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Effect of valproic acid on the urinary metabolic profile of a patient with succinic semialdehyde dehydrogenase deficiency

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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

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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

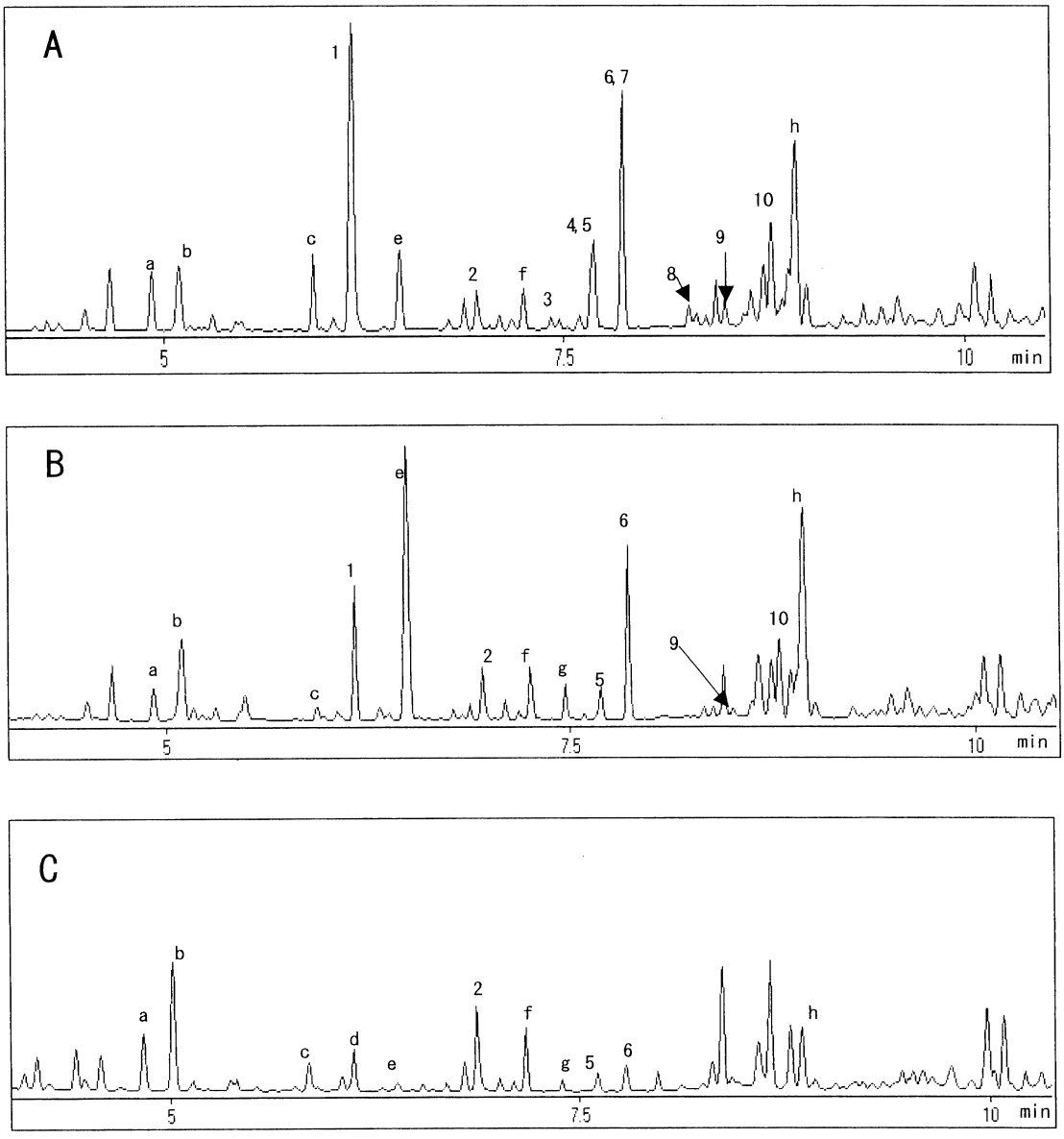

Fig. 1. Total ion current chromatograms of the trimethylsilyl (TMS) derivatives of the urinary metabolites in the patient with SSADH deficiency before and after valproate administration (relative abundance on the *y*-axis). (A) After VPA treatment (1 month after), (B) before VPA treatment), (C) control. 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.

