THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 273, No. 25, Issue of June 19, pp. 15528–15532, 1998

Printed in U.S.A.

The Uncoupling Protein UCP1 Does Not Increase the Proton Conductance of the Inner Mitochondrial Membrane by Functioning as a Fatty Acid Anion Transporter*

(Received for publication, December 9, 1997, and in revised form, February 23, 1998)

M. Mar González-Barroso‡, Christophe Fleury§, Frédéric Bouillaud§, David G. Nicholls¶, and Eduardo Rial‡‖

From the ‡Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, 28006 Madrid, Spain, §Centre de Recherche sur l'Endrocrinologie Moléculaire et le Développement, CNRS, Meudon F-92190, France, and the ¶Neurosciences Institute, Ninewells Medical School, Dundee University, Dundee DD1 9SY, United Kingdom

The activity of the brown fat uncoupling protein (UCP1) is regulated by purine nucleotides and fatty acids. Although the inhibition by nucleotides is well established, the activation by fatty acids is still controversial. It has been reported that the ADP/ATP carrier, and possibly other members of the mitochondrial carrier family, mediate fatty acid uncoupling of mitochondria from a variety of sources by facilitating the transbilayer movement of the fatty acid anion. Brown fat mitochondria are known to be more sensitive to fatty acid uncoupling, a property that has been assigned to the presence of UCP1. We have analyzed the transport properties of UCP1 and conclude that fatty acids are not essential for UCP1 function, although they increase its uncoupling activity. In order to establish the difference between the proposed carrier-mediated uncoupling and that exerted through UCP1, we have studied the facility with which fatty acids uncouple respiration in mitochondria from control yeast and strains expressing UCP1 or the mutant Cys-304 → Gly. The concentration of free palmitate required for half-maximal activation of respiration in UCP1-expressing mitochondria is 80 or 40 nM for the mutant protein. These concentrations have virtually no effect on the respiration of mitochondria from control yeast and are nearly 3 orders of magnitude lower than those reported for carrier-mediated uncoupling. We propose that there exist two modes of fatty acid-mediated uncoupling; nanomolar concentrations activate proton transport through UCP1, but only if their concentrations rise to the micromolar range do they become substrates for nonspecific carrier-mediated uncoupling.

The ability of long-chain fatty acids to uncouple mitochondrial respiration from ATP synthesis (see Ref. 1; reviewed in Ref. 2) has been generally assumed to resemble that of other weak acids, which can permeate through the lipid bilayer in both protonated and unprotonated forms. However, recent data have established that fatty acid anion flip-flop across the phospholipid bilayer occurs far too slowly ($t > 1$ min) (3) to allow the anion to complete a classic protonated/deprotonated cycle as for classical uncouplers. The protonophoric activity observed when extremely high fatty acid concentrations (above 0.1 mM) are employed is probably caused by the disruption of the lipid bilayer in a detergent-like mode (1, 4, 5), inasmuch as, for example, effects are not reversed by albumin. The situation is less clear when lower fatty acid concentrations are employed and detergent effects are negligible. It has been shown recently that, under these conditions, fatty acid uncoupling becomes sensitive to inhibitors such as carboxyatractylate, and consequently it has been proposed that permeation of the fatty acid anion is mediated by the ADP/ATP carrier (6–9) and possibly by other anion carriers of the mitochondrial inner membrane (10, 11).

Brown fat is a specialized tissue, the function of which is to produce heat. The thermogenic mechanism is centered around the brown fat uncoupling protein (UCP1),which allows dissipation of the proton electrochemical potential gradient and therefore uncouples respiration from ATP synthesis (12, 13). Recently, three new uncoupling proteins have been identified with homology to UCP1 and termed UCP2 (14), UCP3 (15), and StUCP (16). Free fatty acids, liberated by the noradrenergic stimulation of lipolysis, are the substrate for brown fat thermogenesis and also act as the cytosolic second messengers by which noradrenaline activates UCP1 (17, 18).

