Uptake and metabolism of palmitate by isolated cardiac myocytes from adult rats: involvement of sarcolemmal proteins

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Abstract The precise mechanism of uptake of long-chain fatty acids (FA) by cardiac myocytes is incompletely understood. We examined the involvement of sarcolemmal proteins in the initial uptake of FA by isolated rat cardiac myocytes, and the relation between initial uptake and metabolism. Cardiac myocytes were incubated in the presence of 90 μM [1-^{14}C]palmitate complexed to 300 μM bovine serum albumin (BSA), presenting a physiologically relevant condition. During initial palmitate uptake (3 min), 56% of the intracellularly sequestered palmitate was esterified, and an additional 21% converted into oxidation intermediates. Varying the palmitate/BSA molar ratio revealed saturation kinetics with the apparent K_m for cellular palmitate uptake (435 μM) to be comparable to those for esterification (465 μM) and oxidation (222 μM). Varying the BSA concentration at a fixed palmitate/BSA molar ratio also showed saturation of uptake at increasing concentrations, with an apparent K_m for BSA of 23 μM. Changes in palmitate metabolism induced by changes in glucose utilization were accompanied by identical effects on palmitate uptake. Addition of lactate also inhibited both oxidation and uptake of palmitate, but had no effect on esterification. Virtually complete inhibition of palmitate oxidation by etomoxir inhibited palmitate uptake for 50%, while decreasing esterification by 33%. In the presence of phloretin and trypsin, palmitate uptake and metabolism were inhibited 76–88%, and in the presence of sulfo-N-succinimidyloleate by 53%.■ It is concluded that *a*) the bulk of sarcolemmal palmitate translocation occurs by membrane-associated FA-binding proteins, most likely assisted by albumin binding proteins without regulatory function, and *b*) palmitate uptake is most likely driven by its rapid intracellular metabolic conversion.—**Luiken, J. J. F. P., F. A. van Nieuwenhoven, G. America, G. J. van der Vusse, and J. F. C. Glatz.** Uptake and metabolism of palmitate by isolated cardiac myocytes, from adult rats: involvement of sarcolemmal proteins. *J. Lipid Res.* 1997. **38:** 745–758.

Supplementary key words long-chain fatty acids • albumin binding protein • fatty acid translocase • acyl-CoA synthetase • carnitine acyltransferase I • glucose • lactate

In cardiac myocytes, oxidation of long-chain fatty acids (FA) is the predominant source of energy needed for proper electromechanical function (1). As the capacity of these cells to store FA in lipid pools is limited, cardiac myocytes are dependent on a continuous supply of these substrates from the blood stream. In the vascular space and the interstitium, FA are almost completely complexed to albumin. Prior to their cellular uptake, FA dissociate from albumin and cross the sarcolemma. The driving force for their cellular uptake is considered to be the FA gradient across the plasma membrane (1). However, the mechanism of transsarcolemmal transport of FA is still a topic of controversy. Based on the lipophilic properties of FA and, consequently their ability to dissolve easily into cellular membranes, it has been proposed that their transmembrane transport is governed by simple, non-facilitated diffusion (2–5). However, the observation that cellular uptake of FA displays saturation kinetics, and is inhibited by proteases and by non-metabolizable fatty acid analogues, have led to the suggestion that plasma membrane proteins are involved in this process (6–8).

Till now, five proteins have been put forward as candidates to participate in the transmembrane translocation of FA (reviewed in Refs. 9, 10), of which a 40 kDa plasma membrane fatty acid-binding protein (FABP$_{PM}$) (7, 11), an 88 kDa fatty acid translocase (FAT) (12, 13), and a 63 kDa fatty acid-transport protein (FATP) (14) have been found to be present in the sarcolemma of cardiac myocytes. Recent observations that the sarcolemma of

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cardiac myocytes (15) and the plasma membrane of adipocytes (16) possess high-affinity albumin-binding sites suggest that in addition to these FA binding proteins membrane-associated albumin binding proteins are also involved in cellular FA uptake.

At present no agreement exists on the possible involvement of sarcolemmal proteins in the uptake and subsequent metabolism of FA by cardiac cells (10). In order to explore this issue in more detail, we investigated the initial uptake kinetics of palmitate and its relation to the formation of palmitate metabolites, at physiologically relevant substrate concentrations, and then applied a variety of interventions directed to *a*) inhibit membrane transport processes, and/or *b*) modulate intracellular utilization of FA. Our first aim was to establish the involvement of sarcolemmal proteins in the initial uptake of FA. For this, we explored the importance of albumin binding proteins in FA uptake by studying uptake kinetics at varying BSA concentrations, and also examined the contribution of membrane-associated FA binding proteins to the FA uptake process by using agents with the potency to interfere with protein-mediated membrane transport. Our second goal was to investigate to what extent initial palmitate uptake kinetics are influenced by subsequent metabolism. For this, we measured palmitate metabolism during its initial uptake phase and studied the influence of effectors of FA metabolism and of competitive substrates on palmitate uptake.

The results presented in this study indicate that palmitate uptake by cardiac myocytes occurs mainly via a protein-mediated mechanism and is driven by a rapid intracellular metabolic conversion.

MATERIALS AND METHODS

Materials

[1-^{14}C]palmitic acid was obtained from Amersham International, U.K. BSA (fraction V, essentially fatty acid free), butanedione monoxime, phloretin, trypsin, and collagenase were obtained from Sigma. Sulfo-*N*-succinimidyloleate was kindly provided by Dr. N. A. Abumrad (Stony Brook, NY); etomoxir was a gift from Dr. H. P. O. Wolf (Byk Gulden Pharmaceuticals, Konstanz, BRD). Eicosadiynoic acid was purchased from Affinity Research Products Ltd, Exeter, U.K..

Isolation of cardiac myocytes

Cardiac myocytes were prepared from adult male Lewis rats (200–250 g) according to the procedure described by Fischer, Rose, and Kammermeier (17). For this, hearts were perfused at 37°C using a Langendorff system with Krebs-Henseleit bicarbonate medium (KHB) supplemented with 11 mM glucose and 0.4 mM taurine, and equilibrated with a carbogen (95% O_2, 5% CO_2) gas phase (medium A) in a non-recirculating manner in order to remove the blood from the coronary system. Subsequently, the hearts were perfused in a recirculating manner with medium A supplemented with 0.7% (w/v) bovine serum albumin (BSA), 15 mM butanedione monoxime, and 0.08% collagenase. $CaCl_2$ was added to the perfusion medium in two steps with a 1-min interval to a final concentration of 0.2 mM. After 20 min of recirculating perfusion, the heart was removed from the catheter, carefully dissected and further digested by incubation for 5 min at 37°C in 20 ml medium A containing 0.04% collagenase, 1.4% BSA, and 0.2 mM $CaCl_2$. Incompletely digested pieces of tissue were gently minced. The suspension then was further incubated for 5 min at 37°C while the $CaCl_2$ concentration was gradually raised to 1.0 mM. Subsequently, the suspension was filtered through a 0.2-mm nylon gauze and centrifuged for 1 min at 20 g. The cell pellet was resuspended in medium A supplemented with 1.0 mM $CaCl_2$ and 2% BSA (medium B), washed twice, and suspended in a volume of 15–20 ml. The latter cell suspension was allowed to recover for 1.5–2 h at room temperature in closed and slowly rotating 50-ml tubes with the gas phase being refreshed every 30 min. At the end of the recovery period, cells were washed and suspended in medium B and used for metabolic studies, provided that >80% of the cells were rod-shaped and excluded trypan blue. The yield of cardiac myocytes amounted to 200–300 mg wet mass per g of heart tissue.

