Resistance of Hippocampal CA-1 Interneurons to 20 min of Transient Cerebral Ischemia in the Rat

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Summary. The aim of this morphological study was to determine the vulnerability of hippocampal interneurons to ischemia in the adult rat. Two types of interneurons situated in the CA-1 stratum oriens were investigated, the larger basket cells close to stratum pyramidale and the smaller basket cells close to the alveus.

Male Wistar rats were subjected to 20 min of transient cerebral ischemia by means of 4-vessel occlusion and perfusion fixed 1, 2, 4, or 21 days later. In both Golgi-impregnated and in routinely stained sections the pyramidal cells and interneurons in the hippocampal CA-1 region were examined and counted.

The study clearly demonstrated the selective vulnerability of the CA-1 pyramidal cells, as no ischemic cell damage to or loss of interneurons was found.

Key words: Cerebral ischemia — CA-1 interneurons — Hippocampus

Introduction

Twenty minutes of transient cerebral ischemia in the rat forebrain results in loss of hippocampal CA-1 pyramidal cells, small neurons in the dorsolateral striatum and nerve cells in certain cortical layers (Pulsinelli and Brierley 1979; Balslev Jørgensen and Diemer 1982).

Examination of posts ischemic regional blood flow using autoradiographic techniques has not revealed no-reflow areas (Pulsinelli et al. 1982) and the selective vulnerability of the nerve cells must then depend on biochemical (or morphological) differences (Vogt and Vogt 1922) and not on vascular factors as advocated by Spielmeyer (1925).

Nemoto (1979) suggested that the differences in nerve cell vulnerability could be due to their different transmitter systems. Recently, this possibility has been examined experimentally by Francis and Pulsinelli (1982) who found that GABAergic neurons in the striatum were damaged in contrast to acetylcholinergic and by Weinberger and Cohen (1982) who found that in the gerbil brain, dopaminergic neurons were more sensitive to ischemia than GABAergic and glutamatergic.

In the hippocampal CA-1 region, only two main types of neurons are known: the excitatory (glutamatergic?) pyramidal cells and the inhibitory (GABAergic) basket cells. The morphology and numbers of these two cell types were investigated after ischemia to find any differences in vulnerability.

Materials and Methods

Thirty male Wistar rats (250—300 g body weight), ranging in age from 3 to 6 months, were anesthetized with 2% Halothane in 98% oxygen after 24-h food deprivation. The vertebral arteries were electrocauterized as described by Pulsinelli and Brierley (1979) using a Martin Electroton 30 (setting 4) and a 0.5 mm thick cauterization needle grounded through the skin at the site of incision. With the exception of four rats in the control group the animals were kept food-deprived until the following day, with free access to water. They were then reanesthetized with Halothane in oxygen, and the common carotid arteries were gently exposed before anesthesia was switched off. Two minutes later the common carotids were clamped with metal clamps for 20 min. Rectal temperature was monitored throughout the experiment and maintained at 37°C with a heating lamp. Seven rats developed respiratory arrest and died during the ischemia period.

Five rats were allowed to survive for 1 day, four for 2 days, five for 4 days, and another five for 21 days before they were anesthetized with Halothane in oxygen and killed with a two-step perfusion-fixation as described by Vogt and Peters (1981). The control group survived for 21 days.

The heads of the animals were postfixed in concentrated fixative for 24 h at 4°C before the brains were removed and kept for another 24 h in the concentrated fixative at 4°C.

Their brains were then coronally sectioned in three blocks and prepared for HE, cresyl violet, Klüver-Barrera staining, and Bodian and Golgi impregnation, respectively (Fig. 1). Small triangular tissue blocks were processed for toluidine staining and embedding in Epon.
for later EM studies (Fig. 1). Routine staining procedures were used except in the case of Golgi impregnation which will be described in detail as several modifications were introduced.

Golgi Impregnation

(1) Blocks are cut 4-5 mm thick and washed for 15 min in 0.15 M Na-cacodylate buffer at pH 7.2.
(2) Postfixation in 2.4% potassium dichromate and 0.2% osmium tetroxide in distilled water (at least 20 ml of solution for each block) for 4 days.
(3) Blocks are washed three times for 5 min in 0.75% silver nitrate and left in 0.75% silver nitrate for 2 days in the dark.
(4) Three washings, each 2 min in absolute alcohol followed by dehydration for 24 h in 99% alcohol:acetone (1:1).
(5) Dehydration for 4 h in absolute alcohol:ether (1:1).
(6) Embedding in thin celloidin (28 g Parlodion in 500 ml 99% alcohol:ether 1:1) for 3 days.
(7) Embedding in thick celloidin (56 g Parlodion in 500 ml 99% alcohol:ether 1:1) for 4 days. The blocks are put into small glass dishes 3/4 filled with thick celloidin which are placed in a large Petri dish with chloroform in the bottom with the Petri dish covered. The chloroform will harden the celloidin. This procedure needs careful refilling every day.
(8) Sections, 50 µm-100 µm thick, are cut on a slide microtome with a knife at a 30° angle in relation to the orientated block. Block and knife are kept wet with 99% alcohol.
(9) Sections are cleaned in alpha-terpinol for 5 min, rinsed in xylene for 5 min, softened on the slide in 3/4 methylenbenzoate - 1/4 xylene, rinsed again with xylene, and mounted with Eukitt.

Counting Technique

Interneurons and pyramidal cells in the CA-1 region were counted inside the grids. The number of neurons was determined by counting all nucleolated pyramidal cells without ischemic cell change and interneurons in stratum oriens with the following characteristics: large nucleus with typical chromatin structure (and possible nucleolus) and finely dispersed Nissl substance.

For each animal the mean of ten grid countings was calculated, and for each group of animals the mean value and standard deviation (SD) were estimated. The means of the different groups were compared using Student's t-test.

Results

Macroscopic examination showed no hemorrhages, tumors, or gross demyelination. Microscopic examination confirmed good preservation of the tissue with the capillaries empty of erythrocytes. The neuronal changes described below had a graded character, i.e., the manifestation of neuronal changes in the individual animal in each group had a graded time course. Only the typical manifestations in each group will be described.

Controls

The Golgi impregnations showed the pyramidal cell with a triangular soma, 25 µm in diameter, and with a large apical dendrite extending into stratum radiatum with many spiny arborations (Fig. 2). From the basis of the pyramidal cell soma many spiny dendrites branch into stratum oriens. Two types of interneurons or basket cells situated in stratum oriens were seen (Andersen et al. 1969). A large interneuron lying close to the pyramidal layer with a soma of spherical or triangular shape (Fig. 3). This interneuron was larger than the pyramidal cell, while the other type of interneuron was slightly smaller with its oblong soma lying very close to the border toward the alveus (Fig. 4). Both kinds of interneurons sent their axons to form baskets around the pyramidal cell bodies. The interneuron dendrites were characteristically beaded with swellings resembling pearls on a string and without spines and were generally distributed in stratum oriens.

In the HE- and cresyl violet-stained sections, the cell bodies of the pyramidal cells and the interneurons generally had the same morphological characteristics, except that the interneuron seemed more angular in shape. This and the close relation of the large interneuron to the pyramidal cells made it difficult to identify the large interneuron. The toluidine-stained plastic sections showing the cell membranes and the proximal part of the largest dendrites offered the best conditions to distinguish between the two cell types. The large interneuron was larger than the pyramidal cell and its main dendrite was typically distributed parallel to the stratum pyramidale. The content of Nissl substance in interneurons, best seen with the cresyl violet stain, separated the interneurons from the gli