The mechanism by which fatty acids activate UCP1 is a matter of debate. Two main hypothesis are current. The first is an extension of the observation of carboxyatractylate-sensitive mitochondrial fatty acid uncoupling, and proposes that UCP1 and other mitochondrial anion carriers (19, 20) can catalyze the transport of the fatty acid anion (21, 22). The second model proposes that fatty acids act as a prosthetic group in UCP1 delivering protons to a site from which they are translocated to the other side of the membrane (23). The anion transporting activity would have little physiological importance and thus would be a vestige of its evolutionary relationship with the rest of mitochondrial metabolite carriers.

The present report will examine the characteristics of fatty acid-mediated uncoupling of yeast mitochondria expressing recombinant UCP1. Two questions are addressed. First, does UCP1 function in the absence of both nucleotides and fatty acids? Second, what is the difference between UCP1-mediated uncoupling and that mediated by other carriers? We conclude that UCP1 retains the capacity to transport $H^+(OH^-)$ in the absence of fatty acids and that fatty acids activate UCP1 at much lower concentrations than are required for other mitochondrial carriers.

* This work was supported by Spanish Ministry of Education and Culture Grant PB95-0118 and by grants from the French Direction des Recherches, Etudes et Techniques and the Association de Recherches sur le Cancer. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‖ To whom correspondence should be addressed: Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Velázquez 144, 28006 Madrid, Spain. Tel.: 341-5611800 (ext. 4236); Fax: 341-5627518; E-mail: rial@fresno.csic.es.

EXPERIMENTAL PROCEDURES

Expression Vector, Site-directed Mutagenesis, and Yeast Growth—The coding sequences for wild type UCP1 and the mutant Cys-304 → Gly were introduced in the pYeDP-1/8–10 vector as described previously (24). Gene expression was under the control of the *gal10-cyc1* promoter, induced by galactose and repressed by glucose. The diploid *Saccharomyces cerevisiae* strain W303 was transformed by electroporation, and clones containing the expression vector were plated on SD minimal medium (2% glucose, 0.67% yeast nitrogen base, 0.1% casamino acids, 20 mg/liter tryptophan, 40 mg/liter adenine, 2% agar) and selected for uracil autotrophy. Control yeasts (UCP^-) contained the same vector but with the UCP1 cDNA in the inverse orientation (25). For preparation of mitochondria, yeast were incubated aerobically at 28 °C in SP medium (0.1% glucose, 2% lactic acid, 0.67% yeast nitrogen base, 0.1% KH_2PO_4, 0.12% $(NH_4)_2SO_4$, 0.1% casamino acids, 20 mg/liter tryptophan, 40 mg/liter adenine, pH 4.5), and 14 h before harvesting were transferred to SG medium (2% galactose, 0.67% yeast nitrogen base, 0.1% casamino acids, 20 mg/liter tryptophan, 40 mg/liter adenine).

Isolation of Mitochondria and Analysis of the Palmitate Stimulation of Respiration—Mitochondria were isolated from protoplasts as described previously (25). The final mitochondrial pellet was resuspended in 0.65 M mannitol, 0.5 mM phosphate, 2 mM EGTA, 1 mM EDTA, 32 μM bovine serum albumin (fatty acid content 0.01%, *i.e.* molar ratio of fatty acid/albumin approximately 0.04), 1 mM phenylmethanesulfonyl fluoride, 1 μg/ml pepstatin A, 10 mM Tes,[1] pH 6.8, to a final concentration of 15–20 mg/ml protein. The sensitivity of mitochondrial respiration to fatty acids was evaluated from the effect of varying palmitate:albumin ratios on the NADH oxidation rate. For respiration assays, mitochondria (0.15 mg/ml protein) were incubated at 20 °C in a buffer containing 0.6 M mannitol, 10 mM phosphate, 0.5 mM EGTA, 1 mg/ml bovine serum albumin (fatty acid content 0.01%), 10 mM Tes, pH 6.8. Respiration was initiated by addition of 3 mM NADH. Other additions are described in the text and/or figure legends. Mitochondrial buffers and stock palmitate and nucleotide solutions were prepared fresh every day. Protein was determined by the Biuret method using albumin as standard.

