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SIGNALING AND CELL PHYSIOLOGY

Fatty acids do not activate UCP2 in pancreatic beta cells: comparison with UCP1

Sandrine Galetti · Alexandre Sarre · Hélène Perreten · Nathalie Produit-Zengaffinen · Patrick Muzzin · Françoise Assimacopoulos-Jeannet

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Abstract UCP2 is expressed in pancreatic β cells where its postulated uncoupling activity will modulate glucose-induced changes in ATP/ADP ratio and insulin secretion. The consequences of UCP2 over/underexpression on β-cell function has mainly been studied in the basal state; however, a UCP has no uncoupling activity unless stimulated by fatty acids and/or reactive oxygen species. Here, UCP2 was overexpressed in INS-1 cells and parameters reflecting mitochondrial coupling measured in the basal state and after stimulation by fatty acids. For comparison, UCP1 was expressed to similar levels and the same parameters measured. Neither UCP1 expression nor UCP2 overexpression modified basal or glucose-stimulated metabolic changes. Upon addition of fatty acids, UCP1-expressing cells displayed the expected mitochondrial uncoupling effect, while UCP2 did not elicit any measurable change in mitochondrial function. Taken together, our data demonstrate that, in pancreatic β-cells, UCP2 has no uncoupling activity in the basal state or after fatty acid stimulation.

Keywords UCP2 · UCP1 · INS-1 cells · Fatty acid-induced uncoupling · Mitochondria

S. Galetti · A. Sarre · H. Perreten · N. Produit-Zengaffinen · P. Muzzin · F. Assimacopoulos-Jeannet (✉)
Department of Cell Physiology and Metabolism, Medical Faculty, University of Geneva,
1 rue Michel Servet,
1211 Geneva 4, Switzerland
e-mail: francoise.assimacopoulos@medecine.unige.ch

Introduction

In the pancreatic β-cell, changes in ATP/ADP play a key role in the coordinated mechanism that senses changes in glucose metabolism and transduces its signal in insulin secretion. The presence of UCP2 in pancreatic β-cells and the regulation of its expression by fatty acids, PPARγ and PPARα agonists or glucose [1–5], drew the attention on this protein as a potential modulator of insulin secretion. Its postulated uncoupling activity would dissipate the proton gradient across the mitochondrial inner membrane, thereby attenuating basal and/or glucose-induced ATP generation and downstream effects on the closure of K_{ATP} channel, plasma membrane depolarization, Ca^{2+} entry, and insulin secretion. Studies in UCP2 KO mice [6] or in pancreatic beta cells and cell lines overexpressing UCP2 [7, 8] have suggested that the uncoupling activity of the protein plays an important role in the modulation of ATP generation and insulin secretion. Similarly, other studies correlate changes in UCP2 expression with alterations in insulin secretion. Sirt1 positively regulates insulin secretion and represses UCP2 expression [9]; Foxa1 deficient islets have a decreased ATP synthesis suggestedly explained by an increased expression of UCP2 [10]. Pancreatic islets from type 2 diabetic subjects show increased UCP2 gene expression, abnormal mitochondrial morphology, and impaired glucose-stimulated insulin secretion [11]. Based on these studies, one may conclude that, when present, UCP2 has uncoupling properties proportional to its level and/or that an activator is produced that induces its activity.

Other studies, however, do not support this observation: β-cell-specific overexpression of UCP2 in transgenic mice

neither modified basal nor glucose-stimulated insulin secretion or changes in ATP/ADP ratio [12]. INS-1 cells with a doxycycline-inducible overexpression of UCP2 show no sign of uncoupling measured as change in mitochondrial membrane potential, ATP generation, or oxygen consumption, and display normal glucose-induced insulin secretion [12]. Reversing hyperglycemia in db/db mice restored normal glucose sensitivity in isolated islets without changes in the high level of UCP2 mRNA [13]. Similarly, rat islets cultured in a high glucose medium have a high mitochondrial metabolism and ATP production despite a 1.7-fold increase in UCP2 mRNA [14]. Other studies in the INS-1E cell line show that overexpression of PPARα and RXR results in a three- to fourfold increase in the level of UCP2 protein, without changes in the mitochondrial membrane potential and glucose-induced insulin secretion. The absence of uncoupling effect measured in the latter studies means either that UCP2 will be active only if the proper stimulant is added, or that UCP2 has no uncoupling activity, as suggested by studies in isolated mitochondria from other tissues expressing this protein, naturally or ectopically [15, 16].

These conflicting data reflect the lack of consensus on the physiological role of the “new” uncoupling proteins, UCP2 and UCP3, and the regulation of their activity (reviewed in [17–19]). The behavior of UCP1, the standard uncoupling protein, depends on the condition in which it is studied. In its cellular environment, UCP1 displays no uncoupling activity, due to inhibitory effect of the purine nucleotides present. Addition of fatty acids will activate the protein by relieving this inhibition, thereby dissociating respiration from ATP synthesis [20].

