Cerebral Ischemia/Dodson et al.

Catecholamine and 5-Hydroxytryptamine Levels in Ischemic Brain

Influence of p-Chlorophenylalanine

K. M. A. Welch, M.D., Eva Chabi, B.Sc., James Buckingham, M.D., Barbara Bergin, V. S. Achar, M.D., and J. S. Meyer, M.D.

SUMMARY The effect of ischemia on catecholamine and 5-hydroxytryptamine (5-HT) levels in brain cortex was examined in the gerbil stroke model.

Unilateral common carotid artery occlusion produced bilateral decrease in cortical dopamine levels in gerbils both symptomatic and asymptomatic of cerebral ischemia. The 5-HT progressively decreased only in the occluded hemisphere of ischemic animals. In p-chlorophenylalanine (PCPA)-treated gerbils, dopamine decreased only in the occluded hemisphere of symptomatic animals, but norepinephrine became decreased bilaterally compared with controls. The 5-HT decrease was twice that seen in untreated animals. It is suggested that these results indicate initial release together with reduced synthesis of monoamines in ischemic brain.

The incidence of ischemia induced by carotid occlusion decreased from 44% to 26% in PCPA-treated animals, which also suggests that depletion of 5-HT available for neuronal release prior to the induction of ischemia may reduce stroke incidence by limiting impairment of collateral vasocapacitance. PCPA pretreatment did not influence the development of edema in the occluded hemisphere of ischemic animals once ischemia was established.

Pial Vessel Constriction associated with foci of spreading cortical pallor observed in the primate cerebral cortex after major cerebral artery occlusion might be due to release of some chemical agent or agents from ischemic brain tissue. The validity of this hypothesis has been supported in part by measurement of 5-hydroxytryptamine (5-HT) release from brain into cerebral venous blood after the induction of acute cerebral ischemia in baboons. Increased 5-HT and catecholamine levels in lumbar cerebrospinal fluid have also been recorded in clinical patients early after the onset of cerebral infarction. Although these studies strongly suggest some abnormality of monoamine neurotransmitter metabolism during cerebral ischemia, both the responsible mechanisms and the roles they may play in the pathogenesis of cerebral infarction remain to be established.

To examine further the possible disorder of 5-HT and catecholamine metabolism in cerebral ischemia, we have measured tissue levels of 5-HT, dopamine, and norepinephrine in the cerebral cortex of gerbils. Unilateral common carotid ligation in this animal consistently induces cerebral hemispheric ischemia in 40 to 50% of animals, the incidence of ischemia and eventual infarction apparently depending on vascular anatomical variation.

Since the incidence of eventual infarction in the gerbil seems so dependent on adequacy of collateral circulation, it seemed appropriate to use this model to test the hypothesis that neuronal release of 5-HT with subsequent vasoconstriction may limit collateral vasocapacitance in foci of cerebral ischemia, thereby contributing to the progression of ischemia. Therefore, a separate animal group was pretreated with the tryptophan-hydroxylase inhibitor, p-chlorophenylalanine (PCPA), in order to deplete cerebral tissue 5-HT content and determine if the stroke incidence rate was thus modified.

Finally, because a relationship between accumulation of 5-HT in brain tissue and the development of cerebral edema has also been observed in some recent studies, cerebral hemispheric swelling and water content were measured in both the untreated and PCPA-pretreated series of animals.

Methods

Adult male and female Mongolian gerbils (Meriones unguiculatus) weighing 50 to 80 gm were studied. Animals were caged (three per unit) at constant temperature in simulated day and night conditions and allowed free access to drinking water and chow.

In 188 gerbils the right common carotid artery was dissected free of its accompanying vagus nerve and vein and...
rapidly ligated twice during recovery from brief hypercapnic hypothermia anesthesia. Sixty of the 188 animals were preinjected with 0.9% saline according to the same protocol for PCPA-treated animals (see below).

The percentage of animals that exhibited the clinical signs of splayed contralateral limbs, ipsilateral ptosis, ipsilateral circulating behavior, and rolling fits was termed the stroke incidence rate. Electron microscopic study of animals with these clinical signs provided morphological confirmation of progressive cerebral hemispheric ischemia (results to be published). Animals that exhibited or did not exhibit such signs were respectively termed symptomatic or asymptomatic.

