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Potentiation of Free Radical-induced Lipid Peroxidative Injury to Sarcolemmal Membranes by Lipid Amphiphiles*

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The effects of naturally occurring lipid amphiphiles on free radical-mediated peroxidative injury in isolated canine sarcolemma were studied. Highly enriched canine myocytic sarcolemmal membranes were preincubated for 10 min at 37 °C with or without different amphiphilic lipids before the addition of a free radical-generating system consisting of dihydroxyfumarate and Fe^{3+}-ADP. Lipid peroxidation, assayed as malondialdehyde formation, was catalyzed linearly up to 40 min in the control samples. Pretreatment of the sarcolemma with palmitoyl-CoA, palmitoylcarnitine, or lysophosphatidylcholine accelerated the initial rates (20 min) of peroxidation in a concentration-dependent manner (10–100 μM) and achieved maximal stimulation (240%, 160%, and 210%, respectively, of controls) at 50 μM concentrations of each of these amphiphiles. However, free fatty acids, CoA, and carnitine were without effect. These promoting effects of the amphiphiles persisted over a wide pH range (pH 6.0–7.8) and exhibited additive effects when lower levels of different amphiphiles were combined together. Associated with the accelerated rates of peroxidation produced by palmitoyl-CoA and palmitoylcarnitine were greater losses in the activity of sarcolemmal (Na,K)-ATPase. Since all three kinds of amphiphilic lipids accumulate during ischemia, this study suggests a novel mechanism of potentiation of sacolemmal membrane injury when free radicals are present.

Recently, endogenously generated free radicals have been implicated to play a role in the process of myocardial ischemic injury (Rao *et al.*, 1983; McCord and Roy, 1982; Meerson *et al.*, 1982; McCord 1985). Presumably, cellular and subcellular membranes are major sites of free radical attack due to the presence of polyunsaturated fatty acids in their phospholipids. In addition, altered lipid metabolism in cardiac ischemia may result in significant accumulation of long chain acyl-CoA and acylcarnitine esters (Whitmer *et al.*, 1978; Idell-Wenger *et al.*, 1978) and possibly lysophospholipids (Shaikh and Downar, 1981; Corr *et al.*, 1982). These lipid amphiphiles are capable of disturbing membrane-related functions and enzymatic activities in a number of subcellular organelles (Katz and Messineo, 1981; Corr *et al.*, 1984). However, to our knowledge, no research has been reported on the ability of these accumulated amphiphiles to enhance free radical-induced membrane damage. The sarcolemma is a critical site of free radical reactions and we have previously demonstrated (Kramer *et al.*, 1984) that purified sarcolemmal membranes from canine myocytes are highly susceptible to free radical-induced peroxidation; inactivation in the activity of the membrane-bound (Na,K)-ATPase was temporally correlated with peroxidation. In the present communication, we extent this work to examine the influence of various naturally occurring lipid amphiphiles on free radical-induced peroxidative injury in highly enriched cardiac sarcolemma.

MATERIALS AND METHODS

Chemicals—Dihydroxyfumarate (DHF[1]), ADP, $FeCl_3 \cdot 6H_2O$, 2-thiobarbituric acid, *S*-stearoyl-CoA, palmitic acid, oleic acid, coenzyme A, and L-carnitine were purchased from Sigma. Palmitoyl-CoA, L-palmitoylcarnitine chloride, and lysophosphatidylcholine (bovine) were from P-L Biochemicals.

Preparation and Incubation of Membranes—Adult canine myocytes were isolated from ventricular tissues by digestion with 0.2% collagenase and the sarcolemma was obtained by differential and then sucrose density centrifugations as described previously (Weglicki *et al.*, 1980). The sarcolemmal fractions used for these studies were enriched at least 50-fold in the specific activities of ouabain-sensitive, K^+-dependent *p*-nitrophenyl phosphatase (Skou, 1974) and of (Na,K)-ATPase over that of the myocyte homogenates (Owens *et al.*, 1982). (Na,K)-ATPase activity ($n = 10$), as determined by the spectrophotometric coupled enzyme method (Schwartz *et al.*, 1973; Kramer *et al.*, 1984), was 67.0 ± 6.1 μmol/mg of protein/h at 37 °C, pH 7.2. Protein determinations were performed according to Lowry *et al.* (Lowry *et al.*, 1951).

