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Journal of Chromatography B, 792 (2003) 99–106
JOURNAL OF CHROMATOGRAPHY B
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Effect of valproic acid on the urinary metabolic profile of a patient with succinic semialdehyde dehydrogenase deficiency

Toshihiro Shinka[a,*], Masafumi Ohfu[b], Shinichi Hirose[b], Tomiko Kuhara[a]

[a]*Division of Human Genetics, Medical Research Institute, Kanazawa Medical University, 1-1 Daigaku, Uchinada-machi, Ishikawa 920-0293, Japan*
[b]*Department of Pediatrics, Fukuoka University School of Medicine, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan*

Received 8 November 2002; received in revised form 3 March 2003; accepted 3 March 2003

Abstract

The metabolic changes in a patient with succinic semialdehyde dehydrogenase deficiency were investigated following valproate administration using urease pretreatment and gas chromatography-mass spectrometry. A stable isotope dilution technique was used for quantification of urinary 4-hydroxybutyrate. Urinary levels of 4-hydroxybutyrate were 4-fold higher after 1-month valproate therapy. 4,5-Dihydrohexanoate, 2-deoxytetronate and 3-deoxytetronate were also 1.7–2.7-fold higher. The urinary excretions of 4-hydroxybutyrate in valproate non-medicated controls were age dependence and decreased with age. Relationships between 4-hydroxybutyrate excretion and 4-hydroxyvalproate or 5-hydroxyvalproate excretion were observed in valproate medicated controls. It seems that 4-hydroxyvalproate and 5-hydroxyvalproate as well as valproate are involved with increased excretion of 4-hydroxybutyrate following valproate administrations.

Keywords: Succinic semialdehyde dehydrogenase deficiency; Valproic acid

1. Introduction

Succinic semialdehyde dehydrogenase (SSADH) deficiency (McKusick 271980) is an inborn error of 4-aminobutyrate (GABA) metabolism [1]. The clinical and biochemical findings have been summarized in several reports [2–4]. Increased 4-hydroxybutyrate (GHB) in the patients with SSADH deficiency were found not only in urine but also blood and cerebrospinal fluid using stable isotope dilution technique combined with gas chromatography-mass spectrometry (GC–MS) [5]. The diagnosis of SSADH deficiency is usually based on elevated concentrations of urinary GHB.

We reported the siblings who were the second and third cases of SSADH deficiency in Japan [6,7]. The younger patient was controlling epileptiform attacks by taking valproate (VPA, antiepileptic drug for treating generalized epilepsy), but then began vigabatrin (γ-vinyl GABA, irreversible inhibitor of GABA-transaminase) therapy [7]. Increases in urinary excretion of GHB after administration of VPA to a patient with SSADH deficiency has been reported by Divry et al. [8]. The effects of VPA on GHB metabolism have been discussed and it is suspected that the inhibition of SSADH by therapeutic levels of

*Corresponding author. Tel.: +81-76-286-2464; fax: +81-76-286-2312.
E-mail address: shinka@kanazawa-med.ac.jp (T. Shinka).

1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00276-9

VPA results in enhanced GHB production. However, the mechanism of GHB accumulation is still under investigation. There are no reports on the relationship between GHB accumulation and VPA metabolism. In this report, we examined the urinary GHB levels in patients and controls using a sensitive quantification method, which consists of urease digestion, stable isotope dilution and GC–MS. We

