In Vivo Determination of the Rate of Cerebral Carbonic Anhydrase Reaction

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Introduction

Carbonic anhydrase (CA) catalyzes the interconversion between CO_2 and HCO_3^- anion: $CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$. In brain tissue, CA is primarily expressed in glial and choroid cells. It has been proposed that CO_2 produced under conditions of high neuronal activity is processed mainly in the glia and the conversion of CO_2 into bicarbonate in glia is coupled to its high-affinity uptake of neurotransmitter glutamate (Deitmer, 2002). CA inhibitors possess important clinical applications, for example, in the treatment of glaucoma, epilepsy and other neurological disorders. Many of them also inhibit the growth of several tumor cell lines in vitro and in vivo, thus constituting interesting leads for developing novel antitumor therapies. CA activators, on the other hand, may be used for management of conditions in which learning and memory are impaired such as in ageing and Alzheimer's disease, and for treatment of genetically inherited CA deficiencies. Considering the importance of CA and the on-going active research in designing drugs for its inhibition or activation, a noninvasive method capable of directly measuring the rate of CA reaction in vivo is clearly of significant value. Here, we report in vivo ^{13}C magnetization transfer effect of the carbonic anhydrase reaction and quantification of the pseudo first order rate constant of the bicarbonate dehydration reaction catalyzed by CA in isoflurane-anesthetized adult rat brain. The effect of intralateral ventricular administration of CA inhibitor acetazolamide was also evaluated.

Materials and Methods

Male Sprague–Dawley rats (164–196 g) fasted for 24 hours with free access to drinking water were studied using a Bruker spectrometer interfaced to an 89-mm bore 11.7 Tesla vertical magnet. The animals were divided into control (n = 7) and acetazolamide-treated (n = 6) groups. All rats were orally intubated and mechanically ventilated with a mixture of ~70% N_2O, 30% O_2 and 1.5% isoflurane. The left femoral artery was cannulated where plasma samples were withdrawn for monitoring arterial blood physiological variables. The left femoral vein was also cannulated for continuous infusion of [2-^{13}C]- or [1-^{13}C]glucose. Plasma glucose concentration was maintained at 25.5 ± 6.7 mM. For intralateral ventricular administration, acetazolamide (100 mM, 5 µl, 0.5 µl/min, solubilized in dimethyl sulfoxide) was injected approximately 3 hours prior to in vivo ^{13}C data acquisition. The control rats received the same volume of dimethyl sulfoxide. An in-house transmit/receive concentric surface $^{13}C/^{1}H$ RF coil system and a 90° excitation, surface-coil-localized acquisition scheme were used. When the ^{13}C magnetization transfer spectra were acquired, the undetectable CO_2 at 125.0 ppm was saturated using a train of spectrally selective 2-ms Gaussian pulses with a nominal flip angle of 180° spaced 12 ms apart. When the control spectra were acquired, the saturation pulse train was placed at 196.4 ppm. TR = 43.4 sec. NS = 320-448 per rat. The control and CO_2-saturated spectra were interleaved every FID. T_1 of bicarbonate was measured from a separate group of rats using the inversion-recovery null method without saturating CO_2 (n = 4).

