Hunting for cerebral [2-^{13}C]GABA signal derived in vivo from glia-specific substrate [2-^{13}C]acetate

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Introduction

Acetate has been demonstrated to be a glia-specific substrate. Prolonged incubation of neuron culture with ^{13}C-labeled acetate showed no enrichment of ^{13}C in glutamate, glutamine, or GABA [1]. [2-^{13}C]GABA is produced by the GABA-synthesizing enzyme glutamic acid decarboxylase from [4-^{13}C]glutamate. The brain [2-^{13}C]GABA signal derived in vivo from glia-specific substrate [2-^{13}C]acetate therefore is a measure of the glutamine-GABA cycle, which carries the glial [4-^{13}C]glutamine to GABAergic neurons to be metabolized into [2-^{13}C]GABA. Since [2-^{13}C]GABA derived in vivo from infused ^{13}C-labeled glucose can be spectrally resolved from the nearby [4-^{13}C]glutamate at 11.7 Tesla [2] here we evaluate the feasibility of detecting the [2-^{13}C]GABA signal derived from [2-^{13}C]acetate using proton-detected POCE method.

Methods

Male Sprague-Dawley rats (n = 14) were studied using POCE spectroscopy method and an 11.7 Tesla 89-mm bore vertical magnet. Control group (n = 7) and vigabatrin-treated group (n = 6, 500 mg/kg, i.p., 24 hrs prior to data acquisition) were infused with [2-^{13}C]acetate for a period of 180 minutes. One gabaculine-treated rat (100 mg/kg, i.v.) was infused with [1-^{13}C]glucose for a period of 120 minutes. All rats were orally intubated and mechanically ventilated with a mixture of ~70% N_2O, 30% O_2 and 1.5% isoflurane, which was discontinued after surgery with anesthesia maintained using α-chloralose instead (initial dose: 80 mg/kg supplemented with a constant infusion of 26.7 mg/kg/hr throughout the experiment). The spectroscopy voxel with a size of either 4.5 x 2.5 x 4.5 mm^3 or 4.0 x 2.5 x 4.0 mm^3 was centered along the brain midline in the rat neocortex. Proton-detected spectra were acquired using a fully adiabatic POCE method [3, and refs therein]. Three rhesus monkeys were also studied using direct ^{13}C MRS method and a 4.7 Tesla 295-mm bore horizontal magnet [4]. The monkeys were anesthetized using propofol and infused with either [1-^{13}C]glucose or [2-^{13}C]acetate. ^{13}C spectroscopy data were acquired from a 35 x 25 x 35 mm^3 voxel located in the monkey brain.

