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High-affinity GABA uptake and GABA-metabolizing enzymes in pig forebrain white matter: A quantitative study

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Abstract

GABA receptor activation in central nervous white matter may be protective during white matter hypoxia in the adult, and it may modify axonal conduction, especially in the developing brain. GABA uptake is important for the shaping of the GABA signal, but quantitative data are lacking for GABA uptake and GABA-metabolizing enzymes in central nervous white matter. We report that high-affinity uptake of GABA in adult pig corpus callosum, fimbria, subcortical pyramidal tracts, and occipital white matter is ~20% of that in temporal cortex gray matter. Tiagabine (0.1 μM), an antiepileptic drug that specifically inhibits the GAT-1 GABA transporter inhibited GABA uptake 50% in temporal cortex and 60–68% in white structures. This finding indicates that GAT-1 is an important GABA transporter in white matter and suggests that white matter GABA uptake is inhibited during tiagabine therapy. GABA transaminase activity in white structures was ~20% of neocortical values. Glutamate decarboxylase (GAD) activity in white structures was only 4% of that in neocortex (7–12 pmol/mg tissue × min^{-1} versus ~200 pmol/mg tissue × min^{-1}). Since white matter activity of citrate synthase of the tricarboxylic acid cycle was ~25% of neocortical values (~0.4 nmol/mg tissue × min^{-1} versus ~1.5 nmol/mg tissue × min^{-1}), the low GAD activity suggests a slower metabolic turnover of GABA in white than in gray matter.

Keywords: High-affinity transport; GABA; GABA transaminase; Glutamate decarboxylase; Metabolism; Tiagabine

1. Introduction

GABAergic neurons and GABA-containing axons and nerve terminals are present in white structures of the central nervous system (Schmechel et al., 1984; Davanger et al., 1991; Wang et al., 2001; Fabri and Manzoni, 2004; Ikeda et al., 2004; Misaki et al., 2004; Tomioka et al., 2005); therefore, GABAergic neurotransmission probably occurs in white matter. Andersen (1975) described the occurrence of nerve terminals with flattened, presumably GABA-containing, vesicles that abutted nodes of Ranvier of myelinated axons in the hippocampus. $GABA_A$ and $GABA_B$ receptor subunits have been detected by immunohistochemistry on both neurons and astrocytes in white matter (Gu et al., 1992, 1993; Rosier et al., 1993; Hendry et al., 1994; Huntsman et al., 1994).

In studies on the functional roles of GABA receptors in white matter Sun and Chiu (1999) found that $GABA_A$ receptor activation reduced the amplitude of compound action potential in optic nerve from newborn rats, whereas $GABA_B$ receptor activation reduced axonal Ca^{2+} influx. Fern et al. (1995) found that $GABA_B$ receptor activation in adult rat optic nerve protected axonal function after hypoxia. Gilbert et al. (1984) showed that GABA depolarizes immature, cultured oligodendrocytes, a finding that was repeated in the corpus callosum slice (Berger et al., 1992), and, more recently, activation of oligodendrocyte precursor cells by GABAergic interneurons in the hippocampus was demonstrated (Lin and Bergles, 2004).

With respect to GABA transport across the plasma membrane the GAT-1 high-affinity GABA transporter predominates in the brain (Guastella et al., 1990; Conti et al., 1998). Minelli et al. (1995) described the presence of GAT-1 high-affinity GABA transporters in rat cerebral cortex and, at lower density, in the underlying white matter, especially on nerve fibers. Howd et al. (1997) detected GAT-1, GAT-2, and GAT-3 mRNA in adult rat optic nerve, and Pow et al. (2005) recently detected GABA transporter proteins in oligodendrocytes in human, monkey, and cat brain. However, no

Abbreviations: GABA, γ-amino-butyric acid; GABA-T, GABA transaminase; GAD, glutamate decarboxylase; GAT (1–3), GABA transporter (1–3); TCAcycle, tricarboxylic acid cycle

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information exists on the actual transport activity of high-affinity GABA uptake in central nervous white matter. This issue is important, because high-affinity GABA uptake would be a crucial element in the shaping of the GABA signal as suggested by previous studies (Frahm et al., 2001; Willmore, 2003; Sipila et al., 2004; Safiulina et al., 2006). Further, high-affinity GABA uptake is a target for antiepileptic drug therapy, and an effect of antiepileptic drugs on white matter GABA signaling might be important for the control of seizure propagation through white matter. Propagation of seizure activity from one hippocampus to the other through myelinated tracts is well documented in humans (Spencer et al., 1987; Gloor et al., 1993; Spanedda et al., 1997).

The present study was undertaken to shed light on quantitative aspects of high-affinity GABA uptake and metabolism in central nervous white matter. We studied the brain of the domestic pig because it lends itself favorably to the harvest of large amounts of pure white matter. High-affinity GABA uptake was analyzed with proteoliposomes into which GABA transporters from various brain regions had been reconstituted, a model which reproduces the high-affinity transport activity of fresh tissue and at the same time allows analysis of frozen tissue (Danbolt et al., 1990; Trotti et al., 1995, 1997; Hassel et al., 2001, 2003).

2. Materials and methods

2.1. Animals and tissue

Pigs were killed at a local slaughterhouse by electrocution and exsanguination. Brains were obtained 15 min after death and were dissected on ice. The poles of the frontal and temporal lobes were sampled; these consisted of each their gyrus, and care was taken to avoid the underlying white matter. A cerebellar cortical folium was sampled from the middle cerebellar lobe. The hippocampal formation was dissected out and freed from attached white matter. The middle part, 2 cm wide, which included the dentate gyrus, was sampled. Fimbria was removed from the hippocampus. Corpus callosum was dissected free, and the induseum griseum was avoided. Pyramidal tracts were sampled subcortically, prior to their entry into the internal capsule, and subcortical white matter was sampled subjacent to the occipital cortex. The tissue specimens were stored at −70 °C until analysis, when they were weighed in the frozen state and homogenized to 10% (w/v) homogenates in ice-cold sucrose, 0.32 M, containing dithiotreitol, 1 mM, to prevent oxidation.

