Cerebral Ischemia in Gerbils: Differential Vulnerability of Protein, RNA, and Lipid Syntheses

T. Yanagihara, M.D.

SUMMARY  In order to compare the difference in the vulnerability of macromolecular syntheses, protein, RNA, and lipid syntheses were studied with ischemic brain tissue three hours following unilateral carotid ligation in gerbils. Precursor incorporation was measured in various subcellular fractions following in vitro incorporation with brain slices. There was marked inhibition of protein synthesis, but DNA and phospholipid syntheses showed little or no change. On the basis of available information on rapid deterioration of polypeptide synthesis, a hypothesis was proposed that messenger ribonucleic acid (RNA) at the polysomal level is promptly affected in this pathophysiologic condition.

Although the alteration of the energy state has been studied extensively in anoxic or ischemic brain in the past, the effect of the depletion of oxygen and the energy source on the macromolecular metabolism has drawn relatively less attention. Since the macromolecules such as nucleic acids, protein, and lipid have a significant role in the cellular regulatory mechanism and are the constituents of various subcellular structures, understanding of the molecular

References
remains unsettled. In order to compare these two conditions, the ischemic condition was produced in gerbils (Meriones unguiculatus) by ligation of the right carotid artery, and protein, RNA, and phospholipid syntheses were investigated.

The biochemical procedures were carried out three to three and one-half hours after ligation to demonstrate clear difference of vulnerability when animals show definite clinical signs. The results indicated selective vulnerability of protein synthesis similar to that shown in cerebral anoxia.

## Methods

Each gerbil (60 to 70 gm) was anesthetized by intraperitoneal injection of chloral hydrate (0.25 mg per gram of body weight) and supplementary inhalation of ether. The right common carotid artery was exposed through a midline incision in the neck and, by retracting the paratracheal muscles, freed from connective tissue and the vagus nerve, doubly ligated with 4-0 silk suture material, and finally sectioned between the ligations. The wound was closed with a surgical skin clip. Three to three and one-half hours after the operation the animals in which acute cerebral ischemia developed were identified by restlessness, circling movement, torsion of the neck, and drowsiness. The animals in which seizure developed were excluded to avoid the possible effect of seizure on biochemical parameters to be investigated. The animals that remained free of clinical signs after the identical surgical procedure were used as controls.

Brains were removed by decapitation after light ether anesthesia and cooled rapidly in ice; the subcortical structures were then removed. Each hemisphere was sliced at 300 μm with a McIlwain tissue slicer (Brinkmann Instruments) and the resulting brain slices from each hemisphere were transferred to a flask containing 5 ml of incubation medium consisting of 38 mM Tris-HCl buffer (pH 7.4), 2.0 mM sodium phosphate buffer (pH 7.4), 5 mM KC1, 100 mM NaCl, 1 mM MgCl₂, 1.8 mM CaCl₂, and 10 mM glucose. The flask was then saturated with oxygen by flushing, sealed with a rubber cap, and incubated at 35°C for 30 minutes under constant shaking at 140 rpm. For protein synthesis, the nuclei and microsomes were precipitated with an equal volume of cold 10% TCA. Each precipitate was washed three times with cold 5% TCA, the last one being heated at 90°C for 15 minutes, extracted with alcohol-ether (1:1), and dried with ether. The dried sample was dissolved in 1 N NaOH and an aliquot was taken for the determination of protein content according to Lowry et al. with bovine serum albumin as standard. Another aliquot was solubilized with Soluene-100 (Packard Instrument) and the radioactivity was counted in scintillation solution consisting of Permablend III (Packard Instrument) in toluene. The specific radioactivity was expressed as dpm/mg of protein content.

The homogenate, nuclei, and microsomes were studied for RNA synthesis. The homogenate was precipitated with an equal volume of cold 10% TCA. The nuclei and microsomes were homogenized in standard sucrose and precipitated with an equal volume of cold 10% TCA after addition of bovine serum albumin. Each precipitate was washed three times with cold 5% TCA. The homogenate and nuclei were suspended in 0.5 N perchloric acid and hydrolyzed by heating at 80°C for 20 minutes. The supernatant containing the hydrolysate of RNA and deoxyribonucleic acid (DNA) was used for determination of DNA content according to Burton, with calf thymus DNA as standard. Another aliquot was dissolved in Insta-Gel and the radioactivity was determined. The specific radioactivity was expressed as dpm/μg DNA content. Less than 5% of the total radioactivity from the hydrolysate was recovered from DNA fraction when the radioactivity of RNA and DNA was determined after separate hydrolysis of RNA and of DNA according to the method described previously. After the washing with TCA, the precipitate from the microsomes was extracted with alcohol-ether (1:1) and dried with ether. The dried sample was hydrolyzed with 0.3 N KOH for 15 hours, and protein and DNA were removed by precipitation with perchloric acid also as described previously. An aliquot of RNA hydrolysate was taken for the determination of RNA content according to Mejbaum with purified Torula RNA as standard. Another aliquot was dissolved in Insta-Gel for...
determination of radioactivity. The specific radioactivity was expressed as dpm/μg RNA content.