Palmitate binding to yeast mitochondria was determined under conditions identical to those maintained during respiration experiments. Mitochondria (0.15 mg/ml) were added to respiration buffer that also contained 40 nM [9,10-^{3}H]palmitate (1.5 μCi/ml) and 0.4 μCi/ml [^{14}C]sucrose. 90 s after the addition of NADH, two aliquots were withdrawn and spun for 1 min at 14,000 rpm in an Eppendorf centrifuge. Subsequently, palmitate additions were made to achieve the desired molar ratio to albumin and left to incubate for another 90 s before two additional aliquots were withdrawn and centrifuged. This period was sufficient to measure an stable rate of respiration in a parallel experiment in the oxygen electrode.

To investigate the removal by albumin of membrane-bound palmitate, 140 μM palmitate was added to an aliquot of stock mitochondria (adjusted to 10.5 mg/ml protein), mixed for 30 s and then added to respiration buffer to give a final concentration of 0.15 mg/ml protein. NADH was then added, and, after another 2 or 4 min, aliquots were withdrawn and spun. Supernatants were removed immediately after centrifugation and pellets dissolved in 25 μl of 5% SDS. Radioactivity was determined both in pellets and the original incubation and from these data bound palmitate determined. Two to three independent experiments were performed with each strain (UCP^-, UCP^+, and Cys-304 → Gly); because there were no significant differences, the binding data were pooled. Unbound palmitate was estimated from the isotherms for the binding of palmitate to bovine serum albumin described in Ref. 26.

RESULTS

In order to establish how the presence of UCP1 modifies the bioenergetic properties of mitochondria, it is necessary to characterize appropriate controls without UCP1. This has been performed previously with warm- and cold-acclimated animals because the brown fat mitochondria of the two appear only to differ in their UCP1 content (18, 27, 28). The recombinant expression of UCP1 in yeasts allows not only the comparison of mitochondria incorporating UCP1 with appropriate negative controls but also the use of mutants to further characterize transport functions. We have recently described that mutations in the C-terminal end of the protein modify the K_m for the activation of ion transport by fatty acids (24). We will use one of these mutants, Cys-304 → Gly, to re-examine the transport properties of UCP1 both in the presence and absence of fatty acids.

UCP1 Transports $H^+(OH^-)$ in the Absence of Fatty Acids—We have reported previously (24) that, when mitochondria are isolated from yeasts expressing wild type UCP1 (UCP^+ strain), they demonstrate diminished coupling relative to those from control yeast containing antisense UCP1 cDNA (UCP^- strain). Thus, the respiratory control ratio was 2.67 ± 0.07 (n = 12) for UCP^- and 1.75 ± 0.03 (n = 28) for UCP^+. GDP addition lowers the state 4 rate by 33 ± 1.7% (n = 17) in UCP^+ mitochondria, consistent with an inhibition of the uncoupling protein, while having no effect in UCP^- mitochondria. The -fold stimulation by FCCP in UCP^- is 5.1 ± 0.6 (n = 12), a value close to that observed in UCP^+ if GDP is present (4.6 ± 0.2, n = 5). We can conclude that differences in bioenergetic properties between the two yeast strains are consistent with the presence of active UCP1.

A fundamental question is whether the lower coupling observed with UCP^+ mitochondria in the absence of external nucleotides is due to UCP1 activity stimulated by endogenous fatty acids or if it represents the basal, fatty acid-independent, $H^+(OH^-)$ transport activity of the carrier. The conditions used for the isolation of yeast mitochondria and assay of respiration have been designed to minimize the presence of free fatty acids. Thus, the isolation buffer contains 32 μM bovine serum albumin (less than 0.04 mol of fatty acid/mol of albumin) and mitochondria are diluted 70–100 times when added to the oxygen electrode chamber where the albumin concentration is 16 μM. To eliminate the remote possibility that the tight binding sites on the albumin present in the respiration buffer become rapidly saturated with endogenous fatty acids from the mitochondrial stock, we evaluated the effect on the state 4 rate of higher albumin concentrations in the incubation medium. No further reduction in respiration was observed when up to three successive additions of albumin (final concentrations of 32, 48, and 64 μM) were made (data not shown).

