J. Physiol. Biochem., **61** (2), 389-394, 2005

Ectopic UCP1 gene expression in HepG2 cells affects ATP production

P. González-Muniesa, F. I. Milagro, J. Campión and J. A. Martínez

Department of Physiology and Nutrition, University of Navarra, 31008 Pamplona, Spain

(Received on November 2, 2004)

P. GONZÁLEZ-MUNIESA, F. I. MILAGRO, J. CAMPIÓN and J. A. MARTÍNEZ. *Ectopic UCP1 gene expression in HepG2 cells affects ATP production.* J. Physiol. Biochem., **61** (2), 389-394, 2005.

The UCP1 is an uncoupling protein located in the inner mitochondrial membrane of brown adipocytes, which has a well-documented role in diet-induced thermogenesis. The current study assessed whether UCP1 transfected liver cells demand more fuel substrates in the oxidative phosphorylation processes. Therefore, the purpose of this experiment was to achieve an ectopic expression of UCP1 in HepG2 cells to significantly decrease the production of ATP. The UCP1 gene was transferred into the hepatic cells by using a calcium phosphate precipitation protocol. The efficiency of the transfection was tested, 48 hours later, by bioluminescence of luciferase previously transfected, while the expression of mRNA of UCP1 was demonstrated by RT-PCR. In addition, measuring the production of ATP by using a bioluminescence procedure assessed the functionality of this protein. Transfected liver cells with UCP1 showed a decrease of 23% in ATP production in comparison with control cells without expression of UCP1 (2.23 vs. 2.90 RLU/pg protein, p=0.015). In conclusion, the ectopic expression of UCP1 decreased the production of ATP, possibly uncoupling the oxidative phosphorylation, which could be a novel approach for understanding thermogenic processes and eventually for energy metabolism and body weight management.

Keywords: UCP1, ATP, HepG2 cells, Gene therapy, Obesity.

Obesity is a common metabolic disorder that can be defined as an excessive accumulation of body fat (19). The increasing worlwide prevalence of obesity is a major public health problem, since it has risen to epidemic levels in the U.S. and other high and low income countries. The increasing rates of obesity prevalence are causing devastating and costly health problems and reducing life expectancy by constituting a risk factor in a number of

Correspondence to J. A. Martínez (Tel.: 948 425 600 ext. 6424; Fax: 948 425 649; e-mail: jalfmtz@unav.es).

chronic diseases as type 2 diabetes or metabolic syndrome (2).

In recent years, a great number of molecules participating in energy homeostasis and fuel metabolism have been described (1), making some of them putative and suitable tools for gene therapy (11). Gene therapy can be broadly defined as the transfer of defined genetic material (DNA sequences encoding specific genes) to specific target cells for the ultimate purpose of preventing, treating or curing a particular dysfunction in a diseased state. Thus, gene transfer is a potentially useful strategy for the treatment of different illnesses that have a genetic component, and obesity may be one of these targets. Therapeutic genes can be introduced into target cells in a variety of ways: viral vectors, synthetic vectors or even naked DNA (3).

In this context, the family of mitochondrial uncoupling proteins (UCPs) has been extensively related with energy metabolism control in recent years (5) and especially with obesity (6, 14). Although the function of different UCPs is still under discussion, the most important component of UCPs family, and the most studied one, is UCP1 (7). This family of proteins is important for the inhibition of reactive oxygen species (ROS) formation or for the prevention of atherosclerosis and inflammation (7). And, even though the proteins UCP2, UCP3, UCP4 and UCP5 can provide a mild uncoupling activity (7), the uncoupling protein 1 (UCP1), an inner mitochondrial membrane protein specific of brown adipose tissue, seems to be the only physiologically relevant UCP for cold- and diet-induced thermogenesis (12). Indeed, UCP1 catalyses thermogenic net transfer of protons from the mitochondrial intermembrane space to the matrix (15, 16), thus uncoupling mitochondrial respiration from ATP synthesis, and making an important contribution to resting energy expenditure and body weight regulation in small mammals (9). Thus, some studies of UCP1 (9) and UCP2 gene transfer (10) have showed a relationship between these proteins and the bioenergetic processes in the mitochondria.

