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Short communication

Simultaneous determination of γ-aminobutyric acid and glutamic acid in the brain of 3-mercaptopropionic acid-treated rats using liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

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Abstract

The measurement of γ-aminobutyric acid (GABA) and glutamic acid (Glu) in the whole brain and in various regions of the brain in 3-mercaptopropionic acid (3-MPA)-treated rats has been developed using liquid chromatography–mass spectrometry with an atmospheric pressure ionization interface system. The recoveries of these compounds were 94.90±4.18% for GABA, 95.60±2.86% for Glu after ion-exchange treatment. The detection limits for GABA and Glu were 2.5±0.3 μg/ml and 5.0±0.8 μg/ml, respectively, when 20μl sample were injected. GABA concentration in the whole brain decreased gradually to 5 min and reached 63% of normal value after administration of 3-MPA, and the concentration increased gradually thereafter until 60 min. Conversely, the concentration of Glu in the whole brain increased gradually to 10 min and reached 154% of normal value, and after that decreased gradually and reached almost normal level at 60 min after administration of 3-MPA. GABA concentration in various regions of brain decreased to 5 min in all regions after administration of 3-MPA, and reached normal levels at 60 min as in the whole brain. This method was found to be useful for studies of metabolism of GABA and Glu in biological samples.

Keywords: γ-Aminobutyric acid; Glutamic acid

1. Introduction

γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter in the mammalian central nervous system [1]. The glutamate decarboxylase (GAD, EC 4.1.1.15) catalyzes formation of γ-aminobutyric acid from glutamate [2]. The enzyme is present in the mammalian brain in two forms coded by two different genes. 3-Mercaptopropionic acid (3-MPA) is a competitive, relatively specific inhibitor of glutamate decarboxylase. The ratio of the two forms may be changed during ontogenesis [3], and it seems to be different in various parts of rat brain [4]. Sprice et al. [5] have reported that 3-MPA and 4-mercaptobutyric acid produced convulsions in mice. Lamar [6] confirmed this effect and demonstrated that the inhibition of GAD by this compound is realized by

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competition with glutamate. Karlsson et al. [7] found strong GAD inhibition and a decrease in GABA levels in different regions of rat brain as early as 4 min after intraperitoneal injection of 3-MPA (50 mg/kg). Löscher et al. [8] found a decrease in GABA concentration in whole tissue and synaptosomal fractions in most of eleven rat brain regions prepared even 3 min after intraperitoneal administration of 3-MPA (50 mg/kg). Mares et al. [9–11] found that when 3-MPA was administered, tonic–clonic seizures in the immature were more latent then adult rats, and GAD inhibition in the immature was more pronounced than that in the adult .

To the best of our knowledge, the time course of GAD activity in brain after 3-MPA administration has been investigated, but the time course of Glu in whole brain and six regions of brain has not been studied yet. Although Alsip et al. [12] examined GABA levels in four regions (cerebellum, hypothalamus, medullapons, cerebral cortex) after systemic administration of 3-MPA, the time course of GABA levels in hippocampus, mesencephalon and caudatus has not been studied.

In the present study, we developed a simple method of simultaneous determination of the contents of GABA and Glu in whole brain and six regions of brain in 3-MPA treated rats using liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (LC–APCI–MS). The temporal relationship seizure and the amino acid neurotransmitter levels was also studied.

2. Experimental

2.1. Reagents

GABA, Glu and 3-Mercaptopropionic acid (3-MPA) were obtained from Wako Pure Chemical Ind. (Osaka, Japan). All other chemicals used were analytical reagents.

2.2. Animals and brain samples

Male Wistar rats weighing 200±5 g were used and fed a MF diet of Oriental Yeast (Tokyo, Japan). The rats were administered with 3-MPA (5 mg/200 g body weight) dissolved in 0.9% NaCl intraperitoneally. Physiological saline solution (the same volume with that of 3-MPA) was injected into control rats (three rats). 3-MPA-treated rats were killed by decapitation at 1, 3, 5, 10, 15, 20, 30 and 60 min after administration of 3-MPA.

The brains were then removed, blotted, separated into six regions (cerebral cortex, hippocampus, caudatum, thalamus and hypothalamus, cerebellum and mesencephalon) as previously described [13] and stored at −80°C if not analyzed immediately.

2.3. Determination of GABA and Glu

For the determination of GABA and Glu in the whole brain and six regions of brain, each brain tissue (1.45–1.71 g) was homogenized in four volumes of 2% sulfosalicylic acid respectively and the homogenate was centrifuged at 3000 *g* for 15 min. The supernatant was applied to a column containing 5 ml of Diaion SK-1 (H^+-form of sulfonated cation exchanger, 100 mesh; Mitsubishi Kasei, Tokyo, Japan), washed with 30 ml of deionized water and eluted with 30 ml of 2 *M* NH_4OH. The effluent was evaporated to dryness under reduced pressure with a rotary evaporator at 40°C. The dried residue was dissolved in an aliquot of water and analyzed by LC–APCI–MS and an amino acid analyser.