The effects of different lipid amphiphiles on free radical-induced lipid peroxidation in the purified sarcolemma were determined in a reaction buffer consisting of 120 mM KCl, 50 mM sucrose, and 10 mM potassium phosphate, pH 7.2. Samples with 0.075–0.100 mg of protein/ml of sarcolemma in the reaction buffer were preincubated with each lipid amphiphile at specified concentrations for 10 min at 37 °C. The peroxidation reactions were initiated by the final additions of Fe^{3+}-ADP (0.025 mM $FeCl_3$ chelated by 0.250 mM ADP) and 0.83 mM DHF. Other conditions or additional components in the incubation mixture are described in the text. The rates of lipid peroxidation were assayed as malondialdehyde formation, which was measured by the thiobarbituric acid method as described previously (Mak *et al.*, 1983).

Statistical Analysis—Statistical analyses were performed by unpaired Student's *t*-test and/or least squares linear regression analysis.

RESULTS

DHF, in the presence of Fe^{3+}-ADP, is a powerful and convenient *in vitro* free radical-generating system, which we have utilized to study free radical-induced peroxidative damages in various subcellular organelles (Kramer *et al.*, 1984; Mak *et al.*, 1983; Weglicki *et al.*, 1984). Fig. 1 displays the early time course of induced lipid peroxidation in the sarcolemmal membranes with or without (controls) added lipid

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[1] The abbreviations used are: DHF, dihydroxyfumarate; MDA, malondialdehyde; lyso-PC, lysophosphatidylcholine.

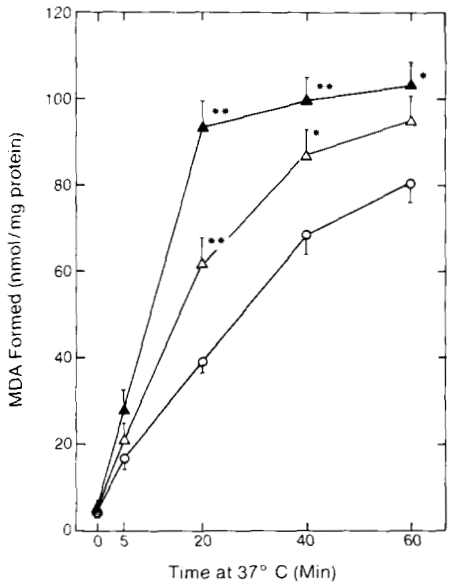

FIG. 1. **Promotion effect of palmitoyl-CoA and palmitoylcarnitine on sarcolemmal lipid peroxidation induced by DHF + Fe^{3+}-ADP.** Sarcolemmal membranes (75–100 μg of protein/ml) were preincubated at 37 °C without (O——O, control) or with either 50 μM palmitoyl-CoA (▲——▲) or 50 μM palmitoylcarnitine (△——△) for 10 min before the additions of Fe^{3+}-ADP (0.025 mM $FeCl_3$, 0.250 mM ADP) and DHF (0.83 mM) in a medium of 0.12 M KCl, 0.05 M sucrose, 0.01 M potassium phosphate, pH 7.2. At indicated times of incubation, samples were assayed for MDA formation. Values are means ± S.E. of 4–8 separate preparations. $^*p < 0.05$, $^{**}p < 0.01$ comparing to controls of the corresponding time of incubation.

amphiphiles. At given concentrations of the free radical-generating components, lipid peroxidation in the control samples as indicated by MDA formation, proceeded quasilinearly at least up to 40 min at 37 °C. When the sarcolemma was preincubated with palmitoyl-CoA or palmitoylcarnitine (50 μM each) before the addition of the free radical system, the initial rates of peroxidation in both samples were significantly ($p < 0.01$) stimulated. After 20 min of incubation, palmitoyl-CoA and palmitoylcarnitine enhanced the levels of MDA formation by 240% (93.9 ± 5.7 nmol/mg of protein) and 160% (62.2 ± 4.9 nmol/mg of protein), respectively, when compared to MDA formation (39.1 ± 2.0 nmol/mg of protein) in the control samples. Separate control aliquots, into which a 50 μM concentration of each lipid amphiphile was included at the time of MDA determination, exhibited similar levels of peroxide formed, thus ruling out artifact in the MDA determination.