The aim of the current study was to assess whether UCP1 transfected liver cells would require more fuel substrates in order to produce the same amount of ATP. Therefore, an assay was devised to achieve an ectopic expression of UCP1 in HepG2 cells by using a non-viral *in vitro* gene transfer (17, 18) in order to significantly increase energy expenditure.

Material and Methods

Cell culture.– HepG2 human hepatoblastoma cells were cultured as monolayer in Dulbecco´s modified Eagle´s medium (DMEM) with Glutamax, supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% (v/v) of penicillin-streptomycin (10000 units/ml) provided by commercial company (Gibco BRL Life Technologies). Cells were housed in a humidified incubator at 37 °C with 5% CO_2, 95% air.

Plasmid and transient transfection.– A plasmid (pXAV2) containing the UCP1 gene under the control of the CMV promoter or an empty plasmid as control (pXAV2) were transferred into the hepatic cells (Hep G2 at 75% of confluence) by using a Calcium phosphate precipitation protocol (17). We have compared ten wells with UCP1 transfected cells (UCP1 cells) versus eight wells with empty plasmid transfected cells (control cells).

Measurements.– The efficiency of the transfection was tested by luciferase bio-

luminescence (Luciferase Assay System; Promega E1500) previously transfected in the same kind of cell line under similar conditions.

RNA was extracted according to a standardised procedure with Trizol using the instructions of the supplier (Life Technologies, Inc). The expression of UCP1 mRNA was demonstrated by RT-PCR using the following primers: 5´-CTCACCTTTGAGCTCCTC-3´(sense) and 5´-CTGATTTGCCTCTGGATG-3´(antisense).

ATP production was measured using a bioluminescence procedure (Enliten rLuciferase/Luciferin reagent; PROMEGA FF2021) assessed the functionality of this protein. Protein has been quantified by piragolol method with a Cobas Mira Autoanalizer (Roche; Geneva).

Statistical analysis.– Differences between UCP1 cells and Control cells were established by the t-Student test taking the $p < 0.05$ level as statistically significant, with a SPSS (versión 11.0) software package (Missouri; USA).

Results

The results of bioluminescence 48 hours after the transfection of different levels of the luciferase gene into HepG2 cells by the calcium phosphate precipitation protocol showed good transfection efficiency (Fig. 1).

UCP1 mRNA expression was analysed in four UCP1 transfected cell cultures and two control cell cultures. Whereas UCP1 mRNA was not found in control cells, in UCP1-transfected cells mRNA expression was detected by RT-PCR, as well as in brown adipose tissue, which was used as positive control (Fig. 2).

The data of ATP production (Fig. 3) are expressed in Relative light units (RLU)/pg of protein, which are directly related to ATP levels. We measured the ATP production 48 hours after the transfection and we observed that UCP1-transfected cells (n=10) showed a decrease of 23% in ATP production in comparison with control cells (n=8), not expressing UCP1.

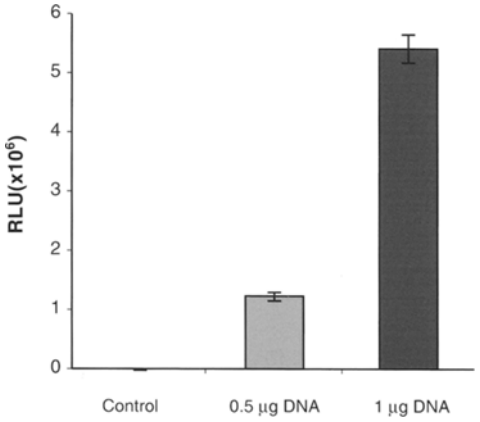

Fig. 1. *Transfection efficiency in HepG2 cells measured by luciferase activity expressed as RLU* (relative light units).

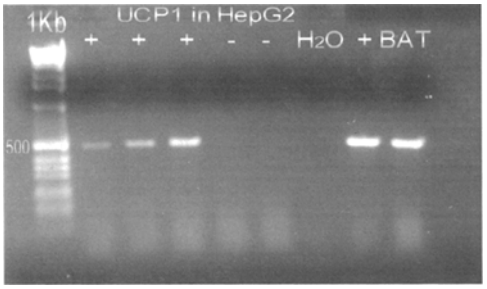

Fig. 2. *UCP1 mRNA expression by RT-PCR in control cells (-) and UCP1 cells (+).*
Brown adipose tissue (BAT) was considered as positive control and MilliQ autoclaved water (H_2O) as negative control.

