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Kiyofumi Kobayashi* Yayoi Koide† Tohru Doi‡

Mutsutoshi Kohsaka** Kiyoshi Hosokawa†† Yuzuru Oku‡‡

*Institute for Neurobiology,

†Institute for Neurobiology,

‡Institute for Neurobiology,

**Institute for Neurobiology,

††Okayama University,

‡‡Kansai Rosai School,

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Kiyofumi Kobayashi, Yayoi Koide, Tohru Doi, Mutsutoshi Kohsaka, Kiyoshi Hosokawa, and Yuzuru Oku

Abstract

The homovanillic acid (HVA) concentrations in the lumbar cerebrospinal fluid (CSF) were determined in 38 epileptic and 39 control patients. The mean concentration of HVA was 23.9 ng/ml +/- 2.8 SEM for the epileptic group and 30.2 ng/ml +/- 2.1 SEM for the control group, respectively. Thus, HVA was significandly reduced in the patients with epilepsy compared with the controls. The mean HVA in the female patients was higher than in the male patients in both groups but this failed to reach statistical significance. There was no apparent relationship between the degree of reduced HVA concentration and other clinical indexes of the epilepsy (age, type and frequency of seizures, and anticonvulsant medication). For the determination of concentration gradient of HVA three fractions of the spinal CSF were obtained from 11 patients. A pronounced gradient of HVA concentration was found with a ratio of 1 : 1.46 : 1.97 for the first, second and third fractions. This suggests that a standardized conditions for collecting CSF should be employed to study HVA levels in humans.

KEYWORDS: CSF, HVA, concentration gradient, epilepsy

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HOMOVANILLIC ACID IN HUMAN CEREBROSPINAL FLUID —ITS CONCENTRATION GRADIENT AND REDUCED LEVELS IN PATIENTS WITH EPILEPSY—

Kiyofumi KOBAYASHI, Yayoi KOIDE, Tohru DOI, Mutsutoshi KOHSAKA, Kiyoshi HOSOKAWA* and Yuzuru OKU**

*Institute for Neurobiology (Director: Prof. M. Kohsaka); *Department of Neuropsychiatry (Director: Prof. S. Otsuki), Okayama University Medical School, Okayama 700, Japan and **Division of Neurosurgery, Kansai Rosai Hospital, Amagasaki 660, Japan*

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Abstract. The homovanillic acid (HVA) concentrations in the lumbar cerebrospinal fluid (CSF) were determined in 38 epileptic and 39 control patients. The mean concentration of HVA was 23.9 ng/ml ± 2.8 SEM for the epileptic group and 30.2 ng/ml ± 2.1 SEM for the control group, respectively. Thus, HVA was significantly reduced in the patients with epilepsy compared with the controls. The mean HVA in the female patients was higher than in the male patients in both groups but this failed to reach statistical significance. There was no apparent relationship between the degree of reduced HVA concentration and other clinical indexes of the epilepsy (age, type and frequency of seizures, and anticonvulsant medication). For the determination of concentration gradient of HVA three fractions of the spinal CSF were obtained from 11 patients. A pronounced gradient of HVA concentration was found with a ratio of 1 : 1.46 : 1.97 for the first, second and third fractions. This suggests that a standardized conditions for collecting CSF should be employed to study HVA levels in humans.

Key words: CSF, HVA, concentration gradient, epilepsy

An increasing number of reports on the cerebrospinal fluid (CSF) concentration of homovanillic acid (HVA), the major metabolite of dopamine, have been appearing in recent literature. The reason for measuring the concentration of HVA in clinical studies can be judged from the following evidence concerning the origin of HVA in the CSF. The HVA concentration in animal and human lumbar CSF reflects the metabolism of dopamine in the brain but not in the spinal cord (1-3). HVA derived from neural structures adjacent to the lateral ventricles, mainly the caudate nucleus, is transported from the lateral ventricles through the third and fourth ventricles to the spinal subarachnoid space by diffusion and CSF flow (4, 5), and is removed from CSF to the blood by an active transport mechanism (6). When humans are given [^{14}C]DOPA, [^{14}C]HVA in

the cisternal CSF reaches a maximum after 2–4 h, but does not attain its maximum concentration in the lumbar CSF until 8 h (7). Peripherally administered HVA does not penetrate appreciably into the CSF (8). Thus, HVA concentration in animal and human CSF decreases from the ventricular through the cisternal to the lumbar compartments (1, 6, 9, 10). The compartmental ratio of HVA in humans is approximately 9 : 4.5 : 1 for the ventricular, the cisternal and the lumbar CSF, respectively (3). This evidence suggests that HVA concentration in human lumbar CSF could provide important information on the dopaminergic neuronal activity in the brain, mainly the nigro-striatal system.