Fig. 1 also shows that the accelerated rates of lipid peroxide produced by palmitoyl-CoA or palmitoylcarnitine appear to diminish after 20 min of incubation. Levels of peroxides formed in samples, treated with lipid amphiphiles, and incubated for more than 60 min were not statistically different from controls (data not shown). At 90 min of incubation, peroxide formation in the samples with or without amphiphiles reached a similiar plateau level probably due to depletion of all the peroxidizable polyunsaturated fatty acids in the samples. Thus, the data suggest that the lipid amphiphiles accelerate primarily the initial rate of peroxidation of sarcolemmal membrane when exposed to free radicals. In additional experiments, the effects of the amphiphiles on much lower rates of sarcolemmal lipid peroxidation were examined. For this study, the peroxidation reactions were initiated by 0.006 mM $FeCl_3$ (chelated by 0.062 mM ADP) and 0.21 mM DHF. With the chosen lower concentrations of the free radical-generating components, incubation of the control samples (no amphiphile) for 60 min resulted in only 3.8 ± 1.6 nmol/mg of protein MDA formation ($n = 3$ ± S.D.). However, pretreatments of the samples with palmitoyl-CoA or palmitoylcarnitine (50 μM each) substantially increased the formation of peroxide to, respectively, 17.0 ± 3.0 and 10.6 ± 3.7 nmol of MDA/mg of protein over the same time period of incubation. These data indicate that although the time frame of stimulation of the initial rate of peroxidation might depend on the level of free radicals present, the enhancing effects of the amphiphiles clearly persisted even at much lower rates of peroxidation.

The concentration-dependent effects of palmitoyl-CoA and palmitoylcarnitine on sarcolemmal lipid peroxidation at 20 min were examined. Fig. 2 shows that preincubation of the samples with a level of palmitoyl-CoA as low as 10 μM already produces a moderate but significant degree (126 ± 5.7% of control, $p < 0.05$) of enhancement of peroxidation. Higher levels of amphiphile increase the relative rate of peroxidation further until maximal stimulation (about 240% of control) is achieved at 50 μM palmitoyl-CoA. Similarly, palmitoylcarnitine also enhanced the relative rate of production of peroxide to reach statistical significance ($p < 0.05$ or less) at 25 μM or higher; the maximal rate of stimulation by palmitoylcarnitine was 160–170% of control at 20 min. Concentrations higher than 100 μM did not further enhance the rate of MDA formation; in fact, both amphiphiles at concentrations higher than 200 μM diminished these stimulatory effects (data not shown).

Since we previously showed that peroxidation of the sar-

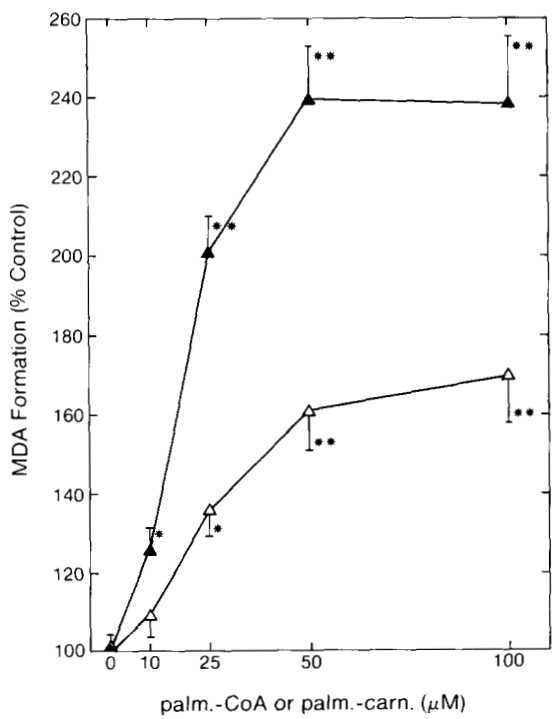

FIG. 2. **Concentration-dependent promotion effect of palmitoyl-CoA and palmitoylcarnitine on sarcolemmal lipid peroxidation.** Sarcolemmal samples were preincubated at 37 °C with various levels of palmitoyl-CoA (▲——▲) or palmitoylcarnitine (△——△) for 10 min before the additions of Fe^{3+}-ADP and DHF. After 20 min of incubation, samples were assayed for MDA formation and expressed as per cent relative to the controls in the absence of amphiphiles. Other conditions were as described in Fig. 1. Values are means ± S.E. of 4–8 separate preparations. $^*p < 0.05$, $^{**}p < 0.01$ comparing to controls.

