Experimental Stroke in Gerbils: Correlation of Clinical, Pathological and Electroencephalographic Findings and Protein Synthesis

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SUMMARY Cerebral ischemia was produced in gerbils by ligation of the right common carotid artery and the resulting clinical manifestations and pathological alterations, along with electroencephalographic findings, were followed from 30 minutes to 24 hours. Protein synthesis was evaluated with brain slices in vitro and subsequent cellular and subcellular fractionations. One group of animals developed clinical signs of cerebral ischemia and stroke very rapidly and often died within 12 hours. In these animals cerebral infarction was diffuse in the right side of the brain within a few hours postoperatively and there was persistent suppression in the electroencephalographic recordings. Amino acid incorporation into proteins of subcellular fractions was decreased to 50% of the opposite side at 30 minutes and further declined to less than 10% in 8 to 10 hours.

Another group of animals survived to 24 hours in spite of severe neurological manifestations, and protein synthesis was about 15% of the control side at 24 hours. The suppression of protein synthesis was observed both in the neuronal and neuroglial fractions indicating similar vulnerability of these cellular elements toward cerebral ischemia as shown with cerebral anoxia in the past. It was emphasized that the correlation of clinical manifestations and biochemical data is very important to extract meaningful information from biochemical investigations in this model.

Materials and Methods

During the early period of the present investigation mongolian gerbils (Meriones unguiculatus) were anesthetized with intraperitoneal injection of chloral hydrate (0.25 mg/kg) and supplementary inhalation of ether. Later, ether inhalation was the only anesthesia used, particularly when observation periods were shorter than 2 hours. The right common carotid artery was exposed through a midline skin incision in the neck. The artery was isolated from the vagus nerve, and supplementary inhalation of ether. Later, ether inhalation was the only anesthesia used, particularly when observation periods were shorter than 2 hours. The right common carotid artery was exposed through a midline skin incision in the neck. The artery was isolated from the vagus nerve, and supplementary inhalation of ether was the only anesthesia used, particularly when observation periods were shorter than 2 hours.

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mosphere and constant shaking as described previously. 14 At the end of incubation 2 brain slices were taken for the determination of acid-soluble radioactivity and the remaining brain slices were recovered by brief centrifugation. The pellet was resuspended in 0.32 M sucrose in 10 mM tris-HCl buffer (pH 7.4), homogenized and further fractionated into nuclei, microsomes and the soluble fraction by sucrose density gradient centrifugation. 15 For the preparation of the neuron-enriched (neuronal) and glia-enriched (glial) fractions, the pellet was suspended in 0.32 M sucrose in 10 mM tris-HCl buffer (pH 7.4) containing 0.5 mM EDTA and 100 mM NaCl. A crude cell fraction was prepared by tissue disruption, sieving through a series of nylon meshes and brief centrifugation. 16 The neuronal and glial fractions were obtained by further fractionation of the crude cell suspension through discontinuous ficoll density gradient centrifugation as described except ficoll gradients on top of the cell suspension were 16, 14 and 10%, and the glial fraction was collected between 16 and 14% ficoll layer. 16

The homogenate, cell fractions and subcellular fractions were rehomogenized and precipitated with an equal volume of cold 10% trichloroacetic acid (TCA), washed 3 times with cold 5% TCA, heated for 15 minutes at 90°C, extracted with alcohol-ether (1:1) and dried with ether. The dried sample was dissolved with 1.0 N NaOH and an aliquot was taken for the determination of protein according to Lowry et al. 16 with bovine serum albumin as standard. Another aliquot was digested with Soluene-100 (Packard Instruments) and the radioactivity measured in toluene-based scintillation solution. The specific radioactivity was expressed as disintegration per minute (DPM) per μg protein. Brain slices for the determination of the acid soluble radioactivity were weighed and homogenized in 1.0 ml of cold 10% TCA. The supernatant was used for the determination of radioactivity in Insta-Gel (Packard Instruments). The specific radioactivity was expressed as DPM per μg wet tissue weight.

