[Chem. Pharm. Bull. 36(9)3589—3594(1988)]

Effect of Sodium Valproate (VPA) on Cerebral Amino Acids: Mechanism of γ-Aminobutyric Acid (GABA) Elevation and Possible Causal Relation of VPA-Induced Encephalopathy and Glutamine Level

CHOICHIRO MIYAZAKI,[a] KENJI MATSUYAMA,*,[a] MASATAKA ICHIKAWA[a] and SHIGERU GOTO[b]

Department of Hospital Pharmacy, Nagasaki University Hospital,[a] Sakamoto-cho 7–1, Nagasaki 852, Japan and Faculty of Pharmaceutical Sciences, Kyushu University,[b] Maidashi 3–1–1, Higashi-ku, Fukuoka 812, Japan

(Received February 10, 1988)

Changes of aspartate, γ-aminobutyric acid (GABA), glutamate, taurine, glutamine and glycine were examined after the intraperitoneal administration of sodium valproate (VPA) to mice at a dose of 200 mg/kg. Brain levels of glutamate and aspartate were significantly reduced by VPA, while the level of GABA was significantly increased. Higher doses of VPA significantly increased the brain levels of glutamine and glycine. These changes seem to be correlated to VPA-induced encephalopathy.

Keywords——valproate; GABA; aspartate; glutamate; glutamine; glycine; encephalopathy; Reye's syndrome

Since Meunier *et al.*[1] first reported that valproate (VPA: *n*-dipropylacetic acid) had anticonvulsant properties, it has been widely used in the treatment of several types of epilepsy.[2,3] Its mechanism of action is generally believed to involve an elevation of brain γ-aminobutyric acid (GABA) levels, caused by inhibition of degradative enzymes of the GABA shunt, namely γ-aminobutyric acid transaminase (GABA-T)[4–6] and succinic semialdehyde dehydrogenase (SSADH),[7] and by enhancement of the activity of the GABA synthetic enzyme, glutamate decarboxylase (GAD).[8,9]

Despite an accumulating literature on the effect of VPA on GABA-mediated neurotransmission, relatively little is known about its effect on glutamate (Glu) and aspartate (Asp). Both Asp and Glu oppose the action of GABA, and the levels of Glu are significantly increased in the cerebrospinal fluid (CSF) of epileptic patients.[10] The anticonvulsant action of VPA might be associated with its effects on the levels of Asp and Glu as well as GABA.

This background led us to investigate the *in vivo* changes of cerebral amino acids, GABA, Glu, Asp, taurine (Tau), glycine (Gly) and glutamine (Glu), after VPA administration. In addition, the dose dependence of the VPA effects on these six kinds of amino acids was examined. The causal relation between VPA and encephalopathy is discussed on the basis of the changes in these cerebral amino acids.

Experimental

Apparatus——Fluorescence liquid chromatographic experiments on *o*-phthalaldehyde (OPA)–amino acid derivatives were performed on a model 510 pump (Waters, Milford, USA) with a model 420-AC fluorescence detector (Waters). Gradient separations utilized a model 680 automated gradient controller (Waters), Waters 740 data module and WISP 710B Waters intelligent sample processor. All chromatography was performed with a Waters Resolve 5 μm C-18 column (150 × 3.9 mm) at 40 °C. Ammonia determination was performed with a Hitachi U-3200

spectrophotometer.

Reagents——Sodium valproate (VPA; lot. no. 70523-00) was a gift from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. OPA, 5-amino-*n*-valeric acid (AVA), 2-mercaptoethanol and amino acids were obtained from Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. All other reagents used were special reagent grade products from Nakarai Chemicals, Ltd., Kyoto, Japan. Ammonia-Test Wako, ammonia determination reagent, was purchased from Wako Pure Chemical Ind., Ltd., Osaka, Japan.