colemmal membrane was temporally correlated with losses of the (Na,K)-ATPase activity (Kramer *et al.*, 1984), we have sought to determine whether potentiation by the lipid amphiphiles could produce further inhibition of the enzyme activity (Fig. 3). Incubation of the samples with the free radical-generating system alone for 20 min resulted in a 40% loss of enzyme activity (60.6 ± 2.6% specific activity of the paired samples incubated in buffer alone (untreated controls)). Treatment of the samples with 25 and 100 μM palmitoyl-CoA decreased the enzymatic activity to 40.9 ± 4.4% and 27.6 ± 4.3%, respectively, of the untreated-control samples; both are significantly lower ($p < 0.01$) than the activities in samples exposed to the free radical system alone. Likewise, 25 μM and 100 μM palmitoylcarnitine decreased the enzymatic activity to 52.4 ± 4.2% and 38.3 ± 6.3%, respectively ($p < 0.05$), of the untreated-controls. Importantly, incubation of the sarcolemmal membranes with these lipid amphiphiles at the indicated concentrations, without exposure to the free radicals, did not change the enzyme activities significantly (Kramer and Weglicki, 1985; Weglicki *et al.*, 1985). Linear regression analysis indicates a positive correlation exists between MDA production and per cent inhibition of enzyme activity for sarcolemmal membranes treated with both the free radical-generating system and various concentrations of lipid amphiphiles (plots not shown). The data presented in Figs. 2 and 3 as well as additional values obtained for per cent inhibition of enzyme activity in the presence of free radicals and alternate levels of palmitoyl-CoA (10 and 50 μM led to 56.5 and 65.7% inhibition, respectively) and palmitoylcarnitine (10 and 50 μM led to 38.6 and 54.9% inhibition, respectively) were used in the statistical analysis. The positive linear correlation coefficients (r) determined from membrane samples exposed to palmitoyl-CoA ($r = 0.91$) and palmitoylcarnitine ($r = 0.978$) demonstrated that direct linear relationships exist between the level of MDA production and enzyme inhibition as a function of lipid amphiphile concentration.

To further examine the specificity of these lipid compounds for stimulating sarcolemmal peroxidation, other lipids were similarly tested and their potencies were compared with that of palmitoyl-CoA and palmitoylcarnitine (Fig. 4). It appears that both stearoyl-CoA (18:0 CoA) and oleoyl-CoA (18:1 CoA), to a lesser extent, are as potent as palmitoyl-CoA; lysophosphatidylcholine (lyso-PC) is intermediate between palmitoyl-CoA and palmitoylcarnitine in stimulating the induced peroxidation. Concentrations of all amphiphiles higher than 50 μM did not further stimulate peroxide formation (data not shown). The data in Fig. 4 also show that long chain free fatty acids have no significant effect. In addition, a related experiment (data not shown) indicated that 50 μM coenzyme A or L-carnitine alone, or 50 μM coenzyme A or L-carnitine plus 50 μM palmitic acid did not have any effect on sarcolemmal peroxidation. Since lysophosphatide levels have also been reported to accumulate in ischemic myocardium and in effluents from isolated, perfused ischemic hearts (Shaikh and Downar, 1981; Snyder *et al.*, 1981), the dose-response of the lyso-PC was studied. As indicated in Fig. 5, all levels of lyso-PC, at or higher than 10 μM, produced significant ($p < 0.05$ or less) promotion of the induced membrane peroxidation and achieved a maximum effect (206% of control) at 50 μM. The time course study, using 50 μM lyso-PC (Fig. 5, *inset*), showed a temporal pattern similar to that for palmitoyl-CoA in Fig. 1.

Since the pH of ischemic cardiac tissue may fall to as low as 5.8–6.0 (Cobbe and Poole-Wilson, 1980), the effect of pH on lipid amphiphile-stimulated lipid peroxidation was studied (Table I). Compared to pH 7.2, pH 6.0 and pH 7.8 decreased and increased, respectively, by less than 10% the apparent level of MDA formed in the control samples. Nevertheless, the potentiation effect and the relative potency of the lipid amphiphiles persisted; the potency of lyso-PC and palmitoylcarnitine were slightly decreased at pH 6.0.

Since different lipid amphiphiles may accumulate simultaneously during ischemia, the effect of combining some of the compounds on peroxidation were examined in the final series of experiments. Treatment with either 25 μM palmitoylcarnitine or 25 μM lyso-PC alone stimulated peroxide formation +37% and +75%, respectively (Table II). However, when both amphiphiles (each at 25 μM) were added together, an additive

