Down-regulation of uncoupling protein-3 and -2 by thiazolidinediones in C2C12 myotubes

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Abstract Uncoupling proteins (UCPs) are mitochondrial membrane proton transporters that uncouple respiration from oxidative phosphorylation by dissipating the proton gradient across the membrane. We studied the direct effect of several peroxisome proliferator-activated receptor (PPAR) ligands on UCP-3 and UCP-2 mRNA expression in C2C12 myotubes for 24 h. In the absence of exogenous fatty acids, treatment of C2C12 cells with a selective PPARα activator (Wy-14,643) or a non-selective PPAR activator (bezafibrate) did not affect the expression of UCP-3 mRNA levels, whereas UCP-2 expression was slightly increased. In contrast, troglitazone, a thiazolidinedione which selectively activates PPARγ, strongly decreased UCP-3 and UCP-2 mRNA levels. Another thiazolidinedione, ciglitazone, had the same effect, but to a lower extent, suggesting that PPARγ activation is involved. Further, the presence of 0.5 mM oleic acid strongly increased UCP-3 mRNA levels and troglitazone addition failed to block the effect of this fatty acid. The drop in UCP expression after thiazolidinedione treatment correlated well with a reduction in PPARα mRNA levels produced by this drug, linking the reduction in PPARα mRNA levels with the down-regulation of UCP mRNA in C2C12 myotubes after thiazolidinedione treatment.

Key words: UCP; PPAR; Thiazolidinedione; Troglitazone; Ciglitazone; C2C12

1. Introduction

Uncoupling proteins (UCPs) are mitochondrial carriers that are localized in the inner mitochondrial membrane. They act by dissipating the proton gradient generated by the respiratory chain. As a result, they uncouple respiration from oxidative phosphorylation and convert fuel substrates to heat. Two recently discovered uncoupling protein genes, UCP-2 and UCP-3, share a high sequence homology with the gene for uncoupling protein-1 (UCP-1), which plays an important role in the control of thermogenesis and is specific for brown adipose tissue (BAT) [1]. UCP-2 is ubiquitously expressed in all the tissues examined, whereas UCP-3 is highly expressed only in skeletal muscle and in BAT [2–6]. The biological functions of UCP-2 and UCP-3 are not well understood. Genetic studies suggest that these UCPs may be involved in energetic expenditure [7]. In addition, they seem to be implicated in the regulation of lipids as fuel substrate in skeletal muscle, since their mRNA levels are up-regulated by the mobilization of fat stores in the fasted state. The high levels of free fatty acids achieved in the fasting have been proposed to be responsible for the increased UCP-3 expression during starvation [8,9]. The induction of UCP by fatty acids is most probably mediated by the activation of peroxisome proliferator-activated receptor (PPAR). Three different PPAR subtypes (α, δ/β, and γ) have been identified to date. PPARα is expressed primarily in tissues that have a high level of fatty acid catabolism such as liver, brown fat, kidney, heart and skeletal muscle [10,11]. PPARβ is ubiquitously expressed, whereas PPARγ has a restricted pattern of expression, mainly in white and brown adipose tissues [12]. PPARγ is also expressed, although at low levels, in skeletal muscle [13,14]. PPAR binds to DNA as a heterodimer with the retinoid X receptor and it is activated by ligands such as naturally occurring fatty acids, which are ligands of all three PPAR subtypes [15,16]. In addition to fatty acids, several synthetic compounds bind and activate specific PPAR subtypes. Antidiabetic thiazolidinediones (troglitazone, ciglitazone, rosiglitazone) bind and selectively activate PPARγ [17,18], whereas (4-chloro-6-(2,3-xylidine)-pirimidinylthio)acetic acid (Wy-14,643) is selective for PPARα [19,20]. In contrast, bezafibrate (BFB) activates PPARα, PPARγ and PPARβ with comparable EC_{50} values [21].

