Palmitoylcarnitine, an endogenous long-chain fatty acyl ester, produced marked changes in the structure and function of cardiac sarcoplasmic reticulum and sarcosommmal Na,K-ATPase isolated from canine ventricular muscle. Low concentrations (5 to 50 μM) of palmitoylcarnitine enhanced Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) binding to sarcoplasmic reticulum and enhanced \(^{3}H\) ouabain binding to Na,K-ATPase above equilibrium binding levels but inhibited Na,K-ATPase hydrolytic activity. Increasing concentrations of palmitoylcarnitine (50 to 200 μM) further inhibited Na,K-ATPase activity and markedly decreased the binding of \(^{3}H\) ouabain to this enzyme. Sarcoplasmic reticulum Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) binding are similarly inhibited by these higher concentrations of palmitoylcarnitine.

Palmitoylcarnitine also produced concentration-dependent changes in the fluorescence intensity of a lipid-bound fluorescent probe, dansyl (5-dimethylaminonaphthalene-1-sulfonyl) phosphatidylethanolamine, which was incorporated into sarcoplasmic reticulum and Na,K-ATPase membranes. Palmitoylcarnitine induced an enhancement of fluorescence in both membrane systems and this enhancement of fluorescence was linearly related to the inhibition of Na,K-ATPase activity.

The biphasic action of palmitoylcarnitine on the functional properties of sarcoplasmic reticulum and Na,K-ATPase resembles the actions of detergents on these membrane systems. Palmitoylcarnitine is considered to act as a naturally occurring detergent and a mechanism is proposed to explain its actions in vitro.

Long chain fatty acids represent the major substrate for energy production in cardiac muscle. Under normal, aerobic conditions, oxidation of fatty acids by mitochondria generates acetyl coenzyme A and ATP via the tricarboxylic acid cycle ([1, 2]). This process is dependent upon extramitochondrial esterification of fatty acids to acyl-CoA esters and their subsequent transport into the mitochondria matrix for \(\beta\) oxidation ([3]). In addition to providing energy for cellular function, fatty acyl esters may play a significant role in the regulation of membrane transport by altering membrane structure ([4]). During episodes of myocardial ischemia, \(\beta\) oxidation of fatty acids is rapidly and markedly inhibited ([5, 6]) and it has been suggested that there is a concomitant increase in tissue metabolite intermediates, particularly long chain acyl-CoA and acylcarnitine esters ([7-9]). Associated with these and other metabolic derangements are the well-known alterations in myocardial performance, i.e. decline in contractile force development and enhancement of cardiac excitability ([10, 11]).

The physicochemical basis for these pathophysiological aspects of myocardial ischemia appear to involve several factors. With respect to the observed decline in contractile force, it is thought that altered calcium availability to or utilization by the cardiac muscle during ischemia, or both, play an important role ([5, 12]). The hyperexcitability and susceptibility of ischemic myocardium to arrhythmia, on the other hand, has been attributed to alterations in intracellular as well as extracellular electrolyte concentrations, particularly the Na\(^+\)/K\(^+\) ratios ([11-13]) and the intracellular Ca\(^{2+}\) content ([14]). Consequently, ischemia-associated defects in membrane systems which regulate calcium availability as well as passive and active movement of sodium and potassium have been reported ([5, 12, 15-18]). While the underlying biochemical or structural cause(s) for these defects has not been elucidated, in recent years much attention has been paid to the role fatty acid metabolism might play in the degeneration of membrane function ([10]). Increased levels of acylcarnitine and other fatty acid esters have been implicated in the pathology of cardiac ischemia since it has been demonstrated that the in vitro function of a number of membrane-bound enzymes is altered by these metabolic products. Shrago and his co-workers ([6, 20]) found that long chain acyl CoA inhibited adenine nucleotide translocase of mitochondrial membranes. Ahmed and Thomas ([21]) and Lamers and Hulsman ([22]) found that long chain fatty acids inhibited Na,K-ATPase of brain and heart, respectively and, more recently, we demonstrated that palmitoylcarnitine is a potent inhibitor of isolated cardiac Na,K-ATPase as well as an inhibitor of digitalis glycoside binding to this enzyme ([23, 24]). In a preliminary report from this laboratory, Cohen et al. ([25]) presented evidence that palmitoylcarnitine had marked effects on the functional properties of cardiac sarcoplasmic reticulum in vitro. Recently, those
findings have been confirmed by Pitts et al. (26).

