Downregulation of uncoupling protein-3 is linked to changes in muscle mitochondrial energy metabolism in vivo as a result of capsiate administration

B. Faraut[1], B. Giannesini[1], V. Matarazzo[2], T. Marqueste[1], G. Rougon[2], P. Cozzone[1], and D. Bendahan[1]

[1]Centre de Résonance Magnétique Biologique et Médicale (CRMBM), UMR CNRS 6612, Marseille, France, [2]Institut de Biologie du Développement de Marseille Luminy, UMR CNRS 6216, Marseille, France

INTRODUCTION

The uncoupling protein-3 (UCP3) is a mitochondrial inner membrane protein which is predominantly expressed in skeletal muscle. Based on its closed sequence homology with the UCP1 protein which uncouples respiration from ATP production, UCP3 has been suggested to be involved in energy expenditure control but its role is still poorly understood. Considering the potential role of UCP3 in skeletal muscle energetics, we were interest in characterizing the physiological function of UCP3 in an integrative approach *in vivo*. We aimed in the present study at investigating energy metabolism, UCP3 gene expression and free fatty acid (FFA) metabolism changes occurring in exercising muscle as a result of capsiate treatment.

METHODS

Female Sprague-Dawley rats (n=38; 275-300 g) were orally administrated capsiate (100 mg.kg-1) or its vehicle used as control at t0 and analyses were performed at t0+2h. Magnetic resonance investigations were performed at 4.7 T (Brüker, Biospec Avance 47/30) with an experimental setup developed in our laboratory. This setup integrates a ^{31}P-magnetic resonance spectroscopy (31P-MRS) surface coil (10x14 mm), a 30-mm-diameter Helmholtz imaging coil and a system allowing transcutaneous stimulation of the gastrocnemius muscle. Force production was measured with an ergometer including a pedal and a pressure transducer. ^{31}P-MR spectra (28.5 s duration; 16 accumulations) were continuously acquired during 6 min at rest, 5.7 min during the stimulation period (isometric contractions electrically induced at 3 Hz) and during 16 min after the stimulation period. MR images (5-slices multiecho sequence; 8 echoes spaced 16 ms; acquisition time, 4.57 min) were recorded at rest and immediately after the 16 min recovery period. Absolute concentrations of phosphorylated compounds were expressed relative to [ATP] measured by HPLC. UCP3 gene expression was measured using quantitative real-time RT-PCR analysis and normalized to 18S rRNA used as an internal control. RT-PCR was carried out on an Applied Abi prism 7000 cycler (Applied Biosystem). Plasma free fatty acid level from transcardiac blood samples was quantified using an enzymatic colorimetric method (Roche).

RESULTS

Capsiate ingestion reduced 9.4 fold UCP3 gene expression in rat gastrocnemius muscle while mitochondrial ATP production and phosphocreatine level significantly increased at rest. During muscle stimulation of the capsiate-treated group, the rate of oxidative ATP production was larger while the phosphocreatine level was maintained higher than in the control group. Similarly, ATP concentration decreased slightly but in a lesser extent than in untreated rats while ADP accumulation was lower. The larger oxidative ATP production did not account for a proportional decrease of the anaerobic contribution to ATP production i.e. glycolysis and phosphocreatine breakdown. In addition, the mechanical performance was not affected by capsiate administration. Finally, we found that in capsiate-treated rats, plasma FFA level increased nearly twofold. Muscle stimulation in the control group did not modify FFA level but enhanced UCP3 mRNA expression suggesting that changes in FFA and UCP3 level might not be mechanistically correlated.

CONCLUSIONS

Overall, we have shown that capsiate administration induced a UCP3 downregulation coupled with an increased mitochondrial ATP synthesis whereas the muscle force-generating capacity was unchanged. This would suggest that additional non-contractile ATP-consuming mechanisms and/or a decrease in muscle efficiency result from UCP3 downregulation. These results also demonstrate the ability of noninvasive ^{31}P-MRS to study *in vivo* energy metabolism modifications resulting from chemically-induced alteration in gene expression.