Here, we report the effects of several PPAR ligands on the expression of UCP-2 and UCP-3 in C2C12 cells. This system, in contrast to in vivo studies, allows us to assess the direct effects of drugs and compounds that regulate UCP expression. In C2C12 cells, a widely used myocyte cell line, the selective PPARγ activators troglitazone and ciglitazone strongly repressed UCP-3 and UCP-2 mRNA expression. UCP mRNA down-regulation correlated well with a PPARα decreased mRNA expression after thiazolidinedione treatment, suggesting that the impaired expression of this transcription factor was involved in the effects caused by these antidiabetic drugs. In the presence of oleic acid in the culture medium, the effects of troglitazone on UCP and PPARα mRNA levels were reversed.

2. Materials and methods

2.1. Chemicals

Troglitazone was kindly provided by Glaxo Wellcome (UK) and atorvastatin by Parke Davis (Spain). Ciglitazone was from Biomol (Plymouth Meeting, PA, USA), bezafibrate and oleic acid albumin were from Sigma (St. Louis, MO, USA) and (4-chloro-6-(2,3-xyl-

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idine)-pirimidinylthio)acetic acid (Wy-14,643) was from Cayman Chemicals (Ann Arbor, MI, USA).

2.2. Cell culture

Mouse C2C12 myoblasts (ATCC) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), 50 units/ml penicillin and 50 μg/ml streptomycin. When cells reached confluence, the medium was switched to the differentiation medium containing DMEM and 2% horse serum, which was changed every other day. After 4 additional days, the differentiated C2C12 cells had fused into myotubes, which were then treated in serum-free DMEM with either vehicle (0.1% DMSO) or the drugs tested for 24 h. After the incubation, RNA was extracted from myotubes as described below.

2.3. RNA preparation and analysis

Total RNA was isolated by 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 5 μl 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 annealing, 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′; M-CPT-I, 5′-TTCACTGTGACCCCAGACGGG-3′ and 5′-AATGGACCAGCCCCATGGAGA-3′; MCAD, 5′-TCGAAAGCGGCTCACAAGCAG-3′ and 5′-CACCGCAGCTTTCCGGAATGT-3′; PPARα, 5′-GGCTCGGAGGGCTCTGTCATC-3′ and 5′-ACATGCACTGGCAGCAGTGGA-3′; PPARβ, 5′-GAGGAAGTGGCCACGGGTGAC-3′ and 5′-CCACCTGAGGCCCCATCACAG-3′; PPARγ, 5′-TGGGGATGTCTCACAATGCCA-3′ and 5′-TTCCTGTCAAGATCGCCCTCG-3′; and APRT (adenosyl phosphoribosyl transferase), 5′-AGCTTCCCGGACTTCCCCATC-3′ and 5′-GACCACTTTCTGCCCCGGTTC-3′. 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 20 (MCAD), 21 (UCP-2), 26 (UCP-3), 27 (PPARα, β and γ) or 33 (M-CPT-I) 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 single band of the expected size (UCP-3: 179 bp, UCP-2: 471 bp, M-CPT-I: 222 bp, MCAD: 216 bp, PPARα: 645 bp, PPARβ: 142 bp, PPARγ: 200 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*).

2.4. Statistical analyses

Results are usually expressed as means ± S.D. of three experiments. Significant differences were established by Student's *t*-test or ANOVA, according to the number of groups compared. When significant variations were found, the Tukey–Kramer multiple comparisons test was