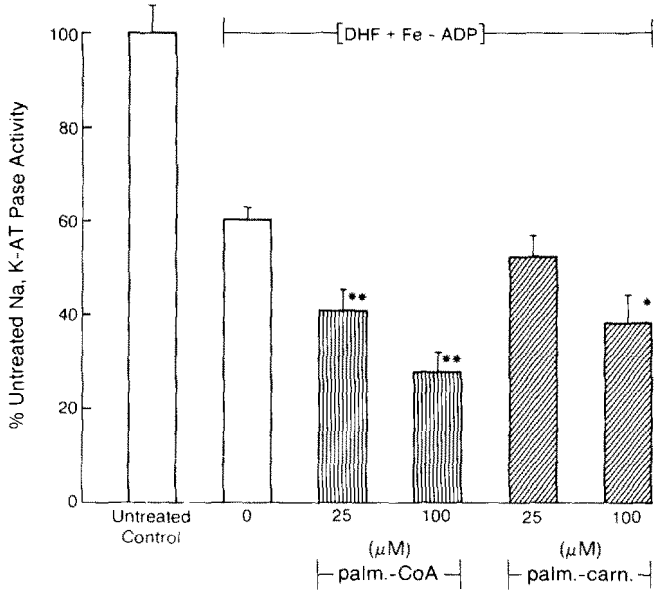

FIG. 3. **Combined effect of lipid amphiphiles and free radicals on sarcolemmal (Na,K)-ATPase activity.** Sarcolemmal samples were preincubated at 37 °C with palmitoyl-CoA (*palm.-CoA*) (▥) or palmitoylcarnitine (*palm.-carn.*) (▨) at concentrations indicated for 10 min before the additions of Fe^{3+}-ADP and DHF. After 20 min of incubation, samples were diluted 30-fold into assay medium for determinations of (Na,K)-ATPase activity. Results are means ± S.E. of 3–9 preparations and were expressed as per cent change from untreated control activity (100%). Other conditions were as described in the text and Fig. 1. $^*p < 0.05$, $^{**}p < 0.01$ compared to samples treated with [DHF + Fe^{3+}-ADP] alone.

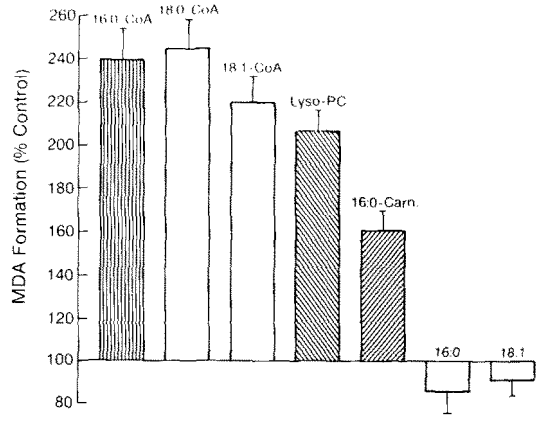

FIG. 4. **Effects of various lipid amphiphiles on sarcolemmal lipid peroxidation.** Sarcolemmal samples were preincubated at 37 °C with the indicated amphiphiles (each at 50 μM) for 10 min before the additions of Fe^{3+}-ADP and DHF. After 20 min of incubation, samples were assayed for MDA formation and expressed as per cent relative to the controls in the absence of amphiphiles. Values are means ± S.E. of 3–8 preparations.

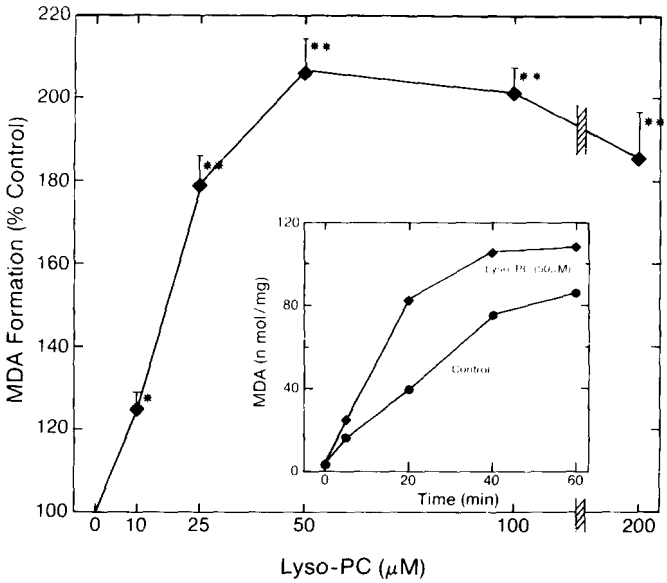

FIG. 5. **Concentration-dependent promotion effect of lyso-PC on sarcolemmal lipid peroxidation.** Sarcolemmal samples were preincubated at 37 °C with various levels of lyso-PC for 10 min before the additions of Fe^{3+}-ADP and DHF. After 20 min of incubation, samples were assayed for MDA formation and expressed as per cent control. Other conditions were as described in Fig. 2. Values are means ± S.E. of 4–6 preparations. *Inset*, time course of lipid peroxidation in samples without (●, control) or with 50 1M lyso-PC (♦). $^*p < 0.05$, $^{**}p < 0.01$ comparing to controls at 20 min.

TABLE I

Effect of pH on DHF-Fe^{3+}-ADP-induced sarcolemmal lipid peroxidation in the presence of lipid amphiphiles

Sarcolemma were preincubated with a 50 μM concentration of each amphiphile for 10 min at 37 °C before the additions of Fe^{3+}-ADP and DHF. After 20 min of incubation, samples were assayed for MDA formation and expressed as per cent relative to the controls at pH 7.2. Values are means of two to four separate preparations.

