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The Mechanisms of Fatty Acid-Induced Proton Permeability of the Inner Mitochondrial Membrane

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Nonesterified long-chain fatty acids have long been known as uncouplers of oxidative phosphorylation. They are efficient protonophores in the inner mitochondrial membrane but not so in artificial phospholipid membranes. In the un-ionized form, they undergo a rapid spontaneous transbilayer movement (flip-flop). However, the transbilayer passage of the dissociated (anionic) form is hindered by the negatively charged hydrophilic carboxylic group. In the inner mitochondrial membrane, the transfer of fatty acid anions is mediated by the adenine nucleotide translocase, the dicarboxylate carrier, and the glutamate/aspartate carrier. As a result, the passage of protons and electric charges is a concerted effect of the spontaneous flip-flop of the undissociated (protonated) form in one direction and carrier-facilitated transfer of the ionized (deprotonated) form in the other direction. In addition, fatty acids also promote opening of the mitochondrial permeability transition pore, presumably due to their interaction with one of its constituents, the adenine nucleotide translocase, thus forming an additional route for dissipation of the proton gradient. Structural prerequisites for these proton-conducting mechanisms are (1) a weakly ionized carboxylic group and (2) a hydrocarbon chain of appropriate length without substituents limiting its mobility and hydrophobicity.

KEY WORDS: Fatty acid; uncoupling; proton permeability; adenine nucleotide translocase; dicarboxylate carrier, glutamate/aspartate carrier; permeability transition pore; mitochondria.

INTRODUCTION

Disturbance of mitochondrial energy coupling by lipidic extracts from microsomes and other organic materials has been known since the early 1950s. It has also been observed that these uncoupling effects can be prevented as well as the yield of oxidative phosphorylation in isolated mitochondria substantially improved by serum albumin (for reviews see Wojtczak, 1976; Wojtczak and Schönfeld, 1993) whose high binding capacity for fatty acids was recognized at that time. Subsequently, this "natural" uncoupling agent was identified as a mixture of long-chain nonesterified fatty acids (Pressman and Lardy, 1956; Scholefield, 1956; Hülsmann *et al.*, 1960; Wojtczak and Wojtczak, 1960; Wojtczak and Lehninger, 1961; Borst *et al.*, 1962). In particular, large quantities of fatty acids accumulated in, or trapped by, isolated mitochondria were found to be responsible for poor P/O ratios of "aged" rat liver mitochondria (Hülsmann *et al.*, 1960; Chefurka and Dumas, 1966) and freshly prepared insect mitochondria (Wojtczak and Wojtczak, 1960).

Fatty acids, either generated endogenously in isolated mitochondria or added to mitochondrial preparations, have also been found to produce large amplitude swelling of these organellae (Lehninger and Remmert, 1959; Lehninger, 1962; Wojtczak and Lehninger, 1961). It then appeared that the swelling-inducing potency (Zborowski and Wojtczak, 1963) was dependent on the chain length and the degree of unsaturation in a similar way as was their ability to disrupt energy-coupling processes (Pressman and Lardy, 1956).

Fatty acids were found to differ from other uncouplers in their effect on mitochondrial ATPase. Whereas

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chemical uncouplers, like 2,4-dinitrophenol, activated mitochondrial "latent" ATPase, fatty acids at low concentration were found to abolish that activation (Bos and Emmelot, 1962; Chefurka and Dumas, 1966; Wojtczak *et al.*, 1969). This paradoxical effect was clarified by the finding (Wojtczak and Załuska, 1967; Wojtczak *et al.*, 1969) that fatty acids are potent inhibitors of the adenine nucleotide translocase (ANT).

Summarizing these early investigations, it is evident that three apparently separate effects of long-chain nonesterified fatty acids on mitochondria have been noted: (1) disruption of energy-coupling machinery (uncoupling), (2) permeabilizing the inner mitochondrial membrane to solutes (swelling), and (3) inhibition of the adenine nucleotide translocase. Achievements of last few years allowed the recognition of a common link between these functions—the subject of this article.

