The Membrane-Perturbing Properties of Palmitoyl-Coenzyme A and Palmitoylcarnitine. A Comparative Study†

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Received January 10, 1995; Revised Manuscript Received June 1, 1995⊗

ABSTRACT: Fatty acyl-coenzyme A's are temporarily converted into fatty acylcarnitines while transferred across the inner mitochondrial membrane, in their catabolic pathway. In search of an explanation for the need of this coenzyme exchange, the present work describes comparatively the abilities of both kinds of fatty acyl derivatives (represented by palmitoyl-coenzyme A and palmitoylcarnitine) in binding to and perturbing the structure of phosphatidylcholine bilayers in the form of large unilamellar vesicles. Both palmitoyl-coenzyme A and palmitoylcarnitine partition preferentially into the bilayer lipids, so that their free concentration in water is in practice negligible. However, palmitoylcarnitine is able to disrupt the membrane barrier to solutes, leading to vesicle leakage, and, at higher concentrations, it produces complete membrane solubilization, while palmitoyl-coenzyme A produces neither leakage nor solubilization. Palmitoylcarnitine has the properties of many commonly used biochemical detergents. The different behavior of both fatty acyl derivatives helps to explain the need for the transitory coenzyme A/carnitine exchange, and provides a pathogenic mechanism for some genetic defects of mitochondrial fatty acid transport. Other pathophysiological processes in which palmitoylcarnitine has been putatively involved are examined in light of the above results.

When fatty acids are imported into the mitochondrial matrix for catabolic purposes, they exist as coenzyme A derivatives (thioesters) both inside and outside the mitochondria. However, for their passage across the inner mitochondrial membrane, they are transitorily converted into carnitine esters. Although it is generally assumed that the coenzyme A moiety is too large to allow passage across membranes, the fact is that biophysical studies intended to explain the temporary coenzyme A/carnitine exchange in terms of the respective properties of acyl-CoA's and acylcarnitines are not available. Moreover, long-chain acylcarnitines have been involved in a variety of physiological effects for which the physicochemical basis is far from being established, e.g., induction of cell currents in myocytes (Wu & Corr, 1994), activation of the Ca^{2+} release channel in skeletal muscle sarcoplasmic reticulum (El-Hayek *et al.*, 1993), fall of mitochondrial transmembrane potential (Siliprandi *et al.*, 1992), or regulation of phospholipid turnover in erythrocytes (Arduini *et al.*, 1992).

Previous workers have reported the critical micellar concentrations of fatty acyl-CoA's (Constantinides & Steim, 1985; Smith & Powell, 1986; Das & Hajra, 1992) and fatty acylcarnitines (Yalkowski & Zografi, 1970a,b). Also, the interactions of oleoyl-CoA with small unilamellar lecithin vesicles have been characterized by NMR (Boylan & Hamilton, 1992). In order to improve our understanding of the molecular mechanisms of fatty acid import into mitochondria, and especially the reasons for the exchange of coenzymes, we have carried out a series of studies, starting from an evaluation of the micelle-forming properties of fatty acyl-CoA's and fatty acylcarnitines under comparable conditions (Requero *et al.*, 1993). The penetration of phospholipid monolayers by both kinds of fatty acyl derivatives has also been examined (Requero *et al.*, 1995), with the result that palmitoylcarnitine (PaCar),[1] but not palmitoyl-CoA (Pa-CoA), interacts with positive cooperativity with the phospholipids at the air−water interface, thus reducing the surface free energy. This higher affinity of PaCar for the phospholipids might facilitate its diffusion across the inner mitochondrial membrane, or impede its moving back to the cytosol.

In the present paper, we have compared the abilities of Pa-CoA and PaCar, as representative examples of fatty acyl-CoA's and fatty acylcarnitines, respectively, in perturbing the integrity of phospholipid bilayers. Large unilamellar vesicles composed of egg phosphatidylcholine and obtained by the extrusion procedure have been used as reliable membrane models. Amphiphile partition, leakage of vesicle aqueous contents, and solubilization of the lipid bilayers have been independently assessed. There is a well-known methodology, from this and other laboratories, for studying the interaction of surface-active molecules with model membranes (Lichtenberg, 1985, 1993; Goñi *et al.*, 1986; Partearroyo *et al.*, 1992). According to our data, both kinds of amphiphiles partition preferentially in the bilayer, but only the carnitine derivatives impair the permeability barrier and lead to bilayer solubilization.

† Supported in part by Grant PB91/0443 from DGICYT and Grant PGV 9002 from the Basque Government.

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⊗ Abstract published in *Advance ACS Abstracts,* August 1, 1995.

[1] Abbreviations: ANTS, 8-aminonaphthalene-1,3,6-trisulfonate; DPX, *p*-xylylenebis(pyridinium bromide); cmc, critical micellar concentration; D_w, concentration of free detergent in water; K, partition equilibrium constant of an amphiphile; LUV, large unilamellar vesicle(s); PC, phosphatidylcholine; PaCar, palmitoylcarnitine; Pa-CoA, palmitoyl-coenzyme A; R_e^{SAT}, effective surfactant/lipid ratio producing the onset of bilayer solubilization; R_e^{SOL}, effective surfactant/lipid ratio producing full bilayer solubilization.