Preparation of the palmitate–albumin complex

Tracer amounts of [1-^{14}C]palmitate and known quantities of unlabeled palmitate, both dissolved in ethanol, were mixed with 10 ml water containing KOH (1.5 times the amount of palmitate on molar basis). After evaporation of ethanol at 45°C under nitrogen gassing, the palmitate solutions were slowly added to a stirred concentrated BSA-containing solution (40 ml) and then diluted to the desired palmitate and BSA concentrations in medium A supplemented with 1.0 mM $CaCl_2$.

Incubation conditions

Cells (1.5 ml; 5–8 mg wet mass/ml), suspended in medium A supplemented with 1.0 mM $CaCl_2$ and varying BSA concentrations, were preincubated in capped 20-ml glass vials for 15 min under shaking conditions at 37°C. At the start of the incubation, 0.5 ml of the

desired substrate solution was added. Incubations were performed for variable time intervals at 37°C.

Assays of uptake and metabolism of [^{14}C]palmitate

In order to study both uptake and intracellular metabolic conversion of palmitate by cardiac myocytes, two identical incubations were performed in triplicate for each condition tested. From one incubation samples were withdrawn for determination of cellular uptake and for assessment of palmitate incorporation into cellular lipid pools. The other incubation served to measure the oxidation of palmitate into CO_2 and (acid-soluble) oxidation intermediates.

For determination of cellular uptake of palmitate, 0.5-ml aliquots of one cell incubation were quickly transferred to 4.5 ml ice-cold medium A containing 1.0 mm $CaCl_2$, 0.1% BSA, and 0.2 mm phloretin, as described by Stremmel and Berk (18). Cells then were centrifuged at 60 g for 1 min and washed three times with the dilution medium. The final cell pellet was dissolved in 5 ml formula 989 scintillation fluid (Packard, Meriden, CT) and assayed for radioactivity. The validity of this stop procedure was indicated by the finding that prolonged incubation of cell samples for time periods up to 2 h in ice-cold dilution medium before centrifugation and washing did not significantly alter the amount of radioactivity measured in the cell pellet (data not shown).

For determination of incorporation of palmitate into cellular lipid pools, a 1.0-ml aliquot of the same cell incubation was quickly transferred to 9.0 ml ice-cold medium A containing 1.0 mm $CaCl_2$ and 0.5% BSA as modified from DeGrella and Light (2). Subsequently, cells were centrifuged and washed as described above. The final cell pellet was subjected to lipid extraction and thin-layer chromatography performed according to Van Der Vusse and Roemen (19). Spots were detected by spraying plates with rhodamine 6G, scraped from the plate, and mixed with 0.5 ml methanol. Thereafter, 5 ml formula 989 scintillation fluid was added, and radioactivity was determined. The recovery of radioactivity as determined with an internal standard was found to be 85% (20). Therefore, experimental values obtained with this procedure were multiplied by 100/85. The validity of this stop procedure was established analogous to that described for the determination of cellular palmitate uptake (data not shown).

Oxidation of palmitate was determined in a parallel incubation after addition of perchloric acid (final concentration 0.5 m) to the cell suspension at the end of the incubation period. As [1-^{14}C]palmitate was applied in this study, labeled chain-shortened fatty acid-intermediates were not formed, and the labeled oxidation products consisted of acetyl-CoA, citric acid cycle intermediates, and CO_2. Production of $^{14}CO_2$ was measured after base trapping according to Glatz, Jacobs, and Veerkamp (21). Production of labeled acetyl-CoA and citric acid cycle intermediates was determined in the acid-soluble supernatant obtained after centrifugation for 2 min at 15,000 g. In order to remove the remaining traces of labeled palmitate, and thus make the recovery of oxidation products complete, 0.9 ml of the latter supernatant was extracted once with 4.5 ml chloroform–methanol 2:1 (by volume).

As the labeled CO_2 produced initially was taken up by cells as palmitate but, after its formation, immediately escaped from the cell, it was not retrieved in the assay for determination of palmitate uptake. Therefore, the term total palmitate uptake is introduced, which refers to the sum of measured palmitate uptake (radioactivity in cell pellet) and of $^{14}CO_2$ production.

Assay of uptake of [^{3}H]deoxyglucose

Cells (1.5 ml; 5–8 mg wet mass/ml) were preincubated in medium B without glucose under conditions exactly as described for incubations with [^{14}C]palmitate. At the start of the incubation, [^{3}H]deoxyglucose in 0.5 ml medium B was added to a final concentration of 100 μM. Incubations were performed for 20 min at 37°C.

For determination of cellular uptake of deoxyglucose, exactly the same procedure was applied as described for uptake of palmitate. Deoxyglucose uptake was found to be linear with time for at least 40 min and linear with the deoxyglucose concentration up to at least 300 μM (data not shown).

Other procedures

For determination of ATP, cell samples were acidified with ice-cold perchloric acid (final concentration 15%). After removal of protein by centrifugation, the extract was neutralized to pH 7 with a solution consisting of 2 N KOH and 0.3 m MOPS. ATP was determined fluorimetrically according to Williamson and Corkey (22).

Cellular wet mass was determined from cell samples taken during the preincubation period, after centrifugation for 2–3 s in a microcentrifuge and subsequent removal of the supernatant.

Protein was quantified according to the procedure of Lowry et al. (23). The amount of total protein per g of wet mass of cardiac myocytes was found to be 178 ± 44 mg (mean ± SD; n = 8).

Calculations and statistics

Non-protein bound palmitate concentrations were calculated from the total palmitate concentration and the fatty acid/BSA molar ratio according to Richieri,

Anel and Kleinfeld (24). Values for apparent K_m and V_{max} for cellular palmitate uptake and metabolism were determined using initial palmitate uptake rates or rates of palmitate metabolism plotted against the (calculated) non-protein bound fatty acid concentration, according to Michaelis-Menten (25).

All data are presented as means ± SD for the indicated number of myocyte preparations. Statistical difference between groups of observations was tested with a paired Student's t-test. P values equal to or less than 0.05 were considered significant.

RESULTS

Viability of cardiac myocytes

Cellular ATP was determined to monitor the viability of cardiac myocytes during cell incubation. Under control conditions, i.e., in the presence of 90 µM palmitate and a molar palmitate/BSA ratio of 0.3 without any further additions, the ATP content of cells was determined at the start of the incubation period and amounted to 10.3 µmol/g wet mass. After 30 min of incubation this value remained essentially the same, indicating that there was no decrease in cell viability during the entire incubation period (data not shown). Upon changing the concentration of BSA and/or palmitate, or upon addition of various modulators of FA uptake and metabolism during cell incubations, the cellular ATP content, as measured after 30 min of incubation, did not deviate more than 10% from the value measured under control conditions, indicating that the effects, if any, of the different treatments were not due to gross changes in cell viability. The latter conclusion was also supported by microscopic inspections, made after 30 min of incubation, which revealed that the percentage of rod shaped cells and/or of cells excluding trypan blue was not significantly altered by the various treatments.