It is conceivable that a fraction of the endogenously generated fatty acids remain tightly associated with the membrane and not readily removable by albumin. To investigate this possibility, it is first necessary to analyze the characteristics of the partition of palmitate between albumin and the lipid bilayer. Fig. 1*A* shows the binding of palmitate to yeast mitochondria using different palmitate:albumin molar ratios to buffer the concentration of free palmitate. Experiments were performed under conditions identical to those used in respiration experiments, except for the presence of radioisotopes. When the palmitate:albumin molar ratio was varied from 1:1 to 5:1, mitochondrially bound palmitate increased from 0.8 to 19 nmol/mg. The concentration of unbound palmitate is extremely low over this range; using the palmitate/albumin binding constants obtained by Richieri *et al.* (26), mitochondrially bound palmitate correlated linearly with the free palmitate, which varied from 5 to 98 nM (Fig. 1*B*).

The second step was to obtain a maximal estimate of the endogenously bound fatty acids. For this purpose, [^{3}H]palmitate (140 μM final concentration) was added to the mitochondrial stock at a molar ratio of 4.5:1 palmitate:albumin in order to saturate the high affinity sites present in albumin and thus mimic mitochondria maximally contaminated with endogenous fatty acid. This concentration should not produce detergent-like effects (4, 5). Mitochondria were then diluted 69-fold in respiration buffer containing 16 μM albumin to decrease the

[1] The abbreviation used is: Tes, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

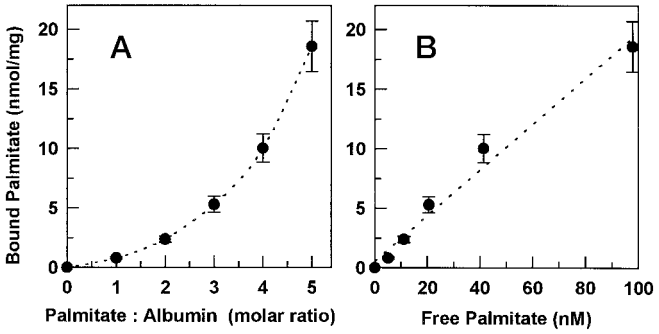

FIG. 1. **Palmitate binding to yeast mitochondria.** *A*, yeast mitochondria were incubated with increasing concentrations of [^{3}H]palmitate, in the presence of 16 μM fatty acid-free albumin, and the amount bound to mitochondria was determined. Because results obtained with the three yeast strains (UCP$^+$, UCP$^-$, and Cys-304 → Gly) showed no difference, data have been pooled; thus, data points represent the mean value (± S.E.) of 8–10 independent determinations performed in quadruplicate. *B*, correlation between the palmitate bound to yeast mitochondria and the concentration of free palmitate as estimated from the binding constants determined by Richieri *et al.* (31). *Dotted line* represents the regression line (r^2 = 0.982).

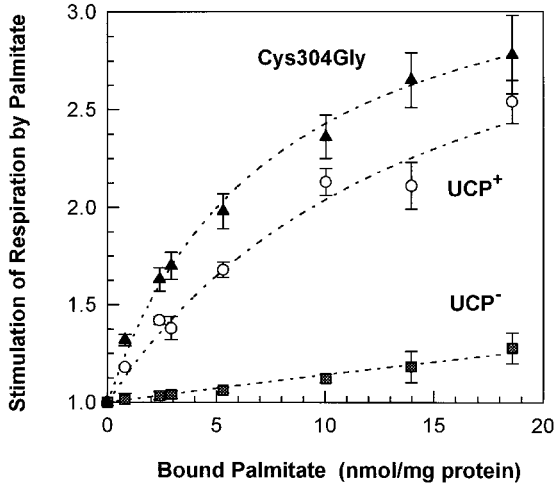

FIG. 2. **Kinetics for the activation of respiration by palmitate.** Respiration rates were measured in the presence of increasing concentrations of palmitate with yeast mitochondria oxidizing NADH in medium containing 16 μM fatty acid-free albumin. Data points represent the mean values (± S.E.) of the -fold stimulation of respiration from 3 (UCP$^-$), 10 (UCP$^+$), and 9 (Cys-304 → Gly) independent determinations performed in duplicate. The K_m calculated from these data were 15 ± 4.8 for UCP$^+$ and 7.5 ± 0.8 nmol/mg for the mutant Cys-304 → Gly. *Dotted lines* represent the curves fitted to those values.

