Biochemical and Biophysical Research Communications **263,** 87–93 (1999)
Article ID bbrc.1999.1332, available online at http://www.idealibrary.com on IDEAL®

Etomoxir, Sodium 2-[6-(4-Chlorophenoxy)hexyl]oxirane-2-carboxylate, Up-Regulates Uncoupling Protein-3 mRNA Levels in Primary Culture of Rat Preadipocytes

Àgatha Cabrero, Marta Alegret, Rosa Sánchez, Tomás Adzet, Juan Carlos Laguna, and Manuel Vázquez

Unidad de Farmacología, Departamento de Farmacología y Química Terapéutica, Facultad de Farmacia, Núcleo Universitario de Pedralbes, Barcelona E-08028, Spain

Received August 6, 1999

Uncoupling proteins (UCPs) are mitochondrial membrane proton transporters that uncouple respiration from oxidative phosphorylation by dissipating the proton gradient across the membrane. Treatment of primary culture of rat preadipocytes for 24 h with 40 μM etomoxir, an irreversible inhibitor of carnitine palmitoyltransferase I (CPT-I), up-regulated UCP-3 mRNA levels (3.6-fold induction), whereas changes in UCP-2 mRNA levels were not significant. As a consequence of increased UCP-3 expression, a fall in the mitochondrial membrane potential was detected by flow cytometry. Etomoxir treatment modified neither L-CPT-I (liver-type) nor PPARα mRNA levels in preadipocytes. In contrast, mRNA expression of acyl-CoA oxidase (ACO), the rate-limiting enzyme of peroxisomal fatty acid β-oxidation, whose transcription is controlled by PPARα, was significantly induced (1.3-fold induction, P = 0.015). These findings suggest that the effects of etomoxir were mediated by PPARα. Since it has been reported that the intracellular accumulation of lipids following the inhibition of CPT-I by etomoxir leads to a PPARα-mediated metabolic response that increases the expression of genes involved in alternate fatty acid oxidation pathways, these results seem to implicate UCP-3 in this protective metabolic response. It remains to be studied whether reductions in the expression of UCP-3 could compromise this response, giving rise to lipotoxic effects on cells.

Uncoupling proteins (UCPs), which are inner mitochondrial proton transporters, stimulate heat production by uncoupling respiration from ATP synthesis (1). Three UCP subtypes have now been identified. UCP-1, the first uncoupling protein identified, is expressed exclusively in brown adipose tissue (1–3). Recently, two new uncoupling proteins, UCP-2 and UCP-3, have been discovered (5–7). Like UCP-1, these new UCPs seem to function as uncouplers of oxidative phosphorylation in mitochondria (5,7,8), although their expression pattern is different. UCP-2 mRNA is widely expressed in many human and rat tissues, including white adipose tissue (WAT) and skeletal muscle. UCP-3 mRNA is expressed in rat skeletal muscle and brown adipose tissue, and to a lesser extent in white adipose tissue and heart, whereas in humans its expression is restricted to skeletal muscle, an important site of energy expenditure (5–9).

UCP-3 has been implicated in thermogenesis, as it diminishes the mitochondrial membrane potential when expressed in yeast or the C_2C_{12} myoblast cell line (8,10). However, since the increase in plasma free fatty acids (FFA) observed after fasting, a well-established condition of suppressed thermogenesis (11), up-regulates UCP-3 mRNA levels in skeletal muscle (12,13), it has been suggested that UCP-3, in addition to its thermogenic capacity, is also involved in lipid metabolism. The molecular mechanism by which FFA activate the expression of UCP-3 mRNA levels seems to involve peroxisome proliferator activated-receptors (PPARs). Ligands of these receptors, which are also activated by fatty acids, increase UCP-3 mRNA levels in muscle and WAT (14–16). Furthermore, three putative peroxisome proliferator response elements (PPREs) have been found in the 5′ flanking region of the human UCP-3 gene, pointing to the involvement of PPARs in the expression of this gene (17). In addition, increased glucose influx up-regulates UCP-3 mRNA expression in muscle and WAT (18), although the molecular mechanism responsible has not been elucidated.

Carnitine palmitoyltransferase I (CPT-I) plays an important role in the homeostasis of fuel substrates. It catalyzes the entry of long-chain fatty acids to the mitochondrial matrix, where they can undergo β-oxidation as a source of energy (19). CPT-I activity

0006-291X/99 $30.00

and the availability of fatty acids are the main factors that determine the flux of β–oxidation, which, in turn, provides the reducing equivalents for the mitochondrial uncoupling reaction in a given tissue. CPT-I has two isoforms encoded by separate genes: liver-type (L-CPT-I) and muscle-type (M-CPT-I). L-CPT-I is expressed not only in liver, but also in human fibroblasts (20), in intestine (21) and at lower levels in rat heart (22) and white adipocytes (23). M-CPT-I, which is the sole isoform in skeletal muscle, is also predominant in rat heart and in both brown and white adipocytes (23).

