Effect of Experimental Ischemia on Neurotransmitter Amines in the Gerbil Brain

M.J.G. HARRISON, D.M., F.R.C.P., C.D. MARSDEN, M. SC., F.R.C.P.,
AND P. JENNER, B. PHARM., PH.D., M.P.S.

SUMMARY In the gerbil cerebral infarction was produced by unilateral carotid ligation. 3.5 hours later, when the neurological deficit was fully developed, hemisphere dopamine (DA) showed little change from normal. It seems unlikely that changes in DA are the direct cause of the turning behavior shown by these animals. Slight changes in norepinephrine (NE) occurred on the operated side but 4 hydroxy-3-methoxy phenylethylenglycol sulphate (MOPEG-SO4) levels were not affected. Significant falls in 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) were found on the operated side but there was also a trend for both 5HT and 5-HIAA to fall on the unoperated side. These changes occurred in clinically affected and unaffected animals and their clinical significance is unproven.

IN AREAS of ischemic brain a variety of pathological disturbances of metabolic function occur. Energy metabolism fails with falls in energy charge and creatine phosphate. Glycogen is depleted and lactate rises with an accompanying decrease in tissue pH. Free fatty acids are released and there are detectable increases in the levels of NADH, adenosine, cyclic AMP, and ammonia. It has been suggested that many of these changes may of themselves contribute to the extent or reversibility of tissue damage.

More recently the role of neurotransmitter amines has been discussed. Their synthesis, storage, release and re-uptake might all theoretically be affected in an area of cerebral ischemia. Wurtman and Zervas suggested that disturbed neurotransmitter behavior might be a cause of increased neurological deficit in patients with ischemic brain damage, adding to the morbidity of cerebral infarcts.

Some animal studies have already suggested that changes occur in the major neurotransmitters dopamine (DA), norepinephrine (NE) and serotonin (5-hydroxytryptamine) (5-HT) after experimental cerebral ischemia. The present study involved an investigation of the changes of DA, NE, 5-HT and the metabolites of NE and 5-HT, 4 hydroxy-3-methoxy phenylethylenglycol sulphate (MOPEG-SO4) and 5-hydroxyindole acetic acid (5-HIAA) in the cerebral hemispheres of the Mongolian gerbil 3.5 hours after ligation of one carotid. This time was chosen because by then neurological abnormalities are obvious in susceptible animals. If changes in neurotransmitter amines do contribute to the morbidity of carotid ligation, such changes should be evident at this time.

Methods

Under brief diethyl ether anesthesia the right common carotid artery was ligated through a ventral midline cervical incision. The animals recovered quickly from the anesthetic and were observed over the next 3.5 hours for evidence of neurological abnormality. Splaying of contralateral limbs or, more commonly, circling behavior was taken as evidence of neurological deficit. The animals turned repeatedly nearly always towards the side of the ligated carotid artery. Many animals also had rolling seizures but these were not separately studied and were simply included as proof of neurological disturbance due to carotid ligation. Animals with frequent seizures often failed to survive throughout the period of observation.

Sham operated animals were anesthetized, the incision made, and the vessel exposed but not mobilized or ligated.

At 3.5 hours surviving animals, whether exhibiting neurological abnormality or not, were decapitated under chloroform anesthesia and the brain rapidly removed. The hemispheres were then separated from each other and from the hind brain on a cooled surface. Frozen samples of right and left hemispheres (whole hemisphere) were analyzed for NE, DA, 5-HT, 5-HIAA and MOPEG-SO4 using fluorometric assays employing an Aminco Bowman spectrofluorometer with ratio mode photometer. DA and NE were isolated from individual hemispheres according to the procedure of Atack.DA was assayed according to this method and NE by the method of Weil-Malherbe and Bigelow.5 5-HT and 5-HIAA were measured in individual samples by the method of Curzon and Green. For MOPEG-SO4 2 hemispheres were pooled for each analysis according to the procedure of Meek and Neff. All assay procedures were carried out by comparison with standards carried through the entire sequence, so the values have not been corrected for absolute recovery. Differences were assessed by Student's t-test and Wilcoxon-White 2-sample rank test for paired data and Student's r- and Mann Whitney U-test for unpaired data.

Results

Cerebral DA levels tended to be lower in the infarcted hemisphere but were not consistently altered; there was a wide scatter of individual results and no significant differences were detectable when a whole hemisphere was used for measurement (table 1). There...
TABLE 1 Levels of DA in the Right and Left Cerebral Hemispheres 3.5 Hours after Carotid Ligation on the Right

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of pairs</th>
<th>DA ng/g Right hemisphere</th>
<th>DA ng/g Left hemisphere</th>
<th>± S.E. Right hemisphere</th>
<th>± S.E. Left hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>8</td>
<td>882.8</td>
<td>919.4</td>
<td>137.2</td>
<td>131.0</td>
</tr>
<tr>
<td>Operated but</td>
<td>4</td>
<td>888.8</td>
<td>760.5</td>
<td>191.6</td>
<td>98.0</td>
</tr>
<tr>
<td>Operated and</td>
<td>12</td>
<td>838.5</td>
<td>1025.0</td>
<td>95.0</td>
<td>162.1</td>
</tr>
</tbody>
</table>

*Student's t- or Wilcoxon rank test for paired data. None of the differences between operated and sham animals is significant at the p < 0.05 level (Student's t- or Mann Whitney U-Test for unpaired data).