Additions	MDA formation (% of controls at pH 7.2)		
	pH 6.0	pH 7.2	pH 7.8
Controls (DHF + Fe^{3+}-ADP)	91.5	100	109
+Palmitoyl-CoA	248	246	230
+Palmitoylcarnitine	136	160	172
+Lyso-PC	166	201	203

TABLE II

Additive and saturable effects of combining two different amphiphiles on DHF-Fe^{3+}-ADP-induced sarcolemmal lipid peroxidation

Sarcolemma were preincubated with the amphiphile(s) for 10 min at 37 °C before the additions of Fe^{3+}-ADP and DHF. After 20 min of incubation, samples were assayed for MDA formation and expressed as net per cent increase over the controls. Values are means of two to four separate preparations.

Additions	Net % increase in MDA formation
Palmitoylcarnitine (25 μM)	+37
Palmitoyl-CoA (25 μM)	+108
Lyso-PC (25 μM)	+75
Palmitoylcarnitine (25 μM) + lyso-PC (25 μM)	+114
Palmitoylcarnitine (25 μM) + palmitoyl-CoA (25 μM)	+142
Palmitoylcarnitine (50 μM)	+60
Palmitoyl-CoA (50 μM)	+148
Lyso-PC (50 μM)	+103
Palmitoyl-CoA (50 μM) + palmitoylcarnitine (50 μM)	+136
Palmitoyl-CoA (50 μM) + lyso-PC (50 μM)	+140

stimulation (+104%) of peroxidation resulted. Similar effects were observed when palmitoylcarnitine and palmitoyl-CoA (both at 25 μM) were combined together. However, 50 μM palmitoylcarnitine plus 50 μM palmitoyl-CoA (or 50 μM lyso-PC plus 50 μM palmitoyl-CoA), which separately produce maximal effect, did not generate any higher stimulation than that which was achieved independently by 50 μM palmitoyl-CoA alone. The slightly lower levels of peroxide formation in the presence of either palmitoyl-CoA plus palmitoylcarnitine or palmitoyl-CoA plus lyso-PC (each at 50 μM) suggest some degree of competition between amphiphiles.

DISCUSSION

Due to the difficulties inherent in techniques for the study of free radical reactions *in situ*, we have established a subcellular model system to investigate free radical-induced injury in lysosomes, microsomes, and sarcolemma (Mak *et al.*, 1983; Kramer *et al.*, 1984; Weglicki *et al.*, 1984; Mak and Weglicki, 1985). In this *in vitro* study, we have demonstrated that lyso-PC, long chain acyl-CoA, and acylcarnitine esters are capable of enhancing the susceptibility of the sarcolemma to free radical-induced lipid peroxidation. The long chain acyl-CoA ester was the most potent in producing this effect. Tissue levels of fatty acyl-CoA are normally low but increase 2–3-fold during ischemia (Whitmer *et al.*, 1978; Idell-Wenger *et al.*, 1978). However, since 95% of the total cellular CoA has been found to be associated with the mitochondria and since the cytosolic concentration of total CoA has been estimated to be only about 15 μM (Idell-Wenger *et al.*, 1978), interactions of critical amounts of these acyl-CoA esters with sarcolemma *in situ* may be less likely. In contrast, accumulations of acylcarnitine occur primarily in the cytosol and may have direct access to the sarcolemmal membrane (Whitmer *et al.*, 1978; Idell-Wenger *et al.*, 1978; Corr *et al.*, 1984). It has been estimated that acylcarnitine can achieve concentrations in the millimolar range during early myocardial ischemia (Idell-Wenger *et al.*, 1978); however, the proportion that would be free to interact with membranes may be considerably lower due to binding to proteins and other cellular components. Nevertheless, in our study, low concentrations (25–100 μM) of palmitoylcarnitine were found to significantly stimulate sarcolemmal peroxidation.

Lysophosphatide levels in myocardium are reported to rise within the first few minutes after the onset of ischemia, although the magnitude of the increase and the specific site of production is not yet clarified (Shaikh and Downar, 1981; Katz and Messineo, 1981; Snyder *et al.*, 1981). It has been implied that much of the endogenously elevated lysophospholipids occur in the extracellular compartment which may thus be accessible to the external surface of the sarcolemma (Corr *et al.*, 1984; Snyder *et al.*, 1981). During ischemia, the concentration of lyso-PC in interstitial fluid was estimated to be at least 0.170 mM, which is within the ranges of the amphiphile (10–200 μM) demonstrated in the present study to produce significant enhancement of sarcolemmal lipid peroxidation. The additive nature of the effects of different amphiphiles at suboptimal concentrations raises the consideration that combined modest increases of several classes of lipid amphiphiles may produce substantial consequences. Thus, each of these lipid amphiphiles, alone or in concert with each other, may accelerate membrane injury when free radicals are present.