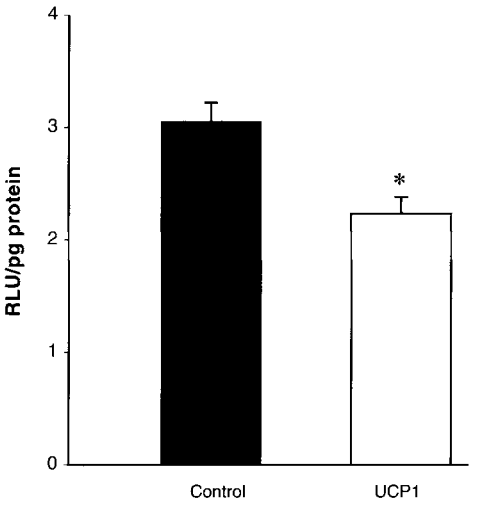

Fig. 3. *Decrease of ATP production in cells transfected with UCP1 expressed as RLU / picogram of protein.* *p < 0.02.

Discussion

The current experimental work reports the successful expression of UCP1 in HepG2 cells after using a Calcium phosphate precipitation protocol and the subsequent changes in bioenergetic properties of isolated hepatic mitochondria.

As expected, our data suggest that calcium phosphate precipitation is a suitable method to transiently express UCP1 or any other gene into HepG2 cells, as previous studies had demonstrated in other different cell lines (17, 18). Our data concerning this cell line let us to conclude that the ectopic expression of UCP1 in these UCP1-lacking cells significantly diminishes ATP production. This reduction in ATP levels, due to the UCP1-mediated uncoupling of mitochondrial oxidative phosphorylation, could appear as a consequence of the increased mitochondrial proton leak affecting the efficiency of cell respiration.

Although nonshivering thermogenesis is a well-defined mechanism that contributes to resting energy expenditure in small mammals, its role in large mammals such as human beings appears to be of relatively low importance at least in the adult (8). The major reason resides in the scarcity of brown adipose tissue, and consequently of UCP1 protein, in adult humans (15). However, as our data suggest, gene transfer techniques leading to expression of the UCP1 protein in tissues could contribute to impaired cell energy efficiency and a subsequent triggering of the metabolite oxidation in order to increase mitochondrial ATP synthesis for maintaining cell metabolism.

In this sense, hepatic cells, as HepG2, which constitutionally lack UCP1 expression, are metabolically very active and have a large amount of mitochondrias. Furthermore, liver is one of the most irrigated organs of the animals, including humans, suggesting that it could be a very interesting target for metabolism-focused gene therapy. Additionally, as we have proved, HepG2 cells are well suited for non-viral gene transfer like calcium phosphate precipitation technique (18). Therefore, liver appears to be a suitable target for achieving a successful outcome in stimulating thermogenesis and nutrient oxidation by gene transfer using this experimental strategy.

In summary, the current approach could be very useful for future experiments with animals and even could be an innovative tool for understanding thermogenic processes and eventually for a future management of fuel bioenergetics as well as in the metabolic disorder management in diseases with a genetic basis.

Acknowledgements

The pXAV2 plasmid and Hep G2 cells were kindly provided by Dr. Novo (Department of

Genetics, University of Navarra) and Dr. Tros de Ilarduya (Department of Galenics, University of Navarra), respectively. This work was financially supported by Special Line of Investigation: Nutrition and Obesity (University of Navarra). P. González-Muniesa holds a predoctoral grant from Danone Institute. The technical assistance of V. Ciaúrriz and A. Lorente is gratefully acknowledged.

P. GONZÁLEZ-MUNIESA, F. I. MILAGRO, J. CAMPIÓN y J. A. MARTÍNEZ. *La expresión ectópica del gen de la UCP1 en células HepG2 afecta a la producción de ATP.* J. Physiol. Biochem., **61** (2), 389-394, 2005.