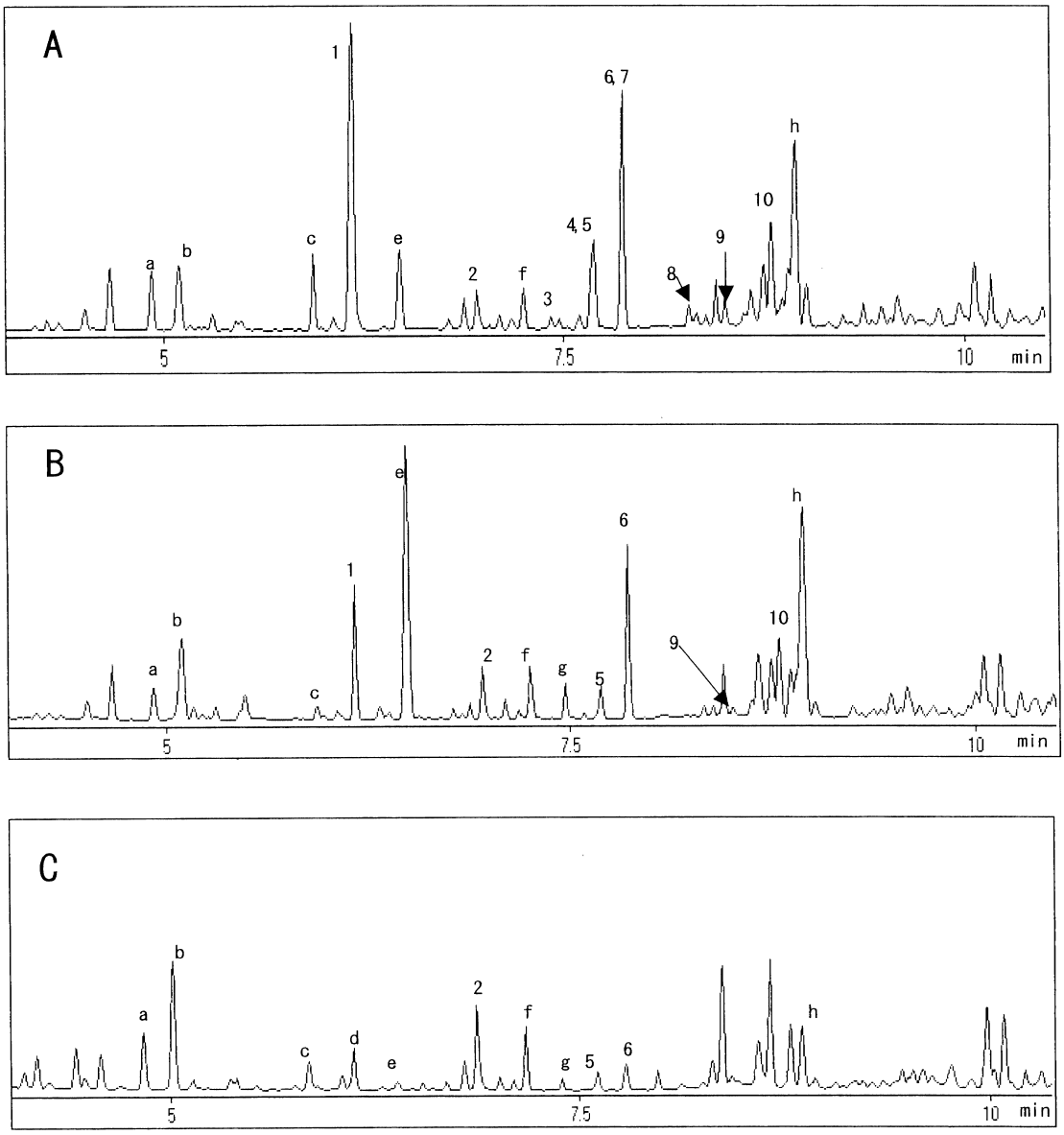

Fig. 1. Total ion current chromatograms of the trimethylsilyl (TMS) derivatives of the urinary metabolites in the patient with SSADH deficiency before and after valproate administration (relative abundance on the *y*-axis). (A) After VPA treatment (1 month after), (B) before VPA treatment), (C) control. Peaks are: (1) GHB; (2) 2,2-dimethylsuccinate (IS); (3) 4HVPA; (4) glutarate (GA); (5) 3DT; (6) 2DT; (7) 3KVPA; (8) 5HVPA; (9) adipate (AD); (10) 4,5DH; (a) alanine; (b) glycine; (c) β-aminoisobutyrate; (d) urea; (e) phosphate; (f) serine; (g) threonine; and (h) creatinine.

