insertion of these "wedge-shaped" moieties can result in an abnormal shape or curvature of the membrane (Lucy, 1970), and thereby alter ionic movement and enzyme activity of integral membrane proteins. In addition, these moieties can decrease electrical resistance in isolated membranes (Weltzien, 1979), although in heart, incorporation of relatively low amounts of lysophospholipids increases membrane resistance (Arnsdorf and Sawicki, 1981). Thus, results from several studies suggest that amphiphiles can alter membrane function by altering the structure or conformation of the lipid bilayer or associated membrane proteins, and that they can alter binding of essential ionic constituents.

**Conclusions**

Several amphiphatic moieties have been implicated in arrhythmogenesis in ischemic myocardium. Diverse findings in many systems suggest that modest elevations of concentrations of amphiphiles at critical loci within the cell membrane or at the cell surface contribute to membrane dysfunction and electrophysiological derangements. Such derangements may be potentiated by other metabolic concomitants of ischemia, such as intracellular acidosis. Identification of the specific moieties responsible and the altered flux in specific metabolic pathways accounting for their accumulation offers particular promise as a focus for development of pharmacological interventions designed to prevent or abort otherwise lethal arrhythmias associated with ischemia.

References


Bremer J, Wojtczak AB (1972) Factors controlling the rate of fatty acid β-oxidation in rat liver mitochondria. Biochim Biophys Acta 280: 13-530


Cras MF III (1972) Exogenous substrate effects on endogenous lipid metabolism in the working rat heart. Biochim Biophys Acta 280: 71-81


Epps DE, Mandle F, Schwartz A (1982a) The alterations of rabbit skeletal sarcoplasmic reticulum function by N-acetylglutamimine, a lipid associated with myocardial infarction. Cell Calcium 3: 531-543

Epps DE, Palmer JW, Schmid HHO, Pfeiffer DR (1982b) Inhibition of permeability dependent Ca2+ release from mitochondria by N-acetylmglutamimines, a class of lipids synthesized in ischemic heart tissue. J Biol Chem 257: 1383-1391


Folts JD, Shug AL, Koke JR, Bittar N (1978) Protection of the ischemic dog myocardium with carnitine. Am J Cardiol 41: 1209-1214


Gross RW (1983) Purification of rabbit myocardial cytosolic acyl CoA hydrolase, identity with lysophospholipase, and modulation...
tion of enzymatic activity by endogenous cardiac amphibians. Biochemistry 22: 5641–5646
Hearse DJ, Humphrey SM, Naylor WG, Slade A, Border D (1975) Untrastructural damage associated with reoxygenation of the anoxic myocardium. J Mol Cell Cardiol 7: 315–324
Liedtke AJ, Nelles SH (1979) Effects of carnitine in ischemic and
Messineo FC, Pinto PB, Katz AM (1980) Palmitic acid enhances calcium sequestration by isolated sarcoplasmic reticulum. J Mol Cell Cardiol 12: 725–732
Molstad P, Bohmer T, Eklid K (1977) Specificity and character-
Shlafer M, Kane PF, Kirsh MM (1982b) Superoxide dismutase.


Vik-Mo H, Mjos OD, Riemersma RA, Oliver MF (1981) Improvement...
Wildenthal K, Decker RS, Poole R, Griffin EE, Dingle JT (1978) Sequential lysosomal alterations during cardiac ischemia. Lab Invest 38: 656–661
INDEX TERMS: Lysophosphatides • Phospholipids • Myocardial infarction • Sudden cardiac death
Amphipathic metabolites and membrane dysfunction in ischemic myocardium.
P B Corr, R W Gross and B E Sobel

doi: 10.1161/01.RES.55.2.135

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/55/2/135.citation