SUMMARY Serum dopamine-β-hydroxylase (DBH) activity was measured in 34 patients with acute cerebrovascular disease. The serum level of DBH activity showed its highest value soon after the onset of stroke and then gradually decreased over the next few days. After reaching its lowest level, the DBH activity again showed a slight increase. There was no direct relationship between serum DBH activity and total serum protein, or blood pressure. In 8 of 12 patients, DBH activity in the cerebral venous blood was higher than that in the arterial blood. These results suggest that rapid release of DBH into the circulating blood occurred after stroke, presumably from sympathetic nerve endings in the vessels or organs, including the brain.

STROKE, Vol 10, No 2, March-April 1979

Serum Dopamine-β-Hydroxylase Activity in Acute Stroke

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DOPAMINE-β-HYDROXYLASE (DBH), the enzyme needed for the final step of norepinephrine synthesis, is known to be present in synaptic vesicles in sympathetic nerve endings1 and to be released together with norepinephrine by a process of exocytosis from the stimulated nerve endings.2 Thus, DBH in circulating blood may be derived mainly from sympathetic nerve terminals3 and the level of DBH activity in the blood may reflect alterations in sympathetic nerve activity.4,5 In clinical studies of migraine, in which a cerebral vasomotor disturbance is known to exist, elevated levels of DBH activity in the circulating blood have been seen in headache-free intervals.6

In patients with stroke, elevated levels of plasma7 and urinary catecholamines8 have been reported during the acute state of cerebrovascular disease, suggesting increased activity in the sympathetic nervous system after ictus. Recent observations9 of experimental subarachnoid hemorrhage have suggested the possible participation of cerebrovascular nerves in the generalized response of the autonomic nervous system. Since the autonomic nervous system may play a role in the regulation of cerebral blood flow10,11 the cerebral vasomotor disturbance observed at the acute stage of stroke could be associated with the release of norepinephrine from nerve endings in cerebral vessels. Measurements of DBH levels in arterial and cerebral venous blood, as well as in the systemic venous blood of patients with stroke, may thus provide some insight into the pathophysiology of stroke.

The purpose of the present study was to determine whether or not perceptible amounts of DBH were released into the circulating blood from sympathetic nerve endings in the vessel of the brain or other organs during an acute stroke.

Subjects and Methods

Serum DBH activity was measured in 34 patients with various types of acute cerebrovascular disease, admitted within 24 hours of onset. Their ages ranged from 28 to 89 (mean 53) years. Ten patients were found to have cerebral infarction, 17 intracerebral hemorrhage, and 7 subarachnoid hemorrhage resulting from ruptured intracranial aneurysms. The diagnosis was based on the clinical examinations, lumbar puncture, cerebral angiography and/or computed tomography. Four patients died within 4 days after the onset and 2 died between 5 to 30 days.