Animal Experiment——Male ddY mice weighing 20 to 30 g (Kyudo Co., Ltd., Kumamoto, Japan) were used. The mice were kept on a commercial diet (Clea Japan Ind. Co., Ltd., Tokyo, Japan) but fasted for 12 h prior to the experiment. Water was given *ad libitum*. Each dose of VPA solution (10, 20, 30, 40 and 80 mg/ml) was prepared using 0.9% (w/v) NaCl, the pH being adjusted to pH 7.0 immediately before use. VPA solution was administered intraperitoneally in a volume of 0.1 ml/10 g body weight. Mice were sacrificed by decapitation, and the brains were weighed and homogenized in 4 ml of 0.2 M trichloroacetic acid containing 800 μg of AVA as an internal standard for high performance liquid chromatography (HPLC) detection. The homogenate was filtered to remove brain tissue and protein using a membrane filter (Centriflo CF-25, Amicon). Derivatization of amino acids and HPLC assay were done according to the methods described in the previous paper.[11]

Determination of Ammonia——According to the improved method of Okuda and Fujii,[12] ammonia was derived to indophenol by using Ammonia-Test Wako reagents, followed by detection at 630 nm with Hitachi U-3200 spectrophotometer.

Results and Discussion

Cerebral amino acids in ddY mice were determined at various times after the intraperitoneal administration of VPA, 200 mg/kg. Table I showed the effect of VPA on brain Asp, Glu, GABA, Gln, Gly and Tau concentrations. A significant difference between the VPA-treated group and the control was seen in brain Asp, Glu, GABA and Gly levels, but not in brain Gln and Tau levels at a dose of 200 mg/kg of VPA. Time course curves for Asp, GABA and Glu are shown in Figs. 1, 2 and 3, respectively. Asp, the excitatory neurotransmitter, was significantly decreased within 15 min and reached a minimum (2.31 ± 0.32 μmol/g wet wt.) at 30 min, remaining depressed for up to 8 h after VPA treatment (200 mg/kg). In contrast, GABA, the inhibitory neurotransmitter, increased significantly from 2.32 ± 0.12 μmol/g wet wt. in the control to 2.89 ± 0.23 μmol/g wet wt. at 30 min after VPA.

It is interesting that the symmetric changes in brain Asp and GABA levels were obtained. Porter and Martin[13] reported that Asp reduces brain GAD activity by converting holo -GAD to apo-GAD at an ordinal treatment dose. According to their report, a decrease in cerebral

TABLE I. Effect of VPA, 200 mg/kg i.p., on Brain Amino Acid Levels as a Function of Time after Administration

Time (h)	Control	0.25	0.5	0.75	1.0	2.0	3.0	5.0	8.0
Asp	3.03	2.56[b]	2.31[b]	2.36[b]	2.56[b]	2.56[b]	2.81	2.74[a]	2.67[b]
S.D.	0.20	0.21	0.32	0.27	0.19	0.21	0.28	0.12	0.07
Glu	11.78	12.34	12.20	12.11	12.09	10.77[a]	11.19[a]	11.42	11.55
S.D.	0.52	0.47	1.02	0.43	0.18	0.44	0.36	0.52	0.40
GABA	2.32	2.56[b]	2.89[b]	2.73[b]	2.84[b]	2.44[a]	2.22	2.18	2.21
S.D.	0.12	0.05	0.23	0.14	0.15	0.05	0.08	0.10	0.06
Gln	5.88	5.93	5.85	5.68	5.57	5.48	6.37	6.12	6.12
S.D.	0.68	0.35	0.46	0.23	0.29	0.29	0.33	0.22	0.37
Gly	1.52	1.82[a]	1.71	1.84[b]	1.87[a]	1.69	1.66	1.69	1.65
S.D.	0.19	0.19	0.41	0.12	0.16	0.10	0.10	0.10	0.09
Tau	10.01	10.57	10.25	10.51	10.03	9.94	10.13	10.46	10.42
S.D.	0.11	0.63	0.82	0.44	0.15	0.47	0.73	0.45	0.32

Each value represents the mean (μmol/g wet wt.) $\pm$ S.D. for 6 animals. Statistical significance in two-tailed Student's *t*-test: *a*) $p<0.05$, *b*) $p<0.01$.

