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Neurochemistry International 48 (2006) 508–514
NEUROCHEMISTRY International
www.elsevier.com/locate/neuint

Acute regulation of steady-state GABA levels following GABA-transaminase inhibition in rat cerebral cortex

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Received 14 October 2005; received in revised form 7 December 2005; accepted 9 December 2005
Available online 3 March 2006

Abstract

Cellular GABA levels are determined by the dynamic balance between synthesis and catabolism and are regulated at the level of glutamate decarboxylase, precursor availability (e.g., glutamate and glutamine), and possibly GABA degradation. GABA levels rise and stabilize within hours in human cortex following orally administered vigabatrin, an irreversible inhibitor of GABA-T, suggesting potential product inhibition of GABA synthesis or enhanced GABA degradation through the non-inhibited GABA-T fraction. In this study time courses of the rise in cortical GABA were measured in anesthetized rats in vivo after vigabatrin treatment using localized ^{1}H magnetic resonance spectroscopy and the times to reach steady-state for a given dose were determined. Rates of GABA synthesis were estimated for the period of constant GABA level from the accumulation of [2-^{13}C]GABA following a short intravenous infusion (20 min) of either [1,6-$^{13}C_2$]glucose or [2-^{13}C]acetate. No evidence of product inhibition of glutamate decarboxylase by the increased GABA concentration or reduced synthesis from [1,6-$^{13}C_2$]glucose (control, 0.031 ± 0.010; vigabatrin-treated, 0.037 ± 0.004 μmol/g/min, $P = 0.30$) or [2-^{13}C]acetate (control, 0.078 ± 0.010; vigabatrin-treated, 0.084 ± 0.006 μmol/g/min, $P = 0.42$) was found. Fractional changes in steady-state GABA levels and GABA-T activities 5–6 h after vigabatrin treatment were approximately equal. The lack of change in GABA synthesis (and GABA catabolic flux for constant GABA levels) suggests that GABA-T has a near-zero flux control coefficient in vivo-capable of greatly altering the steady-state GABA concentration but exerting little or no control on GABA synthesis or GABA/glutamine cycling flux. The findings are consistent with a Michaelis–Menten kinetic model whereby cellular GABA levels increase until flux through the remaining (uninhibited) transaminase equals the rate of GABA synthesis. The findings suggest that astroglia may be the site of continuing GABA catabolism after acute vigabatrin treatment.
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Keywords: GABA; GABA transaminase; NMR Spectroscopy; γ-vinyl GABA; Vigabatrin; ^{13}C isotopes

1. Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (Krnjević, 1987; Roberts, 1988; McCormick, 1989). The cellular GABA level reflects a dynamic balance between synthesis and catabolism, determined respectively by the relative fluxes through two pyridoxal-5'-phosphate dependent enzymes, glutamate decarboxylase (GAD; EC 4.1.1.15) and GABA transaminase (GABA-T; EC 2.6.1.19). Whereas GAD controls the synthesis of GABA from glutamate (Martin and Tobin, 2000), GABA-T catalyzes the first-step in GABA degradation to succinic semialdehyde (SSA) in a transamination reaction with α-ketoglutarate to form glutamate (Bessman et al., 1953; Roberts and Bregoff, 1953). Oxidation of SSA to succinate by NAD^{+}-dependent succinic semialdehyde dehydrogenase (SSADH; EC 1.2.1.24) completes the pathway.

Tissue GABA levels mainly reflect the content of GABAergic neurons because non-GAD expressing cells and extracellular fluid contain only low amounts. Estimates of

Abbreviations: Cr_{tot}, creatine plus phosphocreatine; GABA, γ-Aminobutyric acid; GABA-T, GABA transaminase; GAD, Glutamate decarboxylase; Glu, Glutamate; NMR, Nuclear Magnetic Resonance; SSADH, Succinic semialdehyde dehydrogenase; STEAM, Stimulated echo acquisition mode; TCA, Tricarboxylic acid; VGB, vigabatrin

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0197-0186/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuint.2005.12.024