Based on the observation that: (1) in pancreatic β-cell, an increase in UCP2 is not always accompanied by decreased glucose-stimulated insulin secretion, (2) the proposed uncoupling activity of the “new UCPs” in particular is still debated, (3) UCP1, the original uncoupling protein, needs an activator to exhibit uncoupling properties; UCP2 was overexpressed in a pancreatic beta cell line and several indicators of mitochondrial coupling measured in the absence and presence of fatty acids in intact cells. Similar experiments were performed in the same cell line expressing UCP1 at comparable levels. The data show that, in the basal state, neither UCP2 overexpression nor UCP1 expression modified mitochondrial coupling. Addition of fatty acids to activate the protein resulted in a lowering of the mitochondrial membrane potential and acceleration of oxygen consumption in UCP1-expressing cells, but not in cells overexpressing UCP2. Taken together, the data demonstrate that in intact cells, UCP1, but not UCP2, can trigger uncoupling when activated by fatty acids.

Materials and methods

Establishment of inducible INS-1 stable cell lines

The parental INS-1 cell line expressing the reverse tetracycline-dependent transactivator (INS-1-r9 cells) was kindly provided by P. Iynedjian and H. Wang. The plasmid used for the second stable transfection was constructed by subcloning the human UCP1 cDNA isolated from human perirenal brown adipose tissue [21] into the expression vector pTRE2pur. INS-1r9 stable transfection, clone selection, and screening were as described in [22] except that the selection was performed with 1 µg/ml puromycin. Three positive clones were obtained and one of them was used in the present study. Establishment of the inducible cell line expressing hUCP2 has been described previously [12].

Cell culture

The INS-1 cells were cultured in RPMI 1640 medium containing 11 mM glucose supplemented with 5% heat-inactivated FCS, 10 mM HEPES, 1 mM pyruvate, 50 µM β-mercaptoethanol, 100 U/ml penicillin, and 100 µg/ml streptomycin as described in [12].

Cells were plated into 24-well plates or in 100-mm Petri dishes and cultured under standard conditions. After 1–2 days, 500 ng/ml doxycycline (Sigma Chemical, St. Louis, MO, USA) was added for 48 h to induce UCP1 or UCP2 expression. For the measurement of UCP1 and UCP2 half-lifes, cells in 100-mm Petri dishes were or not induced with 500 ng/ml doxycycline for 48 h, then the medium was removed and the cells incubated with fresh medium without doxycycline, and cycloheximide (10 µg/ml) added. Cells were collected at 0, 30, 60, 120, 180, and 360 min and mitochondria isolated and used for Western blotting.

Insulin secretion

Cells were washed, preincubated for 60 min in KRBH containing 2.8 mM glucose and 0.2% BSA, incubated for 30 min in the same medium with the additions mentioned, and insulin concentration determined in the medium. Total cellular insulin content, measured after cold acid/ethanol extraction, and insulin secretion were quantified by radioimmunoassay using rat insulin as standard [23]. DNA was measured as in [24].

ATP/ADP measurements

The cells were treated as for insulin secretion, except that, 10 min after glucose addition, the medium was rapidly removed and 0.75 ml of 0.6 M $HClO_4$ added to the cells.

The extract was centrifuged and an aliquot of the supernatant neutralized with 2.7 M K_2CO_3. ATP was assayed using luciferase. For ADP measurement, ATP was first hydrolyzed to AMP using ATP sulfurylase [25]. After inactivation of the sulfurylase, ADP was converted to ATP in the presence of pyruvate kinase and phosphoenolpyruvate and was measured by luciferase [1].

Mitochondrial membrane potential ($\Delta\Psi_m$)

Mitochondrial membrane potential was measured using rhodamine 123 (Molecular Probes, Eugene, OR, USA). Cells in 24-well plates were preincubated for 1 h in KRBH containing 2.8 mM glucose, loaded with 10 μg/ml rhodamine 123 for 20 min, washed, incubated in KRBH, and transferred to a thermostated plate reader (Fluostar Optima, BMG Labtechnologies, Offenburg, Germany). The $\Delta\psi_m$ was monitored with excitation and emission set of 485 and 525 nm, respectively. Glucose (12 mM final concentration), or BSA (0.12% final concentration), or BSA-oleate (0.1 mM final concentration) and CCCP 30 μM were added successively as described in [26].

DAPI and mitotracker staining

INS-1 cells were seeded on poly-L-ornithine-coated glass coverslip in cell culture medium and cultured 3 days. Mitochondria were then stained by incubation in RPMI medium containing 500 nM mitotracker orange (CMTMRos, Molecular Probes) for 30 min. Cells were then washed two times and fixed in 4% para-formaldehyde for 20 min at room temperature. Nuclei were then stained by incubation with 5 μg/ml DAPI for 3 min. Cells were visualized by fluorescence microscopy (Axioskop 2 equipped with an Axiocam color CCD camera). Images were recorded and treated through the Axiovision software (Zeiss, Germany).