A minor proportion of cells (less than 20%) in the cell incubations usually was accessible for trypan blue. To test whether these, apparently damaged, cells were able to participate in palmitate utilization, rod-shaped cardiac myocytes were freeze–thawed prior to incubation. This treatment resulted in a complete loss of trypan blue exclusion by the cells. Palmitate uptake and metabolism were not detectable using this cell preparation, indicating that nonspecific binding of palmitate to membranes or palmitate oxidation by cell-free mitochondria derived from damaged cells did not likely interfere with measurements of palmitate utilization by their viable counterparts.

Kinetic studies

Palmitate uptake and metabolism by cardiac myocytes as a function of time. When the time course of palmitate uptake was studied under a physiologically relevant condition, i.e., at exogenous concentrations of palmitate and BSA of 90 and 300 µM, respectively, a rapid initial phase was observed that was linear with time for at least 3 min, with a rate of 21.7 ± 3.0 nmol/min per g wet mass (mean ± SD; n = 5; **Fig. 1A**). Thereafter, the rate of uptake gradually declined (Fig. 1B). Based on these findings, which will be discussed below, we will refer to the period between 0 and 3 min after palmitate addition as the initial uptake phase.

After 30 s of palmitate uptake, 50% of the palmitate label, sequestered by the cardiomyocytes, was already found to have been esterified into cellular lipid pools. This percentage remained virtually constant, varying between 47 and 56%, during the entire initial uptake phase (3 min; Fig. 1A). An additional 16% of the sequestered palmitate label was present as oxidation intermediates, most likely acetyl-CoA and citric acid cycle intermediates, which percentage also remained constant (between 16 and 21%) during the initial uptake phase (Fig. 1A). Within this latter period there was hardly any production of labeled CO_2 (Fig. 1A). Assuming levels of palmitoyl-CoA and palmitoylcarnitine to be low (1), together these results indicate that within 30 s approximately 66%, and, at the end of the initial uptake phase (3 min), about 78% of the sequestered palmitate had already been metabolized.

When the entire 30 min of total palmitate uptake is considered, the percentage of labeled palmitate that was esterified increased slightly from 56% at the end of the initial uptake phase to 59% after 30 min of palmitate uptake (Fig. 1B). At each time point studied during the 30-min incubation period, triacylglycerols accounted for about 70%, and phospholipids for about 30% of esterified palmitate (data not shown). The rate of production of radiolabeled oxidation products remained at the level observed after 3 min, while $^{14}CO_2$ production from labeled palmitate was detectable after 10 min and increased linearly with time for at least 2 h (Fig. 1B and data not shown). The delay in $^{14}CO_2$ production most likely relates to the fact that the citric acid cycle first has to be equilibrated with label. The amount of palmitate that is taken up but not metabolized by cells, and thus remains unesterified, was maintained at a low but constant level of 6–8 nmol/g wet mass, which level was already attained within less than 1 min after palmitate addition (Fig. 1A). After 30 s of palmitate uptake, the amount of unesterified palmitate contributed to 36% of the sequestered palmitate, which contribu-
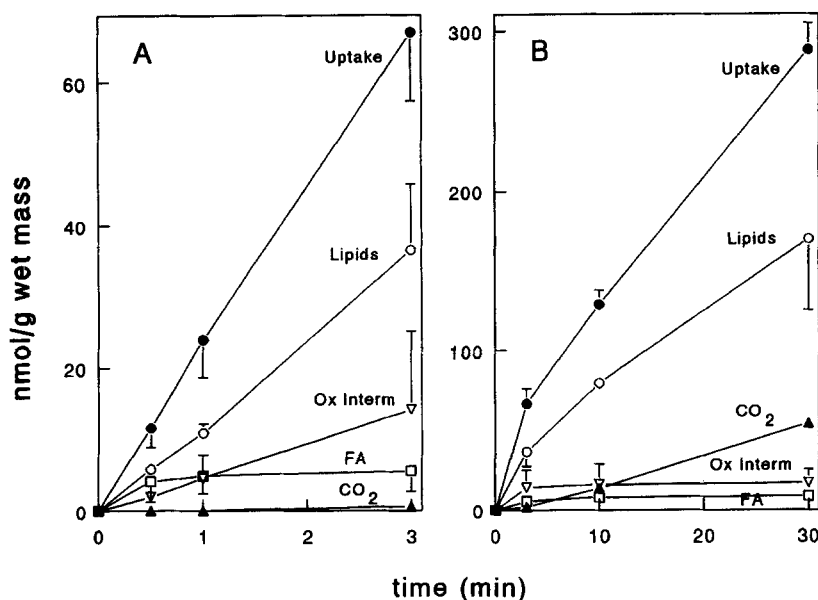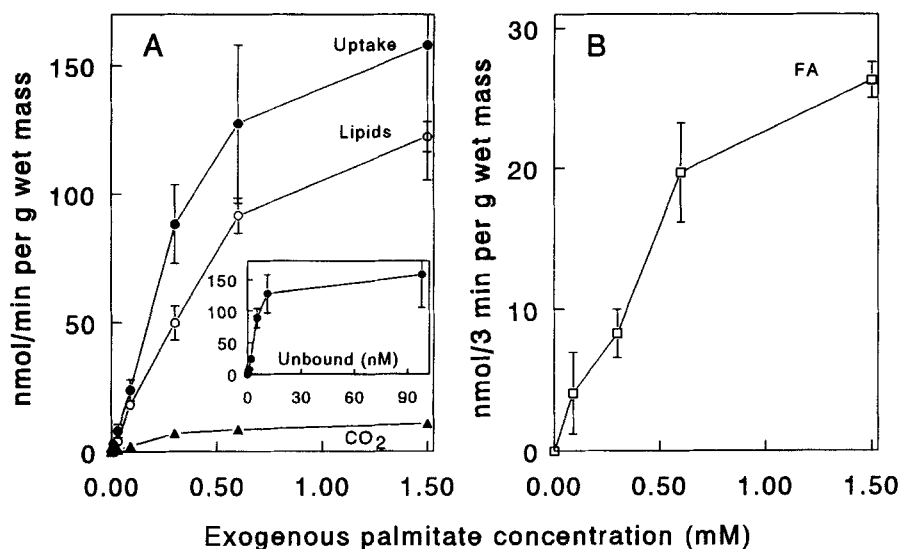
Fig. 1. Time course of palmitate uptake and utilization by cardiac myocytes. Cardiac myocytes in medium B were incubated as described under Materials and Methods with palmitate bound to BSA at final concentrations of 90 and 300 μM, respectively (palmitate/BSA molar ratio 0.3), and analyzed on cellular uptake of palmitate (●) esterification into cellular lipid pools (○), formation of oxidation intermediates (▽) and of CO_2 (▲), and on the cellular level of unesterified palmitate (□). Panel A displays incubation times up to 3 min; panel B displays incubation times between 3 and 30 min. Data are means ± SD of 3–5 experiments carried out with different cardiomyocyte preparations. Uptake, total amount of palmitate uptake; Lipids, amount of palmitate that is esterified into phospholipids and into triacylglycerols; Ox Interm, amount of palmitate converted into oxidation intermediates; CO_2 amount of radiolabeled palmitate converted into $^{14}CO_2$; FA, amount of intracellularly sequestered, unesterified palmitate.

tion declined to 9% and 3% after 3 and 30 min of palmitate uptake, respectively (Figs. 1A and 1B). For all time points studied, the sum of esterified, oxidized, and unesterified palmitate was calculated to vary between 87 and 102% of the total amount of palmitate taken up by the cells (i.e., sum of measured uptake and CO_2 production; see Materials and Methods).