FATTY ACIDS AS PROTONOPHORES

A rational approach to the mechanism of the uncoupling became possible only on the basis of the "chemiosmotic" concept of energy coupling (Mitchell, 1966). Bielawski *et al.* (1966) were the first to demonstrate protonophoric properties of the most popular uncoupler, 2,4-dinitrophenol. Cunarro and Weiner (1975) showed a correlation between the stimulation of mitochondrial respiration by uncouplers (including fatty acids) and their protonophoric properties. The increase by long-chain fatty acids of proton conductance of phospholipid bilayer membranes was subsequently demonstrated by Gutknecht (1988).

The protonophoric mechanism requires that the active compound crosses the membrane in both protonated and deprotonated forms. This is true for most typical synthetic uncouplers, like dinitrophenol and CCCP, because of their highly lipophilic structure and delocalized negative charge of the anionic forms. For long-chain fatty acids, a fast transbilayer movement of the undissociated (protonated) form is possible because of its nonpolar character (Kamp and Hamilton, 1992). However, the transmembrane transfer of fatty acid anion is strongly hindered by the presence of highly polar carboxylic group bearing a localized negative charge. In fact, it has been shown that the half-time for the transbilayer passage of un-ionized long-chain fatty acids in phospholipid bilayers of small unilamellar vesicles is extremely short—in the order of 10 ms or less (Kamp *et al.*, 1995)—whereas the passage of long-chain fatty acid anions requires minutes (Kamp and Hamilton, 1992)—too long to account for effective dissipation of the protonmotive force in respiring mitochondria.

Yet, the protonophoric action of fatty acids in mitochondria is well documented (Luvisetto *et al.*, 1987; Schönfeld *et al.*, 1989; Luvisetto *et al.* 1990; Macrì *et al.*, 1991; Petrussa *et al.*, 1992). Schönfeld (1992) showed that fatty acid-induced uncoupling was limited by the permeation of fatty acid anions. On the other hand, fatty acids at low concentration do not increase proton conductance of liposomes with reconstituted proton-pumping cytochrome oxidase (Labonia *et al.*, 1988). These findings are compatible with the postulation (Wojtczak and Schönfeld, 1993) that fatty acids do not function as protonophores as such, but specifically increase proton conductance in mitochondria due to a concerted action with some component(s) of the inner mitochondrial membrane.

ROLE OF THE ADENINE NUCLEOTIDE TRANSLOCASE

Observations of the Skulachev's group that ADP as well as inhibitors of the adenine nucleotide translocase partly abolish the uncoupling effect of palmitate (Andreyev *et al.*, 1988, 1989; Dedukhova *et al.*, 1991) point to this carrier protein as a likely agent in fatty acid-mediated uncoupling. The following mechanism for the fatty acid-mediated proton transfer in the inner mitochondrial membrane has been therefore proposed (Skulachev, 1991): (1) a spontaneous transfer of the undissociated fatty acid molecule from the outer leaflet of the inner membrane to its inner leaflet (flip-flop); (2) dissociation of proton from the carboxylic group into the matrix compartment; (3) transfer of the fatty acid anion thus formed from the inner side of the membrane to the outer side with participation of ANT. These events result in a net transfer of proton, including its electric charge (Fig. 1).

Validity of this mechanism was confirmed by Schönfeld (1990) who demonstrated a correlation between the activity of ANT in various types of mitochondria and the protection by carboxyatractyloside against fatty acid-induced uncoupling. More recently, a similar parallelism was found for mitochondria whose expression of ANT was modified by the thyroid hormone status of the animals (Schönfeld *et al.*, 1997). The highest content of the transporter and its highest activity in mitochondria from hyperthyroid rats coin-