Groups of gerbils were sacrificed under liquid nitrogen at three and six hours after carotid occlusion, and brain tissue was obtained either for biochemical analysis or edema studies. The number of animals surviving for longer time intervals was insufficient for statistical analysis of biochemical data. However, all animals were included in the analysis of stroke incidence rate. The remaining animals (N = 27) were either symptomatic of ischemia and did not survive to appropriate intervals of sacrifice or were observed for survival to confirm clinical impression of the presence or absence of ischemia. Sham-occluded animals (N = 20) were also sacrificed immediately and at six hours after operation. Normal nonanesthetized and nonoperated animals were also sacrificed for biochemical analysis (N = 6) and edema studies (N = 20).

After the frozen brains were chiseled out under liquid nitrogen, samples of cerebral cortex from the right and left hemispheres were analyzed for 5-HT, dopamine, and norepinephrine. Tissue for this purpose was homogenized in 0.4 M perchloric acid. Catecholamines were extracted from the tissue homogenate on activated alumina at pH 8.6. After prior organic extraction from the column effluent, 5-HT was assayed fluorometrically, using a modification of the methods of Curzon and Green and Maickel and Miller. Catecholamines were eluted from the alumina columns with acetic acid and the eluate halved. In one portion dopamine was analyzed fluorometrically according to modifications of the methods of Curzon and Green and of Anton and Sayre. The remaining portion was placed on amberlite columns from which norepinephrine was eluted by 2/3 M boric acid to be analyzed fluorometrically using the trihydroxyindole reaction according to modifications of the methods of Renzini et al. and Valori et al. An Aminco-Bowman spectrophotofluorometer was used for all measurements.

Values were corrected for recovery (82% ± 13 SD for dopamine, 65% ± 11 SD for norepinephrine, 65% ± 12 SD for 5-HT). Reproducibility on duplicate analysis was considered satisfactory at ± 4.4% for 5-HT, 4.1% for dopamine, and 5.8% for norepinephrine.

Hemispheric brain water content was measured by wet and dry weighing and calculated according to the equation:

\[ \text{% brain water content} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100 \]

A separate animal group (N = 182) was pretreated with PCPA in doses of 300 mg/kg given intraperitoneally 48 hours and 24 hours prior to occlusion of the right common carotid artery. Animals were sacrificed as before except that in some cases time intervals of sacrifice after ligation were prolonged up to 48 hours. Cortical monoamine levels and water content were measured as in untreated animals. As before, some animals (N = 18) were not sacrificed in order to confirm impressions of clinical signs.

In a number of untreated (N = 10) and treated (N = 10) animals, blood pressure was monitored on a polygraph, either by a femoral artery catheter attached to a Statham pressure transducer (P21A), or by an electrophysiograph with automatic cycling cuff pump (Narco Biosystems, Inc.), or both.

Statistical analysis was performed, using the chi-square and Student's t-test.

### Results

#### Untreated Group

Stroke incidence, biochemical parameters, and hemispheric water content in the 60 saline-injected animals did not significantly differ from uninjected animals so that for statistical analysis these animals were included in the untreated group. A stroke incidence rate of 44% was observed in the total 188 untreated animals studied.

There was a variable incidence of rolling fits in animals symptomatic of ischemia. For the most part the incidence increased with the length of survival time. Seizures were infrequent during the first hour after occlusion but were observed in the majority of symptomatic animals that survived to the 6-hour period of sacrifice.

The 5-HT, dopamine, and norepinephrine levels in sham-operated animals did not differ between hemispheres, were unaltered when animals were sacrificed immediately or six hours after operation, and were not significantly different from levels in nonanesthetized, nonoperated animals. Values in right and left hemispheres of immediately sacrificed sham-operated animals were therefore pooled and used as a single control value.

At three hours there was a significant decrease of 5-HT in occluded hemispheres of symptomatic animals with further decrease at six hours (fig. 1). This decrease was significant compared with values in the contralateral nonoccluded hemisphere, with values in the occluded hemisphere of asymptomatic animals sacrificed at the same time interval, and with values in sham-operated controls.

Dopamine was decreased in both hemispheres of symptomatic and asymptomatic animals when compared with sham-operated controls. However, values in the occluded hemisphere of symptomatic animals were further decreased compared with values in the contralateral nonoccluded hemisphere and with values in the occluded hemisphere of asymptomatic animals.

Decrease in norepinephrine was observed only at three hours in the nonoccluded hemisphere of asymptomatic animals when compared with sham-operated controls, although of low significance.