Based on these results, it was decided for subsequent experiments to calculate the initial rates of both palmitate uptake by cells and incorporation into cellular lipids from the amounts of palmitate sequestered or esterified by cells, respectively, during the period between 0 and 3 min after palmitate addition. As, within this period, the production of $^{14}CO_2$ was negligible compared to the amount of palmitate measured with the palmitate uptake assay, CO_2 production was not taken into consideration when determining initial uptake rates. Concerning the intracellular level of unesterified palmitate, the value obtained at 3 min reflects a steady state concentration, rather than an appearance rate, as this level is already maximal within less than 1 min after palmitate addition. The rate of CO_2 production was decided to be calculated from the amount of $^{14}CO_2$ formed in the period between 10 and 30 min after palmitate addition.

Uptake and metabolism of palmitate as a function of palmitate and albumin concentrations. The cellular uptake of palmitate was studied as a function of the exogenous palmitate concentration but at a constant BSA concentration of 300 μM. When the palmitate concentration was increased from 0 to 1.5 mM, which results in an increase in the palmitate/albumin molar ratio from 0 to 5, initial palmitate uptake appeared to be a saturable process (**Fig. 2A**). Under the conditions applied, initial uptake never exceeded 2% of the total amount of added substrate. When palmitate uptake was studied as a function of the unbound palmitate concentration, as calculated from the palmitate/albumin molar ratio according to Richieri et al. (24), the saturable nature of the fatty acid uptake process was preserved (Fig. 2A, inset). For both the total and the unbound palmitate concentrations, the uptake versus concentration curve could be fitted to the Michaelis-Menten equation. The apparent K_m for this process as a function of the total palmitate concentration was calculated to be 435 μM, and that for the unbound palmitate concentration to be 9 nM, while the V_{max} amounted to 160 nmol/min per g of wet mass.

Similar to the cellular uptake, both esterification and

Fig. 2. Rate of palmitate uptake by cardiac myocytes as a function of the exogenous palmitate concentration. Cardiac myocytes in medium B were incubated with varying concentrations of palmitate bound to BSA (final BSA concentration 300 μM) as described in Materials and Methods, resulting in palmitate/BSA molar ratios ranging from 0 to 5. In panel A, cellular uptake of palmitate (●) and palmitate esterification (○) were determined at 3 min after substrate addition, and formation of $^{14}CO_2$ (▲) was determined within a time interval from 10 to 30 min after substrate addition. In the inset, palmitate uptake is plotted against the unbound palmitate concentration, which is calculated from the palmitate/BSA molar ratio according to Richieri et al. (24). In panel B, the cellular steady state level of unesterified palmitate was determined 3 min after substrate addition. Data are means ± SD of 3 experiments carried out with different cardiomyocyte preparations. Uptake, total amount of palmitate uptake; Lipids, amount of palmitate that is esterified into phospholipids and into triacylglycerols; CO_2, amount of radiolabeled palmitate converted into $^{14}CO_2$; FA, amount of intracellularly sequestered, unesterified palmitate.

oxidation of palmitate as a function of the total exogenous palmitate concentration displayed saturation kinetics (Fig. 2A). The apparent K_m for esterification was 465 μM and that for oxidation 222 μM. When plotted as function of the calculated non-protein bound palmitate concentration, the apparent K_m values were 11 nM for esterification and 5 nM for oxidation. The cellular content of unesterified palmitate also appeared to be saturable with increasing concentrations of exogenous palmitate (Fig. 2B). The corresponding K_m value, when plotted against the extracellular non-protein bound palmitate concentration, was found to be 12 nM.

When studying uptake of palmitate at varying concentrations of the palmitate–BSA complex at a fixed molar ratio of 0.3 (**Fig. 3**), it was observed that the initial uptake rate increased linearly with the concentration of the complex up to a BSA concentration of about 30 μM. Thereafter, the uptake rate increased only marginally up to a BSA concentration of 300 μM. The apparent saturation of the initial uptake at increasing concentrations of the palmitate–BSA complex allowed the calculation of an apparent K_m value for BSA. This value amounted to 23 μM.

Inhibition studies

Effects of inhibitors of protein-mediated sarcolemmal FA transport. In order to assess the possible involvement of sarcolemmal proteins in cellular uptake of palmitate, cardiac myocytes were incubated in the presence of the following agents: *a*) trypsin, to remove hydrolytically peptide chains at the extracellular leaflet of the sarcolemma; *b*) phloretin, a non-selective inhibitor of carrier-mediated processes (26, 27), and *c*) sulfo-N-succinimidyl-oleate (SSO), an FA derivative that covalently modifies FA binding proteins by virtue of its highly reactive sulfo-N-succinimidyl moiety and that does not penetrate biological membranes, as was shown for adipocytes (12). Treatment of cells with 0.05% trypsin did not significantly decrease palmitate uptake (**Table 1**). Treatment with 0.25% trypsin, however, resulted in 79% inhibition of palmitate uptake, suggesting that the plasma membrane protein component(s) involved in palmitate uptake are relatively resistant to proteolytical treatment. Phloretin decreased palmitate uptake by 79% when added at a concentration of 0.4 mM. This concentration is sufficient to exert a maximal effect, as addition of 1.0
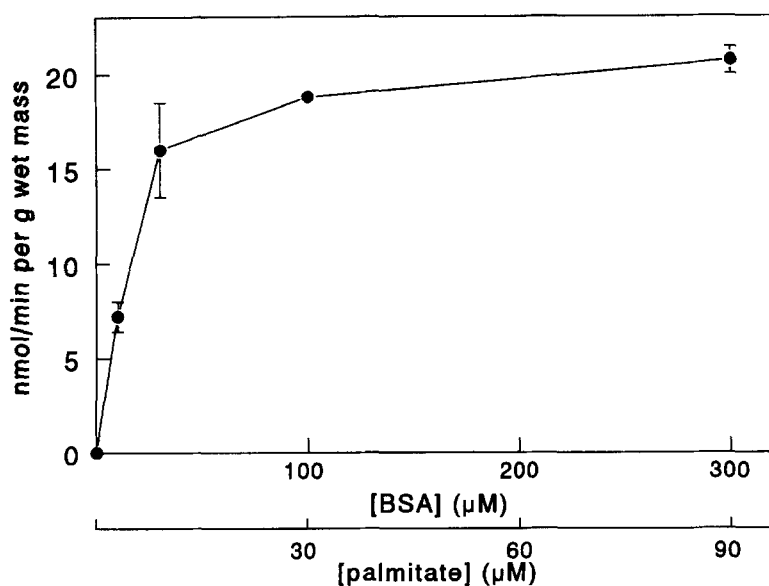
Fig. 3 Palmitate uptake by cardiac myocytes at parallel changes in palmitate and BSA concentration at a fixed palmitate/BSA molar ratio of 0.3. Cardiac myocytes were incubated in medium A supplemented with 1.0 mM $CaCl_2$ with dilutions of a concentrated palmitate–BSA stock solution (palmitate/BSA molar ratio 0.3) as described in Materials and Methods. Cellular uptake of palmitate was determined at 3 min after addition of the substrate solution. For each concentration of the palmitate/BSA ratio applied, the estimated exogenous non-protein bound FA concentration was 18 nM, calculated according to Richieri et al. (24). Data are means ± SD of 3 experiments carried out with different cardiomyocyte preparations.

