CATECHOLAMINE AND 5-HYDROXYTRYPTAMINE LEVELS IN ISCHEMIC BRAIN

INFLUENCE OF P-CHLOROPHENYLALANINE

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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 con-
trols. 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 induc-
tion of ischemia may reduce stroke incidence by limiting impair-
ment of collateral vasocapacitance. PCPA pretreatment did not in-
fluence 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 occlusion1 might be due to
release of some chemical agent or agents from ischemic
brain tissue. The validity of this hypothesis has been sup-
ported 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.2 Increased
5-HT and catecholamine levels in lumbar cerebrospinal fluid have
also been recorded in clinical patients early after the
onset of cerebral infarction.3 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 norepineph-
rine in the cerebral cortex of gerbils. Unilateral common

carotid ligation in this animal consistently induces cerebral
hemispheric ischemia in 40 to 50% of animals,4 the incidence
of ischemia and eventual infarction apparently depending on
vascular anatomical variation.5

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 vasoconstric-
tion may limit collateral vasocapacitance in foci of cerebral
ischemia, thereby contributing to the progression of
ischemia.6 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,7 the 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 un-
guiculatus) 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 dis-
sected 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 Carlsson 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 electrosphygmograph 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 un.injected 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>Water content (%)</th>
<th>Asymptomatic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right H</td>
<td>Left H</td>
<td>Right H</td>
</tr>
<tr>
<td>Untreated occluded group</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>
</tr>
<tr>
<td>6 hours</td>
<td>81.36 ± 1.66†</td>
<td>79.62 ± 1.42†</td>
<td>78.54 ± 6.38</td>
</tr>
<tr>
<td>PCPA-treated occluded group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hours</td>
<td>80.06 ± 1.18†</td>
<td>78.97 ± 0.64</td>
<td>78.05 ± 2.34</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>
</tr>
<tr>
<td>Sham-operated control group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hours</td>
<td>—</td>
<td>—</td>
<td>78.67 ± 0.53</td>
</tr>
<tr>
<td>6 hours</td>
<td>—</td>
<td>—</td>
<td>78.65 ± 0.73</td>
</tr>
</tbody>
</table>

†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).

Cortical 5-HT levels in sham-operated controls were significantly reduced (P < 0.001) compared with those in the untreated group, thus confirming tryptophan hydroxylase inhibition by PCPA. In symptomatic animals sacrificed between 3 and 48 hours, hemispheric 5-HT levels were bilaterally reduced compared with sham-operated controls and decreased in comparison with asymptomatic animals also sacrificed up to 48 hours after occlusion (table 2). The degree of decrease was approximately twice that observed in untreated animals.

Dopamine levels in occluded hemispheres of symptomatic animals were again reduced compared with levels in contralateral nonoccluded hemispheres and with values in the occluded hemispheres of asymptomatic animals (table 2). Hemispheric norepinephrine levels in symptomatic animals were also reduced bilaterally compared with sham-operated controls, but differences were again of low significance (table 2).

Significant increase in brain water content was recorded in the nonoccluded hemisphere at 6 hours. In asymptomatic animals an increase in brain water content of occluded hemispheres was observed in animals sacrificed at three but not six hours after occlusion.

PCPA-Treated Group

In 182 PCPA-pretreated animals stroke incidence (26%) was significantly reduced compared with that in untreated animals (χ² = 12.88, P < 0.001). Animals also appeared to survive longer (up to 48 hours before death), whereas untreated animals usually died within 12 hours of carotid ligation. Seizure incidence varied early after occlusion in symptomatic, treated animals. The time of initial onset seemed delayed, but seizure appeared in the majority of longer surviving animals, although reduced in frequency compared with untreated animals.

Coercing the brain to uptake or expel water (an osmotic pump) may be an effective method of treating cardioочclusive stroke.

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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 Lavyne 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 ischemic hemorrhage 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

<table>
<thead>
<tr>
<th>Monoamines</th>
<th>Sham-operated</th>
<th>PCPA-treated groups*</th>
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<tbody>
<tr>
<td></td>
<td>Symptomatic</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Dopamine μg/g-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right (occluded)</td>
<td>1.366 ± 0.635</td>
<td>0.693 ± 0.346,1,4,7</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(12)</td>
</tr>
<tr>
<td>Left hemisphere</td>
<td>1.413 ± 0.476</td>
<td>1.266 ± 0.544</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(12)</td>
</tr>
<tr>
<td>Norepinephrine μg/g-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right (occluded)</td>
<td>0.557 ± 0.102</td>
<td>0.375 ± 0.183</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(12)</td>
</tr>
<tr>
<td>Left hemisphere</td>
<td>0.686 ± 0.210</td>
<td>0.402 ± 0.141</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(12)</td>
</tr>
<tr>
<td>5-Hydroxytryptamine μg/g-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right (occluded)</td>
<td>0.165 ± 0.048</td>
<td>0.054 ± 0.035</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(12)</td>
</tr>
<tr>
<td>Left hemisphere</td>
<td>0.159 ± 0.060</td>
<td>0.058 ± 0.044</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(12)</td>
</tr>
</tbody>
</table>

*Values are mean ± SD; ( ) = number of animals.
†Significantly different from ipsilateral hemisphere of sham-operated animals (P <0.01).
‡Significantly different from nonoccluded contralateral hemisphere (P <0.001).
§Significantly different from ipsilateral hemisphere of occluded-asymptomatic animals (P <0.001).
∥Significantly different from ipsilateral hemisphere of occluded-asymptomatic animals (P <0.01).
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 methysgeride, 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-β-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

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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.

These observations led us to test the hypothesis that a cerebral neurogenic venoarterial reflex may regulate the CSF-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 venoarterial 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.
Cathecholamine and 5-hydroxytryptamine levels in ischemic brain. Influence of p-chlorophenylalanine.

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