For the preparation of anoxic brain tissue, the in vitro model described for rabbit 14 was used for normal gerbil brain slices of 0.3 mm thick. Subsequent leucine incorporation was carried out in the same manner as described for ischemic brain slices.

Results

Clinical Course

Approximately 40% of the animals became symptomatic. Among those, 60 animals were taken for histopathological investigation and 66 for biochemical study. Symptomatic animals were identified by neck and trunk torsion mostly in the clockwise direction (toward the ligated side), circling movement at various speeds and radii mostly clockwise in direction, restlessness, jumping, left hemiparesis particularly of the hind limb, generalized seizures manifested by corkscrew rotation of the body, decreased response to stimuli and coma. Some animals developed clinical signs within 5 minutes after ligation but some remained asymptomatic for over 1 hour. Seizures were rare in the first 2 hours but were observed in 75% of symptomatic animals between 3 to 12 hours after ligation. At 18 to 24 hours some animals were comatose or semi-comatose while others were only moderately slow. Three groups of animals could be identified, the first group of animals became symptomatic within 30 minutes, developed violent seizures within 5 hours and died within 12 hours. The second became symptomatic within 3 hours, often had seizures later but survived to 24 hours at which time they were semicomatic or comatose. The third group of animals included those which were mildly symptomatic at several hours and moderately symptomatic at 24 hours.

Pathology

This description is limited to the above first 2 groups. No definite abnormality was observed on the surface of the brain at 30 minutes, but the surface of the right cerebral hemisphere was sometimes slightly pale and swollen to 60 minutes. Dusky color was observed at about 2 hours, along with increasing swelling at 3 to 4 hours. Swelling and dusky color became more profound, and the right side of the brain, including subcortical structure, became soft around 5 to 8 hours after ligation. At 24 hours the right cerebral hemisphere was markedly swollen, pale and soft, while the left cerebral hemisphere was only slightly to moderately swollen.

Although only small scattered foci of infarction were visible in the right cerebral hemisphere and thalamus at 2 hours in hematoxylin-eosin stained thin sections, they became multifocal and confluent in a few hours. Comatose animals at 24 hours showed very advanced infarction of almost the entire right side of brain. With light microscopy, the earliest evidence of cerebral ischemia was small foci of vacuolization in neuropil of the cerebral hemisphere and thalamus at 30 minutes, which became mixed with pyknotic neurons and increase of the perineural space at 2 hours. In the cerebral hemisphere, these changes were most visible in the parietal region but they also extended to the occipital and frontal regions. These changes were also visible in the hippocampus and corpus striatum by 2 hours. The infarcted area became more confluent and advanced by 5 to 8 hours. At 24 hours, visible cells were either pyknotic, shrunken or eosinophilic. There was generalized vacuolization (fig. 1).

FIGURE 1. Microscopic finding of infarction in cerebral hemisphere at 24 hours postoperatively. The control (left) side was taken from the same animal as the infarcted (right) side. The photograph was taken at ×100 magnification with hematoxylin-eosin staining.
CEREBRAL ISCHEMIA AND PROTEIN SYNTHESIS/Yanagihara

EEG 1

EEG 2

EEG 3

25 µV 1 sec

FIGURE 2. Electroencephalographic recordings at various stages of cerebral infarction. The upper tracing is from the left side and the lower from the right. The scale for the speed and the amplitude are shown in the lower right side. (1) at 2 hours; (2) at 5 hours in the immediately postictal period and (3) at 24 hours during the interictal period.

Electroencephalography

There was slight slowing and decreased amplitude on the right side at 2 hours (fig. 2-1). At 5 hours, the EEG showed marked amplitude suppression on the right side and some slowing on the left (fig. 2-2). During the immediate postictal period recording from the right side remained suppressed with continuous slow waves in the delta range. On the left side high voltage slow delta activities were intermingled with sharp waves. At 24 hours the EEG activities on the right side were persistently suppressed. In some animals with occasional clinical seizures, sharp waves and slow delta activities were intermingled at interictal periods (fig. 2-3).