Results

The arterial blood physiological parameters for the control and acetazolamide-treated rats are pH = 7.27 ± 0.06, pCO_2 = 32 ± 8 mmHg, MABP = 124 ± 19 mmHg and pH = 7.20 ± 0.10, pCO_2 = 39 ± 13 mmHg, MABP = 112 ± 18 mmHg, respectively. Fig. 1 shows the saturation transfer effect due to rapid exchange between CO_2 and HCO_3^- in the control rats infused with [2-^{13}C]glucose. LB = 15 Hz. The difference spectra show a significant change in the bicarbonate signal at 160.7 ppm (Fig. 1c). M^{sat}/M^{nosat} was determined to be 0.37 ± 0.07 (mean ± SD, n = 7) in the control rats, while M^{sat}/M^{nosat} was significantly increased in acetazolamide-treated rats (0.71 ± 0.07, mean ± SD, n = 6). Glutamate C5+GABA C1, NAA C1, C4 and C5, glutamine C5+aspartate C4, glutamate C1, and glutamine C1+aspartate C1 were also observed (Fig. 1a, b). T_1 of bicarbonate was determined to be 9.8 ± 0.4 sec (mean ± SD, n = 4). The pseudo first-order rate constant of bicarbonate dehydration reaction (k) was determined to be 0.19 ± 0.04 sec^{-1} (mean ± SD, n = 7) in control rats. The pseudo first order rate constant k was found to be significantly decreased in acetazolamide-treated rats (0.043 ± 0.005 sec^{-1}, mean ± SD, n = 6, p = 0.001).

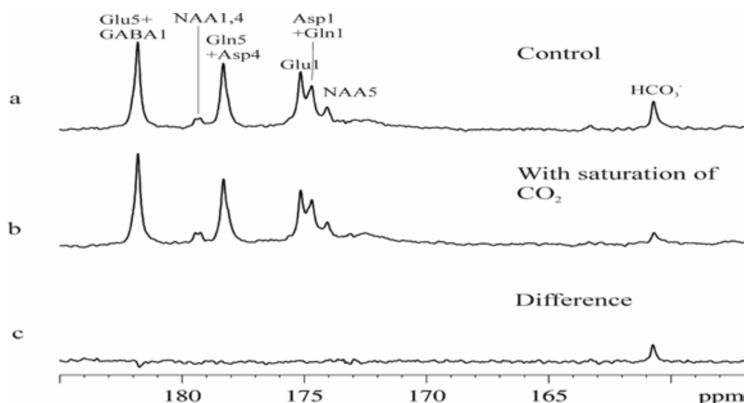

Fig.1. (a) Symmetric control, (b) saturation of carbon dioxide and (c) difference spectra from rats infused with [2-^{13}C]glucose but without acetazolamide (n = 4).

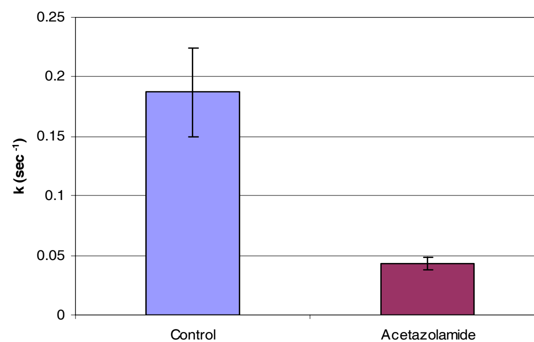

Fig.2. Pseudo first-order rate constants of bicarbonate dehydration reaction with and without acetazolamide treatment.

Discussion

The hydration of CO_2 and dehydration of bicarbonate can proceed without catalysis by CA. k for the uncatalyzed bicarbonate dehydration reaction at 37 °C and pH = 7.0 is 0.005 sec^{-1} based on previously determined first order rate constant of CO_2 hydration in solution (2). In comparison, the current study found that brain CA in control rats elevates k to 0.19 sec^{-1} in vivo. Based on previously determined relationship between pCO_2 and brain tissue [HCO_3^-] (3), [HCO_3^-] was estimated to be 15.5 mM in the brain of control rats. Therefore, the estimated unidirectional bicarbonate dehydration flux rate in the brain of control rats is 174 mM/min. In acetazolamide treated rats, k was reduced by more than four fold. In conclusion, the results in this study demonstrate that the combination of long ^{13}C T_1 of bicarbonate and fast exchange between CO_2 and bicarbonate catalyzed by CA causes a large and quantifiable magnetization transfer effect in vivo. Since bicarbonate is detectable following administration of hyperpolarized [1-^{13}C]pyruvate, it is therefore possible to image the CA reaction in vivo using hyperpolarized ^{13}C imaging.

References

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