Western blotting

Mitochondria were prepared from cultured cells using the Mitochondria/Cytosol fractionation kit (BioVision Inc., Mountain View, CA, USA) according to the manufacturer's instructions. Proteins were measured using the Bio-Rad protein assay (Bio-Rad Laboratories GmbH, München, Germany), with BSA as standard.

Fifteen micrograms of mitochondrial proteins was subjected to electrophoresis on a 12% polyacrylamide gel, electrotransfered to Immobilon P membranes (Millipore Corporation, Bedford, MA, USA), blocked with 2.5% Top Block (VWR International AG, Luzern Switzerland) in phosphate-buffered saline, 0.1% Tween, and incubated overnight at 4°C in the same buffer containing polyclonal antibody to UCP1 or UCP2 (6528 and 6526 for UCP1 and UCP2 respectively, Santa Cruz Biotechnology, Santa Cruz, CA, USA). After washing, the membranes were incubated with a horseradish peroxidase-coupled IgG antibody (Amersham, UK) for 1 h at room temperature. Cytochrome oxidase and prohibitin were detected on the same membrane, using a monoclonal antibody against subunit IV of cytochrome oxidase (Molecular Probes Inc.) or prohibitin (Research Diagnostics Inc., Flanders, USA) and horseradish peroxidase-coupled anti-mouse IgG (Santa Cruz Biotechnology) as secondary antibody. The resolved bands were visualized by UptiLight HRP blot chemiluminescent substrate (Uptima, Interchim, Montluçon, France) and exposed to Hyperfilm™ ECL™ (Amersham, UK) for 1 to 10 min.

Statistical analysis

Results are presented as mean ± SE of the indicated number of independent experiments. Statistical analysis was performed using Student's t test. Differences were considered significant for $p<0.05$.

Results

Inducible expression of UCP1 and UCP2 in INS-1 cells

To compare the consequences of similar levels of UCP1 and UCP2 on mitochondrial properties, INS-1 cells with a doxycycline-inducible expression of UCP1 were generated and compared to cells with inducible overexpression of UCP2 generated as previously described [12]. Addition of 500 ng/ml doxycycline for 48 h resulted in an 11.3±1.2-fold (±SE, n=4) increase in UCP2 (Fig. 1a). A similar induction was used for UCP1. This resulted in comparable levels of UCP1 and UCP2 as quantified with known amounts of recombinant UCP1 and UCP2 (Fig. 1a,b). The estimated concentration was between 1 and 1.5 ng uncoupling protein/μg protein of crude mitochondrial preparation for both uncoupling proteins. The two uncoupling proteins were found exclusively in the mitochondria and not in the cytosolic fraction (data not shown). UCP1 antibody exhibited minimal cross reactivity with UCP2 as assessed by the very faint signal in lane 1 of Fig. 1b and c, representing mitochondria from non-induced cells, containing UCP2. UCP2 antibody did not show any signal when tested with recombinant UCP1 (data not shown). Expression of UCP1 did not significantly modify the level of UCP2 (Fig. 1c). Induction of UCP2 or UCP1 did not induce changes in amount of mitochondria, as indicated by the constant level of two mitochondrial protein, cytochrome oxidase (Fig. 1a–c) and prohibitin (data not shown).

