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FATTY ACID-MEMBRANE INTERACTIONS IN ISOLATED CARDIAC MITOCHONDRIA AND ERYTHROCYTES

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The effects of long-chain fatty acids on mitochondrial functions and red cell stability were studied. In albumin-containing incubation media, fatty acid distribution between the albumin-bound and the unbound fraction was estimated by calculation. When fatty acids are compared to one another on the basis of identical unbound concentrations, their effectiveness differs by orders of magnitude. Fatty acids stimulate mitochondrial basic oxygen consumption, thus lowering the respiratory control index, without changing the ATP/O ratio at lower concentrations. Lower concentrations increase Ca^{2+} uptake velocity, but decrease maximal Ca^{2+} storage capacity. The order of effectiveness of different fatty acids is the same for both oxidative phosphorylation and Ca^{2+} uptake. The influence of fatty acids on red cell stability in hypotonic media is similar to these effects both in concentration range and in order of effectiveness. The influence of fatty acids on red cell stability and their critical micellar concentrations were investigated because these are general characteristics of 'detergent-like' compounds. Critical micellar concentrations of the fatty acids in physiological salt buffers are, in general, at least 10-fold higher than the concentrations exhibiting membrane effects in vitro. Based on these findings it is suggested that, of the various concentrations reported in literature for myocardial non-esterified fatty acids, only the lowest values are physiologically possible.

Introduction

Mitochondria are of central importance during the development of cellular damage in myocardial infarction, first, because they are the source of respiratorily generated ATP (for a review see Ref. 1), and second, because they represent a huge storage capacity for intracellular calcium [2,3] comprising more than 90% of calcium-transporting membranes of the myocardial cell [4]. In hypoxia the cytosolic calcium balance becomes disturbed [5]. Possibly the reported increase in mitochondrial calcium content [6–8] that might result in electron-dense matrix granules [9–11] is an expression of an overloaded 'survival function' of mitochondria as a sink for cytosolic excess calcium.

Repeatedly, it has been asked whether an intracellular increase of long-chain fatty acids and their carnitine and coenzyme A ester derivatives might enhance tissue damage in myocardial infarction [12–24]. Fatty acids are disadvantageous substrates under oxygen limitations, since their ATP/O yields are lower than those of glucose or lactate. A second effect discussed recently may consist of futile ATP-consuming cycles activated by high fatty acid concentrations during restricted β-oxidation [25]. However, apart from these metabolic effects, accumulating fatty acids may

Abbreviation: POCA, sodium 2-(5-(4-chlorophenyl)pentyl)-oxirane-2-carboxylate.

cause membrane damage by physical interaction with the lipid phase of biological membranes, since they and their carnitine and CoA derivatives are amphiphilic in character [18,24,26–29]. This study is concerned with this last hypothesis.

Considerable efforts have been undertaken to determine the contents of non-esterified fatty acids in normal and hypoxic hearts [30–38], but with results which differ up to a factor of 1000 (cf. Refs. 38 and 34). The authors who have given the most attention to analytical details are those who report the lowest values. If indeed the lower values are correct, a large amount of the literature in this area is severely in doubt. This study tries to answer the question as to the range of fatty acid concentrations in which pathological effects appear in isolated mitochondria. With some assumptions about compartmentation, from this the magnitude of the corresponding tissue contents can be estimated.

Like other amphilic substances, fatty acids may interfere with the physical stability of biological membranes. These effects have been named 'detergent-like effects' [24]. If functional alterations induced by different fatty acids in mitochondria are caused by the general property of acting as a detergent on biological membranes, then their different effects on mitochondrial functions might be mirrored in the differences in their ability to alter the mechanical stability of other biological membranes, e.g., the erythrocyte membrane.

In this study the action of several physiologically important long-chain fatty acids on mitochondrial membranes is investigated. Purified isolated mitochondria from fresh myocardium were incubated in albumin-containing fatty acid salt solutions, whose contents of unbound fatty acids can be calculated [39]. Thus mitochondria were exposed to defined concentrations of unbound fatty acids which cannot be metabolized unless converted to their coenzyme A esters. At equal unbound concentrations, the effects of different fatty acids on mitochondrial respiration and calcium uptake were compared. Finally, the observed orders in effectiveness were compared with fatty acid influence on red cell stability and their critical micellar concentrations, as parameters of the supposed 'detergent-like' action of fatty acids on the myocardial cell.

