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Determination of L-glutamic acid and γ–aminobutyric acid in mouse brain tissue utilizing GC–MS/MS

Christine A. Farthing[a], Don E. Farthing[a,b], Ronald E. Gress[b], Douglas H. Sweet[a,*]

[a] Virginia Commonwealth University, Department of Pharmaceutics, Richmond, VA 23298, USA
[b] National Institutes of Health, National Cancer Institute, Experimental Transplant and Immunology Branch, Bethesda, MD 20892, USA

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ABSTRACT

A rapid and selective method for the quantitation of neurotransmitters, L-Glutamic acid (GA) and γ–Aminobutyric acid (GABA), was developed and validated using gas chromatography-tandem mass spectrometry (GC–MS/MS). The novel method utilized a rapid online hot GC inlet gas phase sample derivatization and fast GC low thermal mass technology. The method calibration was linear from 0.5 to 100 μg/mL, with limits of detections of 100 ng/mL and 250 ng/mL for GA and GABA, respectively. The method was used to investigate the effects of deletion of organic anion transporter 1 (Oat1) or Oat3 on murine CNS levels of GA and GABA at 3 and 18 mo of age, as compared to age matched wild-type (WT) animals. Whole brain concentrations of GA were comparable between WT, Oat1$^{-/-}$, and Oat3$^{-/-}$ 18 mo at both 3 and 18 mo of age. Similarly, whole brain concentrations of GABA were not significantly altered in either knockout mouse strain at 3 or 18 mo of age, as compared to WT. These results indicate that the developed GC–MS/MS method provides sufficient sensitivity and selectivity for the quantitation of these neurotransmitters in mouse brain tissue. Furthermore, these results suggest that loss of Oat1 or Oat3 function in isolation does not result in significant alterations in brain tissue levels of GA or GABA.

1. Introduction

L-Glutamic acid (GA) and γ-aminobutyric acid (GABA) are neurotransmitters found in the brains of mammals (Fig. 1). GABA is synthesized from GA by glutamate decarboxylase, yet the functions of these two neurotransmitters have opposing effects. GA is considered a major excitatory neurotransmitter in the CNS, while GABA is considered a major inhibitory neurotransmitter. Quantitation of these two neurotransmitters is important to the field of neuropharmacology, as they have been associated with learning and memory, the sleep cycle, and clinical conditions including schizophrenia, Alzheimer's disease, Parkinson's disease, depression, anxiety and obsessive-compulsive disorder [1–9].

High performance liquid chromatography with electrochemical detection (HPLC-ECD) techniques have previously been used to study amino acid concentrations in the brain [10–13]. Since the amino acids are not naturally electroactive, these methods typically require a derivatization step (usually using o-phthalaldehyde in the presence of a thiol, e.g., β-mercaptoethanol, or a sulfite group). However, despite the sensitivity that can be achieved using HPLC-ECD, the electroactive derivatives are often unstable, and it has been reported that resolution may be problematic due to unknown peaks of biological origin that elute closely to GABA [14–16].

In addition, there have been several publications using liquid chromatography-tandem mass spectrometry (LC–MS/MS) for the analysis of GA and GABA in biological samples [17–19]. However, to our knowledge, there are no currently published methods utilizing gas chromatography-tandem mass spectrometry (GC–MS/MS) offering quantitation of both GA and GABA in brain tissue. In this article, we describe a rapid and selective GC–MS/MS method for the detection and quantitation of GA and GABA in mouse brain homogenate. Stable isotopes of each compound were used as internal standards. The use of MethElute™ reagent (as described in [20]) rapidly derivatized GA and GABA and their isotopologues in the heated GC injection port, which required less than 20 s for completion and utilization of the low thermal mass (LTM) technology provided high resolution and fast chromatography (Fig. 1). The method demonstrated excellent linearity from 0.5 to 100 μg/mL, with limits of detections of 100 ng/mL and 250 ng/mL for GA and GABA, respectively. The method was successfully used to assess the effects of deletion of the Solute Carrier 22 family members organic anion transporter 1 (Oat1) or Oat3 on mouse brain tissue concentrations of GA and GABA.

* Corresponding author at: Department of Pharmaceutics, Virginia Commonwealth University, 410 N 12th St, PO Box 980533, Richmond, VA 23298, USA.
E-mail address: dsweet@vcu.edu (D.H. Sweet).