La UCP1 es una proteína desacoplante localizada en la membrana interna mitocondrial del adipocito pardo, que tiene un papel importante en la termogénesis inducida por la dieta. El presente estudio fue diseñado para verificar si las células hepáticas transfectadas con el gen de la proteína UCP1 utilizan más substratos energéticos en los procesos de fosforilación oxidativa. Así, el propósito del experimento era lograr una expresión ectópica de la proteína UCP1 en células HepG2 para disminuir significativamente la producción de ATP. El gen de la UCP1 fue transferido a las células hepáticas usando un protocolo de precipitación del fosfato cálcico. La eficiencia de la transfección se demostraba 48 horas más tarde por bioluminiscencia de la luciferasa previamente transfectada, y la expresión del RNA mensajero de la UCP1, por RT-PCR. Además, la funcionalidad de la proteína se comprobaba mediante la medida de la producción de ATP usando un procedimiento de bioluminiscencia. Los resultados demuestran un descenso en la producción de ATP del 23% en las células hepáticas transfectadas con UCP1 respecto de las células control (2,23 frente a 2,90 RLU/pg proteína, p=0,015). En suma, la expresión ectópica de la UCP1 redujo la producción de ATP, posiblemente por desacoplamiento de la fosforilación oxidativa, lo cuál puede ser de interés para estudiar la importancia de los procesos termogénicos implicados en la regulación del metabolismo energético y el peso corporal.

Palabras clave: UCP1, ATP, Células HepG2, Terapia génica, Obesidad.

References

1. Bays, H. E. (2004): *Obes. Res.*, **12**, 1197-1211.
2. Bray, G. A. and Champagne, C. M. (2004): *J. Am. Diet. Assoc.* **104**, 86-89.
3. Campión, J., Milagro, F. I. and Martínez, J. A. (2004): *Nutr. Rev.* **62**, 321-330.
4. Cheng, S. H. and Smith, A. E. (2003): *Gene Ther.* **10**, 1275-1281.
5. Erlanson-Albertsson, C. (2003): *Acta Physiol. Scand.* **178**, 405-412.
6. Giacobino, J. P. (2002): *Ann. N. Y. Acad. Sci.* **967**, 398-402.
7. Jezek, P., Zackova, M., Ruzicka, M., Skobisova, E. and Jaburek, M. (2004): *Physiol. Res.* **53** Suppl. 1, S199-211.
8. Klingenspor, M. (2003): Exp Physiol **88**, 141-148.
9. Larrarte, E., Margareto, J., Novo, F. J., Marti, A. and Martínez, J. A. (2002): *Arch. Biochem. Biophys.* **404**, 166-171.
10. Marti, A., Larrarte, E., Novo, F. J., García, M. and Martínez, J. A. (2001): *Int. J. Obes. Relat. Metab. Disord.* **25**, 68-74.
11. Marti, A., Novo, F. J. and Martínez, J. A. (1999): *J. Physiol. Biochem.* **55**, 98A.
12. Nedergaard, J. and Cannon, B. (2003): *Exp. Physiol.* **88**, 65-84.
13. Palou, A., Pico, C., Bonet, M. L. and Oliver, P. (1998): Int. J. Biochem. Cell Biol. **30**, 7-11
14. Rodríguez, A. M. and Palou, A. (2004): *Int. J. Obes. Relat. Metab. Disord.* **28**, 500-502.
15. Rolfe, D. F. and Brown, G. C. (1997): *Physiol. Rev.* **77**, 731-758.
16. Rolfe, D. F., Newman, J. M., Buckingham, J. A., Clark, M. G. and Brand, M. D. (1999): *Am. J. Physiol.* **276**, C692-699.
17. Roy, I., Mitra, S., Maitra, A. and Mozumdar, S. (2003): *Int. J. Pharm.* **250**, 25-33.
18. Tros de Ilarduya, C., Arangoa, M. A., Moreno-Aliaga, M. J. and Duzgunes, N. (2002): *Biochim. Biophys. Acta* **1561**, 209-221.
19. WHO/OMS Consultation group (2000): *World Health Organ. Tech. Rep. Ser.* **894**, 1-253.