Methods and Materials

1. Isolation of mitochondria

Cardiac mitochondria were isolated from guinea pigs (250 g) using a modification of the procedure described by Sordahl and Schwartz [40]: 4–6 g of minced heart tissue were suspended in 30 ml of buffer A (180 mM KCl/10 mM EGTA/5 mM Tris/2% bovine serum albumin, pH 7.4) and homogenized with an Ultra-Turrax tissue homogenizer (Jahnke und Kunkel, Stauffen i. Br., F.R.G.). This suspension was centrifuged at $500 \times g_{max}$ for 10 min, and the supernatant was filtered through cheesecloth and centrifuged at $10\,000 \times g_{max}$ for 15 min. The pellet was resuspended in buffer B (180 mM KCl/1 mM EGTA/5 mM Tris/0.5% human serum albumin, pH 7.4). This centrifugation step was repeated twice. The pellet finally obtained was resuspended in buffer B to approximately 20 mg/ml mitochondrial protein. For Ca^{2+} uptake measurements EGTA was left out of the last two resuspensions. Protein content was determined from a double washed sample by Bradford's method [41]. All steps were carried out in the cold (4°C); mitochondrial suspensions were stored on ice.

2. Calculation and preparation of fatty acid-albumin buffer systems

A quantitative description of fatty acid binding to human serum albumin has been given by Ashbrook et al. [39] in terms of stepwise equilibrium constants for pH 7.4 and 37°C. With the help of a computer program similar to the one proposed by Wosilait et al. [42] we calculated from these data the concentrations of unbound fatty acids at different fatty acid to albumin ratios. These calculations could be carried out for those fatty acids whose binding constants have been reported, i.e.: myristic acid (14 : 0), palmitic acid (16 : 0), stearic acid (18 : 0), oleic acid (18 : 1) and linoleic acid (18 : 2). Preparation of these buffers was started by dissolving sodium salts of these fatty acids (obtained from Sigma Chemie, Taufkirchen, F.R.G.) in methanol and subsequently drying off the methanol under a stream of nitrogen. The albumin (75 μM) -containing salt solution was added then to the precipitate and stirred for at least 5 h. Clear stock solutions of 10^{-4} or

10^{-5} M unbound fatty acids were prepared by this procedure. Solutions containing unsaturated fatty acids were held under nitrogen. According to the calculated unbound concentrations, resulting from defined ratios of total fatty acid to total albumin content, these stock solutions were appropriately diluted with 75 μM albumin solution, then stirred again, in order to obtain the desired unbound concentration. It has been shown by Soltys and Hsia [43] that this dilution procedure leads to identical results with separate preparations. The albumin used was human serum albumin 'reinst' from Behringwerke (Marburg, F.R.G.); all other reagents were analytical grade.

3. Measurement of mitochondrial respiratory parameters

For the determination of O_2 consumption mitochondria (2 mg of protein) were incubated at 37°C in 3 ml of a buffer containing 270 mM sucrose, 1 mM EGTA, 10 mM Tris, 11 mM KH_2PO_4, 5 mM glutamate, 75 μM human serum albumin and different amounts of fatty acids as determined by calculation, at pH 7.4. Oxygen tension in this suspension was continuously recorded with a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). After 5 min of incubation 250 μM ADP was added to the suspension. ADP-induced state 3 respiration (Q_3), the following state 4 respiration (Q_4), respiratory control index (RCI: ratio of O_2 consumption in the presence of ADP to that after phosphorylation to ATP) and the ATP/O quotient (nmol ADP used per natoms O_2 consumed) were determined according to Estabrook [44]. The O_2 consumption rate at a control index of 1, when a difference between state 3 and state 4 respiration could not longer be seen, was named 'Q_0'. In order to prove that the observed effects of increasing concentrations were not influenced by oxidation of fatty acids (as they might be if residual amounts of carnitine and coenzyme A were still adherent to the isolated mitochondria) all experiments were run twice, with the acyl-carnitine transferase I inhibitor POCA (sodium 2-(5-(4-chlorophenyl)-pentyl)oxirane-2-carboxylate, 10^{-4} M) present in one series [45]. POCA was a generous gift from Dr. H. Wolf from Byk Gulden Pharmazeutika, Konstanz, F.R.G.