The homogenate, mitochondria, synaptosomes, and microsomes were taken for phospholipid synthesis. Lipid from each fraction was extracted with 15 volumes of chloroform-methanol (1:1) as described previously. The deproteinization and washing procedures were carried out according to Folch et al. The washing procedure with the solvent upper phase was repeated twice. A washed lipid sample was dissolved in chloroform-methanol (2:1), and an aliquot was taken for determination of phosphorus content according to Fiske and Subbarow after digestion with 60% perchloric acid for three hours. Spectrophotometric reading was carried out either at 650 nm or at 830 nm. Monosodium phosphate was used as standard. Another aliquot was used for determination of radioactivity in scintillation solution (Permablend III in toluene). The specific radioactivity was expressed as counts per minute per milligram (cpm/mg) of phospholipid.

Results

Three hours after operation, the right cerebral hemisphere of the symptomatic animal was pale and swollen. Under light microscope examination, after staining with hematoxylin and eosin, scattered neurons with pyknotic nuclei and eosinophilic cytoplasm as well as scattered vacuolization of neuropils were identified. Although the left cerebral hemisphere was often slightly swollen, no significant microscopic abnormality was detected. The brains from asymptomatic animals were normal both grossly and microscopically. Under the stated experimental conditions, normal brain slices showed l-leucine uptake of 9.95 × 10⁶ μmol/mg protein weight and incorporation into protein of 1.12 × 10⁷ μmol/mg protein; uridine uptake of 1.64 × 10⁷ μmol/mg protein weight and incorporation into RNA of 1.61 × 10⁷ μmol/μg DNA; phosphorus uptake of 7.23 × 10⁴ μmol, and incorporation into phospholipid of 9.18 × 10⁴ μmol/mg phospholipid. To eliminate the variation of specific radioactivities from one experiment to another, the specific radioactivity from the right cerebral hemisphere was expressed as a percentage of the value from the left hemisphere, for both the symptomatic and the asymptomatic animals. The specific radioactivity of the left cerebral hemispheres of the symptomatic and asymptomatic animals was similar in protein, RNA, and lipid syntheses at the postoperative time chosen for the present investigation.

The effect of cerebral ischemia on protein and RNA synthesis in the right cerebral hemisphere is shown in table 1. The difference in radioactivity of the acid-soluble fraction between control and ischemic animals was not significant. On the other hand, 80% inhibition of protein synthesis was observed in the homogenate of the ischemic tissue and similar changes existed in all the subcellular fractions studied. However, inhibition was not observed for RNA synthesis in the homogenate, nuclei, or microsomes. In fact, some tendency toward higher values was noted in the right side. Since the radioactivity of the acid-soluble fraction of the ischemic hemisphere also showed a similar tendency, a higher rate of uridine incorporation into RNA of the ischemic hemisphere may be the reflection of the availability of the radioactive precursor. When phospholipid synthesis was compared with the control value (table 2), the ischemic hemisphere showed some tendency toward inhibition that was more prominent in the mitochondria. However, since there was simultaneous decrease of the radioactivity of the acid-soluble fraction in the ischemic hemisphere, the observed alteration of phospholipid synthesis may not be valid. At any rate, the inhibition was not as striking as in protein synthesis. The values for protein, RNA, and phospholipid syntheses in the right and left hemispheres were similar in the asymptomatic animals.