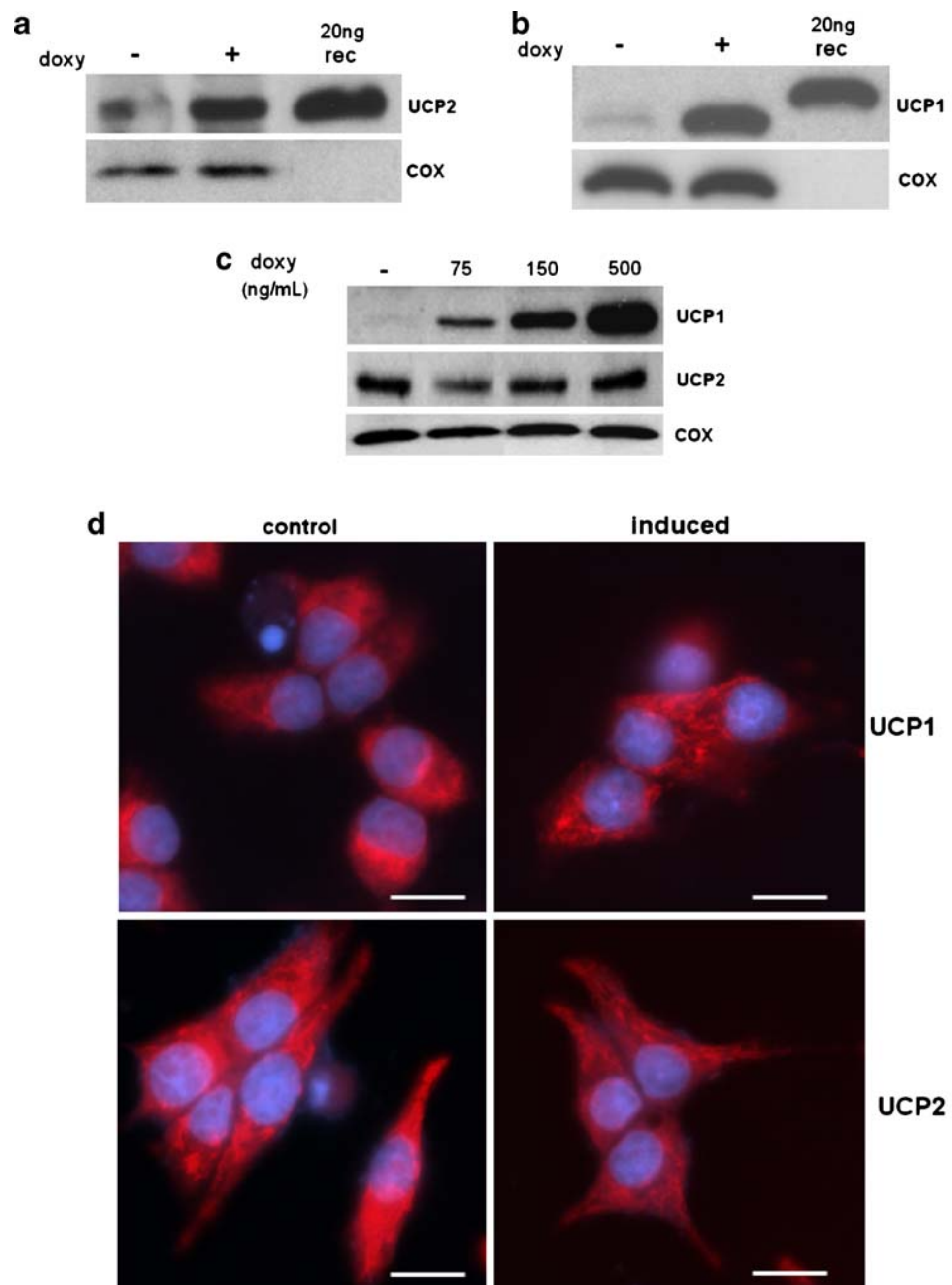

Fig. 1 UCP2 overexpression or UCP1 expression in INS cells. **a** Basal and doxycycline-induced (*doxy*) expression of UCP2 in INS-1 cells. For comparison, 20 ng of recombinant (*rec*) UCP2 are shown. **b** Doxycycline-induced (*doxy*) expression of UCP1 in INS-1 cells. For comparison, 20 ng of recombinant (*rec*) UCP1 are shown. **c** Doxycycline-induced expression of UCP1 does not alter UCP2 level. Cells were cultured and induced with different concentration of doxycycline. After 48 h, mitochondria were isolated, 15 μg mitochondrial protein separated by SDS-PAGE, and UCP2, UCP1, and cytochrome oxidase (*COX*) measured by Western blotting as described in the "Materials and methods" section. **d** UCP2 overexpression or UCP1 expression in INS-1 cells does not alter mitochondrial shape and distribution. Cells were cultured and induced with doxycycline to express UCP2 or UCP1 as described in the "Materials and methods" section. After 48 h induction, control and induced cells were loaded with mitotracker, fixed, nuclei colored with DAPI, and the cells visualized by fluorescence microscopy (Axioskop 2) at 100× magnification, *bar*=5 μm

The staining pattern of the mitochondria with mitotracker, a mitochondrial fluorescent marker, shows no visible difference between control and induced cells, indicating that neither UCP2 nor UCP1 modified mitochondrial shape or number (Fig. 1d). Compared to UCP1, UCP2 is known to have a very short half-life [27]. To examine whether this is also observed in INS-1 cells, the half-life of endogenous and endogenous plus overexpressed UCP2 as well as that of expressed UCP1 were measured after treatment with the translation inhibitor cycloheximide. Figure 2 shows that both endogenous and overexpressed UCP2 decrease rapidly after addition of 10 μg/ml cycloheximide, while the level of UCP1 remained stable over the 6 h of measurement. The half-life of endogenous and of overexpressed plus endogenous UCP2 was 94±3 (n=3) and 98±4 min (n=4), respectively.