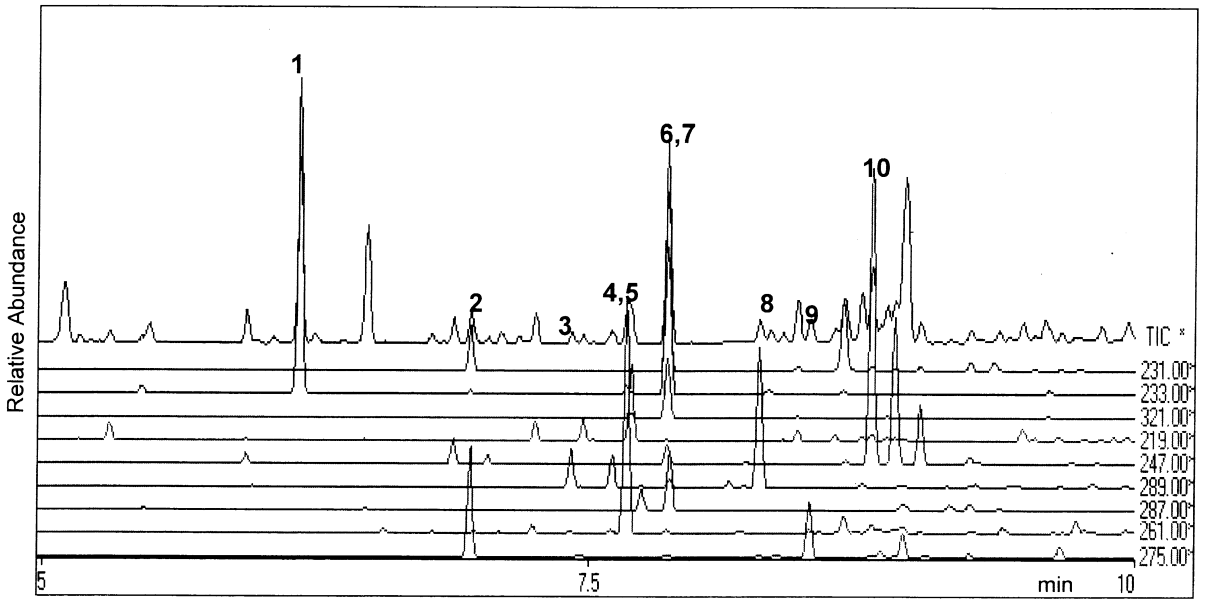

Fig. 2. Mass chromatograms of the trimethylsilyl (TMS) derivatives of the urinary metabolites in the patient with SSADH deficiency. The ions targeted were m/z 231 for 2,2-dimethylsuccinate (IS), m/z 233 for GHB, m/z 321 for 2DT, m/z 219 for 3DT, m/z 247 for 4,5DH, m/z 289 for 4HVPA and 5HVPA, m/z 287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