Recovery experiments were performed using the whole brain extracts. A mixture containing 10 μg/20 μl each of GABA and Glu was added into 0.2 ml of a sulfosalicylic acid homogenate of the whole brain. The solution was applied to a Diaion SK-I (H^+-form) column. The column was washed with 30 ml of deionized water and thereafter eluted with 30 ml of 2 *M* NH_4OH. The eluate of NH_4OH was dried under reduced pressure with a rotary evaporator at 40°C. The residue was dissolved in 0.2 ml of deionized water and 20 μl of the solution was analysed by LC–APCI–MS.

2.4. Instrumentation

The apparatus used was a Hitachi L-6200 high-performance liquid chromatography (HPLC) instrument, equipped with a 5-μm Inertsil ODS-2 packed column (150×4.6 mm I.D.) from Gasukuro Kogyo (Tokyo, Japan), connected to a Hitachi M80B mass

spectrometer–computer system through the APCI interface (LC–APCI–MS) [14,15]. The nebulizer and vaporizer temperatures were 280 and 390°C, respectively. The determinations of GABA and Glu were carried out with a mobile phase composed of 10% acetonitrile in 100 mmol/l ammonium acetate at a flow-rate of 0.9 ml/min, and scanned using a mass spectrometer at a rate of 4 s per scan.

The amino acid analyser used was a Hitachi Model 835 liquid chromatograph.