0006-2960/95/0434-10400$09.00/0

MATERIALS AND METHODS

Materials. Egg phosphatidylcholine (PC) was grade I from Lipid Products (South Nutfield, England). 8-Aminonaphthalene-1,3,6-trisulfonate (ANTS) and *p*-xylylenebis-(pyridinium bromide) (DPX) were purchased from Molecular Probes (Eugene, OR). Palmitoyl-CoA and palmitoylcarnitine were supplied by Sigma (St. Louis, MO) and used without further purification. Radiolabeled derivatives were supplied by New England Nuclear (Stevenage, Herts, U.K.).

Vesicle Preparation and Amphiphile Treatments. Large unilamellar vesicles (LUV) of egg PC were prepared by the extrusion method of Mayer *et al.* (1986), using Nuclepore filters of 0.1 μm pore size. Trapped volume measurements, according to those authors, were compatible with a figure of 1.4 lamellae/vesicle. The actual surface of the phospholipid vesicles is calculated from the measured trapped volume, and their theoretical surface is estimated from the number of lipid molecules in the sample, assuming an average molecular area of 0.60 nm^2. The ratio theoretical/actual surface corresponds to the average number of lamellae per vesicle. Measurements carried out with a Malvern Zetasizer gave an average vesicle diameter of 102 nm. The buffer was, unless otherwise stated, 100 mM PIPES, 140 mM NaCl, pH 7.4.

Aliquots containing the appropriate amounts of Pa-CoA or PaCar in small volumes of the above buffer were added as required to the LUV suspension with vigorous stirring, and the mixtures were left to equilibrate at 37 °C for 24 h. The critical micellar concentrations of Pa-CoA and PaCar, measured as described previously (Requero *et al.*, 1993), were respectively 2.5×10^{-5} and 1.5×10^{-5} M at 37 °C in the above buffer. The final phospholipid concentration was adjusted after determination of lipid phosphorus according to Bartlett (1959).

Measurements of Amphiphile Binding. Amphiphile (Pa-CoA or PaCar) binding to LUV was estimated using radiolabeled derivatives, [^{14}C]Pa-CoA, [^{14}C]PaCar, and [^{3}H]PC. Stock solutions of radiolabeled compounds were 7.4 μM Pa-CoA (0.4 μCi/mL), 18 μM PaCar (1 μCi/mL), and 25 μM PC (1 mCi/mL). These were diluted as appropriate with the corresponding unlabeled compounds prior to use. Amphiphile-treated suspensions of LUV (0.2 mL, 1 mM in PC) were centrifuged in order to separate free from bound amphiphile. LUV sedimentation was facilitated by loading them with a dense sucrose solution, as indicated by Rebecchi *et al.* (1993). Briefly, LUV were prepared from [^{3}H]PC containing lipid in 170 mM sucrose, 100 mM PIPES, pH 7.4, and then diluted 5-fold in the usual 100 mM NaCl, 100 mM PIPES, pH 7.4, buffer. Previous measurements with an Osmomat 30 osmometer (Gonotec, Berlin) had shown that both buffers were virtually isotonic. After ^{14}C-labeled amphiphile treatment and equilibration, as indicated above, the suspensions were centrifuged for 1 h at 25 °C and 90 000 rpm (≈350000*g*) in a TLA 100 Beckman rotor. Virtually all the radioactivity (>95%) was recovered in the pellets, that were dispersed in scintillation liquid and measured for ^{14}C and ^{3}H. Measurements were corrected for the energy overlap between both isotopes. In control experiments in which [^{14}C]PaCar or [^{14}C]Pa-CoA was centrifuged in the absence of liposomes, no radioactivity was pelletted.

Assessment of Bilayer Solubilization. Liposome solubilization can be conveniently measured as a decrease in turbidity, or light scattering, of the suspension (Urbaneja *et al.*, 1988; González-Mañas *et al.*, 1994). In our case, light scattering of the amphiphile-treated LUV suspensions was measured at 90° in a Perkin Elmer LS 50 spectrofluorometer with both monochromators adjusted at 280 nm. Effective detergent:lipid ratios producing the onset or completion of solubilization, respectively R_e^{SAT} and R_e^{SOL}, were estimated as described by Lichtenberg (1985) and by Partearroyo *et al.* (1992). Briefly, there is a linear relationship between the total detergent concentrations (D_T) producing the onset or completion of solubilization and the lipid concentration (L) in the system:

$$D_T = R_e[L + 1/K(R_e + 1)]$$

where the slope R_e corresponds to the *effective* detergent:lipid ratio producing the effect (onset or completion of solubilization). The y intercept corresponds to the concentration of free detergent, D_w, equivalent to its cmc (Lichtenberg, 1985, 1993). In addition, a partition equilibrium constant relates R_e^{SAT} and D_w, so that $R_e^{SAT} = KD_w/(1 - KD_w)$ (Lichtenberg, 1985, 1993). The meaning of K (dimensions = concentration^{-1}) is approximately similar to a (dimensionless) lipid−water partition coefficient.