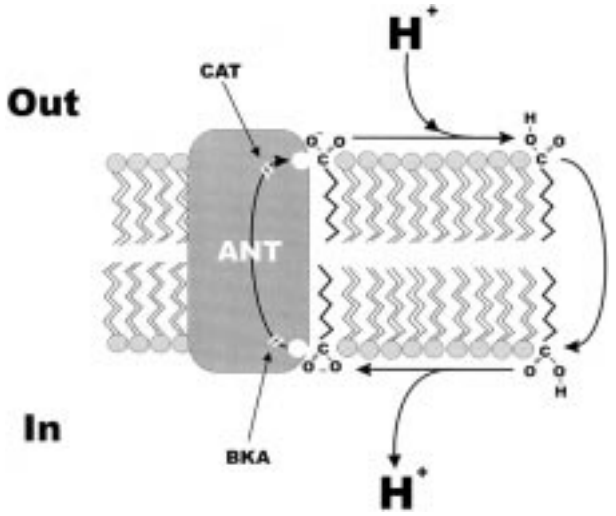

Fig. 1. Fatty acid cycling model of proton conductance in the inner mitochondrial membrane according to Skulachev (1991). Fatty acid molecule in the protonated (undissociated) form performs a spontaneous transbilayer movement (flip-flop) in the membrane, dissociates a proton in the matrix compartment, and the fatty acid anion is electrophoretically transported to the external leaflet of the membrane by the adenine nucleotide translocase (ANT). The latter transport can be inhibited by carboxyatractyloside (CAT) and bongkrekic acid (BKA).

cided with the highest stimulation of resting state respiration by fatty acids and its highest sensitivity to carboxyatractyloside. On the other hand, mitochondria from ANT-deficient yeast mutant appeared to be insensitive to the uncoupling by fatty acids (Polčic *et al.*, 1997). An elegant argument in favor of the role of ANT in fatty-acid induced uncoupling was provided by Brustovetsky and Klingenberg (1994) by their reconstitution experiments. Participation of ANT in the fatty acid-induced uncoupling has also been demonstrated in plant mitochondria (Vianello *et al.*, 1994).

The protonophoric and ionophoric (see further) action of fatty acids is efficiently prevented by millimolar concentrations of Mg^{2+} in the external medium. It has been proposed (Shinohara *et al.*, 1995) that this is due to the formation of magnesium complexes with fatty acids within the membrane that are thus immobilized at its surface.

The fatty acid cycling mechanism (Skulachev, 1991) (see also Fig. 1) requires that the anionic form of fatty acid transiently binds to ANT. Such binding has, indeed, been demonstrated by photolabeling experiments using azido derivatives of fatty acids (Schönfeld *et al.*, 1996). First, it was shown that irreversible inactivation of the translocator by a covalent binding of azido fatty acid resulted in an increased resistance of mitochondria to the uncoupling effect of fatty acids. Second, photolabeling of the translocator protein by tritiated azido-hexadecanoic acid was directly visualized.

The proposed mechanism requires that ANT not be absolutely specific for ATP and ADP. In fact, its affinity to phosphoenolpyruvate (Shug and Shrago, 1973), pyrophosphate (Asimakis and Aprile, 1980; D'Souza and Wilson, 1982) and creatine phosphate (Soboll *et al.*, 1997) has been described. Thus, long-chain fatty acids might form another category of anionic compounds, which are transported by ANT. Such assumption is also compatible with the observation (Wojtczak and Załuska, 1967; Schönfeld *et al.*, 1996) that long-chain fatty acids inhibit the translocation of adenine nucleotides, presumably as competing substrates.

On the other hand, however, fatty acids differ from other substrates of ANT by forming monovalent anions whereas all other substrates of this carrier are three- or four-valent anions. Moreover, the proposed mechanism implies that ANT functions as a uniporter of fatty acid anions and not an obligate exchanger, as is the case with adenine nucleotides and, possibly, with other substrates. The uniport mode action of this carrier as well as of some other mitochondrial transport proteins has been observed so far only as a result of modification of their thiol groups (Dierks *et al.*, 1990a, b; Stappen and Krämer, 1993). It might, therefore, be assumed that highly hydrophobic chain of the fatty acid produces a modification of the carrier protein enabling a unidirectional mode of action.