palmitate:albumin ratio to 0.12:1. Residual membrane-bound palmitate was determined 2 and 4 min after NADH addition, which is the time interval generally used to measure the state 4 respiration rate. The residual bound palmitate amounted to 0.77 ± 0.08 nmol/mg mitochondrial protein after 2 min, and this was further lowered to 0.54 ± 0.05 nmol/mg after 4 min. The state 4 rates measured with these palmitate pre-treated mitochondria were identical to those observed with untreated mitochondria: 177 ± 6 *versus* 185 ± 6 nmol of "O" min^{-1} mg^{-1} (n = 11 and 6), respectively. It is evident, therefore, that under our standard conditions any residual fatty acids bound to the mitochondrial membrane will be equal to or less than that found after palmitate.

The final question is whether any residual bound fatty acids would be sufficient to cause significant activation of UCP1. In order to answer this, we determined the concentrations of bound palmitate required to stimulate respiration in our yeast strains. Fig. 2 shows the stimulation of respiration obtained with the same range of palmitate:albumin ratios. The amount of bound palmitate required to observe a half-maximal stimulation of respiration in UCP$^+$ mitochondria is 15 ± 4.8 nmol/mg. These data are in excellent agreement with those published previously by Cunningham *et al.* (27), where the apparent K_m for the uncoupling of pyruvate oxidation in brown fat mitochondria from the guinea pig was 10–20 nmol/mg. Even if the endogenous fatty acids that cannot be removed by albumin amounted to 0.5 nmol/mg (as in the isotopic experiment), they would stimulate state 4 respiration by less than 3%. However, because under these conditions the GDP-sensitive respiration amounts to 33% of the state 4 rate, it is clear that this conductance cannot be attributed solely to fatty acids and must represent a fatty acid-independent mode of proton conductance.

We can confirm this assumption by using the Cys-304 → Gly mutant, which, according to a previous report, is stimulated with lower palmitate concentrations than UCP$^+$ (24). Fig. 2 shows that the apparent K_m for the activation for palmitate with this mutant is lowered to 7.5 ± 0.8 nmol/mg. If the observed initial state 4 rate were to depend on the endogenous fatty acids, we should observe that Cys-304 → Gly mitochondria are more uncoupled. However, data are identical to those observed with UCP$^+$ mitochondria, with a respiratory control ratio of 1.73 ± 0.07 (n = 8) and GDP inhibition of the state 4 respiration of 32.5% ± 1.09 (n = 10). We must therefore conclude that the uncoupling activity observed cannot be attributed to endogenous fatty acids and therefore UCP1 must catalyze the direct transfer of $H^+(OH^-)$.

UCP1-mediated Versus Carrier-mediated Uncoupling—Recent publications have established that the ADP/ATP carrier can also contribute to the uncoupling of mitochondrial respiration induced by fatty acids (6–9), and this proposal has been extended to other metabolite carriers of the mitochondrial inner membrane (10, 11). Because we now have the protein incorporated into recombinant yeast mitochondria, we can test the UCP1-independent uncoupling and compare it to that in its presence. It is to be expected that the other transporters of the mitochondrial inner membrane are present in equal amounts in both cases. The yeast are grown under aerobic conditions, and thus mitochondrial oxidative phosphorylation is fully operative. Fig. 2 shows the effect on respiration of the same range of palmitate:albumin ratios, and it becomes apparent that UCP$^-$ mitochondria are far less sensitive to palmitate. It should be noted that, in UCP$^-$ mitochondria, the palmitate-stimulated respiration is not sensitive to atractylate over the range 10–40 μM, concentrations that do prevent ADP phosphorylation (data not shown). Recently, it has been demonstrated that oleate uncouples *S. cerevisiae* mitochondria at concentrations ranging from 10 to 50 μM and this uncoupling could be partially inhibited by bongkrekate (9).