CPT-I is uniquely inhibited by malonyl-CoA, the product of acetyl-CoA carboxylase. Thus, in the fed state, or in conditions of increased glucose flux into skeletal muscle and WAT, malonyl-CoA levels are elevated, causing the inhibition of CPT-I and thus of fatty acid oxidation (24,25). In fasting, however, malonyl-CoA levels decrease and β–oxidation can proceed.

Increased fatty acid β–oxidation, as a result of elevated plasma FFA, may contribute to insulin resistance (26). This hypothesis led to the development of inhibitors of fatty acid oxidation for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). Etomoxir, sodium 2-[6-(4-chlorophenoxy)hexyl]oxirane-2-carboxylate, is converted to etomoxir-CoA, an irreversible inhibitor of CPT-I. The inhibition by this drug of the transport of long-chain acyl-CoA compounds into the mitochondria increases the long-chain fatty acyl-CoA/long-chain fatty acyl-carnitine ratio in the cytoplasm and inhibits β-oxidation.

Here we examine the effects of etomoxir on UCP-3 and UCP-2 mRNA levels in primary monolayer cultures of undifferentiated rat preadipocytes. These cells have been shown to be precursors of adipocytes both *in vitro* and *in vivo* (27). We report that treatment with etomoxir for 24 h up-regulated UCP-3 mRNA expression. In contrast, UCP-2 mRNA levels were not significantly modified. The increase in UCP-3 expression induced by etomoxir reduced the mitochondrial membrane potential of rat primary preadipocytes, which is consistent with the role proposed for UCPs.

MATERIALS AND METHODS

Cell isolation and primary culture of rat preadipocytes. Fibroblastic preadipocytes were isolated from adipose tissue following Robdell (28), with some modifications. The epididymal adipose tissue from 200–250g male Sprague-Dawley rats was removed in sterile conditions and washed in Hank's balanced salt solution (HBSS) (Life Technologies). Minced tissue was transferred to a sterile polypropopylene Erlenmeyer flask containing collagenase (2 mg/ml, type II, Sigma) and 5% bovine serum albumin. Following incubation at 37°C for 45 min in a shaking water bath, the digest was filtered through a sterile 250 μm nylon mesh. The digested tissue was then centrifuged at 250×*g* for 10 min and mature adipocytes were removed by aspiration. The pellet was resuspended in HBSS, filtered through 25 μm nylon mesh and centrifuged again. The pellet obtained consisted mainly of preadipocytes. It was resuspended in MEM Alpha medium (Life Technologies) supplemented with penicillin, streptomycin and fungizone (100 units/ml, 100 μg/ml and 2.5 μg/ml, respectively; Life Technologies), and refiltered. To eliminate red blood cells, preadipocytes were incubated with an erythrocyte lysing buffer consisting of 0.154 M NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA for 5 min at room temperature. In these conditions, >95% of red blood cells were lysed without damaging the nucleus-containing cells as assessed by Trypan blue exclusion. The suspension was centrifuged (250×*g* for 10 min) and the cells were counted using a haemocytometer. Cells were plated in 60 mm culture dishes at a density of 1.5×10^5 cells/ml in MEM Alpha medium with 10% fetal calf serum (FCS). Cells were maintained in this medium until 80% confluence was reached (usually 2 days). Preadipocytes were then treated in serum-free MEM Alpha medium with either vehicle (0.1% DMSO) or 40 μM etomoxir for 24 h. After the incubation, RNA was extracted from the preadipocytes as described below.

RNA preparation and analysis. Total RNA was isolated using the Ultraspec reagent (Biotecx). Relative levels of specific mRNAs were assessed by the reverse transcription-polymerase chain reaction (RT-PCR). Complementary DNA was synthesized from RNA samples by mixing 1 μg of total RNA, 125 ng of random hexamers as primers in the presence of 50 mM Tris-HCl buffer (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 200 U. Moloney murine leukemia virus reverse transcriptase (Life Technologies), 20 U RNAsin (Life Technologies) and 0.5 mM of each dNTP (Sigma) in a total volume of 20 μl. Samples were incubated at 37°C for 60 min. A 5 μl aliquot of the RT reaction was then used for subsequent PCR amplification with specific primers.

