Synaptic Alterations in Developing Cortical Infarction: An Experimental Investigation in Monkeys

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Abstract: The sequential synaptic alterations which occur during the early phase of developing cortical infarction in the squirrel monkey were studied with the electron microscope. Mild swelling of a few dendritic terminals and clumping of synaptic vesicles were the earliest detectable changes, being present at 45 minutes. The two basic patterns of degeneration of the terminal boutons were shrinkage and swelling, with shrinkage being the predominant response. Many of the shrunken boutons became electron-dense, resembling the changes present in anterograde axonal degeneration. The alterations which developed in the dendritic terminals were relatively unimpressive. Disruption of axosomatic synapses occurred at an earlier stage and was more severe than that of axodendritic synapses. Reduction in the number of synaptic vesicles was first observed at six hours and appeared to be progressive. The edema which developed was morphologically distinct from both "vasogenic" and "cytotoxic" edema in that there was early and progressive enlargement of the extracellular space.

Additional Key Words
cerebral ischemia
cerebral edema

Introduction

Detailed descriptions of the ultrastructural alterations in the cerebral cortex produced by ischemia have been reported previously. These studies have dealt mainly with the microvasculature, neuronal perikarya and glia, with little attention being given to the synapses. The object of this investigation has been to study the electron microscopic alterations of synapses in the cerebral cortex during the evolution of an ischemic infarct.

Methods

The techniques used for the production of the ischemic lesions, fixation, and tissue selection have been described in detail in previous reports. The tissue was obtained from 12 squirrel monkeys, killed in groups of two, after ischemic periods of 45 minutes, 90 minutes, 3 hours, 6 hours, 12 hours, and 24 hours.

Results

A. CONTROL TISSUE

The synapses in the nonischemic cortex of all 12 animals maintained a normal appearance and none of the alterations to be described in the ischemic tissue were detected. For the most part, two distinct morphological types of synapse, corresponding to the classification of Gray, were present. The type 1 synapses were located on dendritic spines and small dendrites. They were characterized by a wide synaptic cleft (about 300 Å) which contained some dense extracellular material. A large accumulation of dense material was present next to the postsynaptic membrane. The type 2 synapses were present on the large dendrites and perikarya. They were characterized by a narrower synaptic cleft and were less extensive. Small accumulations of dense material were present next to the presynaptic and postsynaptic membranes (fig. 1A).

B. ISCHEMIC TISSUE

45 Minutes

Most of the synapses were unaltered. In a few terminal boutons, the synaptic vesicles appeared to be clumped together. A small number of dendritic terminals were slightly swollen.

90 Minutes

The majority of terminal boutons were unaltered; however, some appeared to be either slightly shrunken or swollen. The cytoplasm in a few of the shrunken terminals was a little more electron-dense than normal. The synaptic vesicles in some of the boutons were clumped together. The mitochondria in the terminal boutons appeared to be normal and there was no evidence of swelling.

Little alteration of dendritic terminals was noted aside from occasional mild swelling.
A slight but definite increase in the extracellular space was detected with separation of the constituents of the neuropil. Retraction of a few terminal boutons from the perikarya was seen with contact being maintained only in the region of the synaptic cleft (fig. 1B). Similar retraction of axodendritic synapses also was observed. A few swollen astrocytic processes were distributed at random throughout the neuropil.

**3 Hours**

Most of the terminal boutons were shrunken. The electron density of the contained cytoplasm was

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**FIGURE 1A**

Control tissue. Two axodendritic synapses (a-d) and one axosomatic synapse (a-s) are present. The terminal boutons (b) contain numerous synaptic vesicles. Note the very narrow extracellular space.

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**FIGURE 1B**

90 minutes. The terminal bouton (b) appears to be retracting from the perikaryon; however, contact is maintained in the region of the membrane specialization (crossed arrow). The mitochondrion (m) within the perikaryon is swollen, but the mitochondrion within the terminal bouton appears normal. Note the slight enlargement of the extracellular space (arrows). The profile (a) adjacent to the plasma membrane of the perikaryon may represent a mildly swollen astrocytic process.
slightly increased in many and markedly increased in a few of the processes. A small number of boutons were swollen and contained low density cytoplasm. The distribution of synaptic vesicles was essentially normal in most instances; however, clumping of vesicles either in the center of the profile or adjacent to the area of membrane specialization was occasionally seen. There was no evidence of a substantial reduction in the number of vesicles, nor was there any evidence to suggest release of the vesicles into the expanded extracellular space. There was an increase in the number of vesiculated processes (i.e., processes without associated membrane specialization in the plane of section in which synaptic vesicles are found). A few of the contained mitochondria appeared to be slightly swollen. The mitochondria in the dense boutons contained an electron-dense matrix.