4. Measurement of mitochondrial Ca^{2+} uptake

Ca^{2+} uptake of mitochondria was followed in a medium containing 200 mM sucrose, 70 mM KCl, 10 mM Tris, 1 mM K_2HPO_4, 0.5 μg/ml rotenone, 75 μM human serum albumin and 1 mg/ml mitochondrial protein at pH 7.4 and 37°C. Ca^{2+} depletion of the bulk phase of the medium was followed by absorbance changes of 50 μM murexide in the double-wavelength mode (507 vs. 542 nm) with a DW 2 spectrophotometer (American Instruments, Silver Springs, MD, U.S.A.) according to Scarpa and Graziotti [46]. After 2 min of incubation 1000 μM Ca^{2+} was added to the solution, then mitochondria were energized by addition of 5 mM succinate. In the concentration range tested changes of bulk-phase Ca^{2+} are in linear relationship with changes in the indicator's absorbance.

5. Determination of osmotic fragility of red cells

Human erythrocytes were obtained from heparinized blood samples by centrifugation and two-fold resuspension in NaCl solution A (138.6 mM NaCl/8.7 mM Na_2HPO_4/1.4 mM NaH_2PO_4. pH 7.4) or B (as A with 75 μM human serum albumin additionally). Finally the hematocrit was adjusted to 50%.

Curves of osmotic resistance were determined with erythrocytes washed in either solution according to Parpart et al. [47]. Hemoglobin content in the supernatant was determined as hemoglobinocyanide spectroscopically. For 100% lysis a sample was sonicated for maximal hemoglobin release. For each fatty acid its effect on osmotic hemolysis was tested by incubating erythrocytes in a dilution of solution A of 125 mosM/kg with 75 μM human serum albumin and fatty acid content as determined by calculation.

6. Determination of critical micellar concentration

The critical micellar concentrations of fatty acids were determined following the procedure of Kovatchev et al. [48]. This method uses the fluorescent indicator *N*-phenylnaphthylamine (Kodak, Rochester, NY, U.S.A.), the fluorescence intensity of which increases exponentially if the concentration of lipids is raised over the critical concentration for micelle formation. Since the indicator interferes with albumin, the critical micellar con-

centrations were determined in an albumin-free salt solution (100 mM NaCl/10 mM NaH_2PO_4 at pH 7.4 and 37°C). The critical micellar concentration of the compound under investigation can be read from a plot of its logarithmic concentration values against measured fluorescence intensity, since the critical micellar concentration is marked as the point of intercept of the two linear branches of the graph.

Results

1. Oxidative phosphorylation

The effects of unbound long-chain fatty acids (UFA) were tested in the range 10^{-7}–10^{-4} M. As shown in Figs. 1–3, this range almost completely covers the unbound concentrations from those with just detectable effects up to those that fully destroy mitochondrial functions. The respiratory control index is heavily suppressed by increasing unbound concentrations of all fatty acids under investigation, but concentrations necessary to lower the index to 1 differ by more than two orders of magnitude (Fig. 1). Chain elongation from 14 : 0 to 18 : 0 results in an increasing detrimental effect, but unsaturation can make a compound more (18 : 1) or much less harmful (18 : 2) than the saturated fatty acid (18 : 0). 18 : 2 is even less effective than 14 : 0 and 16 : 0.

The fact that the respiratory control index of the control samples is as low as 7 is mostly due to the fact that for application of the equilibrium constants conditions of pH 7.4 and 37°C had to be chosen, whereas commonly standard conditions of 25°C and lower pH are used. Lowering the temperature to 25°C led to values of 12, possibly due to less proton leakage. External NADH did not stimulate respiration. As shown in Fig. 2, ATP/O ratios do not change much unless the control index has almost reached a value of 1. All fatty acids stimulate oxygen consumption if concentrations are raised up to the level where the control index becomes 1 (Fig. 3). With the exception of 18 : 1 and 18 : 2 all fatty acids lead to a Q_0 value that is about twice as high as Q_4 under control conditions. They keep Q_0 at this value for some orders of magnitude in concentration. 18 : 2 is exceptional, since Q_0 is 9-times as great as the control Q_4 when the control index reaches 1. 18 : 1

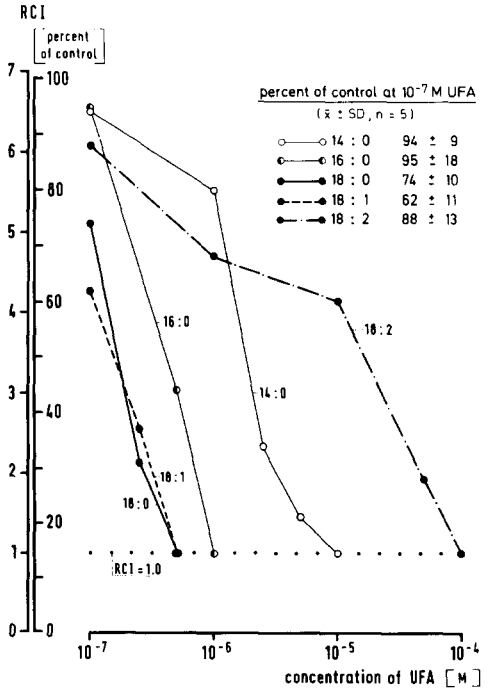

Fig. 1. Respiratory control index (RCI) of mitochondria under the influence of different unbound fatty acids (UFA).