Each 25-μl PCR reaction contained 5μl of the RT reaction, 1.2 mM $MgCl_2$, 200 μM dNTPs, 1.25 μCi [^{32}P]-dATP (3000 Ci/mmol, Amersham), 1 unit of Taq polymerase (Ecogen, Barcelona, Spain), 0.5 μg of each primer and 20 mM Tris-HCl, pH 8.5. To avoid unspecific annealling, cDNA and Taq polymerase were separated from primers and dNTPs by using a layer of paraffin (reaction components contact only when paraffin fuses, at 60°C). The sequences of the sense and antisense primers used for amplification were: UCP-3, 5′-GGAGCCATGGCAGTGACCTGT-3′ and 5′-TGTGATGTTGGGCCAAGTCCC-3′; UCP-2, 5′-AACAGTTCTACACCAAGGGC-3′ and 5′-AGCATGGTAAGGGCACAGTG-3′; L-CPT-I, 5′-TATGTGAGGATGCTGCTT-3′ and 5′-CTCGGAGAGCTAAGCTTG; ACO, 5′-ACTATATTTGGCCAATTTTGTG-3′ and 5′-TGTGGCAGTGGTTTCCAAGCC-3′; PPARα, 5′-GGCTCGGAGGGCTCTGTCATC-3′ and 5′-ACATGCACTGGCAGCAGTGGA-3′ and APRT (adenosyl phosphoribosyl transferase), 5′-AGCTTCCCGGACTTCCCCATC-3′ and 5′-GACCACTTTCTGCCCCGGTTC-3′. The *aprt* gene was used as internal control in the PCR to normalize the results, except for UCP-3 and L-CPT-I, where co-amplifications for these genes and *aprt* were performed in separate tubes and in duplicate. PCR was performed in an MJ Research Thermocycler equipped with a peltier system and temperature probe. After an initial denaturation for 1 min at 94°C, PCR was performed for 23 (UCP-2, ACO, PPARα), 25 (L-CPT-I) or 30 (UCP-3) cycles. Each cycle consisted of denaturation at 92°C for 1 min, primer annealing at 60°C, and primer extension at 72°C for 1 min and 50 s. A final 5-min extension step at 72°C was performed. Five microliters of each PCR sample was electrophoresed on a 1-mm-thick 5% polyacrylamide gel. The gels were dried and subjected to autoradiography using Kodak X-ray films to show the amplified DNA products. Amplification of each gene yielded a unique band of the expected size (UCP-3: 179 bp, UCP-2: 471 bp, L-CPT-I: 629 bp, ACO: 195 bp, PPARα: 654 bp, APRT: 329 bp). Preliminary experiments were carried out with various amounts of cDNA to determine non-saturating conditions of PCR amplification for all the genes studied. Thus cDNA amplification was performed in comparative and semiquantitative conditions. Radioactive bands were quantified by video-densitometric scanning (Vilbert Lourmat Imaging). The results for the expression of specific mRNAs are always presented relative to the expression of the control gene (*aprt*).

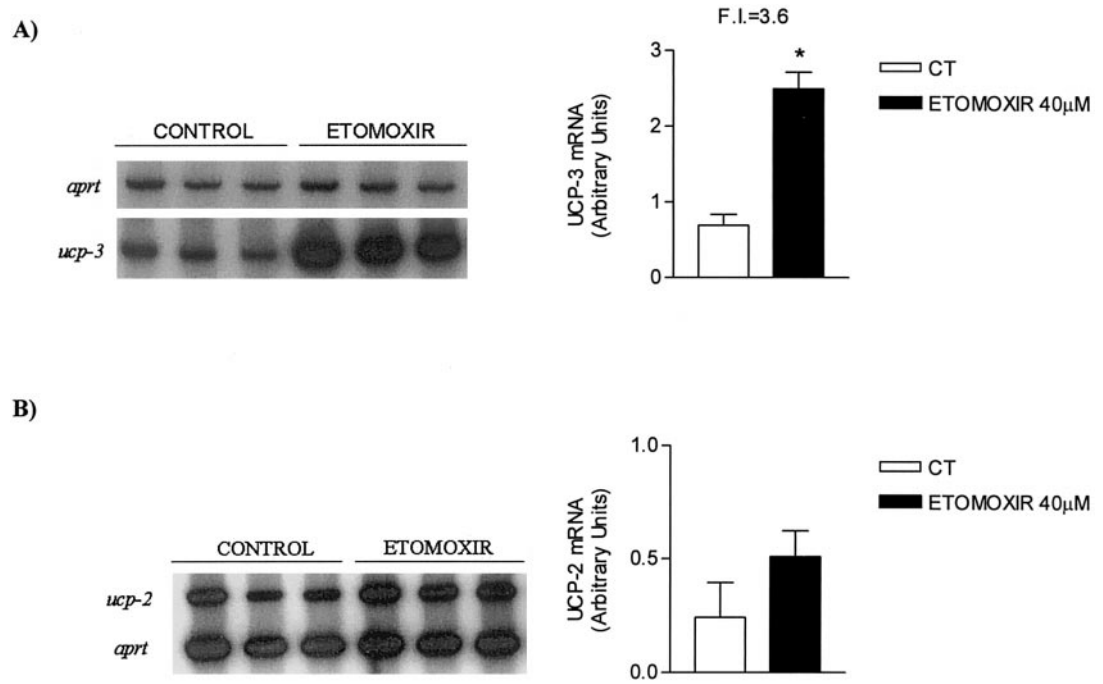

FIG. 1. Effect of etomoxir on the expression of UCP-3 (A) and UCP-2 (B) mRNA in primary culture of rat preadipocytes. Cells were incubated for 24 h with or without 40 μM etomoxir. All cells were exposed to 0.1% DMSO. 0.5 μg of total RNA was analyzed by RT-PCR. A representative autoradiogram and the quantification of the aprt-normalized UCPs mRNA levels are shown. Data are expressed as mean ± S.D. of 3 experiments. *P = 0.0003 compared with control experiments. FI, fold induction.

