Homovanillic acid and 5-hydroxyindoleacetic acid in the ventricular CSF of comatose patients with obstructive hydrocephalus

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Ventricular cerebrospinal fluid (CSF) levels of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were determined every 2 to 4 hours over a period of 1 to 4 days in 12 patients, consisting of seven cases of brain tumor, two cases of cerebrovascular disease, and three cases of head injury. The concentrations of HVA and 5-HIAA varied with time in all cases, and significant correlations were found between the two values in eight cases. However, the relationship between variations of HVA and 5-HIAA levels and rhythms of sleep and waking could not be clarified. Both HVA and 5-HIAA concentrations varied at high levels in two patients whose CSF flow was completely blocked by tumor at the site of the fourth ventricle and aqueduct, respectively. On the contrary, in a case with craniopharyngioma in the third ventricle which blocked the bilateral foramina of Monro, although the HVA values were high, the 5-HIAA values varied at low levels. Of five comatose patients, two had cerebrovascular lesions and three had sustained head injury, and, in four of the five, the values of either one or both of HVA and 5-HIAA were low, but in the fifth case the 5-HIAA value was high.

Estimation of HVA and 5-HIAA concentrations in ventricular CSF may be a valuable tool in the investigation of brain monoamine metabolism. However, many factors must be considered in the interpretation of results of clinical studies.

KEY WORDS • cerebrospinal fluid • monoamine metabolite • hydrocephalus • coma

It is generally accepted that monoamines, such as dopamine, noradrenaline, and 5-hydroxytryptamine (5-HT), act as neurotransmitters in the central nervous system. In animal experiments, it has been shown that the monoamine concentrations in the brain may be profoundly altered by brain injury, hypoxia, and vascular occlusion. Homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) are the main metabolites of dopamine and 5-HT, respectively. In clinical studies, attempts have been made to investigate the brain monoamine metabolism in various pathological conditions, such as disturbance of cerebrospinal fluid (CSF) circulation and coma after head injury, by measurement of HVA and 5-HIAA values in CSF obtained by lumbar puncture. Recently, some investigators have reported variations in HVA and 5-HIAA levels in the ventricular CSF of patients with coma following severe head injury, blockage of CSF flow, and cerebral angiospasm. Determination of the ventricular CSF values of HVA and 5-HIAA may be more useful than the lumbar CSF values in the study of monoamine metabolism in the brain, because HVA originates mainly at the lateral ventricular level, but 5-HIAA is produced also at the spinal level.

Since the HVA and 5-HIAA values of the ventricular CSF change with time as mentioned in a preliminary report, patients cannot be evaluated on the basis of a single determination. In this study, we measured HVA and 5-HIAA values to ascertain whether there were circadian rhythms in the two values, and further examined the levels of the two metabolites in cases with CSF flow blockage and those in patients made comatose due to head injury or cerebrovascular disease in whom the values are said to vary markedly.

Clinical Material and Methods

Summary of Cases

Studies were performed on 12 patients, including seven cases of brain tumor, two cases of cerebro-
vascular disease, and three cases of head injury, who had been admitted to the Department of Neurosurgery, Hiroshima University Hospital. The mean age was 42 years, and there were two women. The diagnoses of the patients are presented in Tables 1, 2, and 3. The patients were divided into three groups at the time of CSF sampling.

Group A consisted of conscious patients without internal hydrocephalus (Table 1). Consciousness of the patients in this group was unequivocal, and it was confirmed by computerized tomography (CT) or ventriculography that the ventricular system was not dilated. In Case 1, left-sided suboccipital craniectomy was performed for a left acoustic neurinoma, the tumor was removed subtotally, and a ventricular drain was installed. The postoperative course was uneventful, and CSF collection was commenced before ventriculography. In Case 3, a tumor of the fourth ventricle was diagnosed, but dilatation of the lateral ventricles could not be demonstrated by ventriculography or CT scan.

Group B included conscious patients with internal hydrocephalus because of blockage of CSF flow (Table 2). Again consciousness of patients was unequivocal. The lateral ventricles in all cases were dilated because of CSF flow blockage by tumors involving the fourth ventricle, aqueduct, or the third ventricle. Neither direct attack on the tumor nor ventriculography was performed before CSF sampling.

Group C patients were comatose (Table 3). Patients in this group had lapsed into coma as a result of cerebrovascular disease or head injury and subsequently failed to regain consciousness. In Cases 9 and 11, craniotomy and removal of hematoma were performed. The lateral ventricles were slightly dilated due to obstructive hydrocephalus in Case 9 and to brain atrophy in Cases 10 and 11.

