The Role of Cardiac Carbonic Anhydrases In Vivo: A Hyperpolarised ^{13}C MR Study

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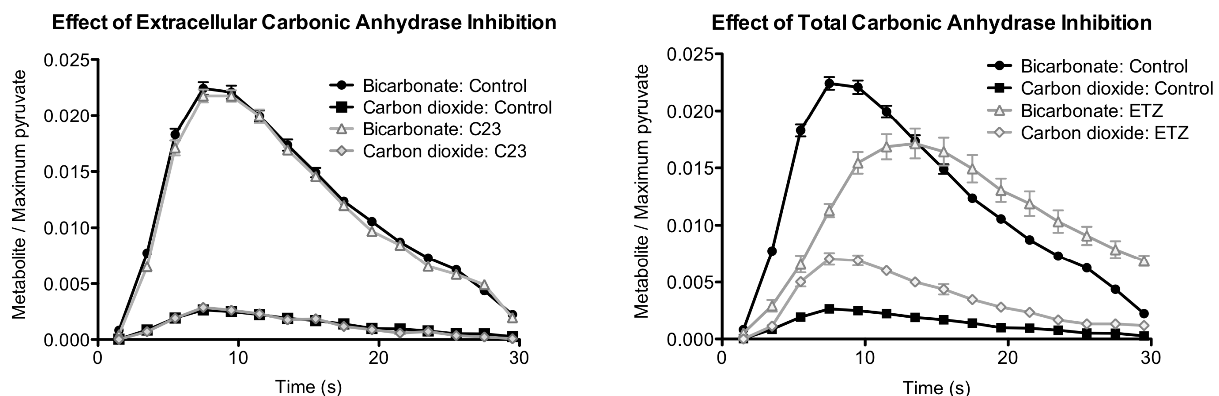
Introduction CO_2/HCO_3^- is a major buffer system in blood plasma and inside cells. It reduces pH changes during acid/base disturbances and contributes to the regulation of intracellular and extracellular pH (pH_i and pH_e). However, the kinetics of CO_2/HCO_3^- buffering, particularly the hydration of CO_2 to HCO_3^- and H^+, are very slow, unless catalysed by the enzyme carbonic anhydrase (CA). In the heart, modest levels of intracellular CA have been detected functionally[1] and immunohistochemically[2], and sarcolemmal isoforms of CA with extracellular function have been suggested[3]. Myocardial oxidative metabolism generates large quantities of CO_2 via the enzyme complex pyruvate dehydrogenase (PDH) and the Krebs cycle. CO_2 is highly membrane-permeant, though the CO_2 hydration products, HCO_3^- and H^+, are membrane-impermeant. Therefore, intracellular CO_2 hydration could lead to 'trapping' of H^+ and HCO_3^-, causing intracellular acidification that would be more difficult to remove than extracellular acidification. It has been hypothesised that the pattern of CA expression in the heart (intracellular versus extracellular) may determine the CO_2 gradient, therefore affecting the rate CO_2 is vented out of respiring cells[3]. A high extracellular/intracellular CA ratio would increase the CO_2 gradient and favour rapid CO_2 removal, by shifting the site of hydration to the blood-perfused extracellular compartment.

To date, cardiac isoforms of CA have only been studied *in vitro*. However, the function of CA *in vivo* may be different for the following reasons: (1) Metabolic generation of CO_2 may be reduced *in vitro* due to decreased cardiac workload, 2) Perfusate composition may differ from blood in terms of substrates/ions/gases. 3) Perfusate delivery and washout may be different *in vitro*, and 4) *In vitro,* the CO_2 gradient that spans mitochondrial CO_2 production to the extracellular space may be disrupted. *In vivo* infusion and metabolism of hyperpolarised [1-^{13}C]pyruvate produces $H^{13}CO_3^-$ and $^{13}CO_2$ at levels which are quantifiable with ^{13}C MRS[4], providing the first technique which may allow direct study of CA *in vivo*. Consequently, the aim of this study was to determine the effects of intra- and extracellular isoforms of CA on CO_2 efflux from the heart, *in vivo*.