The present study is an extension of experiments designed to investigate the in vitro action of fatty acids and fatty acyl esters on cardiac membrane structure and function (25, 27). The determination of the action of palmitylcarnitine on Ca++ binding and Ca++-ATPase activity of cardiac sarcoplasmic reticulum and on sarcolemmal Na,K-ATPase activity and reactivity to ouabain, provides information about how functional and structural properties of these membrane enzyme systems are affected by naturally occurring metabolic substances. As an approach to investigate the mechanism of the fatty acyl ester effects on function and membrane structure, a lipid-bound fluorescent probe, dansyl phosphatidylethanolamine (28), was incorporated into sarcoplasmic reticulum and Na,K-ATPase (sarcolemmal) membranes and its fluorescence was monitored as a function of fatty acyl ester concentration.

METHODS

Preparation of Sarcoplasmic Reticulum—Sarcoplasmic reticulum vesicles were prepared from dog cardiac left and right ventricles according to the procedure of Pitts and Schwartz (29). Approximately 100 g of muscle, cleaned of fat and connective tissue, was cut into small pieces and homogenized at 3°C in a Sorvall Omnimixer, fully (to reduce foam formation) with 10 mM Tris/maleate buffer of pH 6.8. Total homogenization time was 45 s (three full speed runs with 30-s cooling intervals). The homogenate was centrifuged at 15,000 g for 10 min and the supernatant was passed through four layers of cheesecloth and the filtrate was centrifuged at 15,000 × g (Rmax) for 20 min. The supernatant was again filtered through four layers of cheesecloth and centrifuged at 143,000 × g (Rmax) for 30 min. The resultant supernatant was discarded and the pellet was suspended in 100 to 150 ml of 10 mM Tris/maleate containing 0.6 mM CaCl2 using a glass homogenizer and Teflon pestle. The homogenate was then centrifuged at 143,000 × g (Rmax) for 45 min. The supernatant was discarded and the pellet was resuspended in 4 ml of 10 mM Tris/maleate containing 100 mM KCl. Protein content was determined by the biuret procedure (30). This method of isolation consistently gave higher yields, higher activity, and greater stability of the sarcoplasmic reticulum preparation than previous techniques (29).

This sarcoplasmic reticulum preparation was checked for contamination by mitochondrial and sarcolemmal membrane components. The extent of mitochondrial contamination was determined by an assay for cytochrome c oxidase activity (31), which was less than 9% of that in sarcoplasmic reticulum. Contamination by mitochondrial-sensitive Na,K-ATPase, an enzyme marker for sarcosome (32), was less than 1% (0.01 mmol of active Na,K-ATPase/mmol of active Ca++-ATPase) of the total ATPase of this sarcoplasmic reticulum preparation and [H]ouabain binding was generally less than 1.5 pmol/mg of protein when assayed using various ligand conditions (see below).

Assay of Sarcoplasmic Reticulum Ca++-ATPase Activity—Ca++-ATPase activity was measured by the spectrophotometric linked-enzyme method (33) utilizing pyruvate kinase and lactate dehydrogenase and continuous monitoring of the absorbance change at 340 nm (Beckman 35-K spectrophotometer) due to oxidation of NADH. The reaction mixture (final volume 2.5 ml) contained 100 mM KCl, 5 mM MgCl2, 2.5 mM NaATP, 0.5 mM NADH (Sigma), 2 mM phosphoenolpyruvate, 0.15 mM ethylene glycol-bis(2-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), and 0.1 mM CaCl2 (free Ca++ ≈ 14 μM, based on Ks of 4.4 × 10^-9 M at pH 6.8, for EGTA/Ca2+). The pH of the reaction mixture was preincubated for 10 min at 37°C before the addition of palmitylcarnitine and the final concentration of CaCl2 (to a final concentration of 0.06 mM) and NaATP (to a final concentration of 0.33 mM). The final volume of the cuvette was 5 ml.