Analysis of mitochondrial membrane potential by flow cytometry. Subconfluent preadipocytes treated with either vehicle (0.1% DMSO) or 40 μM etomoxir for 24 h were washed, trypsinized, and resuspended in HBSS. Cells were incubated at 37°C for 30 min in the dark with 10 nM tetramethylrhodamine ethyl ester (TMRE, Molecular Probes) in the presence or in the absence of the uncoupler carbonyl-cyanide *m*-chlorophenylhydrazone (CCCP, Sigma) (75 μM). Flow cytometry was carried out using an Epics XL flow cytometer (Coulter Corporation). Excitation of the sample was performed using a standard 488 nm air-cooled argon-ion laser at 15 mW. The instrument was set up with the standard configuration. Forward scatter (FSC), side scatter (SSC) and orange (575 nm) fluorescence for TMRE were acquired. Orange fluorescence was collected with a 600 nm dichroic long filter and a 575 band pass filter. Optical alignment was based on the optimized signal from 10 nm fluorescent beads (Immunocheck, Epics Division). A minimum of 10,000 cells/sample were acquired and analyzed.

Statistical analyses. Results are expressed as means ± s.d. Statistical significance was evaluated using Student's t-test.

RESULTS

The main goal of this work was to study the effects of etomoxir treatment on UCP mRNA levels in rat primary culture of preadipocytes. The addition of 40 μM etomoxir, an irreversible inhibitor of CPT-I, caused a 3.6-fold induction in UCP-3 mRNA levels (P = 0.0003) (Fig. 1A). In contrast, although UCP-2 mRNA levels increased 2-fold after etomoxir treatment, differences were not significant (Fig. 1B).

Fibroblastic preadipocytes mainly express liver type CPT-I (29). Etomoxir treatment did not affect the mRNA expression of L-CPT-I in preadipocytes (Fig. 2A). On the other hand, it has been reported that irreversible inhibition of CPT-I by etomoxir, which leads to an accumulation of intracellular lipids, induces a PPARα-mediated feedback activation of target genes, such as acyl-CoA oxidase (ACO), involved in alternate oxidation pathways (30). Thus, in order to explore a similar response in preadipocytes we studied the effect of etomoxir treatment on ACO, the rate-limiting enzyme of peroxisomal fatty acid β-oxidation, whose transcription is controlled by PPARα (31). Treatment with etomoxir caused an increase in ACO mRNA levels (1.3-fold induction, P = 0.015) (Fig. 2B). The mRNA levels of PPARα in these preadipocytes were not modify by etomoxir (Fig. 2C).

In order to study whether the up-regulation of UCP-3 mRNA levels caused by etomoxir in preadipocytes was correlated with an increase in the activity of this protein, we analyzed the mitochondrial membrane potential by flow cytometry (Fig. 3). The fluorescence intensity of the dye tetramethylrhodamine ethyl ester (TMRE) has been used as an indicator of mitochondrial membrane potential (10). This fluorescent dye is sensitive to the mitochondrial potential, and therefore, to the electrochemical gradient across the mitochondrial inner membrane. Using confocal microscopy studies we confirmed that the fluorescence was specifically localized in mitochondria of the preadipocytes (data not shown), as previously described (10,32). In this study,