Determination of Amphiphile-Induced Vesicle Leakage. Vesicle leakage was measured with the ANTS/DPX system, according to Ellens *et al.* (1985). In these assays, the liposomes contained 12.5 mM ANTS, 45 mM DPX, and 100 mM PIPES, pH 7.4. Nonentrapped fluorescence probes were removed with a Sephadex G-75 column, eluted with 140 mM NaCl, 100 mM PIPES, pH 7.4. Isotonicity of the inner and outer buffers was achieved by adding small amounts of NaCl, according to the data supplied by a Osmomat 30 osmometer (Gonotec, Berlin). LUV loaded with ANTS/DPX were treated with Pa-CoA or PaCar and equilibrated as described above, and then diluted with buffer to a 0.1 mM PC concentration. The fluorescence of the resulting suspension was measured in a Perkin Elmer LS-50 spectrofluorometer, at 37 °C, with continuous stirring. The excitation and emission wavelengths were 355 and 530 nm, respectively. Slit widths were 5 nm. In addition, a 470 nm interference filter was used; 0% fluorescence and 100% fluorescence were established, respectively, from measurements of LUV in pure buffer (which gave a stable reading for hours) and in 4 mM Triton X-100.

RESULTS

Partition of Fatty Acyl Derivatives into PC Membranes. Amphiphiles may partition between solvent and bilayer in an aqueous liposome suspension. It is thus essential to quantitate the amounts of free and membrane-bound palmitoyl-CoA and palmitoylcarnitine in vesicle−amphiphile−water systems as a first step in understanding their interactions. For that purpose, ^{3}H-labeled PC vesicles were treated with ^{14}C-labeled palmitoyl-CoA or palmitoylcarnitine under conditions where no phospholipid solubilization occurs (see section below on Bilayer Solubilization by Palmitoylcarnitine). After 24 h equilibration at 37 °C, the vesicles were spun down by ultracentrifugation and the sediments counted for ^{14}C and ^{3}H radioactivity. The procedure is only an approximate one, since the sediments contain obviously a volume of the aqueous medium, but the latter volume is

Table 1: Composition of Amphiphile-Treated Vesicles Sedimented by Centrifugation[a]

amphiphile:phospholipid mole ratio in suspensions (before centrifugation)	amphiphile:phospholipid mole ratio in sediments	
	amphiphile PaCar	amphiphile Pa-CoA
0.1	0.105	0.089
0.2	0.218	0.178
0.5	0.500	0.475

[a] Average values of four measurements. Estimated experimental error is ±10%. Phospholipid concentration in the suspensions was 1 mM.

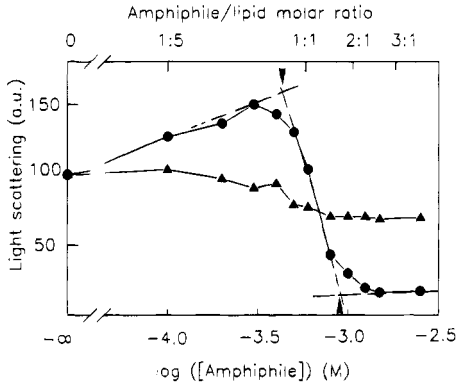

FIGURE 1: Percent change in light scattering of egg phosphatidylcholine large unilamellar vesicle suspensions in the presence of various amphiphile concentrations. PC concentration was 0.5 mM. (●) Palmitoylcarnitine; (▲) palmitoyl-coenzyme A. The arrows indicate the detergent concentrations producing the onset and completion of solubilization.

usually negligible, so that the method is commonly in use in membrane−amphiphile interaction studies (Jackson *et al.*, 1982; González-Mañas *et al.*, 1990). The data in Table 1 show that, for a 1 mM PC concentration and amphiphile concentrations ranging from 0.1 to 0.5 mM, the amphiphile compositions of the vesicles were the same, within experimental error, as those of the original vesicle + amphiphile mixtures in suspension. Since over 95% of the radioactivity is recovered, this means for practical purposes that all of the amphiphile, either PaCar or Pa-CoA, is located in the lipid bilayers. The data on Pa-CoA binding are in good agreement with the NMR observations by Boylan and Hamilton (1992), who suggested that oleoyl-CoA, but not octanoyl-CoA, binds completely to small unilamellar PC vesicles.