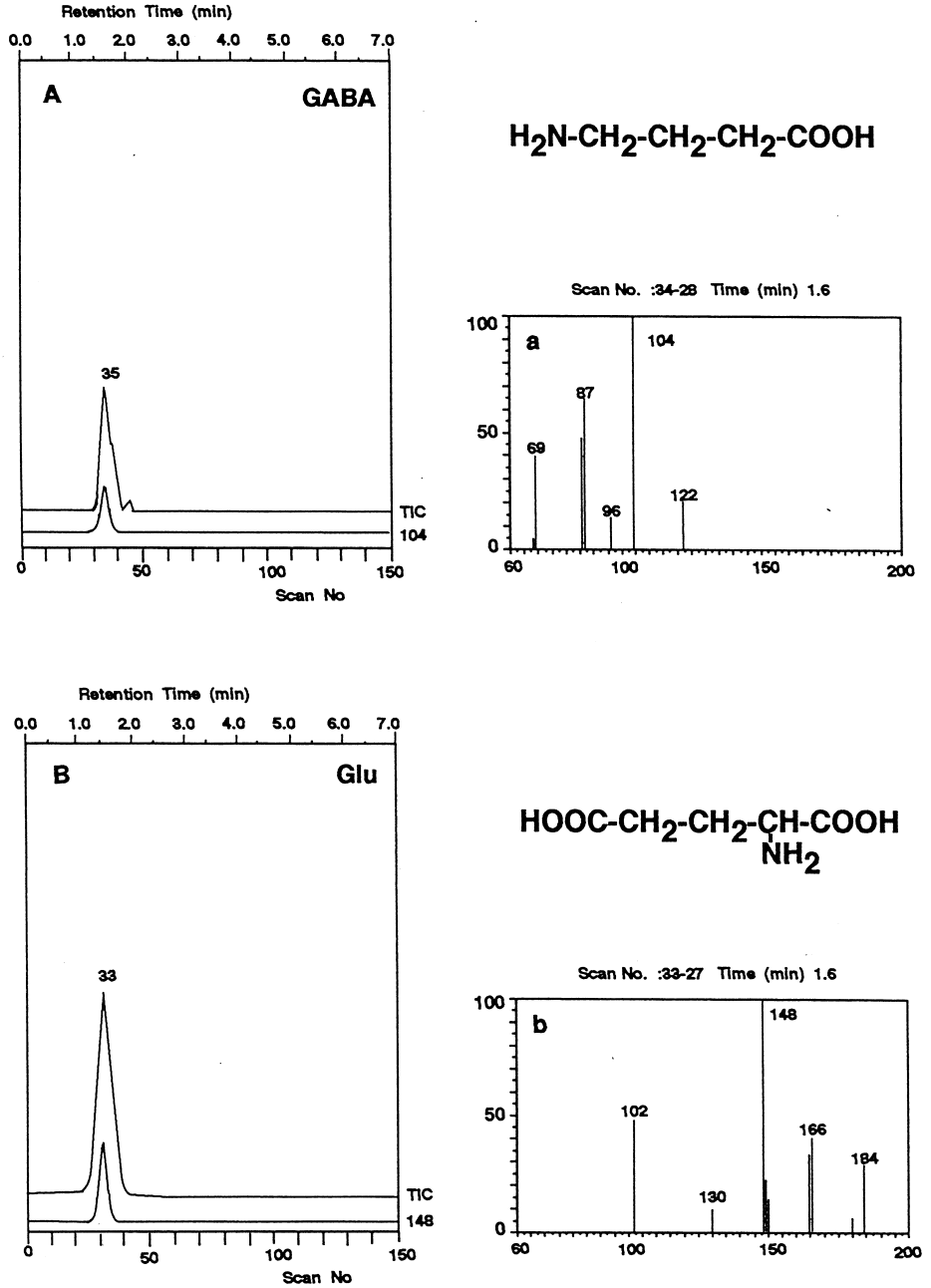

Fig. 1. Mass chromatograms and spectra of synthetic GABA(A,a) and Glu (B,b). The contents of GABA and Glu were 1000 ng. (Concentration was 0.1 μg/μl, 10 μl was injected.)

3. Results and discussion

Simultaneous determination of GABA and Glu in whole brain and six regions of rat brain was carried out using LC–APCI–MS [14,15]. Mass chromatograms and spectra of synthetic GABA and Glu are shown in Fig. 1 (A, a; B, b). In the LC–APCI–MS system, the quasi-molecular ions $[M+H]^+$ of GABA and Glu were observed as base peaks at m/z 104 for GABA and m/z 148 for Glu, respectively. The mass chromatograms of the authentic mixture of GABA and Glu, and GABA and Glu in the whole brain are shown in Fig. 2A and B. The retention times of the peaks of GABA and Glu in whole brain were the same as those of authentic samples. The recoveries ($n=6$) of authentic GABA and Glu added to sulfosalicylic acid homogenate of whole brain after treatment with ion-exchange resins were 94.90±4.18% (range 89.7–98.1) and 95.16±2.86% (range 92.91–100.48), respectively. The limits of detection and quantification were derived from multiple measurements in the low concentration range. The limit of detection is defined as the level three times the noise level, according to the IUPAC convention. Similarly, the limit of quantification is defined as the level ten times the noise level. The limits of detection for GABA and Glu were 2.5±0.3 μg/ml and 5.0±0.8 μg/ml, respectively (20 μl were injected), and the limits of quantification for GABA and Glu were 1.0±0.15 μg/ml and 2.0±0.35 μg/ml, respectively (20 μl were injected). The calibration curves of the two compounds were constructed using a fresh solution of synthetic compounds prepared just before determination. The regression line for GABA was: Y (peak-area)=1.112 Å$\times 10^4 X$ −7.011 Å$\times 10^5$, the range was from 100 to 1500 ng, and that for Glu was: Y (peak-area)=1.617 Å$\times 10^4 X$ −1.293$\times 10^6$, the range was from 200 to 2000 ng, respectively. The correlation coefficients of the two lines were 0.9998 for GABA and 0.9999 for Glu, respectively. The values of these compounds obtained using the present method coincided with the