GABA concentration in GABAergic nerve terminals are high (e.g., 50–100 mM, Fonnum and Walberg, 1973), and while much of this is likely to be entrapped in vesicles, cytoplasmic GABA levels are likely to be saturating with respect to GABA-T (K_m ~1.1 mM; Schousboe et al., 1973) and thus unlikely to regulate GABA catabolism in these cells. However, GABA catabolism could be modulated by GABA availability in surrounding non-GABAergic cells (e.g., postsynaptic neurons and astroglia) where GABA levels are low and closer to K_m and where mass action and mitochondrial milieu (e.g., α-ketoglutarate availability and pH) may potentially influence catabolism (Baxter, 1970; Lopes-Cardozo and Albers, 1979).

GABA levels are altered in a number of neurological and psychiatric disorders (Brambilla et al., 2003; Lewis et al., 2005). In studies of subjects with complex partial epilepsy and depressive disorder, GABA levels are low and therapeutic improvement parallels rising GABA levels (Petroff et al., 1996a; Sanacora et al., 1999). Short-term (days to minutes) fluctuations in occipital cortical GABA levels are observed during the menstrual cycle (Epperson et al., 2002), acute deafferentation (Levy et al., 2002), and visual dark-adaptation (Babak et al., 2001) indicating that GABA levels are responsive to endocrine and sensory cues. While GAD and GABA-T are likely to be involved in the change in GABA level, the mechanism(s) are not understood.

Clinical ^{1}H NMR studies of epileptic subjects treated with vigabatrin (VGB), an irreversible inhibitor of GABA-T (Jung et al., 1977), show that GABA levels rise and stabilize within hours of receiving a single oral dose (Petroff et al., 1996b). The mechanism for this acute stabilization is distinct from the reduction in GABA synthesis and GAD_{67} isoform which follows more prolonged GABA elevation (Rimvall and Martin, 1994; Mason et al., 2001). The acute stabilization of GABA suggests faster kinetic adjustments involving inhibition of synthesis or enhanced degradation.

In the present study, we applied localized ^{1}H NMR spectroscopy in vivo and ^{1}H-[^{13}C] NMR ex vivo to assess the influence of GABA-T activity on GABA levels and rates of synthesis from [1,6-$^{13}C_2$]glucose and [2-^{13}C]acetate. The results support an important role of GABA-T in the regulation of GABA levels in vivo.

2. Materials and methods

2.1. Preparation of animals

Adult male Sprague-Dawley rats (155 ± 18 g, mean ± S.D., n = 16), fasted overnight, were tracheotomized and ventilated under 1.5% halothane anesthesia and 70%N_2O/28.5%O_2. A femoral artery and vein were cannulated to monitor arterial blood parameters (pO_2, pCO_2, pH, pressure), and to administer vigabatrin (VGB) and ^{13}C-labeled compounds. Physiological variables were within normal limits (in mmHg: pCO_2 = 36 ± 4; pO_2 = 145 ± 18; mean pressure = 102 ± 8; pH = 7.33–7.42). The core temperature was maintained near 37 ± 1 °C using a heated water pad. VGB was dissolved (500 mg/2 ml water), centrifuged, and the supernatant injected intravenously at doses of 250 mg/kg to 1000 mg/kg body weight. VGB concentration was verified by ^{1}H NMR. After completion of in vivo and ex vivo experiments the brain was frozen in situ with liquid N_2 (Ponten et al., 1973). Frozen heads were removed and kept at -85 °C until extraction. Animal experiments were performed in accordance with NIH Guidelines for the Care and Use of Laboratory Animals and locally approved protocols.

2.2. In Vivo ^{1}H NMR spectroscopy

In vivo NMR experiments were performed at 7 T (Bruker Instruments) using actively shielded shim/gradient coils (~180 mT/m in 100 μs). A homogeneous saddle-shaped coil was employed as transmitter in conjunction with a surface coil (14 mm) receiver. The spectroscopic volume was positioned using multislice gradient-echo imaging (15° nutation angle; TR/TE, 50/10 ms; 256 × 256 data matrix; 3.2 × 3.2 cm^2 field-of-view; 1.0 mm slice thickness). Magnetic field homogeneity was optimized using the non-iterative FASTMAP algorithm (Gruetter, 1993) resulting in signal line widths of 9–13 Hz for water and 7–10 Hz for metabolites.

