Ultrastructure of the blood-brain barrier of the gyrus hippocampal cortex in an experimental model of febrile seizures and with the use of a new generation antiepileptic drug – topiramate

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Abstract
The ultrastructure of the blood-brain barrier (BBB) of the gyrus hippocampal cortex in an experimental model of febrile seizures in rats and the effect of a new generation antiepileptic drug, topiramate, on the morphological status of this barrier were investigated. Advanced changes indicating a substantial increase in BBB permeability were observed in the animals with induced febrile seizures (FS), with approximately 2/3 of capillaries and perivascular astroglial processes being affected. Almost total occlusion of the capillary lumen was frequently seen, caused by damaged endothelial lining and by external pressure from markedly swollen perivascular astrocytic processes. Mitochondrial changes predominated among the abnormalities found in endoplasmic organelles of endothelial cells. Lesions in the BBB coexisted with damage to pyramidal neurons, mainly with features of aponecrosis (“dark neurons”). The study on topiramate seems to demonstrate its protective action on the BBB components of the ammonal cortex in the group receiving the drug as prevention, i.e. against febrile seizures. It was found to prevent marked BBB damage in over half of the capillaries. However, the application of topiramate directly after FS induction had no distinct beneficial effect on the structural BBB components.

Key words: febrile seizures, topiramate, blood-brain barrier, hippocampal cortex, ultrastructure, rats.

Introduction
Febrile seizures (FS), usually observed in patients in the course of infection with fever (body temperature above 38.5°C), are the most common form of convulsions in childhood. According to literature data, FS have been associated with a markedly increased risk of epilepsy in the future [9]. It is believed that prolonged FS are a frequent cause of temporal lobe epilepsy, e.g. drug-resistant epilepsy of the temporal lobe associated with mesial temporal sclerosis [13,18]. This pathology is characterized by neuronal losses especially in the hippocampal CA1 and CA3 sectors and accompanying microcirculatory abnormalities in the hippocampal gyrus [13].
Although temporal lobe epilepsy is the most prevalent type of epilepsy, its morphogenesis has not been fully elucidated yet.

Neuropathological studies of febrile seizures, especially those concerning the blood-brain barrier (BBB), are rather uncommon due to complex methodology and problematic interpretation of results.

The pathogenesis of changes in the CNS due to hyperthermia has been largely elucidated by the findings reported by Polish researchers Kapuściński and Karczewski [9], who demonstrated that thermal stress is accompanied by hyperventilation (accelerated respiration) that promotes hypercapnia, with subsequent contraction of cerebral vessels and the resulting brain ischaemia.

Since the brains of animals subjected to hyperthermia, especially their cerebral cortex, show a distinct increase in oxygen consumption [1], high temperature-induced morphological changes observed in the cortex are assumed to be most pronounced. It should be added that in normothermia, the cerebral cortex, which is a cell-rich structure, exhibits high oxygen consumption.

Therefore, the objective of the current study was the ultrastructural assessment of the elements of the blood-brain barrier of the gyrus hippocampal cortex in an experimental model of febrile seizures and the evaluation of the potential neuroprotective action of topiramate, a new generation antiepileptic drug in FS-induced damage, upon this barrier.

No similar studies on topiramate, considered to be an effective neuroprotectant, have been conducted so far.

The animal model for the study on the CNS in hyperthermia elaborated in our centre [20] meets the methodological criteria for an experimental model of febrile seizures, which well correspond to this form of convulsions in children.

The current study is a continuation of our histological research into the effect of topiramate on the ammonal cortex in an analogous model of hyperthermic seizures [20,21] and adds to our previous observations of the BBB components in the course of experimental administration of another antiepileptic drug, valproate [19,24-26].

Material and Methods

Model of febrile seizures

The experiment used 18 young male Wistar rats aged 22-30 days. The degree of brain maturity in such animals corresponds to that of 1- or 2-year-old children. The rats used for our studies were preselected according to the standard pharmacological screening tests. Prior to the experiment, they were kept in cages (15 rats in 3 cages, 5 in each, and 3 animals in one cage) with free access to food (standard granulated rat chow) and tap water at 12-hour cycles of light and darkness, in a room at 18-20°C. All procedures were carried out in strict accordance with Helsinki Convention Guidelines for the care and use of laboratory animals. For the needs of the study, the rats were divided into 4 groups (3 experimental and one control), 5 animals in each experimental group and 3 in the control group.