Similar levels of UCP1 and UCP2 do not alter the basal and glucose-induced changes in insulin secretion, ATP/ADP ratio, or mitochondrial membrane potential (Fig. 3a–f). Glucose-induced hyperpolarization, represented by a downshift of the fluorescence, was similar in control and induced cells (Fig. 3e–f). To evaluate the possibility that UCP2 or

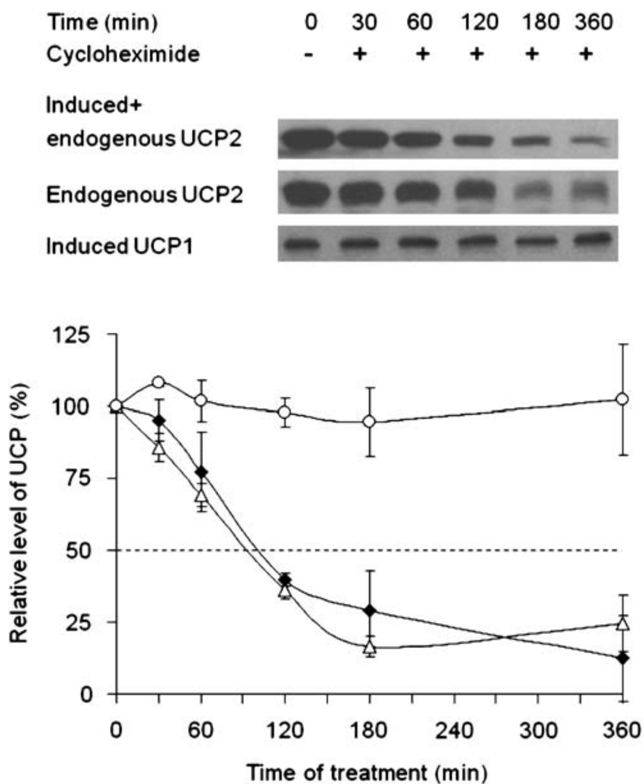

Fig. 2 Half-life of UCP2 and UCP1 in INS-1 cells. Control cells and cells overexpressing UCP2 or cells expressing UCP1 were treated with cycloheximide (10 μg/ml) for 0, 30, 60, 120, 180, and 360 min and UCP2 (*open triangle*, endogenous; *black diamond*, induced + endogenous) or UCP1 (*open circle*) levels determined by Western blotting and expressed as percent of initial values. Results are mean± SD of three to four independent experiments. *Inset* Representative Western blotting of endogenous, endogenous plus overexpressed UCP2, and of induced UCP1. To see all the spots of endogenous UCP2, the blot had to be exposed for 10 min instead of 2 min for the other data

UCP1 could induce small changes in the basal mitochondrial potential, an index of mitochondrial polarization was calculated and expressed as the ratio $(F_{max} - F_{basal})/F_{basal}$ [28] and data from three to four independent experiments pooled. Neither UCP2-overexpressing cells nor UCP1-expressing cells display any significant change of this ratio (Fig. 4g–h).

Taken together, these data indicate that neither UCP1 nor UCP2 triggers any sign of innate uncoupling in INS-1 cells.

Fatty acids induce uncoupling in cells expressing UCP1 but not UCP2

At a cellular level, UCPs are not uncoupling unless activated by fatty acids and/or reactive oxygen species (ROS) [17, 20]. For this reason, oxygen consumption and mitochondrial membrane potential were measured in cells with similar levels of UCP2 or UCP1 before and after addition of 0.1 mM oleate bound to 0.12% BSA (molar ratio BSA/oleate=5.0). These experiments were performed at 12 mM glucose, a concentration at which fatty acid oxidation is minimal and similar in control and induced cells (data not shown). Absolute values of oxygen consumption were not altered by the overexpression of UCP2: 0.182±0.016 and 0.190±0.018 nmol O_2/min×μg DNA (n=4) for control and induced cells, respectively. Figure 4a shows that oxygen consumption is unchanged after BSA addition and increased by 40% ($p<0.05$) after addition of oleate to control cells. This increase was unchanged by UCP2 induction. In contrast, a specific stimulation of respiration was measured in UCP1-expressing cells after oleate addition (Fig. 4b). As for UCP2, absolute values of basal oxygen consumption were not altered by the expression of UCP1: 0.189±0.009 and 0.182±0.023 nmol O_2/min×μg DNA (n=4) for control and UCP1-expressing cells, respectively. In agreement with the fatty acid-induced changes in oxygen consumption, addition of oleate produced a slight decrease in the mitochondrial membrane potential that was unaltered by UCP2 overexpression (Fig. 4c), while this decrease was amplified by the presence of UCP1 (Fig. 4d).

Discussion

The results of the present study clearly show that, when expressed at similar levels in pancreatic INS-1 cells, neither UCP1 nor UCP2 affect basal mitochondrial metabolism as indicated by the lack of effect on cellular oxygen consumption, ATP/ADP ratio, mitochondrial membrane potential, and indirectly by similar glucose-stimulated insulin secretion. Upon addition of fatty acids, only the cells with UCP1 show signs of uncoupling as evidenced by increased oxygen consumption and decreased mitochondrial membrane potential. Cells expressing UCP2 at comparable levels do not respond to the addition of the fatty acid.

