Electrostimulation enhances FAT/CD36-mediated long-chain fatty acid uptake by isolated rat cardiac myocytes

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Luiken, J. J. F. P., J. Willems, G. J. van der Vusse, and J. F. C. Glatz. Electrostimulation enhances FAT/CD36-mediated long-chain fatty acid uptake by isolated rat cardiac myocytes. Am J Physiol Endocrinol Metab 281: E704–E712, 2001.—We investigated palmitate uptake and utilization by contracting cardiac myocytes in suspension to explore the link between long-chain fatty acid (FA) uptake and cellular metabolism, in particular the role of fatty acid translocase (FAT)/CD36-mediated transsarcolemmal FA transport. For this, an experimental setup was developed to electrically stimulate cardiomyocytes in multiple parallel incubations. Electrostimulation at voltages ≥170 V resulted in cellular contraction with no detrimental effect on cellular integrity. At 200 V and 4 Hz, palmitate uptake (measured after 3-min incubation) was enhanced 1.5-fold. In both quiescent and contracting myocytes, after their uptake, palmitate was largely and rapidly esterified, mainly into triacylglycerols. Palmitate oxidation (measured after 30 min) contributed to 22% of palmitate taken up by quiescent cardiomyocytes and, after stimulation at 4 Hz, was increased 2.8-fold to contribute to 39% of palmitate utilization. The electrostimulation-mediated increase in palmitate uptake was blocked in the presence of either verapamil, a contraction inhibitor, or sulfo-N-succinimidyl-FA esters, specific inhibitors of FAT/CD36. These data indicate that, in contracting cardiac myocytes, palmitate uptake is increased due to increased flux through FAT/CD36.

fatty acid translocase; long-chain fatty acids; esterification; β-oxidation; cellular contraction

ALTHOUGH LONG-CHAIN FATTY ACIDS (FA) REPRESENT AN IMPORTANT SOURCE OF ENERGY FOR CARDIAC MYOCYTES (33), THE MECHANISM OF THEIR UPTAKE AND CHANNELING TOWARD MITOCHONDRIAL β-OXIDATION IS INCOMPLETELY UNDERSTOOD. FOR CARDIAC MYOCYTES, EVIDENCE HAS BEEN PRESENTED IN FAVOR OF PASSIVE DIFFUSION (7, 22) AS WELL AS OF CARRIER-MEDIATED UPTAKE OF FA (27, 31). FOR THREE DISTINCT SARCOLEMMAL PROTEINS, THERE IS NOW FUNCTIONAL EVIDENCE THAT THEY SERVE AS FA TRANSPORTERS. THESE ARE A 43-kDa PLASMA MEMBRANE FA BINDING PROTEIN (FABPpm) (28), A 62-kDa FA TRANSPORT PROTEIN (FATP) (26), AND AN 88-kDa HEAVILY GYCOXYLATED FA TRANSLOCASE (FAT), THE RAT HOMOLOG OF HUMAN CD36 (1). RECENTLY, FOUR TYPES OF FATP HAVE BEEN IDENTIFIED, TWO OF WHICH, FATP1 AND FATP4, ARE ALSO EXPRESSED IN HEART (14).

Previously, we studied palmitate uptake by cardiac myocytes incubated in suspension under resting conditions, i.e., in the absence of a trigger for mechanical activity (19). Using this system, we demonstrated that palmitate uptake occurs by both diffusional and carrier-mediated processes, whereby the latter represents ~80% of the sarcolemmal palmitate translocation. On the basis of inhibition studies with sulfo-N-succinimidoxyloleate (SSO), a specific inhibitor of FAT/CD36 (12, 18), it appeared that FAT/CD36 fulfills an important role in the uptake of FA by these myocytes (19). In addition, two lines of evidence strongly suggested that the rate of cellular uptake of palmitate is tightly coupled to its subsequent metabolism. First, upon their cellular uptake, FA were rapidly metabolized, as a consequence of which the intracellular level of unesterified FA remained low. Second, a reduction in the rate of FA metabolism, either by metabolic inhibitors or by competitive substrates such as glucose or lactate, was accompanied by a decrease in the initial uptake rate of FA (19).