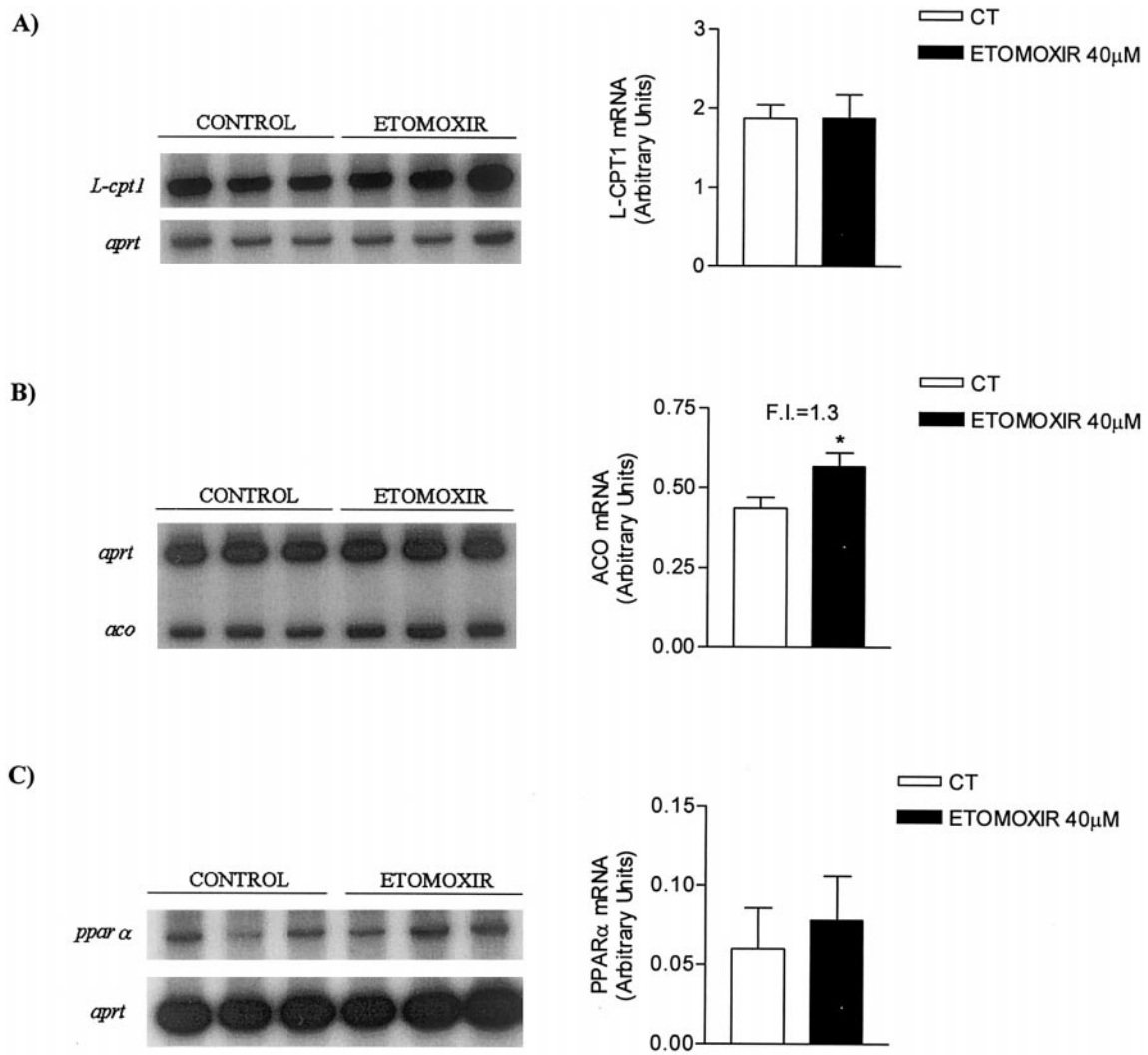

FIG. 2. Effect of etomoxir on the expression of L-CPT-I (A), ACO (B), and PPARα (C) mRNA in primary culture of rat preadipocytes. Cells were incubated for 24 h with or without 40 μM etomoxir. All cells were exposed to 0.1% DMSO. 0.5 μg of total RNA was analyzed by RT-PCR. A representative autoradiogram and the quantification of the aprt-normalized mRNA levels are shown. Data are expressed as mean ± S.D. of 3 experiments. *P = 0.015 compared with control experiments. FI, fold induction.

we used as a positive control carbonylcyanide *m*-chlorophenylhydrazone (CCCP), a very effective uncoupler of mitochondria which decreases the fluorescence emission. The CCCP-dependent fluorescence is normally considered to derive from the mitochondrial contribution. Etomoxir treatment resulted in a shift of the fluorescence peak of untreated cells to the left, which reflects a decrease in the level of fluorescence per cell. The mean values of arbitrary fluorescence units in control and etomoxir-treated cells were 60.5 ± 2.8 (n = 3) and 44.0 ± 2.3 (n = 3) (P = 0.001), respectively. Addition of CCCP induced a large shift to the left in control and treated-cells (values of arbitrary fluorescence units were 13.7 and 12.6, respectively). Thus, since UCP-2 expression was not significantly altered by the treatment, the changes in fluorescence could be attributed to the induction in UCP-3 expression caused by etomoxir.

DISCUSSION

In the present study we show that etomoxir, which is an irreversible inhibitor of CPT-I, and therefore, of mitochondrial β-oxidation of fatty acids, increases the expression of UCP-3 mRNA in rat primary culture of preadipocytes. UCP-2 mRNA expression, however, was not significantly altered by the treatment. According to the role proposed for the UCP family, the increase in UCP-3 mRNA levels induced by etomoxir led to a reduction in the mitochondrial membrane potential.