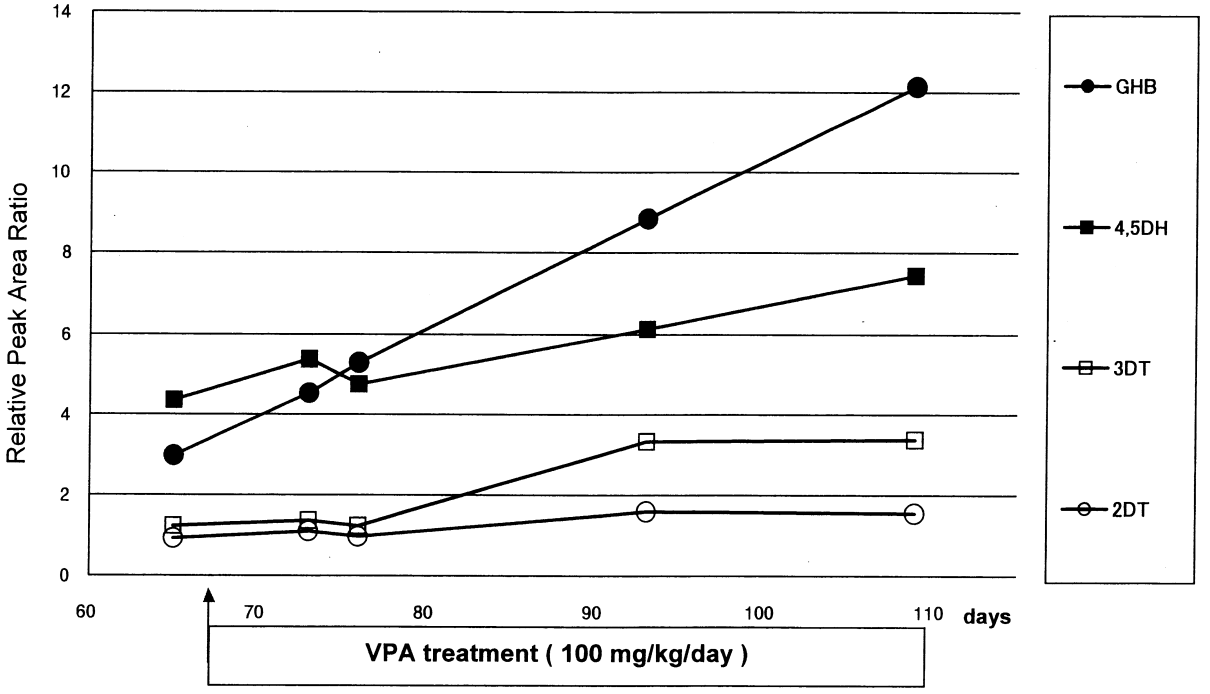

Fig. 3. Urinary excretion of GHB and its related metabolites in the patient with SSADH deficiency after VPA administration. Urease digestion method was used for sample preparation, and 2,2-dimethylsuccinate was used as an internal standard. The ions targeted were same as Fig. 2.