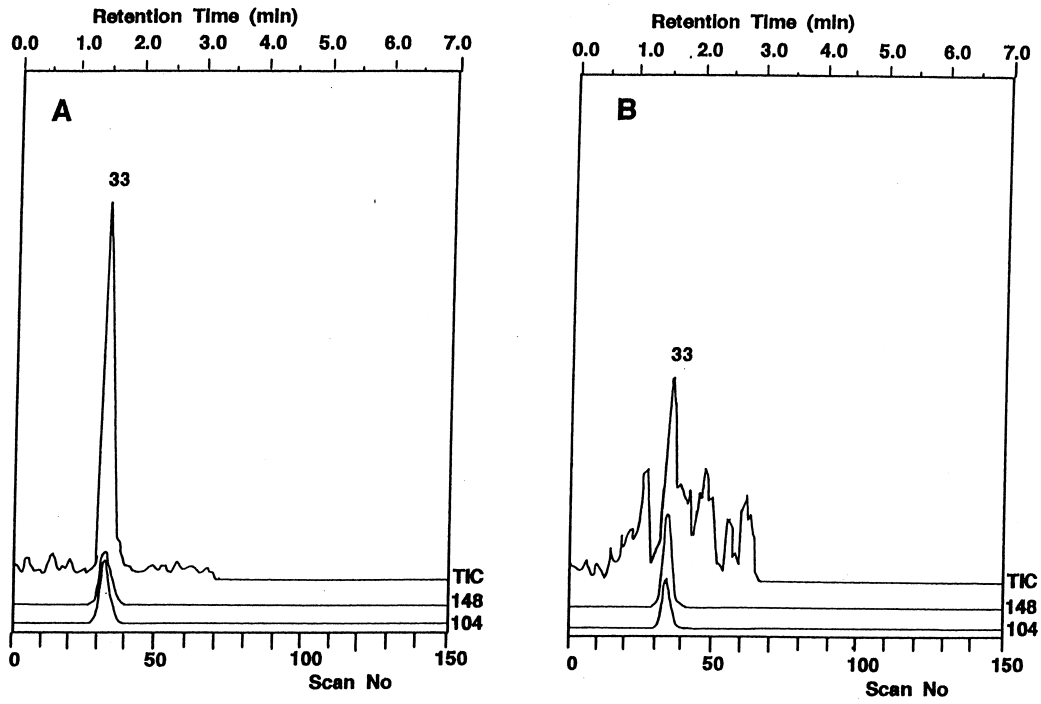

Fig. 2. Mass chromatograms of the mixture (each 1000 ng) of synthetic GABA and Glu (A), and the whole brain sample from 3-MPA non-treated rat (B), the sample corresponding to 0.5 mg of fresh tissue was analysed by LC–APCI–MS.

Table 1
Determination of GABA and Glu in whole brain of 3-MPA- treated rats[a]

Time after 3-MPA injection (min)	GABA (μg/g)		Glu (μg/g)	
	LC–APCI–MS	AAA	LC–APCI–MS	AAA
0	318±43	320±22	1202±139	1309±150
3	249±37	248±29	1621±122	1791±156
5	203±31	206±31	1752±143	1865±124
10	228±32	223±25	1856±169	2039±119
20	267±39	249±24	1403±151	1683±105

[a] Values are expressed as mean ±S.D. of data obtained from five rats.

values obtained by the amino acid analyser as shown in Table 1. These results indicate that the present method is reliable for the measurement of these compounds.

Therefore, in the present study, the contents of GABA and Glu in the whole brain of rats were determined using LC–APCI–MS at 1, 3, 5, 10, 15, 20, 30, and 60 min after the intraperitoneal injection of 5 mg of 3-MPA/200 g of body weight. The GABA concentration level in whole brain decreased until 5 min after the injection of 3-MPA, after that, the level increased gradually until 60 min as shown in Fig. 3. Whereas the level of Glu increased until 10 min after the injection of 3-MPA, the level of Glu decreased gradually until 60 min (Fig. 3) and reached almost control levels. The time profiles of glutamate decarboxylase activity in whole brain of rat after 3-MPA administration reported in the previous paper [11] were almost the same as the time profile of the GABA level in whole brain of rat obtained in the present method. The time profiles of GABA and Glu levels in various regions of brain after 3-MPA administration were also investigated (data not shown). GABA levels in various regions of control rat brains (0 min) are different in each region as reported in the previous papers [16–19]. The GABA levels in six regions of brain (cerebral cortex, hippocampus, caudatus, thalamus and hypothalamus, cerebellum and mesencephalon) decreased until 5 min after injection of 3-MPA, and then the levels increased until 60 min as in the whole brain, and reached almost control levels. The time profiles of Glu levels in other regions, except for caudatum, thalamus plus hypothalamus, were also similar to that of the whole brain. The onset of convulsions was at 5 min after the injection of 3-MPA when GABA concentration was lowest. The GABA levels of mesencephalon at 5 min after 3-MPA administration was the lowest compared with other regions of the brain. This might be due to the different penetration of 3-MPA into various parts of the brain. At present, we have no data supporting problems described above, but the penetration of 3-MPA into brain regions might explain the different GABA level in various regions of brain.

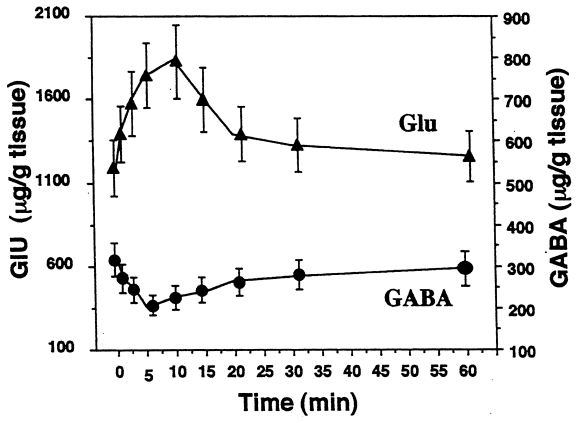

Fig. 3. Time course of relative changes of GABA and Glu levels in whole brain after 3-MPA administration (5 mg/200 g body weight). Values are expressed as mean of data obtained from 10 rats. The details of procedures were described in Experimental.

In conclusion, simultaneous determination of GABA and Glu using LC–APCI–MS were shorter in time than other instruments like amino acid analyser. These results indicate that the present method might be useful for the study of metabolism of GABA and Glu in animal brains.

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