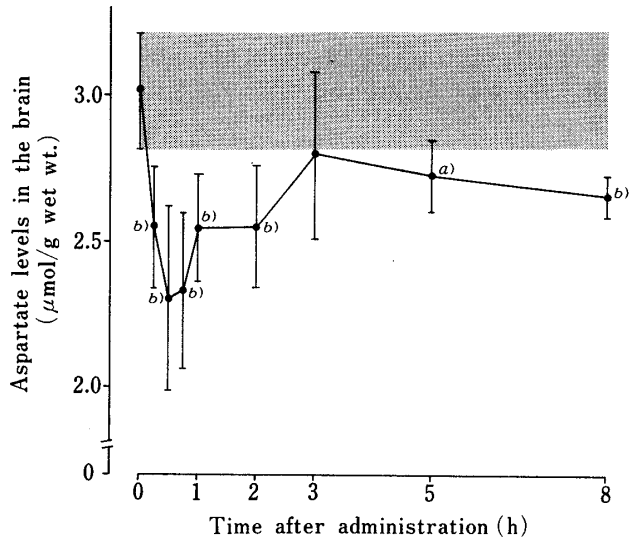

Fig. 1. Time Course of Aspartate Levels in Mouse Whole Brain after Intraperitoneal Administration of 200 mg/kg of VPA

The shadowed area represents the mean ± S.D. for the levels of control aspartate (3.03 ± 0.20 μmol/g wet wt.; $n=6$). Vertical bars indicate standard derivation of the means of 6 animals. Statistical significance in two-tailed Student's t-test: *a*) $p<0.05$. *b*) $p<0.01$.

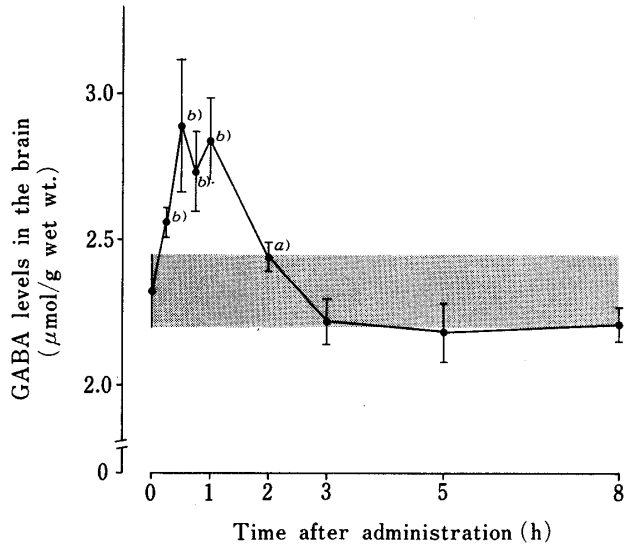

Fig. 2. Time Course of GABA Levels in Mouse Whole Brain after Intraperitoneal Administration of 200 mg/kg of VPA

The shadowed area represents the mean ± S.D. for the levels of control GABA (2.32 ± 0.12 μmol/g wet wt.; $n=6$). Vertical bars indicate standard deviation of the means of 6 animals. Statistical significance in two-tailed Student's t-test: *a*) $p<0.05$. *b*) $p<0.01$.

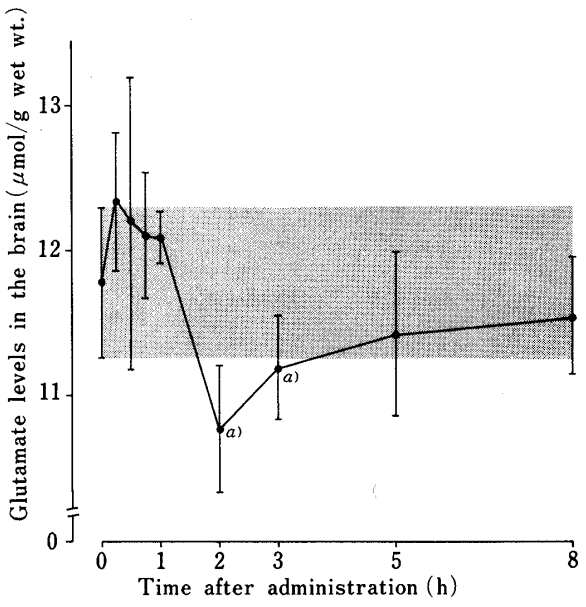

Fig. 3. Time Course of Glutamate Levels in Mouse Whole Brain after Intraperitoneal Administration of 200 mg/kg of VPA

The shadowed area represents the mean ± S.D. for the levels of control glutamate (11.78 ± 0.52 μmol/g wet wt.; $n=6$). Vertical bars indicate standard deviation of the means of 6 animals. Statistical significance in two-tailed Student's t-test: *a*) $p<0.05$.