The present communication deals with HVA concentration in the lumbar CSF of patients with epilepsy. In addition, CSF from different levels in the spinal subarachnoid system was analysed in order to assess the concentration gradient of HVA.

MATERIALS AND METHODS

In order to determine the gradient of HVA concentration three fractions of the spinal CSF were obtained from 11 patients during diagnostic pneumoencephalography (PEG). Preparatory medication was given intramuscularly 30 min before PEG was started. In most cases this consisted of atropine sulfate (0.2–0.5 mg) and hydroxyzine hydrochloride (1 mg/kg). The procedures were performed after intramuscular injection of ketamine hydrochloride (5 mg/kg) in the patients under 7 years of age. Patients with evidence of obstruction of CSF flow were excluded from this series. After the lumbar puncture had been performed while seated, the initial 5–6 ml of CSF was taken for the first sample (fraction I) and the same volume of room air was injected through the puncture needle. This procedure was then repeated twice for the second and third samples (fractions II and III).

HVA concentration in the lumbar CSF was determined in 38 epileptic and 39 various neurological or psychiatric patients. The patients with epilepsy were 18 males and 20 females ranging in age from 5 months to 55 years, with a mean of 21.4 years. They consisted of 6 patients with Lennox-Gastaut syndrome (childhood epileptic encephalopathy with diffuse slow spike-waves), one with atonic seizures, one with tonic, 15 with generalized tonic-clonic, one with psychomotor in addition to generalized, 6 with psychomotor, one with autonomic, 2 with adversive and 5 with focal cortical seizures. Seizure frequency of these patients varied from once a year to over 10 times per day. While five patients were studied before anticonvulsant medication was initiated, the remaining 33 patients were receiving various anticonvulsant drugs such as diphenylhydantoin, phenobarbital, or carbamazepine either alone or in various combinations at the time of the study. On the other hand, because of difficulty in obtaining CSF from the normal subjects, those patients comprising the control group in this study had various central nervous system disorders excluding epilepsy, extrapyramidal diseases and hydrocephalus. Patients with paroxysmal discharges on the electro-

encephalograms were also excluded from the control group. They were 25 males and 14 females ranging in age from one to 71 years, with a mean of 37.1 years. Hence the age distribution in two groups did not match. Neither of the groups were receiving any neuroleptic or other drugs which are well known to affect dopamine metabolism in the brain.

Usually lumbar puncture was carried out between 9 and 10 A. M., and an initial 5-6 ml of CSF flowing out through the needle was collected for HVA determination. The procedures were performed under ketamine anesthesia in the patients under 7 years of age. The CSF samples were placed immediately on ice and then stored at −20°C until analysis. HVA was determined by the fluorimetric method of Curzon *et al.* (11).

Statistical analysis was made using the Mann-Whitney U-test.

RESULTS

The results in Table 1 show an increase of HVA concentration from fraction I to III. The mean ratios of fractions II and III to fraction I were 1.46 (range 1.20–1.77) and 1.97 (range 1.33–3.01), respectively. This indicates an apparent concentration gradient of HVA in the spinal CSF. However, the ratio between fractions varied considerably in individual case. There was no correlation between the ratio and the clinical profiles of patients.

TABLE 1. CONCENTRATION OF HVA IN CSF IN FRACTIONS I-III

Case	Age	Sex	Clinical Diagnosis	HVA (ng/ml) Fraction I	Fraction II	Fraction III
1 K.K.	3	F	Epilepsy	63.2	76.0 (1.20)[a]	95.2 (1.51)[a]
2 M.T.	4	F	Epilepsy	36.0	58.3 (1.62)	63.6 (1.77)
3 A.G.	3	M	Epilepsy	54.0	71.7 (1.33)	71.7 (1.33)
4 M.A.	10	M	Epilepsy	21.9	31.5 (1.44)	40.4 (1.84)
5 N.N.	22	M	Epilepsy	13.4	21.7 (1.62)	40.4 (3.01)
6 I.O.	25	M	Hyperventilation syndrome	25.7	35.1 (1.37)	56.7 (2.21)
7 M.M.	1	F	Hydroencephalo-dysplasia	31.5	40.8 (1.30)	50.6 (1.61)
8 A.F.	26	M	Schizophrenia	23.9	29.1 (1.22)	63.6 (2.66)
9 S.M.	27	F	Schizophrenia	28.9	51.2 (1.77)	59.0 (2.04)
10 S.K.	49	F	Diencephalosis	26.5	—	45.1 (1.70)
11 T.K.	52	F	Presenile dementia	27.1	45.7 (1.69)	54.7 (2.02)

a The ratio of fraction II or III to fraction I is shown in parenthesis.