The importance of putative FA transporters in the uptake of FA by heart and skeletal muscle was verified with giant sarcolemmal vesicles, which allow the study of FA uptake in the absence of metabolism (4, 18). These studies confirmed that FA uptake by these tissues is largely protein mediated and identified FAT/CD36 and FABPpm as functionally important transporters (18). FATP1 most likely is not involved in bulk transsarcolemmal FA uptake (18), whereas FATP4 has not yet been studied. Interestingly, upon short-term electrostimulation of rat hindlimb muscle, FA uptake by giant vesicles was markedly enhanced, which effect could be annulled when giant vesicles from electrostimulated legs were pretreated with SSO, indicating that the increase in FA uptake was mediated by an increased transport function of FAT/CD36 (4). Subsequent fractionation studies in noncontracting muscle revealed an intracellular pool of FAT/CD36 from which this transporter can be recruited to the cell surface upon electrical stimulation (4).

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In the present work, we investigated palmitate uptake and utilization by contracting cardiac myocytes to further explore the link between uptake of FA and subsequent metabolism by cardiac myocytes, in particular the role of FAT/CD36-mediated transsarcolemmal FA transport as a rate-limiting step in cellular FA utilization. Because contractile performance is likely to increase the metabolic demands of cardiac myocytes, our first aim was to investigate whether mitochondrial FA oxidation by electrically stimulated cardiac myocytes is enhanced. This is still a controversial issue, because it has been reported that, in the intact heart, increased workload selectively enhances carbohydrate oxidation (5, 10) and that FA and glucose oxidation increase in parallel (6, 20). Our second goal was to assess whether contractile activity of cardiac myocytes increases their rate of uptake of palmitate, as has been observed in giant vesicles prepared from contracting skeletal muscle (see above). Third, because we have obtained evidence for FAT/CD36 translocation from intracellular stores to the sarcolemma in skeletal muscle upon muscle contractions (4), we explored with cardiac myocytes the involvement of FAT/CD36 in the possible alterations in FA uptake.

For these studies we needed a setup allowing isolated cardiac myocytes in suspension to undergo controlled contractions in an electric field at the pace of the applied frequency. Therefore, and also to allow the simultaneous assessment of other manipulations (e.g., effect of inhibitors), we developed a pulse generator that can introduce an electric field in up to 20 parallel cell incubations.

MATERIALS AND METHODS

Isolation of cardiac myocytes. Cardiac myocytes were isolated from male Lewis rats (200–250 g) with the use of a Langendorff perfusion system and a Krebs-Henseleit bicarbonate medium supplemented with 11 mM glucose and equilibrated with a carbogen (95% O2-5% CO2) gas phase (medium A) at 37°C, according to Fischer et al. (8), as previously described (19). After isolation, the cells were washed twice with medium A supplemented with 1.0 mM CaCl2 and 2% (wt/vol) BSA (medium B) and then suspended in 20 ml of medium B and gassed with carbogen. The isolated cells were allowed to recover for 2–3 h at room temperature. At the end of the recovery period, cells were washed and suspended in medium B. Only when >80% of the cells had a rod-shaped appearance and excluded trypan blue were they used for subsequent tracer uptake studies. The yield of cardiac myocytes amounted to 400–500 mg wet mass/g heart tissue.

