Plasma 3-Methoxy-4-Hydroxyphenylethylene glycol and Homovanillic Acid Concentrations in Patients with Subarachnoid Hemorrhage

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To ask if the determination of central-nervous-system-derived catecholamine metabolites in peripheral circulation could be a useful index of brain dysfunction after subarachnoid hemorrhage, 3-methoxy-4-hydroxyphenylethylene glycol and homovanillic acid concentrations in plasma, together with those of free catecholamines (noradrenaline, adrenaline, and dopamine), were serially measured for up to 3 weeks after the initiation of symptoms in 23 patients with aneurysmal subarachnoid hemorrhage as compared to 17 healthy and 9 patient controls. Catecholamines and their metabolites were determined by using high-performance liquid chromatography with electrochemical detection. Plasma 3-methoxy-4-hydroxyphenylethylene glycol concentrations were markedly elevated in subarachnoid hemorrhage patients with coma compared to those without, and the maximal concentrations observed in comatose patients never occurred in normal subjects or in patients with other neurological disorders. The mean maximal plasma concentrations of free catecholamines did not differ significantly between the comatose and noncomatose groups. Combining 3-methoxy-4-hydroxyphenylethylene glycol with homovanillic acid level data more clearly discriminated between the comatose and noncomatose subarachnoid hemorrhage groups. The results suggest that plasma concentration of 3-methoxy-4-hydroxyphenylethylene glycol, a major metabolite of brain noradrenaline, can be a prognostic discriminator for patients with subarachnoid hemorrhage and its discriminating power can be strengthened by combining it with homovanillic acid data. (Stroke 1987;18:223-228)

During the past decade, convincing evidence has accumulated indicating that free 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), a major metabolite of brain noradrenaline, in the systemic circulation reflects a functional activity of noradrenergic neurons in the central nervous system (CNS). Free MHPG concentration in plasma is considered a reliable index of central noradrenergic turnover from the highly significant relation between plasma and brain levels in primates treated with drugs altering the central noradrenaline metabolism. In humans, a centrally acting α-adrenergic agonist, clonidine, is reported to reduce plasma MHPG level. Maas et al have demonstrated that approximately 60% of total MHPG production in humans is derived from the brain, while others have indicated that the proportion may be lower because of the possible peripheral conversion of MHPG to vanillylmandelic acid. As well as MHPG, plasma concentration of a major dopamine metabolite, homovanillic acid (HVA), seems to fluctuate with changes in dopamine turnover in the CNS. Several studies measuring MHPG or HVA in plasma have shed light on various areas of clinical psychiatric research, but to our knowledge, not on neurosurgical research.

Elevations of peripheral catecholamines have been observed after subarachnoid hemorrhage (SAH), revealing peripheral sympathetic overactivity in SAH patients. Acute cerebrovascular accident or cerebral ischemia can cause substantial damage in certain brain areas. Sympathetic overactivity seen after SAH has been suggested to originate in dysfunction of the hypothalamus, which is located near the common sites of aneurysms. Hypothalamic dysfunction associated with high concentrations of circulating catecholamines and with hypersensitivity of neurons innervating cerebral blood vessels seems to play an important role in the genesis of vasospasm frequently occurring with an episode of neurological deterioration after SAH. In addition, sustained sympathetic overactivity or marked hypertension associated with SAH may augment the incidence of rebleeding, another major risk factor, for SAH patients. By considering MHPG and HVA to be major CNS-derived catecholamine metabolites in the systemic circulation, the determination of these concentrations in plasma could be a useful index of brain dysfunction or damage in SAH patients. This index is assumed to be superior to that of peripheral catecholamines, the plasma levels of which vary, de-
pending on environmental, emotional, and endogenous stimuli provoking a sympathetic response. To examine these hypotheses, we examined the serial changes in plasma concentrations of free catecholamines (noradrenaline, adrenaline, and dopamine) and their metabolites (MHPG and HVA) for up to 3 weeks after the initiation of SAH symptoms in 23 patients compared to 17 healthy and 9 patient controls.