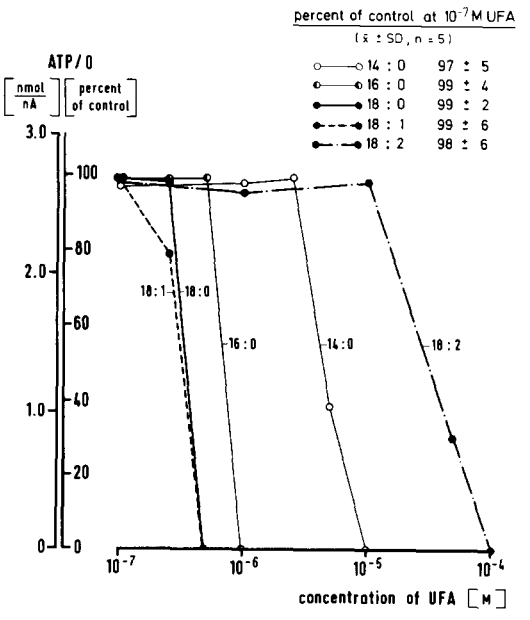

Fig. 2. Alterations of mitochondrial ATP/O ratio in the presence of different unbound fatty acids (UFA).

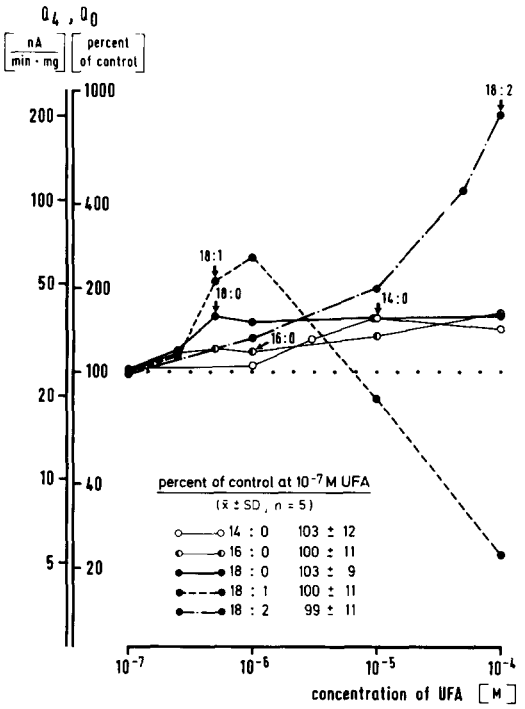

Fig. 3. Effects of unbound fatty acids (UFA) on mitochondrial respiration. Under the influence of increasing fatty acid concentrations state 4 respiration (Q_4) turns into fully uncoupled respiration (Q_0, indicated by arrow).

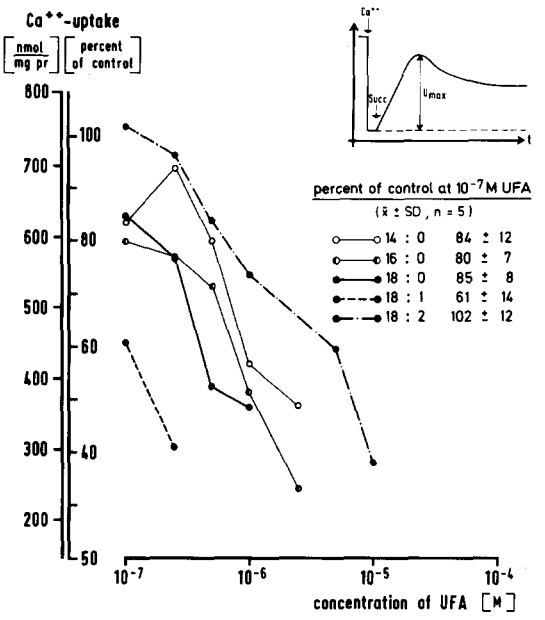

Fig. 4. Influence of unbound fatty acids (UFA) on maximal Ca^{2+} uptake capability of mitochondria. The insert shows schematically a typical absorbance recording for Ca^{2+} uptake measurements using murexide as indicator.

is another exception, although opposite in character: at 10^{-4} M, Q_0 is diminished to about 20% of control Q_4. In this respect 18 : 0 differs markedly from 18 : 1, even if its influence on the control index is similar to that of 18 : 1.