Electrical stimulation of cardiac myocytes in suspension. Cell suspensions were subjected to an electric field via two platinum electrodes connected to a pulse generator capable of generating biphasic pulses up to 250 V (Fig. 1). The frequency of the pulses was commonly 2 or 4 Hz. The monophasic components of the pulses exhibit a block profile. The duration of a monophasic pulse was set at 100 μs, and the time interval between the monophasic components before reversal of the voltage at 10 μs. The biphasic nature of the pulses served to prevent electrolysis of cells (15, 23). The electrodes were connected to the screw cap of a 20-ml glass incubation vial (Packard-Canberra, Meriden, CT) in such a manner that capping of the vial resulted in immersion of the electrodes into the cell suspension (Fig. 1). The caps were equipped with a silicon rubber seal to allow injection of the radiolabeled substrate without compromising the gas-tight sealing. The distance between the electrodes in the cell suspension was 15 mm. The vials were placed in a water bath at 37°C and connected to the pulse generator by a flexible wire to allow electrostimulation during continuous shaking. Because the generator was equipped with 20 outputs placed in parallel, it was possible with this setup to introduce an electric field in up to 20 cell incubations simultaneously.

Palmitate utilization by cardiac myocytes. Cells (1.8 ml; 5–8 mg wet mass/ml), suspended in medium B equilibrated with carbogen, were preincubated in capped 20-ml incubation vials for 15 min at 37°C under continuous shaking. At the start of the incubations, 0.6 ml of the [1-14C]palmitate/BSA complex was added so that the final concentration of palmitate amounted to 100 μM with a corresponding palmitate-to-BSA ratio of 0.3. This palmitate/BSA complex was prepared as previously described (19). For each condition, two parallel incubations were performed for selected time intervals at 37°C. The first incubation served to assess both cellular uptake of palmitate and its esterification into cellular uptake and utilization by contracting cardiac myocytes to further explore the link between uptake of FA and subsequent metabolism by cardiac myocytes, in particular the role of FAT/CD36-mediated transsarcolemmal FA transport as a rate-limiting step in cellular FA utilization. Because contractile performance is likely to increase the metabolic demands of cardiac myocytes, our first aim was to investigate whether mitochondrial FA oxidation by electrically stimulated cardiac myocytes is enhanced. This is still a controversial issue, because it has been reported that, in the intact heart, increased workload selectively enhances carbohydrate oxidation (5, 10) and that FA and glucose oxidation increase in parallel (6, 20). Our second goal was to assess whether contractile activity of cardiac myocytes increases their rate of uptake of palmitate, as has been observed in giant vesicles prepared from contracting skeletal muscle (see above). Third, because we have obtained evidence for FAT/CD36 translocation from intracellular stores to the sarcolemma in skeletal muscle upon muscle contractions (4), we explored with cardiac myocytes the involvement of FAT/CD36 in the possible alterations in FA uptake.

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FA uptake by electrically stimulated cardiomyocytes

In this study, the term palmitate uptake refers to the sum of palmitate-derived CO₂ and (acid-soluble) oxidation intermediates (19).

Intracellular ATP was determined after addition of perchloric acid (final concentration 0.5 M) to the cell suspension according to Glatz et al. (9). ATP was determined fluorimetrically according to Williamson and Corkey (36).

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incubation, the proportion of cells excluding trypan blue and having a rod-shaped appearance amounted to >80 and 90%, respectively, which values were not detectably decreased after 30 min of incubation in either the absence or presence of an electric field of ≤4 Hz (data not shown). In conclusion, no significant changes in cell viability were detected under the applied conditions of electric field stimulation (i.e., 200 V and 0–4 Hz).

**Influence of electrostimulation on palmitate and deoxyglucose uptake by cardiac myocytes.** Palmitate and deoxyglucose uptake were studied at 3 and 10 min after substrate addition, respectively. For palmitate, an incubation time of 3 min provides information about the initial uptake process, because within this initial phase palmitate uptake proceeded linearly with time in both quiescent (19) and stimulated cells (data not shown), whereas thereafter the rate of uptake gradually declined. Uptake of deoxyglucose was linear with time for at least 30 min (19).