HVA concentrations in the lumbar CSF of the epileptic and control groups are shown in Table 2. In the epileptic group HVA levels ranged from 4.5 to

TABLE 2. CONCENTRATION OF HVA IN THE EPILEPTIC AND CONTROL PATIENTS

	Concentration of HVA (ng/ml)	
	Epilepsy	Control
Total	23.9 ± 2.8 (38)[a]	30.2 ± 2.1 (39)
Male	20.3 ± 3.6 (18)[b]	29.5 ± 2.8 (25)
Female	27.1 ± 4.2 (20)[c]	31.4 ± 3.1 (14)

Data given are mean values ± SEM with the number of cases in parenthesis.
a Different from control ($p<0.005$)
b Different from male control ($p<0.005$)
c Different from female control ($p<0.1$)

68.4 ng/ml, with a mean value of 23.9 ng/ml ± 2.8 SEM, which was significantly reduced as compared with the control group ranging from 8.5 to 72.9 ng/ml, with a mean value of 30.2 ng/ml ± 2.1 SEM. Although the mean HVA level in the female patients was higher than in the male patients in both groups, this difference was not statistically significant. As can be seen in Fig. 1, HVA levels

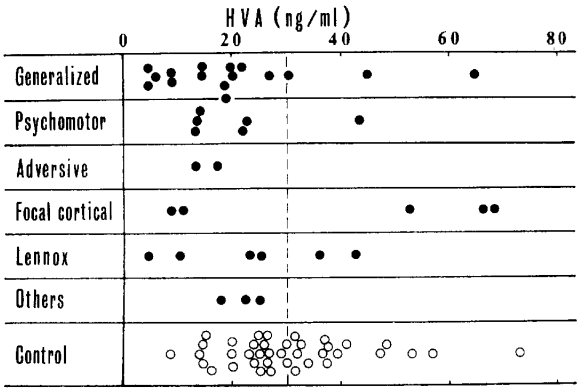

Fig. 1. The relationship between the HVA concentrations and the types of epileptic seizures. Each point represents a specific patient. Dotted line represents the mean concentration of controls.

in 20 (52.6%) cases out of 38 epileptic patients were below 20 ng/ml, while only 9 (23.1%) cases of 39 control patients showed HVA levels less than 20 ng/ml. There was no apparent correlation between the degree of reduced HVA concentration and the type of seizures. HVA levels in five epileptic patients with no antiepileptic medication (3 with generalized, one with psychomotor and one with focal cortical seizures) were 14.4, 26.8, 30.5, 14.2 and 10.7 ng/ml, respectively. There did not appear to be any consistent relationship between age and HVA levels in both groups, as shown in Fig. 2. HVA levels were neither related to seizure frequency nor antiepileptic medication.

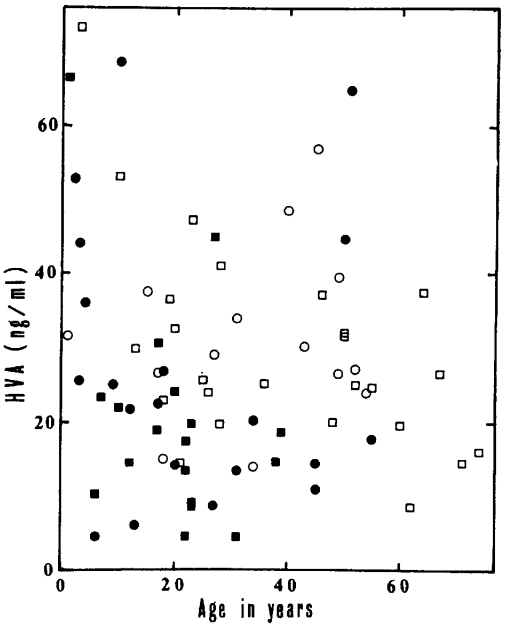

Fig. 2. The relationship between the HVA concentrations and the age of patients. Each point represents a specific patient (■, male epileptic; ●, female epileptic; □, male control; ○, female control).