The mechanism of fatty acid cycling has also been proposed for the protonophoric function of the uncoupling protein (Skulachev, 1991; Ježek *et al.*, 1994; Garlid *et al.*, 1996) which is a homologous protein to ANT (Klingenberg, 1993) and is responsible for the thermogenic function of brown adipose tissue. In relation to this tissue, Winkler and Klingenberg (1994) proposed alternative models whereby fatty acids can participate in the protonophoric action. Apart form the "cycling model" of Skulachev (1991) (Fig.1), they also consider a conformational change of the protein molecule induced by fatty acid molecule and formation by carboxylic groups of fatty acids of a channel within the protein molecule, which facilitates H^+ trafficking. Such possibilities could also apply for ANT (Fig. 2). Their advantage is that they do not require the uniport mode of action. On the other hand, however, the results with photomodification of ANT by azido derivatives of fatty acids (Schöfeld *et al.*, 1996)

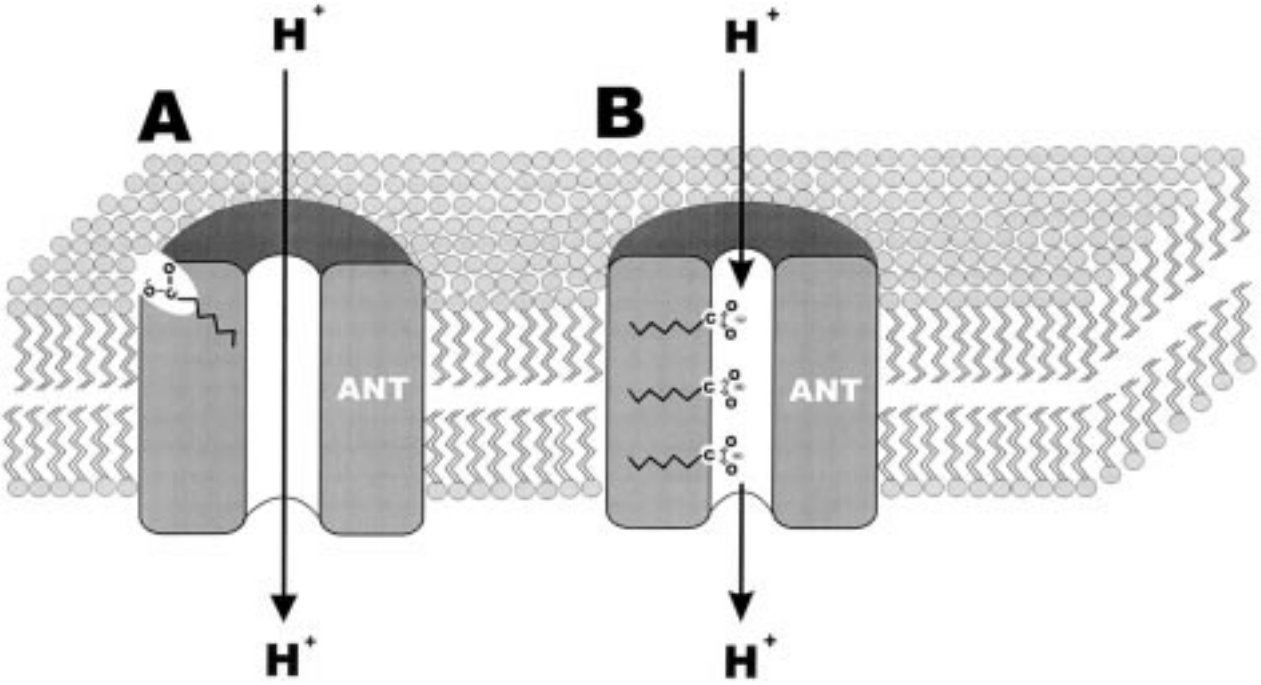

Fig. 2. "Static models" of fatty acid-facilitated proton conductance. (A) "Conformational" model: Binding of fatty acid to ANT induces a conformational change of the translocase, resulting in the formation a proton channel within the carrier molecule. (B) "Channel" model: Fatty acid molecule(s) anchored to ANT form a channel lined with negatively charged carboxylic groups that enables protons to pass through the carrier molecule. According to Winkler and Klingenberg (1994), modified.

strongly argue for the cycling hypothesis. Another argument for the cycling model is provided by studies on the protonophoric effect of fatty acid analogs (Wojtczak *et al.*, 1998), as will be discussed further in this article.

