Disaggregation of Polyribosomes in Intact Gerbils Following Ischemia. An Ultrastructural Study

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Abstract: Disaggregation of Polyribosomes in Intact Gerbils Following Ischemia. An Ultrastructural Study

Adult gerbils were subjected to either unilateral or bilateral common carotid artery ligation for varying periods of time with varying durations of recovery. Selected brain regions were prepared for ultrastructural study after perfusion fixation. Dissociation of polyribosomes was observed in neurons but not in glial cells of the cerebral cortex and thalamus after 30 minutes of unilateral occlusion with no recovery period. At the other extreme, disaggregation was present in all brain regions studied after 60 minutes of bilateral occlusion followed by a 60-minute recovery period. Still longer recovery periods result in the appearance of some neurons with normally aggregated polyribosomes. The results suggest that a relatively long period of ischemia induces a derangement of neuronal protein synthesis that may persist for some time after restoration of circulation.

Additional Key Words: protein synthesis, carotid artery occlusion

Introduction

The apparent inadequacy of the circle of Willis in the Mongolian gerbil (Meriones unguiculatus) permits induction of cerebral ischemia in more than half the animals that undergo unilateral common carotid artery ligation. This convenient animal model of human cerebrovascular disease has been used in a number of studies, none of which has dealt with early ultrastructural changes. We report here on the electron microscopy of selected brain regions of the gerbil following unilateral or bilateral common carotid artery occlusion for varying periods of time and with varying periods of survival.

Methods

A total of 29 adult gerbils of both sexes were used. Anesthesia was induced by intraperitoneal injection of sodium pentothal (150 mg per kilogram body weight) or sodium pentobarbital (160 mg per kilogram body weight). We prefer the former because of the lower incidence of respiratory distress attending its use in our experiments. All animals remained anesthetized throughout the experimental period.

Four animals failed to survive the experimental procedure, and two showed evidence of inadequate fixation. Small artery clips were used to occlude one or both common carotid arteries at about the midcervical region. The occlusions lasted from 20 to 60 minutes in individual experiments. Some animals were killed at the end of the occlusion time; others were allowed to survive for 30 minutes to 5.3 hours after removal of the clips. In all cases, the carotid arteries were inspected at the time the clips were removed to be sure that arterial patency was re-established. This was done to eliminate possible persistent occlusion due to arterial damage which would prevent adequate flow of fixing fluid to the brain. The fixation procedure described below had been tested on a series of six normal animals to provide confidence in its adequacy and reliability for ultrastructural studies.

The thoracic cavity was opened by cutting the sternum in the midline, exposing the heart and ascending aorta. The right atrium was punctured and a slit was made near the tip of the left ventricle. A cannula was introduced through the ventricle into the aorta and perfusion begun. The time for opening the thorax to the start of perfusion was less than one minute.

A peristaltic-type pump designed to deliver perfusate at a constant rate of flow rather than at constant pressure was connected by plastic tubing through a flowmeter to the cannula. The initial flow rate of 30 ml per minute was reduced to 15 ml per minute after the first five minutes and perfusion continued for another 35 minutes. Two concentrations of glutaraldehyde were used, both of them made up in cacodylate buffer at pH 7.4 and both containing 4% (w/V) dextran of molecular weight 37,000 to 43,000. Glutaraldehyde (4%) was used during the phase of faster flow rate, and 2.5% during the slower. The osmolalities of these two
AN ULTRASTRUCTURAL STUDY

fixing fluids as measured by the freezing point depression method were 662 mOsm and 279 mOsm, respectively. The perfused brain was left undisturbed for a few hours after perfusion was ended. The brain was then removed, and desired regions were dissected out and divided into 1-mm cubes for postfixation in 2% osmium tetroxide in cacodylate buffer at pH 7.4 (osmolality 465 mOsm). Brain regions selected for study were cerebral cortex, head of caudate nucleus, thalamus and hippocampus. Because many of the ultrastructural changes seen following experimental ischemia, hypoxia or hypercapnia also can occur as a result of inadequate fixation, samples of cerebellum also were routinely studied to permit evaluation of the overall quality of fixation, since the vertebral-basilar arterial system had not been interfered with. Perfusion fixation was not always successful with the technique described, and two brains judged to be inadequately perfused, either by gross inspection or by subsequent ultrastructural examination, were eliminated from the experimental series.

Results

In common with the experience of others who have used different means of inducing hypoxia or ischemia, we have encountered swelling of neuronal mitochondria, enlargement of astrocytic foot processes and of extracellular space, and distention of neuronal endoplasmic reticulum. Since the time of that publication, in which disaggregation of neuronal polyribosomes was a preliminary finding, we have extended our experimental series in the attempt to determine whether this particular alteration increased in severity with duration of ischemia and whether or not it is reversible.

Table 1 lists the experimental conditions and indicates which of the five brain regions studied had shown disaggregation of polyribosomes in addition to the ultrastructural changes listed above. Figure 1 is a control section and figure 2 is representative of polyribosomal disaggregation as seen in neurons of the experimental tissues. From table 1 it appears that the cerebral cortex is the first brain region to be affected, with other regions becoming involved during the recovery period.

It will be noted from the table that disaggregation was not seen consistently in all brain regions studied until the stage at which 30 minutes of bilateral occlusion were followed by 30 minutes of recovery. When a 60-minute bilateral occlusion was followed by more than 60 minutes of recovery, only about half the neurons encountered showed polysomal disaggregation. It should be mentioned that disaggregation tended to be an all-or-none phenomenon in individual neurons. Thus the plus-minus signs in the table do not indicate an equivocal situation with respect to any individual neuron, but rather an almost equal population distribution of neurons exhibiting and not exhibiting disaggregation.