2.2. High-affinity uptake of [^{3}H]GABA into proteoliposomes and fresh homogenates

Plasma membrane transporters for GABA were reconstituted in proteoliposomes according to the method of Danbolt et al. (1990) as described (Trotti et al., 1995; Hassel et al., 2003), starting from 10% homogenates (w/v) in sucrose, 0.32 M. These proteoliposomes have an internal buffer of KCl, 140 mM. Uptake of [^{3}H]GABA (New England Nuclear, Boston, MA, USA), 0.5 μM, final specific activity 5.6 mCi/μmol, was performed in triplicates in the presence of NaCl, 150 mM, and valinomycin, 1 μM, for 15 min at 30 °C; the sequestered radioactivity was trapped on filter paper and quantified by scintillation counting. Blanks were run in duplicates for each structure and were obtained by keeping the samples on ice and adding the sodium ionophore nigericin, 1 μM. Uptake was linear with time for at least 20 min. Previously, uptake by proteoliposomes made from frozen brain tissue has been shown to be the same as for fresh brain homogenates when [^{3}H]glutamate was the transported compound (Hassel et al., 2003), suggesting that freezing does not affect transport activities.

The sensitivity of high-affinity GABA uptake to tiagabine was investigated in temporal cortex and white structures. Tiagabine (Sanofi-Winthrop, Solna, Sweden), a selective inhibitor of the GAT-1 GABA transporter, with a K*i* of 0.1 μM (Thomsen et al., 1997), was added during incubation at 0.1 or 0.33 μM.

High-affinity uptake of GABA by fresh homogenate of temporal cortex gray matter (10% w/v in sucrose, 0.32 M) was done as described (Fosse et al., 1989), replacing [^{3}H]glutamate with [^{3}H]GABA.

2.3. Determination of enzyme activities and GABA levels

Glutamate decarboxylase (GAD) activity was assayed with a modification of the method of Fonnum et al. (1970). Hundred microliters of a reaction buffer consisting of sodium glutamate, 44 mM, sodium phosphate, 10 mM, pyridoxal phosphate, 0.4 mM, dithiotreitol, 2 mM, pH 7, were mixed with 5 μl of the 10% brain homogenates and incubated at 37 °C for 90 min. The reaction was stopped by addition of 105 μl perchloric acid, 7% (v/v), containing leucine, 0.1 mM. Blanks, in which 5 μl of homogenate were mixed with 105 μl perchloric acid, 7% (v/v), on ice, before the addition of 105 μl reaction buffer, were run for each sample. Protein was removed by centrifugation, and the supernatant was neutralized with KOH to precipitate the perchloric acid. GABA was quantified by HPLC and fluorescence detection after pre-column derivatization with *o*-phthaldialdehyde as described (Hassel et al., 1997). Leucine was used as an internal concentration standard because it elutes 15 min after glutamate, avoiding overlap with this peak, which was very large due to the high concentration of glutamate in the assay. Endogenous leucine levels were too low to interfere with the assay.

GABA transaminase (GABA-T) activity was analyzed as the formation of glutamate from GABA and α-ketoglutarate. Ten microliters of homogenate, 10%, were preincubated with 70 μl of a reaction buffer containing α-ketoglutarate, 5 mM, NAD^+, 2 mM, dithiotreitol, 2 mM, Hepes, 20 mM, pH 8.6, at 37 °C for 45 min. This was done to allow formation of glutamate from aspartate and other amino acids to take place prior to the addition of GABA. Twenty microliters of GABA, 40 mM, pH 8.6, were added and incubation continued for 30 min. The reaction was stopped by addition of 100 μl perchloric acid, 7% (v/v), with leucine, 0.1 mM, as internal standard. Blanks were obtained by adding the perchloric acid, 7% (v/v) after the preincubation period, i.e. prior to addition of GABA. A blank was run for each sample. Removal of protein and perchloric acid, and determination of glutamate with HPLC and fluorescence detection after pre-column derivatization with *o*-phthaldialdehyde was done as described (Hassel et al., 1997).

Citrate synthase was analyzed according to Srere (1969); fumarase was analyzed according to Hill and Bradshaw (1969). Linearity with time and amount of tissue added was established for all enzyme assays.

Immediately after homogenization of the tissue 100 μl of the homogenate were mixed 1:1 with perchloric acid, 7% (v/v), containing α-aminoadipate, 500 μM, as internal standard. Removal of protein and perchloric acid, and quantification of GABA by HPLC and fluorescence detection after pre-column derivatization with *o*-phthaldialdehyde was done as described (Hassel et al., 1997).

2.4. Data presentation and statistics

Data are presented as mean ± S.D. values. Statistical analysis was done by one-way ANOVA and Student–Newman–Keul's test for multiple pair-wise comparisons or with Dunnett's test for comparison of white matter values to values obtained for temporal cortex gray matter.

3. Results

3.1. High-affinity GABA uptake in gray and white structures

The high-affinity uptake of [^{3}H]GABA in gray matter (temporal and frontal cortical poles, hippocampal formation and cerebellar cortex) varied between ∼180 fmol/mg tissue × min^{-1} in the cerebellar cortex and ∼325 fmol/mg tissue × min^{-1} in gray matter of the temporal cortex (Fig. 1A).