In the absence of an electric field and in the presence of 11 mM glucose in the medium, initial palmitate uptake amounted to 22.7 ± 2.7 nmol·min⁻¹·g wet mass⁻¹ and, upon electrostimulation at 200 V, was increased to 30.0 ± 3.9 nmol·min⁻¹·g wet mass⁻¹ (1.3-fold) at 2 Hz and to 33.9 ± 2.3 nmol·min⁻¹·g wet mass⁻¹ (1.5-fold) at 4 Hz (means ± SD for n = 3) (Table 1; see also Fig. 3). Omission of glucose did not significantly alter palmitate uptake either in the absence or in the presence of electrostimulation (Fig. 3). The rate of uptake of 2-deoxy-D-[1,3]H]glucose by quiescent cardiac myocytes was 2.7 ± 0.4 nmol·min⁻¹·g wet mass⁻¹ (n = 3) in the absence of exogenously added palmitate. Electrically stimulated cardiac myocytes showed rates of uptake of this nonmetabolizable glucose species that were also increased 1.3-fold (to 3.7 ± 0.3 nmol·min⁻¹·g wet mass⁻¹) at 2 Hz and 1.5-fold (to 4.0 ± 0.3 nmol·min⁻¹·g wet mass⁻¹) at 4 Hz (means ± SD for n = 3) (Fig. 3). However, in the presence of 100 μM palmitate, incubation of quiescent cardiac myocytes showed a deoxyglucose uptake rate of only 1.90 ± 0.37 nmol·min⁻¹·g wet mass⁻¹, whereas there was no increase in deoxyglucose uptake upon electrostimulation (Fig. 3).

**Influence of electrostimulation on palmitate utilization by cardiac myocytes.** Uptake and metabolism of radiolabeled palmitate were studied after 3 and 30 min of incubation of the cardiac myocytes and under a

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**Table 1. Influence of electrostimulation on [1-14C]palmitate uptake and deposition and oxidation by cardiac myocytes after 3 and 30 min of incubation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3 min</th>
<th>4 Hz</th>
<th>30 min</th>
<th>4 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>68.2 ± 8.2</td>
<td>90.1 ± 11.7</td>
<td>101.7 ± 6.9</td>
<td>354.3 ± 26.2</td>
</tr>
<tr>
<td>Esterification</td>
<td>55.2 ± 5.4</td>
<td>71.8 ± 8.7</td>
<td>78.4 ± 7.3</td>
<td>282.9 ± 14.2</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>19.9 ± 1.3</td>
<td>n.m.</td>
<td>24.5 ± 3.9</td>
<td>78.9 ± 6.2</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>35.3 ± 4.4</td>
<td>n.m.</td>
<td>54.4 ± 3.6</td>
<td>204.8 ± 20.3</td>
</tr>
<tr>
<td>Oxidation</td>
<td>10.1 ± 4.3</td>
<td>11.6 ± 3.4</td>
<td>12.7 ± 3.9</td>
<td>76.8 ± 8.7</td>
</tr>
<tr>
<td>14CO2</td>
<td>3.0 ± 0.6</td>
<td>3.2 ± 0.6</td>
<td>4.1 ± 0.6</td>
<td>63.8 ± 9.4</td>
</tr>
<tr>
<td>Oxidation intermediates</td>
<td>7.1 ± 4.1</td>
<td>8.4 ± 3.0</td>
<td>8.6 ± 3.5</td>
<td>13.0 ± 0.6</td>
</tr>
<tr>
<td>FA (nonesterified)</td>
<td>4.7 ± 2.2</td>
<td>n.m.</td>
<td>4.4 ± 2.3</td>
<td>5.6 ± 1.0</td>
</tr>
</tbody>
</table>

Data are means ± SD of 3–4 experiments carried out with different cardiomyocyte preparations. n.m., Not measured. Cardiac myocytes were incubated as described in MATERIALS AND METHODS and analyzed on production of 14CO2 at 30 min after addition of the palmitate/BSA complex. In quiescent cardiac myocytes, 14CO2 production amounted to 67.5 ± 6.1 nmol/g wet mass (means ± SD, n = 13). A: palmitate oxidation as a function of the voltage. The voltage of the electric field was varied at a fixed frequency of 2 Hz; B: 14CO2 production as a function of the frequency. The frequency of the pulses was varied at a fixed voltage of 200 V. Data are means ± SD of 3–5 experiments carried out with different cardiomyocyte preparations.
Physiologically relevant condition, i.e., at exogenous concentrations of palmitate and BSA of 100 and 300 μM, respectively. We have established previously that, within the initial uptake phase (3 min), there is virtually no production of 14CO2, due to the time lag needed to equilibrate the endogenous lipid stores and the intermediates in the oxidative pathway with 14C from labeled palmitate (18). However, the palmitate oxidation rate can be properly measured as CO2 production at 30 min after palmitate addition, since CO2 is formed proportionally with time between 10 and 30 min after palmitate addition (18).