Bilayer Solubilization by Palmitoylcarnitine. Solubilization of PC large unilamellar liposomes by PaCar and Pa-CoA was tested by the turbidimetric technique described under Materials and Methods. Figure 1 shows the turbidity of a 0.5 mM suspension of PC LUV as a function of PaCar or Pa-CoA concentration. In the case of PaCar, bilayer solubilization is detected as a decrease in turbidity starting at about 0.5 mM PaCar. Turbidity does not change with further additions of PaCar beyond ≈1 mM. The arrows in the figure indicate the points of onset and completion of solubilization that are introduced in Figure 2 for the computation of R_e^{SAT} and R_e^{SOL}. In contrast, Pa-CoA does not produce any marked decrease in turbidity in the same range of amphiphile concentrations (Figure 1), or even at such high amphiphile:lipid mole ratios as 10:1 (not shown). Chemical analysis also failed to reveal the formation of PC/Pa-CoA mixed micelles. These results indicate that, when added externally to preformed LUV, PaCar but not Pa-CoA is able to induce bilayer solubilization. In the subsolubilizing range of PaCar concentrations, the amphiphile produces an increase in the turbidity of the vesicle suspension, with a maximum at near 0.5 mM PaCar. A similar phenomenon has been observed for other surfactants, and shown by electron microscopy to be due to liposome "fusion" or increase in size (Alonso *et al.*, 1981; Jackson *et al.*, 1982).

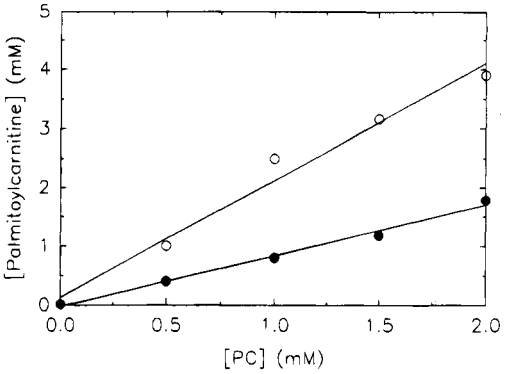

FIGURE 2: Determination of effective detergent:lipid ratios. The total PaCar concentrations producing (●) onset and (○) completion of solubilization at each PC concentration are plotted versus PC concentration. R_e^{SAT} and R_e^{SOL} are estimated respectively from the slopes of the resulting onset and completion straight lines.

When the solubilization assay is performed at different phospholipid concentrations, several interesting parameters may be estimated (Lichtenberg, 1985, 1993; Partearroyo *et al.*, 1992). When the amphiphile concentration producing in each case the onset of solubilization is plotted versus phospholipid concentration (Figure 2), a straight line is obtained, whose slope, in our case 0.78, is the effective amphiphile:lipid mole ratio at which the bilayer becomes saturated with detergent, and solubilization starts. The corresponding line obtained from the amphiphile concentrations leading to complete solubilization has a slope of 1.97 (Figure 2), corresponding to the effective PaCar:lipid mole ratio of mixed micelles. At effective amphiphile:lipid ratios between 0.78 and 1.97, bilayers and micelles coexist. According to theory, both straight lines should intercept the y axis at a value corresponding to the critical micellar concentration of the surfactant. The intercepts for the saturation and solubilization lines are, respectively, 13 μM and 66 μM. The critical micellar concentration of PaCar in pure buffer is 15 μM, measured according to Requero *et al.* (1993). In addition, a bilayer/water partition equilibrium constant K may be obtained from the saturation straight line, such that $R_e^{SAT} = KD_w/(1 - KD_w)$, assuming ideal mixing of lipid and detergent, in dilute aqueous media (Lichtenberg, 1993). The estimated value for PaCar is $K = 34$ mM^{-1}.

Liposomal Leakage Induced by Pa-CoA and PaCar. Release of vesicle aqueous contents in the presence of amphiphiles was tested by the ANTS/DPX method; the results are summarized in Figure 3. Again, Pa-CoA fails to elicit any membrane effect; incubation with PC liposomes for 24 h at 37 °C and a 3.5 Pa-CoA:PC molar ratio does not produce any leakage. PaCar instead leads to the full release of vesicle aqueous contents, almost as an all-or-none phenomenon, at a ≈1:1 PaCar:PC molar ratio, i.e., at the onset of solubilization.

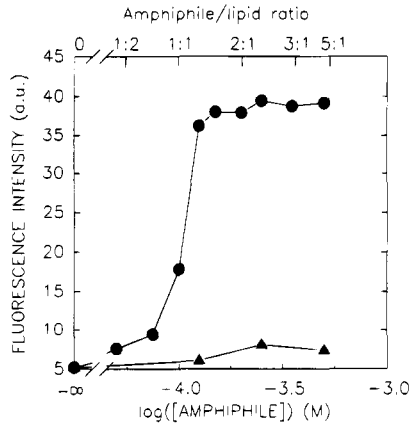

FIGURE 3: Amphiphile-induced liposomal leakage. The fluorescence intensity of a PC LUV suspension (100 μM in lipid) after treatment with various concentrations of (●) PaCar and (▲) Pa-CoA is plotted versus amphiphile concentration.