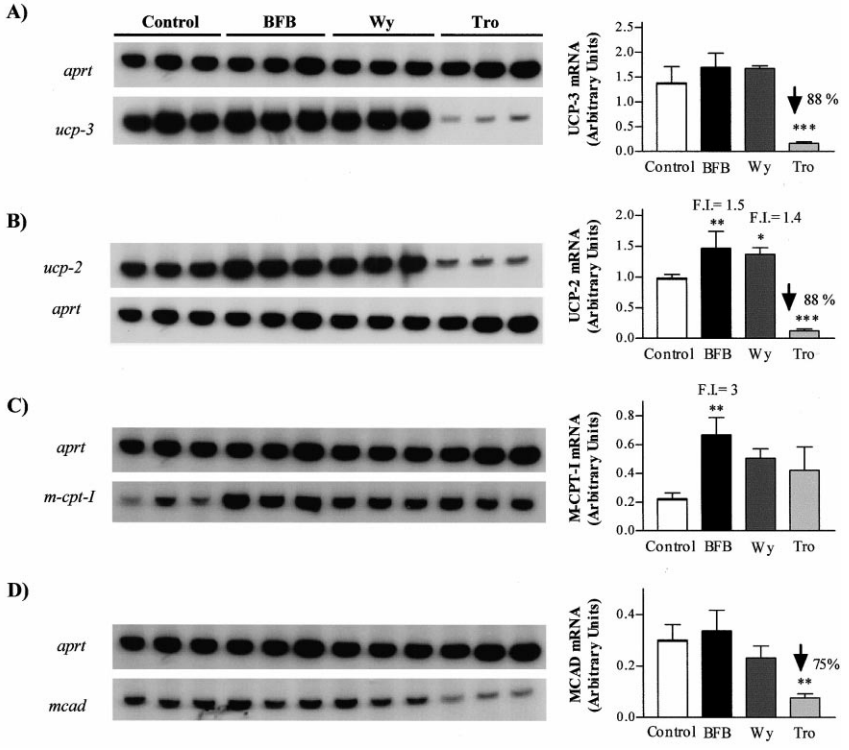

Fig. 1. Effects of bezafibrate, Wy-14,643 and troglitazone on the expression of UCP-3 (A), UCP-2 (B), M-CPT-I (C), and MCAD (D) mRNA in C2C12 myotubes. Cells were incubated for 24 h with vehicle, 500 μM bezafibrate, 10 μM Wy-14,643 or 5 μM troglitazone. 0.5 μg of total RNA was analyzed by RT-PCR. A representative autoradiogram and the quantification of the aprt-normalized mRNA levels are shown. Data are expressed as mean ± S.D. of three experiments. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared with control experiments. F.I., fold induction.

performed. Simple correlation analysis was used to evaluate the relationships among UCP and PPARα mRNA levels. All the statistical analyses were performed using the computer program GraphPad Instat.

3. Results

3.1. Effects of PPAR activators on UCP mRNA levels in C2C12 myotubes

C2C12 myoblasts differentiated morphologically to fuse into myotubes when cultured in the presence of 2% horse serum 4 days after reaching confluence. The effects of several PPAR activators on UCP-3 and UCP-2 mRNA expression were assessed in C2C12 myotubes for 24 h, incubated without exogenous fatty acids. In these culture conditions, 5 μM troglitazone, a selective PPARγ activator [17,18], strongly decreased UCP-3 mRNA levels by 88% ($P < 0.001$), whereas neither bezafibrate, a non-selective PPAR activator [21] used at a concentration within the plasma levels of fibrates reported in humans [22], nor Wy-14,643, which at 10 μM binds and activates only the PPARα subtype [21], modified UCP-3 mRNA expression (Fig. 1A). Similarly to UCP-3, UCP-2 mRNA levels decreased by 88% ($P < 0.001$) after the addition of troglitazone to C2C12 myotubes. In contrast, treatment with bezafibrate and Wy-14,643 caused a 1.5- and 1.4-fold increase in UCP-2 mRNA levels, respectively (Fig. 1B). Since mitochondrial β-oxidation is involved in the generation of the electrochemical gradient of protons, which is dissipated by UCP, we tested whether PPAR activators affected the mRNA expression of two genes of this system, muscle-type carnitine palmitoyl transferase (M-CPT-I) and medium-chain acyl-CoA dehydrogenase (MCAD). The former catalyzes the entry of long-chain fatty acids into the mitochondrial matrix [23] and is regulated by PPARα and PPARγ [24]. Of the three drugs studied, bezafibrate was the only to significantly change the expression of M-CPT-I (3-fold induction, $P < 0.01$) (Fig. 1C). The second, MCAD, catalyzes a rate-limiting step in the mitochondrial oxidation of medium-chain fatty acyl thioesters and its transcription is controlled by PPARα [10]. Neither BFB nor Wy-14,643 affected the MCAD mRNA expression after 24 h of treatment. Interestingly, addition of troglitazone, at a concentration (5 μM) known to activate PPARγ, led to a 70% reduction in the mRNA expression of MCAD (Fig. 1D).