Discussion

The failure to find consistent changes in DA levels in the ischemic hemisphere is of interest. Zervas et al.11 found a significant depletion of DA on the ischemic side 24 hours after carotid ligation in this model. The fall in DA appears, therefore, to develop slowly although Kogure et al.6 have suggested that in the rat the change is biphasic with an early rise in slowly although Kogure et al.6 have suggested that in the same phenomenon occurs in the gerbil it would perhaps account for the failure of the present study to find any abnormality at 3.5 hours. Welch et al.15 however, in a recent study, have reported a significant reduction in dopamine in the gerbil hemisphere cortex at 3 hours. There are methodological differences which may explain this discrepancy. Welch’s data showed a bilateral change in clinically affected and unaffected animals, although the greatest change was recorded in the infarcted hemisphere of symptomatic animals.

It has been suggested that the fall in dopamine detected by Zervas11 is related to the turning behavior of the gerbils with unilateral cerebral infarction because of its role as a neurotransmitter in the basal ganglia. However, the present study shows no clearcut change in DA at a time after carotid ligation when turning behavior is well developed and Welch’s study showed bilateral changes. It is much more prominent at this early stage than at the later time when the more severe DA depletion has been described. The animals turning toward the damaged side may represent the physiological effect of damage to the striopallidal outflow pathways, for both the caudate-putamen and the globus pallidus are involved.

Minor changes in norepinephrine were seen though they are not statistically significant when compared with sham-operated animals. The tendency appeared to be for a small bilateral drop in levels of NE as also found by Welch15 but the clinically normal and abnormal animals were all affected and MOPEG sulphate levels were identical between affected and unaffected animals.

TABLE 2 Levels of NE in Right and Left Cerebral Hemispheres 3.5 Hours After Carotid Ligation on the Right

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of pairs</th>
<th>NE ng/g Right hemisphere</th>
<th>NE ng/g Left hemisphere</th>
<th>± S.E. Right hemisphere</th>
<th>± S.E. Left hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>6</td>
<td>237.9</td>
<td>256.9</td>
<td>19.2</td>
<td>33.7</td>
</tr>
<tr>
<td>Operated but</td>
<td>6</td>
<td>181.7*</td>
<td>p &lt; 0.05</td>
<td>30.6</td>
<td>76.9</td>
</tr>
<tr>
<td>Operated and</td>
<td>6</td>
<td>182.3</td>
<td>210.25</td>
<td>66.4</td>
<td>34.4</td>
</tr>
</tbody>
</table>

None of the differences between operated and sham animals is significant except the level of NE in operated animals showing no deficit compared with sham operated animals (p < 0.05, Mann Whitney U-test for unpaired data).

TABLE 3 Levels of MOPEG-SO4 in the Right and Left Cerebral Hemispheres 3.5 Hours after Carotid Ligation on the Right

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of pairs</th>
<th>MOPEG-SO4 ng/g Right hemisphere</th>
<th>MOPEG-SO4 ng/g Left hemisphere</th>
<th>± S.E. Right hemisphere</th>
<th>± S.E. Left hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>6</td>
<td>49.8</td>
<td>N.S.</td>
<td>4.9</td>
<td>10.5</td>
</tr>
<tr>
<td>Operated but</td>
<td>5</td>
<td>48.9</td>
<td>N.S.</td>
<td>8.97</td>
<td>13.9</td>
</tr>
<tr>
<td>Operated and</td>
<td>7</td>
<td>44.7</td>
<td>N.S.</td>
<td>14.1</td>
<td>10.2</td>
</tr>
</tbody>
</table>

*Each sample obtained by combining hemispheres from 2 animals.

Difference from level in sham operated animals: Student’s t- or Mann Whitney U-test for unpaired data. *p < 0.01; **p < 0.001.
hemispheres in both sets of operated animals. Zervas\textsuperscript{11} found no difference in NE at 24 hours. Kogure\textsuperscript{4} found an initial fall in the ischemic rat brain which tended to recover toward normal by 4 hours, perhaps accounting for the minor differences in the present study at 3.5 hours.

The clinical implication of the NE changes is uncertain since the differences are small, and are equally present in clinically affected and clinically unaffected animals.

Some of the variability in DA and NE findings may relate to the nature of the neurological abnormality produced in individual animals. Thus, Welch et al.\textsuperscript{14} have suggested that DA and NE levels only fall in those animals that develop seizures as a result of infarction. Our study included such animals (with seizures) and others with only circling behavior and no seizures as a single category of neurologically affected animals. In future studies a separation of subgroups according to the nature of the clinical deficit is clearly indicated.