Acute administration of etomoxir and other 2-oxirane-carboxylates, such as clomoxir or palmoxirate, to ani-

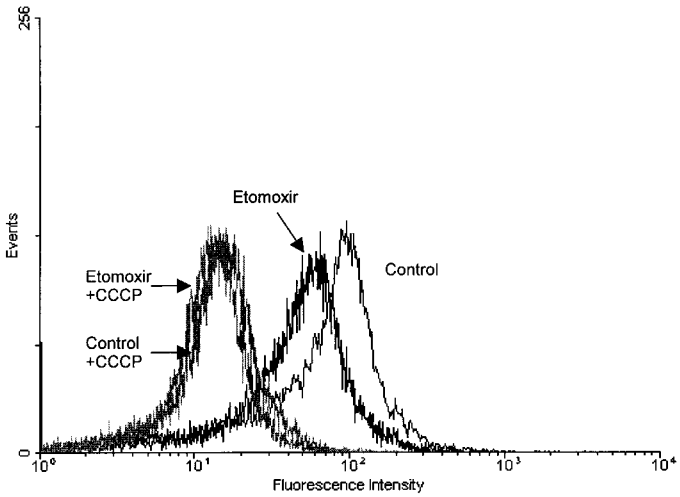

FIG. 3. Etomoxir effect decreases the mitochondrial membrane potential in primary culture of rat preadipocytes. Cells treated for 24 h with or without 40 μM etomoxir were incubated with the mitochondrial potential-sensitive dye TMRE and analyzed by flow cytometry. The chemical uncoupler CCCP (75 μM) was used as a positive control. One experiment representative of three is shown, with a minimum of 10,000 events/sample. A decrease in fluorescence intensity indicates a reduction in mitochondria membrane potential.

mals and man increases plasma FFA (33,34). This makes it difficult to study the direct effects of etomoxir on UCP-3 expression *in vivo*, since FFA are potent inducers of this UCP subtype (12,13). Here we have studied the effects of etomoxir in a rat primary culture of undifferentiated preadipocytes incubated without exogenous fatty acids. Preadipocytes, in addition to adipocytes, express UCP-3 and UCP-2, affording a chance to study their regulation. Further, the metabolic effects of β-oxidation inhibitors have been examined mainly in hepatocytes and muscle, whereas the effects of these drugs on either adipocytes or preadipocytes have remained undocumented.

Changes induced by etomoxir are due to inhibition of CPT-I, leading to the increase in the long-chain fatty acyl-CoA/fatty acyl carnitine ratio in the cytoplasm, which has been reported to affect a large number of cellular systems and functions, including ion channels, ion pumps, translocators, enzymes, membrane fusion and gene regulation (35). Thus, for example, acyl-CoA esters have been shown to be potent inhibitors of the mitochondrial adenine nucleotide translocase (ANT) (36), which catalyses the exchange of ADP and ATP across the mitochondrial inner membrane, considered the overall rate-limiting step in energy metabolism (37). Therefore, etomoxir treatment may induce changes in the ATP/ADP ratio. Since a high ATP/ADP ratio could have harmful effects on the cells, it has been hypothesized that this ratio is kept constant through an increase in UCP-3 activity, which would dissipate the proton gradient across the mitochondrial membrane, uncoupling oxidative phosphorylation (18,39). Our results support the recent hypothesis which indicates that, in addition to thermogenesis (38), the metabolic role of UCP-3 up-regulation by increased substrate (both FA and glucose) flux into tissues (mainly WAT and skeletal muscle) is to regulate cellular ATP and ADP homeostasis by uncoupling oxidative phosphorylation. In the absence of UCP-3 up-regulation, an ADP deficit would appear, causing a reduced state of the electron transport system, which is known to release excessive amounts of superoxide and other toxic reactive oxygen species (40,41). Inhibition of β-oxidation by etomoxir could also shift the respiratory chain into a reduced state. However, this seems unlikely since treatment of preadipocytes for 24 h with 100 μM 4-pentenoic acid, an established inhibitor of long-chain fatty acid β-oxidation at the level of acyl-CoA dehydrogenase (42), did not modify the expression of UCP-3 mRNA (data not shown).

At the level of gene regulation it has been shown that the accumulation of intracellular long-chain fatty acyl-CoA, induced by etomoxir inhibition of mitochondrial fatty acid import, causes a PPARα-mediated activation of target genes involved in alternate oxidation pathways. As a consequence, peroxisomal ACO, a known PPARα target gene, and other similar genes were induced in heart and liver of mice receiving etomoxir (50 μg/g body weight, for 5 days) (30). This induction appears to be consistent with the present study, where we have shown a significant induction of ACO mRNA levels just 24 h after 40 μM etomoxir treatment. Djouadi *et al.* (1998) demonstrated the involvement of PPARα in this response by using mice lacking PPARα, in which the response of PPARα target genes to etomoxir was abolished (30). As a consequence of the lack of this response in these mice, there was a massive accumulation of intracellular lipids in liver and heart.

Several lines of evidence indicate that PPAR is involved in the control of UCP-3 expression. Thus, both PPARα and PPARγ activators, including fatty acids, up-regulate its expression (14–16) and, recently, three putative PPREs have been found in the 5′ flanking region of the human UCP-3 gene (17), converting UCP-3 to a possible new PPAR-target gene. In this study we show that in fibroblastic preadipocytes, which express PPARα, etomoxir would cause similar effects to those described in liver and heart regarding the induction of target genes involved in alternate oxidation pathways, such as the ACO gene. In addition, in these conditions of PPARα activation, UCP-3 mRNA would also be strongly induced as well as its activity, detected by the fall in the mitochondrial membrane potential. Therefore, it seems likely that up-regulation of UCP-3 expression by etomoxir is mediated by PPARs.