Preparation of Na,K-ATPase—Na,K-ATPase was partially purified from bovine heart mitochondria by centrifugation of crude mitochondria on a 20% sucrose cushion and subsequent centrifugation at 100,000 × g (Rmax) for 45 min. The final volume of the pellet was diluted to 10 ml with appropriate buffers and stored at 3°C until

W. Roussin, personal communication.
used. Concentrations of incorporated dansyl PE were determined by absorption spectroscopy assuming an ε_{340} = 3400 M⁻¹ cm⁻¹ for the dansyl fluorophore.

**Fluorescence Measurements of Dansyl PE-Sarcoplasmic Reticulum and Dansyl PE-Na,K-ATPase**—Fluorescence measurements were made using a Perkin-Elmer MFP 44A fluorescence spectrophotometer operated in the ratio mode. Titrations of the palmitoylcarnitine-induced fluorescence increase in dansyl PE-sarcoplasmic reticulum and dansyl PE-Na,K-ATPase were conducted by making continuous additions of microliter increments of palmitoylcarnitine (10 mM) to 100 µg/ml of labeled enzyme in 2.0 ml of 10 mM Tris/Cl (pH 7.4) at 37°C. In parallel control experiments, equal microliter additions of water were made to identical samples of dansyl PE-sarcoplasmic reticulum and dansyl PE-Na,K-ATPase to compensate and correct for the small change in fluorescence intensity due to dilution. The fluorescence intensity of dansyl PE-sarcoplasmic reticulum and dansyl PE-Na,K-ATPase was determined at 510 nm using an excitation wavelength of 340 nm.

**RESULTS**

**Influence of Palmitoylcarnitine on Cardiac Sarcoplasmic Reticulum**

**Ca²⁺-ATPase Activity**—Palmitoylcarnitine produced a concentration-dependent, biphasic effect on sarcoplasmic reticulum Ca²⁺-ATPase activity. The Ca²⁺-ATPase activity was stimulated above control levels in the presence of low concentrations of palmitoylcarnitine (5 to 60 µM) and was inhibited by higher concentrations of the acyl ester (Fig. 1). Ca²⁺-ATPase activity increased maximally to 49% above control levels with increases in the concentration of palmitoylcarnitine up to 20 µM. The activity of the enzyme declined from this maximal level with further increases in palmitoylcarnitine and maximal inhibition (~86% of control) was obtained at approximately 200 µM (Fig. 1).

**Ca²⁺ Binding**—Low concentrations of palmitoylcarnitine also caused a small, but significant increase in the amount of Ca²⁺ bound by sarcoplasmic reticulum (Fig. 2). However, the increase in Ca²⁺ binding was much less marked than was the stimulation of Ca²⁺-ATPase activity and occurred over a much reduced concentration range of palmitoylcarnitine. Enhancement of Ca²⁺ binding was maximal (10% above control levels) after incubation of sarcoplasmic reticulum with 5 mM palmitoylcarnitine. Ca²⁺ binding declined to control levels at 20 µM and was then inhibited in a concentration-dependent manner to ~86% of control in the presence of 200 µM palmitoylcarnitine.

A comparison of the data shown in Figs. 1 and 2 reveals some interesting differences as well as similarities between Ca²⁺ binding and Ca²⁺-ATPase activity as affected by palmitoylcarnitine. Although the stimulatory effect of the acyl ester is much less for Ca²⁺ binding than for Ca²⁺-ATPase activity, inhibition of binding occurred at lower palmitoylcarnitine concentrations (100 to 125 µM) than the inhibition of ATPase (25 versus 60 µM, respectively). The concentration of palmitoylcarnitine that reduced both binding and activity to 50% of control levels is very similar and is in the range of 100 to 125 µM. Furthermore, both the Ca²⁺-ATPase activity and Ca²⁺ binding are inhibited to approximately ~86% of control at 200 µM palmitoylcarnitine.

Although the initial stimulatory and subsequent inhibitory effects of palmitoylcarnitine on Ca²⁺ binding were most pronounced at low and high acyl ester concentrations, respectively, the apparent initial rate of Ca²⁺ binding appeared to be unaffected by any concentration of palmitoylcarnitine studied (Fig. 3). However, it is apparent that with lesser amounts of calcium bound, the time to maximal binding is abbreviated (Fig. 3).