Neither astrocytes nor oligodendroglial cells showed polyribosomal disaggregation under the experimental conditions used. Neuroglial cells also failed to show mitochondrial changes.

### Table 1

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Occlusion</th>
<th>Duration (minutes)</th>
<th>Recovery (minutes)</th>
<th>Cerebellum</th>
<th>Cerebral cortex</th>
<th>Caudate nucleus</th>
<th>Hippocampus</th>
<th>Thalamus</th>
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<td>30</td>
<td>0</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
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<td>B</td>
<td>30</td>
<td>30 minutes</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>B</td>
<td>60</td>
<td>30 minutes</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>B</td>
<td>60</td>
<td>60 minutes</td>
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<td>-</td>
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<tr>
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<td>B</td>
<td>60</td>
<td>2 hours</td>
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<td>+</td>
<td>*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
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<td>60</td>
<td>2 hours</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td>+</td>
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</tr>
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<td>1</td>
<td>B</td>
<td>60</td>
<td>5.3 hours</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

- : No disaggregation in any neurons encountered.
± : Disaggregation present in some neurons encountered.
+ : Disaggregation present in all neurons encountered.

Stroke, Vol. 4, November-December 1973 965
Discussion

The inconsistent results of unilateral occlusion shown in Table 1 could be due to a marginally adequate collateral circulation in some animals. This could be by way of a large anterior communicating artery or a functional posterior communicating artery, or both. Levine and Sohn reported ischemia as occurring in only about half the cases of unilateral ligation, but neither their experiments nor ours were designed to explore individual variations among animals with respect to communicating arteries.

The apparent spreading of polysomal changes from the cerebral cortex to other brain regions is in line with the observations of Kleihues and Hoss mann, who found disaggregation of polyribosomes in neurons of cats in the recovery phase after brief total body ischemia.

We consider it unlikely that a "no-reflow phenomenon" compromised our perfusion fixation to the extent of producing artifactual changes that mimic those resulting from ischemia. The fixative was delivered to the cerebral vessels at constant flow rate instead of constant pressure. Signs of inadequate fixation, such as swelling of astrocytic mitochondria, were not found, and there was no evidence of ultrastructural damage to capillaries such as has been reported following ischemic brain lesions in other experimental animals. It also has been stated that interruption of the arterial supply does not induce the no-reflow phenomenon unless the venous outflow also is occluded—a condition which did not exist in our experiments. Finally, the occurrence of neurons with aggregated polyribosomes after long recovery periods speaks against the possibility of fixation artifact.

It should be stated that our criteria for survival involved only the maintenance of normal heartbeat and respiration for the duration of the experiments, since the animals remained anesthetized throughout. Survival, as thus defined, for as long as 5.3 hours after 60 minutes of bilateral carotid occlusion could be due in part to the well-documented protective
AN ULTRASTRUCTURAL STUDY

FIGURE 2
Neuronal cytoplasm from cerebral cortex of gerbil that had undergone bilateral common carotid artery occlusion for 30 minutes with 30 minutes' recovery. Almost complete disaggregation of polyribosomes is shown. Magnification 58,500 X.

effects of anesthesia and partly to the fact that the brain stem circulation was in all likelihood uncompromised.

It is difficult to interpret the finding that as the recovery period is prolonged, a significant proportion of neurons appear normal with respect to polysomal aggregation, although mitochondrial changes persist. It seems reasonable to interpret the observed disaggregation as a morphological expression of derangement of protein synthesis. Even then, it is not possible to conclude whether the process is slowed or accelerated. If slowed, one might expect fewer ribosomal particles to be attached to messenger ribonucleic acid (mRNA) strands in a given time. If accelerated, the particles could be released from mRNA strands at a faster rate. Either situation could result in the ultrastructural appearance shown in figure 2. One can conclude, however, that the normal equilibrium between attachment of RNA particles to mRNA and their release has been disturbed. A third possibility cannot be excluded at this point. There may be less mRNA available in the cytoplasm for ribosomal attachment, either because of diminished synthesis in the nucleus or because of lowered nucleocytoplasmic transport. Neuronal nuclei are in any case ultrastructurally inscrutable, and showed no obvious changes in our preparations, so this third possibility must remain open.

It is tempting to interpret the occurrence of normally aggregated polysomes in some neurons two hours or more after restoration of cerebral circulation as a sign of reversibility of an abnormal situation, but such an interpretation is not permissible on morphological grounds alone.

Our finding of polyribosomal disaggregation during both the ischemic phase and the recovery phase is somewhat at variance with the results of Kleihues and Hossmann, who observed it in the cat brain only during the postischemic period. This may be due to species differences, but it does not
significantly affect the conclusion that some alteration of protein synthesis can begin early in the ischemic phase and continue well into the recovery phase. If it is indeed an inhibition of protein synthesis continuing after restoration of circulation, it is probably not due to failure of amino acid precursors to pass the blood-brain barrier, for serum albumin enters the brain parenchyma at an increased rate during hypercapnia.26

Clearly, much work remains to be done to find out where the derangement in the protein synthetic pathway occurs, but the present work offers additional evidence that ischemia may in fact have a relatively long-lasting effect on this phase of neuronal metabolism.

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