Control experiments in the presence of the acyl-carnitine transferase inhibitor did not give significantly different results. These findings indicate that the effects observed are not influenced by β-oxidation, as could be if fatty acids were esterified and transported into the matrix space. Preliminary experiments in which mitochondrial protein was varied from 0.5 to 2 mg/ml in the assay volume did not show changes in susceptibility to identical unbound concentrations.

2. Calcium accumulation

Again, it is the range from 10^{-7} to 10^{-4} M of unbound concentrations in which the deleterious effects of long-chain fatty acids on mitochondrial calcium accumulation can be observed. And, as presented by Fig. 4, the fatty acids investigated

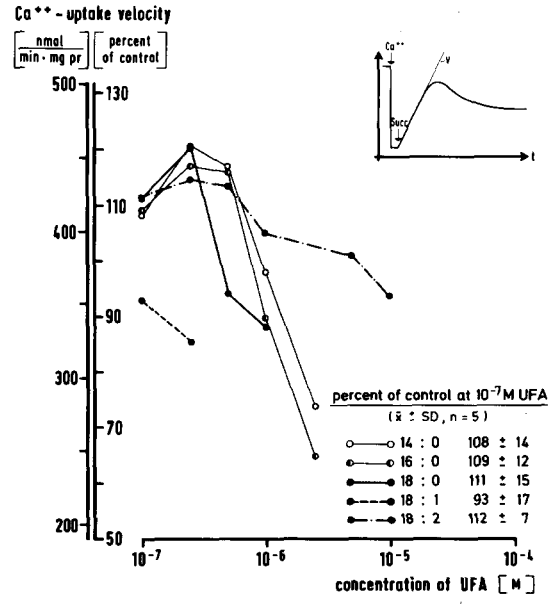

Fig. 5. Initial Ca^{2+} uptake velocity of mitochondria exposed to different unbound fatty acids (UFA).

affect the maximal calcium uptake in the same order of effectiveness as mitochondrial respiration. 18 : 1 needs the lowest, 18 : 2 the highest concentration to abolish specific calcium binding. The graphs in Fig. 4 end at nonzero accumulation. This is because, at higher concentrations, changes in absorbance are irregularly undulating and do not allow a definition of initial uptake velocity. This is possibly due to release of Ca^{2+} by severely damaged mitochondria and reuptake by less damaged ones. The influence of increasing fatty acid concentrations on the initial velocity of calcium uptake markedly differs from all other functional effects that were already mentioned (Fig. 5). With the exception of 18 : 1 all fatty acids investigated significantly improve the Ca^{2+} uptake velocity in the lower concentration range, by at least 10% ($P < 0.01$, one-way analysis of variance using quantitative levels).

3. Effects of fatty acids on red cell membrane stability

All fatty acids examined had a distinct effect on the stability of membranes of red blood cells in hypoosmolar media. At low concentrations they improved mechanical stability; at higher concentrations the red cells were lysed. This biphasic behaviour is demonstrated in Fig. 6. It should be noted that the degree of spontaneous hemolysis in hypotonic NaCl is markedly low if compared with what is generally known for the mechanical stability of normal human erythrocytes [47]. This is caused by washing erythrocytes with purified human serum albumin.

It is evident from Fig. 6 that the order of stabilization of erythrocytes is similar to the order of inhibition of mitochondrial function for the fatty acids investigated. Again, 18 : 1 is the most, 18 : 2 the least powerful agent. Moreover, the range of effective concentrations for membrane alterations is basically the same. But there is not total congruence. Whereas 14 : 0 and 18 : 2 differ distinctly in their action on mitochondrial respiration, they act almost identically on red cell membranes. In addition, after a steep transitional incline, they act lytically at lower concentrations than 16 : 0 and 18 : 0.