After binding of Ca²⁺ to sarcoplasmic reticulum has reached maximum, there is a characteristic spontaneous "release" phase observed in the absence of oxalate (41). In this study, palmitoylcarnitine appeared to have little effect on the initial release phase immediately following maximal binding (Fig. 3).

**Influence of Palmitoylcarnitine on Cardiac Na,K-ATPase Activity**

In the present experiments, two cardiac Na,K ATPase preparations were studied, one with a specific activity of ±80 µmol of P_i/mg of protein/h (citrate fraction) and one with a specific activity of ±20 µmol of P_i/mg of protein/h (deoxycholate fraction). Palmitoylcarnitine inhibited enzyme activity of both preparations in a concentration-dependent manner without evidence of initial stimulation by low concentrations of palmitoylcarnitine. There were, however, slight but statistically significant differences between the two concentration response curves (Fig. 4). In the higher activity preparation, 50% of maximal inhibition of Na,K-ATPase activity occurred at ±40 µM and maximal inhibition of enzyme activity (~90% of control) resulted after incubation of enzyme with 100 µM palmitoylcarnitine. Further increases in acyl ester did not result in greater inhibition of activity in the higher activity preparation. These data confirm earlier findings reported by this laboratory (23, 24) using Na,K-ATPase preparations with specific activities between 90 and 200 µmol of P_i/mg of protein/h. With the lower activity enzyme, 50% of maximal inhibition occurred at ±90 µM palmitoylcarnitine and 100% inhibition of Na,K-

---

**Fig. 1. Effect of palmitoylcarnitine on Ca²⁺-ATPase activity of cardiac sarcoplasmic reticulum.** Ca²⁺-ATPase activity measured in the presence of increasing concentrations of palmitoylcarnitine as described under "Methods." Control Ca²⁺-ATPase activity was 46.2 ± 14.8 µmol of P_i/mg of protein/h. Each data point represents the mean of five experiments. Vertical bars indicate S.E. * = p < 0.025; ** = p < 0.05.

**Fig. 2. Effect of palmitoylcarnitine on Ca²⁺ binding to cardiac sarcoplasmic reticulum.** Ca²⁺ binding was assayed in the presence of increasing concentrations of palmitoylcarnitine as described under "Methods." Maximal binding in the absence of palmitoylcarnitine was 44.3 ± 4.2 nmol of Ca²⁺/mg of protein. Each data point represents the mean of eight experiments. Vertical bars indicate S.E. * = p < 0.05.
palmitylcarnitine appeared to be quantitatively similar and independent of ligand conditions, the concentration range over which this effect occurred was greater using [MgATP]NaNaK compared to [MgATP]NaNaK or [MgF]NaNaK. Inhibition of Na,K-ATPase was evident at 25 μM palmitylcarnitine; further increases in palmitylcarnitine concentration resulted in a reduction of ouabain binding from this maximal level. In the presence of [MgATP]NaNaK, enhancement of [H]ouabain binding above control levels was maximal at 25 μM palmitylcarnitine. Ouabain binding remained elevated above control levels until the concentration of palmitylcarnitine exceeded 60 μM.

Although [H]ouabain binding to cardiac Na,K-ATPase was inhibited (in the presence of all binding media) with increasing concentrations of palmitylcarnitine, the degree of inhibition at any given concentration of acyl ester was dependent upon the ligand conditions (Fig. 5). When [MgF] or [MgATP] binding conditions were used, palmitylcarnitine was most effective. Inhibition of ouabain binding was evident at 25 μM palmitylcarnitine, 50% of maximal inhibition occurred at about 50 μM, and inhibition was complete at 125 to 150 μM palmitylcarnitine. Using [MgATP]NaNaK ligand conditions, inhibition of ouabain binding was not apparent until a concentration of 75 μM palmitylcarnitine was attained. Maximal inhibition using these conditions was ~60% of control binding at 150 μM palmitylcarnitine.