Hemispheric water content was increased in occluded hemispheres of symptomatic animals at three and six hours after occlusion (table 1). Water content was also increased in
CEREBRAL HEMISPHERIC MONOAMINE LEVELS IN GERBILS SYMPTOMATIC OR ASYMPTOMATIC OF ISCHEMIA AFTER RIGHT CAROTID OCCLUSION

FIGURE 1. To show bilateral cerebral hemispheric dopamine decrease (more marked in occluded hemispheres) of gerbils both symptomatic and asymptomatic of ischemia after right common carotid occlusion. The 5-hydroxytryptamine progressively decreased in the occluded hemispheres of symptomatic animals only. The norepinephrine changes were of low significance.

TABLE 1 Cerebral Hemispheric Water Content in Untreated and PCPA-Treated Symptomatic and Asymptomatic Occluded Animals Compared with Sham-operated Controls

<table>
<thead>
<tr>
<th>Time of sacrifice</th>
<th>Symptomatic</th>
<th></th>
<th></th>
<th></th>
<th>Asymptomatic</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right H</td>
<td>Left H</td>
<td>Right H</td>
<td>Left H</td>
<td>Right H</td>
<td>Left H</td>
<td>Right H</td>
<td>Left H</td>
</tr>
<tr>
<td>Untreated occluded group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hours</td>
<td>80.34 ± 1.96†</td>
<td>79.56 ± 2.15</td>
<td>79.60 ± 1.12†</td>
<td>79.38 ± 1.15</td>
<td>(17)</td>
<td>(7)</td>
<td>(33)</td>
<td>(31)</td>
</tr>
<tr>
<td>6 hours</td>
<td>81.36 ± 1.66†</td>
<td>79.62 ± 1.42†</td>
<td>78.54 ± 6.38</td>
<td>78.86 ± 1.15</td>
<td>(20)</td>
<td>(10)</td>
<td>(32)</td>
<td>(31)</td>
</tr>
<tr>
<td>PCPA-treated occluded group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hours</td>
<td>80.06 ± 1.14†</td>
<td>78.97 ± 0.64</td>
<td>78.05 ± 2.34</td>
<td>77.82 ± 1.83</td>
<td>(13)</td>
<td>(10)</td>
<td>(38)</td>
<td>(37)</td>
</tr>
<tr>
<td>6 hours</td>
<td>80.56 ± 1.05†</td>
<td>78.00 ± 1.15</td>
<td>78.36 ± 1.17</td>
<td>77.93 ± 1.11</td>
<td>(14)</td>
<td>(10)</td>
<td>(41)</td>
<td>(39)</td>
</tr>
<tr>
<td>Sham-operated control group</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>3 hours</td>
<td>—</td>
<td>—</td>
<td>78.67 ± 0.53</td>
<td>78.82 ± 0.26</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
</tr>
<tr>
<td>6 hours</td>
<td>—</td>
<td>—</td>
<td>78.65 ± 0.73</td>
<td>78.27 ± 1.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tr>
</tbody>
</table>

H = hemisphere—the occluded hemisphere was always the right; ( ) = number of animals; (—) denotes swelling.

†Values are mean ± SD.

Significantly different from the same hemisphere of sham-operated controls sacrificed at the same time interval (P < 0.02).

Significantly different from the same hemisphere of sham-operated controls sacrificed at the same time interval (P < 0.01).
only the occluded hemisphere of PCPA-treated symptomatic animals (table 1). These increases were not significantly different from those recorded in the occluded hemispheres of untreated symptomatic animals.

The mean arterial blood pressure in PCPA-treated animals did not differ from that of untreated animals.

**Discussion**

**Biochemical Changes**

These experiments demonstrate a reduction in cortical 5-HT and dopamine levels in occluded hemispheres of animals symptomatic of cerebral ischemia, findings which are in accord with those of other researchers who have reported catecholamine depletion during ischemia in the gerbil and rat. However, the exact cause of this reduction has not been directly identified. Previous reports from this laboratory in the baboon and clinical patients with stroke, as well as studies in the gerbil by Layyne and colleagues, have suggested neuronal release of monoamines early after the onset of ischemia. Neuronal membrane depolarization with ionic shifts as well as synaptosomal mitochondrial dysfunction, conditions which might cause abnormal release of neurotransmitters, are well documented in cerebral ischemia. Other factors which would cause eventual intraneuronal monoamine depletion include failure of energy-dependent synaptosomal reuptake as well as synthesis impairment.