Furthermore, inhibition of CPT-I by etomoxir could explain recently published results, in which overexpression of GLUT4 in skeletal muscle and WAT of

transgenic mice harboring a GLUT4 minigene led to up-regulation of UCP3 in these tissues (18). Increased glucose entry to skeletal myocytes and white adipocytes, due to high GLUT4 levels, increases the concentration of malonyl-CoA, a known inhibitor of CPT-I (24). Therefore, forced glucose flux into the cells may lead to CPT-I inhibition by malonyl-CoA, causing an accumulation of intracellular long-chain fatty acyl-CoA, which in turn would activate PPAR, increasing the expression of UCP-3 mRNA.

In summary, we show an up-regulation of UCP-3 mRNA levels after etomoxir treatment in rat primary culture of preadipocytes. Further, UCP-3 activity was also increased, as shown by the fall in the mitochondrial membrane potential. In our opinion, UCP-3 induction could be included in the context of a generalized response of the cells to a major perturbation in fatty acid flux caused by etomoxir, in which PPARα activates target genes involved in alternate cellular fatty oxidation. This response tries to counteract massive accumulation of intracellular lipids in tissues. Thus, UCP-3 would form part of an important cellular response to reduce lipotoxic effects of fatty acids. Since UCP-3 mRNA levels are reduced in skeletal muscle of NIDDM patients (43), it remains to be stablished whether reduced expression of UCP-3 leads to a low capacity of tissues to overcome these toxic effects of fatty acids.

ACKNOWLEDGMENTS

We thank Mr. Rycroft (Language Advice Service of the University of Barcelona) for his helpful assistance. Further, we greatly appreciate Jaume Comas and Susanna Castell from the Serveis Científico-Tècnics of the University of Barcelona for their technical support. We are grateful to Dr. G. Asins (Unidad de Bioquímica, Facultad de Farmacia, Universidad de Barcelona) for generously providing us etomoxir. This study was partly supported by grants from the FPCNL and CICYT (SAF97-0215 and SAF98-0105). We also thank the Generalitat de Catalunya for Grant SGR96-84 and 1998SGR-33. Àgatha Cabrero was supported by a grant from the Ministerio de Educación of Spain.