INVOLVEMENT OF OTHER MITOCHONDRIAL CARRIERS

Inhibitors of ANT usually do not completely abolish the uncoupling effect of long-chain fatty acids, thus indicating that the mechanism described above is not entirely responsible for the dissipation of mitochondrial electrochemical proton gradient. In fact, inhibitors and/or substrates of mitochondrial glutamate/aspartate and dicarboxylate carriers exert a partial recoupling action, which is additive to that of carboxyatractyloside (Bodrova *et al.*, 1995; Samartsev *et al.*, 1997; Więckowski and Wojtczak, 1997) (Fig. 3). In addition, it has been shown (Więckowski and Wojtczak, 1997) that long-chain fatty acids reversibly inhibit mitochondrial dicarboxylate carrier. The inhibition by azido fatty acids becomes irreversible upon illumination with ultraviolet light. It can be assumed, therefore, that, in analogy to ANT, the two other mitochondrial carriers can also export fatty acid anions across the inner mitochondrial membrane from the internal compartment to the external one, thus providing an additional mechanism for the net proton transfer in the opposite direction.

INVOLVEMENT OF THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE

Discovery of the mitochondrial permeability transition pore (PTP) (Hunter *et al.*, 1976) that opens in the inner mitochondrial membrane under specific conditions (for reviews see Bernardi *et al.*, 1994; Zoratti and Szabó. 1995) allowed for a new insight into the problem of mitochondrial integrity. This pore of low specificity enables equilibration of ions between the inner and the outer compartments, thus leading to the uncoupling. Opening of PTP also results in large amplitude swelling of isolated mitochondria because of the influx of low molecular weight solutes from the medium and increased internal osmolarity. Fatty acids

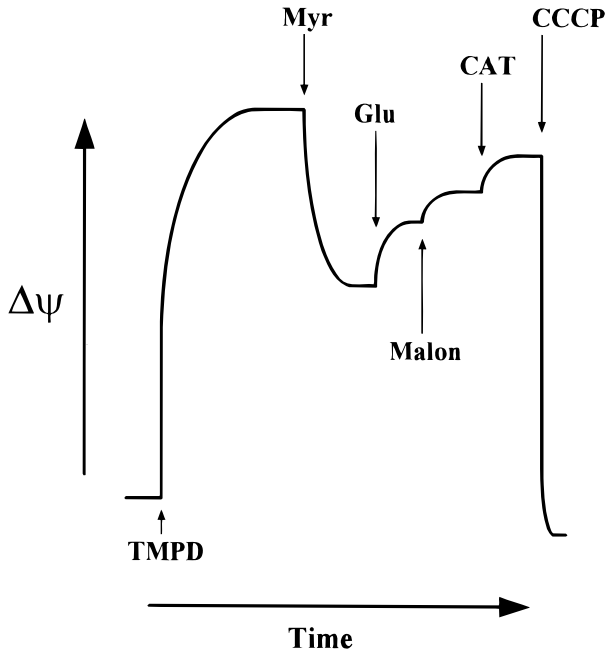

Fig. 3. Reversal of fatty acid-induced uncoupling by substrates and inhibitors of mitochondrial carrier proteins. Rat liver mitochondria were energized with tetramethyl-*p*-phenylenediamine (TMPD) and partly deenergized by myristic acid (Myr). Partial recoupling followed the additions of malonate (Malon), carboxyatractyloside (CAT), and glutamate (Glu). Complete uncoupling was produced by carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). Mitochondrial electric potential ($\Delta\Psi$) was measured with safranin O. Experimental conditions as described by Więckowski and Wojtczak (1997).