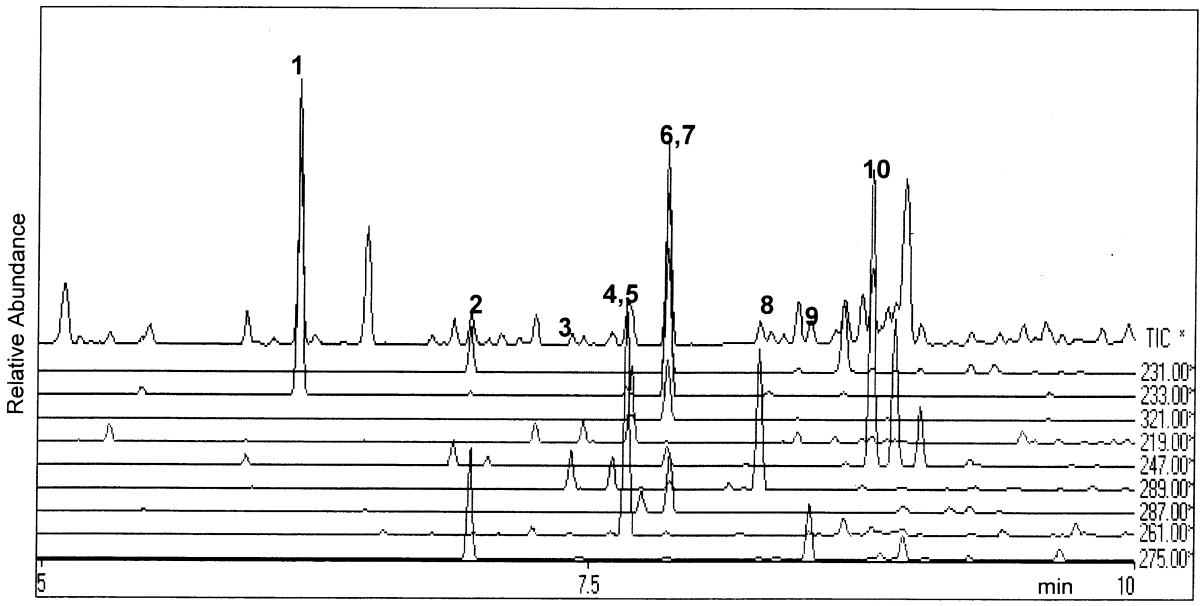

Fig. 2. Mass chromatograms of the trimethylsilyl (TMS) derivatives of the urinary metabolites in the patient with SSADH deficiency. The ions targeted were *m*/*z* 231 for 2,2-dimethylsuccinate (IS), *m*/*z* 233 for GHB, *m*/*z* 321 for 2DT, *m*/*z* 219 for 3DT, *m*/*z* 247 for 4,5DH, *m*/*z* 289 for 4HVPA and 5HVPA, *m*/*z* 287 for 3KVPA, *m*/*z* 261 for glutarate and *m*/*z* 275 for adipate. Peak identifications are same as Fig. 1.