Group I (FS group) consisted of animals with induced febrile seizures. Hyperthermic stress was induced by placing the rats in a 30 × 30 × 60 cm water bath filled with 45°C warm water. Water temperature was maintained at the same level. The animals were put into water for 4 minutes until convulsions appeared and then moved to a separate container lined with lignin. The rats, except for controls, were placed in water for four consecutive days (for more details on seizure induction see our earlier publication [20]).

In group III (FS+TPM group), topiramate (Topamax, f. Jaansen-Cilag; 80/kg b.m. dissolved in 2 ml normal saline) was administered with an intragastric tube, immediately after each convulsive episode (every animal received the drug in the course of the experiment four times altogether).

In group II (TPM+FS group), topiramate was administered in the same way and at the same dose, prior to the induction of febrile seizures, i.e. 90 minutes before the rats were placed in the water bath.

Control animals and the FS group received only normal saline. The dose of the drug was chosen according to literature references, including those listed in our previous paper [4,20,23].

Preparation for electron microscopy

Seventy-two hours after the last convulsive episode, the animals were anaesthetized with Nembutal (25 mg/kg b.w., i.p.) and transcardially perfused with fixative solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4. After removal of the brains, hippocampal samples were taken and fixed in the same solution for 24 h at 20°C. Postfixation was
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completed with 1% osmium tetroxide (OsO₄). After dehydration in ethanol and propylene oxide, small specimens (1 mm³) of the gyrus hippocampal cortex were processed routinely for embedding in Epon 812. Semithin sections were stained with methylene blue and examined in the light microscope. Ultrathin sections (60 nm) were double stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (Opton 900 PC, Zeiss, Oberkochen, Germany). The material obtained from the gyrus hippocampal cortex in the control group was processed using the same techniques as for experimental groups.

Results

FS group

The animals with induced febrile seizures showed variously pronounced quantitative and qualitative changes in structural elements of the blood-brain barrier of the CA3 and CA1 sectors of the gyrus hippocampal cortex. The changes varied from discreet, affecting approximately 1/4 of the capillaries, through moderate to pronounced, seen in about 3/4 of the remaining capillaries and adjacent neuroglia components, especially in perivascular processes of astrocytes.

Fig. 1. Capillary with nearly occluded lumen (L). Nucleus of endothelial cell is stimulated – enlarged, filled up with granular and osmophilic chromatin that accumulates under the nuclear membrane, with visible nucleoli. Tight junctions between endothelial cells – invisible. Neuropil elements, especially axonal endings (a), situated in the vascular vicinity show features of distinct swelling and disintegration; perivascular astrocytic process (pAs) slightly swollen. FS group. Original magn. × 12 000
Advanced submicroscopic changes in the BBB components were seen both at the luminal and antiluminal sides of the barrier and were characterized by significant damage to the endothelial lining, including necrosis, and by substantial swelling of perivascular processes of astroglia. Significant alterations in endothelial cells, with outstanding swelling of astrocytic processes exerting a pressure on these vessels, frequently caused a significant reduction in capillary patency (Figs. 1-3A-B), leading to almost complete lumen occlusion (Fig. 1).

Some fragments of swollen endothelium with numerous micropinocytic vesicles and sometimes with single slightly larger vacuolar structures protruding towards the vascular lumen were seen. In places, however, considerable thinning of endothelial lining or even its complete loss was observed, causing basement membrane denudation. Loosening and disruption of tight intercellular endothelial junctions were noted (Fig. 2); frequently these junctions were completely invisible (Fig. 1).

Endothelial cell nuclei were enlarged, with granular osmophilic chromatin unevenly distributed under the nuclear membrane (Fig. 1).