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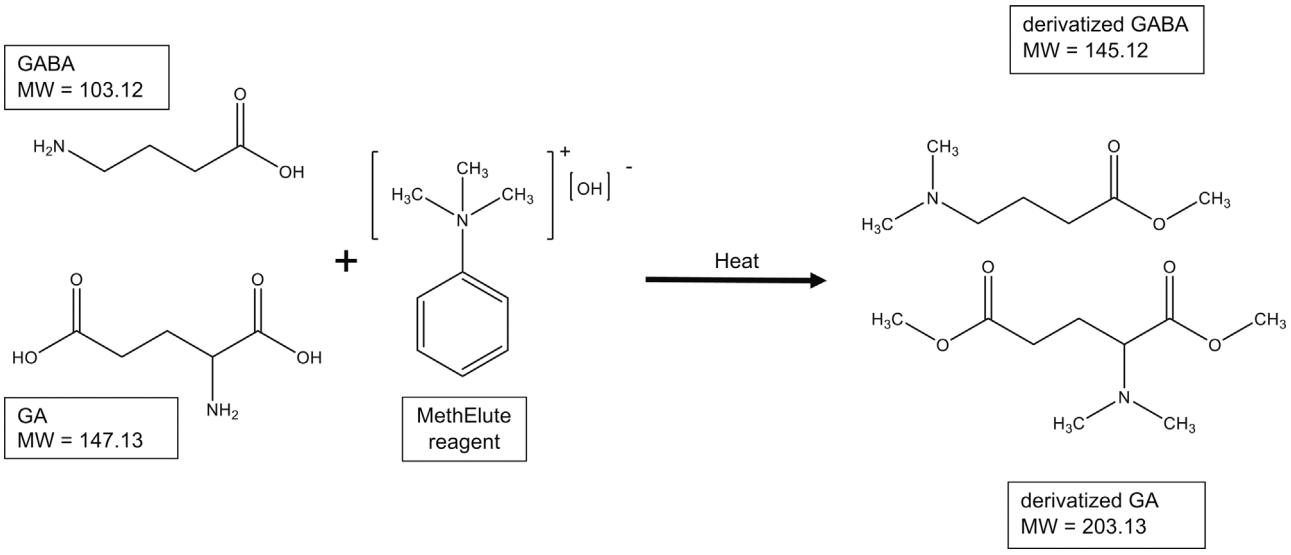

Fig. 1. Structures depicting the methylation sites (i.e., labile hydrogens) for L-Glutamic Acid and γ-Aminobutyric Acid. The MethElute derivatization occurs rapidly in a deactivated GC liner located in the heated GC inlet.

Table 1
Agilent 7890A GC and Autosampler Parameters.

GC Inlet	
Mode	Pulsed Splitless
Temperature (°C)	235
Total Flow (mL/min Helium)	54
Septum Purge Flow (mL/min Helium)	3
Injection Pulse Pressure (psi until 1 min)	25
Purge flow to split vent (mL/min Helium at 1.1 min)	50
Gas saver (mL/min Helium after 3 min)	15
GC Program	
Initial GC Oven Temperature (°C)	30
Initial Oven Hold Time (min)	1
Rate (°C/min)	75
Final GC Oven Temperature (°C)	300
Final Over Hold Time (min)	1.4
Run Time (min)	6
Equilibration Time (min)	0.1
GC Transfer Line (°C)	300
Oven Max Temperature (°C)	340
GC Column	
LTM Column	DB–5 ms, 15 m x 250 μm x 0.25 μm
Head Pressure (psi)	2.14
Flow (mL/min Helium)	1
Average Velocity (cm/sec)	47
Holdup Time (min)	0.5
GC Autosampler	
Syringe Size (μL)	10
Injection Volume (μL)	1
Post Washes A	3 with ethanol
Post Washes B	3 with acetone
Samples Washes	1
Sample Wash Volume (μL)	4
Samples Pumps	3
Viscosity Delay (sec)	3
Air Gap (μL)	0.2
GC Autosampler Barcode Mixer	
Mixer	Enabled
Mixer Cycle	1
Mixer Time (sec)	10
Mixer Speed (rpm)	2000

Table 2
Agilent 7000A MS Parameters.

Helium Quench Gas (mL/min)	2.25
Nitrogen Collision Gas (mL/min)	1.5
Isobutane Reagent Gas (mL/min)	2
Ion Source Temp (°C)	350
Quad 1 Temperature (°C)	150
Quad 3 Temperature (°C)	150
Ion Source	Chemical Ionization (CI)
Mode	Positive Ion (PCI)
Electron Energy Mode	Use tune settings
Solvent Delay (min)	1.0
Run Time (min)	6.0
Time Filter Enabled	
Peak Width	0.7
MS1 Resolution	Unit
MS2 Resolution	Unit
Dwell Time (msec)	50
Scan Rate (cycles/sec)	5
Collision Energy-GA (V)	15
Collision Energy-GABA (V)	20
Electron Multiplier (V)	1600
Delta EMV (V)	400
HED (kV)	−10
Instrument Tuning (Mass Calibration)	
Tune MS weekly or as necessary	
MRM Transitions	
GA derivative (precursor ion/product ion)	*m/z* 204/144
d5-GA derivative (precursor ion/product ion)	*m/z* 209/148
GABA derivative (precursor ion/product ion)	*m/z* 146/101
D4-GABA derivative (precursor ion/product ion)	*m/z* 150/105

2. Materials and methods

2.1. Chemicals, reagents, and gases

Standard material of L-Glutamic acid and γ-aminobutyric acid were purchased from Sigma Aldrich (St. Louis, MO). The $^{13}C_5$-L-Glutamic acid (M + 5 stable isotope, purity 99%) and U-$^{13}C_4$-4-aminobutyric acid (M + 4 stable isotope, purity 98%) internal standards were purchased from Cambridge Isotopes (Andover, MA).

MethElute™ derivatization reagent (0.2 M trimethylanilinium hydroxide in methanol, pH ≥ 10) was purchased from Thermo Scientific (Waltham, MA). Methanol (MeOH) (HPLC grade, 99.9% purity) was purchased from Acros Organics (Fair Lawn, NJ). Ultrapure deionized