Decreased dopamine in nonoccluded hemispheres of symptomatic animals (fig. 1) seems evidence of a remote effect of focal ischemia, i.e., diaschisis. Kogure et al. have also recorded bilateral changes in cortical catecholamine levels in rats subjected to unilateral cerebral embolism, although in their model norepinephrine levels were reduced while dopamine levels increased. However, hemispheric dopamine decrease was also observed in asymptomatic animals. Recent experiments have confirmed regional cerebral blood flow (rCBF) decrease in the occluded hemisphere of asymptomatic animals, although the degree of reduction was less than that measured in symptomatic animals. Dopamine metabolism, therefore, may be particularly sensitive to cerebral hemodynamic shifts, even in the absence of clinical signs of ischemia.

The considerable variability of norepinephrine data in the present study does not permit confident comment on the influence of ischemia on brain norepinephrine metabolism in the gerbil. Reports from other laboratories using the same model indicate a similar inconsistency of norepinephrine results. The significant, although temporary, reduction in norepinephrine levels in the nonoccluded hemispheres of asymptomatic animals is not fully understood, but since norepinephrine is synthesized from dopamine, it might reflect the correspondingly lower dopamine levels in the same hemispheres (fig. 1).

**PCPA**

Reduction in 5-HT, dopamine, and, though less significantly, norepinephrine was also observed in occluded hemispheres of PCPA-pretreated animals symptomatic of ischemia. The results suggest that reduced 5-HT in ischemic brain may be due in part to synthesis impairment since inhibition of tryptophan hydroxylase activity prior to ischemia resulted in even greater 5-HT depletion than that seen in untreated animals. Bilateral 5-HT decrease in symptomatic animals also suggests some synthesis impairment in brain areas remote from the ischemic territory. Bilateral 5-HT reduction occurred in asymptomatic animals, although to a lesser degree. This finding might also be explained by reduced rCBF despite absence of ischemic symptomatology.

**Stroke Incidence**

Unilateral common carotid artery ligation in the gerbil has, in our hands, consistently produced signs of cerebral hemispheric ischemia in 40 to 50% of over 600 normal animals studied to date. Some batch variability in stroke incidence is occasionally noted, making it necessary to study large numbers of animals for incidence rates to be
meaningful. When such large series of animals are studied, the stroke incidence also appears independent of variation in surgical technique of personnel performing the experiment. Furthermore, stroke incidence was not influenced by anesthetic procedures (pentobarbital 42%, N = 29; diethyl ether 44%, N = 45; unpublished observations).

Reduced stroke incidence following PCPA pretreatment supports the hypothesis that if 5-HT available for release onto collateral vessels is reduced prior to induction of brain ischemia, then there will be less impairment of collateral vasocapacitance with reduced incidence of ischemia progressing to infarction. Similar reduction in stroke incidence after treatment with methysergide, which blocks 5-HT vascular receptors, adds strength to this argument. Since blood pressure was identical in both treated and untreated groups, differences in cerebral perfusion pressure cannot explain the difference in stroke incidence. Perfusion pressure, however, is only one of the factors controlling flow in the microvasculature. Among these factors the role of edema in compression of the microvasculature is discussed below. Alternative mechanisms by which PCPA favorably influences stroke incidence also need to be studied.

PCPA may also cause temporary inhibition of tyrosine hydroxylase activity. For this reason PCPA was administered 48 and 24 hours prior to carotid ligation at which time tyrosine hydroxylase activity is said to recover. Nevertheless, some tendency toward reduction in dopamine levels was found in PCPA-treated sham-operated animals, and unlike the findings in untreated animals, norepinephrine levels were significantly reduced in treated animals that were symptomatic of ischemia. These findings may have some relevance since the specific tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine, reportedly also modifies the pathological sequelae of cerebral ischemia.

Cerebral Edema

Increased water content in ischemic hemispheres is interpreted as evidence of edema (confirmed by electron microscopic morphological study), which in the gerbil model apparently develops with reasonable rapidity. The increased water content of brain tissue that also occurred in the non-occluded hemisphere of symptomatic animals sacrificed at 6 hours is again evidence of bilaterality of the effect of unilateral carotid occlusion in the gerbil. Hemodynamic studies in the same model showed no evidence of ischemia in non-occluded hemispheres. This finding might be associated with monoamine changes in the contralateral hemisphere or perhaps is secondary to the generalized seizures that occurred more frequently in longer surviving animals. However, the transient increase of brain water content in occluded hemispheres of asymptomatic animals might be explained on the basis of ischemia since hemodynamic shift occurred in this animal group.

