UCP1 mRNA induction by RU486 in brown adipocytes is followed by marked induction of UCP1 protein levels

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Published online: 21 September 2007
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Keywords UCP1 · Thermogenesis · Obesity · RU486

Introduction

RU486 (mifepristone), a potent antagonist at progesterone and glucocorticoid receptors, is well known for its use in the termination of unwanted pregnancies, the potential development of oral contraceptives, treatment of certain cancers and other activities. Potentially, it could also play a role in thermogenesis and body weight control. RU486 acts on white adipose tissue and reverses hyperlipidic diet induced obesity in rats, or activates oxygen consumption and GDP-binding activity in brown adipose tissue (BAT), and it was suggested that BAT activation is mediated by sympathetic stimulation due to the RU486 effects on the central nervous system [1]. On the other hand, we showed, in a previous work, where RU486 was used as a tool for inhibiting progesterone effects, that it could have a possible direct effect, *in vitro*, on the uncoupling proteins expression in brown adipocytes [2]. Nevertheless, few studies have addressed the relationship between RU486 and brown adipose thermogenesis and they were focused mainly on the central and anti-glucocorticoid effects of RU486.

Brown adipose tissue is highly relevant in the regulation of energy balance in small mammals, as rodents, due to the fact that it is the principal mediator of adaptive thermogenesis, which is mainly mediated by the uncoupling

protein 1 (UCP1), an inner mitochondrial membrane protein which is able to catalyze the reentry of protons into the mitochondrial matrix, thus bypassing ATP synthase, uncoupling oxidative phosphorylation and producing heat. Thus, BAT can efficiently respond to environmental stimulus such as cold or an excessive caloric intake.

Objectives

We aimed to analyse the effects of RU486 on the expression of the main mediator of adaptive thermogenesis, the brown fat uncoupling protein UCP1, both at the mRNA and protein expression level.

Experimental design and methods

Murine brown adipocytes were differentiated in culture and were treated with RU486 (10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} and 10^{-4} M) in the presence or absence of Noradrenaline (NA) 10^{-7} M (NA induces UCP1 mRNA expression) (see Fig. 1). Before treating with RU486, the cells were placed in a serum-free medium to exclude the presence of progesterone or glucocorticoids, thus ensuring that the observed effects would be due to a direct action of RU486 and not to effects derived from the inhibition of progesterone or glucocorticoid actions. UCP1 mRNA levels were analysed by Northern blot and specific UCP1 protein levels were analysed by Western blot.

Results

RU486 enhanced noradrenalin-induced UCP1 mRNA expression in a dose-dependent manner, followed by a

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Fig. 1 Experimental design **Fig. 2** Stimulation by RU486 of UCP1 mRNA and protein expression under NA induction. Significant differences were tested by oneway ANOVA and least-significant difference (LSD) post hoc comparisons ($P < 0.05$): RU indicates RU486 effect, values not sharing a common letter are statistically different **Fig. 3** Representative Northern (a) and Western (b) blots of UCP1 and 18S mRNAs and UCP1 specific protein. The images correspond to the results from 24-h-RU486 plus 6-h-NE treated cells, except the last strip of protein, which is from non-NE treated cells (indicated as “without NE”)

delayed but marked increase in the UCP1 protein levels, much higher than for the mRNA levels (see Fig. 2); these protein levels were also induced in the absence of nor-adrenalin (see Fig. 3).

Conclusion

The results suggest that RU486 could have a direct role stimulating brown adipose tissue thermogenesis by increasing UCP1 levels and that this stimulation could be carried out at different levels of the UCP1 activation cascade

Acknowledgments Thanks to The European NutriGenomics Organisation (NuGO), The Network of Excellence on Nutrition and Genomics (Contract No. FP6-506360). This work was funded by grants from the Spanish Government: BF12003-04439, FIS 01/1379 and Red temática de investigación cooperativa G03/028.

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