Asp gives rise to activation of GAD, followed by the increment of brain GABA. In addition, Nau and Löscher[9] demonstrated that the increment of brain GABA after VPA (200 mg/kg, i.p.) was accompanied with elevation of GAD activity, and GABA-T inhibition was not observed then. Phelan *et al.*[14] also criticized the general concept that an elevation of brain GABA after VPA is due to the inhibition of GABA-T, suggesting that the concentrations required to significantly affect GABA-T are unlikely to be achieved *in vivo*. If GABA-T is inhibited by 200 mg/kg of VPA *in vivo*, Glu, the precursor of GABA, should increase as well as GABA. However, the cerebral levels of Glu were significantly decreased at 2 and 3 h after VPA, as shown in Fig. 3. Our data also support the view of Nau and Löscher.[9]

Table II shows the effect of dose of VPA on cerebral levels of six kinds of amino acids at 2 h. The reason why we selected 2 h as the measuring time was because Glu changed drastically at 2 h. From the toxicological point of view, it is of special interest that 800 mg/kg of VPA

TABLE II. Dose Dependence of Effect of VPA on Brain Amino Acid Levels at 2 h after Administration

Dose (mg/kg)	Control	100	200	300	400	800
Asp	3.03	2.59[a]	2.56[a]	2.11[b]	1.78[b]	1.73[b]
S.D.	0.20	0.27	0.21	0.20	0.24	0.31
Glu	11.78	10.74[a]	10.77[b]	10.28[b]	10.58[b]	9.24[b]
S.D.	0.52	0.46	0.44	0.32	0.58	0.72
GABA	2.32	2.56[a]	2.44[a]	2.77[b]	2.96[b]	3.71[b]
S.D.	0.12	0.15	0.05	0.07	0.22	0.15
Gln	5.88	5.59	5.48	5.38	6.14	7.24[b]
S.D.	0.68	0.49	0.29	0.33	0.27	0.73
Gly	1.52	1.51	1.69	1.69	1.71	1.90[a]
S.D.	0.19	0.12	0.10	0.09	0.11	0.21
Tau	10.01	9.96	9.94	10.83	9.41	10.83
S.D.	0.11	0.50	0.47	0.27	0.51	0.91

Each value represents the mean (μmol/g wet wt.) ± S.D. for 6 animals. Statistical significance in two-tailed Student's *t*-test: *a*) $p<0.05$, *b*) $p<0.01$.