**Influence of Palmitylcarnitine on Fluorescence of Dansyl PE-Labeled Sarcoplasmic Reticulum and Na,K-ATPase**

The cumulative addition of palmitylcarnitine to either dansyl PE-sarcoplasmic reticulum or dansyl PE-Na,K-ATPase caused marked changes in the fluorescence intensity of the membrane-bound probe. Significant increases in the fluorescence intensity of dansyl PE-sarcoplasmic reticulum were

![Graph](http://www.jbc.org/)

**Fig. 6 (left).** Effect of palmitylcarnitine on the fluorescence intensity of dansyl PE-sarcoplasmic reticulum and dansyl PE-Na,K-ATPase. The percentage of maximal increase in dansyl PE fluorescence is shown as a function of increasing palmitylcarnitine concentrations for dansyl PE-sarcoplasmic reticulum (○—○) and dansyl PE-Na,K-ATPase (●—●). The inset shows the fluorescence spectrum of dansyl PE-sarcoplasmic reticulum in the absence of palmitylcarnitine (lower trace) and in the presence of 100 μM palmitylcarnitine (upper trace). See “Methods” for details of experimental procedure. DPE, dansyl PE.

**Fig. 7 (right).** Palmitylcarnitine-induced inhibition of cardiac Na,K-ATPase activity as a function of the enhancement of dansyl PE fluorescence intensity. Each data point was derived by plotting the percentage of increase in dansyl PE fluorescence intensity versus the percentage of inhibition of Na,K-ATPase activity at the same palmitylcarnitine concentration (6 to 200 μM). Maximal increase in dansyl PE-Na,K-ATPase fluorescence intensity was obtained in the presence of 200 μM palmitylcarnitine. Control specific activity of native Na,K-ATPase was 20 μmol of P /mg/h. One hundred percent inhibition of Na,K-ATPase activity occurred in the presence of 200 μM palmitylcarnitine. Data was derived from Figs. 4 (○—○) and 6 (●—●). Method of linear regression analysis yielded a slope of 0.983 and y-intercept of 5.166.

DPE, dansyl PE.
observed in the presence of very low (5 μM) concentrations of palmitylcarnitine. Increasing concentrations of the acyl ester up to 100 μM resulted in further enhancement of fluorescence with a maximal increase of 36% above basal fluorescence levels (Fig. 6). Further increases in the palmitylcarnitine concentration resulted in a decline of dansyl PE-sarcoplasmic reticulum fluorescence to a level of 30% above basal levels at 1 mM palmitylcarnitine.

Palmitylcarnitine caused qualitatively similar changes in the fluorescence intensity of dansyl PE-Na,K-ATPase. A concentration-dependent enhancement of dansyl PE-Na,K-ATPase fluorescence occurred up to 200 μM palmitylcarnitine (Fig. 6). This concentration of acyl ester produced a fluorescence enhancement of the dansyl PE-Na,K-ATPase (42% above basal levels) that was similar to the effect of 100 μM palmitylcarnitine on dansyl PE-sarcoplasmic reticulum. As with the dansyl PE-sarcoplasmic reticulum, further increases in the concentration of palmitylcarnitine above the maximal enhancement level resulted in a decrease in dansyl PE-Na,K-ATPase fluorescence. At 1 mM palmitylcarnitine, dansyl PE-Na,K-ATPase fluorescence was reduced to 37% above basal levels.

The changes in dansyl PE fluorescence intensity induced by palmitylcarnitine presumably reflect changes in membrane structure, particularly of the glycerol head region of the bilayer where the dansyl group of the dansyl PE probe is incorporated (28). The enhancement of dansyl PE-Na,K-ATPase fluorescence appears to correlate well with the inhibition of Na,K-ATPase activity (r = 0.988) caused by increasing concentrations of palmitylcarnitine (Fig. 7). These relationships suggest that the effect of palmitylcarnitine on the functional properties of the enzyme may be mediated via palmitylcarnitine-induced changes in the membrane structure.