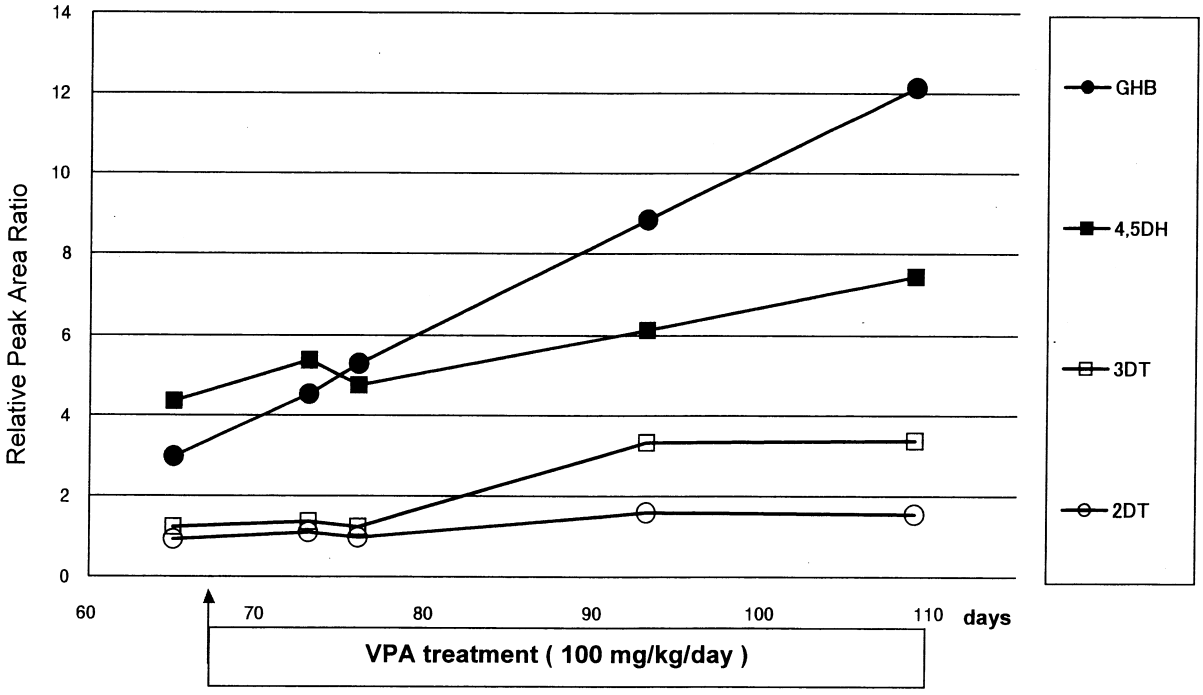

Fig. 3. Urinary excretion of GHB and its related metabolites in the patient with SSADH deficiency after VPA administration. Urease digestion method was used for sample preparation, and 2,2-dimethylsuccinate was used as an internal standard. The ions targeted were same as Fig. 2.

also analyzed VPA metabolites in VPA medicated patients, and discuss the effects of VPA on GHB metabolism.

2. Experimental

2.1. Chemicals

We obtained 4-hydroxybutyric acid sodium salt from Tokyo Kasei Kogyo (Tokyo, Japan), and urease (type C-3: from Jack beans) from Sigma (St. Louis, MO). Gamma-butyrolactone-d_6 (GBL-d_6, 99.5 atom %) was purchased from CDN (Quebec, Canada). GHB-d_6 (0.5 μmol/ml) stock solution was prepared by dissolving GBL-d_6 in 0.1 N NaOH [9]. Other chemicals are same as described [7].

2.2. Samples

The male patient (2-month-old, the younger of the siblings) with SSADH deficiency has already been described [7]. Treatment with VPA was started when the patient was 69 days old (100 mg/kg/day). Urine was collected before VPA administration and on days 4, 7, 24 and 40 after the start of administration. Urine specimens from patients with no metabolic disorders aged from 1-month-old to 7-years-old (VPA medicated; $n=23$, VPA non-medicated; $n=20$) were also examined. All samples were stored at −20 °C until analysis.