TABLE 1. Influence of effectors of FA metabolism and of plasma membrane acting agents on palmitate utilization by cardiac myocytes

Addition	Initial Rate of Palmitate Uptake		Initial Rate of Palmitate Esterification		Rate of $^{14}CO_2$ Production	
	nmol/min per g wet mass					
None, control	22.9 ± 3.5		16.5 ± 2.1		2.11 ± 0.11	
DMSO (0.05%)	22.4 ± 0.9	(98)	15.9 ± 0.8	(96)	2.11 ± 0.38	(100)
Insulin (10 nM)	18.6 ± 0.6[a]	(81)	14.1 ± 0.6	(85)	1.79 ± 0.02[a]	(85)
− Glucose	27.4 ± 2.5[a]	(120)	18.9 ± 0.6	(114)	2.61 ± 0.09[a]	(123)
− Glc + lactate (1.5 mM)	24.9 ± 0.9[b]	[91]	19.0 ± 1.7	[100]	1.68 ± 0.16[b]	[64]
− Glc + lactate (5.0 mM)	24.1 ± 0.7[b]	[88]	19.2 ± 0.2	[103]	1.56 ± 0.05[b]	[59]
Etomoxir (10 µM)	11.4 ± 1.2[a]	(50)	11.1 ± 1.7[a]	(67)	0.10 ± 0.05[a]	(5)
Trypsin (0.05%)[c]	22.7 ± 1.1	(99)	−		−	
Trypsin (0.25%)[c]	4.8 ± 3.9[a]	(21)	2.5 ± 0.6[a]	(15)	0.51 ± 0.14[a]	(24)
Phloretin (0.4 mM)	4.9 ± 4.3[a]	(21)	3.2 ± 1.9[a]	(19)	0.30 ± 0.05[a]	(14)
Phloretin (1.0 mM)	4.7 ± 1.9[a]	(20)	2.4 ± 1.9[a]	(14)	0.25 ± 0.11[a]	(12)
SSO (0.1 mM)[c]	20.4 ± 1.3	(89)	15.7 ± 0.4	(95)	2.02 ± 0.05	(96)
SSO (0.4 mM)[c]	10.8 ± 1.6[a]	(47)	8.6 ± 0.7[a]	(52)	1.28 ± 0.10[a]	(61)
SSO (0.8 mM)[c]	11.9 ± 1.3[a]	(52)	8.5 ± 2.7[a]	(51)	1.38 ± 0.16[a]	(65)

Cardiac myocytes were incubated in medium B (containing 11 mM glucose) with palmitate complexed to BSA to final concentrations of 90 and 300 µM, respectively, (palmitate/BSA molar ratio 0.3) as described in Materials and Methods. Palmitate uptake and esterification were determined at 3 min after addition of the substrate solution, and $^{14}CO_2$ formation within the time interval from 10 to 30 min after substrate addition. Insulin, lactate, etomoxir, and phloretin were added to the cell suspensions 15 min prior to substrate addition in final concentrations as indicated in the table. In the case of trypsin and SSO, cells were pretreated with these agents. For this, cells suspended in medium A supplemented with 30 µM BSA and 1.0 mM $CaCl_2$, were incubated with the respective compound for 15 min at 37°C, centrifuged for 1 min at 20 g, washed twice, and suspended in medium B. Then, palmitate complexed to BSA was added to final concentrations of 90 and 300 µM, respectively. Values are expressed as nmol palmitate sequestered or utilized per min and per g wet mass. The values presented between parentheses are percentages of the control value (no additions) for the respective parameter. The values presented between brackets are percentages of the value in the absence of glucose (−glc). Data are means ± SD of 3–5 experiments carried out with different cardiomyocyte preparations. (−) in the absence of.

[a]Significantly different from control (P < 0.05).
[b]Significantly different from the value in the absence of glucose (−glc) (P < 0.05).
[c]Pretreatment.

mM did not result in further inhibition. This maximal inhibition by phloretin is not significantly different from the observed inhibition by trypsin at 0.25%. SSO also markedly inhibited initial palmitate uptake, but the maximal inhibition, observed at 0.4 mM or higher, amounted to about 50% and thus was less than the maximal inhibition exerted by trypsin or phloretin.

The inhibitory effects of these modulators on esterification and oxidation were of magnitude comparable to the inhibition of palmitate uptake: inhibition of both esterification and oxidation by phloretin and trypsin was between 76 and 88% and inhibition by SSO between 35 and 49% (Table 1).

As hydrophobic compounds such as phloretin and SSO were added together with their solvent dimethylsulfoxide (DMSO), the concentration of which never exceeded 0.05% in the incubation medium, it was tested whether this solvent at this concentration affected palmitate uptake and metabolism. This appeared not to be the case (Table 1).

Influence of modulators of FA metabolism on initial palmitate uptake. To investigate the role of FA metabolism in the uptake of palmitate, it was attempted to alter the flux through FA metabolizing pathways by addition of competitive substrates, such as glucose and lactate, or of inhibitors/modulators of FA utilizing enzymes, like etomoxir (28) and 8,11-eicosadiynoic acid (EDYA) (29).

The effects of alternations in the utilization of glucose on the uptake and metabolism of palmitate were studied, first by omitting glucose (11 mM) from the incubation medium, and second by adding insulin to stimulate both glucose uptake and cellular carbohydrate metabolism. Rates of uptake and metabolism of palmitate were moderately increased (14–23%) in the absence of glucose and moderately decreased (15–19%) in the presence of insulin (Table 1). Comparison of the effects exerted by both manipulations on uptake of palmitate versus esterification and oxidation revealed that these three processes were each affected to the same extent.

The effects of lactate on palmitate uptake and metabolism were studied in the absence of glucose. Lactate, added in physiologically relevant concentrations of 1.5 and 5.0 mM, exerted a marked inhibition of about 40% on palmitate oxidation and a smaller, but significant inhibition of about 10% on palmitate uptake. There was no significant effect of lactate on esterification of palmitate at the concentrations applied (Table 1).

Palmitate metabolism was also directly manipulated by incubation of cardiomyocytes in the presence of etomoxir, which specifically inhibits carnitine acyltransferase I activity, and thus prevents β-oxidation of FA (28). Etomoxir inhibited CO_2 production from palmitate vir-

tually completely, while palmitate uptake was reduced by about 50% and esterification by about 30% (Table 1).