A direct relationship between disordered monoamine metabolism and the development of cerebral edema cannot be established by these studies. However, the increased water content that occurred in the occluded hemispheres of both symptomatic and asymptomatic animals, as described above, coincided with reduced dopamine levels. Pretreatment with PCPA showed only a tendency to limit the increase of water content in the occluded hemisphere of animals symptomatic of ischemia. However, the increased brain water content in contralateral hemispheres of untreated symptomatic animals and in the occluded hemispheres of untreated asymptomatic animals was not observed in treated animals. Electron microscopic study showed that the edema in contralateral hemispheres of symptomatic animals and in the occluded hemispheres of asymptomatic animals was limited to perivascular astrocytic end-foot process swelling. These findings were not present, and morphological integrity was confirmed in these same cortical regions upon ultrastructural study of PCPA-treated animals. An alternative explanation for the reduction of stroke incidence after PCPA treatment may therefore be the prevention of microvascular compression by edema in areas of potential collateral flow.

In conclusion, depleted levels of 5-HT, dopamine, and possibly to a lesser extent norepinephrine in ischemic brain of the gerbil together with a reduced stroke incidence after PCPA pretreatment further support our earlier hypothesis that disordered neurotransmitter function is involved in the pathogenesis of progressive cerebral ischemia.

References

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Effect of Cerebrospinal Fluid Removal on Cerebral Blood Flow and Metabolism in the Baboon

Influence of Tyrosine Infusion and Cerebral Embolism on Cerebrospinal Fluid Pressure Autoregulation

YOHSUKE MIYAKAWA, M.D., JOHN STIRLING MEYER, M.D., NAOKI ISIHARA, M.D., HIROAKI NARITOMI, M.D., KIYOHIKO NAKAI, M.D., MING-CHANG HSU, M.D., AND VINOD D. DESHMUKH, M.D., M.S., PH.D.

SUMMARY Cerebral blood flow (CBF) and metabolism were measured before and after withdrawal of 5 to 6 ml of cerebrospinal fluid (CSF) in 17 baboons. The measurements were made before and after infusion of tyrosine, the precursor amino acid of the putative neurotransmitters, dopamine and norepinephrine, in the brain. The same observations were made in another experimental group, i.e., before and after acute cerebral multiembolization induced by microfil emboli. In the steady state CBF was unaltered following reduction of intracranial pressure by removal of CSF. After infusion of tyrosine, CBF was decreased, and cerebrovascular resistance increased significantly on removal of CSF. Cerebral embolization did not influence changes in CBF at reduced intracranial pressure. It appears that the cerebral resistance vessels constrict following reduction of intracranial pressure by removal of CSF and that cerebrospinal fluid pressure-CBF autoregulatory mechanisms are resistant to cerebral ischemia induced by middle cerebral artery embolization.

RECENTLY, THE EFFECTS OF WITHDRAWAL OF CEREBROSPINAL FLUID (CSF) ON CEREBRAL HEMODYNAMICS IN PATIENTS WITH STROKE AND ALZHEIMER'S DISEASE WERE COMPARED. In that study after CSF removal cerebral blood flow (CBF) decreased in patients with Alzheimer's disease but showed no significant change in patients with stroke. It was postulated that a neurogenic reflex induced by alterations in cerebral venous pressure may vary vasomotor tone of the cerebral arterial system (venoarterial reflex), and this reflex may provide part of a cerebrospinal fluid pressure-cerebral blood flow (CSFP-CBF) autoregulatory mechanism. Furthermore, it was postulated that the excessive vasconstriction demonstrated in Alzheimer's disease might reflect an imbalance of the central neurotransmitter system.

These observations led us to test the hypothesis that a cerebral neurogenic vеноarterial reflex may regulate the CSFP-CBF autoregulatory mechanism before and after cerebral embolization in the baboon. The hypothesis to be tested was based on the following evidence: First, CBF normally remains constant despite wide changes in CSF; secondly, the walls of the cerebral veins are thought to be the site most sensitive to changes in CSF, and thirdly, similar vеноarterial reflexes have been regularly observed in many tissues other than the brain. In addition, it was conjectured that differences in the disorder of the various neurotransmitter systems between patients with stroke and those with Alzheimer's disease might account for the different transmitter systems between patients with stroke and those with Alzheimer's disease.
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