We conclude that fatty acids stimulate proton transport via the UCP1 at concentrations far lower than those required for carrier-mediated translocation of the fatty acid anion. Therefore, the appearance of UCP1 in the brown adipocyte would confer upon these cells a high thermogenic activity in response to low free fatty acid concentrations.

DISCUSSION

The acute control of energy dissipation in brown fat mitochondria has been a subject of debate for more than two decades, preceding the discovery of UCP1 and yet to be resolved. It was recognized in the late 1960s that brown fat mitochondria prepared by conventional methods were uncoupled and that special conditions were needed to observe efficient oxidative phosphorylation, namely removal of endogenous fatty acids and addition of purine nucleotides (29, 30). The mode of action

of purine nucleotides has been less controversial than that of fatty acids. Nucleotides bind to UCP1 from the cytosolic side of the mitochondrial inner membrane and inhibit its transport activity (31). The role of fatty acids in the regulation of energy dissipation and their mechanism of action have been the subjects of continuous controversy.

The first question that is addressed in the present paper relates to the basal activity of UCP1. The earliest studies on the bioenergetic properties of brown adipose tissue mitochondria established that brown adipose tissue mitochondria were uncoupled in the absence of both nucleotides and fatty acids (29, 30). However, recent reports have questioned these findings by suggesting that simple removal of fatty acids is sufficient to abolish H^+ transport and consequently that fatty acids are obligatory for UCP1 activity (23). A separate report (32) questioned the interpretation of nonrespiring swelling experiments performed in potassium acetate plus valinomycin, which requires $H^+(OH^-)$ movement for charge and pH balance, by claiming that the rate observed in the absence of fatty acids was due to acetate anion transport through the UCP1, although this explanation fails to account for the high proton conductance of respiring brown adipose tissue mitochondria in the presence of albumin calculated from the proton current and proton electrochemical potential (33). Results presented in this paper demonstrate that there is a GDP-sensitive conductance that cannot be attributed to endogenous fatty acids, and that an increased affinity for palmitate in a UCP1 mutant does not result in an increased basal activity. The conclusion is that fatty acids are not essential for UCP1 function, although they increase its uncoupling activity. It should be stressed at this point that, if experiments are performed with low and/or transient diffusion potentials, rather than during respiration, UCP1 activity in the absence of fatty acids may not be detected, because in the absence of nucleotide the $H^+(OH^-)$ transport rate depends linearly on the driving force (33).

The ability of fatty acids to uncouple mitochondrial respiration has been known for decades (see Ref. 1; reviewed in Ref. 2) but was generally considered "nonspecific," relying on the translocation across the bilayer of the fatty acid in both protonated and in anionic forms and additionally, at high concentrations, on detergent-like effects. In 1988, Skulachev and coworkers (6) demonstrated that carboxyatractylate inhibited the uncoupling effect of palmitate in liver mitochondria and thus proposed that the protonophoric effect of fatty acids was mediated by the ADP/ATP carrier facilitating the translocation of the fatty acid anion. This finding has been supported subsequently by other laboratories, both with intact mitochondria (9, 34) and in reconstituted systems (8). More recently, it has been shown that other members of the family of mitochondrial transporters also appear to participate in this protonophoric action of fatty acids (10, 11). A general model is now emerging, wherein all these carriers, including UCP1, facilitate the transbilayer movement of the fatty acid anion. The protonophoric cycle would be completed with the flip-flop across the bilayer of the protonated acid (21, 22, 35). This H^+ cycling mechanism would be analogous to the uncoupling by weak acid protonophores.