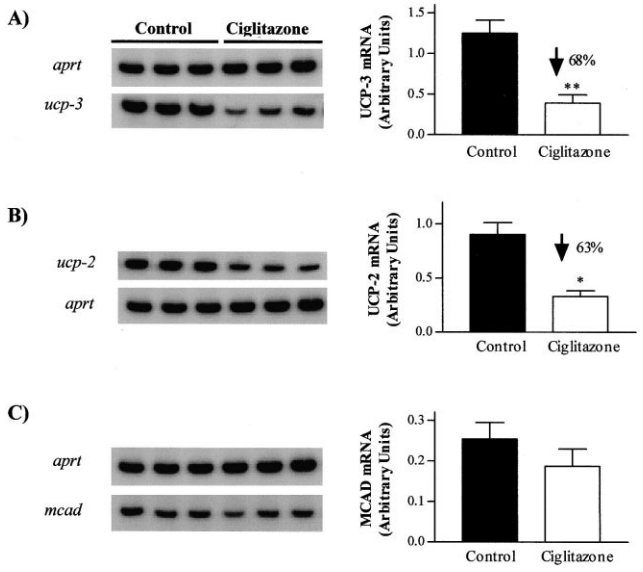

Fig. 3. Effects of ciglitazone on the expression of UCP-3 (A), UCP-2 (B) and MCAD (C) mRNA in C2C12 myotubes. Cells were incubated for 24 h with vehicle or 5 μM ciglitazone. 0.5 μg of total RNA was analyzed by RT-PCR. A representative autoradiogram and the quantification of the aprt-normalized mRNA levels are shown. Data are expressed as mean ± S.D. of three experiments. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared with control experiments. F.I., fold induction.

3.2. Effects of triacsin C and atorvastatin on UCP mRNA levels in C2C12 myotubes

Troglitazone, in addition to its effects mediated through PPARγ activation, possesses several additional effects that are independent of this transcription factor. Thus, it has been reported that in hepatocytes it reduces fatty acid oxidation through inhibition of acyl-CoA synthetase (ACS) activity [25]. Further, it has been shown that troglitazone strongly inhibits cholesterol biosynthesis in muscle cells [26]. In order to study the contribution of these mechanisms to the effects of troglitazone, we assessed the effects of triacsin C, a potent inhibitor of ACS activity, and atorvastatin, an inhibitor of the cholesterol biosynthesis, on UCP-3 mRNA levels. Addition of 5 μM triacsin C, a concentration previously reported

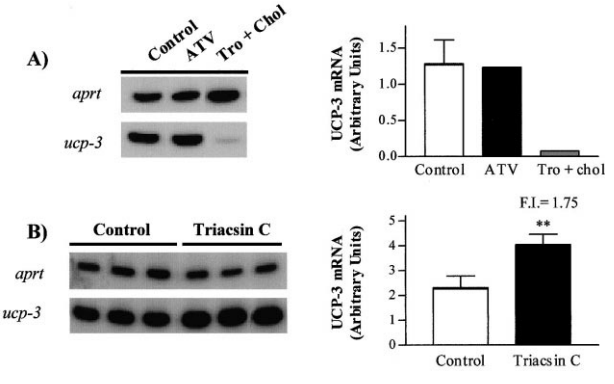

Fig. 2. Effects of triacsin C (A), cholesterol and atorvastatin (B) on the expression of UCP-3 mRNA levels in C2C12 myotubes. Cells were incubated for 24 h with vehicle, 5 μM triacsin C, cholesterol (10 μg/ml) or 5 μM atorvastatin. 0.5 μg of total RNA was analyzed by RT-PCR. A representative autoradiogram and the quantification of the aprt-normalized mRNA levels are shown. Data are expressed as mean ± S.D. of three experiments. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared with control experiments. F.I., fold induction.

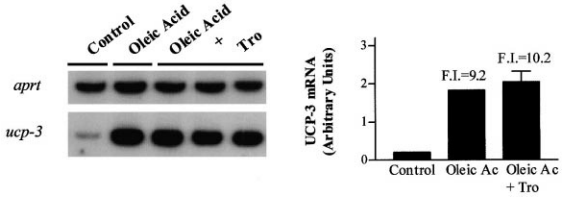

Fig. 4. Effects of oleic acid on the expression of UCP-3 mRNA in C2C12 myotubes. Cells were incubated for 24 h with vehicle or 0.5 mM oleic acid complexed to BSA, in the absence or presence of 5 μM troglitazone. 0.5 μg of total RNA was analyzed by RT-PCR. A representative autoradiogram and the quantification of the aprt-normalized mRNA levels are shown. Data are expressed as mean ± S.D. of three experiments. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared with control experiments. F.I., fold induction.