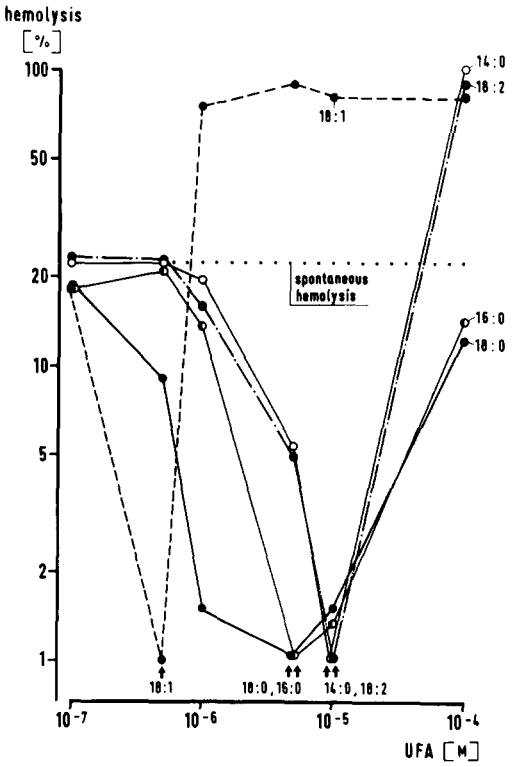

Fig. 6. Influence of unbound fatty acids (UFA) on erythrocytes incubated in hypotonic sodium chloride (125 mosM/kg). Under control conditions 22.6 ± 1.3% of hemoglobin ($\bar{x} \pm SD$, $n = 4$) was released.

4. Critical micellar concentrations

Critical micellar concentrations of all fatty acids used in this study are shown in Table I. With the exception of 18 : 2 all concentrations are higher by at least one order of magnitude than the concentrations that lead to maximal inhibition of mitochondrial functions and erythrocytolysis.

Increasing the number of carbon atoms lowers the critical micellar concentrations for saturated fatty acids. In the group of C_{18} acids, double bonds decrease them; however, 18 : 1 has the lowest value. This order in critical concentrations does not correlate exactly with the order in the biological effects examined in this study, but shows some resemblance. The order of magnitude of the critical concentrations for sodium salts of fatty acids is in accordance with investigations using other methods for their determination [49].

TABLE I

CRITICAL MICELLAR CONCENTRATIONS OF FATTY ACIDS

Critical micellar concentrations were determined with the use of the indicator *N*-phenylnaphthylamine [48] in 100 mM NaCl/10 mM NaH_2PO_4 at pH 7.4 and 37°C (mean values, $n = 5$).

Sodium myristate	(14:0)	$4.6 \cdot 10^{-3}$ M
Sodium palmitate	(16:0)	$3.6 \cdot 10^{-3}$ M
Sodium stearate	(18:0)	$1.6 \cdot 10^{-4}$ M
Sodium oleate	(18:1)	$2.5 \cdot 10^{-5}$ M
Sodium linoleate	(18:2)	$5.2 \cdot 10^{-5}$ M

Discussion

1. General aspects

In 1958, Lehninger and Remmert [50] described an extraction product from sonicated mitochondria which induced an increase in Q_4 respiration without lowering the ATP/O ratio. Borst et al. [51] have shown that oleic acid (18:1) at lower concentrations also has such effects. However, higher concentrations which decreased the respiratory control index nearly to 1 lowered the ATP/O ratio. Palmitic acid (16:0) was reported to be much less effective. The effects of fatty acids could be weakened by addition of serum albumin, which has been known to bind long-chain fatty acids [52].

However, from such studies no quantitative results could be obtained, since the albumin-bound fraction of fatty acids was not specified. From the work of Spector's group [39,42,53] data are now at hand which allow quantification not only of the effects of the one selected, but of several fatty acids in comparison. These can be used reliably in physiological buffered salt solutions at pH 7.4, as long as the ionic strength is not changed drastically [54].

As mentioned in the Introduction, there is little agreement in the literature as to what the actual levels of non-esterified fatty acids in normal myocardium are. We want to make an estimation of cytosolic unbound concentrations, taking into account the lowest values reported. These are of the order of 10^{-8} to 10^{-7} mol/g (wet wt.) [33–35,37,55,56]. For a rough calculation we might take tissue water as about 70% of wet weight, cytosolic space as 20% of total cell volume [57] and the portion of intracellular fatty acids not tightly bound as 70% [58]. From this, not-tightly-bound cytosolic fatty acids can be estimated to be of the order of 10^{-5} to 10^{-4} M. If it is assumed that the ratio of fatty acids loosely bound to cytosolic proteins and membranes [59–61] to those unbound is not less than 100:1 and the relative portion of the most dangerous compound, 18:1, is about 18% [34,37], then this fatty acid, in its unbound form, is in the cytosol at concentrations of the order of 10^{-8} to 10^{-7} M. Our data indicate that unbound concentrations of 18:1 between 10^{-7} and 10^{-6} M completely abolish mitochondrial function, whereas concentrations below 10^{-7} M are almost ineffective. Among the assumptions made, the one of 100:1 binding in the cytosol is the least certain, but it seems to give a reasonable lower-limit estimate for the bound fraction. If the estimation of normal 18:1 contents is basically correct, the lowest values reported seem to be the most probable. On the basis of these calculations a myocardial non-esterified fatty acid content of greater than 10^{-7} mol/g (wet wt.) is damaging and can hardly be found in normal hearts. Van der Vusse et al. [35] reported relative increases in fatty acid contents for 18:1 and 18:2 by factors of 6 and 7 after 2 h of coronary ligation. From our data and from the estimative calculations just performed, this raises 18:1 to damaging concentrations, but not 18:2 (whose relative portion is about 14% normally [34,37]).