Time-Resolved Surfactant Action. The above results have been obtained under equilibrium conditions, i.e., after 24 h equilibration, since preliminary experiments had detected no change after this period of time. In this section, the solubilization and leakage effects induced by PaCar are studied as a function of time. Figure 4 shows two representative cases, namely, $R_e = 0.8$ and $R_e = 2.0$, corresponding to the onset and completion of solubilization, respectively. In both cases, equilibrium is reached in less than 10 h. For $R_e = 2.0$, the kinetics of both leakage and solubilization are very similar, while for $R_e = 0.8$ (as well as for other R_e ratios in the micelle−vesicle coexistence region, data not shown) leakage reaches equilibrium faster than solubilization. For $R_e = 0.8$, the time required for 50% leakage is 2.6 h, vs 4.5 h for 50% solubilization.

DISCUSSION

Palmitoylcarnitine as a Surfactant. Palmitoylcarnitine is a common metabolite that shares with biochemically employed detergents the ability to convert a lipid bilayer into a suspension of phospholipid−surfactant mixed micelles. A comparison of the physical properties of Pa-Car with those of commonly used detergents may be illustrative. The critical micellar concentration of PaCar is in the 10^{-5} M range under a variety of conditions, well below the corresponding values for detergents such as Triton X-100 (3.7×10^{-4} M), CHAPS (3.5×10^{-3} M), or Hecameg (1.65×10^{-2} M) (Requero *et al.*, 1993). The shorter chain analogues of PaCar display significantly higher cmc values, e.g., $\approx 10^{-3}$ M for dodecylcarnitine (Requero *et al.*, 1993). A low value of cmc is generally indicative of a high degree of intermolecular hydrophobic interaction. In the presence of membranes, it should lead to a high degree of partition into the lipid phase, as is indeed the case. The binding experiments (Table 1) indicate that there is virtually no surfactant in the water phase; in good agreement with those, the data derived from Figure 2 indicate a very low concentration of a free detergent in the buffer, actually very near the cmc. Also the membrane/water partition equilibrium constant K (34 mM^{-1}) is much higher than those of, e.g., Triton X-100 (1.9 mM^{-1}; Partearroyo *et al.*, 1992) or Hecameg (0.08 mM^{-1}; Ruiz *et al.*, 1994).

Gruver and Pappano (1993) found for PaCar and sarcoplasmic reticulum a membrane/water partition coefficient of

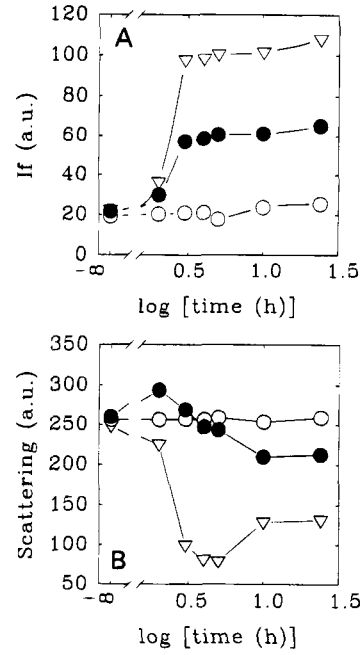

FIGURE 4: Time-resolved membrane effects of palmitoylcarnitine. (A) Leakage is plotted as an increase in fluorescence, and (B) solubilization is presented as a decrease in light scattering, both versus time. The PaCar:PC R_e effective molar ratios were 0 (○), 0.8 (●), and 2.0 (▽).

1.2×10^5, expressed as "(mass of PaCar in pellet/mass of lipid)/(mass of PaCar in supernatant/mass of buffer)". From our experimental data, we can similarly estimate average partition coefficients of 6.5×10^3 and 3.8×10^3 for PaCar and Pa-CoA, respectively, in egg PC bilayers. The observed relatively smaller affinity of these amphiphiles for the membranes may be explained by the different phospholipid headgroups in the sarcoplasmic reticulum and liposomal phospholipids or, more probably, to the fact that the proteins in the cell membranous system will also bind some PaCar, thus increasing the apparent partition coefficient. In any case, our experimental values still indicate a very high affinity of PaCar and Pa-CoA for the lipid phase. This has the practical consequence that the total amount of surfactant in a membrane suspension is virtually equal to the amount of membrane-bound surfactant. In turn, this allows direct comparison of experiments with similar "total surfactant/lipid" ratios, even at differing lipid concentrations.

