**Effect of Experimental Ischemia on Neurotransmitter Amines in the Gerbil Brain**

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**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 phenylethyleneglycol 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.

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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 phenylethyleneglycol sulphonate (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 the unoperated hemisphere but were not consistently altered; 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 phenylethyleneglycol sulphonate (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.

**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-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 t- 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
Operated but no neurological deficit
Operated and showing deficit

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

Animals No. MOPEG-SO₄ ng/g R. v L. ± S.E. Left hemisphere

Sham operation 6 2276.3** N.S. 2498
Operated but no neurological deficit 6 2355.0* N.S. 2440
Operated and showing deficit 6 2152.3 243

*Each sample obtained by combining hemispheres from 2 animals.

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. have suggested that in the rat the change is biphasic with an early rise in dopamine, perhaps due to release.¹² The time course of the biphasic change in the rat was such that normal figures were likely at approximately 3 to 4 hours. If 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.,¹³ 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 Welch¹³ but the clinically normal and abnormal animals were all affected and MOPEG sulphate levels were identical between affected and unaffected groups.

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The pathogenic effect is slender. The reduction of cerebral vessels. While there is general agreement about the variables to be studied further. Our study revealed so far.

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 serotonergic neurones, 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 neurones 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 gerbils 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. 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**

Serum Dopamine-β-Hydroxylase Activity in Acute Stroke

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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.

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 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, lumber 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

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