2.3. Sample preparation

Samples were prepared and derivatized as described [6,7,10] additional use of GHB-d_6 as the internal standards. In brief, 0.1 ml of urine was digested with 20 units of urease at 37 °C for 10 min. After adding 5 nmols of GHB-d_6 and 25 nmoles of 2,2-dimethylsuccinic acid as the internal standard, the urine was deproteinized with 1 ml ethanol. The precipitate was removed by centrifugation, and then the supernatant was concentrated under reduced pressure and evaporated to dryness under nitrogen

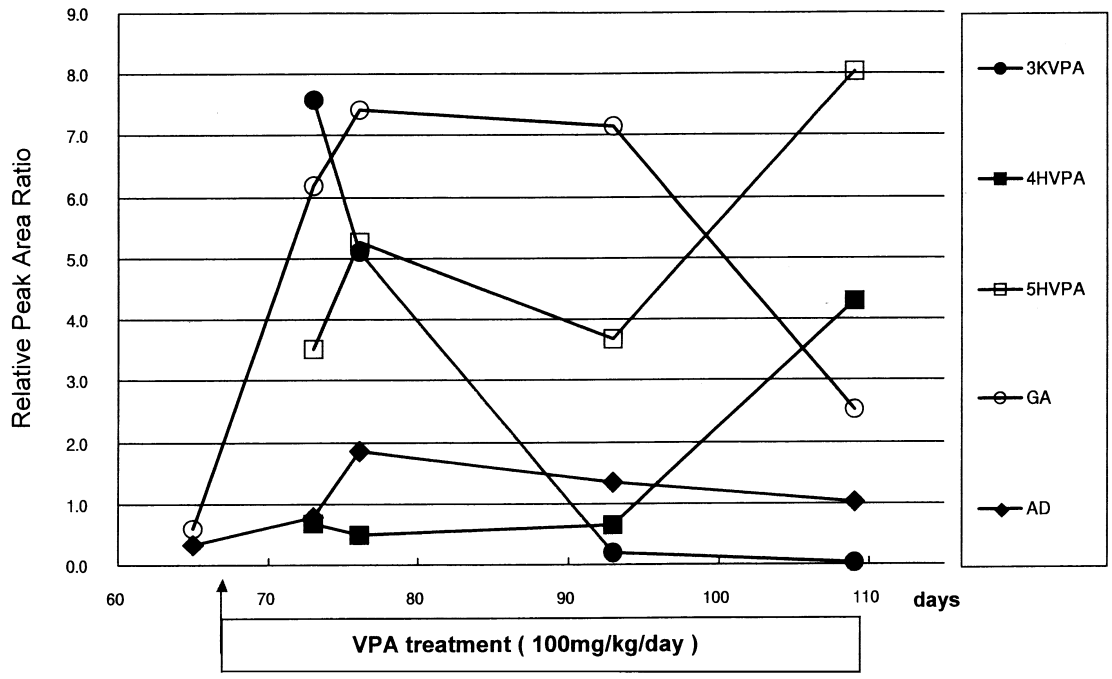

Fig. 4. Variations in urinary excretion of VPA metabolites and medium chain dicarboxylic acids in the patient with SSADH deficiency after VPA administration. Organic acid extraction method was used for sample preparation, and 2,2-dimethylsuccinate was used as an internal standard. The ions targeted were same as Fig. 2.

gas. The residue was trimethylsilylated using 100 μl of BSTFA plus 10% TMCS at 80 °C for 30 min, then 1 μl of the reaction mixture was analyzed by GC–MS. Urinaly organic acids were also extracted by organic solvent extraction method. To an amount of urine equivalent to 1 μmol creatinine (total volume 1 ml), 25 nmoles of 2,2-dimethylsuccinic acid was added as an internal standard. After acidification to pH 1 with 2 N HCl, the sample was extracted three times with 3 ml diethyl ether. The organic phase was dried on anhydrous Na_2SO_4 and evaporated to dryness under nitrogen stream. Trimethylsilylation was same as urease digestion method. Urinary creatinine was enzymatically measured using a Beckman Synchron CX5CE auto analyzer (Beckman Instruments, Brea, CA).