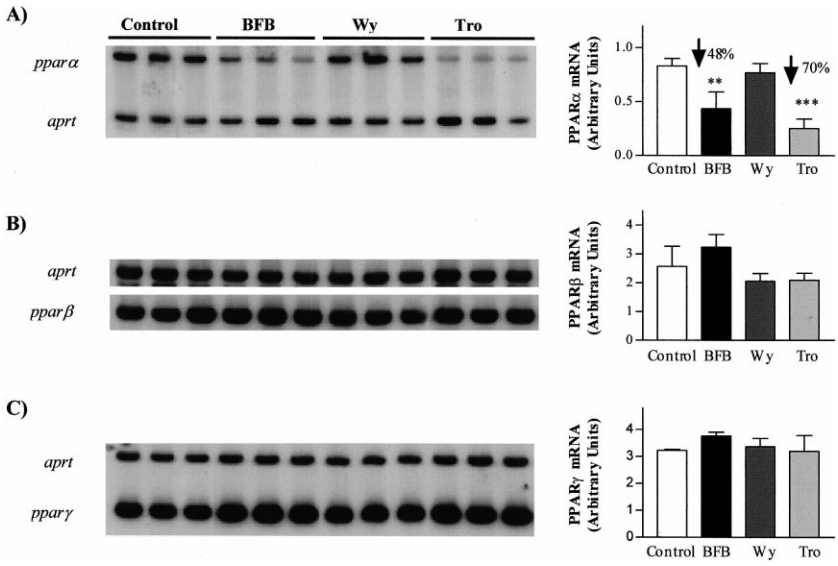

Fig. 5. Effects of bezafibrate, Wy-14,643 and troglitazone on the expression of PPARα (A), PPARβ (B) and PPARγ (C) mRNA in C2C12 myotubes. Cells were incubated for 24 h with vehicle, 500 μM bezafibrate, 10 μM Wy-14,643 or 5 μM troglitazone. 0.5 μg of total RNA was analyzed by RT-PCR. A representative autoradiogram and the quantification of the aprt-normalized mRNA levels are shown. Data are expressed as mean ± S.D. of three experiments. $*P<0.05$, $**P<0.01$ and $***P<0.001$ compared with control experiments. F.I., fold induction.

to inhibit ACS [27], resulted in a 1.8-fold induction of UCP-3 mRNA expression ($P<0.01$), which is consistent with the activation of PPARα by free fatty acids [28] (Fig. 2A). The down-regulation of UCP-3 mRNA levels caused by troglitazone was not reversed by the addition of cholesterol (10 μg/ml) (Fig. 2B). Likewise, 5 μM atorvastin, a concentration reported to inhibit cholesterol biosynthesis [29], did not modify UCP-3 mRNA levels in C2C12 cells.

3.3. Effects of ciglitazone on UCP-3 and MCAD mRNA levels in C2C12 myotubes

In order to determine whether the effects of troglitazone on UCP expression were specific of this compound or can be extended to other thiazolidinediones, we tested the effect of ciglitazone, another compound of this class of antidiabetic drugs. After 24 h of treatment, ciglitazone decreased the UCP-3 mRNA expression by 68% ($P<0.01$) (Fig. 3A) and UCP-2 mRNA expression by 63% ($P<0.05$) (Fig. 3B), whereas MCAD mRNA levels were not significantly decreased (Fig. 3C). These results suggests that ciglitazone had a lesser potency than troglitazone.

3.4. Effects of oleic acid on UCP-3 mRNA levels in C2C12 myotubes

It has been shown that addition of oleic acid to C2C12 cells results in up-regulation of UCP-3 mRNA levels [30]. Maximal induction (8-fold) of UCP-3 mRNA levels was reported between 0.6 and 0.9 mM of oleic acid, which approximates the level of fatty acids in the blood of fasted rats [31]. To test whether fatty acids reverse the effect evoked by troglitazone on UCP-3 mRNA expression, we treated C2C12 cells with 0.5 mM oleic acid. As shown in Fig. 4, oleic acid addition caused a 9.2-fold induction in UCP-3 mRNA levels and abolished the effect of troglitazone (Fig. 4).