Little is known about the interaction of cytosolic fatty acids with binding proteins and low-affinity membrane binding sites. This is, however, of special importance for quantitative considerations of the meaning of fatty acid accumulation in tissue. The cytosolic fatty acid binding protein seems to be an effective regulator for normal fatty acid metabolism [61–64] and may protect cells against accumulating lipids that act harmfully in vitro [65,66].

2. Mitochondrial function and structure

It has been reported several times that oleic acid (18:1) is the most effective among physiologically occurring fatty acids in disturbing mitochondrial function [51,67–70]. The relative differences

between the other fatty acids are not so clear. Fatty acid 'buffer systems' stabilize the external levels of unbound fatty acids (and thereby the membrane-bound fraction) with an albumin-bound fraction that is at least 100-fold higher under our conditions. This gives a more reliable basis for comparisons than the total amount of fatty acids in the assay volume per mg mitochondrial protein, because in the latter system the degree of saturation of mitochondrial binding sites remains uncertain.

The investigated fatty acids differ in their deleterious effects by orders of magnitude. At high concentrations, they are even opposite in their ability to increase basic O_2 consumption. The effect of 18 : 2 exceeds all others by a factor between 4 and 6. In the case of 18 : 1, going beyond the point of total uncoupling leads to almost total inhibition of O_2 consumption.

The capacity of mitochondria to bind ionized calcium is inhibited by fatty acids in the same relative order and by amounts comparable to those found effective on oxidative phosphorylation. In contrast to the distinct damaging influence of fatty acids on maximal calcium storage, calcium uptake velocity is not inhibited by lower unbound concentration, except with 18 : 1. This discrepancy might be partly explained by a 'premature' release of accumulated Ca^{2+} [71]. It is not yet clear whether such hyperpermeability is an expression of definite membrane damage or has some physiological meaning [72]. Low unbound concentrations even improve initial calcium uptake velocity. This might be due to a functional improvement of the uptake mechanism through alteration of the lipid environment [73]. As we have reported recently [74], acylcarnitines also improve the Ca^{2+} uptake velocity of mitochondria at lower, but inhibit it at higher, concentrations. It is not known, however, whether the observed relative improvement in vitro corresponds to a beneficial effect in situations of moderate fatty acid accumulation in vivo, since Ca^{2+} uptake competes with ATP formation for respiratorily generated energy [75]. Additionally, there may be other agents accumulating coincidentally which induce Ca^{2+} leakage [72].

Many reports [71,76–80] demonstrate that state 3 and state 4 respiration are inhibited in mitochondria isolated from ischemic myocardium. These findings may be due to one of the following reasons. 1. There is no respiratory stimulation in ischemic hearts because there are more potent and inhibitory influences than fatty acids or simply because there is no rise in unbound fatty acids. 2. The stimulatory effect of accumulating fatty acids is not seen in mitochondria isolated from ischemic tissue because mitochondria become depleted of adhering lipids by the conventionally performed washings in albumin-containing media.

When mitochondria were incubated with increasing concentrations of unbound fatty acids that led to functional damage, alterations of mitochondrial ultrastructure could be detected. At unbound concentrations that lowered the respiratory control index, cristae became more diffuse in structure and more rarefied in number. These changes were similar to those shown by Wojtczak et al. [69] in comparable experiments with liver mitochondria. Amorphous densities, which have been reported to be typical after prolonged low-flow hypoxia with high palmitate concentrations [81,82], were not seen regularly in our samples.

