An extra-mitochondrial domain rich in carbonic anhydrase activity improves myocardial energetics

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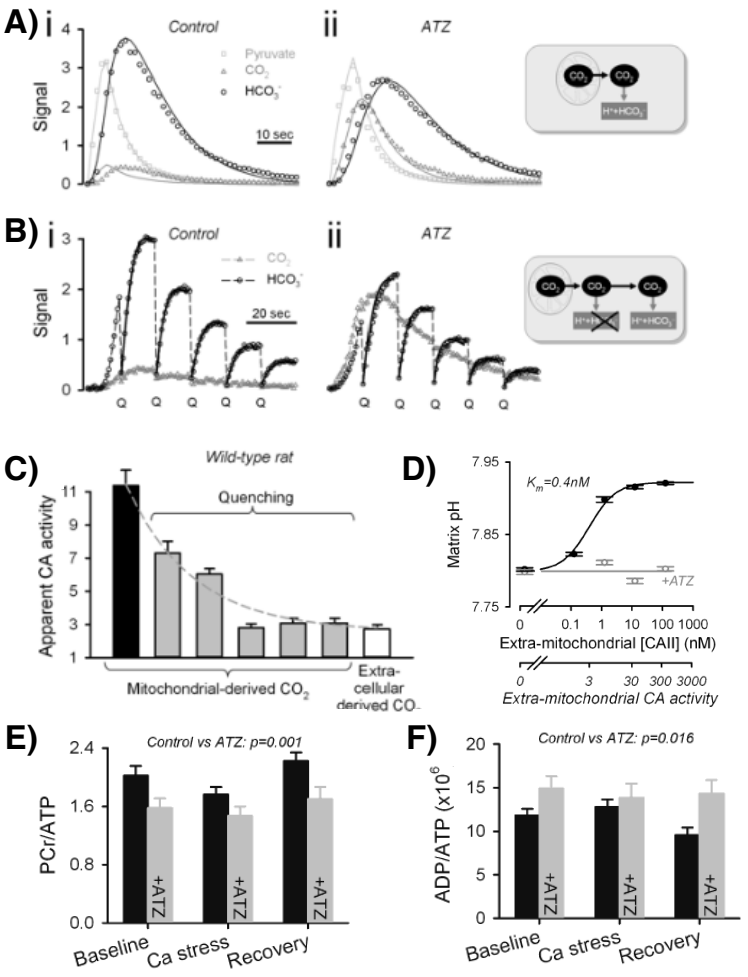
Introduction

CO_2 is produced in vast quantities by cardiac mitochondria and efficient means of its venting are required to support metabolism. A range of metabolic and physiological adaptations for improving energy provision has been identified[1], yet little is known about mechanisms for improving CO_2 venting. Carbonic anhydrases (CAs), expressed at various sites in ventricular cardiomyocytes, may affect mitochondrial CO_2 clearance by catalyzing CO_2 hydration (to H^+ and HCO_3^-) and changing trans-membrane $[CO_2]$-gradients for diffusion. In this study, we investigated the hypothesis that mitochondrial CO_2 venting is facilitated by concentrating CA activity near (but not within) mitochondria, and that this distribution improves myocardial energetics.

Methods and Results

Using fluorescent dyes to measure pH-changes arising from the intracellular hydration of CO_2 introduced from outside cells, overall CA activity in the cytoplasm of isolated ventricular myocytes was found to be modest (2.7-fold above spontaneous kinetics). Experiments on isolated ventricular mitochondria demonstrated negligible intra-mitochondrial CA activity. *In vivo* cardiac CA activity was also investigated by hyperpolarized ^{13}C magnetic resonance spectroscopy (MRS) from the rate of production of $H^{13}CO_3^-$ from $^{13}CO_2$, released by mitochondrial metabolism of hyperpolarized [1-^{13}C]pyruvate[4]. CA activity measured upon [1-^{13}C]pyruvate infusion was 4-fold higher than the cytoplasm-averaged value (11-fold above spontaneous kinetics, Fig. A). However, after the $^{13}CO_2$ resonance was repeatedly quenched with a saturation pulse to allow CO_2 to dissipate away from its mitochondrial source, the apparent CA activity decreased (Fig. B). A fluorescent CA-ligand co-localized with the mitochondrial marker TMRE, indicating that mitochondria are near a CA-rich domain. Based on immunoreactivity, this domain may comprise of CAXIV and, to a lesser extent CAII, which remained closely associated with purified mitochondria. Extra-mitochondrial CA activity raised matrix pH (~0.1 units; flow-cytometry of isolated mitochondria, Fig. D) and improved cardiac energetics indexed by increased phosphocreatine-to-ATP (PCr/ATP) ratio and decreased [ADP] (^{31}P MRS of intact hearts, Figs. E&F).



A) *(i)* Time course of ^{13}C-labelled pyruvate, CO_2 and HCO_3^- measured by MRS of rats infused with hyperpolarized [1-^{13}C]pyruvate (N=8). *(ii)* Experiment repeated on rats pre-treated with acetazolamide (ATZ, a CA inhibitor), 15 min before infusion of [1-^{13}C]pyruvate (N=6). Continuous traces show the best-fit model simulation of the data. **B)** Experimental protocol of A repeated, but with the $H^{13}CO_3^-$ signal quenched every 20 sec to measure $^{13}CO_2$ hydration rate as $^{13}CO_2$ diffused away from the mitochondria. **C)** Apparent CA activity was highest when measured near mitochondria. **D)** Relationship between mitochondrial matrix pH and extra-mitochondrial CA activity. Best-fit Hill plot (K_m=0.4 nM CAII). **E)** Cardiac energetics measured in Langendorff-perfused hearts using ^{31}P MRS under baseline conditions, during Ca-stress and upon recovery. ATZ reduced PCr/ATP ratio at baseline, and increased **(F)** ADP/ATP ratio.

Discussion

These data provide evidence for a functional domain of high CA activity around mitochondria that facilitates CO_2 venting, thus supporting the activity of the heart's mitochondria and improving energetics by means of streamlined waste removal. Aberrant CA activity or distribution may reduce the heart's energetic efficiency, an important finding as reduced PCr/ATP is characteristic of heart failure and correlates with the New York Heart Association classes of heart disease[5] and predicted prognosis[6]. Certain cardiac disease states involve altered CA expression levels[7,8], and the effect that this has on the state of their extra-mitochondrial CA-rich domain and energetics warrants further investigation.

References

1. Neubauer S (2007) N Engl J Med 356: 1140-1151.
2. Leem, Vaughan-Jones (1998) J Physiol (Pt 2): 471-485.
3. Dodgson et al. (1980) PNAS USA 77: 5562-5566.
4. Schroeder et al. (2008) PNAS USA 105: 12051-12056.
5. Neubauer et al. (1992) Circulation 86: 1810-1818.
6. Neubauer et al. (1997) Circulation 96: 2190-2196.
7. Vargas, Alvarez (2012) J Mol Cell Cardiol 52: 741-752.
8. Dzeja et al. (1999) Mol cell biochem 201: 33-40.

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