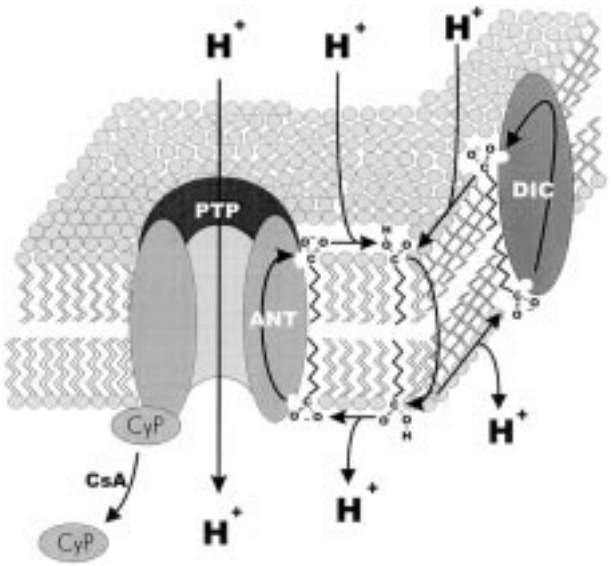

Fig. 4. Expanded model of fatty acid-induced uncoupling, including participation of the adenine nucleotide translocase (ANT), dicarboxylate carrier (DIC), glutamate/aspartate carrier (not indicated in the figure), and the permeability transition pore (PTP). PTP opens presumably as effect of ANT modification due to binding of a fatty acid molecule. Opening of PTP can be prevented by cyclosporin A (CsA), which binds another component of the pore assembly, cyclophilin (CyP).

have long been known as a potent mitochondrial swelling agent (Wojtczak and Lehninger, 1961; Lehninger, 1962) and their effect on the opening probability of PTP has been documented more recently (Petronilli *et al.*, 1993; Broekemeier and Pfeiffer, 1995). In fact, it has been shown (Brustovetsky *et al.*, 1993; Starkov *et al.*, 1994; Amerkhanov *et al.*, 1996; Więckowski and Wojtczak, 1998) that dissipation of the mitochondrial membrane potential produced by fatty acids can be partially reversed by cyclosporin A, a potent blocker of the permeability transition pore (Broekemeier *et al.*, 1989; Bernardi *et al.*, 1994). These results show that long-chain fatty acids not only function as protonophores, but also open the permeability pore enabling protons and other ions to cross the inner mitochondrial membrane (Fig. 4).

It is well documented (Bernardi, 1992) that high mitochondrial membrane potential keeps the permeability pore in its closed configuration, whereas lowering of this potential increases the open probability. Therefore, one of the ways by which fatty acids may promote PTP opening is by dissipating the membrane potential because of the aforementioned protonophoric mechanism mediated by mitochondrial carrier proteins (Schönfeld and Bohnensack, 1997). In this way, the protonophoric action of fatty acids becomes amplified. The same authors, however, observed, that the pore-opening capacity of fatty acids was higher than that of typical uncouplers, like carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). Further results from our laboratory (Więckowski and Wojtczak, 1998) corroborated these observations and showed that, in mitochondria that had accumulated a certain amount of Ca^{2+}, fatty acids directly promoted cyclosporin-sensitive pore opening (Więckowski and Wojtczak, 1998) in a mechanism that was independent of the protonophoric activity. The assembly of PTP is assumed to include ANT (Brustovetsky and Klingenberg, 1996; Beutner *et al.*, 1996; Halestrap *et al.*, 1997). Since long-chain fatty acids are known to exhibit a high affinity to this carrier protein (Schönfeld *et al.*, 1996), it seems likely that they modify PTP in a similar way as, for example, carboxyatractyloside, another pore-opening ANT ligand (Halestrap and Davidson, 1990; Novgorodov *et al.*, 1994).

FATTY ACIDS AS MONOVALENT METAL IONOPHORES

Long-chain fatty acids are also able to facilitate transmembrane flux of some other cations, namely monovalent alkali metal cations. This observation made for mitochondria more than two decades ago (Wojtczak, 1974) has been recently confirmed for artificial lipid membranes (Zeng *et al.*, 1998). The characteristics of this transport appeared to be very similar in both kinds of the membranes: (1) it can be driven by the transmembrane electric potential (Wojtczak, 1974) or concentration gradient (Zeng *et al.*, 1998); (2) it is not ion-specific as it can mediate the transfer of Li^+, Na^+, K^+, Rb^+, and Cs^+; (3) it is inhibited by Mg^{2+}; (4) it depends on the chain length of the fatty acid and its unsaturation. Both groups of authors propose that these cations are transferred in form of undissociated fatty acid salts (soaps). This assumption was supported by showing that potassium (Wojtczak, 1974) and rubidium (Wojtczak, unpublished) salts of long-chain fatty acids can be extracted from water phase by organic solvents.