Initial blood samples were drawn 40 minutes to 23 hours after onset. The second and third samples were then obtained at 3–4 days and 5–10 days after onset. In 12 patients, simultaneous blood samples were obtained within 48 hours after onset from the femoral
artery and internal jugular vein for measurement of cerebral veno-arterial (V-A) differences in DBH activity. The venous samples for controls were also obtained at similar intervals following admission from 7 patients with neurological diseases other than cerebrovascular disease. The brachial blood pressure was determined when blood samples were taken. All blood samples were cooled in ice, until centrifuged at 4°C within 60 minutes. The sera were stored at −40°C and enzyme assay was performed in duplicate within 3 weeks. Serum DBH activity was measured according to the method of Nagatsu and Udenfriend. The reaction mixture (total volume, 2 ml) contained: 100 μl serum, 50 μl of 0.4 M tyramine, 100 μl of 0.2 M sodium fumarate, 100 μl of 0.2 M ascorbic acid, 1500 units of catalase in 100 μl of aqueous solution, 100 μl of 20 mM pargyline, 300 μl of 0.2 M N-ethylmaleimide, and 400 μl of 1M sodium acetate buffer (pH 5.0). The reaction mixture was incubated at 37°C for 60 min in a water bath with agitation. The incubation was stopped by adding 0.4 ml of 3M trichloroacetic acid and was centrifuged at 2,000 rpm for 10 min. The supernatant fluid was transferred to a small column containing Dowex —50 (H +, 200-400 mesh), (packed volume, 0.4 ml). After washing, the adsorbed octopamine was eluted with 2.0 ml of 4M NH₄OH. Octopamine in the elute was converted to p-hydroxybenzaldehyde by addition of 0.2 ml of 2% NaIO₄. Excess periodate was reduced by addition of 0.2 ml of 10% NaS₂O₆. The absorbance of p-hydroxybenzaldehyde was determined at 333 nm using a Hitachi spectrophotometer (Model 124). A standard curve was obtained by subjecting known amounts of octopamine to the procedure. In this method, N-ethylmaleimide at a concentration of 10 mM/liter or greater gives complete inactivation of endogenous enzyme inhibitors in serum. The enzyme activity is equal to that obtained in the presence of the optimum Cu²⁺ concentrations. One unit of DBH activity represents the formation of 1 μmol of octopamine per liter of serum per minute. The mean activity of serum DBH in 40 healthy subjects measured by this method in our hands was 24.9 ± 2.4 units (range 2 to 64). Total serum protein was determined by the method of Lowry et al.

Results

1) Time Course of Serum DBH Activity after Stroke

Fig. 1 illustrates typical changes in serum DBH and creatine phosphokinase (CPK) activity (normal range 0-12 units/ml), total serum protein and the blood pressure of a 68-year-old male patient with cerebral hemorrhage. On the day of admission, he suddenly developed left hemiplegia and became somnolent. The DBH activity of the initial blood sample of the series collected 2 hours after onset showed the highest value. The DBH activity of the initial blood sample of the series collected 2 hours after onset showed the highest value. The DBH of activity gradually decreased over the next few days, with his recovery of consciousness, while the serum level of CPK activity showed a delayed increase. After reaching its lowest level, the DBH activity again showed a slight increase.

The time course of serum DBH activity in various types of cerebrovascular disease are shown in fig. 2. In patients with cerebral hemorrhage, the mean DBH value of 39.7 ± 7.9 units/ml for the serum obtained within 24 hours of the onset was significantly higher than that of 29.7 ± 8.3 units/ml for the serum obtained 5 to 10 days after stroke (p < 0.001, p value was determined by Student's paired t test). In patients with subarachnoid hemorrhage and cerebral infarction, the mean values for the former were also significantly higher than those for the latter (p < 0.001 and p < 0.005, respectively). The mean value for control subjects without diagnosed cerebrovascular disease did not change significantly (table 1, fig. 2). The mean

![Figure 1. Time course of serum levels of dopamine-β-hydroxylase (DBH) and creatine phosphokinase (CPK) activity, total serum protein and blood pressure (BP) in a patient with cerebral hemorrhage.](http://stroke.ahajournals.org/)
40
30
20
10
0

Cerebral hemorrhage (N = 13)
Subarachnoid hemorrhage (N = 7)
Cerebral infarction (N = 10)
Control (N = 7)
Mean ± S.E.M.

FIGURE 2. Time courses of serum DBH activity after onset of stroke in groups of patients with cerebral hemorrhage, subarachnoid hemorrhage and cerebral infarction, and control subjects. (*p < 0.005, **p < 0.001, Student's paired t test)

value of 25.3 ± 2.6 units for control subjects was close to that of 24.9 ± 2.4 units for 40 healthy subjects.

The individual time courses of the level of serum DBH activity in 9 patients with cerebral hemorrhage and subarachnoid hemorrhage who were in the hospital beyond 2 weeks after stroke, are shown in fig. 3. The activity of DBH in the serum obtained immediately after the onset of stroke showed the highest value and then the level began to decrease rapidly, reaching a minimum within 2 weeks. Thereafter, it increased slightly and attained a plateau level within 2 months after onset of stroke. Similar changes were also observed in 7 patients with cerebral infarction as shown in fig. 4. However, in a majority of patients the activity of the enzyme reached the minimum level within a week, which is earlier than that in hemorrhagic group.