**Subjects and Methods**

This study included 23 patients (8 men and 15 women) with recent rupture of an intracranial saccular aneurysm admitted to the Section of Neurosurgery, St. Marianna University Hospital. The age of the patients ranged from 35 to 83 years. Their clinical condition was assessed daily and graded according to the system of Hunt and Hess. Computer tomography (CT) was performed in all patients between the 1st and 3rd day after admission. Subarachnoid hemorrhage due to ruptured aneurysm was detected by CT, angiogram, and/or diagnostic lumbar puncture in all patients. No patient receiving catecholamines or drugs that affect plasma catecholamine levels prior to or during sample collection is included in this report. All patients were evaluated independently by the same neurosurgeon, who was unaware of the assay results. The SAH patients were divided into two groups: 11 with coma who were assigned to Grade 4 or 5, and 12 without coma (Table 1). In addition, 17 normal healthy volunteers (7 men and 10 women, ranging in age from 23 to 38 years) and 9 patients (6 men and 3 women, ranging in age from 16 to 64 years) with other neurological disorders (cerebral hemorrhage in 7; germinoma and extradural hematoma in 1 each) served as reference groups. Informed consents were obtained from guardians in cases where the patients were mentally and/or physically incapacitated. Otherwise, informed consents were obtained from the patients or normal subjects themselves.

Blood samples were serially drawn for the measurement of monoamine metabolites until 3 weeks after the initiation of SAH symptoms at a few day intervals (129

| S.A. | M | 35 | Basilar tip | 1 | Vasospasm | Dead |
| C.W. | F | 68 | Right MCA | 0 | Initial coma | Dead |
| M.J. | F | 54 | Left MCA | 1 | Vasospasm | Dead |
| T.C. | F | 54 | Basilar tip | 0 | Operational problem | Dead |
| H.M. | M | 42 | Basilar tip + left MCA | 0 | Initial coma | Vegetative |
| S.K. | M | 50 | Left MCA | 1 | Initial coma | Dead |
| A.T. | F | 53 | Left MCA | 0 | Initial coma | Recovered |
| H.O. | F | 52 | Ant. comm. A | 1 | Operational problem | Recovered |
| M.S. | F | 67 | Right MCA | 0 | Rebleeding | Dead |
| K.T. | F | 46 | Left ICA | 0 | Rebleeding | Dead |
| T.I. | F | 54 | Ant. comm. A | 0 | Vasospasm | Recovered |

**Table 1. Clinical Characteristics and Outcome of Patients with SAH**

MCA = middle cerebral artery; Ant. comm. A = anterior communicating artery; ICA = internal carotid artery; and 0 = within 24 hours.
samples collected from 23 SAH patients, 49 from 9 other neurological patients, and 51 from 17 normal subjects. All patients were at complete bed rest throughout the sampling periods. Samples were collected from normal subjects after a 60-minute supine rest. Blood was collected in a cooled tube containing 0.1% EDTA 2Na and 0.1% sodium m-bisulphite, and put on ice. Plasma was separated by centrifugation at 600g for 10 minutes at 4°C and then stored at −80°C until analyzed. Plasma concentrations of monoamine metabolites were determined by using high-performance liquid chromatography with electrochemical detection (HPLC-ECD) according to our newly developed method.22,23 Plasma concentrations of catecholamines were measured with the reported HPLC-ECD method24 with alumina adsorption and subsequent elution with weak acids.25

Results

Clinical characteristics of the 11 comatose and 12 noncomatose SAH patients are shown in Table 1. There were no significant differences between the groups in mean values for age, sex distribution, time to admission after the initiation of SAH symptoms, or number of patients with or without surgery.

The most striking difference between the comatose and noncomatose SAH groups was in changes of plasma MHPG concentrations among catecholamines and their metabolites. In noncomatose patients plasma MHPG did not fluctuate much during the whole course of sampling (Figure 1B). The upper limit of plasma MHPG level in normal adult subjects has been reported to be <9 ng/ml by previous investigators.26,27 Only 3 of the noncomatose patients had plasma MHPG levels exceeding the reported upper limit of 9 ng/ml — and only once each during the sampling period. In contrast, MHPG levels in all comatose patients varied widely, and values above 9 ng/ml were observed more than twice in each patient during the sampling period irrespective not only of whether surgery was performed but also of what neurological grade was assessed at sampling (Figure 1A).