Although we have demonstrated that long chain acyl-CoA and carnitine esters and lyso-PC at micromolar concentrations (10–100 μM) are capable of promoting free radical-mediated peroxidation in the isolated sarcolemma, the molecular interactions within the membranes remain unclear. We

have carried out a preliminary study to determine the effect of palmitoyl-CoA on the sarcolemmal lipid peroxidation induced by ascorbate + iron-ADP, another well established *in vitro* free radical-generating system (Mak and Weglicki, 1985; Wills, 1969). In these experiments, purified sarcolemma (85 μg/ml), in the same buffer described in Fig. 1, were incubated with or without 25 μM palmitoyl-CoA for 10 min before the additions of Fe^{3+}-ADP (0.1 mM $FeCl_3$, 1 mM ADP) and ascorbate (0.1 mM). After 30 min of incubation, lipid peroxidation was induced modestly as indicated by the formation of 18–23 nmol of MDA/mg of protein in the control samples. However, peroxide formation in the samples with palmitoyl-CoA treatment was increased to 35–40 nmol of MDA/mg of protein (190% of control). Thus, the promoting effect of the lipid amphiphiles described in the present study is not unique to the DHF-Fe^{3+}-ADP peroxidation system; the sarcolemma may be attacked by free radicals generated from other systems and would probably be affected similarly by the lipid amphiphiles.

Palmitoylcarnitine, lyso-PC, and palmitoyl-CoA, to a lesser extent, bear certain degrees of structural similarity. The lack of effect of free fatty acids, coenzyme A, and carnitine alone or in combination suggests a requirement for an intact ester (or ether) molecule with both a polar head group and a nonpolar aliphatic chain. All three amphiphiles possess detergent-like properties and aggregate into micelles at high concentrations (Powell *et al.*, 1981; Yalkowsky and Zografi, 1970; Weltzien, 1979; Utsumi *et al.*, 1978). The critical micellar concentrations for pamitoyl carnitine and lyso-PC are estimated to be 15–100 μM (Yalkowsky and Zografi, 1970; Weltzien 1979; Utsumi *et al.*, 1978), whereas palmitoyl-CoA has recently been determined to achieve critical micellar concentration at 30–60 μM (Powell *et al.*, 1981). Since significant promotion of peroxidation by the amphiphiles was observed at concentrations of 25 μM or lower, the interactions of the amphiphiles may involve binding of their monomeric forms to the sarcolemmal membrane at, as suggested by the data of combining different amphiphiles, specific saturable sites. The diminished effects of lyso-PC, palmitoyl-CoA, and palmitoylcarnitine at higher levels (>100 μM) may result from partial micellization and subsequent solubilization of the membrane lipids (Weltzien, 1979; Utsumi *et al.*, 1978). Presumably, incorporation of the amphiphilic monomers into the membrane lipid bilayer as wedge-shaped structures can change the physical properties of the lipid bilayer which, in our study, might result in enhanced accessibility of free radicals to the phospholipid unsaturated fatty acids and the subsequent increased initial rates of peroxide formation.

The data (Fig. 3) also suggest that these surface-active lipid intermediates may increase the susceptibility of the lipid-dependent (Na,K)-ATPase to free radical injury. We observed significantly greater free radical-induced inhibition of enzyme activity when sarcolemmal membranes were exposed to non-inhibitory concentrations of both palmitoyl-CoA and palmitoylcarnitine. The combined interaction of the lipid amphiphiles and free radicals at the sarcolemma led to inhibition having synergistic rather than additive characteristics. This was evident by the fact that significant ($p < 0.05$) loss of activity (60–72%) due to the combined effects far exceeded that observed by the sum (40%) of the independent actions of the free radicals (40% inhibition) and lipid amphiphiles (no inhibition) alone. Linear regression analysis provided positive linear correlation coefficients (r) for membrane samples exposed to both exogenous free radicals and either palmitoyl-CoA ($r = 0.91$) or palmitoylcarnitine ($r = 0.978$). This suggests that a direct linear relationship exists between the extent of MDA production and loss of enzyme activity as a function of lipid amphiphile concentration. Thus, it appears that intercalation of the amphiphiles into the sarcolemmal lipid bilayer potentiates lipid peroxidation and inhibition of the (Na,K)-ATPase. However, the data do not preclude the possible contribution toward enzyme inhibition of direct free radical-mediated inactivation of the sarcolemmal protein.