2) Correlation between Serum DBH Activity and Total Serum Protein

The correlation between changes in serum DBH activity and changes in total serum protein was tested in 16 patients. The initial blood samples were compared with the second or third samples in each patient. No statistical correlation existed between the changes in total serum protein and alterations of serum DBH activity (r = 0.315, p > 0.2) (fig. 5).

3) Correlation Between Serum DBH Activity and Blood Pressure

The correlation between changes in serum DBH activity and changes in systolic blood pressure after stroke was examined. The initial blood samples were compared with the second or third samples in each patient. The changes in systolic blood pressure and serum DBH activity were similar in 17 of the 24 patients. The changes in systolic blood pressure were not necessarily accompanied by a proportional degree of alteration of serum level of DBH activity, and no statistical correlation existed (r = 0.123, p > 0.5). No statistical correlation held between the changes in diastolic pressure and alterations of serum DBH activity (r = 0.282, p > 0.1).

FIGURE 3. Individual time courses of serum DBH activity after the onset of stroke in 7 patients with cerebral hemorrhage and 2 patients with subarachnoid hemorrhage. Note a slight increase in serum DBH activity after reaching the lowest level.
4) Cerebral V-A Differences in DBH Activity

Differences in DBH activity between cerebral venous and arterial blood were measured within 48 hours of onset in a group of 12 patients with acute symptoms and, for comparison, in a group of 8 patients with chronic symptoms of more than 3 weeks duration. In the acute group, the cerebral V-A differences exhibited a broad range of values and the venous blood levels of DBH activity were higher than the arterial levels in 8 of the 12 patients. On the other hand, the chronic patients showed only a limited distribution of V-A differences (table 2, fig. 6).

Discussion

Serial measurements of serum DBH activity made after stroke in this study demonstrated that the activity was at its highest level soon after the onset of stroke and that it gradually decreased over the next few days. Since changes in serum DBH activity did not parallel alterations in total serum protein, it was
unlikely that shifts in plasma volume were responsible for the fall in serum DBH.

It is known that the resting levels of serum DBH in healthy subjects exhibit a wide variation. In individual subjects, however, the levels are markedly constant over successive days and months. Since it is generally impossible to determine the baseline level of serum DBH activity in any patient prior to the onset of stroke, the question thus arises as to whether the serum DBH activity initially increases or decreases from the baseline level following onset. Based on our findings, the latter possibility appears unlikely since the mean activity of serum DBH in the acute state was significantly higher than that in the chronic state (figs. 3, 4). It is, therefore suggested that as a result of DBH release triggered by stroke, the serum DBH level may rapidly increase, reaching a maximum within a short period of time. Thereafter it decreases, reaching a minimum within 5 to 10 days, followed by a gradual increase toward the baseline levels.

It has been demonstrated in in vitro studies that stimulation of postganglionic sympathetic nerves results in DBH release by exocytosis, together with a proportional amount of norepinephrine. Subsequent studies have suggested that the circulating DBH level might be derived mainly from sympathetic nerve terminals. The results of the present study indicated that an excessive sympathetic nerve discharge may have occurred in the acute state of a cerebrovascular accident.

It is interesting to note that in the majority of patients, the serum level of DBH activity showed a slight increase after reaching a minimum. Since the serum DBH activity level became stable a month after onset, the increase seemed to be toward the baseline level. Although the precise reason for the slight increase in serum DBH activity is not clear, the following mechanism may be suggested. If DBH in synaptic vesicles is continuously released and the DBH available for the release is almost exhausted following sustained nerve stimulation, the DBH in the circulating blood may decrease below the baseline level and remain so until the DBH released from the synaptic vesicles is restored. The reduction in serum DBH activity below the expected baseline level thus implies that the patient may fall into a state of diminished sympathetic activity as a result of the massive release of DBH triggered by stroke. However, as the validity of