Methods Male Wistar rats (~250 g) were studied with hyperpolarized [1-^{13}C]pyruvate in the control state, and in one of two states of CA inhibition: (i) inhibition of all intracellular and extracellular CAs, using a 100 μM dose of the broad spectrum, membrane-permeant CA inhibitor 6-ethoxyzolamide (ETZ); and (ii) inhibition of extracellular CAs only, using a 50 μM dose of the membrane-impermeant CA inhibitor C23 synthesised in-house[5]. Infusion of each CA inhibitor was commenced 15 min prior to assessment with hyperpolarized [1-^{13}C]pyruvate. Each rat was positioned at the isocentre of a 7 T Varian horizontal bore MR scanner, with a dual-tuned $^{1}H/^{13}C$ coil localised over the animal's chest. Aqueous hyperpolarised [1-^{13}C]pyruvate (80 μmol) was then infused into the rat via the tail vein, and cardiac ^{13}C spectra were acquired every 2 s for 1 min[6]. All cardiac ^{13}C spectra were analysed using the AMARES algorithm in the jMRUI software package[7], and the $H^{13}CO_3^-$ and $^{13}CO_2$ peak areas were normalised to maximum [1-^{13}C]pyruvate signal. The effect of each CA inhibitor (ETZ and C23) was determined, based on changes to the area under the $H^{13}CO_3^-$ and $^{13}CO_2$ curves for the first 30 s after pyruvate infusion, the maximum metabolite/maximum pyruvate ratio, and the initial slope of HCO_3^- and $^{13}CO_2$ production over the first 6 s following pyruvate infusion. Statistical differences between control (n = 8), C23 (n = 6), and ETZ (n = 4) groups were assessed using one-way ANOVA with a post-hoc Tukey's multiple comparison test. Statistical significance was considered at $p < 0.05$. A system of ordinary differential equations was formulated to model the effects of diffusion, reaction with intracellular and extracellular isoforms of CA, and convection on *in vivo* CO_2 venting from the myocardium.

Results C23 (50 μM) had no significant effect on the production of either $H^{13}CO_3^-$ or $^{13}CO_2$ following infusion of hyperpolarised [1-^{13}C]pyruvate (Figure 1A). 100 μM ETZ had no effect on the area under the $H^{13}CO_3^-$ plus $^{13}CO_2$ curve, or on the peak ($H^{13}CO_3^-$ plus $^{13}CO_2$)/maximum pyruvate ratio, but did reduce the initial slope of $H^{13}CO_3^-$ plus $^{13}CO_2$ production by 57%. Additionally, ETZ significantly reduced the peak $H^{13}CO_3^-$/[1-^{13}C]pyruvate ratio and the initial slope of $H^{13}CO_3^-$ production by 24% and 85%, respectively, compared with controls (Figure 1B). Further, compared with controls, ETZ significantly increased the peak $^{13}CO_2$/[1-^{13}C]pyruvate ratio by 88%, the area under the $^{13}CO_2$ curve by 76%, and the initial slope of $^{13}CO_2$ production by 70%.



Discussion Our results have indicated that extracellular isoforms of CA have no effect on the removal of CO_2 from the healthy heart *in vivo*. Diffusion-reaction-convection modeling has proposed a role for extracellular CAs only under conditions of reduced blood flow, such as myocardial ischemia, where convective CO_2 removal is impared[3]. Under these conditions, extracellular CA may have a significant effect on the trans-membrane CO_2 gradient, removing extracellular CO_2 by reactive means rather than via convection. The observation that ETZ, but not C23, had a significant effect on $^{13}CO_2$ and $H^{13}CO_3^-$ detection within the heart suggests both that our measurements reflected predominantly intracellular $^{13}CO_2$ and $H^{13}CO_3^-$, and that intracellular CA isoforms have a more pronounced effect on $^{13}CO_2$ venting than extracellular isoforms (under normal perfusion conditions). When intracellular isoforms of CA were inhibited and the steady-state intracellular $^{13}CO_2/H^{13}CO_3^-$ ratio was increased, the outward CO_2 diffusion gradient also increased, enabling rapid CO_2, HCO_3^-, and H^+ removal from myocytes. This was evidenced by the reduced initial slope of $H^{13}CO_3^-$ plus $^{13}CO_2$ production measured *in vivo*, indicative of reduced accumulation (i.e. faster venting) of respiratory products. Normal intracellular CA activity trapped $H^{13}CO_3^-$ and H^+ within myocytes, slowing $^{13}CO_2$, $H^{13}CO_3^-$, and H^+ removal. Physiological levels of cardiac intracellular CA must reflect a balance between retaining sufficient levels of myocardial bicarbonate to buffer potential acid/base disturbances, without causing excessive intracellular acidification.

References

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