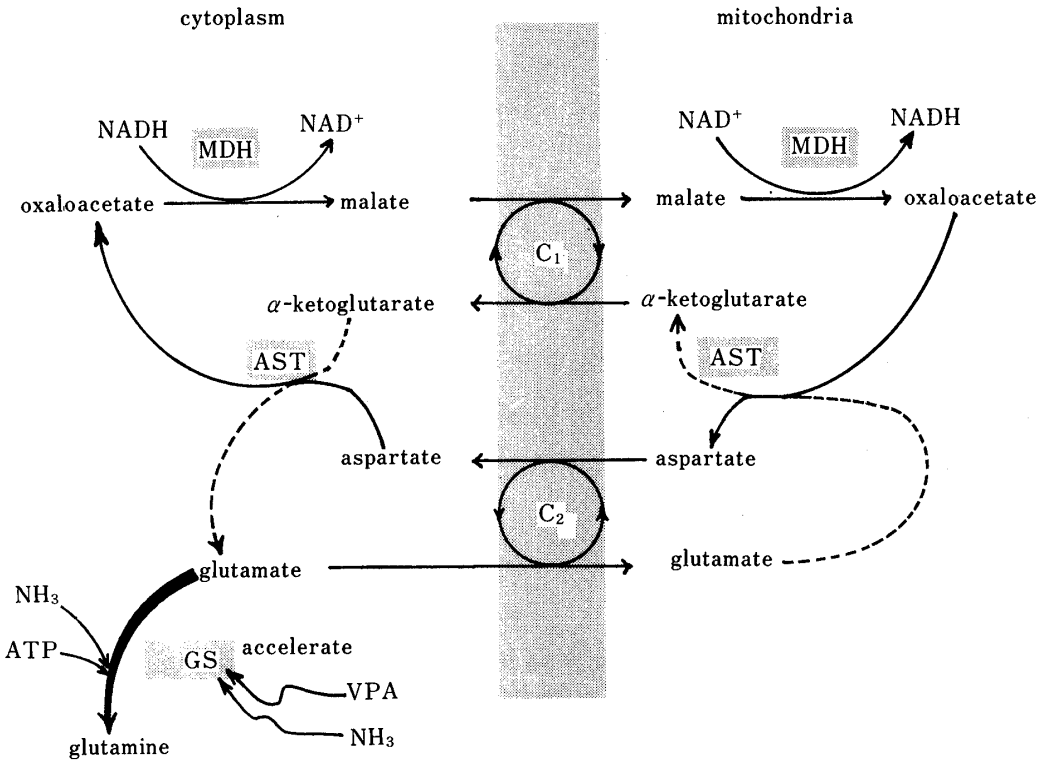

Fig. 4. Transport of Reducing Equivalents from Cytoplasm to Mitochondria *via* the Malate–Aspartate Shuttle

C_1 and C_2 indicate membrane carrier system for malate–α-ketoglutarate and aspartate–glutamate transport, respectively. Both VPA and ammonia accelerate glutamine synthetase (GS) activity, leading to imbalance of ATP. This system depends on intramitochondrial and extramitochondrial transminating enzymes (malate dehydrogenase (MDH) and aspartate aminotransferase (AST)). Modification from Hindfelt *et al.*[15]

TABLE III. Ammonia Levels in the Brain and Arterial Blood at 2 h after the Intraperitoneal Administration of VPA (200 mg/kg)

	Arterial blood	Brain
Control	0.067±0.013	0.883±0.062
VPA (800 mg/kg)	0.062±0.008	0.769±0.050[a)]

Each value represents the mean±S.D. (μmol/g wet wt. for brain or μmol/ml for blood) of 6 animals. Statistical significance in two-tailed Student's *t*-test: *a*) $p<0.01$.

elevated Gln level from 5.88±0.68 μmol/g wet wt. in the control to 7.24±0.73 μmol/g wet wt. The changes of Asp, Glu and Gln after 800 mg/kg of VPA were found to be quite similar to those after ammonium acetate (AA). AA injection (5.3 mmol/kg, i.p.) also gives rise to a significant reduction of both Asp and Glu, though it causes a significant increase in Gln level.[15)]

These changes suggest a defect or block in the transport of reduced equivalents from cytoplasm to mitochondria through the malate–aspartate shuttle (Fig. 4).

In the case of ammonia encephalopathy, ammonia interferes with the shuttle by reacting with cytoplasmic Glu to form Gln with consumption of brain ATP, leading to cerebral energy failure through depletion of nicotinamide adenine dinucleotide (NADH), mitochondrial substrate for oxidative phosphorylation.

Single administration of 800 mg/kg of VPA (i.p.) seems to accelerate Gln synthesis judging from the increased level of Gln and decrease levels of Glu and ammonia (Table III).

Phelan *et al.*[14)] also clarified the enhancing effect of VPA on Gln synthetase. It is notable that both ammonia and a high dose of VPA enhance Gln synthetase. This suggests that VPA is able to give rise to encephalopathy in a quite similar manner to ammonia.