**DISCUSSION**

The normal function of the heart is dependent upon the concerted activities of numerous subcellular membrane systems. Membranes containing lipid-bound proteins, such as Na,K-ATPase within the sarcoplasm, and Ca²⁺-ATPase within the sarcoplasmic reticulum, play critical roles in the maintenance of intracellular ion distributions which modulate cardiac performance. During episodes of myocardial ischemia, severe alterations in myocardial contractility and electrical excitability are known to occur (10), but the pathophysiological basis for these alterations is not well characterized. In recent years, subcellular membrane systems have been considered as primary sites of dysfunction during ischemia.

In previous studies conducted in this (5, 42) and other laboratories (12, 15, 43), alterations in the functional properties of subcellular enzyme systems have been described in association with various types of experimental myocardial ischemia or hypoxia. Defects which have been found in the Ca²⁺ transport capacity of sarcoplasmic reticulum vesicles isolated from ischemic and from anoxic myocardium include reduced Ca²⁺ binding, reduced uptake, and reduced Ca²⁺-ATPase activity (12, 15, 42), as well as reduced Ca²⁺ release (5, 42). In view of the well established regulatory role of muscle sarcoplasmic reticulum in contraction and relaxation, attempts have been made to correlate the decrement in Ca²⁺ transport with the decline in contractile force during myocardial ischemia and to understand the physicochemical basis for the dysfunction of cardiac sarcoplasmic reticulum (5, 12).

Functional properties of the Na,K-ATPase (in vitro) and of the Na⁺,K⁺ pump (in vivo) have also been reported to be altered during myocardial ischemia. The marked efflux of potassium from the intracellular to the extracellular space (12, 13, 44) and a net influx of sodium (12, 13) that occurs shortly after initiation of ischemic insult could result from inactivation of the sarcoplasmal Na⁺ pump, although passive permeability changes of the membrane to Na⁺ and K⁺ with concomitant flux of these ions down their respective concentration gradients may be the basis for these events (45). Several investigators have, however, reported that the activity of Na,K-ATPase isolated from ischemic myocardium was markedly depressed in comparison to enzyme from nonischemic tissue of the same heart (16-18). Associated with a reduction in enzyme activity, Beller et al. (16, 17, 46) found a reduction in the capacity of ischemic myocardium to bind [¹H]digoxin. Similar alterations in the apparent reactivity of Na,K-ATPase to digitalis glycosides during ischemia have been reported by Hopkins and Taylor (47) and Kuhlmann et al. (48) who suggested that the observed increased arrhythmogenic as well as reduced inotropic properties of digitalis during ischemia (49, 50) could be attributed to these factors.

Altered ion transport across the cardiac sarcolemma (and altered intracellular electrolyte composition) appears to be the underlying cause of many electrophysiological changes observed in ischemic myocardium (10, 11, 51), perhaps secondary to Na,K-ATPase/pump inhibition. Although several explanations for such an inhibition have been presented (10, 11, 16), the mechanism remains unclear. A decline in intracellular pH (52) and the accumulation of toxic metabolites of fatty acids (19) have been suggested as key factors in alteration of ion transport and the development of cardiac arrhythmia during ischemia. In view of the numerous metabolic alterations associated with acute myocardial ischemia (5, 6, 10, 13, 51), it seems likely that some cellular factor(s) or metabolic product(s) occurring as a consequence of reduced coronary perfusion could contribute to the observed degeneration of membrane function cited above.

In the present study, it has been demonstrated that the in vitro effects of an endogenously occurring long chain fatty acyl ester, i.e. palmitylcarnitine, are deleterious to the function of the key membrane-bound enzymes. Furthermore, these in vitro effects are observed at concentrations of palmitylcarnitine that could occur during ischemia (7-9, 26) to produce the kind of alterations in sarcoplasmic reticulum Ca²⁺-ATPase and Ca⁺⁺ transport as well as Na,K-ATPase activity and reactivity to digitalis glycosides observed in vivo. The in vitro inhibition of Ca²⁺-ATPase activity and Ca⁺⁺ binding, and the inhibition of Na,K-ATPase activity and [¹H]ouabain binding to the enzyme all occur at concentrations of palmitylcarnitine (50 to 200 μM) well within range of the levels of acylcarnitine (0.38 to 2 mM) reported by Idell-Wenger et al. (9) to occur in the cytosol during myocardial ischemia. These concentrations of palmitylcarnitine, i.e. 0.38 mM in normal and up to 2 mM in ischemic tissue were reported as total levels of extramitochondrial acyl carnitine. But, as pointed out by Idell-Wenger and colleagues (9), fatty acylcarnitine binds readily to membrane lipids and proteins so that the effective concentration of this fatty acyl ester must be considerably less than the total measured. However, as palmitylcarnitine levels rise during ischemia, it is probable, due to the abundance and availability of sarcoplasmic reticulum and sarcosomal membranes within the cardiac cell, that these organelles would serve as binding sites for palmitylcarnitine.