EDYA was earlier reported to inhibit acyl-CoA synthetase in detergent-solubilized calf brain preparations, and arachidonate uptake by platelets (29). When cardiac myocytes were preincubated with 100 μM EDYA for 15 min at 37°C and subsequently washed, according to the procedure described by Laposata et al. (29), we observed a markedly (2.0-fold) increased initial palmitate uptake rate (46.9 ± 9.9 nmol/min per g wet mass; n = 3) accompanied by an initial esterification rate of 27.0 ± 6.7 nmol/min per g wet mass (n = 3) and an oxidation rate of 6.03 ± 0.75 nmol/min per g wet mass (n = 3), which were increased 1.9-fold and 2.9-fold, respectively. The mechanism underlying the unexpected stimulation of FA uptake and metabolism in cardiac myocytes by EDYA is, at present, incompletely understood.

Deoxyglucose uptake by cardiac myocytes

In order to determine the specificity of the effects exerted by the compounds described in Table 1 on cellular FA uptake processes, their effects on the uptake of [³H]deoxyglucose by cardiac myocytes were monitored in similar incubations. These experiments were conducted under identical incubation conditions as described for investigation of palmitate uptake, except that glucose routinely was omitted to avoid competition between glucose and deoxyglucose species for cellular uptake. This latter competition was apparent from the undetectable rate of deoxyglucose uptake in the presence of 11 mM glucose (**Table 2**). Insulin caused the cellular deoxyglucose uptake to increase more than 3-fold (Table 2). In the presence of etomoxir, deoxyglu-

TABLE 2. Influence of glucose and effectors of FA metabolism and plasma membrane acting agents on uptake of deoxyglucose by cardiac myocytes

Addition	Initial Rate of Deoxyglucose Uptake
	nmol/min per g wet mass
None, control	0.98 ± 0.32 (100)
Glucose (11 mM)	0.00 ± 0.00[a] (0)
Insulin (10 nM)	3.26 ± 0.04[a] (333)
Etomoxir	1.21 ± 0.05 (123)
Phloretin (0.4 mM)	0.07 ± 0.01[a] (8)
SSO (0.4 mM)[b]	1.31 ± 0.01[a] (134)

Cardiac myocytes suspended in medium B without glucose were incubated with deoxyglucose as described under Materials and Methods. Cellular uptake of deoxyglucose was determined at 20 min after addition. Preincubations with the applied agents were performed as described in the legend to Table 1. The values presented between parentheses are percentages of the control value. Data are means ± SD of 3 experiments carried out with different cardiomyocyte preparations.

[a]Significantly different from the control (*P* < 0.05).
[b]Pretreatment.

cose uptake was slightly, but not significantly, increased. Treatment with SSO did not diminish deoxyglucose uptake but rather moderately stimulated it, whereas phloretin inhibited this process by more than 90% (Table 2).

DISCUSSION

Validation of the methods

This study aimed at characterizing the process of transmembrane translocation of palmitate by cardiac myocytes. For this purpose, we were interested in unidirectional palmitate influx rather than net palmitate uptake. It is assumed that unidirectional influx is mainly reflected by the rapid linear uptake phase. As a representative of initial uptake, the amount of palmitate that was sequestered during the first 3 min after addition was taken as a measure. Two observations confirm the justification of this approach. First, during this 3-min period, uptake of palmitate increased linearly with time. Second, backflux most likely does not play a quantitatively important role, because the intracellular (unesterified) palmitate concentration reached a maximal value within less than 1 min after palmitate addition and, thereafter, remained at this low steady-state level (Fig. 1A). The cellular concentration of palmitate (6–8 μM, assuming 1 g wet mass is equal to 1 ml) is comparable to that found in intact myocardium (30, 31), and is 11- to 15-fold lower than the extracellular concentration (90 μM). This gradient is in accordance with earlier studies in which the ratio of the content of FA from blood plasma to rat cardiac muscle (31) and dog skeletal muscle (19) were each found to be about 17 in favor of arterial plasma. In addition, we have studied the uptake process at a BSA concentration of 300 μM, which reflects the albumin concentration of the interstitial space (1), and at a total substrate concentration of 90 μM, which is well below the apparent K_m of palmitate uptake, found to be 435 μM. The calculated V_{max} of palmitate uptake (160 nmol/min per g wet mass) is in close agreement with the value (205 nmol/min per g) reported by Rose, Hennecke, and Kammermeier (4) for similar conditions. Based on the foregoing, we selected this condition as standardized protocol to further characterize the palmitate uptake process.

With respect to the measurement of cellular FA uptake, the type of assay used does not permit the discrimination between transport across the membrane and binding of FA to the membrane. However, there are several lines of evidence to suggest that in our studies the bulk of palmitate detected in the cell pellet after the incubation will have been translocated across the

sarcolemma. First, the percentage of this amount of palmitate that had been metabolized was as high as 66% already after 30 s of palmitate uptake and increased to about 97% after 30 min of palmitate uptake (Fig. 1). As a result, the remaining amount of (nonmetabolized) palmitate decreased from 34% (30 s) to 3% (30 min) of the sequestered palmitate, but was relatively constant at 6–8 nmol/g of wet mass. This unesterified palmitate will be partly bound to cytoplasmic FABP (10) and partly partition into (sub)cellular membranes. However, it is not excluded that in the initial uptake phase these FA may all be present in the sarcolemma. As the phospholipid content of cardiac myocytes is about 20 μmol/g of wet mass, of which an estimated 10% resides in the sarcolemma (i.e., 2 μmol/g of wet mass) (1), even in this latter case the palmitate to phospholipid ratio would be sufficiently low (i.e., 1:250 to 1:350) to make it unlikely that the palmitate partition would elicit major changes in membrane structure and/or function.

Involvement of sarcolemmal proteins in fatty acid uptake

Putative fatty acid translocating proteins. Studying uptake of palmitate as a function of the exogenous palmitate concentration can provide information on the properties of the transport process. Passive diffusion would be indicated in principle by a linear relationship of uptake with the external palmitate concentration. However, we observed a saturable FA uptake, in agreement with earlier studies performed on cardiac myocytes (4, 7, 8). Because under these conditions, initial uptake never exceeded 2% of the total amount of added substrate, the observed saturation kinetics are not due to extracellular substrate depletion. Saturability of uptake is generally regarded as an indication that the uptake process is carrier mediated (7, 8), but, theoretically, could also be explained by a passive diffusion mechanism in combination with a rate limitation in the subsequent intracellular FA metabolism.

Assuming that the various inhibitors of membrane processes studied exert their reported specific effects, our observations that phloretin and trypsin each inhibit the initial palmitate uptake rate by ca. 80% and that SSO inhibits this process by ca. 50% are in support of the involvement of sarcolemmal proteins in the transmembrane translocation of palmitate. For phloretin and trypsin, the observed inhibition is in accordance with earlier studies using adipocytes (6), hepatocytes (32) or cardiac myocytes (7), in which the extent of inhibition varied from 60 to 80%. The observed inhibition of initial palmitate uptake by cardiac myocytes upon SSO addition is also of a similar magnitude compared to studies of Harmon et al. (12) with adipocytes, where

an inhibition of 65% was observed. Using tritiated SSO in order to radiolabel FA-binding membrane proteins, these authors could only identify one heavily glycosylated protein with a molecular mass of 88 kDa, which was named fatty acid translocase (FAT). Also in rat myocardium, with the aid of a tritiated sulfo-*N*-succinimidyl derivative of palmitate, one protein was detected having a molecular mass of 80–90 kDa and was identified as FAT (13). Compared to trypsin and phloretin, the smaller effect of SSO on palmitate uptake could be explained by the assumption that SSO is specific for FAT and does not block the action of other putative fatty acid transporters such as $FABP_{PM}$ or FATP, which were identified using the techniques of affinity chromatography (7, 33) and expression cloning (14), respectively. Apparently, the latter two proteins could also participate in FA uptake by cardiac myocytes. Indeed, the possible involvement of $FABP_{PM}$ in FA uptake was indicated in experiments with antibodies directed to this protein, which were found to inhibit oleate uptake in cardiac myocytes, but by not more than 40% (7). Although, on the mRNA level, FATP appears to be present in the myocardium (14), up to now no data are available to elucidate the quantitative contribution of FATP to FA uptake in cardiac cells.