Zeng *et al.* (1998) speculate that this transport occurs as bidirectional flip-flop of undissociated fatty acid salts. Such electroneutral mechanism may explain, for example, the exchange of K^+ against Li^+ as observed by these authors. However, it can not account for the observed increase of electric conductance of planar phospholipid membranes. In mitochondria, the electrophoretic accumulation of monovalent cations (Wojtczak, 1974) might be explained in a similar way as fatty acid-mediated proton influx, i.e., by unidirectional flip-flop of undissociated fatty acid salts compensated by countercurrent of fatty acid anions mediated by mitochondrial carrier proteins. Another possibility is opening of the permeability transition pore. Differentiation between these two possibilities may only be possible by using specific inhibitors of the two pathways.

DEPENDENCE OF THE PROTONOPHORIC ACTIVITY ON FATTY ACID STRUCTURE

According to the fatty acid cycling model, the prerequisites for the protonophoric efficiency are (1) the ability of the undissociated fatty acid to perform flip-flop in the inner mitochondrial membrane and (2) the ability of the anionic form to be transferred by the adenine nucleotide translocator and/or other carrier proteins. Both processes may depend on the size and the structure of the fatty acid molecule. The highest potency to uncouple oxidative phosphorylation (Pressman and Lardy, 1956; Schönfeld *et al.*, 1989), to induce mitochondrial swelling (Zborowski and Wojtczak, 1963), and to promote energy-dependent accumulation of monovalent cations in mitochondria (Wojtczak, 1974) was found for saturated fatty acids of 12 to 16 carbon atoms and *cis* mono- and diunsaturated 18-carbon atom acids. It has been speculated (Wojtczak and Schönfeld, 1993) that the transbilayer mobility of the undissociated molecule of the fatty acid is the highest when the hydrodynamic length of its molecule is close to half of the thickness of the hydrophobic core of the membrane. This proposal has recently been corroborated by Zeng *et al.* (1998) who studied fatty acid-promoted transport of monovalent alkali metal cations through artificial membranes of various composition.

A systematic study on transbilayer movement within phospholipid model membranes of a range of fatty acid derivatives and analogs has been recently made by Ježek *et al.* (1997a). They divided fatty acid analogs into two groups: “active” and “inactive.” In general, to the first one they classified long-chain fatty acids bearing no hydrophilic groups at the other end of the carbohydrate tail. A OH group in the close vicinity of the carboxylic group presented no obstacle for the flip-flopping. In contrast, OH group(s) or a second carboxylic group at the end of the chain prevented the transbilayer movement. Ježek *et al.* (1997a) hypothesized that such bipolar analogs have a nonstandard U-shape conformation within the membrane that makes flip-flop movements impossible. A similar hindrance was produced by phenyl moieties attached to the hydrophobic chain, probably because of their bulky and flat structure. Interestingly, hydrophilic moieties (e.g., OH groups), that are shielded by vicinal hydrophobic structures, such as, for example, in 12-hydrostearic acid, did not prevent flip-flopping. Ježek *et al.* (1997b) have shown that only “active” fatty acid analogs were able to increase proton conductance of mitochondria in brown adipose tissue.

We have reexamined these analogs and derivatives using mitochondrial membranes instead of artificial lipid films and came to the same classification (Wojtczak *et al.*, 1998). In particular, we found the highest rate and extent of the flip-flop for unsubstituted fatty acids of carbon atom chain length of 14 to 16 atoms and we extended the list of “active” derivatives over thia and oxa fatty acids, i.e., fatty acid analogs in which one or more methylene groups in the carbon atom chain is substituted by sulfur or oxygen atoms,

respectively. On the other hand, fatty acid analogs with strongly ionized acidic moieties, like alkyl long-chain sulfonates and sulfates, were found unable to flip-flop. Since at neutral pH such compounds are almost completely ionized, this finding supports the assumption that a fast transbilayer movement (flip-flop) is possible for the undissociated form only.