Assuming a relatively high affinity of palmitylcarnitine for these subcellular membranes (9), the effects of the acyl ester on membrane structure were examined using the lipid-bound fluorescence probe, dansyl phosphatidylethanolamine. The dansyl fluorophore of dansyl PE is a sensitive probe of changes in the rigidity or the hydrophobicity, or both, in the glycerol
head region of the membrane bilayer (40). The palmitoylcarnitine-induced changes in dansyl PE-sarcoplasmic reticulum and dansyl PE-Na,K-ATPase fluorescence must, therefore, result from a change of membrane structure near the phospholipid head groups. The mechanism by which low concentrations (5 to 200 μM) of palmitoylcarnitine interact with the membranes to enhance dansyl PE fluorescence is uncertain. It is plausible, however, that due to the hydrophobic nature of the acyl fatty acid chains of palmitoylcarnitine, the acyl ester may insert into the lipid matrix of the sarcoplasmic reticulum and Na,K-ATPase-associated membrane lipids. Such an insertion mechanism has been documented for detergents in similar systems (53-56).

Although the dansyl PE probe does report palmitoylcarnitine-induced changes in the glycerol head group region of the lipids of the membrane, it does not rule out the possibility that greater and more extensive perturbations of the membrane may occur. At palmitoylcarnitine concentrations near or above those which produced the maximal fluorescence enhancement in dansyl PE-sarcoplasmic reticulum and dansyl PE-Na,K-ATPase, a clearing of the slight turbidity of the membrane suspensions was observed (data not shown; see Ref. 53). This suggests the possibility of a marked disruption of the membranes which mimics the action of detergents such as sodium dodecyl sulfate and sodium dodecyl sulfate on plasma membranes (53-56). We have recently reported additional physicochemical evidence which suggests that palmitoylcarnitine acts like a detergent (53).

If palmitoylcarnitine is indeed a "naturally occurring detergent," then we suggest a simple mechanism for its effect on enzyme function. The presence of detergent-like action of palmitoylcarnitine could be incorporated into the membrane lipids causing gradual structural and functional changes such as increased permeability (59, 60). The stimulation of sarcoplasmic reticulum Ca²⁺-ATPase activity and of Ca²⁺ binding, as well as the stimulation of ouabain binding to Na,K-ATPase which occurred in the presence of low palmitoylcarnitine concentrations, could result from such a mechanism, i.e. "uncovering of latent sites." Similar effects have been demonstrated with sarcoplasmic reticulum of sarcoplasmic reticulum in eliciting increased fluorescence of dansyl PE-Na,K-ATPase (61, 62) and membrane vesicles containing Na,K-ATPase (63) treated with low concentrations of non-ionic detergents and Pitts et al. (26) reported an enhancement of Ca²⁺-ATPase activity after treatment with 10 μM palmitoylcarnitine. The finding that Na,K-ATPase activity was not stimulated at the same palmitoylcarnitine concentrations that stimulated ouabain binding is not clear, but may reflect a greater sensitivity of hydrolytic activity to the detergent effects of palmitoylcarnitine. Other investigators using various detergents as well as other perturbations have reported inactivation of Na,K-ATPase hydrolytic activity without parallel inactivation of enzyme reactivity to ouabain as measured by [³H]ouabain binding or inhibition of enzyme activity (64-66). These studies indicated that detergent treatment or delipidation of the enzyme, or both, may affect the ATP binding-hydrolytic site without affecting a more stable, or more distant, ouabain binding site on the enzyme. Palmitoylcarnitine-induced increased permeability of sarcoplasmic reticulum membranes may also explain the apparent "uncoupling" of Ca²⁺ binding and Ca²⁺-ATPase activity observed in this study. Inhibition of Ca²⁺ binding occurred at concentrations of palmitoylcarnitine that caused an apparent stimulation of Ca²⁺-ATPase activity. If the sarcoplasmic reticulum membranes were made more permeable, or "leaky" to Ca²⁺, then loss of luminal Ca²⁺ would occur or an inability of the sarcoplasmic reticulum to sequester Ca²⁺ could result. Thus, in spite of an enhanced Ca²⁺-ATPase activity, an apparent inhibition of Ca²⁺ binding would occur (67-69).