The effective detergent:lipid ratios between which bilayers and micelles coexist, i.e., R_e^{SAT} and R_e^{SOL} (Lichtenberg, 1993), are for PaCar 0.78 and 1.97, respectively. These values are close to the corresponding ones for biochemically used detergents, e.g., Triton X-100 (0.71 and 3.0; Partearroyo *et al.*, 1992) or Hecameg (1.4 and 2.6; Ruiz *et al.*, 1994). The similarity of R_e values for surfactants with otherwise different properties, notably cmc and K, has been pointed out previously (Lichtenberg, 1993). PaCar also resembles other known detergents in that it produces an increase in light scattering by the liposomal suspension prior to solubilization (Figure 1), that, at least for other surfactants, has been shown to be due to an increase in vesicular size (Urbaneja *et al.*, 1988). Vesicle growth in size occurs in the absence of major leakage (compare Figures 1 and 3 for a 1:2 PaCar:PC ratio); this might suggest that size growth occurs via a nonleaky vesicle fusion mechanism. The fact that vesicle leakage occurs at lower PaCar concentrations

(Figure 3) and reaches equilibrium in some cases faster than solubilization (Figure 4) confirms that leakage and solubilization are separate phenomena caused by the surfactant, as suggested previously (Ruiz *et al.*, 1988). Also since complete leakage (in 24 h) occurs at the lower end of the range of coexistence of vesicles and micelles (Figure 3), within this range the reversible vesicle−micelle transformation is dynamic in nature.

Also in conjunction with other known amphiphiles, PaCar has an antihemolytic effect when added to osmotically stressed human erythrocytes (Gruver & Pappano, 1993), as shown previously for other surface-active molecules (Seeman, 1972; Helenius & Simons, 1975).

One striking property of PaCar as a surfactant is the very slow equilibration time (of several hours) with a virtually unilamellar vesicle preparation (Figure 4). Kinetic studies of membrane−surfactant interaction are scarce, but the available data indicate that equilibrium is reached in seconds for Triton X-100 (Alonso *et al.*, 1987) or minutes for lysolecithin (Elamrani & Blume, 1982) and "unilamellar" (i.e., average number of lamellae per vesicle <2) liposomes. The origin of our slow kinetics cannot be explained at present.

Differences between PaCar and Pa-CoA. Various differences had been detected in our previous comparative studies between carnitine and coenzyme A acyl derivatives: acyl-CoA's were often more sensitive than acylcarnitines to environmental factors, with respect to their micellization properties (Requero *et al.*, 1993). Palmitoylcarnitine was also observed to interact with phospholipid monolayers with a degree of cooperativity that was not found for palmitoyl-CoA (Requero *et al.*, 1995). The differences between both metabolites are more apparent in the present work: although both of them partition completely with the lipid phase in a liposome−amphiphile−water system (Table 1), Pa-CoA, as opposed to PaCar, appears to be totally unable to bring about the transition from the lamellar to the micellar phase or to break down the membrane barrier (Figures 1 and 3). At least two different reasons may be proposed to explain such a difference; *either* the polar headgroup of Pa-CoA is too large for the molecule to act as a proper detergent *or* the insertion of Pa-CoA in the bilayer is such that it does not disturb the lamellar architecture. It has been shown for cell (Egan *et al.*, 1976) and model (Partearroyo *et al.*, 1995) membranes that an optimum hydrophile/lipophile balance exists for detergents, so that molecules far from this optimum cannot solubilize lipid bilayers. It is very reasonable to assume that Pa-CoA has an excessively large hydrophilic moiety with respect to the hydrophobic palmitoyl residue. Also relevant in this respect is the ability of PaCar to undergo lamellar−hexagonal (H_I) transitions (Stinson, 1990), a behavior that has not been observed for Pa-CoA. Moreover, it is accepted that detergents exert their effect because their "wedge-like" or "inverted conical" shape impairs the balance between hydrophobic and hydrophilic interactions that stabilize the lamellar phase (Alonso *et al.*, 1981; Grunner *et al.*, 1988). The insertion of Pa-CoA in bilayers has not been studied in detail as yet, but recent infrared spectroscopic studies suggest that it may bind the phospholipid bilayers in such a way that the bilayer architecture is not modified at large (Echabe *et al.*, 1995).

Boylan and Hamilton (1992) have reported formation of PC/oleoyl-CoA mixed micelles above 15 mol % oleoyl-CoA. However, sonicated PC vesicles were used in those studies, and such vesicles are known to be particularly sensitive to the action of amphipathic agents (Alonso *et al.*, 1981, 1982).

Pathophysiological Correlations. The exchange of fatty acyl-bound coenzymes during fatty acyl transport across the inner mitochondrial membrane, from coenzyme A to carnitine and back to coenzyme A, has been traditionally associated with the idea that fatty acylcarnitines, but not their coenzyme A analogues, would be able to cross the inner membrane through passive diffusion. Even recent biochemistry textbooks rely on that idea, despite the fact that already in 1975 evidence was presented for a translocase system for carnitine and acylcarnitines in mitochondria (Pande, 1975). Our results in this and a preceding paper (Requero *et al.*, 1995) show indeed that the membrane-perturbing abilities of palmitoylcarnitine are remarkably higher than those of palmitoyl-CoA. However, at least two lines of evidence prevent an interpretation of our data in support of the passive diffusion hypothesis. First, recent findings of carnitine−acylcarnitine translocase deficiency in humans reveal that the lack of this transporter is not compatible with life (Pande & Murthy, 1994), and second, careful measurements of radioactively-labeled palmitoylcarnitine diffusion across plane lipid bilayers were unable to detect any translocation of radioactive material (F. Homblé and M. A. Requero, unpublished results).