Protein Synthesis

Among asymptomatic animals, with the present assay system for amino acid incorporation in vitro, the homogenate showed specific radioactivities of 22 DPM, nuclei 34 DPM, microsomes 28 DPM and the soluble protein fraction 28 DPM/µg protein, while the neuronal fraction demonstrated 40 DPM and the glial fraction 14 DPM/µg protein. The acid-soluble radioactivity was in the range of 18 DPM/µg wet tissue weight. Among these animals there was no difference of the specific radioactivities between the right and left cerebral hemisphere at 10 and 24 hours in the homogenate, nuclei, microsomes, and the soluble protein as well as the acid-soluble radioactivity, while there was a slight tendency of higher specific radioactivity in various subcellular fractions from the right cerebral hemisphere at 2 hours.

Among symptomatic animals (fig. 3) there was about a 50% decline of leucine incorporation in the homogenate at 30 minutes, which rapidly declined further to 20% of the left side at 3 hours. The lowest level of leucine incorporation was found at 5 or 8 hours postoperatively but the suppression of

FIGURE 3. Leucine incorporation into proteins of subcellular fractions. The results are expressed as percent of the specific radioactivity of the right cerebral hemisphere in comparison to the left side. All values are mean ± S.E. Hour indicates the postoperative period.
leucine incorporation became less prominent at 24 hours. As indicated by standard errors, the specific radioactivities were widely scattered at 10 and 24 hours. The same tendency observed in the homogenate was seen in various subcellular fractions with the lowest value being about 10% of the left side in each fraction. The results from the homogenate of thalamus also showed a similar tendency (data not shown). The acid-soluble radioactivity remained normal until 5 hours postoperatively but at 8 and 10 hours the acid-soluble radioactivity of the right cerebral hemisphere was 77 to 79% of the left side in each fraction. The results from the homogenate of thalamus also showed a similar tendency (data not shown). The same tendency observed in the homogenate was seen in various subcellular fractions with the lowest value being about 10% of the left side in each fraction. The results from the homogenate of thalamus also showed a similar tendency (data not shown).

Since there was considerable heterogeneity of clinical manifestations in the late stage of cerebral infarction, the data presented in figure 3 were further divided according to the severity of clinical signs. The results (fig. 4) demonstrated a distinct difference between moderately and severely affected animals at 5 hours and thereafter for the homogenate and 3 subcellular fractions evaluated in the present study. The decline of the acid-soluble radioactivity was more notable in the severely affected animals after 8 hours (data not shown). There was no difference in leucine incorporation into protein or in the acid soluble radioactivity between the animals with seizures and without (table 1).

The effect of cerebral ischemia and cerebral anoxia on the neuronal and glial fractions are shown in table 2. Since 6 hemispheres had to be pooled for a single cellular fractionation procedure, the degree of clinical manifestations of cerebral ischemia was not uniform. The average duration of cerebral ischemia was 90 minutes, while the anoxic period was 10 minutes. In both situations, the neuronal and the glial fractions were affected to a similar degree.

### Discussion

In the previous investigations reported in the literature dealing with the gerbil model for cerebral ischemia, the clinical criteria described by Kahn have often been used although his paper mainly described hemiparesis and not other clinical signs. During the initial part of the present investigation, it became apparent that the clinical manifestations of cerebral ischemia and infarction are very variable. Among the clinical signs of ischemia, torsion of neck was often the initial one. Since the rotating movement can be experimentally produced by a lesion in a nigrostriatal tract, the observed torsion of neck was believed to be due to the destruction of this tract in the thalamic area in view of the early pathological change in the thalamus. The fact that 5

### Table 1 Influence of Seizure on Protein Synthesis

<table>
<thead>
<tr>
<th>Time</th>
<th>Seizure (number)</th>
<th>No seizure (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Hours</td>
<td>Homogenate 21.2 ± 3.6 (3)</td>
<td>23.6 ± 7.2 (3)</td>
</tr>
<tr>
<td></td>
<td>Acid-soluble 120.7 ± 14.5 (3)</td>
<td>119.7 ± 15.2 (3)</td>
</tr>
<tr>
<td>5 Hours</td>
<td>Homogenate 11.6 ± 4.7 (3)</td>
<td>12.1 (2)</td>
</tr>
<tr>
<td></td>
<td>Acid-soluble 115.1 ± 6.1 (3)</td>
<td>93.6 (2)</td>
</tr>
</tbody>
</table>