Localized ^{1}H NMR spectra were obtained using a 3,1-DRYSTEAM pulse sequence (TR/TE/TM = 2500/5/25ms; detection volume = 100 μl, 5 × 4 × 5 mm^3; NEX = 256) (de Graaf, 1998). TE and TM crushers were executed as 1 ms (140 mT/m) and 5 ms (110 mT/m) trapezoidal gradients, respectively. Water suppression employed the chemical-shift-selective (CHESS) method (Haase et al., 1985) using 10 ms Gaussian radiofrequency pulses, truncated at 10%, followed by a 5 ms (100 mT/m) magnetic field gradient.

^{1}H NMR spectra were measured serially before and after administration of vigabatrin. Free-induction decays were zero-filled to 8192 data points, apodized (2 Hz Gaussian line broadening), Fourier transformed, phase corrected, and frequency shifted. GABA was determined from the difference of spectra acquired after VGB administration (averaged over ~7 min) and baseline (averaged over ~28 min) appropriately scaled to match the acquisition time of the subtracted spectrum. The intensities of the GABA resonances in the difference spectra were quantified using the LCmodel algorithm (Provencher, 1993) with GABA, glutamate, glutamine and a low-order, low-amplitude baseline as input functions.

2.3. Measurement of GABA synthesis

The rate of GABA synthesis was determined in separate groups of rats (Sprague Dawley, 160 ± 21 g, mean ± S.D., n = 16) treated similarly to those studied in vivo but infused intravenously with either [2-^{13}C]acetate (n = 6) or [1,6-^{13}C]glucose (n = 10) (99 atom%; Cambridge Isotopes) and measured ex vivo. Rats were administered vigabatrin (700 mg/kg, i.v.) or 0.9% saline and after 6 h, administered [1,6-$^{13}C_2$]glucose or [2-^{13}C]acetate intravenously using bolus-variable rate infusions (Patel et al., 2005). The infusions resulted in a rapid increase in blood substrate levels and enrichments which remained nearly constant over the respective infusion intervals. [1,6-$^{13}C_2$]glucose was infused as a 0.25 ml bolus (per 200 g body weight) of 0.75 M solution in water over 15 s and followed by a progressively decreasing rate. Sodium [2-^{13}C]acetate (3M in water, pH 7) was infused at a rate of 1.25 mmol/min/kg (0–5 min), 0.625 mmol/min/kg (5–10 min) and 0.25 mmol/min/kg (10–20 min). Arterial blood samples were collected periodically, centrifuged and plasma separated for subsequent measurement of glucose (Beckman Glucose Analyzer II) and acetate concentration and ^{13}C isotopic enrichment by NMR. After infusion the rat brain was frozen in situ using liquid N_2.

2.4. Preparation of extracts

Frozen cortical tissue (100–150 mg) was ground with 0.1M HCl/methanol (2:1 vol/wt) at −40 °C and homogenized with ice-cold 90% ethanol (6:1 vol/wt) (Patel et al., 2005). After centrifugation the supernatant was lyophilized and dissolved in 85% D_2O containing phosphate buffer (0.1 M) and 3-trimethylsilyl[2,2,3,3-D_4]-propionate (0.5 mM). [2-^{13}C]glycine (0.5 μmol) was added as an internal concentration reference during extraction in some experiments ([2-^{13}C]acetate infusions). The total (^{12}C + ^{13}C) and ^{13}C concentrations of the extracted amino acids were determined using ^{1}H-[^{13}C] NMR.

2.5. Measurement of GABA-transaminase activity

The activity of GABA-transaminase was measured in cortical homogenates (10:1 vol/wt) containing protease inhibitors using the spectrophotometric assay

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