These data would appear to question the specificity of the fatty acid uncoupling of brown fat mitochondria. However, in all the reports involving the mitochondrial carriers, the unbound fatty acid concentrations required to observe uncoupling are in the micromolar range (6, 9–11). Thus, for example, in the ADP/ATP carrier of *S. cerevisiae* mitochondria, the system closest to the one presented in this paper, this form of fatty acid uncoupling requires concentrations in excess of 10 μM (9). The data presented in this paper show that the concentration of free palmitate required for half-maximal stimulation of respiration of UCP^+ mitochondria is around 80 nM, a value that is in perfect agreement with that reported for brown fat mitochondria from cold-adapted guinea pigs (27). The extension of the cycling model to the UCP1 has been based mainly on direct measurements of the transport of the fatty acid anion and/or the subsequent H^+ delivery across the membrane. Under those conditions, the K_m for the anion is around 20 μM (21) and is thus similar to values obtained with other members of the carrier family.

A second model, termed "local H^+-buffering," proposes that the fatty acid acts as a prosthetic group for H^+ transport and thus the carboxyl group delivers H^+ to a site inside UCP1 from which translocation occurs (23). This model would resemble the H^+ translocating mechanism in bacteriorhodopsin (23, 36). We consider that the physiological relevance of the two models can be discriminated by the concentrations (K_m) required to observe the effects and may represent two separate functions of the UCP1. Nanomolar concentrations of free fatty acids such as can be generated by noradrenaline-evoked lipolysis within brown adipocytes (27) would activate proton transport by UCP1 by the "local H^+-buffering" mechanism. Only if unbound fatty acid concentrations were to rise to several micromolar would they themselves become transportable substrates for the UCP1, and presumably for the other mitochondrial carriers. It is important to recall the physiological context of the brown fat UCP1, which was shown several years ago to confer high sensitivity of intact brown adipocytes to fatty acid uncoupling and to be reversibly activated within adipocytes by endogenously generated fatty acids after noradrenaline stimulation of the cells (27). Until similar criteria can be demonstrated for the much less sensitive carrier-mediated uncoupling, the physiological role of the latter, which may provide a mechanistic explanation for what has been known as nonspecific fatty acid uncoupling, remains contentious.

REFERENCES

1. Pressman, B. C., and Lardy, H. A. (1956) *Biochim. Biophys. Acta* **21,** 458–466
2. Wojtczak, L., and Schönfeld, P. (1993) *Biochim. Biophys. Acta* **1183,** 41–57
3. Kamp, F., and Hamilton, J. A. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 11367–11370
4. Wojtczak, L., and Zaluska, H. (1967) *Biochem. Biophys. Res. Commun.* **28,** 76–81
5. Vaartjes, W. J., and Van den Bergh, S. G. (1978) *Biochim. Biophys. Acta* **503,** 437–449
6. Andreyev, A. Yu., Bondareva, T. O., Dedukhova, V. I., Mokhova, E. N., Skulachev, V. P., and Volkov, N. I. (1988) *FEBS Lett.* **226,** 265–269
7. Andreyev, A. Yu., Bondareva, T. O., Dedukhova, V. I., Mokhova, E. N., Skulachev, V. P., Tsofina, L. M., Volkov, N. I., and Vygodina, T. V. (1989) *Eur. J. Biochem.* **182,** 585–592
8. Brustovetsky, N., and Klingenberg, M. (1994) *J. Biol. Chem.* **269,** 27329–27336
9. Polcic, P., Sabová, L., and Kolarov, J. (1997) *FEBS Lett.* **412,** 207–210
10. Wieckowski, M. R., and Wojtczak, L. (1997) *Biochem. Biophys. Res. Commun.* **232,** 414–417
11. Samartsev, V. N., Smirnov, A. V., Zeldi, I. P., Markova, O. V., Mokhova, E. N., and Skulachev, V. P. (1997) *Biochim. Biophys. Acta* **1319,** 251–257
12. Nicholls, D. G., and Locke, R. M. (1984) *Physiol. Rev.* **64,** 1–64
13. Nedergaard, J., and Cannon, B. (1992) in *New Comprehensive Biochemistry: Molecular Mechanisms in Bioenergetics* (Ernster, L., ed) Vol. 23, pp. 385–420, Elsevier, Amsterdam
14. Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. F., Surwit, R. S., Ricquier, D., and Warden, C. H. (1997) *Nat. Genet.* **15,** 269–272
15. Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J. S., and Lowell, B. B. (1997) *Biochem. Biophys. Res. Commun.* **235,** 79–82
16. Laloi, M., Klein, M., Riesmeier, J. W., Müller-Röber, B., Fleury, C., Bouillaud, F., and Ricquier, D. (1997) *Nature* **389,** 135–136
17. Locke, R. M., Rial, E., Scott, I. D., and Nicholls, D. G. (1982) *Eur. J. Biochem.* **129,** 373–380
18. Locke, R. M., Rial, E., and Nicholls, D. G. (1982) *Eur. J. Biochem.* **129,** 381–387
19. Walker, J. E., and Runswick, M. J. (1993) *J. Bioenerg. Biomembr.* **25,** 435–446
20. Palmieri, F., Indiveri, C., Bisaccia, F., and Krämer, R. (1993) *J. Bioenerg. Biomembr.* **25,** 525–535
21. Garlid, K. D., Orosz, D. E., Modriansky, M., Vassanelli, M., and Jezek, P. (1996) *J. Biol. Chem.* **271,** 2615–2620
22. Skulachev, V. P. (1991) *FEBS Lett.* **294,** 158–162
23. Winkler, E., and Klingenberg, M. (1994) *J. Biol. Chem.* **269,** 2508–2515
24. González-Barroso, M. M., Fleury, C., Arechaga, I., Zaragoza, P., Levi-