Mitochondria were the most affected cell organelles. They frequently filled up cytoplasmic protrusions of endothelial cells and showed varied morphological abnormalities – from slight swelling to degenerative changes manifested as the formation of characteristic myelin structures within the matrix (Fig. 3B). Markedly swollen mitochondria, resembling double-contoured vacuoles, showed fragmentation and destruction of mitochondrial crests, loss of matrical granules and the presence of floccular microfibrillar material within the matrix (Fig. 3A-B).
Fig. 3A-B. Fragment of a vessel lined with damaged endothelium that forms protrusions directed towards the vascular lumen (L) and narrowing it considerably. The protrusions contain degenerating mitochondria (m) – markedly swollen, deprived of crests, with fine myelin structures (→) being formed within the matrix; in the vicinity of one of the mitochondria – irregular vacuolar structure (v) and micropinocytic vesicles can be seen. pAs – swollen perivascular astrocytic process. The vessel surrounding neuropil components – swollen and loosened. FS group. Original magn. × 20 000
Disintegration of granular endoplasmic reticulum was quite common, manifesting as loss of ribosomes in short fragments of this reticulum and an increased number of free ribosomes and polysomes. Damaged capillaries were frequently surrounded by markedly swollen perivascular processes of astroglia, more seldom oligodendroglia and microglia. The cytoplasm of such processes displayed considerably reduced electron density (even electron-translucency), and frequently contained fine floccular material and residual markedly damaged cell organelles, mainly abnormal mitochondria and fragments of disintegrating granular endoplasmic reticulum (Fig. 2). The cytoplasm also contained water-light vacuoles of various size, possibly originating from widened smooth and granular endoplasmic reticulum deprived of ribosomes.

Sometimes single disintegrating capillaries seen as capillary shadows (Fig. 4) could be found in the gyrus hippocampal cortex; this was, however, a rare phenomenon.

In the vicinity of markedly damaged capillaries, stimulated microglial cells were found (Fig. 4).

It should be emphasized that in this experimental group, considerable neuronal abnormalities were observed close to damaged BBB elements, mainly as aponecrotic changes in neuronal perikaryons. The changes usually affected pyramidal neurons and were manifested as dark neurons (Fig. 5).

Moreover, distinct alterations were observed in astroglia situated at a variable distance from markedly swollen capillaries (Fig. 5).

Damage to the gyrus hippocampal cortex neuropil elements was also observed (Figs. 1, 3A-B), especially...
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to axodendritic synaptic endings (Fig. 1), dendritic processes of pyramidal neurons and glial processes lying loosely in neuropil.

A careful ultrastructural analysis of pyramidal neurons, astroglial perikaryons and synaptic endings in the course of the current experiment is in preparation.

**FS + TPM group**

The ultrastructural picture of the BBB components of the hippocampal cortex in the rats which after experimentally induced febrile seizures received TPM had changes qualitatively similar to those observed in the FS group (Fig. 6). However, they were less common than after convulsions.

**TPM + FS group**

A beneficial effect of topiramate administration on the electron-microscopic picture of the BBB components was observed in more than half of the microcirculatory capillaries of the hippocampal cortex of the animals receiving TPM prior to FS. The BBB structure, especially the capillary endothelial lining, showed relatively slight abnormalities as compared to the control group or was occasionally normal; the lumen of such capillaries was wide (Figs. 7-9). Astrocytic processes around such vessels often did not show any significant lesions (Figs. 8, 9).

However, the remaining capillaries and/or the astrocytic processes enclosing the vessels showed moderate swelling. Submicroscopic abnormalities were seldom observed in the BBB components; they corresponded to advanced changes in the FS group described above.

Neuronal changes in the form of dark pyramidal neurons and damaged neuropil were much less common.

**Discussion**

Our earlier histological studies with morphometric analysis, performed on the ammonal cortex in

*Fig. 5. A considerably degenerated dark pyramidal neuron showing features of aponecrosis, with adhering swollen astrocytic cell. FS group. Original magn. × 4400*
experimental hyperthermia-induced convulsions, demonstrated variously pronounced neuronal changes as the major pathology, mainly manifested as sclerosis of pyramidal neurons (dark shrunken neurons), less commonly as a chronic disorder, with accompanying extensive neuronal loss. These abnormalities were located in 3 zones of the ammonal cortex: in the pyramidal layer of sectors CA1 and CA3 and in the hilar zone of the hippocampal gyrus [20,21].

Semiquantitative morphometric examinations revealed a loss of nearly 60% of neurons in the CA1 sector and death of approximately 50% in the CA3 sector [20].

It is interesting that in histological analysis in the same model of febrile seizures in rats receiving TPM, sclerotized neurons were much less common in the gyrus hippocampal cortex, both before and directly after FS; also the loss of neurons was considerably smaller (approximately 20% of pyramidal neurons of CA1 and CA3), which confirms the neuroprotective properties of the drug [20].

Literature data concerning studies on the blood-brain barrier in the experimental model of febrile seizures are scarce. The effect of topiramate on the morphological and functional status of the BBB in this model has not been elucidated either.

The current ultrastructural study of the BBB of the gyrus hippocampal cortex in the FS group, i.e. in rats with convulsions induced by water bath at a temperature of 45°C for 4 min, showed a number of significant microscopic changes in the barrier components. Severe abnormalities, mainly suggesting a considerable increase in BBB permeability, were observed in approximately 2/3 of the capillaries and astrocytic processes surrounding them.