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

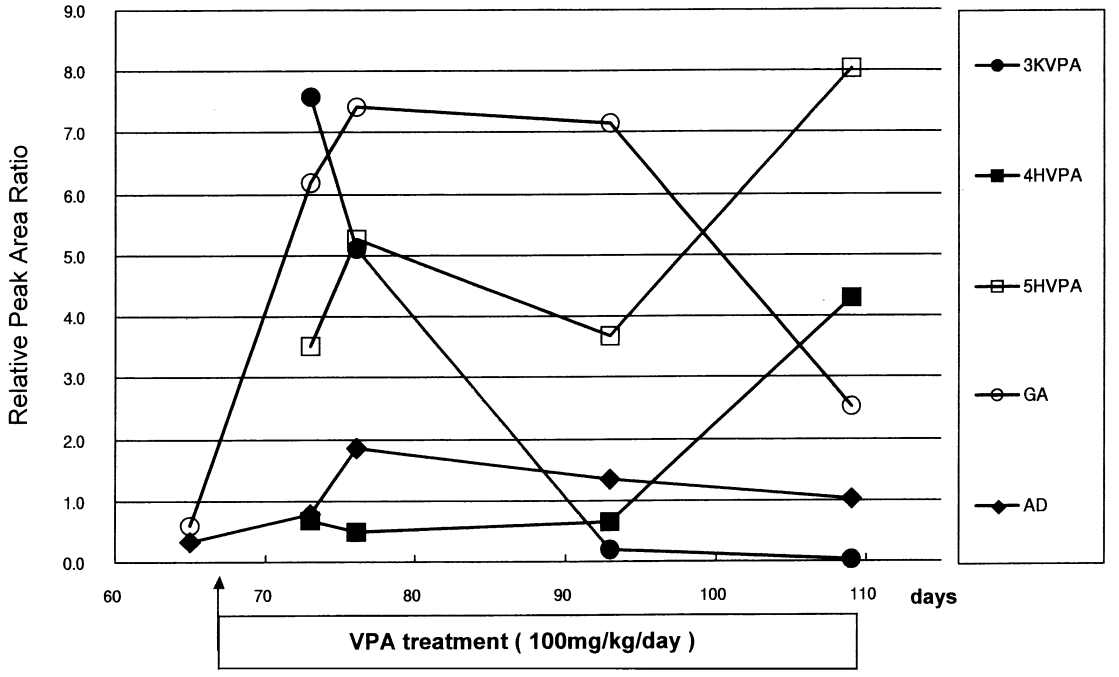

Fig. 4. Variations in urinary excretion of VPA metabolites and medium chain dicarboxylic acids in the patient with SSADH deficiency after VPA administration. Organic acid extraction method was used for sample preparation, and 2,2-dimethylsuccinate was used as an internal standard. The ions targeted were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

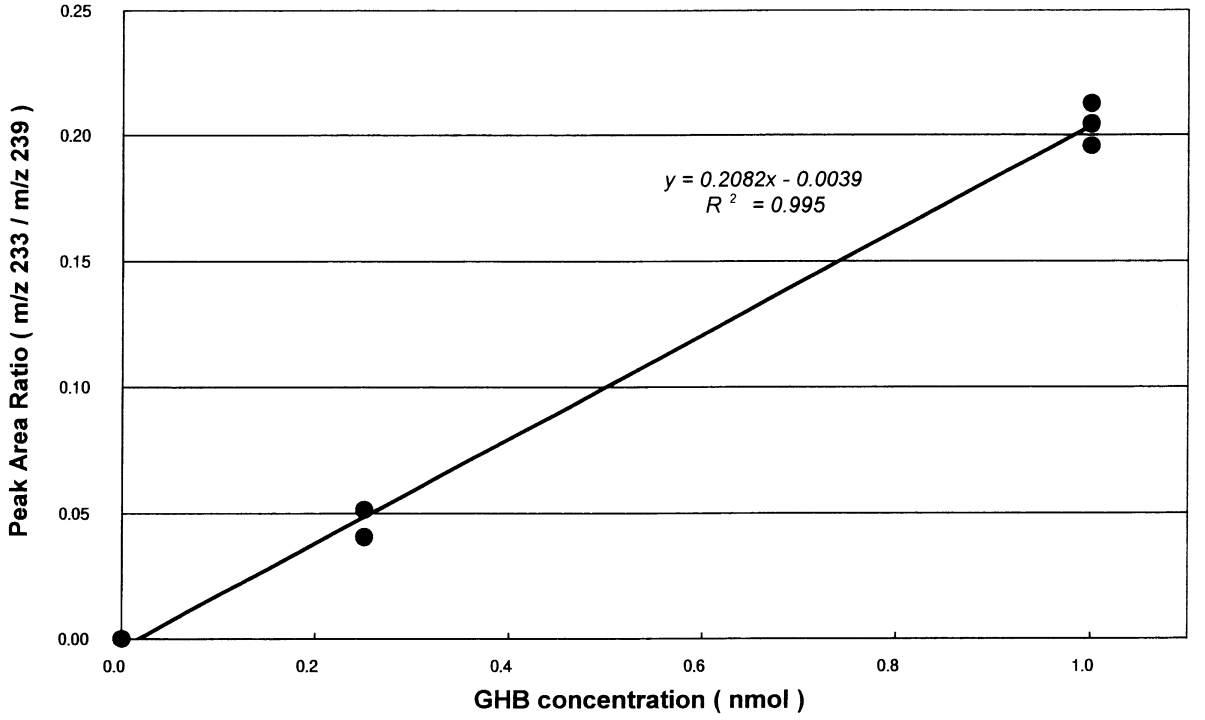

Fig. 5. Calibration curve for GHB in urease digestion method. GHB-d_6 (5 nmol) was used as an internal standard. The ions targeted for GHB-d_6 and GHB were m/z 239 and m/z 233, respectively.