In our previous paper, it was pointed out that the observed cooperative binding of PaCar but not of Pa-CoA to phospholipids might secure the fatty acyl residues inside the membrane, preventing them from "swimming back" to the cytosol (Requero *et al.*, 1995). This would explain the advantage of exchanging the bulky, polar coenzyme A for the much smaller carnitine. The present observation that PaCar may impair the membrane barrier properties explains that PaCar is only a transient intermediate within the inner mitochondrial membrane, its possible *local* accumulation (and subsequent mitochondrial uncoupling) being prevented both by the inner carnitine:coenzyme A palmitoyltransferase and by the reversible operation of the translocase (Pande & Murthy, 1994). The surfactant properties of PaCar may be also at the origin of some of the severe disorders arising from the deficiency of the inner carnitine palmitoyltransferase (Demaugre *et al.*, 1991; Hug *et al.*, 1991; El-Hayek *et al.*, 1992), although the characteristic muscle necrosis appears to be due rather to a direct deregulating effect of PaCar on sarcoplasmic reticulum Ca^{2+} release (El Hayek *et al.*, 1993).

The results in this paper may also be discussed in light of other recently published pathophysiological effects of PaCar or Pa-CoA. In particular, the role of long-chain acylcarnitines in the pathogenesis of myocardial infarction may be of interest. Fatty acylcarnitines increase over 3-fold in ischemic myocardium within 2 min (DaTorre *et al.*, 1991). More recently, Wu and Corr (1994) have shown that PaCar modifies sodium currents and elicits a transient inward current in isolated rabbit ventricular cells. According to the latter authors, the primary effect of PaCar would be to induce a Na^+ inward current, which would in turn elicit an increase in intracellular Ca^{2+} via the Na^+/Ca^{2+} exchanger, leading to the development of delayed after-depolarizations and triggered activity. Wu and Corr (1994) point out that similar "Na^+ channel openings" have been observed with lysophosphatidylcholine (Undrovinas *et al.*, 1992). Of course our data on vesicle leakage induced by PaCar are in agreement

with this observation and may explain the role of fatty acylcarnitines in acute myocardial ischemia. El Hayek *et al.* (1993) also show an increased ion flow in the presence of PaCar, namely, Ca^{2+} release from sarcoplasmic reticulum. However, the mechanism appears in this case to be one of direct interaction with the channel (ryanodine receptor), since the effect is observed at low PaCar concentrations (50 μM) and, above all, a similar effect is detected with Pa-CoA. In a different context, both Pa-CoA and PaCar appear to be similarly active in dissipating mitochondrial membrane potentials at 50−100 μM (Siliprandi *et al.*, 1992); the authors explain the effect in terms of disruption of membrane architecture, although it is not clear, in light of the evidence in this paper, why Pa-CoA should impair the mitochondrial membrane structure. Finally, the proposed existence of a fatty acyl reservoir in the form of acylcarnitines, in red blood cell phospholipid metabolism (Arduini *et al.*, 1992), could also be criticized as endangering membrane stability, after the data in the present paper.

ACKNOWLEDGMENT

M.A.R. held a predoctoral studentship from the Basque Government. We are grateful to Dr. Fabrice Homblé (Université Libre de Bruxelles) for his help with the measurements using plane lipid bilayers.