It was found that, when studied at 3 min after palmitate addition, the electrostimulation-induced increase in palmitate influx (1.3-fold at 2 Hz and 1.5-fold at 4 Hz; Table 1) is accompanied by a similar increase in palmitate esterification into cellular lipid pools (1.3-fold at 2 Hz and 1.5-fold at 4 Hz; Table 1) and increased palmitate metabolism (19). Moreover, when total cellular lipid was split into triacylglycerols and phospholipids, it appeared that, upon electrostimulation at 4 Hz, esterification into triacylglycerols was enhanced 1.5-fold (P < 0.05) and esterification into phospholipids 1.2-fold, with the latter being not significant (Table 1). Thus, during the initial uptake phase of the increased palmitate influx into cardiac myocytes (101.7 – 68.2 = 33.5 nmol·min⁻¹·g wet mass⁻¹), 70% is incorporated into triacylglycerols (increase: 78.4 – 55.2 = 23.2 nmol/g wet mass) (Table 1). At this initial phase and under resting conditions, only ~15% of the palmitate taken up by cardiac myocytes is converted into oxidation products (sum of oxidation intermediates and produced CO2), and this percentage does not increase upon electrostimulation. Finally, in both the absence and presence of electrostimulation, the amount of palmitate that was taken up by cardiac myocytes but not metabolized (i.e., unesterified FA; Table 1) was found to contribute to ~6% of the total amount of palmitate taken up.

When palmitate uptake and metabolism were studied at 30 min after palmitate addition, palmitate uptake was enhanced 1.4-fold at 2 Hz and 1.5-fold at 4 Hz, whereas esterification into cellular lipid pools was not significantly altered either at 2 Hz or at 4 Hz. In addition, the proportional contribution of phospholipids and triacylglycerols to the incorporation of celluarly sequestered palmitate was unaltered (Table 1). After 30 min of incubation, the oxidation of [1-14C]palmitate (into 14CO2 and acid-soluble oxidation intermediates) was easily detectable in quiescent cells and accounted for 22% of the palmitate that was taken up. At 2 and 4 Hz of electrostimulation, palmitate oxidation was increased 1.9- and 2.8-fold, respectively, and contributed to 30 and 39% of palmitate utilization. These latter increases resulted fully from increases in 14CO2 production, as the amount of oxidation intermediates was not significantly altered upon electrostimulation (Table 1). Hence, the extra incoming palmitate was completely oxidized to CO2 and acid-soluble oxidation intermediates (Fig. 4A) or oxidation (Fig. 4B). In electrically stimulated myocytes, it completely blocked the electrostimulation-induced increase in palmitate uptake, so that palmitate uptake in quiescent and electrostimulated cells treated with this compound were not significantly different (Fig. 4A). In addition, it largely (66%) abolished the electrostimulation-induced increase in palmitate oxidation (Fig. 4B).

Role of FAT/CD36 in FA uptake by cardiac myocytes. To test whether the electrostimulation-induced increases in palmitate uptake and metabolism are due to passive diffusion or to protein-mediated transport, we used phloretin, an inhibitor of protein-mediated membrane transport processes (2). Palmitate uptake and oxidation were inhibited by phloretin by 87 and 76% in
quiescent myocytes, respectively [in agreement with our earlier observations (19)], and by 90 and 89% in electically stimulated myocytes. Furthermore, the remaining palmitate uptake and oxidation rates measured in cardiac myocytes incubated with phloretin were not significantly different in the presence and absence of electrostimulation (Fig. 4).