3. Detergent-like activity

It is difficult to defend or refute the assumption that deleterious effects of free fatty acids are due to their 'detergent-like activities'. Being amphiphilic these substances have such properties, as is demonstrated by their ability to destroy erythrocytes. However, the concept 'detergent' is not always used in a clearly defined manner. The critical micellar concentration is a physical measure for the amphiphilic character of a substance in aqueous solutions. We found a discrepancy between these physical characteristics of fatty acids and their functional effectiveness in mitochondria in regard to the range and order of their concentrations effective on mitochondrial functions (Fig. 7). This may be because functional changes are caused by insertion of fatty acids into the lipid layer of mitochondrial membranes [73,83–87]. For this process the regulatory circumstances might be quite different from those for micelle formation of a single substance.

If the supposed 'detergent-like activity' were a general property of these compounds it could be expected that other biological membranes, even with quite different lipid composition, would also

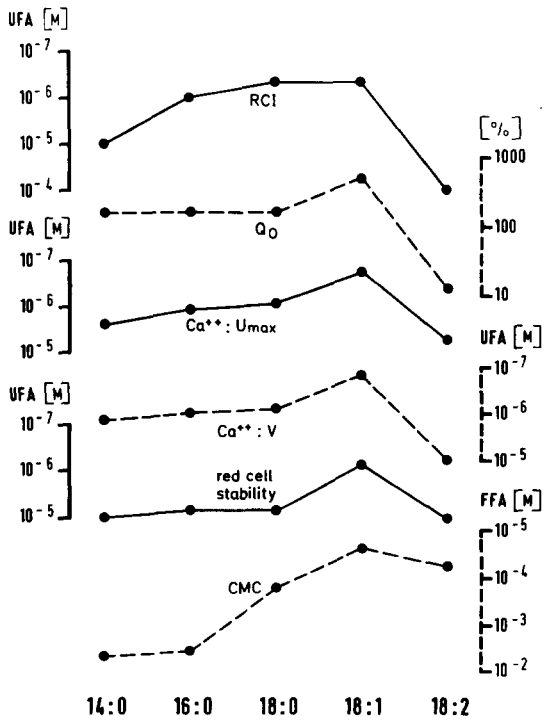

Fig. 7. Comparison of fatty acid effects on different parameters. From top: (1) unbound concentration (UFA) at which the respiratory control index reaches the value 1; (2) Q_0 respiration at 10^{-4} M UFA, expressed in percent of control Q_4 respiration; (3) unbound concentration at which maximal Ca^{2+} uptake becomes 50% of control; (4) unbound concentration at which initial uptake velocity becomes 90% of control; (5) unbound concentration at which red cell stability is maximal; (6) critical micellar concentrations.

be affected. Now, there is indeed some similarity between the effects of fatty acids on red cell stability and on mitochondrial functions (Fig. 7), but the congruence is not absolute. Fatty acids exert a lytic influence on red blood cells at unbound concentrations slightly above those at which the control index reaches 1.

Raz and Livne [88] have also demonstrated quantitative differences in the biphasic action of fatty acids on red cell membranes, but in the absence of albumin. For 18:0, 18:1 and 18:2 they found maximal stabilizing effects between 10^{-6} and $2 \cdot 10^{-5}$ M. Their results agree with ours in the finding that 18:1 and 18:0 tend to stabilize at lower concentrations than 18:2. But they detected only slight differences between these fatty acids, probably because they did not use buffered unbound concentrations. In previous studies it has been shown that several amphiphilic drugs as well as technically used detergents (such as Triton X-100 or SDS) exhibit this biphasic effect on red cells in hypoosmolar media [89–91]. But not all detergents show this stabilizing effect at low concentrations (e.g. saponines do not; see Ref. 84).

Conclusions

Physiologically occurring fatty acids differ by orders of magnitude in their detrimental effects on mitochondrial functions. Probably because of steric reasons linoleic acid is much less damaging than oleic acid. There are similarities between effects of fatty acids on mitochondrial functions and physical chracteristics of fatty acids such as their critical micellar concentrations and their influence on osmotic hemolysis. However, these similarities are not close enough to explain their action on functional membrane properties.

We are still very limited in our knowledge about the fatty acid contents of normal and hypoxic tissue. Our model calculations together with our experimental results show that only the lowest values reported for normal myocardial non-esterified fatty acid contents are in agreement with the hypothesis that normal contents are not harmful. Whether elevated contents of a certain fatty acid may result in a damaging effect seems to depend on how much is actually unbound. Still almost nothing is known about the action of cytosolic fatty acid binding proteins in heart. However, from the data available it is doubtful whether fatty acids by detergent-like effects play a decisive role in earlier ischemia.

Acknowledgements

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