In conclusion, although the molecular mechanism remains unknown, our results clearly demonstrate enhanced susceptibility of sarcolemmal membranes to free radical-induced injury due to three major lipid amphiphiles which have been reported to accumulate during cardiac ischemia. Since free radicals are believed to exist *in vivo* (Freeman and Crapo, 1982) and may increase to levels overcoming the endogenous cellular defense mechanisms during cardiac ischemia (McCord and Roy, 1982; Meerson *et al.*, 1982; McCord, 1985; Freeman and Crapo, 1982), it is reasonable to consider that a similar combined detrimental effect may occur *in vivo*.

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REFERENCES

Cobbe, S. M., and Poole-Wilson, P. A. (1980) *J. Mol. Cell. Cardiol.* **12,** 745–760
Corr, P. B., Snyder, D. W., Lee, B. I., Gross, R. W., Keim, C. R., and Sobel, B. E. (1982) *Am. J. Physiol.* **243,** H187–H195
Corr, P. B., Gross, R. W., and Sobel, B. E. (1984) *Circ. Res.* **55,** 135–154
Freeman, B. A., and Crapo, J. D. (1982) *Lab Invest.* **47,** 412–426
Idell-Wenger, J. A., Grotyohann, L. W., and Neely, J. R. (1978) *J. Biol. Chem.* **253,** 4310–4318
Katz, A. M., and Messineo, F.C. (1981) *Circ. Res.* **48,** 1–16
Kramer, J. H., and Weglicki, W. B. (1985) *Am. J. Physiol.* **248,** H75–H81
Kramer, J. H., Mak, I. T., and Weglicki, W. B. (1984) *Circ. Res.* **55,** 120–124
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275
Mak, I. T., and Weglicki, W. B. (1985) *J. Clin. Invest.* **75,** 58–63
Mak, I. T., Misra, H. P., and Weglicki, W. B. (1983) *J. Biol. Chem.* **258,** 13733–13737
McCord, J. M. (1985) *N. Engl. J. Med.* **312,** 159–163
McCord, J. M., and Roy, R. S. (1982) *Can. J. Physiol. Pharmacol.* **60,** 1346–1352
Meerson, F. Z., Kagan, V. E., Kozlov, Yu P., Belkins, L. M., and Arkhipenko, Yu V. (1982) *Basic Res. Cardiol.* **77,** 465–485
Owens, K., Kennett, F. F., and Weglicki, W. B. (1982) *Am. J. Physiol.* **242,** H456–H461
Powell, G. L., Grothusen, J. R., Zimmerman, J. K., Evans, C. A., and Fish, W. W. (1981) *J. Biol. Chem.* **256,** 12740–12747
Rao, P. A., Cohen, M. V., and Mueller, H. S. (1983) *J. Mol. Cell. Cardiol.* **15,** 713–716
Schwartz, A., Wood, J. M., Allen, J. C., Bornet, E. P., Entman, M. L., Goldstein, M. A., Sordahl, L. A., and Suzuki, M. (1973) *Am. J. Cardiol.* **32,** 46–61
Shaikh, N. A., and Downar, E. (1981) *Circ. Res.* **49,** 316–325
Skou, J. C. (1974) *Biochim. Biophys. Acta* **339,** 258–273
Snyder, D. W., Crafford, W. A., Glashow, J. L., Rankin, D., Sobel, B. E., and Corr, P. B. (1981) *Am. J. Physiol.* **241,** H700–H707
Utsumi, H., Inoue, K., Nojima, S., and Kwan, T. (1978) *Biochemistry* **17,** 1990–1996
Weglicki, W. B., Owens, K., Kennett, F. F., Kessner, A., Harris, L., Wise, R. M., and Vahouny, G. V. (1980) *J. Biol. Chem.* **255,** 3605–3609
Weglicki, W. B., Dickens, B. F., and Mak, I. T. (1984) *Biochem. Biophys. Res. Commun.* **124,** 229–235
Weglicki, W. B., Kramer, J. H., Franson, R. C., Pang, D. C., and Owens, K. (1985) in *Pathobiology of Cardiovascular Injury* (Stone, H. L., and Weglicki, W. B., eds) pp. 258–272, Martinus Nijhoff Publishing, Boston, MA
Weltzien, H. U. (1979) *Biochim. Biophys. Acta* **559,** 259–287
Whitmer, J. T., Idell-Wenger, J. A., Rovetto, M. J., and Neely, J. R. (1978) *J. Biol. Chem.* **253,** 4305–4309
Wills, E. D. (1969) *Biochem. J.* **113,** 315–324
Yalkowsky, S. H., and Zografi, G. (1970) *J. Pharmacol. Sci.* **59,** 798–802