of this enzyme by VPA is believed to be another reason for accumulation of GHB [13]. To elucidate the relationships between GHB accumulation and VPA metabolism, the urinary metabolic profiles of the patients were investigated following VPA treatment.

Total ion current chromatogram and mass chromatograms of urinary metabolites in our patient with SSADH deficiency are shown in Figs. 1 and 2. GHB, 2-deoxytetronate (2DT, β-oxidation product of GHB), 3-deoxytetranate (3DT, α-oxidation product of GHB) and 4,5-dihydroxyhexanoate (4,5DH, condensation product of succinic semialdehyde with a 2-carbon fragment) were observed in the patient urine in large amounts [14]. The relationships between GHB excretion and several metabolites before and after VPA treatment are shown in Figs. 3 and 4. After valproate therapy, excretion of GHB and other SSADH deficiency related metabolites increased by factors ranging from 1.7 to 4 times (Fig. 3). On the other hand, levels of 3-keto-valproate (3KVPA; β-oxidation product of VPA [15]) was decreased sharply, while levels of 4-hydroxyvalproate (4HVPA) and 5-hydroxyvalproate (5HVPA), ω-oxidation products of VPA [16], were elevated following VPA administration (Fig. 4). This suggests that ω-oxidation of VPA was stimulated but β-oxidation of VPA was suppressed in the patient.

If GHB accumulation influences fatty acid β-oxidation [14], it is likely that fatty acid ω-oxidation is also stimulated and medium-chain dicarboxylic aciduria is observed in SSADH patients. SSADH-deficient patients are known to occasionally exhibit dicarboxylic aciduria [3]. However, dicarboxylic aciduria was variable and was not a parallel phenomenon with GHB accumulation in our patient. Fatty acid β-oxidation enzymes and SSADH are located in mitochondria, but fatty acid ω-oxidation enzymes are