Strikingly, manipulations meant to inhibit protein-mediated membrane transport in the present and other studies (6, 7, 12, 32) failed to inhibit palmitate uptake completely. The remaining part, in the present study contributing to about 20% of total palmitate uptake, is likely due to passive diffusion. Hence, the findings suggest that FA uptake by cardiac myocytes is accomplished by both passive and carrier-mediated transmembrane transport. The carrier-mediated component is likely responsible for the majority of the FA influx and could be the result of the concerted action of FAT together with other membrane-associated proteins.

Further support for this concept comes from the experiments in which we studied the uptake of deoxyglucose by cardiac myocytes in order to test the specificity of the various compounds on inhibition of FA metabolism. Phloretin, in addition to its effects on palmitate uptake, blocked the uptake rate of deoxyglucose almost completely. The larger effect of phloretin on deoxyglucose uptake compared to that on FA uptake is likely due to the lack of a diffusive component in the uptake of hydrophilic substrates, such as glucose. Finally, the observation that SSO did not inhibit deoxyglucose uptake stresses the notion that this compound specifically affects the FA transporting process.

Putative albumin-binding proteins. Our experiments, in which palmitate uptake was studied at increasing exogenous palmitate concentrations in parallel with increasing BSA concentrations (Fig. 3), is indicative of the existence of an albumin binding protein (albumin receptor) in cardiac myocytes. As the exogenous palmitate concentration was varied at a fixed palmitate/BSA molar ratio, the unbound palmitate concentration, which is solely a function of the palmitate/albumin molar ratio (24, 34) remained constant. Hence, the only variable in this setting is the concentration of BSA. Under these conditions, the palmitate uptake appeared to be saturable at increasing BSA concentrations, indicating the involvement of an albumin binding protein in the FA uptake process. Alternatively, the accessibility of the sarcolemma for BSA may be limited, possibly due to a physical limitation of the number of albumin molecules that can be present in close vicinity of the sarcolemma (cf. 35).

Another explanation for the observed kinetics is that palmitate uptake is governed by the extracellular unbound palmitate concentration rather than by the total palmitate concentration. The decrease in uptake at low palmitate and BSA concentrations then could be explained in the following manner. First, at very low concentrations of BSA, the unbound FA concentration is no longer determined by the palmitate/BSA molar ratio but decreases to zero as the total FA concentration decreases to zero. However, the BSA concentrations at which a decrease in palmitate uptake was observed are far too high to support this explanation (24). Second, the dissociation of palmitate from albumin may become rate limiting at these low BSA concentrations. Dissociation rates of FA from albumin have been reported to vary between 0.03 and 0.175 s^{-1} depending on the method applied to measure such rates (36). Assuming a dissociation rate of 0.1 s^{-1}, a BSA concentration of 300 μM in an incubation volume of 2 ml, would allow $2.2 \cdot 10^{18}$ dissociations/min to occur. The cell incubations contained on average 10 mg wet mass of cells (see Materials and Methods). This fact, combined with the observed palmitate uptake rate amounting to 20 nmol palmitate/min per g wet mass at this molar ratio, corresponds to $1.2 \cdot 10^{14}$ molecules of palmitate to be sequestered by the cells per min. Thus, even at BSA concentrations near the apparent K_m, the number of dissociations far exceeds the amount of FA to be taken up by the cells, strongly arguing against the dissociation being a limiting factor.

In line with this kinetic evidence for the existence of albumin-binding proteins on the sarcolemmal surface of cardiac myocytes is the identification of high affinity albumin binding sites in sarcolemmal fractions from rat and rabbit ventricular myocardium (15). However, because the apparent K_m for BSA (23 μM), as observed in our studies, is far below physiologically relevant concentrations of albumin in the interstitial space (approximately 300 μM), it is unlikely that this albumin binding

protein would be involved in rapid modulation of FA uptake by cardiac myocytes. This notion does not exclude the possibility that the putative albumin binding protein exerts a permissive role in overall FA extraction.

In one model of fatty acid uptake (37), an unstirred fluid layer surrounding the cellular membrane is postulated, imposing a diffusion constraint on the palmitate-albumin complex. A function of the high affinity albumin binding sites could be to improve the diffusion rate of the complex through this layer, and in this way enhance the concentration of the unbound fatty acid fraction in the close vicinity of the sarcolemma. The present findings do not exclude this possibility.

Interaction between uptake of fatty acids and their subsequent metabolism

A decisive role of metabolism in governing the FA uptake process by cardiac myocytes was first suggested by DeGrella and Light (2, 3). These authors proposed that the uptake process was caused by passive diffusion. As the present study together with other studies (6, 7, 32) provide evidence that the bulk uptake of FA by cardiac myocytes is mediated by proteins, the involvement of metabolism must be reconsidered. In view of the present data, there are two lines of evidence indicating that initial FA uptake by cardiac myocytes is a protein-mediated process which is driven by the rapid metabolic conversion of palmitate.

First, during the initial uptake phase a substantial amount of the sequestered FA is rapidly metabolized. At 30 s after palmitate addition, which time point is also taken in earlier studies as representative for studying initial uptake in cardiac myocytes (4, 7), at least 60% of the sequestered palmitate is channeled into metabolic pathways. The rapid entrapping of palmitate by the metabolizing machinery allows the establishment of a steady state between cellular influx of palmitate and its subsequent conversion to palmitoyl-CoA in less than 1 min after palmitate addition. The consequence of this is that the intracellular concentration of (unesterified) palmitate remains low.

Second, changes in palmitate metabolism, caused by either metabolic effectors or competitive substrates, markedly affected palmitate uptake, which strongly suggests that palmitate metabolism is the driving force for palmitate uptake.

Metabolic inhibitors. The observation that the carnitine acyltransferase I inhibitor, etomoxir, which inhibits β-oxidation of palmitate virtually completely, is able to inhibit the initial palmitate uptake rate by 50%, points towards a close link between metabolism and initial uptake of FA. A direct inhibitory effect of etomoxir on the transmembrane translocation of palmitate is not likely as, *a*) the chemical structure of this compound does not

resemble that of FA (28), excluding competition as a possibility, and *b*) etomoxir did not decrease the uptake of deoxyglucose, indicating that this compound cannot be regarded as a general inhibitor of carrier-mediated processes. In the presence of this compound, the palmitate taken up by the cells is almost completely esterified into cellular lipid pools. However, compared to control conditions, esterification is reduced by approximately 30%. As etomoxir, at the concentration used, is reported to be a specific inhibitor of carnitine acyltransferase I (28), a direct effect on esterification is not likely. A possible explanation is that the reduction in esterification is secondary to the decrease in cellular uptake caused by the complete blockade in oxidation. Alternatively, the complete block in β-oxidation could render cardiac myocytes more dependent on glucose utilization for ATP production, so that less dihydroxyacetone-phosphate will be converted into glycerol-3-phosphate required for synthesis of phospholipids and triacylglycerols.