The majority of dendritic terminals were slightly

![Image of Figure 1C]

**FIGURE 1C**

*Three hours. The swollen astrocytic processes (a) appear to be displacing a terminal bouton (b) from the plasma membrane of a shrunken, dense neuron (n). A vesiculated process (v) is also present. Note the substantial enlargement of the extracellular space.*

![Image of Figure 1D]

**FIGURE 1D**

*Six hours. The terminal boutons (b) contain abundant synaptic vesicles. An intact axodendritic synapse (a-d) is present in the center of the figure. Swelling of an associated axonal process is present (arrow).*
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shrunken. The electron density of the cytoplasm in these structures was slightly increased in most instances.

There was a significant increase in the size of the extracellular space; however, it tended to be somewhat variable in distribution. Numerous swollen astrocytic processes were present throughout the neuropil, with many of them aggregating around shrunken neurons. There was a definite reduction in the number of intact axosomatic synapses. Electron micrographs of individual neurons usually demonstrated only two or three intact axosomatic synapses. In some instances it appeared as if the terminal boutons had been forced away from the plasma membrane of the perikaryon by

![Figure 1E](image)

12 hours. A mildly swollen bouton containing a small number of clumped, distorted vesicles is present in the center of the figure (arrow). A dense terminal bouton (b) containing numerous synaptic vesicles is present in the upper left corner. A number of vesiculated processes and shrunken, dense axons and dendrites also are present. Note the marked enlargement of the extracellular space.

![Figure 1F](image)

12 hours. A shrunken, dense terminal bouton (b) appears to have retained its contact with the plasma membrane of a swollen neuron. An intact axodendritic synapse is present on the right. The terminal bouton is swollen and contains clumped synaptic vesicles (arrow); the dendritic terminal (d) appears shrunken. (Calibrations equal 1 μ.)
expanding astrocytic processes (fig. 1C). The axodendritic synapses for the most part appeared to be intact, although contact was usually preserved only in the region of the membrane specialization.

6 Hours
Numerous shrunken electron-dense terminal boutons were distributed throughout the neuropil. A small number of swollen, pale terminals were observed. There was little apparent change in the size, shape or distribution of synaptic vesicles (fig. 1D); however, a few empty boutons were identified. Rupture of the limiting membranes was not seen. Numerous vesiculated processes were present. The mitochondria were essentially unchanged from three hours.

Most of the dendritic terminals were shrunken. A few of them contained a very dense cytoplasmic matrix.

Expansion of the extracellular space was more uniform than at three hours. Marked swelling of astrocytic processes was present — especially around the shrunken neurons (fig. 2). In a few instances, one or two swollen terminal boutons could be identified among these distended astrocytic processes. Intact axosomatic synapses were infrequently observed. The integrity of the majority of the axodendritic synapses was preserved.

12 Hours
The majority of terminal boutons were markedly shrunken and approximately half of these were extremely electron-dense (figs. 1E and F). Occasional swollen boutons containing clumped synaptic vesicles also were observed. Many of the synaptic vesicles were swollen or distorted and there appeared to be a definite decrease in the number of these structures. Some terminal boutons contained only a few vesicles or none at all (i.e., empty boutons). Numerous boutons were present which appeared to lack any synaptic contact. The mitochondria in the shrunken terminals frequently were partially collapsed. Swollen mitochondria seldom were observed.

There were few changes in the dendritic terminals as compared with those seen at six hours.

The extracellular space had increased in size. Many of the swollen astrocytic processes had ruptured, spilling their contents into the already expanded extracellular space. Electron micrographs of individual neurons seldom demonstrated intact axosomatic synapses. There also appeared to be a reduc-
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In the number of intact axodendritic synapses, and many unattached axon and dendritic terminals were observed in the neuropil.

24 Hours

The findings at 24 hours were essentially the same as those at 12 hours except that fragmentation of the limiting plasma membranes of some terminal boutons had taken place.