There is a general agreement about 5-HT in that most workers have confirmed a fall in ischemic cerebral tissue with or without seizures. There are important discrepancies between the present results and those of Welch et al.\textsuperscript{15} however. Welch's study revealed a fall in 5-HT but only on the operated side of symptomatic animals. In our study the changes were bilateral and accompanied by a fall in 5-HIAA as seen in the rat model (Brown et al.).\textsuperscript{15}

These differences may, in part, be due to different methods of sacrifice and sample handling and some of these variables are to be studied further. Our study has, for example, involved assaying monoamine levels in the whole infarct-bearing hemisphere while Welch et al. use cortical tissue samples. There are advantages and disadvantages to either approach but a study of regional differences with histological control will, hopefully, throw some light on the discrepancies revealed so far.

Welch, Hashi and Meyer\textsuperscript{16} have suggested that a fall in 5-HT reflects release of transmitter 5-HT into the extracellular fluid where it may have the effect of limiting capillary perfusion by vasoconstricting small vessels. While there is general agreement about the fall in tissue 5-HT level, the evidence for an important pathogenic effect is slender. The reduction of cerebral 5-HT was accompanied in our animals by a corresponding fall in the level of its metabolite 5-HIAA, which suggests the 5-HT is not being released for subsequent metabolism. These changes could be explained by a reduced synthesis and release of 5-HT in damaged serotoninergic neurones,\textsuperscript{17} in which case they would have no significance in relation to secondary effects on the cerebral circulation. This seems likely, if only because the changes in brain 5-HT and 5-HIAA were similar in both hemispheres but infarction is largely confined to the ischemic hemisphere. (The bilateral drop in cerebral 5-HT and 5-HIAA may reflect damage to the midbrain raphe nuclei which are the major source of cerebral 5-HT neurone projections. These nuclei lie in the midline of the midbrain and are included in the demonstrable area following carotid ligation in the gerbil.) Furthermore, in our study there was no difference in cerebral 5-HT and 5-HIAA levels between those animals that exhibited clinical evidence of brain damage and those that did not.

Welch has also reported that the number of gerbilss showing signs of hemisphere damage after carotid ligation was reduced by pretreatment of the animals with para-chlorophenylalanine which inhibits tryptophan hydroxylase, thereby preventing 5-HT synthesis and leading to lower levels of cortical 5-HT. This follows the earlier evidence of a similar effect on the morbidity of carotid ligation in this model of methysergide.\textsuperscript{18} The mechanism of this clinical sparing effect may not simply relate to the altered 5-HT level and needs more study before further considering its relevance to the human clinical situation.

References


### Table 5: Levels of 5-HIAA in the Right and Left Cerebral Hemispheres 3.5 Hours After Carotid Ligation on the Right

<table>
<thead>
<tr>
<th>Animals</th>
<th>Right hemisphere</th>
<th>SHIAA ng/g</th>
<th>S.E.</th>
<th>Left hemisphere</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>1204</td>
<td>77.1</td>
<td>177.5</td>
<td>N.S.</td>
<td>1105</td>
</tr>
<tr>
<td>Operated but no neurological deficit</td>
<td>744.8*</td>
<td>74.8</td>
<td>101.4</td>
<td>N.S.</td>
<td>798.8</td>
</tr>
<tr>
<td>Operated and showing deficit</td>
<td>754.0*</td>
<td>74.6</td>
<td>93.9</td>
<td>N.S.</td>
<td>746.6</td>
</tr>
</tbody>
</table>

Difference from level in sham animals: Student's t- or Mann Whitney U-test, unpaired data. *p < 0.01.
Serum Dopamine-\(\beta\)-Hydroxylase Activity in Acute Stroke

TADASHI KANDA, M.D., FUMIO GOTOH, M.D., MASAHIRO YAMAMOTO, M.D., FUMIHIKO SAKAI, M.D., TSUNEYUKI TAKEOKA, M.D., and YASUYUKI TAKAGI, M.D.

SUMMARY Serum dopamine-\(\beta\)-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.

DOPAMINE-\(\beta\)-HYDROXYLASE (DBH), the enzyme needed for the final step of norepinephrine synthesis, is known to be present in synaptic vesicles in sympathetic nerve endings and to be released together with norepinephrine by a process of exocytosis from the stimulated nerve endings. Thus, DBH in circulating blood may be derived mainly from sympathetic nerve terminals and the level of DBH activity in the blood may reflect alterations in sympathetic nerve activity. 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.

In patients with stroke, elevated levels of plasma and urinary catecholamines have been reported during the acute state of cerebrovascular disease, suggesting increased activity in the sympathetic nervous system after ictus. Recent observations 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 flow, 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 occlusion. J Neurol Neurosurg Psychiatry 36: 724-735, 1973.
Effect of experimental ischemia on neurotransmitter amines in the gerbil brain.
M J Harrison, C D Marsden and P Jenner

Stroke. 1979;10:165-168
doi: 10.1161/01.STR.10.2.165

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/10/2/165

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/