Results and Discussion

Fig. 1 shows the summed POCE spectra from (i) the rat treated with gabaculine (bottom trace, 4.0 x 2.5 x 4.0 mm^3 voxel, NS = 1024, lb = -4, gb = 0.2, acquired ~ 2 hrs after gabaculine injection) which provides a chemical shift reference for [2-^{13}C]GABA; (ii) the rats treated with vigabatrin (middle trace, 4.5 x 2.5 x 4.5 mm^3 voxel, NS = 4608, lb = -4, gb = 0.2, acquired 2 hrs after [2-^{13}C]acetate infusion); (iii) the control rats (upper trace, 4.5 x 2.5 x 4.5 mm^3 voxel, NS = 5888, lb = -4, gb = 0.2, acquired 2 hrs after [2-^{13}C]acetate infusion). The [2-^{13}C]GABA signal derived in vivo from [2-^{13}C]acetate was clearly observed in the vigabatrin-treated rats (middle trace). However, in the control rats, the [2-^{13}C]GABA signal is not significantly above the noise level. At 500 mg/kg, vigabatrin is known to increase GABA concentration by approximately 100-200% at 24 hrs after administration. From Fig. 1 middle trace, we found FE([2-^{13}C]GABA) << FE([4-^{13}C]glutamate). The low intensity of the [2-^{13}C]GABA signal observed in vivo indicates that the glutamine-GABA cycle should be a relatively small metabolic flux inaccessible to in vivo MRS unless the concentration of GABA is significantly elevated. In vivo detection of [3-^{13}C]GABA or [4-^{13}C]GABA using POCE is difficult because of the overlapping [2-^{13}C]acetate signal at 1.9 pm and the residual creatine signal at 3.0 ppm due to its natural abundance and potential movement-related POCE subtraction errors. ^{13}C-labeled glucose synthesized from [2-^{13}C]acetate in the liver also contributes to the labeling of [2-^{13}C]GABA. Fig. 2 upper trace shows the summed ^{13}C spectra of monkey brain detected between 65-150 min after the start of [1-^{13}C]glucose infusion (NS = 3600). At 4.7 Tesla [2-^{13}C]GABA is clearly resolved from the nearest [3,4-^{13}C$_2$]glutamate downfield satellite signal. In contrast, the expected [2-^{13}C]GABA signal at 35.2 ppm was not significantly above the noise level despite a 2-hr signal averaging during [2-^{13}C]acetate infusion (see summed spectrum in Fig. 2 lower trace). The acetate data were acquired between 120-180 min after the start of acetate infusion (NS = 3600). In contrast to the intact brain, this study shows that [2-^{13}C]GABA derived from [2-^{13}C]acetate can be used to study the effect of elevated endogenous GABA concentration on the glutamate-GABA cycle in vivo.

Fig. 1

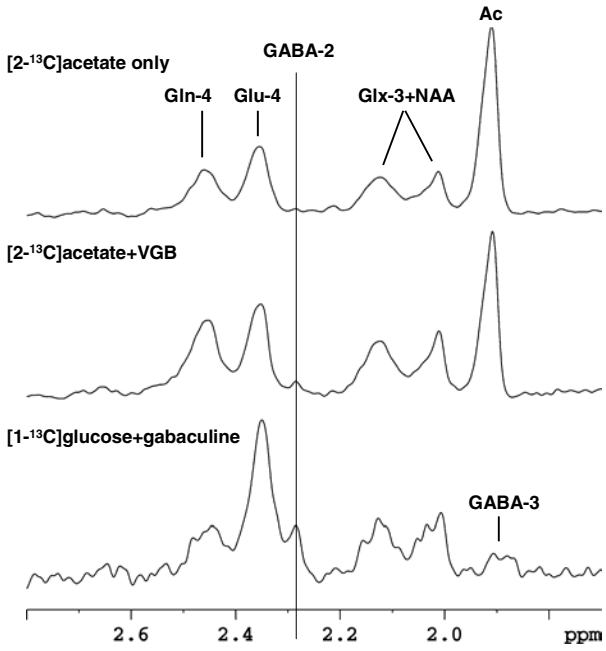
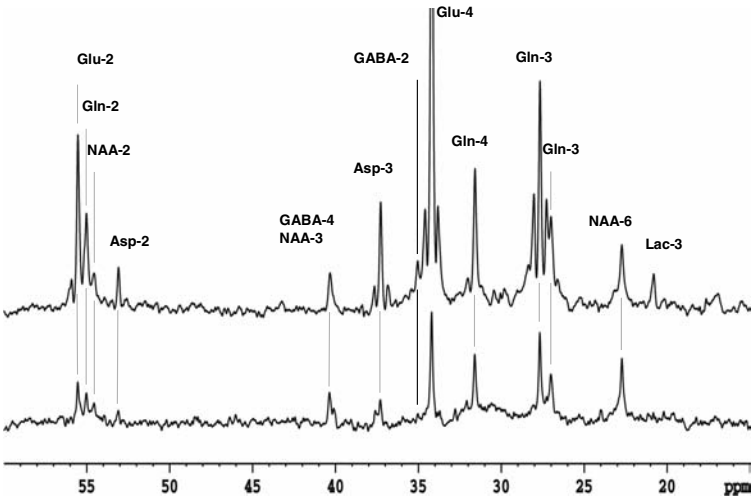


Fig. 2



References

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