In fact, Reye's syndrome in patients with VPA has become a subject of increasing medical attention.[16–20)] Coulter and Allen[16)] proposed the hypothesis that the increment of blood propionate caused by continuous administration of VPA may inhibit carbamyl phosphate synthetase I (CPS-I), resulting in accumulation of ammonia and secondary ammonia encephalopathy (Reye's syndrome). As a result of continuous administration of VPA, acyl coenzyme A (CoA) derived from VPA might interfere with the formation of *N*-acetyl-L-glutamate,[21)] the co-factor of CPS-I, or formed *N*-acyl-L-glutamate might inhibit the action of *N*-acetyl-L-glutamate as the allosteric effector of CPS-I, followed by hyperammonemia. In addition, it was also reported as a cause of VPA-induced encephalopathy that acyl CoA derived from VPA depletes carnitine,[22,23)] which prevents penetration of acyl CoA derived from endogenous fatty acid through the inner mitochondria membrane to the site of the fatty acid β-oxidation enzyme system, also resulting in hyperammonemia.

As described above, much attention has been given to dysfunction of ammonia metabolism and subsequent encephalopathy as the cause of Reye's syndrome. However, judging from the fact that a single administration of 800 mg/kg of VPA reduced Glu and Asp levels in the brain and increased Gln level as well as ammonia, it might be possible to consider that VPA itself developes encephalopathy in a person whose Gln synthetase is particularly susceptible to VPA.

Genetic differences in drug-metabolizing enzymes are commonly seen as strain differences in animals, and as racial and interindividual variability in man, become of great importance in toxicology. There are many examples of human subjects showing idiosyncratic reactions to pharmacological or toxicological actions of drugs. VPA-induced Reye's syndrome might be one such example.

References

1) H. Meunier, G. Carray, Y. Meunier, P. Eymard and M. Aimard, *Therapie*, **18**, 435 (1963).
2) R. M. Pinder, R. N. Brodgen, T. M. Speight and G. S. Avery, *Drugs*, **13**, 81 (1977).
3) D. Simon and J. K. Penry, *Epilepsia*, **16**, 549 (1975).
4) L. J. Fowler, J. Beckford and R. A. John, *Biochem. Pharmacol.*, **24**, 1267 (1975).
5) G. Anlezark, R. W. Horton, B. S. Meldrum and M. C. V. Sawaya, *Biochem. Pharmacol.*, **25**, 413 (1976).
6) J. W. Van der Laan, Th. Boer and J. Bruinvels, *J. Neurochem.*, **32**, 1769 (1979).
7) M. Maitre, L. Ossola and P. Mandel, *FEBS Lett.*, **72**, 53 (1976).
8) W. Löscher, G. Bohme, H. Schafer and K. Kochen, *Neuropharmacology*, **20**, 1187 (1981).
9) H. Nau and W. Löscher, *J. Pharmacol. Exp. Ther.*, **220**, 650 (1982).
10) C. M. Plum, *J. Neurochem.*, **23**, 595 (1974).
11) C. Miyazaki, M. Ogasawara, M. Ichikawa, K. Matsuyama and S. Goto, *J. Parm. Dyn.*, **11**, 202 (1988).
12) T. Okuda and S. Fujii, *Saishinigaku*, **21**, 622 (1966).
13) T. G. Porter and D. L. Martin, *J. Neurochem.*, **48**, 67 (1987).
14) P. Phelan, C. Regan, C. Kilty and A. Dunne, *Neuropharmacology*, **24**, 895 (1985).
15) B. Hindfelt, F. Plum and T. E. Duffy, *J. Clin. Invest.*, **59**, 386 (1977).
16) D. L. Coulter and R. J. Allen, *Lancet*, i, 1310 (1980).
17) J. A. Sills, R. H. Trefor Jones and W. H. Taylor *Lancet*, ii, 260 (1980).
18) S. Rawat, W. J. Borkowski and H. M. Swick, *Neurology*, **31**, 1173 (1981).
19) B. S. Zaret, R. R. Beckner, A. M. Wagle and C. Passarelli, *Neurology*, **32**, 206 (1982).
20) J. V. Murphy and K. Marquardt, *Arch. Neurol.*, **39**, 591 (1982).
21) T. Shafai, L. Sweetmen, W. Wegler, S. I. Goodman, P. V. Fennessey and W. L. Nyhan, *J. Pediat.*, **92**, 84 (1978).
22) H. Bohles, K. Richter, E. Wagner-Thiessen and H. Schafer, *Eur. J. Paediatr.*, **137**, 185 (1982).
23) J. V. Murphy, K. M. Marquardt and A. L. Shug, *Lancet*, **i**, 820 (1985).