Developmental Alterations in the Histochemical Structures of Brain Capillaries: A Histochemical Contribution to the Problem of the Blood-Brain Barrier

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Summary. Activity of the non-specific alkaline phosphatase appears in brain capillaries in the course of intrauterine development, whereas that of the pseudocholinesterase (butyrylcholinesterase) appears only after birth. This latter enzyme is located in a perinuclear localization at the beginning and occupies later the entire cytoplasm of the endothelial cell. The definitive enzymo-histochemical structure of the capillary is achieved on the 21st day of postnatal development. By means of electron histochemistry, the reaction product of the butyrylcholinesterase reaction appears to be located in and around pinocytotic vesicles and in the perinuclear cysternae of endothelial cells. The results obtained are in harmony with the idea that butyrylcholinesterase is somehow involved in the function of the blood-brain barrier.

Introduction

In spite of numerous histochemical and ultrastructural studies, the structural basis of the blood-brain barrier (BBB) is still far from being completely understood. In this present paper, several enzymo-histochemical alterations in the light- and electron histochemical structures of brain capillaries during ontogenetic development, will be described and discussed.

Material and Methods

Our studies were performed on the parietal cortex of albino rats. 5 embryos, ranging from the 10th intrauterine day up to the birth, 3 newborn and 20 baby rats (1 to 30 days) as well as 20 young and mature animals were used. The animals were killed by decapitation, without anesthesia.

For light microscopic histochemistry, frozen sections (30 μ) obtained from the parietal cortex, fixed in 10% neutral formalin at 4o for 4--6 hours, were used. However, even this relatively mild fixation "knocks" out the slight enzyme activities in embryonic and newborn material, therefore we used cryostat sections in such cases. Butyrylcholinesterase (BuChE) activity of the capillaries was stained by the Koelle-Friedenwald technique (1949), as modified by Gekko (1959), using formalin-fixed frozen sections; and by the Koelle technique (1951), employing a hypersaturated sodium sulphate medium (for cryostat sections). Incubation was carried out at pH = 6.0 at 37o for 1 to 6 hours, using butyrylthiocholine substrate. After incubation, the sections were rinsed in distilled water and post-treated by a 2% aqueous solution of yellow ammonium sulphide. Sections were mounted in gum arabic or (after dehydration) in canada balsam.

Non-specific alkaline phosphatase activity of brain capillaries has been studied by means of the Fredricson modification (1952) of the original Gömöri technique (1939).

Electron histochemical methods. Fixation by means of immersion in formalin or glutaraldehyde resulted in a poor ultrastructural preservation. Therefore, perfusion with the following solution has been introduced: 10% formalin adjusted to pH = 7.4 by means of 0.1 M sodium cacodylate. Thin slices (0.1—0.2 mm) of the parietal cortex were obtained by free hand dissection, using a razor blade. The slices were fixed in the aforementioned fixative
for one hour at 4°C, and rinsed subsequently in sodium cacodylate solution (0.14 M) containing
0.25 M sucrose. BuChE activity was demonstrated in these slices by means of the lead thio-
choline technique described by us earlier (Joó, SÁVAY and CSILLIK (1965), KÁSA and CSILLIK
(1966), CSILLIK, Joó, KÁSA and SÁVAY (1966)).

After incubation, the sections were briefly rinsed and posttreated in yellow ammonium
sulphide (2%), rinsed again and postfixed in buffered osmic acid. The subsequent technique
was identical with the routine electron microscopic procedure: dehydration in graded alcohols,
embedding in Durcupan (Fluka), sectioning (using an LKB ultratome), application of silver
interference colour sections on non-coated (300 mesh) grids and observation under a Tesla
242 D table type electron microscope. Some of the sections were stained also according to
the Reynolds lead citrate technique (1963).

Results

There is no BuChE activity in brain capillaries of rat embryos, new-born
rats and up to the third day of post-natal development. The first signs of such
enzyme could be observed in cryostat sections obtained on the third postnatal
day. At the first time, the enzyme appears in the shape of fine granules, located
in a narrow border around the endothelial nucleus (the latter being itself inactive,
as seen in Fig. 1). When using a phase contrast system, or simply increasing the
contrast of the regular light microscope, the entire capillary “wall” can be observ-
ed, in the form of a non-reactive structure. At the age of 5 days, the number of
endothelial cells exhibiting a perinuclear reaction, increases considerably (Fig. 2).
Later the same kinds of enzyme-active granules can be observed in the endo-
thelial cytoplasm (“capillary wall”) even at areas more remote from the peri-
nuclear region (see e.g. the capillaries of a 10 days old rat, Figs. 3 and 4). In the
latter figure, the difference between the strong perinuclear and the much weaker
internuclear BuChE activity can readily be observed. In the course of later
development, the enzyme activity of the brain capillaries increases and, at the
21st day of postnatal development, the characteristic pattern of branching
capillaries is more or less completed and it can already be demonstrated in for-
malin-fixed frozen sections, too (Figs. 5 and 6). In young rats (Figs. 7 and 8) the
BuChE activity appears in the shape of 0.2—0.5 μ granules located randomly in
the capillary “wall” (= endothelial cytoplasm) leaving endothelial nuclei entirely
blank. The enzyme activity of the perinuclear region appears to be the strongest,
even in adult animals. Here (Fig. 9) capillaries show a tortuous course, exerting
a rich BuChE activity.

Non-specific alkaline phosphatase of brain capillaries could be demonstrated
already in pre-natal periods (Fig. 10). At the beginning, a slight enzyme activity,
located at the entire length of the capillary could be observed, that showed an
increasing tendency during the days after birth (Fig. 11). The branching system
of capillaries can readily be observed on the 21st day of postnatal development
(Fig. 12), giving rise to the patterns characterizing young and adult (Fig. 13)
animals.

In electron histochemical sections of the brain capillaries of adult animals, the
end-product of the BuChE reaction was found in the cytoplasm of the endo-
thelial cells. No activity could be observed in the astrocyte (glial) end-feet, attached
to the subendothelial basement membrane (Fig. 14); neither could any activity be
observed in the pericytes (Fig. 15). The fine structure of the endothelial cell can
readily be studied in the photomicrograms. Pinocytotic vesicles were present in