3.5. Effects of PPAR activators on PPARα, β and γ mRNA levels in C2C12 myotubes

Finally, we determined the effects of the several PPAR activators used in this study on the mRNA levels of PPARα, β and γ. In contrast to L6 myotubes [32], which only express PPARβ mRNA, C2C12 myotubes express transcripts of the three PPAR subtypes. Regarding PPARα mRNA levels, bezafibrate treatment caused a 48% reduction ($P<0.01$), Wy-14,643 did not affect its expression, whereas troglitazone decreased it by 70% ($P<0.001$) (Fig. 5A). These results suggest that impaired expression of PPARα was involved in the effects caused by thiazolidinediones. In fact, a high correlation coefficient was obtained when we performed linear regression between UCP-2 and PPARα ($r^2=0.94$, $P=0.0013$, $n=6$) and between UCP-3 and PPARα mRNA levels ($r^2=0.84$, $P=0.010$, $n=6$). None of the PPAR activators studied modified the mRNA expression of PPARβ or PPARγ (Fig. 5B and C). The effects of troglitazone on PPARα mRNA levels were reversed by the addition of oleic acid (Fig. 6A). Similarly to

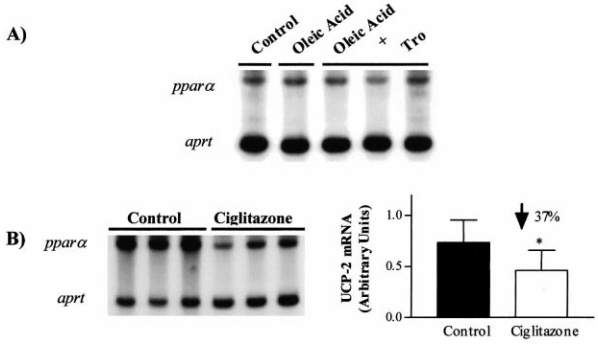

Fig. 6. Effects of oleic acid (A) and ciglitazone (B) on the expression of PPARα mRNA in C2C12 myotubes. Cells were incubated for 24 h with vehicle, 0.5 mM oleic acid complexed to BSA, in the absence or presence of 5 μM troglitazone or 5 μM ciglitazone. 0.5 μg of total RNA was analyzed by RT-PCR. A representative autoradiogram and the quantification of the aprt-normalized mRNA levels are shown. Data are expressed as mean ± S.D. of three experiments. $*P<0.05$, $**P<0.01$ and $***P<0.001$ compared with control experiments. F.I., fold induction.

troglitazone, ciglitazone significantly decreased the PPARα mRNA expression by 37% ($P < 0.05$) (Fig. 6B).

4. Discussion

PPARs have been involved in the regulation of UCPs [1], which is consistent with the presence of several potential peroxisome proliferator response elements (PPRE) in the 5′ flanking region of UCP-3 [33] and UCP-2 [34] genes. Interestingly, of the three PPAR subtypes, PPARα seems to play an important role in UCP-3 regulation in muscle, since it is responsible for the switch on of UCP-3 at birth [14]. Premature newborns, which do not express PPARα in muscle, have thus impaired responsiveness to PPARα activators [14]. The present study shows that thiazolidinediones, in the absence of exogenous fatty acids, down-regulate the mRNA expression of UCP-3 and UCP-2 in C2C12 cells through impairment of PPARα expression. The down-regulation of UCP-3 and UCP-2 by thiazolidinediones correlates well with a reduced expression in PPARα mRNA levels, suggesting that impaired expression of this transcription factor was involved in the effects caused by these antidiabetic drugs.