Results are expressed as percent ± SD of the specific radioactivity (DPM/μg protein) of the right cerebral hemisphere.
animals with grossly identifiable hemispheric infarction were found among approximately 160 asymptomatic animals up to 3 months after carotid ligation, indicated that some animals probably did not suffer the destruction of the nigrostriatal tract and the more subtle clinical signs went undetected. However, hemiparesis or progressive dullness was observed in many of the animals with hemispheric infarction. When seizures occurred, they often consisted of corkscrew rotation of the trunk. Unfortunately the surface recording of EEG was not able to localize the epileptogenic focus. The EEG findings from the right brain were persistent suppression with only occasional burst of sharp waves reminiscent of periodic lateralized epileptiform discharges, while the findings from the left side were indicative of the involvement of the left cerebral cortex and/or pressure on the brain stem with additional sharp wave activities during the immediately postictal period.

The observed improvement of protein synthesis at 24 hours in comparison to the earlier periods is best explained by heterogeneity of the clinical conditions as clearly shown in figure 4 between severely affected and moderately affected animals. The animals with rapid progression of cerebral infarction also showed rapid decline of leucine incorporation. However, even among the severely affected animals, leucine incorporation was higher at 24 hours than at 8 or 10 hours. This probably reflects the fact that the animals which survived for 24 hours were less severely affected than those which had died by 12 hours. The changes seen among 3 subcellular fractions were very similar to the change in the homogenate. The findings are the same as observed with the in vitro model of cerebral anoxia with rabbit brain.14 The diffuse suppression among various subcellular fractions indicated the involvement of the neuronal and neuroglial elements together as demonstrated in the past.18 For this reason, the neuronal and glial fractions were prepared from ischemic gerbil brains and compared to the findings from anoxic gerbil brains. As shown in table 2, protein synthesis, both in neuronal perikarya and glial cells, was similarly affected for both cerebral ischemia and anoxia, suggesting that the basic insult on the polyribosomal function is the same for anoxia and ischemia. Although the precursor availability was tested only with the alteration of the acid-soluble radioactivity, a recent study from this laboratory (Yanagihara and Tyce, unpublished data) indicated that over 90% of radioactivities remained in radioactive leucine at least with brain tissue obtained 2 hours postoperatively. The decline of the acid-soluble radioactivity at the advanced stage of cerebral infarction may indicate the decline of the active uptake of amino acids into cells.

It has been shown that protein synthesis is very vulnerable to the reduction of energy state in comparison to ribonucleic acid or lipid synthesis both in cerebral anoxia17 and cerebral ischemia.14 Furthermore, functional and structural alteration of polyribosomes have been implicated in these conditions.6,12,15 An attractive hypothesis is that either synthesis or nucleo-cytoplasmic transport of messenger RNA is suppressed, or there is destruction of messenger RNA at the polyribosomal site.

From the present investigation one group of gerbils emerged which sustained very rapidly progressive stroke with an onset of clinical manifestations within 30 minutes after ligation and often died within 12 hours. Those animals showed histological evidence of diffuse cerebral infarction by 6 hours with suppression of electroencephalographic activities and rapid decline of protein synthesis. Another group of animals survived to 24 hours in spite of severe seizures and other neurological signs, and the decline of protein synthesis was less severe than the first group. Considerable variation of the evolution of stroke was likely due to variation in the extent of the existence of carotid-basilar and anterior communicating anastomoses, and also the extent of collateral microcirculation. It is important to correlate clinical manifestations and alteration of biochemical states when gerbils are chosen as the model for cerebral ischemia, particularly when the effectiveness of a therapeutic measure is to be evaluated.

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References

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