Previous studies have shown that the functional properties of UCP1 described in brown adipose tissue mitochondria in response to fatty acids [29] are also observed when the protein is expressed in other cell types like skeletal muscle cells [30] or CHO cells [31]. The present study shows that this is also the case when UCP1 is expressed in the pancreatic β-cell line INS-1.

One of the main criticisms addressed to experiments with overexpression of UCPs is a non-specific uncoupling due to an artifact of supraphysiological levels of the protein. This artifact results in an activity of the protein in the basal state (i.e., without activation by fatty acids) that is not inhibited by purine nucleotides when studied in isolated

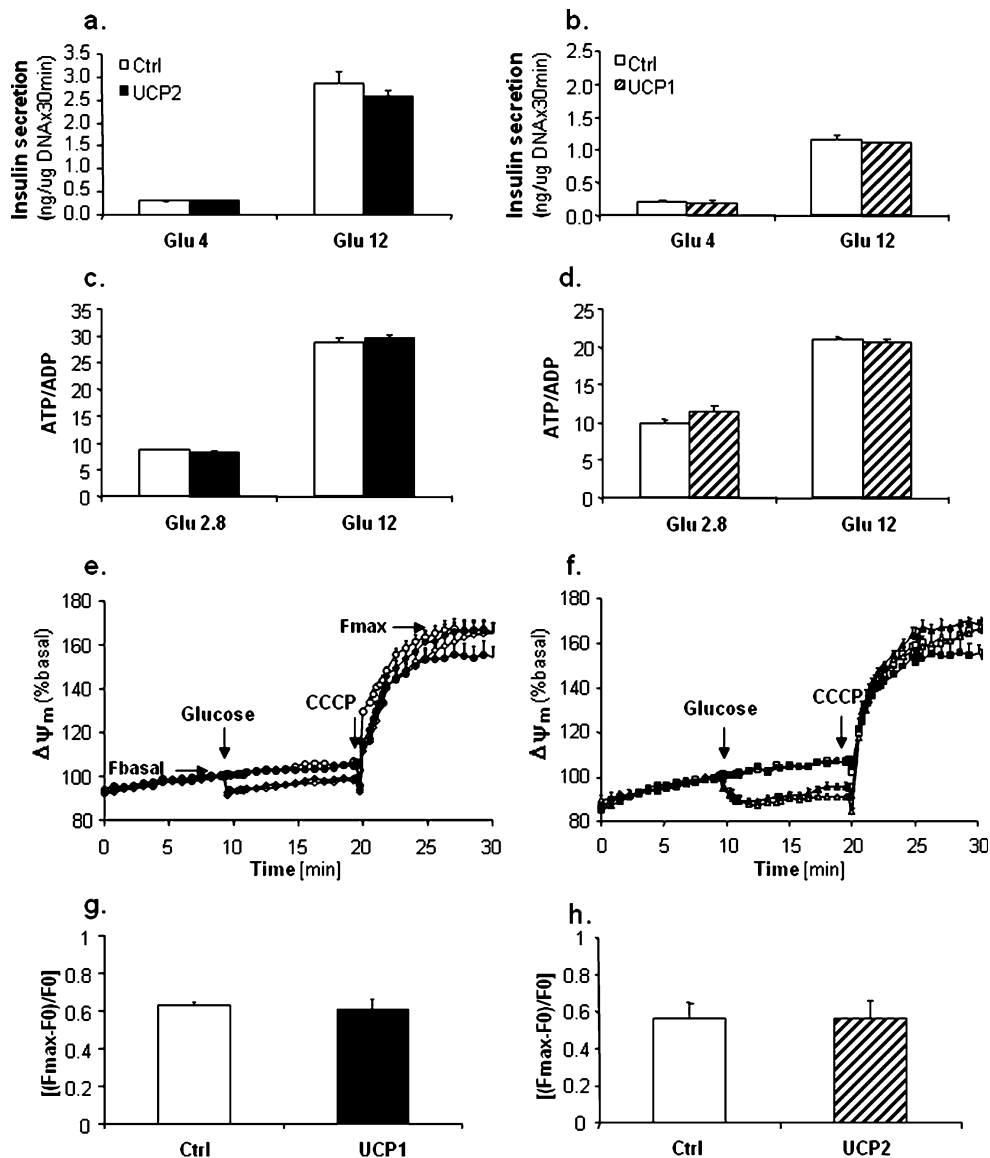

Fig. 3 UCP2 overexpression or UCP1 expression in INS-1 cells does not affect glucose-induced changes in insulin secretion, ATP/ADP ratio, or mitochondrial membrane potential. **a** Insulin secretion in response to glucose in control and UCP2-overexpressing cells. **b** Insulin secretion in response to glucose in control and UCP1-expressing cells. **c** Basal and glucose-induced changes in ATP/ADP ratio in control and UCP2-overexpressing cells. **d** Basal and glucose-induced changes in ATP/ADP ratio and in control and UCP1-expressing cells. All the results are mean±SE of four to six independent experiments performed as described in the "Materials and methods" section. **e** Mitochondrial membrane potential ($\Delta\Psi_m$) at 2.8 mmol/l glucose (F_{basal}) and 12 mmol/l glucose and after maximal depolarization (F_{max}) with 10 μmol/l CCCP in control cells and in cells overexpressing UCP2. **f** Mitochondrial membrane potential ($\Delta\Psi_m$) at 2.8 mmol/l glucose (F_{basal}), after addition of glucose to 12 mmol/l final concentration, and at maximal depolarization (F_{max}) with 10 μmol/l CCCP in control cells and in cells expressing UCP1. **g** Index of mitochondrial polarization as measured by the ratio ($F_{max}-F_{basal}$)/F_{basal} in control and UCP2-overexpressing cells. **h** Index of mitochondrial polarization as measured by the ratio ($F_{max}-F_{basal}$)/F_{basal} in control and UCP1-expressing cells. For **g** and **h**, results are mean±SE of quadruplicate measurements from three independent experiments performed as described in the "Materials and methods" section. *White column* and *open circle* control cells; *black column* and *black diamond* UCP2-overexpressing cells; *hatched column* and *black triangle* UCP1-overexpressing cells

mitochondria [32]. In the present study, the lack of uncoupling in the basal conditions, indicated by similar mitochondrial membrane potential, oxygen consumption, and ATP/ADP ratio is an indirect indication that the insertion of UCP1 and UCP2 in the mitochondrial membrane is not causing nonspecific proton leaks. Indeed, mammalian UCP2 expressed in yeast at a level comparable to that reached in our study did not result in basal or fatty