REFERENCES

Alonso, A., Villena, A., & Goñi, F. M. (1981) *FEBS Lett. 123*, 200−204.
Alonso, A., Sáez, R., Villena, A., & Goñi, F. M. (1982) *J. Membr. Biol. 67*, 55−62.
Alonso, A., Urbaneja, M. A., Goñi, F. M., Carmona, F. G., Cánovas, F. G., & Gómez-Fernández, J. C. (1987) *Biochim. Biophys. Acta 902*, 237−246.
Arduini, A., Mancinelli, G., Radatti, G. L., Dottori, S., Molajoni, F., & Ramsay, R. R. (1992) *J. Biol. Chem. 267*, 12673−12681.
Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466−468.
Boylan, J. G., & Hamilton, J. A. (1992) *Biochemistry 31*, 557−567.
Constantinides, P. P., & Steim, J. M. (1985) *J. Biol. Chem. 260*, 7573−7580.
Das, A. K., & Hajra, A. L. (1992) *J. Biol. Chem. 267*, 9731−9736.
DaTorre, S. D., Creer, M. H., Pogwizd, S. M., & Corr, P. B. (1991) *J. Mol. Cell. Cardiol. 23*, 11−22.
Demaugre, F., Bonnefont, J. M., Colonna, M., Lepanec, C., Leroux, J. P., & Sandobray, J. M. (1991) *J. Clin. Invest. 87*, 859−864.
Echabe, I., Requero, M. A., Goñi, F. M., Arrondo, J. L. R., & Alonso, A. (1995) *Eur. J. Biochem.* (in press).
Egan, R. W., Jones, M. A., & Lehninger, A. L. (1976) *J. Biol. Chem. 251*, 4442−4447.
Elamrani, K., & Blume, A. (1982) *Biochemistry 21*, 521−526.
El-Hayek, R., Valdivia, C., Valdivia, H. H., & Coronado, R. (1992) *Biophys. J. 61*, 137A.
El-Hayek, R., Valdivia, C., Valdivia, H. H., Hogan, K., & Coronado, R. (1993) *Biophys. J. 65*, 779−789.
Ellens, H., Bentz, J., & Szoka, F. C. (1985) *Biochemistry 24*, 3099−3106.
Goñi, F. M., Urbaneja, M. A., Arrondo, J. L. R., Alonso, A., Durrani, A. A., & Chapman, D. (1986) *Eur. J. Biochem. 160*, 659−665.
González-Mañas, J. M., Virto, M. D., Gurtubay, J. I. G., & Goñi, F. M. (1990) *Eur. J. Biochem. 188*, 673−678.
González-Mañas, J. M., Kaschny, P., & Goñi, F. M. (1994) *J. Phys. Chem. 98*, 10650−10654.
Grunner, S. M., Tate, M. W., Kirk, G. L., So, P. T. C., Turner, D. C., Keane, D. T., Tilcock, C. P. S., & Cullis, P. R. (1988) *Biochemistry 27*, 2853−2866.
Gruver, C., & Pappano, A. J. (1993) *J. Mol. Cell. Cardiol. 25*, 1275−1284.
Helenius, A., & Simons, K. (1975) *Biochim. Biophys. Acta 415*, 29−79.
Hug, G., Bove, K. E., & Sonkop, S. (1991) *N. Engl. J. Med. 325*, 1862−1864.
Jackson, M. L., Schmidt, C. F., Lichtenberg, D., Silinan, B. J., & Albert, A. D. (1982) *Biochemistry 21*, 4576−4582.
Lichtenberg, D. (1985) *Biochim. Biophys. Acta 821*, 470−478.
Lichtenberg, D. (1993) in *Biomembranes. Physical Aspects* (Shinitzki, M., Ed.) pp 63−95, VCH, Weinheim.
Mayer, L. O., Hope, M. J., & Cullis, P. R. (1986) *Biochim. Biophys. Acta 858*, 161−168.
Pande, S. V. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 883−887.
Pande, S. V., & Murthy, M. S. R. (1994) *Biochim. Biophys. Acta 1226*, 269−276.
Partearroyo, M. A., Urbaneja, M. A., & Goñi, F. M. (1992) *FEBS Lett. 302*, 138−140.
Partearroyo, M. A., Alonso, A., Goñi, F. M., Tribout, M., & Paredes, S. (1995) *J. Colloid Interface Sci.* (submitted for publication).
Rebecchi, M., Peterson, A., & McLaughlin, S. (1992) *Biochemistry 31*, 12742−12747.
Requero, M. A., Goñi, F. M., & Alonso, A. (1993) *J. Colloid Interface Science 161*, 343−346.
Requero, M. A., González, M., Goñi, F. M., Alonso, A., & Fidelio, G. (1995) *FEBS Lett. 357*, 75−78.
Ruiz, J., Goñi, F. M., & Alonso, A. (1988) *Biochim. Biophys. Acta 937*, 127−134.
Ruiz, M. B., Prado, A., Goñi, F. M., & Alonso, A. (1994) *Biochim. Biophys. Acta 1193*, 301−306.
Schurtenberger, P., Mazer, N. A., & Kansig, W. (1985) *J. Phys. Chem. 89*, 1042−1059.
Seeman, P. (1972) *Pharmacol. Rev. 24*, 583−655.
Siliprandi, D., Biban, C., Testa, S., Toninello, A., & Siliprandi, N. (1992) *Mol. Cell. Biochem. 116*, 117−123.
Smith, R. H., & Powell, G. L. (1986) *Arch. Biochem. Biophys. 244*, 357−360.
Stinson, R. H. (1990) *Chem. Phys. Lipids 52*, 29−39.
Undrovinas, A. I., Fleidervish, I. A., & Makielski, J. C. (1992) *Circ. Res. 71*, 1231−1241.
Urbaneja, M. A., Goñi, F. M., & Alonso, A. (1988) *Eur. J. Biochem. 173*, 585−588.
Wu, J., & Corr, P. B. (1994) *Am. J. Physiol. 266*, H1034−H1046.
Yalkowski, S. H., & Zografi, G. (1970a) *J. Pharm. Sci. 59*, 798−802.
Yalkowski, S. H., & Zografi, G. (1970b) *J. Colloid Interface Sci. 34*, 525−533.

BI950051M