The unexpected stimulation of palmitate uptake, esterification, and oxidation by EDYA is in disagreement to its proposed ability to inhibit acyl-CoA synthetase (29). Yet, interestingly, the concomitant increase in the three parameters illustrates the link between uptake and cellular metabolism. Preliminary studies performed in our laboratory and aimed at increasing the energy demand of the myocytes by electric field stimulation revealed that both palmitate uptake and oxidation are increased under these conditions (data not shown), further indicating a close relationship between these latter processes.

Competitive substrates. Changes in glucose utilization were brought about by either omission of glucose from the incubation medium or by addition of insulin. The latter resulted in a more than 3-fold increase in deoxyglucose uptake, as was reported earlier by Fischer et al. (17), and indicates that cardiac myocytes are competent to react towards changes in hormonal and nutritional conditions. The insulin effect relates to the well-characterized transfer of glucose transporters from intracellular sites to the sarcolemma (38). Addition of insulin caused the uptake as well as the esterification and oxidation of palmitate to decrease by about 20%. On the other hand, omission of glucose caused these processes to increase correspondingly by about 20%. As it is very unlikely that glucose directly interferes with uptake of palmitate by competition for translocation sites at the sarcolemma, it is reasonable to assume that glucose inhibits intracellular FA metabolism, and because of this particular action decreases uptake of palmitate. According to Randle et al. (39), utilization of glucose and FA are reciprocally regulated. The sites of regulation of glucose utilization by FA are well described and are

pinpointed to inhibition of both phosphofructokinase and pyruvate dehydrogenase. However, with respect to sites of inhibition of FA metabolism by glucose, data are scarce. The similar extent of inhibition of both esterification and oxidation of palmitate suggests that the activation of palmitate by acyl-CoA synthetase, catalyzing the first metabolic step in cellular FA utilization, is affected. This is in agreement with the suggestion made by Neely and Morgan (40) that glucose is able to compete with FA for the intracellular pool of coenzyme A.

When lactate was applied as a competitive substrate, palmitate oxidation was decreased by 36–41% and uptake by 9–12%, while there was no effect on esterification. The ability of lactate to effectively compete with FA for cardiac oxygen consumption has been described in whole animal studies and in perfused hearts (for a review, see 41) but not yet in isolated cardiac myocytes. The inability of lactate to interfere with palmitate esterification suggests that a step downstream of the activation by acyl-CoA synthetase is affected, presumably carnitine acyltransferase I, as was hypothesized by Bielefeld, Vary, and Neely (42). Thus, etomoxir and lactate probably affect FA utilization by a related mechanism. The absence of an effect of lactate on palmitate esterification could be related to the fact that in the presence of this alternative oxidizable substrate cardiac myocytes are less dependent on glucose metabolism, so that there is no limitation in the availability of glycerol-3-phosphate for FA esterification.

Apparently, the rate of palmitate metabolism is an important regulator of its initial uptake rate. Based on the presented evidence, the most important metabolic step in determining the extent of palmitate uptake probably is the activation step carried out by acyl-CoA synthetase. Conversion of palmitate to its CoA ester most likely acts as a sink for fatty acids upon cellular sequestration, and allows the establishment of a fatty acid gradient, which is regarded as the driving force for fatty acid uptake (31).

Although we obtained strong indications that its metabolic entrapping is important for the initial uptake of palmitate, less information has become available about the rate-limiting step in the utilization of palmitate. The observation that palmitate oxidation and esterification as a function of the externally added palmitate concentration display saturation kinetics, in which there is no marked change in the apparent K_m values when compared to the initial palmitate uptake, point in the direction of a fine-tuning between uptake and metabolism. This delicate balance could very well be regulated at the site of acyl-CoA synthetase. The observation that at high, externally added palmitate concentrations, when the initial uptake becomes saturated, there is no drastic increase in the cellular steady state level of unesterified fatty acids (Figs. 2A and B), suggests that under these conditions uptake becomes limited. If, under these same conditions, metabolism of palmitate would be rate limiting, such an increase in the cellular FA concentration is likely to be expected, provided that the degree of backflux does not markedly increase.

Concluding remarks

In this study, evidence has been presented that FA uptake by isolated cardiac myocytes is due to both passive and protein-mediated processes (**Fig. 4**), whereby the latter component accounts for the majority of FA uptake and comprises albumin binding proteins (Alb. BP) and one or more membrane-associated FA-binding proteins (membrane FABPs, such as $FABP_{PM}$, FAT, or FATP). In addition, FA uptake appears to be facilitated by rapid activation by acyl-CoA synthetase to acyl-CoA, which most likely causes the intracellular unesterified FA concentration to be low and, hence, the FA gradient across the sarcolemma remains high.

The presence of membrane-associated FABPs has only recently been recognized in cardiac myocytes (7, 11, 13, 14). In contrast, the presence of cytoplasmic FABP in heart has been firmly established since the early seventies (43) (for reviews, see 9,10). Despite this fact, there still is no direct evidence whether cytoplasmic FABP is involved in regulation of the flux through FA utilizing pathways. However, the ability of
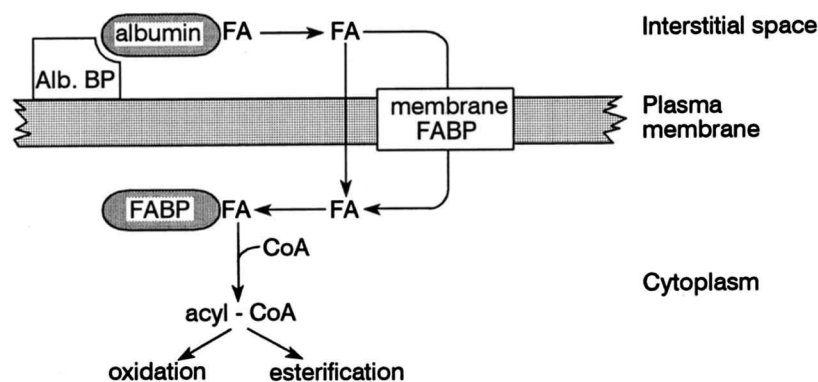
Fig. 4. Schematic presentation of the putative mechanism of cellular fatty acid uptake illustrating the possible roles of membrane-associated and cytoplasmic proteins in this process. Transmembrane transport of FA could take place by simple diffusion or could be mediated by membrane FABPs. Intracellularly, FA will be bound by cytoplasmic FABP. FA, long-chain fatty acid; Alb.BP, albumin binding protein; membrane FABP, membrane-associated fatty acid-binding protein; FABP, cytoplasmic fatty acid-binding protein.

FABP to enhance the cytoplasmic solubility of FA more than 700-fold (44) indicates that these proteins could serve as a cytoplasmic buffer for intracellularly sequestered FA. A recently observed physical interaction between FAT and cytoplasmic FABP provides further indications for a role for the latter FABP in intracellular FA transport (45).█

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