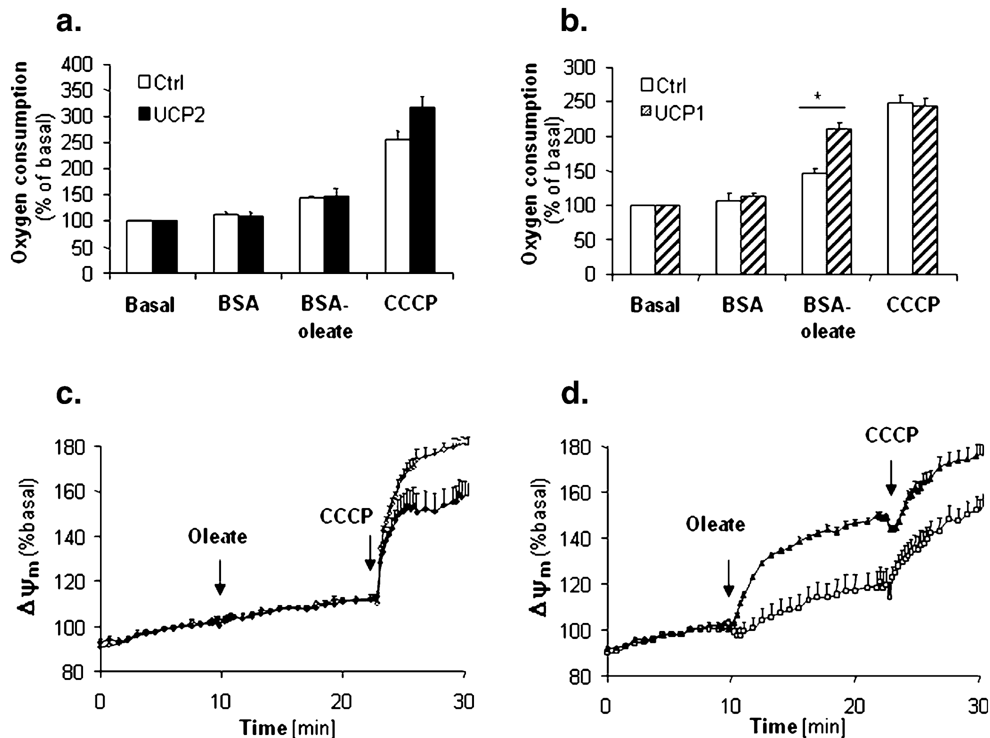

Fig. 4 Fatty acids induce uncoupling in cells expressing UCP1 but not UCP2. **a** Effects of BSA, BSA-oleate, or CCCP on oxygen consumption in control cells and in cells overexpressing UCP2. **b** Effects of BSA, BSA-oleate, or CCCP on oxygen consumption in control cells and in cells expressing UCP1. **c** Effect of oleate on mitochondrial membrane potential ($\Delta\Psi_m$) in control (*white diamonds*) and UCP2-overexpressing cells (*black diamond*). **d** Effect of oleate on mitochondrial membrane potential ($\Delta\Psi_m$) in control (*white square*) and UCP1-overexpressing cells (*black triangle*). For **a** and **b**, cells were cultured and doxycycline added as described in the "Materials and methods" section. After 48-h induction, cells were detached and oxygen consumption measured in the thermostated cuvette of a Clark-type electrode. Basal oxygen consumption was measured at 12 mmol/l glucose then BSA (final concentration 0.12%) or BSA-oleate (final concentration 0.1 mM) were added, followed by 10 µM CCCP. Results are mean±SE of at least four independent measurements. *$p<0.05$ between control and UCP1-expressing cells. For **c** and **d**, cells were cultured and doxycycline added as described in the "Materials and methods" section. After 48-h induction, cells were loaded with rhodamine 123 and mitochondrial potential measured before and after oleate and CCCP addition. Results are mean±SE of quadruplicate measurements of one representative experiment. *$p<0.05$ between control and UCP1-expressing cells

acid-induced uncoupling [16]. Similarly, no basal or stimulated uncoupling was measured in UCP2-containing mitochondria of spleen or lung, the two organs expressing UCP2 protein at the highest level [15]. However, a proton leak proportional to the overexpression of UCP2 was measured in mitochondria isolated from INS-1 cells [33]. The difference between the present study and that of Fink et al. [33] could be explained either by higher levels of UCP2 or by the measurements in isolated mitochondria instead of whole cells. Our results also diverge from those of Affourtit and Brand [34]. These authors showed that a knock-down of UCP2 in INS-1E cells recouples the mitochondria and improves glucose-induced insulin secretion. Additional indirect indication for similar behavior of endogenous and overexpressed protein is provided by the equally short half-life of UCP2 measured in mitochondria from noninduced and induced cells. A short half-life of the protein has been first described in lung, spleen and intestine, and in UCP2 expressed in yeast [27]. Similarly, the longer half-life of UCP1 found in brown adipocytes [35] is also measured when this protein is expressed in INS-1 cells.