**Sampling of Cerebrospinal Fluid**

Ventricular CSF samples were collected every 2 to 4 hours over a period of 1 to 4 days in all patients. In Case 11, CSF was aspirated by puncture of an Omaya reservoir placed under the scalp and connected to a tube in the anterior horn of the lateral ventricle; in the other 11 cases, CSF samples were obtained through a drainage tube inserted into the lateral ventricle. The fluid in the tube was discarded, and only that drawn from the ventricle was analyzed. The volume of CSF collected ranged from 5 to 8 ml per patient, but care was taken to draw the same amount each time. The daily outflow volume of CSF obtained by ventricular drainage was 170 to 450 ml. In Case 5, volume of daily efflux of CSF was diminished because of dysfunction of ventricular drainage.

In Cases 1 to 7, meals were taken orally, whereas liquid food was provided for Cases 8 to 12 by gastric tube three times daily, at 8 a.m., 12 noon, and 5 p.m. Also the sleep-awake cycle in the conscious group, Cases 1 to 7, was checked.

**Biochemical Determinations**

Samples of CSF were frozen with dry ice immediately after collection, and kept at −20°C until analysis, which was performed within 1 month at the latest. Preliminary experiments showed no substantial decomposition of HVA and 5-HIAA under storage conditions. Samples contaminated with blood were discarded. The volume of samples used for determination of HVA and 5-HIAA values was 5 and 3 ml, respectively. When the total volume of sample was less than 8 ml, it was diluted with distilled water to this volume. Determination of HVA was done fluorimetrically with a modification of the method of Curzon, et al.® Protein was precipitated from 5 ml CSF by adding 1 ml of 10% zinc sulphate followed by 0.2 ml of 2.5 N sodium hydroxide. After centrifugation, supernatant was pipetted into a centrifuge tube, acidified by

**Table 1**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>Delay (days)</th>
<th>CSF Outflow Volume/Day (ml)</th>
<th>HVA &amp; 5-HIAA Concentrations (ng/ml)</th>
<th>Correlation §</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>1st Day</td>
<td>2nd Day</td>
</tr>
<tr>
<td>1</td>
<td>56, F</td>
<td>Lt acoustic neurinoma</td>
<td>11</td>
<td>220–250</td>
<td>HVA: 12(12) 117 ± 9 (12) 125 ± 10(12) 122 ± 7 (36)</td>
<td>HVA 5-HIAA: 64 ± 8 (12) 46 ± 5 (12) 60 ± 3 (12) 63 ± 3 (36)</td>
</tr>
<tr>
<td>2</td>
<td>41, M</td>
<td>Rt thalamic tumor</td>
<td>4</td>
<td>250–300</td>
<td>HVA: 12(12) 148 ± 17 (12) 142 ± 14 (12) 148 ± 10 (36)</td>
<td>HVA 5-HIAA: 42 ± 4 (12) 49 ± 6 (12) 52 ± 4 (12) 48 ± 3 (36)</td>
</tr>
<tr>
<td>3</td>
<td>55, M</td>
<td>4th ventricle tumor</td>
<td>3</td>
<td>170–220</td>
<td>HVA: 12(12) 190 ± 16 (12) 151 ± 12 (12) 152 ± 9 (36)</td>
<td>HVA 5-HIAA: 32 ± 4 (12) 61 ± 5 (12) 43 ± 3 (12) 45 ± 3 (36)</td>
</tr>
</tbody>
</table>

*HVA = homovanillic acid; 5-HIAA = 5-hydroxyindoleacetic acid. Cerebrospinal fluid (CSF) samples were obtained through a drainage tube every 2 hours in all cases.

†Lapse of time between ventricular drainage and the start of CSF sampling.

‡Mean ± standard error. Numbers in parentheses indicate number of measurements. Mean of three cases: HVA 140 ± 5 (108) ng/ml; 5-HIAA 52 ± 2 (108) ng/ml.