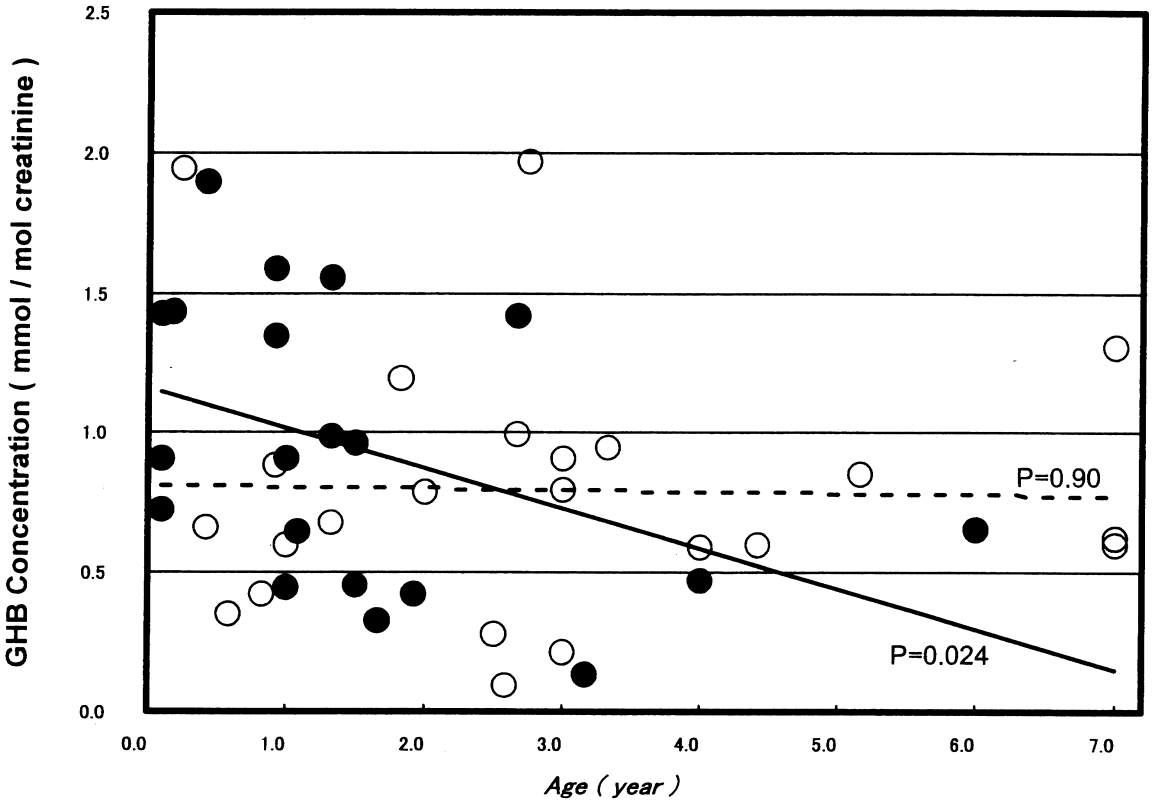

Fig. 6. Relationships between urinary GHB concentration and age in VPA medicated (-○-) and VPA non-medicated (-•-) patients without metabolic disorders. Urease digestion method was used for sample preparation, and GHB-d_6 was used as an internal standard.

in cytosol. Therefore, it seems that the accumulation of GHB is not concern with dicarboxylic aciduria directly, but the capacity of β-oxidation in these patients is reduced and some factor stimulates ω-oxidation.

To elucidate the relationships between GHB accumulation and VPA metabolism, the metabolic profile in the urine of the patients who have no metabolic disturbance were also investigated following VPA treatment. Although the concentrations of GHB in SSADH deficiency are typically 880–3630 mmol/mol creatinine [7], but those in normal individuals are lower than 5 mmol/mol creatinine. An improved method for GHB quantification, which was consisted with stable isotope dilution technique combined with urease digestion-GC–MS method, was used in this study. GHB-d_6 was used as an internal standard in this experiment. This method successfully detects GHB with a sensitivity of 0.05 nmol/0.1 ml urine (Fig. 5). Detection limit was improved about 10 times compared with former report [7] and recovery of GHB from urine is more than 93% and reproducibility (C.V.%) was 8% at 5 nmol. The concentrations of GHB in control are shown in Fig. 6. Age dependent excretion of GHB was observed in VPA non-medicated patients without metabolic disorders, Spearman's correlation coefficient, $P=0.024$ [17]. On the other hand, no statisti-