Since sampling times and time courses of changes in plasma MHPG concentrations as well as other measures varied interindividually, observed maximal levels up to 3 weeks were considered the most appropriate indices for analyzing their changes. The respective mean (± SEM) maximal levels of noradrenaline, adrenaline, and dopamine were 1.310 ± 0.478 vs. 0.682 ± 0.142, 0.453 ± 0.132 vs. 0.974 ± 0.561, and 0.126 ± 0.034 vs. 0.149 ± 0.049 ng/ml in the comatose vs. the noncomatose patients. These mean values did not differ significantly between the two groups. However, the mean maximal MHPG concentration was significantly (r = 3.311, p < 0.01) greater in the comatose group (26.01 ± 5.57 ng/ml) than in the noncomatose group (7.39 ± 0.75 ng/ml). Although the difference in the mean maximal HVA concentration between the comatose (25.39 ± 4.82 ng/ml) and noncomatose patients (16.37 ± 2.03 ng/ml) did not reach statistical significance, plotting maximal MHPG against maximal HVA values more clearly discriminated between these two groups (Figure 2A). This was confirmed by discriminate analysis (Mahalanobis D-square = 3.112, F (2, 20) = 8.505, p < 0.003) as compared to MHPG data alone (D-square = 2.085, F (1, 21) = 11.969, p < 0.003). As shown in Figure 2A, all comatose patients fell into the hatched area, and 10 of the 11 comatose patients had a maximal plasma MHPG level not only above the normal upper range reported26,27 but also above 12 ng/ml. Maximal plasma MHPG above 12 ng/ml was never observed in the noncomatose patients, while only one comatose patient had a maximal plasma MHPG level between 9 and 12 ng/ml. This patient had a maximal HVA value above 20 ng/ml, which is the upper limit previously reported from normal adult subjects.26,28 None of our healthy subjects had maximal HVA values exceeding 20 ng/ml (Figure 2C). All noncomatose patients fell into the nonhatched area of Figure 2A. Distribution of MHPG values over the three ranges (<9, 9–12, and >12 ng/ml) was significantly different between the two groups ($\chi^2 = 19.99, df = 2, p < 0.001$). Neither the 17 normal subjects nor the 9 control patients with other neurological disorders showed maximal plasma MHPG concentrations exceeding 9 ng/ml (Figures 2B and 2C).
FIGURE 2. Diagrammatic plots of maximal plasma MHPG and HVA concentrations observed up to 3 weeks after initiation of symptoms in patients with SAH (A), in those with other neurological disorders (B), and in normal healthy subjects (C). Closed (●) and open (○) circles indicate comatose (n = 11) and noncomatose SAH patients (n = 12), respectively. Hatched area indicates the proposed risk area for developing coma in SAH patients. V and D designate comatose patients who at discharge were vegetative or dead, respectively. All noncomatose patients who recovered afterward and were discharged fall into nonhatched area. Three comatose SAH patients, not designated with V or D, also recovered afterward and were discharged. Dotted horizontal and vertical lines indicate the upper limits of plasma HVA (20 ng/ml)26-28 and MHPG concentrations (9 ng/ml) reported in normal adults,26-27 respectively. No noncomatose patients showed plasma MHPG concentration exceeding 12 ng/ml as shown by another dotted vertical line.

and 2C), although maximal HVA concentrations were somewhat elevated in 2 of the patients (Figure 2B).

In all comatose patients, a maximal plasma MHPG level exceeding 9 ng/ml had already been observed by 7 days after the initiation of SAH symptoms, in contrast to a level that high in only 1 of the noncomatose group (11/11 vs.1/12, \( \chi^2 = 15.82, \ df = 1, \ p < 0.0001 \)).