The mechanism by which troglitazone down-regulates UCP mRNA expression seems to involve PPARγ activation but not additional effects not related to PPARγ activation (inhibition of fatty acid oxidation and cholesterol biosynthesis). This is consistent with the fact that ciglitazone, another compound of thiazolidinedione family, which also activates PPARγ, elicited the same effects although with lesser intensity, which is in agreement with the lower potency of ciglitazone in activating PPARγ [26]. In fact, ciglitazone reduced PPARα mRNA expression by only 37%, whereas troglitazone reduced it by 75%. This slighter reduction in PPARα mRNA expression caused by ciglitazone reduced UCP-3 and UCP-2 mRNA levels, whereas MCAD expression was not significantly affected. BFB, which activates the three PPAR subtypes [21], significantly reduced PPARα mRNA levels, probably by activating the PPARγ subtype. However, no reduction was observed in UCP-3, UCP-2 or MCAD mRNA levels after BFB treatment, showing that PPARα activation by BFB can compensate its reduced expression. BFB increased M-CPT-I mRNA levels, whereas Wy-14,643 had no effect, suggesting that this induction could be associated with the reported inhibition of M-CPT-I by BFB [35] but not with PPARα activation. In agreement to this hypothesis, etomoxir, a well-known inhibitor of this enzyme activity, up-regulates M-CPT-I mRNA levels in C2C12 cells (data not shown).

Wy-14,643 treatment for 24 h did not modify mRNA levels of MCAD, a well-known PPARα target gene [10]. This is consistent with a previous study, which reported that MCAD mRNA levels were not induced in the heart of mice treated with the PPARα activator etomoxir for 24 h [36]. However, in the same study a longer treatment with etomoxir for 5 days induced MCAD mRNA levels. Therefore, it is likely that longer times of treatment of C2C12 cells with PPARα activators would result in the induction of mRNA levels of this PPARα target gene.

In contrast to the present study, it has been reported that another thiazolidinedione, rosiglitazone (BRL49653), strongly increased UCP-3 mRNA levels in C2C12 cells [30]. This discrepancy may be attributed to the higher PPARγ binding potency of rosiglitazone compared to troglitazone [37] or to differential activation of PPARγ by troglitazone and rosiglitazone, as recently reported [38]. Indeed, troglitazone acted as a partial agonist for PPARγ in transfected C2C12 cells, producing a submaximal transcriptional response compared with rosiglitazone. Further, troglitazone antagonized rosiglitazone-stimulated PPARγ transcriptional activity. In another study, troglitazone treatment did not modify UCP-3 mRNA levels in L6 myotubes [32]. This lack of effect of troglitazone on L6 myotubes compared to C2C12 cells was attributed to the absence of PPARγ in the former. In the present work, troglitazone treatment for 24 h did not change PPARγ expression, which is in contrast to the 2.5-fold induction reported in PPARγ mRNA levels after 4 days of treatment in human skeletal muscle cells [39]. Although differences in the cellular type might be the reason of this discrepancy, it is likely that longer treatments of C2C12 with thiazolidinediones may result in PPARγ up-regulation.

Here, thiazolidinediones down-regulated UCP expression in C2C12 cells in the absence of exogenous fatty acids, but in the presence of glucose in the culture media. When oleic acid was added to the cells, a strong induction was observed in UCP-3 mRNA levels, similarly to previous studies [30], and troglitazone did not reverse this induction. UCP-3 down-regulation has been reported in vivo after thiazolidinedione treatment (pioglitazone) in skeletal muscle of hyperglycemic KK mice [40]. In this study, a high correlation was found between reduced glucose plasma levels and UCP-3 down-regulation, whereas plasma free fatty acids were not reported. Since thiazolidinediones improve the use of glucose as substrate [41], when glucose is the main substrate available, these drugs may strongly increase the use of glucose and decrease the expression of UCP. In contrast, when fatty acids are present in the media, they are used, leading to increased expression of UCP. In these conditions it is believed that UCP up-regulation may be involved in adaptation of cellular metabolism to an excessive supply of lipid substrates in order to regulate the ATP level, the NAD^+/NADH ratio and to contain superoxide production [1].

In summary, thiazolidinediones down-regulate the expression of UCP-3 and UCP-2 mRNA levels in C2C12 myotubes cultured in the absence of exogenous fatty acids. The decrease in UCP expression correlated well with an impaired expression of PPARα mRNA levels. In the presence of oleic acid, UCP-3 expression was up-regulated and thiazolidinediones did not reverse this induction. Although further evidence is required, these results suggest that the presence of different fuel substrates in the culture media modifies the effects of PPAR ligands on UCP expression.

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