REFERENCES

1. Jacobson, A., Stadler, U., Glotzer, M. A., and Kozak, L. P. (1985) *J. Biol. Chem.* **260,** 16350–16254.
2. Bouillad, F., Weissenbach, J., and Ricquier, D. (1986) *J. Biol. Chem.* **261,** 1487–1490.
3. Klingenberg, M. (1990) *Trends Biochem. Sci.* **27,** 781–791.
4. Lowell, B. B., Susulic, V. S., Hamann, A., Lawitts, J. A., Himms-Hagen, J., Boyer, B. B., Kozaki, L. P., and Flier, J. S. (1993) *Nature* **366,** 740–742.
5. Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi, M. C., Bouillaud, F., Seldin, M. F., Surwitt, R. S., Ricquier, D., and Warden, C. H (1997) *Nat. Genet.* **15,** 269–272.
6. Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J. S., and Lowell, B. B. (1997) *Biochem. Biophys. Res. Commun.* **235,** 79–82.
7. Boss, O., Samec, S., Paoloni, G. A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P., and Giacobino, J. P. (1997) *FEBS Lett.* **408,** 39–42.
8. Gong, D.-W., He, Y., Karas, M., and Reitman, M. (1997) *J. Biol. Chem.* **272,** 24129–24132.
9. Gimeno, R. E., Dembski, M., Weng, X., Deng, N., Shyjan, A. W., Gimeno, C. J., Iris, F., Ellis, S. J., Woolf, E. A., and Tartaglia, L. A. (1997) *Diabetes* **46,** 900–906.
10. Boss, O., Samec, S., Kühne, F., Bijlenga, P., Assimacopoulos-Jeannet, F., Seydoux, J., Giacobino, J. P., and Muzzin, P. (1998) *J. Biol. Chem.* **273,** 5–8.
11. Keesey, R. E. (1998) *in* Handbook of Eating Disorders. 2. (Burrows, G. D., Beaumont, P. G., and Casper, R. C., Eds.), pp. 87–102. Elsevier Science, Amsterdam.
12. Millet, L., Vidal, H., Andreeli, F., Larrouy, D., Riou, J. P., Ricquier, D., Laville, M., and Langin, D. (1997) *J. Clin Invest.* **100,** 2665–2670.
13. Weigle, D. S., Selfridge, L. E., Schwartz, M. W., Seely, R. J., Cummings, D. E., Havel, P. J., Kuipjer, J. L., and BeltrandelRio, H. (1998) *Diabetes* **47,** 298–302.
14. Matsuda, J., Hosoda, K., Itoh, H., Son, C., Doi, K., Hanaoka, Y., Inoue, G., Nishimura, H., Yoshimasa, Y., Yamori, Y., Odaka, H., and Nakao, K. (1998) *Diabetes* **47,** 1809–1814.
15. Brun, S., Carmona, M., Mampel, T., Viñas, O., Giralt, M., Iglesias, R., and Villarroya, F. (1999) *Diabetes* **48,** 1217–1222.
16. Cabrero, A., Llaverías, G., Roglans, N., Alegret, M., Sánchez, R., Adzet, T., Laguna, J. C., and Vázquez, M. (1999) *Biochem. Biophys. Res. Commun.* **260,** 547–556.
17. Acín, A., Rodríguez, M., Rique, H., Canet, E., Boutin, J. A., and Galizzi, J-P. (1999) *Biochem. Biophys. Res. Commun.* **258,** 278–283.
18. Tsuboyama-Kasoaka, N., Tsunoda, N., Maruyama, K., Takahashi, M., Kim, H., Cooke, D. W., Lane, D., and Ezaki, O. (1999) *Biochem. Biophys. Res. Commun.* **258,** 187–193.
19. McGarry, J. D., and Brown, N. F. (1997) *Eur. J. Biochem.* **244,** 1–14.
20. Britton, C. H., Schultz, R. A., Zhang, B., Esser, V., Foster, D. W., and McGarry, J. D. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 1984–1988.
21. Asins, G., Serra, D., Arias, G., and Hegardt, F. G. (1995) *Biochem. J.* **306,** 379–384.
22. Weis, B. C., Esser, V. Foster, D. W., and McGarry, J. D. (1994) *J. Biol. Chem.* **269,** 18712–18715.
23. Esser, V., Brown, N. F., Cowan, A. T., Foster, D. W., and McGarry, J. D. (1996) *J. Biol. Chem.* **271,** 6972–6977.
24. McGarry, J. D., Mannaerts, G. P., and Foster, D. W. (1977) *J. Clin. Invest.* **60,** 265–270.
25. Sidossis, L. S., Stuart, C. A., Shulman, G. I., Lopashuck, G. D., and Wolfe, R. R. (1996) *J. Clin. Invest.* **98,** 2244–2250.
26. Randle, P. J. (1963) *Lancet* **1,** 785–789.
27. Ailhaud, G., Grimaldi, P., and Négrel, R. (1992) *Annu. Rev. Nutr.* **12,** 207–233.
28. Rodbell M. (1964) *J. Biol. Chem.* **239,** 375–380.
29. Brown, N. F., Hill, J. K., Esser, V., Kirkland, J. L., Corkey, B. E., Foster, D. W., and McGarry, J. D. (1997) *Biochem J.* **327,** 225–231.
30. Djouadi, F., Weinheimer, C. J., Saffitz, J. E., Pitchford, C., Bastin, J., Gonzalez, F. J., and Kelly, D. P. (1998) *J. Clin. Invest.* **102,** 1083–1091.
31. Wahli, W., Braissant, O., and Desvergne, B. (1995) *Chem. Biol.* **2,** 261–266.
32. Farkas, D. L., de Wei, M., Febbroriello, P., Carson, J. H., and Loew, L. M. (1989) *Biophys. J.* **56,** 1053–1069.
33. Koundakjian, P. P., Turnbull, D. M., Bone, A. J., Younen,

S. I. M., and Sherrat, H. S. A. (1984) *Biochem. Pharmacol.* **33,** 465–473.

34. Reaven, G. M., Chang, H., and Hoffman, B. B. (1988) *Diabetes* **37,** 28–32.
35. Faergeman, N. J., and Knudsen, J. (1997) *Biochem. J.* **323,** 1–12.
36. Shrago, E., Woldegiorgis, A. E., and DiRusso, C. C. (1995) *Prostaglandins Leukotrienes Essential Fatty Acids* **52,** 163–166.
37. Lemasers, J. J., and Sowers, A. E. (1979) *J. Biol. Chem.* **254,** 1248–1251.
38. Hinz, W., Faller, B., Grüninger, S., Gazzotti, P., and Chiesi, M. (1999) *FEBS Lett.* **448,** 57–61.
39. Hwang, C-S., and Lane, M. D. (1999) *Biochem. Biophys. Res. Commun.* **258,** 464–469.
40. Negre-Salvayre, A., Hirtz, C., Carrera, G., Cazenave, R., Troly, M., Salvayre, R., Penicaud, L., and Casteilla, L. (1997) *FASEB J.* **11**(10), 809–815.
41. Kroemer, G., Petit, P., Zamzani, N., Vayssiere, J. L., and Mignotte, B. (1995) *FASEB J.* **9** (13), 1277–1287.
42. Bjorge, S. M., and Baillie, T. A. (1985) *Biochem. Biophys. Res. Commun.* **132,** 245–252.
43. Krook, A., Digby, J., O'Rahilly, S., Zierath, J. R., and Wallberg-Henriksson, H. (1998) *Diabetes* **47,** 1528–1531.