Subsequently, to investigate a possible role for FAT/CD36, we used two specific inhibitors, SSO and SSP (12, 19). SSO inhibited palmitate uptake and oxidation by quiescent myocytes by 65 and 52%, respectively [in agreement with our earlier observations (19)] and by electrically stimulated myocytes by 74 and 81%, respectively. SSP showed similar effects on palmitate uptake and oxidation by isolated cardiac myocytes. Notably, in the presence of either one of these succinimimidyl-FAs esters, the rates of FA uptake and oxidation were not significantly different between quiescent and electrically stimulated cardiac myocytes (Fig. 4), indicating that these FAT/CD36-specific inhibitors can fully block the electrostimulation-induced increases in both uptake and oxidation of palmitate.

**DISCUSSION**

This study documents that subjecting rat cardiac myocytes in suspension to regular contractions by applying electric field stimulation results in an enhanced rate of long-chain FA uptake (≥1.5-fold) and an enhanced rate of mitochondrial FA oxidation (≥2.8-fold). Importantly, the enhanced uptake appears to be mediated by the membrane-associated FA transporter FAT/CD36. These findings point toward a pivotal role of FAT/CD36-mediated transsarcolemmal FA transport as a rate-governing step in FA utilization by cardiac myocytes.

To make these studies possible, it was necessary to develop a system in which isolated cardiac myocytes, in parallel incubations, could be stimulated to contract regularly in an electric field at the pace of the applied frequency. Therefore, we will discuss both the merits of this model system and the interpretation of the study data.

**Electrical stimulation device.** Electric field stimulation of cardiac myocytes in vitro has been used mainly in microscopic studies on contraction parameters and in studies on calcium fluxes and ion channels, requiring an experimental setup in which cells can be studied on individual responses to electrical stimulation (3, 11, 16, 21, 25). However, such a setup is not suitable for metabolic studies on utilization of labeled substrates. Up to now, studies on the metabolic effects of electrically induced contractions in isolated cardiac myocytes in suspension have been scarce and hampered by the limitations in the experimental setup, i.e., a pulse generator connected to a single stimulation chamber, allowing one measurement at a time (13, 15, 32). This restricts the making of a detailed inventory of kinetics of substrate handling by contracting cardiac myocytes, for instance in combination with hormones, competing substrates, or metabolic agents. Therefore, we developed a setup in which up to 20 cell incubations can be electrically stimulated simultaneously, as schematically shown in Fig. 1.

For cardiac myocytes to contract, an electric potential difference of sufficient magnitude between one end of the cell and the other is required to induce membrane depolarization. Because of the relatively small size of the cardiac myocytes and the high conductance of the incubation medium, it was previously reported by Kammermeier and Rose and coworkers (15, 23, 24) that large electrical pulses are necessary to accomplish excitation. In our experimental system, pulses ≥170 V were necessary to exceed the threshold for contraction, as was observed microscopically and was apparent from an increased rate of oxidation of palmitate (Table 1 and Fig. 2A). Under these conditions, there was no decrease in cell viability, as can be deduced from the fact that cellular ATP content was maintained and the percentage of rod-shaped cells excluding trypan blue did not decline.
Metabolic responses in stimulated cardiac myocytes. Palmitate uptake and metabolism by cardiac myocytes were studied at 100 μM palmitate complexed to 300 μM BSA (palmitate/BSA molar ratio 0.3), as also applied previously (19). This palmitate concentration is well below the apparent Michaelis-Menten value of palmitate uptake of 435 μM (19), whereas the BSA concentration reflects the albumin concentration of the interstitial space (33). This condition was chosen because it is physiologically relevant and allows the study of regulatory aspects of FA uptake and metabolism.

