A quantitative study of bioenergetics in carbonic anhydrase III knockout mice by in vivo 31P magnetic resonance spectroscopy

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INTRODUCTION:

The purpose of this study was to provide *in vivo* experimental evidence for the biological importance of carbonic anhydrase (CA) III in energy metabolism of skeletal muscle. The CA gene family plays an important role in many physiological processes including renal acidification, respiration, and signal transduction. However, its role in skeletal muscle has not been well established. In order to assess quantitatively the importance of CA III in skeletal muscle, changes in phosphate metabolite concentrations and intracellular pH were measured at rest, during ischemia, and recovery.

METHODS:

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. A one turned standard ^{1}H coil was placed underneath the hindlimb to perform localized shimming. An inflatable blood pressure cuff was positioned around the animal's thigh. Spectra were acquired with a 50 µs square pulse, a pulse repetition time of 2 sec, and data were averaged into 30 second bins. The sweep width was 10,000 Hz and 8,000 complex data points were used. Phosphorus spectra were obtained at rest (10 min), ischemia (30 min), and recovery (30 min). Intracellular pH was calculated based on the chemical shift of Pi. The Pi and PCr concentrations were determined using area integration and assuming a resting ATP concentration of 8.2 mM. Recovery rates of PCr were calculated from the data points at the end of ischemia and in the first 5 min of recovery. At the termination of the NMR experiment, the gastrocnemius muscles were dissected, frozen in liquid nitrogen for subsequent biochemical analysis. *In vitro* force mechanics were also performed on the perfused extensor digitorum longus and soleus muscle to determine differences in tetanic force production and fatigue resistance.

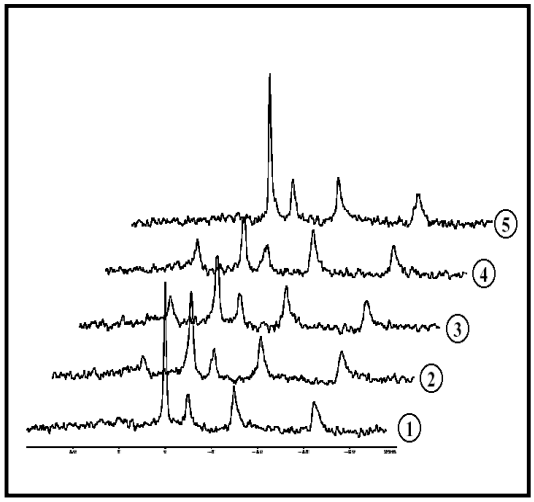

Figure 1: ^{31}P spectra obtained from a CA III knockout mouse at rest (1), after 10, 20, 30 min of ischemia (2-4) and after 10 min of recovery (5)

Table 1: Basal metabolite content and intercellular pH at rest

Variable	Group	
	CA III knockout mice	C57BL6
n	8	3
Pi (mM)	1.93±0.19	2.69±0.61
PCr (mM)	25.39±1.2	28.99±1.81
Pi/PCr	0.08±0.01	0.09±0.2
pH	7.19±0.01	7.13±0.02

RESULTS:

^{31}P spectral analysis on mutants (n=8) showed a PCr concentration of 25.39±1.2 mM and Pi concentration of 1.93±0.19 mM at rest. The resting intercellular pH was 7.19±0.01. During 30 minutes of ischemia, the PCr levels decreased by 45 to 55% while the pH remained relatively unchanged. Following ischemia, the PCr concentration returned to pre-ischemic levels within 5 minutes with a rate of 2.04±0.18 mM/min. Pi levels recovered concomitantly. Compared with control mice (C57BL6, n=3), there are no significant differences observed in either the resting, ischemia or recovery results. Initial analysis of the force mechanics data showed no apparent difference in muscle strength or fatigue resistance in CAIII knockout mice.

CONCLUSION:

This study supports the contention that CA III may not play an important role for the system to be functional under a mild ischemic stress condition. Our quantitative study of muscle bioenergetics in CA III knockout mice showed no abnormalities at rest, during ischemia or recovery. Future studies may require the investigation of CA III under more metabolically demanding conditions, such as exercise, to further elucidate the function of CA III in muscle energy metabolism.

ACKNOWLEDGEMENTS:

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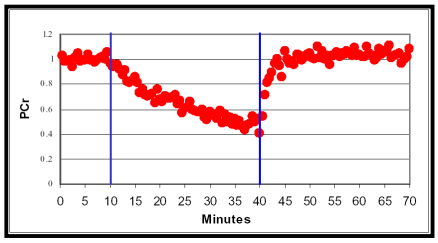

Figure 2: Representative PCr kinetics at rest, during ischemia and recovery from a CA III knockout mouse.

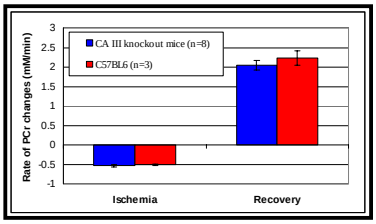

Figure 3: The rate of PCr changes during ischemia and recovery