The capillary patency was significantly delimited; frequently the lumen was almost completely occluded by damaged endothelial lining or by the

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**Fig. 6.** Fragment of a capillary lined with swollen endothelium containing numerous micropinocytic vesicles and markedly swollen mitochondrion (m). The perivascular astrocytic process (pAs) is considerably swollen. L – vascular lumen. FS + TPM group. Original magn. × 20 000.
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external pressure exerted by markedly swollen perivascular astrocytic processes, which caused ischaemia and oedema of the surrounding tissue. Mitochondrial changes were found to predominate in endothelial cell cytoplasmic organelles, indicating that febrile seizures are an effective inhibitor of oxidative phosphorylation.

The ultrastructural changes in the BBB in the ammonal cortex observed in the experimental model of febrile seizures and indicating markedly increased permeability of the barrier seem to correspond to the abnormalities in the cerebral cortex found in other experiments with hyperthermic stress by e.g. Dydyk and Pluta [3] in rabbits or Urakawa et al. [28] in rats. The changes were not specific and resembled those found in human psychomotor epilepsy [2,16], in many experimental models of cerebral ischaemia and/or anoxia [8,12,27], or in experimental valproate encephalopathy investigated in our previous studies [19,24-26].

The changes observed in the BBB components of the hippocampal cortex (“vasogenic factor”) correspond to dark neurons found in our present and earlier histological studies [20,21].

According to many authors, the morphogenesis of dark neurons (defined as ischaemic) situated in various regions of grey matter of the CNS, especially in the hippocampal cortex, is closely connected with ischaemic conditions of the nervous tissue [8,14,29].

Gadomski and Lasocki [5], explaining the morphogenesis of neuronal changes due to experimental hyperthermia, pointed out the important role of the vascular factor, apart from changes in enzymatic activity in the neuron, related to acid phosphatase and thiamine pyrophosphatase. They emphasized the significance of metabolic acceleration, increased oxygen consumption by overstimulated neurons and CNS ischaemia caused by cerebral vessel contraction induced by decreased molecular pressure of CO₂ in arterial blood.

Fig. 7. Fragment of a relatively well preserved capillary; wide vascular lumen (L). Tight intercellular junctions – visible. Axonal ending (a) adhering to the basement membrane of the vessel markedly swollen. TPM + FS group. Original magn. × 12 000
The current findings seem to indicate a beneficial effect of topiramate on the morphological status of the BBB. Prophylactic administration of the drug, i.e. prior to febrile seizures (TPM+FS group), prevented substantial damage to structural elements of the BBB of the ammonal cortex in over half of the capillaries. The protective effect of the drug was mainly observed in the endothelial lining, which suggests its high compensatory potential, and which may thus have practical clinical implications.

However, administration of the antiepileptic drug directly after the induction of febrile seizures (FS+TPM group) had no distinct beneficial effect on the BBB components.

There are numerous reports on the neuroprotective effect of TPM in various experimental models of CNS damage due to epilepsy, epileptic state, and brain ischaemia [4,9,11,14], but not to febrile seizures. The experimental model of hyperthermia-induced convulsions has been used only in our morphological research [20,21].

According to literature data, topiramate has several mechanisms of action that may contribute to its anticonvulsant and neuroprotective activity [22], including antagonistic effects on glutamine receptors of kainite/AMPA subtype, which play an essential role in excitotoxic neuronal damage [6,17,30]. Hippocampal neurons are particularly sensitive to a variety of excitatory amino acid-mediated cerebral damage. This is assumed to relate to prominent glutamatergic input and a high density of glutamate receptors.

Our submicroscopic study using TPM seems to indicate that the drug triggers mechanisms that strengthen the morphological BBB elements, due to which the barrier, especially the endothelial lining, becomes more resistant to hyperthermic stress-induced convulsions.
It can be assumed that in children with recurrent and prolonged FS, prophylactic administration of the drug would prevent BBB damage and hippocampal sclerosis, and may inhibit the development of symptomatic epilepsy. This however requires further research.

References


Fig. 9. Fragment of a well preserved capillary lined with a thin normal endothelial cell. Tight intercellular junction (J) – clearly visible. Neuropil elements adhering to the basement membrane with no significant changes. L – vascular lumen. TPM + FS group. Original magn. × 20 000