Assuming that the second prerequisite for a fatty acid to function as a mitochondrial protonophore is its ability to be transported, in its anionic form, by mitochondrial carrier proteins, we also checked these compounds with respect to their interaction with ANT. Practically all "active" derivatives were found to inhibit this carrier and most of the "inactive" ones were not inhibitory. Interestingly, however, a few compounds that did not flip-flop were inhibitory to some extent, among them the strongly ionized dodecyl sulfonate and dodecyl sulfate (Wojtczak *et al.*, 1998). It was found, furthermore, that only those derivatives and analogs that exhibited both properties, i.e., the ability to flip-flop and to interact with the adenine nucleotide transporter, were able to dissipate mitochondrial membrane potential and to increase the resting state respiration. This finding provides a strong support to the fatty acid cycling model and makes the two other, "stationary," models (Fig. 2) less likely.

An interesting example of hydrophobic shielding is presented by β,β′-methyl-substituted hexadecane α, ω-dioic acid (MEDICA-16). This symmetric compound with a potential antilipidemic action (Bar-Tana *et al.*, 1985) uncouples mitochondrial energy conservation process (Hermesh *et al.*, 1998). We have found that it is capable to flip-flop in the inner mitochondrial membrane and to interact with ANT (Wojtczak *et al.*, 1998) in spite of two carboxylic groups at both ends of the chain. Apparently, because of strong shielding by vicinal methyl groups, only one of the carboxylic groups can dissociate at a time at neutral pH, the other one behaving as a hydrophobic structure. However, shifting methyl substituents to positions closer to or more distant from the terminal carboxylic groups potentiates or weakens the uncoupling properties, respectively (J. Bar-Tana, personal communication), that agrees well with the shielding concept.

THE OCCURRENCE AND IMPORTANCE OF *IN VIVO* UNCOUPLING, AND CONCLUSIONS

It has been pointed out (Stucki, 1980) that the maximum efficiency of ATP production proceeds at the degree of energy coupling somewhat lower than unity. Physiological importance of partial uncoupling, e.g., for thermogenesis, prophylaxis against obesity, prevention of the formation of reactive oxygen species, etc., will be dealt with elsewhere in this issue. Therefore, it seems highly likely that nonesterified long-chain fatty acids may play the role of a natural regulator of oxidative phosphorylation. However, conflicting data on this point have been reported (see e.g., Soboll *et al.*, 1984; Soboll and Stucki, 1985; Nobes *et al.*, 1990; for a more detailed discussion see also the previous review by Wojtczak and Schönfeld, 1993).

The actual content of nonesterified fatty acids in mitochondrial membranes within intact cells is not known. Values of 2 to 20, 10, and 50 nmol/mg protein have been reported for freshly isolated mitochondria from liver (Wojtczak *et al.*, 1968; Boime *et al.*, 1970), kidney (Smith *et al.*, 1980), and heart (Wojtczak *et al.*, 1968), respectively. The level of nonesterified fatty acids in blood plasma is known to increase under certain physiological (e.g., fasting, high-fat diet, excessive exercise) and pathological (e.g., diabetes, peroxisomal disorders) conditions. Such an increase may also propagate over particular tissues and their mitochondria (Boime *et al.*, 1970; Smith *et al.*, 1980). According to some estimations, the content of nonesterified fatty acids in cell membranes under stress conditions may amount to 5 to 15% of total lipids (Zeng *et al.*, 1998). These values are within the range in which fatty acids exert their protonophoric effect in isolated mitochondria.

The fact that the protonophoric action of fatty acids is mediated by at least three important mitochondrial carrier proteins and the Ca^{2+}-dependent permeability pore suggests that proton conductance of the inner mitochondrial membrane may be subject to a subtle regulation to meet variable and changing requirements of the cell.

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