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Analysis of amino acid neurotransmitters from rat and mouse spinal cords by liquid chromatography with fluorescence detection

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ABSTRACT

A RP-LC-FL detection method has been developed to identify and quantitate four amino acid neurotransmitters including glutamic acid, glycine, taurine and γ-aminobutyric acid in rat and mouse spinal cord tissue. 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde (CBQCA) was employed for the derivatization of these neurotransmitters prior to RP-LC-FL analysis. Different parameters which influenced separation and derivatization were optimized. Under optimum conditions, linearity was achieved within the concentration ranges of 0.50–50.00 μM for all analytes with correlation coefficients from 0.9912 to 0.9997. The LODs ranged from 0.03 μM to 0.06 μM. The proposed method has been successfully applied to the determination of amino acid neurotransmitters in biological samples such as rat and mouse spinal cord with satisfactory recoveries.

1. Introduction

Amino acids play a major role in energy metabolism, neurotransmission, and lipid transport and their quantitative analysis is increasingly important in disease diagnostics, and in elucidating nutritional influences on physiology [1]. Over the past several years, amino acid neurotransmitters have been the focus of much attention in biomedical research, medical diagnostics, clinical chemistry, and the pharmaceutical industry, because they play essential roles in control and regulation of various functions in the central and peripheral nervous system [2,3]. The most studied amino acid neurotransmitters are glutamic acid (Glu), γ-aminobutyric acid (GABA), glycine (Gly) and taurine (Tau) (Fig. 1). As the major excitatory neurotransmitters in the mammalian central nervous system (CNS), Glu is present in more than half of all CNS synapses, which underscores their important involvement in learning, memory, sleep, movement, and feeding [4]. GABA, Gly, and Tau are inhibitory neurotransmitters in the CNS [5]. In fact, as many as 10–40% of nerve terminals in the hippocampus and cerebral cortex may use GABA as a neurotransmitter to transmit "closure" signals [6]. Gly, plays key roles in postsynaptic inhibition, sensorimotor function, and abnormal startle responses. The inhibitory amino acid Tau is an osmoregulator and neuromodulator, and also exerts neuroprotective actions in neural tissue [7,8].

In addition, evidences showed that the changes in amino acid neurotransmitter levels in biological samples was correlated with a number of neurological diseases such as Alzheimer's [9], Parkinson's [10], stroke [11], epilepsy [12], and schizophrenia [13]. Hence, determination of these neurotransmitter levels in biological samples may provide a means of diagnosis and possible treatment of neuropsychiatric diseases. Moreover, the components of these biological samples are quite complex (from amino acids, peptides, to protein). It is therefore necessary to develop an improved, new, rapid, selective, accurate, precise, sensitive, fully validated technique for the determination of neurotransmitters in rat spinal cord tissues.

There are multiple separation methods reported for these amino acid neurotransmitters including separation approaches such as gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) coupled with different detection techniques [14–16]. HPLC is commonly used for quantification, because these systems are widely commercially available and can be very robust. In recent years, several papers have reported the use of HPLC coupled with fluorescence detection (FLD) [17] or mass spectrometric (MS) detection [18] to resolve mixtures.

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0731-7085/

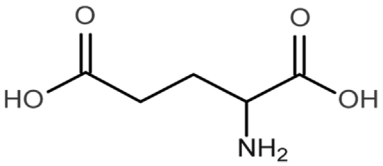
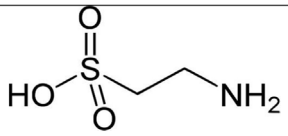
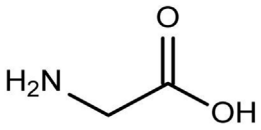
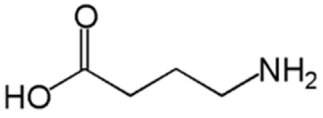
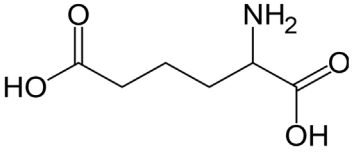
Compound	Chemical Structure
Glutamic acid (Glu) 2-Aminopentanedioic acid pK_{a1} = 2.10 (carboxyl group) pK_{a2} = 4.07 (side chain) pK_{a3} = 9.47 (amino group) CAS No: 617-65-2	 $C_5H_9NO_4$ MW: 147.13 g mol^{-1}
Taurine (Tau) 2-aminoethanesulfonic acid pK_{a1} = 1.5 pK_{a2} = 9.06 CAS No: 107-35-7	 $C_2H_7NO_3S$ MW: 125.15 g mol^{-1}
Glycine (Gly) Aminoacetic acid pK_{a1} = 2.35 (carboxyl group) pK_{a2} = 9.78 (amino group) CAS No: 56-40-6	 $C_2H_5NO_2$ MW: 75.07 g mol^{-1}
γ-aminobutyric acid (GABA) pK_{a1} = 4.23 (carboxyl group) pK_{a2} = 10.43 (amino group) CAS No: 56-12-2	 $C_4H_9NO_2$ MW: 103.12 g mol^{-1}
2- aminoadipic acid (2-AAP) IS 2-aminohexanedioic acid pK_{a1} = 2.14 (carboxyl group) pK_{a2} = 4.21 (side chain) pK_{a3} = 9.77 (amino group) CAS No: 542-32-5	 $C_6H_{11}NO_4$ MW: 161.16 g mol^{-1}

Fig. 1. Chemical structures of studied compounds.

Derivatization (pre- or post-separation) is often necessary due to the lack of a chromophore for UV–vis and fluorescence detection [19,20]. The main advantage of manual derivatization is the relatively free choice of the reaction conditions. Most of the research on amino acids in biological samples has been performed with six different labeling agents: NDA (naphthalene dicarboxaldehyde) [21,22], OPA [23], FQ (3-(2-furoyl)quinoline-2-carboxaldehyde) [24], CBQCA (3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde) [25], fluorescein derivatives as FITC (fluorescein-5-isothiocyanate) or CFSE (carboxyfluorescein succinimidyl ester) [26].

The CBQCA reagent is virtually nonfluorescent in aqueous solution; however, in the presence of cyanide, it reacts with primary amines at room temperature such as those found in spinal cord to form highly fluorescent derivatives. The reaction scheme for derivatizing primary amines with CBQCA is given in Fig. 2. The high sensitivity, freedom from background and long-wavelength excitability makes this a potential reagent for research and biological sample applications. Compared to fluorescein based reagents, less interference in chromatograms can be expected because of the decreased number of side products and minimized effects of the excess reagent.

This work describes, a rapid, selective, accurate, precise, sensitive, fully validated RP-LC-FL method to quantify the Glu, GABA, Tau, Gly in rat and mouse spinal cord tissues as well as its application to real sample analysis.