It is now generally accepted that, within a cell, any uncoupling protein needs an activator to increase proton conductance; this activator can be fatty acids [36], reactive oxygen species [37], or still undetermined molecules. Fatty acids are known to act as weak uncouplers of mitochondrial respiration in several cell types including hepatocytes which neither express UCP1 nor UCP2 (reviewed in [38]). A similar UCP-independent uncoupling effect in pancreatic beta cells could explain the small effect of oleate on the mitochondrial membrane potential and on oxygen consumption in noninduced cells. Alternatively, but less likely, the effect could suggest that the low basal level of UCP2 is sufficient to trigger a maximal uncoupling effect of the protein. The lack of fatty acid-induced uncoupling in cells with increased UCP2 levels is in agreement with studies using UCP chimera expressed in yeast and tested for their response to fatty acids. Yeast mitochondria, with

UCP1 recombinantly expressed, display a high sensitivity to uncoupling induced by fatty acids, which is not observed in mitochondria with UCP2 expression [39]. Using protein chimera, this group showed that the introduction of the central matrix loop of UCP1 into UCP2 is necessary and sufficient to confer fatty acid-induced uncoupling. It should however be mentioned that, when inserted in lipid bilayers, UCP1 and UCP2 display similar fatty acid-induced proton conductance [40]. Such discrepant conclusions obtained from different experimental settings highlight the need to also study the uncoupling proteins in their cellular environment.

The role of UCP2, its mechanism of action and regulation, as that of the other novel uncoupling proteins, is still under study and contradictory findings reported. The assumption that UCP2 and UCP3 act on proton leak is challenged by several studies (reviewed in [17]) due to the properties of the proteins and/or to their very low degree of expression [15, 16, 19, 41]. Other functions have been proposed, related or not to their putative uncoupling function and very low level of expression. In particular, UCP2 could play a role in the control of mitochondrial ROS production [37, 42] and cell viability [43, 44] as an essential component of mitochondrial Ca^{2+} uptake [45] or as a determinant of substrate utilization by the mitochondria [46]. In a previous study, we showed that when cells with increased UCP2 are exposed to cytokines, they produce less ROS [12] and less nitric oxide (NO) [47, and Produit-Zengaffinen (manuscript in preparation)]. The exact mechanism leading to changes in ROS and NO generation is not yet determined, but these observations can be related to the increased level of iNOS and NO production in macrophages of UCP2 KO mice [48, 49] and decreased LPS-induced iNOS expression in the macrophage cell line RAW264 with UCP2 overexpression [50].

In summary, the present study demonstrates that an increased level of UCP2 in pancreatic β-cells does not induce any changes in basal and fatty acid-stimulated mitochondrial function. When expressed at similar levels in the same cell line, UCP1 displays the expected fatty acid-induced uncoupling. While our data do not confirm a role of UCP2 in the regulation of mitochondrial membrane potential, our previous data provide evidences that UCP2 may decrease ROS production [12]. These data, together with the short half-life of the protein and the translational regulation of its expression [51, 52], suggest an important function for this protein in pancreatic β cells.

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