Under this condition, palmitate oxidation, measured after 30 min of palmitate uptake as the sum of the production of 14CO2 and of 14C-labeled acid-soluble oxidation intermediates, was increased 1.9-fold at 2 Hz and 2.8-fold at 4 Hz (Table 1). Moreover, palmitate oxidation increased proportionally with the pulse frequency up to 4 Hz (Fig. 2B). Hence, with this setup, enhanced mechanical activity causes palmitate oxidation by isolated cardiac myocytes to increase, which is in agreement with earlier observations in intact heart, whereby a twofold difference was observed between a Langendorff-perfused heart and a working heart in favor of the latter (6). Interestingly, other studies, in which an increase in workload is achieved by exposing working hearts to epinephrine (5, 10), demonstrated no change in palmitate oxidation but a selective increase in carbohydrate oxidation. A possible explanation between these differing observations could include an epinephrine-mediated activation of pyruvate dehydrogenase in working hearts through Ca2+-dependent dephosphorylation of this enzyme, as hypothesized by Collins-Nakai et al. (5), leading to a preferential carbohydrate utilization (Randle cycle) and prevention of an increase in FA oxidation.

A novel observation in this study is that electrically induced contractions of cardiac myocytes increased the initial rates of uptake of both palmitate and deoxyglucose and did so to a similar extent (1.3-fold at 2 Hz and 1.5-fold at 4 Hz). Enhancement of glucose transport upon electrical stimulation of cardiac myocytes in suspension has recently been described by Till et al. (32) and has been found to be due to the well-characterized contraction-induced translocation of GLUT-4 to the cell surface. However, we observed that, in the presence of physiological concentrations of palmitate in the incubation medium, the contraction-induced increase in deoxyglucose uptake was abolished. In contrast, the reverse, i.e., the presence of glucose as competing substrate, did not prevent increased uptake of palmitate in the presence of electrostimulation. Apparently, electro-stimulation of cardiac myocytes favors FA utilization above glucose, but when FA are absent, glucose will be used to meet with the increase in metabolic demands.

The data collected for the initial (3 min) uptake phase (Table 1, left) indicate that, in quiescent cardiac myocytes, ~80% of the radiolabeled palmitate extracted by the cells from the medium was esterified by incorporation into both triacylglycerols (50%) and phospholipids (30%). Upon (4 Hz) electrical stimulation, the 1.5-fold increase in initial palmitate uptake was accompanied by a similar (1.4-fold) increase in esterification of radiolabeled palmitate into triacylglycerols, whereas there were no significant changes in incorporation of radiolabel into other fractions (phospholipids, citric acid cycle and β-oxidation intermediates, CO2, and nonesterified FA). Thus, in both quiescent and contracting cardiac myocytes, after their uptake, FA are largely (and rapidly) esterified, predominantly into triacylglycerols. The absence of a rise in nonesterified FA in the presence of electrostimulation indicates that, during initial FA uptake, esterification is sufficiently rapid to keep pace with the increased influx of palmitate and thus suggests that FA uptake is a rate-governing step in FA utilization by cardiac myocytes.

The measurements made after 30 min of incubation with radiolabeled palmitate (Table 1, right) indicate that, in quiescent myocytes, the rate of FA uptake has declined (from 22.7 to, on average, 11.8 nmol·min⁻¹·g wet mass⁻¹) but that still ~80% of the radiolabeled palmitate taken up is esterified, mainly into triacylglycerols. Hence, incoming FA continue to be preferentially incorporated into the cellular lipid pool. Electrical stimulation (4 Hz) of the myocytes induces a markedly increased energy demand, as appears from a 2.8-fold increased rate of palmitate oxidation and which corresponds to a 2.9-fold increase in O2 consumption (measured with a Clark-type oxygen electrode; data not shown). Because in quiescent cardiac myocytes a relatively small proportion (22%) of the extracted palmitate is used for β-oxidation, and because electrical stimulation affects only the rate of β-oxidation and not the rate at which radiolabel is incorporated into the esterified FA pool, a relatively large increase in β-oxidation is accompanied by a relatively modest increase in cellular FA uptake.