§Correlation coefficient between HVA and 5-HIAA.
**TABLE 2**

Ventricular CSF levels of HVA and 5-HIAA in conscious patients with internal hydrocephalus caused by blockage of CSF flow (Group B)*

| Case No. | Age (yrs), Sex | Diagnosis               | Site & Degree of CSF Flow Blockage | Delay† (days) | CSF Outflow Volume/Day (ml) | HVA & 5-HIAA Concentrations (ng/ml)‡ | Correlation|| |
|----------|----------------|-------------------------|------------------------------------|--------------|----------------------------|-------------------------------------|-----|-----|-----|-----|
| 5        | 18, M          | Pineal tumor            | complete block of 3rd ventricle     | 3            | 200-250                    | HVA: 538 ± 38 (12) 646 ± 43 (11) 573 ± 50 (12) 584 ± 26 (35)§ 5-HIAA: 86 ± 4 (12) 110 ± 9 (11) 108 ± 11 (12) | 0.828 < 0.001 |
| 6        | 53, F          | 3rd ventricle           | complete block of both foramina of Monro | 20           | 300                        | HVA: 273 ± 12 (12) 5-HIAA: 39 ± 2 (12) | 0.247 NS |
| 7        | 28, M          | 3rd ventricle           | partial block of 3rd ventricle      | immedia-     | 350                        | HVA: 133 ± 12 (8) 5-HIAA: 32 ± 3 (8) | 0.694 NS |

*HVA = homovanillic acid; 5-HIAA = 5-hydroxyindoleacetic acid. Cerebrospinal fluid (CSF) samples were obtained through a drainage tube every 2 hours in Cases 5 and 6, and at 3 hours in Cases 4 and 7.
†Delay indicates length of time between ventricular drainage and start of CSF sampling.
‡Mean ± standard error. Numbers in parentheses indicate number of measurements.
§Significant difference as compared with Group A (p < 0.001).
||Correlation coefficient between HVA and 5-HIAA. NS = not significant.

**TABLE 3**

Ventricular CSF levels of HVA and 5-HIAA in comatose patients (Group C)*

| Case No. | Age (yrs), Sex | Diagnosis                  | Duration of Coma | Delay† (days) | CSF Outflow Volume/Day (ml) | HVA & 5-HIAA Concentrations (ng/ml)‡ | Correlation|| |
|----------|----------------|---------------------------|------------------|--------------|----------------------------|-------------------------------------|-----|-----|-----|-----|
| 8        | 39, M          | Brain-stem hemorrhage     | 6 days           | 6 days       | 300-350                    | HVA: 60 ± 12 (6) 70 ± 11 (6) 62 ± 13 (3) 64 ± 7 (15)§ 5-HIAA: 17 ± 2 (6) 23 ± 3 (6) 18 ± 5 (3) 19 ± 2 (15)§ | 0.573 < 0.05 |
| 9        | 61, M          | Intracerebral and intracerebellar hematoma | 1 mo             | 2 wks        | 250                        | HVA: 126 ± 17 (6) 170 ± 17 (6) 134 ± 10 (6) 143 ± 9 (18) § 5-HIAA: 18 ± 4 (6) 34 ± 5 (6) 24 ± 4 (6) 26 ± 3 (18)§ | 0.310 NS |
| 10       | 49, M          | Brain contusion           | 1 mo             | 4 days       | 300                        | HVA: 94 ± 13 (12) 5-HIAA: 51 ± 6 (12) | 0.762 < 0.01 |
| 11       | 45, M          | Acute subdural and intracerebral hematoma | 2 mos            |              | 250                        | HVA: 23 ± 1 (12) 16 ± 1 (12) 15 ± 2 (12) 18 ± 1 (36)§ 5-HIAA: 38 ± 2 (12) 34 ± 2 (12) 30 ± 3 (12) 34 ± 1 (36)§ | 0.760 < 0.001 |
| 12       | 48, M          | Brain contusion           | 2 mos            | 1 mo         | 250                        | HVA: 143 ± 10 (12) 115 ± 21 (4) 136 ± 9 (16) 5-HIAA: 99 ± 4 (16) 83 ± 8 (4) 95 ± 4 (16)§ | 0.892 < 0.001 |

*HVA = homovanillic acid; 5-HIAA = 5-hydroxyindoleacetic acid. In Case 11, cerebrospinal fluid (CSF) was aspirated by puncture of an Ommaya reservoir connected to the tube placed in the lateral ventricle, while in the other four cases, CSF samples were obtained through a drainage tube. Samples of CSF were collected every 2 hours in Cases 10-12, and at 4 hours in Cases 8 and 9.
†Delay indicates length of time between ventricular drainage and start of CSF sampling.
‡Mean ± standard error. Numbers in parentheses indicate number of measurements.
§Significant difference as compared with Group A (p < 0.001).
||Correlation coefficient between HVA and 5-HIAA. NS = not significant.
adding 1 ml of 1 N hydrochloric acid saturated with sodium chloride, and shaken mechanically for 10 minutes with 15 ml ethyl acetate. After centrifugation, the organic phase was taken in a centrifuge tube and back-extracted into 5 ml of pH-8.6 0.1 M borate buffer by shaking mechanically for 10 minutes. After centrifugation, two 2.0-ml portions of the aqueous phase were pipetted into test tubes. To one tube were added 1 ml of 5 N ammonia and 0.2 ml of 0.01% potassium ferricyanide. Exactly 4 minutes later, 0.2 ml of 0.1% L-cysteine was added, and fluorescence was read immediately. The second 2.0-ml sample was used as a blank by adding the potassium ferricyanide after the ammonia and the L-cysteine. Determination of 5-HIAA was by the method of Ashcroft, et al.¹