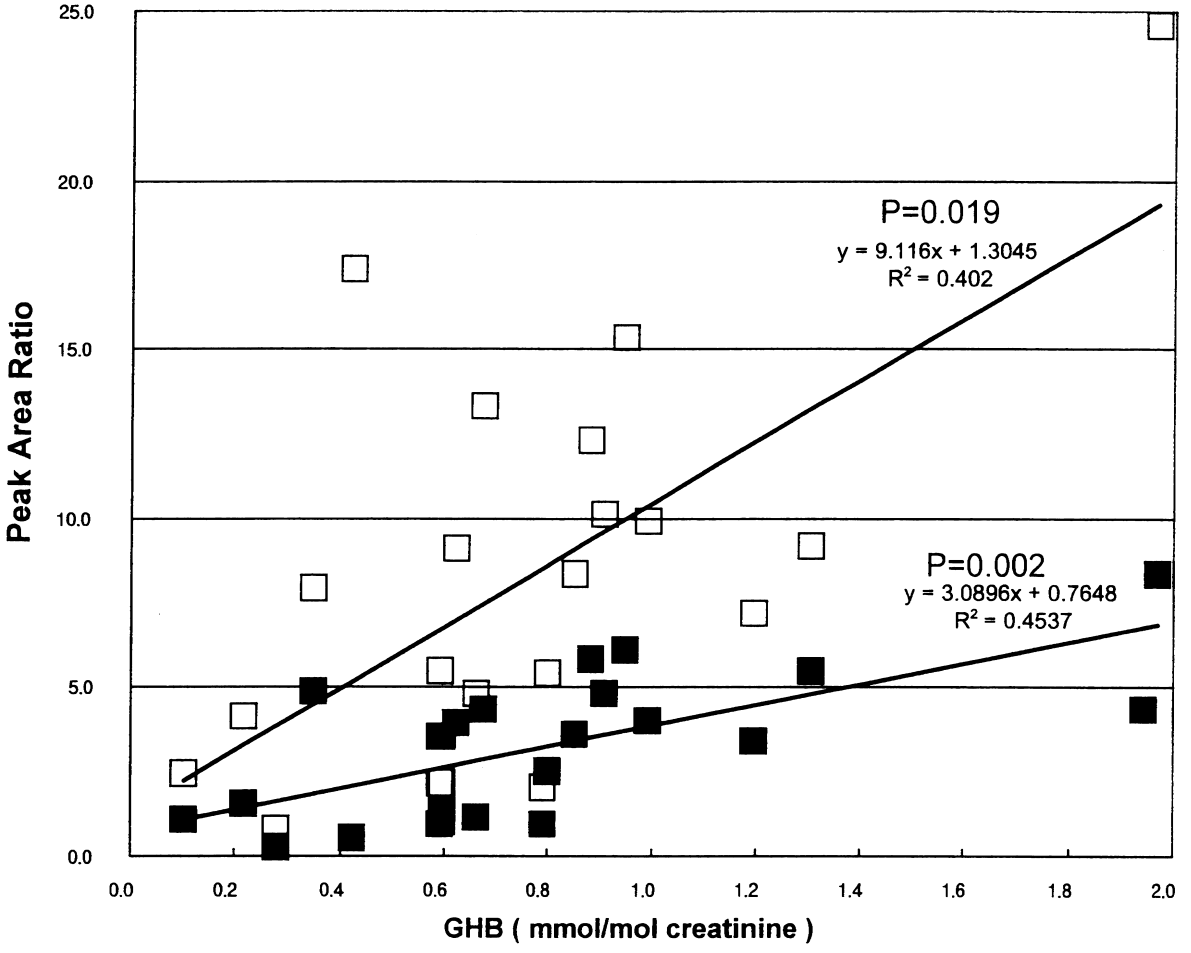

Fig. 7. Relationships between urinary GHB concentration and urinary 4HVPA (-■-) or 5HVPA (-□-) levels in VPA medicated patients without metabolic disorders. Urease digestion method was used for sample preparation. The ions targeted were m/z 289 for 4HVPA and 5HVPA, and m/z 239 for GHB-d_6 (internal standard).

cally significant age dependence was observed in VPA medicated patients.

We compared the relationship between GHB and VPA metabolites in VPA medicated control. The levels of 4HVPA and 5HVPA excretion had both statistically significant relationships with GHB (Fig. 7).

Our data reveals that accumulation of 4HVPA and 5HVPA is correlated with GHB levels. These VPA metabolites are expected to have the competitive inhibition on GHB non-specific reductase because their chemical structures are very similar to that of GHB. The mechanism of GHB accumulation in SSADH deficient patient after VPA administration is thought to be follows: VPA inhibits the SSADH reaction and succinic semialdehyde levels are increased, but VPA dose not inhibit succinic semialdehyde reductase and thus SSA is converted to GHB. This pathway is reversible, but the conversion of GHB to SSA is catalyzed by cytosolic non-specific reductase. This non-specific reductase is competitively inhibited by 4HVPA and 5HVPA, resulting in GHB accumulation.

Acknowledgements

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