### Table 2: Venous-Arterial Differences in DBH Activity in Acute Stroke

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Time after Onset (hours)</th>
<th>Cerebral venous blood (units)</th>
<th>Arterial blood (units)</th>
<th>(V-A) difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hemorrhage</td>
<td>18</td>
<td>26.7</td>
<td>32.4</td>
<td>-5.7</td>
</tr>
<tr>
<td>2</td>
<td>Infarction</td>
<td>8</td>
<td>4.2</td>
<td>4.8</td>
<td>-0.6</td>
</tr>
<tr>
<td>3</td>
<td>Hemorrhage</td>
<td>43</td>
<td>9.9</td>
<td>8.1</td>
<td>1.8</td>
</tr>
<tr>
<td>4</td>
<td>SAH</td>
<td>17</td>
<td>27.3</td>
<td>27.6</td>
<td>-0.3</td>
</tr>
<tr>
<td>5</td>
<td>SAH</td>
<td>22</td>
<td>18.0</td>
<td>16.5</td>
<td>1.5</td>
</tr>
<tr>
<td>6</td>
<td>Infarction</td>
<td>47</td>
<td>43.8</td>
<td>39.9</td>
<td>3.9</td>
</tr>
<tr>
<td>7</td>
<td>Hemorrhage</td>
<td>23</td>
<td>20.1</td>
<td>19.8</td>
<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>Hemorrhage</td>
<td>23</td>
<td>46.6</td>
<td>48.4</td>
<td>-1.8</td>
</tr>
<tr>
<td>9</td>
<td>Hemorrhage</td>
<td>40</td>
<td>28.1</td>
<td>17.9</td>
<td>10.2</td>
</tr>
<tr>
<td>10</td>
<td>Hemorrhage</td>
<td>30</td>
<td>12.2</td>
<td>11.2</td>
<td>1.0</td>
</tr>
<tr>
<td>11</td>
<td>SAH</td>
<td>4</td>
<td>47.1</td>
<td>44.1</td>
<td>3.0</td>
</tr>
<tr>
<td>12</td>
<td>SAH</td>
<td>24</td>
<td>34.8</td>
<td>29.1</td>
<td>5.7</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>26.6</td>
<td>24.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Values are mean ± standard error. Control samples were obtained at similar intervals after admission. Asterisks indicate significant difference (p < 0.005, **p < 0.001) from respective samples obtained 0-24 hours after onset.

SAH = subarachnoid hemorrhage.
serum DBH activity as an index of acute sympathetic activation remains to be confirmed. The evaluation of changes in DBH activity after stroke must be, at best, speculative until the precise relationship between serum DBH level and sympathetic nerve activity is known.

Since DBH has been reported to exist in human and animal cerebral vessels and its distribution corresponds well with the localization of norepinephrine, elevated DBH in the blood might be derived, at least in part, from cerebral vessels, if the nerves on cerebral vessels participate in generalized responses of the sympathetic nervous system. In fact, the present studies of cerebral V-A differences in DBH activity showed higher venous than arterial levels of the enzyme in more than half of the patients.

The pathogenesis of a diffuse reduction in cerebral blood flow associated with the acute state of stroke has been a subject of interest and discussion. Some of the views suggest release of neurotransmitter from the damaged brain as being responsible for the impaired cerebral circulation. In clinical and experimental studies, the sympathetic nervous system has been reported to play a role in regulating cerebral blood flow. Therefore, it is suggested that changes in DBH activity in the arterial and venous cerebral blood observed in the present study may represent a deranged state of sympathetic nervous activity, and that a part of released DBH may reflect a pathogenic role for released norepinephrine in the cerebral hemodynamic disorders associated with acute stroke. In order to study this further, it would be desirable to determine cerebral blood flow concurrently with the measurement of norepinephrine concentrations as well as DBH activity in the circulating and cerebral venous blood.

Supported in part by grants for study of vascular diseases of the central nervous system from the Ministry of Health and Welfare, Japan.

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Stroke. 1979;10:168-173
doi: 10.1161/01.STR.10.2.168

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