A QUANTITATIVE STUDY OF BIOENERGETICS IN SKELETAL MUSCLE LACKING CARBONIC ANHYDRASE III BY ^{31}P MAGNETIC RESONANCE SPECTROSCOPY

M. Liu (UF, Physical Therapy); G.A. Walter (UF, Physiology); N. Pathare (UF, Physical Therapy); U-J. Zimmerman (UPENN, Physiology); R. E. Forster (UPENN, Physiology); K. Vandenborne (UF, Physical Therapy)

Introduction

Oxidative slow skeletal muscle contains carbonic anhydrase III in a concentration as high as 2% of wet weight, but its primary function remains in question. In order to determine if the lack of this enzyme handicaps energy metabolism and/or acid elimination under stress, we measured the intracellular pHi and energy phosphates by ^{31}P magnetic resonance spectroscopy (MRS) in hind limb muscles of wild type and CAIII knockout mice during and after ischemia and intense exercise (electrical stimulation).

Experimental

The MR measurements were performed inside an 11T/470 MHz spectrometer. Spectra were acquired using a 6-mm x 12-mm oblong phosphorus (190.5 MHz) surface coil, placed over the belly of the gastrocnemius muscles. Spectra were acquired with a 50 μs square pulse, a pulse repetition time of 2 sec, and data were averaged into 30 second bins. Phosphorus spectra were obtained at rest (5 min), during ischemia (30 min) or electrical stimulation (2 min), and the following recovery (30 min). Intracellular pH was calculated based on the chemical shift of Pi. The Pi and PCr concentrations were determined using area integration.

Results and Discussion

Thirty minutes of ischemia caused phosphocreatine (PCr) to fall and inorganic phosphate (Pi) to rise, while pH and ATP remained constant in both strains of mice. PCr and Pi kinetics during ischemia and recovery were not significantly different between the two genotypes. From this we conclude that under neutral pH conditions resting muscle anaerobic metabolism, the rate of the creatine kinase reaction, intracellular buffering of protons, and phosphorylation of creatine by mitochondrial oxygen metabolism are not influenced by the lack of CA III. Two minutes of intense stimulation of the mouse gastrocnemius caused PCr, ATP and pH_i to fall and ADP and Pi to rise and these changes, with the exception of ATP, were all significantly larger in the animals lacking CA III. The rate of return of pH_i and ADP to control values was the same in wild type and mutant mice, but in the mutants PCr and Pi recovery were delayed in the first minute after stimulation (Fig).

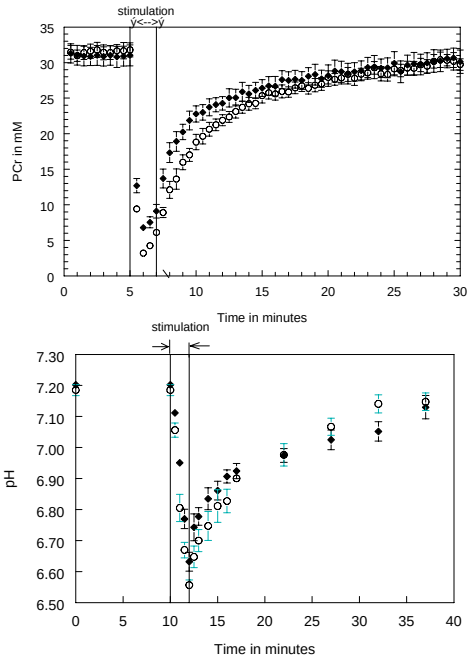

Fig: PCr and pH kinetics in CA III knockout and wild type mice following electrical stimulation.

Conclusions

Our quantitative study of muscle bioenergetics in CA III knockout mice showed a significant decrease in PCr recovery rate following electrical stimulation. We conclude that a lack of CA III impairs mitochondrial ATP *in vivo* synthesis.

Acknowledgements

This research was supported by NIH grant AR45394. The mutant mice were obtained from G. Kim and R. L. Levine at NIH. MR data were supported through the National High Magnetic Field Laboratory and obtained at the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility in the McKnight Brain institute of the University of Florida.