# Investigation of the Role of GABA Reuptake in GABAergic Neurotransmission

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**INTRODUCTION:** A recent $^{13}C$ MRS study reported that GABA neurotransmission and glucose oxidation contributes a significant fraction (~20%) of total glutamate (Glu) plus GABA neurotransmitter cycling ($V_{cyc}$) and glucose oxidation ($CMR_{glc(ox)N}$) in rat cortex (1). However, previous estimates of the capacity of GABA uptake by GABAergic neurons and astroglia suggest that 80% or more of synaptically released GABA is returned to GABAergic neurons by reuptake through GABA transporters (2) which in neurons is dominated by GAT-1. The measurement of GABA/Gln cycle flux in the $^{13}C$ MRS experiment will underestimate total synaptic GABA release to the extent that neuronal reuptake occurs because $^{13}C$ labeling patterns for released GABA followed by reuptake cannot be differentiated from intracellular GABA shunt activity. The purpose of the present study was to assess the potential contribution of the GABA reuptake pathway to total GABA release. We hypothesized that if GABA reuptake is major fraction of the total GABA release, then pharmacologic block of GABA reuptake by a GAT-1 inhibitor will result in either lower intracellular (and tissue) GABA levels and/or increased cycling from astroglial glutamine to maintain homeostasis.

**MATERIALS AND METHODS:** Two groups (A, control; B, tiagabine-treated) of overnight fasted, halothane-anesthetized Wistar rats (160-180 g) were studied. Rats were tracheotomized and ventilated (30%$O_2$/68.5% $N_2O$, 1.5% halothane), and arterial and venous catheters placed for measurement of blood gases, blood pressure, and the infusion of [1,6-$^{13}C_2$]glucose. *In vivo* experiments were performed at 9.4T (Bruker AVANCE spectrometer) using a surface transceiver coil placed on the rats head for $^{1}H$ detection and an orthogonal pair of coils, coupled in quadrature, for $^{13}C$ RF transmission. Shimming was optimized using FASTMAP (3). $^{1}H$-[$^{13}C$]-NMR spectra were obtained every 40s from a localized volume (7x4x7mm$^3$) during the infusion of [1,6-$^{13}C_2$]glucose (4). Tiagabine (30 mg/kg, i.p.) was administered (Group B) 45 min before the start of [1,6-$^{13}C_2$]glucose infusion. Arterial blood samples were taken periodically for the analysis of plasma glucose concentration and $^{13}C$ enrichment. In addition rats in both groups were also infused with [2-$^{13}C$]acetate for a period of 2.5 hours on the bench for the estimation of the ratio, $V_{cyc}/V_{TCA}$, for glutamatergic and GABAergic neurons (1). At the end of the experiment, the brain was frozen *in situ* with liquid nitrogen. Metabolites were extracted from frozen cortical tissue (5). The concentrations and percent $^{13}C$ enrichment of metabolites were determined from the $^{1}H$-[$^{13}C$]-NMR spectrum of the extract recorded at 11.7T (Bruker AVANCE spectrometer). The percentage $^{13}C$ enrichment of plasma glucose-C1 was measured using $^{1}H$ NMR from glucose-C1$\alpha$ at 5.2ppm. A three compartment (glutamate neuron, GABA neuron, astrocyte) metabolic model was fitted to the measured $^{13}C$ time courses of cortical amino acids from [1,6-$^{13}C_2$]glucose yielding estimates of the metabolic fluxes (1).

**RESULTS AND DISCUSSION:** No significant changes (P>0.10) in the levels of the measured cortical amino acids (Ala, Asp, Cre, GABA, Glu, Gln, Lac, and NAA) were observed after tiagabine treatment. However, tiagabine treatment led to a small but statistically insignificant decreases in glucose oxidation and neurotransmitter cycling (Fig.1). For glutamatergic neurons: $CMR_{glc(ox)N}$ (control, 0.294±0.070; *vs.* tiagabine-treated, 0.229±0.042 μmol/g/min, P=0.07) and $V_{cyc}$ (control, 0.259±0.062 *vs.* tiagabine-treated, 0.219±0.039 μmol/g/min; P=0.23). Similarly, for GABAergic neurons: $CMR_{glc(ox)N}$ (control, 0.0890±0.025 *vs.* tiagabine-treated, 0.069±0.030 μmol/g/min, P=0.44) and $V_{cyc}$ (control, 0.069±0.022 *vs.* tiagabine-treated, 0.062±0.020 μmol/g/min, P=0.37). The contribution of GABAergic neurons to total (glutamate plus GABA) neuronal glucose oxidation and neurotransmitter cycling was similar (~22%) in both groups of rats. The small decrease in glutamatergic and GABAergic fluxes were proportional suggesting a high degree of coupling, possibly due to reduced glutamatergic activity (and drive on GABAergic neurons) of the elevated extracellular GABA expected with tiagabine treatment. The absence of changes in GABA and glutamate levels together with no significant increase in GABA/glutamine cycle flux in tiagabine treated rats suggests that the contribution of reuptake to synaptically released GABA is less than previously expected. However, a more definite conclusion will require additional studies to establish the extent of inhibition of GAT-1 by the current dose of tiagabine.

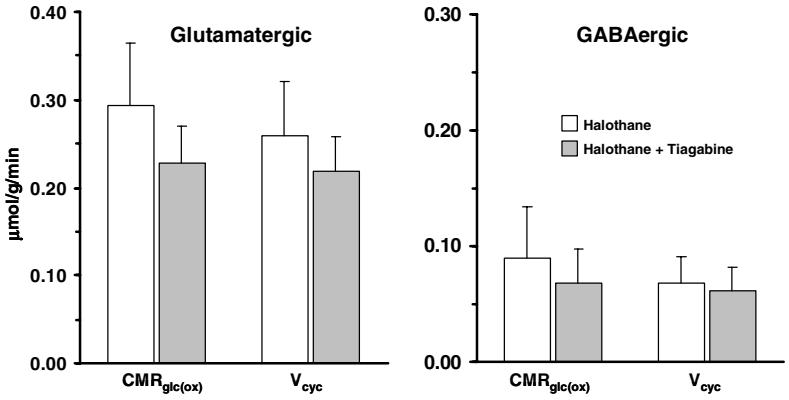


**Fig. 1** Cerebral metabolic rates estimated in control (□)and tiagabine treated (▨)rats

**REFERENCES:** (1) Patel AB et al. *Proc Natl Acad Sci* 102:5588, 2005; (2) Hertz L and Schousboe A. *Model Systems of Development and Aging of the Nervous System (eds Vernadakis et al) M Nijhoff Publ Comp Boston*, pp 19, 1987; (3) Gruetter R. *Magn Reson Med* 29:804, 1993; (4) de Graaf RA et al. *Magn Reson Med* 49:37, 2003; (5) Patel AB et al. *Brain Res* 919:207, 2001.

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