2. With increasing concentrations of palmitoylcarnitine (those approaching the critical micelle concentration), incorporation of greater amounts of the fatty acyl ester into the membrane could occur and result in disruption of the basic membrane structure; loss of bulk lipids would result and mixed micelles of lipid, lipoprotein, and palmitoylcarnitine would be formed.

3. Further increases in palmitoylcarnitine concentration could cause separation of boundary lipids from protein and eventual delipidation of the enzymes would occur. These latter two phases (2 and 3) of action could explain the concentration-dependent inhibition of Ca²⁺-ATPase activity and Ca²⁺ binding by the sarcoplasmic reticulum and the inhibition of Na,K-ATPase hydrolytic activity and reactivity to ouabain. Palmitoylcarnitine was more effective as an inhibitor of [³H]ouabain binding to Na,K-ATPase when in the presence of [MgP] or [MgATP] than when in the presence of [MgATPNa] or [MgATPNaK]. Although the reason for this difference in ouabain binding is unclear, it does not appear to be due to an ionic strength effect on the critical micelle concentration of palmitoylcarnitine (since choline chloride did not substitute for NaCl) but rather to a protective effect of sodium on the enzyme (24).

For this proposed mechanism of action of palmitoylcarnitine (i.e. palmitoylcarnitine acts like a detergent to disrupt and delipidate membrane-bound enzymes to cause inactivation of enzyme function) to be plausible, a lipid dependency for the function of Ca²⁺-ATPase and Na,K-ATPase is required. Such is the case for both enzymes. Delipidation of Na,K-ATPase by phospholipase A treatment results in inactivation of catalytic activity (68) and treatment of Ca²⁺-ATPase with detergents, which results in solubilization of the enzyme, causes a reduction in activity (61, 62). Total delipidation with higher concentrations or longer detergent treatment times, results in total and irreversible inhibition (69, 70-72). If palmitoylcarnitine is acting to produce these types of structural changes in the membranes of Ca²⁺-ATPase and Na,K-ATPase preparations, one should be able to correlate them with the observed functional changes. A correlation of this nature was established in this study. The enhancement of fluorescence intensity of dansyl PE-Na,K-ATPase appears to correlate with the loss of Na,K-ATPase activity caused by increasing concentrations of palmitoylcarnitine. The increase in fluorescence probably represents some aspect of the delipidation process as detailed by Johnson et al. (53).

In the preceding discussion, the in vitro effects of palmitoylcarnitine on plasma membrane Na,K-ATPase and sarcoplasmic reticulum Ca²⁺-ATPase functional properties have been characterized with an attempt to identify related changes in membrane structure. Although it is not possible at this time to ascribe any of the in vitro effects of palmitoylcarnitine to an in vivo situation, several investigators have suggested that elevated levels of palmitoylcarnitine which occur during myocardial ischemia (7-9) could act to alter membrane permeability or transport, or both (7-9, 73-75). It is apparent from the present data that palmitoylcarnitine can produce marked changes in two strategic cardiac membrane-enzyme systems in vitro, probably by virtue of its detergent-like action.

Acknowledgments—We wish to gratefully acknowledge Cindy Edelbrock, Jean Taoi, and Denise Robinson for their valuable technical assistance, Gwen Kraft for her expert artwork, and Linda Miner, Publications Assistant.
REFERENCES


Palmitoylcarnitine Effects on Membrane Na,K-ATPase, and Ca²⁺-ATPase


Access the most updated version of this article at http://www.jbc.org/content/254/24/12404.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/254/24/12404.citation.full.html#ref-list-1