Results

Variations in HVA and 5-HIAA Values with Time

The HVA and 5-HIAA values varied with time in all cases (Figs. 1, 2, and 3). Significant correlations were found between ventricular HVA and 5-HIAA levels in eight cases (Tables 1, 2, and 3). However, the presence or absence of a correlation between HVA and 5-HIAA values was not related to the state of CSF flow or the level of consciousness.

Study was made using correlogram analysis to ascertain if there were circadian rhythms in HVA and 5-HIAA values, but a statistically significant finding was noted in Case 2 only (Fig. 4, p < 0.05); the others failed to demonstrate a uniform rhythm. A relationship of HVA and 5-HIAA values to the sleep-awake cycle and meals could not be established in any case, including Case 2.

HVA and 5-HIAA Levels in Ventricular CSF

The daily mean HVA and 5-HIAA concentrations were almost uniform in all cases except Case 3. In Case 4, CSF sampling was commenced immediately after ventricular drainage, but the daily mean values checked on the 1st and 4th days failed to show any difference. Normal control ventricular CSF samples cannot be obtained in clinical studies for obvious reasons. Therefore, we used Group A as the control, and statistically analyzed the HVA and 5-HIAA values in all cases of Group B and C (Student’s t-test).

Patients with Blockage of CSF Flow: In two patients (Cases 4 and 5), whose CSF flow was completely blocked by tumor occupying the fourth ventricle and aqueduct, respectively, both HVA and 5-HIAA concentrations showed variations at a higher level (Table 2) than in Group A. This was particularly remarkable in Case 5, in which the CSF outflow was disturbed by ventricular drainage dysfunction (Fig. 2 right). In Cases 6 and 7, the tumor occupied the third ventricle. The HVA value in Case 6, in which the bilateral foramina of Monro were completely blocked by the tumor, was higher than that of Group A, but in

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![FIG. 1. Variation of HVA and 5-HIAA levels in ventricular CSF in a Group A patient (Case 2). The HVA and 5-HIAA concentrations vary with time, but there is no relationship between variations of the two values and the sleep-awake rhythm.](image1)

![FIG. 2. Variation of HVA and 5-HIAA levels in ventricular CSF in Group B patients (Case 4, left, and Case 5, right). Both HVA and 5-HIAA concentrations vary at higher levels than in Group A. In Case 4, HVA and 5-HIAA values do not decrease with lapse of time after ventricular drainage.](image2)
Monoamine metabolism in hydrocephalus and coma

Case 7, with the third ventricle only partially obstructed by the tumor, no difference was observed. On the contrary, the 5-HIAA concentrations were lower than those of Group A in both cases.

Comatose Patients. Both HVA and 5-HIAA concentrations in Cases 8 and 11, 5-HIAA in Case 9, and HVA in Case 10 were lower than those of Group A (Fig. 3 left), but in Case 12 the 5-HIAA values showed variations at a rather higher level (Fig. 3 right). Definite relationships were not found between duration of coma and HVA or 5-HIAA levels.

Discussion

Variations in HVA and 5-HIAA Values with Time

The HVA and 5-HIAA values varied with time in all cases. Wyatt, et al. reported that ventricular 5-HIAA concentrations during non-rapid eye movement sleep were higher than when the subjects were awake or during rapid eye movement sleep. However, in the present study, variations of HVA and 5-HIAA values were observed in conscious patients while awake as well as throughout the day in comatose patients. It may be suggested that there is no relationship between variations of HVA and 5-HIAA values and the sleep-awake rhythm.

The mechanism by which the HVA and 5-HIAA values vary in parallel with each other is considered to be that these amine metabolites are equally regulated during their respective courses of 1) production, 2) circulation together with the CSF flow, and 3) process of absorption. However, points (1) and (2) can be ruled out because there was a significant correlation between the values of HVA and 5-HIAA even in comatose patients in whom one or both HVA and 5-HIAA values showed decreases and in the patient in whom there was complete blockage of the CSF flow. Parallel variation of HVA and 5-HIAA values may be due to the activity of the reabsorption system which is said to be located in the choroid plexus. Studies using probenecid which blocks active transport of these acid metabolites from CSF to blood, may help to elucidate this point.