Meyrueis, C., Raimbault, S., Ricquier, D., Bouillaud, F., and Rial, E. (1996) *Eur. J. Biochem.* **239,** 445–450
25. Arechaga, I., Raimbault, S., Prieto, S., Levi-Meyrueis, C., Zaragoza, P., Miroux, B., Ricquier, D., Bouillaud, F., and Rial, E. (1993) *Biochem. J.* **296,** 693–700
26. Richieri, G. V., Anel, A., and Kleinfeld, A. M. (1993) *Biochemistry* **32,** 7574–7580
27. Cunningham, S. A., Wiesinger, H., and Nicholls, D. G. (1986) *Eur. J. Biochem.* **157,** 415–420
28. Rial, E., and Nicholls, D. G. (1984) *Biochem. J.* **222,** 685–693
29. Rafael, J., Ludolph, H.-J., and Hohorst, H.-J. (1969) *Hoppe-Seyler's Z. Physiol. Chem.* **350,** 1121–1131
30. Hohorst, H.-J., and Rafael, J. (1968) *Hoppe-Seyler's Z. Physiol. Chem.* **349,** 268–270
31. Nicholls, D. G. (1976) *Eur. J. Biochem.* **62,** 223–228
32. Jezek, P., Orosz, D. E., Modriansky, M., and Garlid, K. D. (1994) *J. Biol. Chem.* **269,** 26184–26190
33. Nicholls, D. G. (1977) *Eur. J. Biochem.* **77,** 349–356
34. Schönfeld, P., Jezek, P., Belyaeva, E. A., Borecky, J., Slyshenkov, V. S., Wieckowski, M. R., and Wojtczak, L. (1996) *Eur. J. Biochem.* **240,** 387–393
35. Jezek, P., Modriansky, M., and Garlid, K. D. (1997) *FEBS Lett.* **408,** 166–170
36. Lanyi, J. K. (1993) *Biochim. Biophys. Acta* **1183,** 241–261

The Uncoupling Protein UCP1 Does Not Increase the Proton Conductance of the Inner Mitochondrial Membrane by Functioning as a Fatty Acid Anion Transporter

M. Mar González-Barroso, Christophe Fleury, Frédéric Bouillaud, David G. Nicholls and Eduardo Rial

J. Biol. Chem. 1998, 273:15528-15532.
doi: 10.1074/jbc.273.25.15528