Discussion

The two basic patterns of degeneration of the terminal boutons were shrinkage and swelling, with shrinkage being the predominant response. The neuronal perikarya were noted in a previous report to respond to ischemia in a similar fashion. These findings suggest that the type of degeneration which develops in a terminal bouton is the same as that which develops in its perikaryon. The changes which occur in many of the shrunken terminal boutons, that is, condensation of their contents and marked increase in electron density, resembled those changes described in the electron-dense form of degeneration secondary to axonal sectioning.

The alterations which developed in the dendritic terminals were relatively unimpressive. Some degree of shrinkage and swelling was seen, but it was not as severe as that of the axon terminals. We did not observe the marked and widespread swelling of the dendritic terminals as described previously by Garcia.

Disruption of axosomatic synapses occurred at an earlier stage and was more severe than that of axodendritic synapses. A definite reduction in the number of intact axosomatic synapses was noted as early as three hours. In a study of synaptic density on spinal neurons of dogs with hindlimb rigidity produced by transient ischemia, Gelfan and Rapisarda demonstrated a selective reduction in the number of axosomatic synapses. Van Harreveld and Khattab reported similar changes in the cat spinal cord following asphyxia. These findings suggest a greater vulnerability of the axosomatic synapse to ischemia.

The thickenings of the apposed synaptic membranes are thought to be the regions of firm attachment. Consequently, the apparent sensitivity of the axosomatic synapses to ischemia may be related in part to the fact that their synaptic zones are less extensive than those of the axodendritic synapses. Terminal boutons which end upon the plasma membrane of the perikarya also appear to be displaced by the great enlargement of perineuronal astrocytic processes.

Other factors which may be significant with regard to the loss of synaptic contact include: (1) shrinkage of the axons and dendrites, and (2) the inability of axons and dendrites to maintain an adequate ength across an acutely distended, edematous neuropil.

Williams and Grossman studied the ultrastructural features of cortical synapses in the cat during failure of synaptic transmission produced by systemic hypotension. These authors stated that an altered pattern of distribution of synaptic vesicles was observed after presynaptic afferent fiber terminal activity was abolished by 3/4 to 4 minutes of cerebral ischemia. Clumping of vesicles in a region away from the synaptic cleft was seen in about 10% of synaptic endings and there was more than a twofold increase in the number of presynaptic profiles devoid of vesicles in ischemic cortex. Van Harreveld and Khattab, in an ultrastructural investigation of asphyxiated spinal cords in cats, described swelling of terminal boutons and empty boutons in animals fixed 30 minutes after 50 minutes of asphyxiation.

In our study, occasional clumping of synaptic vesicles was an early finding. Reduction in the number of synaptic vesicles was a relatively late change, but it suggested that some release of active neurotransmitter substances may be taking place. The effects upon the nervous tissue of these transmitter could be of some importance in the extension of the zone of infarction as suggested by Wurtman and Zervas, Osterholm and Mathews, Meyer et al., and MacDonald et al. Extrusion of the contents of neuronal lysosomes (especially the residual bodies) into the neuropil has been observed recently by Little et al. in the squirrel monkey following 12 hours of ischemia. The release of lysosomal enzymes also may be of importance with regard to extension of injury into the surrounding tissue as well as exacerbating the cerebral edema; however, because lysosomal alterations occur at a relatively late stage, it is doubtful that their hydrolytic enzymes play a role in the initiation of these processes.

Klatzo classified brain edema into two different types — vasogenic and cytotoxic edema. Vasogenic edema was characterized by enlargement of the extracellular and intracellular spaces of the white matter as well as by enlargement of the intracellular space, predominantly astrocytic, of the gray matter. Enlargement of the extracellular space of the gray matter was thought to occur at a late stage as the result of the rupture of greatly distented astrocytic processes. Cytotoxic edema was characterized by swelling of various tissue components only, without enlargement of the extracellular space. The edema which developed in the ischemic cortex in this study was morphologically unique in that there was early enlargement of the extracellular space. This occurred concomitantly with the swelling of astrocytes and did not appear to be the result of rupture of the swollen astrocytic processes. This type of edema probably reflects a severe disturbance of membrane transport which results from the rapid depletion of energy reserves and the development of lactic acidosis. The morphological changes in the cerebral white matter were similar to those observed with vasogenic edema. Previous histological studies of developing in-
farction in the squirrel monkey have demonstrated that the edema fluid in the white matter tends to spread along the fiber tracts beyond the area of the most intense ischemic injury.

References

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