DISCUSSION

Bernheimer *et al.* (12) and Barolin and Hornykiewicz (13) first reported on the reduced HVA concentrations in the spinal CSF of epileptic patients. Recently, Shaywitz *et al.* (14) suggested the possibility of reduced turnover of dopamine in the brain of children with epilepsy, since HVA concentrations in those patients were significantly lower even after the administration of probenecid, a drug that blocks the active transport of HVA from CSF (9). Papeschi *et al.* (15) noted reduced HVA concentrations in the ventricular CSF of patients with temporal lobe epilepsy. We also found low HVA concentrations (139 ng/ml ± 82 S. D.) in the ventricular CSF obtained during stereotaxic operation on 8 children with intractable epilepsy (6 with Lennox-Gastaut syndrome and 2 with tonic-clonic seizures) (16). On the other hand, however, Garelis and Sourkes (17) and Fujii *et al.* (18) failed to find HVA differences between the epileptic and control groups. Further, Chadwick *et al.* (19) found that HVA levels tended to be raised in anticonvulsant-treated and well-controlled epileptic patients.

It is difficult to account for these conflicting results. Whereas an inhibitory effect of phenobarbital on dopamine metabolism has been reported (20), the effect of anticonvulsants on HVA levels in CSF is currently unknown. Three

cases in the report of Shaywitz *et al.* (14) and 4 of 5 cases in this study investigated prior to initiating anticonvulsant medication had low concentrations of HVA. In addition, Shaywitz *et al.* (14) could not find any consistent relationship between HVA concentrations in CSF and the blood levels of diphenylhydantoin or phenobarbital. From these results it is unlikely that the reduced HVA concentration in CSF can be attributed to the effect of anticonvulsant medication.

Another possibility which could explain such a discrepancy might be that epilepsy is a syndrome manifesting various types of epileptic seizures caused by different etiologic factors or lesions at different sites in the brain. It should be kept in mind that the subjects of the previous and present studies are epileptic patients of different seizure types. It is not clear at the present time whether or not all types of epileptic seizures are regulated by the same neurochemical mechanism. Although, in this study, we failed to find a significant relationship between the degree of reduced HVA concentrations and the type of seizures, further studies along this line are necessary to elucidate the relationship of reduced HVA concentration to epilepsy.

The relationship between brain monoamines and susceptibility to epileptic seizures has been studied in experimental animals by a number of investigators (21, 22). The results of these works suggest that the pharmacological treatments lowering monoamine levels in the brain generally increase the susceptibility to experimental seizures, while treatments that increase monoamines decrease the susceptibility to seizures. At the present time, however, there is little agreement as to which monoamine of the brain (dopamine, norepinephrine or serotonin) is most intimately involved in seizure susceptibility. Of these monoamines, the inhibitory action of dopamine to seizure susceptibility has been reported by several authors (23-27). If dopamine plays an important role in regulating seizure susceptibility even in the human brain, the decreased activity of dopaminergic neurons, suggested by the reduced HVA concentrations in the CSF, must result in susceptibility to seizures in epileptic patients. As is well known, however, the reduced HVA concentrations are not specific for epileptic patients. It is necessary to clarify whether the decreased activity of dopaminergic neurons relates directly or not to the seizure susceptibility of epileptic patients.

We have reported the first case in which L-DOPA, precursor of dopamine, had a definite therapeutic effect on the psychomotor and adversive seizures (28). The effect of L-DOPA on the epileptic patients with low levels of HVA was reported recently by Cools *et al.* (29) and Sugie *et al.* (30).

A pronounced concentration gradient of HVA was found with a ratio of 1 : 1.46 : 1.97 between three fractions of the spinal CSF. A similar caudocranial concentration gradient has been reported elsewhere (10, 17). The ratios obtained in the present study are somewhat higher than those reported by Sjöström *et al.*

(10). This might be due to mixing of CSF in different compartments by air injected during PEG. However, a significant gradient of HVA exists certainly even in the spinal CSF. Therefore, it is necessary to standardize the conditions for obtaining CSF in the clinical investigation on HVA levels of various neurological and psychiatric diseases.

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