HVA and 5-HIAA Levels in Ventricular CSF

Patients with Blockage of CSF Flow. As it has been shown that HVA and 5-HIAA are not transported from the blood system to the CSF, they presumably originate in the central nervous system. It is generally assumed that HVA enters the lateral ventricle from the periventricular brain parenchyma, especially the striatum, and descends from the ventricular system to the subarachnoid space. However, there is not necessarily agreement among the various authors on the specific site of 5-HIAA production. As to the origin of 5-HIAA in lumbar CSF, Curzon, et al., and Weir, et al., have suggested considerable contribution from the brain, whereas Bulat reported that it derives exclusively from the spinal cord. Furthermore, it has been reported that 5-HIAA concentrations in ven-
tricular or cisternal CSF tend to parallel those in the cerebral parenchyma. However, the exact site of origin of ventricular CSF 5-HIAA is still unknown.

In Cases 4, 5, and 6, with a complete blockage of CSF flow due to the presence of tumor, the ventricular CSF level of HVA was high. As factors other than the blockage of CSF flow are responsible for such elevated HVA values, the following may also be considered: 1) monoamine metabolism of the tumor per se, and 2) damage to the cerebral parenchyma. However, it has been found that the glioma monoamine level is low, and generally the intracerebral dopamine and HVA are decreased in dopamine pathway damage. The elevation of HVA value is probably due to the fact that its transport to the lower level is obstructed. On the other hand, although the 5-HIAA value was high in Cases 4 and 5, in which the CSF flow into the fourth ventricle and aqueduct was completely blocked, respectively, it was interesting that the 5-HIAA values were low in Cases 6 and 7, in which the foramina of Monro were blocked by craniopharyngiomas of the third ventricle. The decrease in 5-HIAA in Cases 6 and 7 is thought to be due to damage sustained by the periventricular structure of the third ventricle, which is rich in 5-HT nerve terminals. These findings may suggest that most of the ventricular CSF 5-HIAA is derived from the periventricular structure of the third ventricle.

Before this study, we had assumed that, in patients in whom there was blockage of CSF flow, the HVA and 5-HIAA concentrations would decrease with time after ventricular drainage. However, in Case 4, the HVA and 5-HIAA values did not decrease with lapse of time. Also in Case 6, in whom there was blockage of CSF flow, the HVA value was still elevated. It is thus necessary to determine whether the HVA and 5-HIAA values decrease after longer intervals.

Comatose Patients. During recent years, variations in HVA and 5-HIAA values in CSF collected mainly by lumbar puncture have been reported in patients with head injury with disturbance in consciousness. However, as evident from this study, research on cerebral amine metabolism should include determinations of HVA and 5-HIAA values in the ventricular fluid after a lapse of time.

In the present study, four of the five comatose patients showed low levels in either one or both of the HVA and 5-HIAA values. Decreases in HVA and 5-HIAA levels after head injury or cerebrovascular disease are thought to be due to direct or indirect damage of dopamine or 5-HT pathways. The decreases in HVA and 5-HIAA values in Case 8 were probably due to direct injury to dopamine and 5-HT cell groups of the brain stem. On the other hand, in Cases 9, 10, and 11, either or both dopamine and 5-HT pathways, which have different intracerebral distributions, might have been injured directly or indirectly, resulting in permanent decrease in brain monoamine metabolism.

The increase in 5-HIAA value noted in Case 12 is of interest. There are several reports that the 5-HT content in brain may become elevated in the acute stage after head injury, brain infarction, or hypoxia. The 5-HIAA level in Case 12 was determined 2 months after head injury. Therefore, it is not known whether elevation of the 5-HT level persisted from the acute stage in this case, but it is possible that, in contrast to the other comatose patients, the increased brain 5-HT metabolism may have had some ill effect upon the consciousness level. If 5-HT plays an aggravating role during posttraumatic coma, the administration of drugs that inhibit 5-HT synthesis or such preparations as L-dopa, which have arousal effects on hepatic coma (which indicates the presence of increase in 5-HT turnover), may prove to be effective.

Last, the CSF values of HVA and 5-HIAA may be greatly affected by the choroid plexus function which transports these amine metabolites to the blood from CSF. This is a subject that requires further study.

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