2.4. Gas chromatography–mass spectrometry

Samples were analyzed using a QP-5000 gas chromatograph–mass spectrometer (Shimadzu, Kyoto, Japan) with a fused-silica capillary column (J&W DB-5MS, 30 m×0.25 mm×0.25 μm). The GC–MS conditions were the same as described previously [7]. The temperature was programmed to increase at a rate of 17 °C/min from 60 to 325 °C, which was finally maintained for 10 min. Electron impact mass spectra were obtained by repetitive scanning at a rate of 0.25 s intervals from m/z 50 to 650. We quantified GHB by mass chromatography. The targeted ions for quantification were follows; m/z 233 for GHB and m/z 239 for GHB-d_6.

3. Results and discussion

It has been reported that VPA inhibits SSADH but not GABA-transaminase and succinic semialdehyde reductase [11–13]. The ‘‘valproate-effect’’ has been discussed by Divry et al. and Johannessen [8,13]. They mentioned that SSADH inhibition by valproate was the reason for higher production of GHB. GHB is converted to succinic semialdehyde by the reverse reaction with non-specific reductase, and inhibition

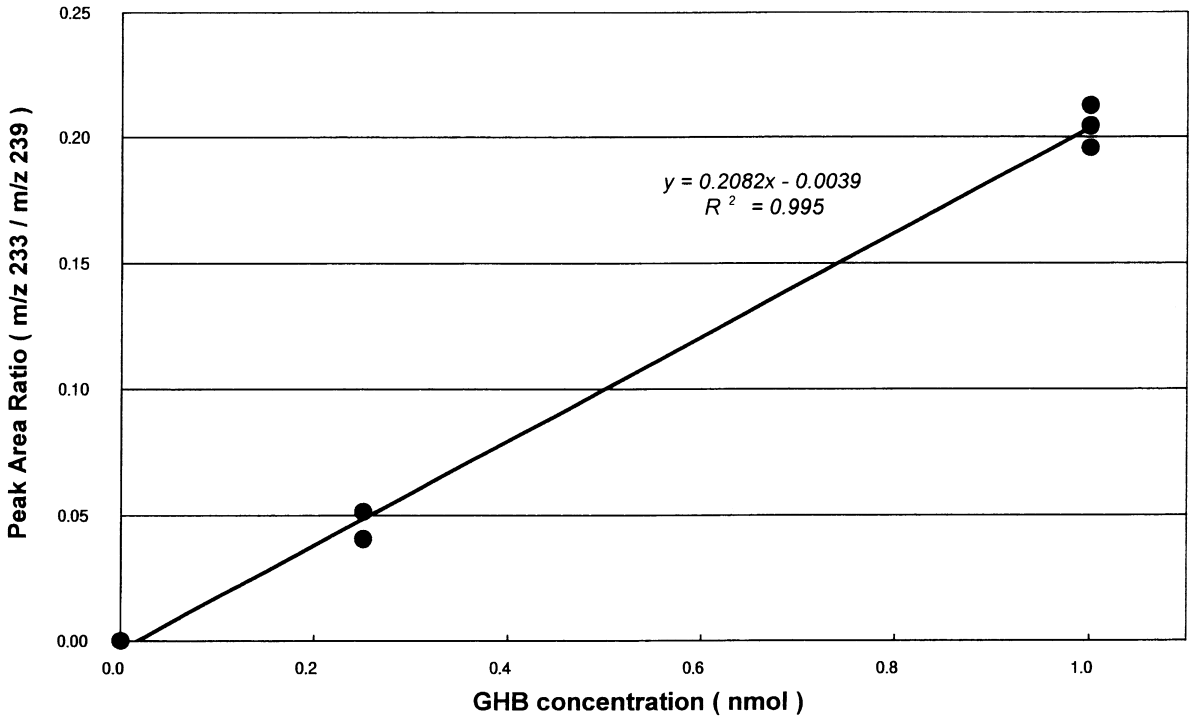

Fig. 5. Calibration curve for GHB in urease digestion method. GHB-d_6 (5 nmol) was used as an internal standard. The ions targeted for GHB-d_6 and GHB were m/z 239 and m/z 233, respectively.

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