In Vivo Detection of Altered GABA Levels Following Acute Administration of Antidepressant/Antipanic Drug Phenelzine

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Introduction

Phenelzine is a non-selective monoamine oxidase inhibitor which is efficacious in the treatment of depression, social phobia and panic disorders. As a potent inhibitor of both MAO-A and MAO-B, administration of phenelzine increases brain levels of the amine neurotransmitters noradrenaline, dopamine, and serotonin, which has been the focus of many previous studies of the mechanisms of the therapeutic action of phenelzine. In vitro studies have shown that phenelzine also causes a significant increase in the levels of brain's major inhibitory neurotransmitter GABA (1). In animal model studies, phenelzine affects memory in a task-dependent manner and produces anxiolytic and antipanic effects. The elevation of brain GABA level by phenelzine has been shown to be essential in producing its anxiolytic effects in the plus-maze model. Administration of phenelzine, like vigabatrin and gabaculine, leads to dose-dependent inhibition of GABA-T, elevated GABA levels and augmented GABA release. Recently, a novel approach for in vivo spectral editing has been proposed (2), in which the signal to be edited (target signal) is completely regenerated anew from the corresponding J-coupled thermal equilibrium magnetization of a source signal. In the case of GABA editing, the edited GABA-4 resonance is derived from the thermal equilibrium GABA-3 peak using a novel doubly selective homonuclear polarization transfer approach. For each single scan, the thermal equilibrium signal of the GABA-4 resonance and its overlapping signals (creatine, glutathione, and macromolecules) are completely suppressed prior to polarization transfer from GABA-3. The purpose of this study is to measure changes in GABA concentration in vivo in rat neocortex following acute administration of phenelzine using the polarization transfer editing method.

Materials and Methods

All experiments were performed on a microimaging spectrometer interfaced to an 11.7 T 89-mm bore vertical magnet. Male Sprague-Dawley rats (160-200 g, n = 9) were anesthetized using intravenous infusion of α-chloralose. The metabolite linewidth $\Delta\upsilon_{1/2}$ = ~ 10-13 Hz after in vivo shimming (3). In the control group (n = 4), basal GABA was measured. In the phenelzine-treated group (n = 5), GABA was measured four hours after acute phenelzine treatment (10 mg/kg, i.p.). In the pulse sequence (Fig. 1), a slice-selective 90° pulse (five-lobe sinc, 500 μs) was applied along the *x*-axis (rotating frame, with slice selection gradient on the *x*-axis). An optimized doubly selective 180° refocusing pulse based on the Hermite pulse (along the *x*-axis in rotating frame, 15 ms, B_{1max} = 375 Hz, centered at GABA-3 at 1.91 ppm and GABA-4 at 3.02 ppm) together with a pair of gradient crushers were used to allow the GABA-3 resonance to evolve under the GABA-3, 4 J coupling while refocusing the spin evolution due to the GABA-2, 3 J coupling. The optimized double band Hermite-based pulse generated minimal excitation at the macromolecule resonating frequency of 1.72 ppm. The nominal evolution period t_1 = 1/4J. Then the antiphase GABA-3, 4 spin state was acted on by a 90° pulse on the *y*-axis (rotating frame, five-lobe sinc, 500 μs) to perform homonuclear polarization transfer. During the rephasing period (t_2 = 1/4J), the inphase GABA-4 doublet was formed while spatial selection along the *y*- and *z*-axes was achieved using using slice-selective 180° refocusing pulses (a pair of identical sech pulses per axis, 2 ms, μ = 5, 1% truncation). The localized voxel was centered in the brain midline with a dimension of 4.5 mm x 2.5 mm x 4.5 mm. Quantification of GABA concentration based on the GABA-to-NAA ratio measured in vivo (2).

Fig. 1

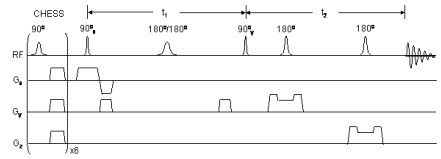
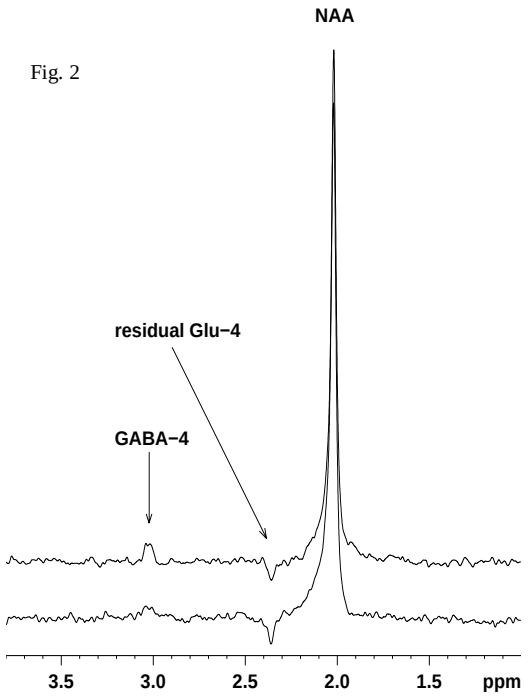


Fig. 2



Results and Discussion

Fig. 2 shows the result of GABA-4 editing using the homonuclear polarization transfer scheme from the control group (lower trace) and the phenelzine-treated group (upper trace) (TR/TE = 2000/68 ms, 4.5 mm x 2.5 mm x 4.5 mm, NS = 256, LB = 5 Hz). Both spectra were phased using zero order phase only without any baseline corrections. In Fig. 2, a clear GABA doublet is observed at 3.02 ppm with a clean baseline indicating excellent suppression of the overlapping Cr methyl resonance at 3.03 ppm and GSH cysteinyl methylene resonance at 2.87 – 2.96 ppm as well as outer volume signals. Complete suppression of macromolecules was verified using the metabolite null method (data not shown). The selective polarization transfer spectrum also clearly demonstrates the phase relationship between NAA and GABA. The negative peak at 2.35 ppm was the Glu-4 signal resulted from partial polarization transfer from Glu-3 at 2.11 pm. As discussed previously (2), the in vivo GABA-to-NAA intensity ratio was measured using the doubly selective polarization transfer method. In the control group, [GABA] was determined to be 1.0 ± 0.2 μmol/g wet weight (mean ± SD, n = 4). Four hours after phenelzine treatment, [GABA] was determined to be 2.1 ± 0.2 μmol/g wet weight (mean ± SD, n = 5). Even at 11.7 T, we have found that macromolecule baseline and partial spectral overlapping around the GABA-2 resonance make reliable quantification of basal and small changes in GABA concentration in vivo using LCModel rather difficult. We have found that spectral editing was necessary to obtain a flat baseline and a clean GABA peak for accurate in vivo quantification of cortical GABA and GABA synthesis. Although this new GABA-4 editing method has only been demonstrated for in vivo measurement of GABA in the rat brain at 11.7 T, it could also be very useful for human studies at lower field strength because DQ-based GABA-4 editing using similar doubly selective refocusing pulses has already been demonstrated at 2-3 Tesla (4, 5).

Conclusion

We have shown that acute phenelzine treatment causes a significant increase in GABA levels in the rat neocortex using an in vivo spectral editing which achieves GABA editing and complete suppression of all overlapping resonances in a single shot with simultaneous detection of the dominant NAA signal at 2.02 ppm. The results provided the first direct in vivo evidence of augmented GABA pool due to acute administration of the antidepressant / antipanic drug phenelzine.

References

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