Discussion

It has been documented that SAH patients with poor outcome showed an elevated noradrenaline and/or adrenaline concentration in the systemic circulation.11-13 In our study, maximal plasma noradrenaline, but not adrenaline, concentrations tended to be elevated in the comatose group compared with the noncomatose group. However, noradrenaline concentration ranges observed in the poor and good outcome groups of previous studies11-13 and of the present study overlapped. Therefore, it does not seem to be feasible to predict the outcome of an individual SAH patient solely from this information. In the present study, peripheral catecholamine levels did not significantly differ between the two SAH groups. In addition, elevation of plasma MHPG levels was not found in the comatose patients with other neurological disorders. Thus, our findings on MHPG result neither from a general stress response to the acute disorder nor from nonspecific factor(s) modulating the sympathetic activity.20

Among the 11 comatose SAH patients, 4 became comatose shortly after the initiation of symptoms, 2 deteriorated postoperatively, 3 had vasospasm, and the remaining 2 had rebleeding (Table 1). The elevation of MHPG concentrations in plasma was not always associated with simultaneous neurological deterioration, but rather preceded it except for cases where patients had been comatose immediately after the initiation of symptoms. Therefore, the present results suggest not only that an elevated plasma free MHPG reflects the underlying damage to brain noradrenergic neurons (e.g., hypothalamus) commonly seen in SAH but also that the release of neurotransmitters from cerebral tissues in severely ill SAH patients may play a role in exacerbating the illness. Hypothalamic lesions in SAH patients have been documented as correlating with the myocardial lesion or dysfunction of the autonomic nervous system.16 In accordance with the above hypothesis, Wurtman and Zervas29 have suggested that the resulting inappropriate loss of several monoamine neurotransmitters after ischemia due to SAH may further exacerbate the pathophysiological changes caused by the initial symptoms. However, we cannot deny another possibility, i.e., an involvement of the cerebrovascular adrenergic neurons. The transient decrease in the histochemical fluorescence of the adrenergic nerve plexus supplying the cerebral arteries has been found in SAH patients.30 This may be responsible for the supersensitivity of arterial noradrenergic neurons observed in experimental SAH models.31 Mean-while, \( \beta \)-adrenoceptor blocking agents have been reported effective in prophylaxis of vasospasm and in therapy of refractory hypertension after SAH,19,31,32
due at least in part to effects on central noradrenergic neurons. This also supports our hypothesis.

Monoaminergic function(s) in humans cannot be measured directly. Even if assessment of monoamine metabolites in CSF is preferable, it can be permitted only under certain restrictions. Under usual clinical circumstances, successive determinations of monoamine metabolites in CSF is difficult — for ethical reasons. On the other hand, although plasma catecholamine levels have been considered an index of sympathetic nervous activity, sampling for determination of plasma catecholamines requires rigidly controlled environmental circumstances because of the lability of plasma catecholamines in response to emotional and physical stimuli. Thus, the assessment of monoamine metabolites in plasma offers several practical advantages, and this strategy may be applicable to patients at various appropriate clinical stages. Determination of the metabolites from serial samplings can be performed without much difficulty and does not require strictly controlled circumstances.

Based on the present results, we are tempted to warrant that an SAH patient whose MHPG and HVA levels up to 3 weeks after the initiation of symptoms fall into the hatched area of Figure 2A has a high risk for developing more severe neurological deterioration or coma. Though we assessed MHPG levels for 3 weeks, MHPG levels exceeded 9 ng/ml in all comatose patients within a week after the ruptured aneurysm, but this occurred in only 1 noncomatose patient. Therefore, plasma free MHPG monitored until a week after the initiation of symptoms can also be useful as a prognostic discriminator for SAH. Finally, MHPG levels of 3 recovered patients in the comatose group were around 12 ng/ml, which is near the borderline level separating the two SAH groups (Figure 2A). It seems likely that a comatose patient with SAH who shows a maximal plasma MHPG level of near the borderline as shown in Figure 2A might have a rather good outcome, although this is not conclusive from the small number of such patients in the present study.

Further study may elucidate whether these measures are of a critical value for estimating the ultimate outcome in comatose SAH patients.

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