Discussion

In the previous investigation of cerebral anoxia, the vulnerability of protein synthesis was demonstrated with isolated microsomes and in comparison to RNA synthesis with DNA-dependent RNA-polymerase assay with isolated nuclei. This approach gave more definitive results than the methods used in the present investigation. However, the in vitro assay of total phospholipid with isolated microsomes is difficult, and it was necessary to use the procedures with

### Table 1: Protein and RNA Synthesis in Gerbil Brain After Ligation of the Right Carotid Artery

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Symptomatic</th>
<th>Asymptomatic</th>
<th>SD</th>
<th>Symptomatic</th>
<th>Asymptomatic</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-soluble</td>
<td>111.5 ± 22.2</td>
<td>104.0 ± 9.9</td>
<td>9.9</td>
<td>113.7 ± 12.9</td>
<td>96.7 ± 1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Homogenate</td>
<td>23.8 ± 6.9</td>
<td>21.8 ± 7.4</td>
<td>14.1</td>
<td>121.4 ± 18.9</td>
<td>100.9 ± 6.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Nuclei</td>
<td>20.7 ± 2.4</td>
<td>111.5 ± 19.3</td>
<td>19.3</td>
<td>122.1 ± 32.4</td>
<td>102.4 ± 11.6</td>
<td>11.6</td>
</tr>
<tr>
<td>Microsomes</td>
<td>22.9 ± 15.5</td>
<td>102.4 ± 31.0</td>
<td>31.0</td>
<td>116.1 ± 25.9</td>
<td>102.8 ± 8.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Supernatant</td>
<td>18.8 ± 9.5</td>
<td>102.5 ± 20.5</td>
<td>20.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The results are expressed as percent of the specific radioactivity of the right cerebral hemisphere compared with the left hemisphere and are based on four experiments for each symptomatic and asymptomatic group. The specific radioactivity of the acid-soluble fraction was expressed as dpm/μg wet tissue weight; the subcellular fractions for protein synthesis as dpm/μg protein content, the homogenate and nuclei for RNA synthesis as dpm/μg DNA content, and microsomes for RNA synthesis as dpm/μg RNA content.

### Table 2: Lipid Synthesis in Gerbil Brain After Ligation of the Right Carotid Artery

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Symptomatic</th>
<th>Asymptomatic</th>
<th>SD</th>
<th>Symptomatic</th>
<th>Asymptomatic</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-soluble</td>
<td>73.9 ± 17.9</td>
<td>93.1 ± 1.6</td>
<td>1.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Homogenate</td>
<td>86.2 ± 16.8</td>
<td>89.8 ± 8.7</td>
<td>8.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>71.6 ± 10.0</td>
<td>120.8 ± 11.9</td>
<td>11.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Synaptosomes</td>
<td>89.1 ± 11.4</td>
<td>93.1 ± 20.9</td>
<td>20.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Microsomes</td>
<td>95.2 ± 9.0</td>
<td>89.4 ± 6.2</td>
<td>6.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The results are expressed as percent of the specific radioactivity (cpm/mg wet tissue weight) for the acid-soluble fraction and cpm/mg phospholipid content for the subcellular fractions of the right side compared with the left side, based on four experiments for the symptomatic group and three experiments for the asymptomatic group.
brain slices in order to make comparisons between protein, RNA, and phospholipid syntheses. This system minimizes the problem of precursor availability, which is encountered in systemic administration of precursors that do not cross the blood-brain barrier easily. If utilized with relatively short incubation time such as 30 minutes as used in the present investigation, during which time the incorporation is linear, this system also minimizes the active metabolism of precursors encountered with their systemic administration. The expression of the acid soluble radioactivity on a wet tissue weight might give erroneous results if the water content of tissue is different. However, the edematous tissue slices (right side) actually have higher radioactivity if they are corrected to a dry weight and therefore the interpretation of the results for protein and phospholipid would not be affected.

Results of the present investigation demonstrated clearly the differential vulnerability of macromolecular syntheses after cerebral ischemia. The observed vulnerability of protein synthesis is in accordance with the previous investigation for cerebral anoxia. This vulnerability can be reinforced by the fact that the inhibition of protein synthesis with brain slices and with microsomes corresponded well in the case of cerebral anoxia, and that preliminary data (unpublished) show considerable inhibition of protein synthesis with isolated microsomes but intact DNA-dependent RNA-polymerase activity in the ischemic gerbil brain three hours after ligation. However, the present results do not necessarily indicate that RNA and lipid syntheses are not affected, since the present investigation dealt only with total proteins, RNAs, and phospholipids. Possibly further detailed investigation may show that certain species of RNA or certain classes of lipid are affected more promptly. The present investigation points out the different sensitivities of various subcellular structures and biochemical constituents toward the deprivation of oxygen and the decline of the energy sources such as ATP and glucose.

Either synthesis or transport of messenger RNA may be affected promptly in cerebral ischemia, resulting in disaggregation of polyribosomes. This phenomenon has been observed in the laboratory with the model for cerebral anoxia and hypoxia.

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References
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