Role of FAT/CD36 in contraction-enhanced palmitate uptake. Using phloretin, we established that the contraction-induced increase in FA uptake is a protein-mediated process. Furthermore, the ability of SSO and SSP to completely block the additional uptake of FA under stimulating conditions indicates that, among the various FA transporters, FAT/CD36 is responsible for the major part of increased sequestration of FA by cardiac myocytes during increased mechanical activity. This notion is based on the fact that there is convincing evidence that sulfo-N-succinimidyl esters of FA are specific inhibitors of FAT/CD36. First, incubation of adipocyte plasma membranes, in which both FABPpm and FATPs are ubiquitously present (14, 35), with [3H]SSO shows selective incorporation of radiolabel in an 85-kDa protein corresponding with FAT/CD36 (12). Second, with giant vesicles derived from liver tissue, which enclosing membranes do contain both FABPpm, FATPs, caveolin, and a variety of anion transporters (14, 35) but, most importantly, lack the presence of FAT/CD36 (35), FA uptake is not inhibited by addition of SSO (18). The similar inhibitory actions of SSO and SSP on both uptake and oxidation of palmitate show that, under these conditions of greatly diminished up-
take of FA, their channeling into oxidation vs. esterification is not affected by SSO or SSP. Therefore, it is likely that SSO and SSP affect FA utilization solely at the level of the sarcolemmal uptake process. Thus the complete inhibition by SSO of contraction-induced FA uptake suggests that a main function of FAT/CD36 is to allow greater FA influx when elevated oxidation rates are demanded to support mechanical activity.

The involvement of FAT/CD36 in the uptake of FA on the onset of mechanical activity is in line with our earlier study with skeletal muscle, which tissue demonstrated a marked increase in FA uptake upon electrically induced contractions (4). The underlying mechanism for this increase in FA uptake includes a translocation of FAT/CD36 from a yet-unidentified intracellular compartment (presumably endosomes) to the sarcolemma. One may speculate that electrical stimulation of cardiac myocytes in suspension also causes redistribution of FAT/CD36 from this compartment to the cell surface. From the effects of verapamil on FA uptake by cardiac myocytes (no effect in quiescent myocytes and inhibition of FA uptake in stimulated myocytes), we further speculate that contractile activity and, hence, increased metabolic demand is the trigger for FAT/CD36 translocation and that metabolic drive of the electric pulse through the sarcolemma is not sufficient for activating this phenomenon. Because electrostimulation of cardiac myocytes also induces GLUT-4 translocation (32), it would then be of interest to study which components of the cellular translocation machinery are shared to mobilize both FAT and GLUT-4 at the onset of cellular contractions. However, the inability of contractions to stimulate glucose uptake when cardiac myocytes are electrically stimulated in the presence of palmitate as the sole exogenously added substrate indicates a lack of GLUT-4 mobilization in the presence of ongoing recruitment of FAT/CD36. This apparent uncoupling of FAT/CD36 translocation from that of GLUT-4 in this special condition is evidence in favor of the notion that both transporters are, at least in part, localized in distinct endosomal compartments.

In conclusion, we developed an experimental setup to study substrate handling by cardiac myocytes in the presence of electric field stimulation. The significance of this model is that experiments with contracting cardiac myocytes allow the investigation of the relation between effects of various agents (e.g., hormones, inotropic agents, inhibitors) and of contractile activity on substrate metabolism so as to delineate whether such effects are mutually dependent or occur independently of each other. With this setup, we observed that an increase in metabolic demand as elicited by electrostimulation of the myocytes gives rise to an enhanced initial palmitate uptake rate. Virtually all of the extra incoming palmitate is immediately deposited into intracellular triacylglycerols. Experiments with specific inhibitors suggested that the increased palmitate uptake rate is mediated primarily by membrane-associated FAT/CD36. In analogy with our observations in skeletal muscle, a possible mechanism explaining this increase in FA uptake by electrostimulated cardiac myocytes could include a contraction-induced translocation of FAT/CD36 from intracellular stores to the sarcolemma.

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