

The neuroprotective effect of topiramate on the ultrastructure of pyramidal neurons of the hippocampal CA1 and CA3 sectors in an experimental model of febrile seizures in rats

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Abstract

The objective of the current ultrastructural study was to explore the potentiality of the neuroprotective effect of TPM against damage of pyramidal neurons in the hippocampal CA1 and CA3 sectors in an experimental model of febrile seizures (FS) in rats. The FS group exhibited variously pronounced submicroscopic lesions of the neuronal perikarya, including total cell disintegration. Advanced changes induced by hyperthermic stress were manifested by marked degenerative abnormalities, such as substantial swelling of the mitochondria, dilation, degranulation and disintegration of the granular endoplasmic reticulum, and vacuolar changes in the Golgi complex. The most substantially damaged pyramidal neurons showed features of aponecrosis (so-called "dark neurons"), resulting in a marked neuronal loss in the explored areas of the hippocampal cortex. The neurodegenerative changes were accompanied by distinct damage to the blood-brain barrier components. The administration of topiramate at a dose of 80/kg b.m. prior to the induction of hyperthermic stress (as prevention against febrile seizures) caused a substantial neuroprotective action – the drug efficiently lightened the neuronal damage, basically reduced cell aponecrosis and enhanced cell viability. However, TPM applied directly after FS induction did not exert any distinct neuroprotective effect on the perikarya of pyramidal neurons in the hippocampal cortex.

Key words: febrile seizures, hyperthermic stress, topiramate, hippocampal pyramidal neurons, aponecrosis, ultrastructure, rats.

Introduction

Febrile (fever-induced) seizures (FS) are still the most common problem in paediatric neurology. Brain damage caused by repeated febrile seizures during development is not only associated with an increased risk of epilepsy later in life, including temporal

lobe epilepsy [2,12,19,20], but according to some authors is also harmful to the intellectual development of a child [9,17]. Thus, it is very important to reduce the related seizure-induced damage.

The current study was inspired by our histological research associated with a semiquantitative mor-

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phometric analysis of the population of pyramidal neurons performed on the rat ammonal cortex in an experimental model on warm water induced febrile seizures and after application of a novel anti-epileptic drug – topiramate (TPM) [15]. Our observations revealed that FS caused advanced neurodegenerative changes in the pyramidal neurons of the hippocampal CA1 and CA3 sectors with over 50% neuronal loss. Topiramate, especially when administered before the seizures, considerably improved pyramidal cell survival in these sectors, reducing the loss of pyramidal neurons to 20%. A markedly lesser degree of the neuroprotective effect of this drug was observed when it was administered after the febrile seizures [15].

We were also encouraged to extend our neuropathological study with ultrastructural analysis by the latest report of Chinese authors Huang *et al.* [6] concerning *in vitro* investigations of the protective action of topiramate on primary dopaminergic neuronal cell cultures exposed to 6-OHDA toxicity. The authors showed that the neuroprotective effect of TPM in studied neuronal cell cultures involves cell apoptosis reduction and enhancement of cell viability [6].

The present study aimed at exploring the potentiality of the neuroprotective effect of TPM against ultrastructural damage of pyramidal neurons in the hippocampal CA1 and CA3 sectors caused by FS in rats. This is a continuation of our electron-microscopic research on the effect of topiramate on selected structural CNS components, namely the blood-brain barrier (BBB) [10] and astrocytes [11] in the cortex of the hippocampal gyrus and in the neocortex of the temporal lobe in an analogous experimental model of hyperthermic seizures.

The current study is the first to provide evidence for the effect of topiramate on the ultrastructure of CNS neurons in an experimental model of febrile seizures.

Material and methods

The study used 18 young male Wistar rats aged 22–30 days. Brain maturity in such animals corresponds to that of 1- or 2-year-old children [15]. The rats were divided into four groups – three experimental and one control (five rats in each experimental group and three in the control). The animals were pre-selected according to the standard pharmacological screening tests. All procedures were perfor-

med in strict accordance with Helsinki Convention Guidelines for the care and use of laboratory animals. The study was approved by the Ethical Committee of the Medical University of Białystok.

The FS group contained rats with induced febrile seizures. Hyperthermic stress was evoked by placing animals in a 45°C water bath. Water temperature was maintained at the same level. The rats were put into water for 4 minutes until convulsions appeared and then moved to a separate container lined with lignin. The animals, except for controls, were placed in the water bath for four consecutive days.

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

In the 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 animals were put into water.

Control animals and the FS group received only normal saline. The dose of topiramate was chosen according to literature references [1,14].

A detailed description of the methodology has been presented in our previous papers [10,15].

Preparation for electron microscopy

Seventy-two hours after the last convulsion episode, the rats were anaesthetized with Nembutal (25 mg/kg b.w., i.p.) and transcardially perfused with a fixative solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4. After removal of the brains, hippocampal and temporal lobe samples were taken and fixed in the same solution for 24 h at 20°C. Postfixation was completed with 1% osmium tetroxide (OsO₄). After dehydration in ethanol and propylene oxide, small specimens (1 mm³) of the gyrus hippocampal cortex (from the hippocampal CA1 and CA3 areas) were processed routinely for embedding in Epon 812. Semithin sections were stained with methylene blue and examined under a light microscope. Ultrathin sections were double stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (Opton EM 900, Zeiss, Oberkochen, Germany). The material obtained from the gyrus hippocampal cortex and neocortex of the temporal lobe

in the control group was processed using the same techniques as for the experimental groups.

Results

FS group

In the FS group, the electron-microscopic investigations revealed variously pronounced degenerative abnormalities in the perikarya of the hippocampal pyramidal neurons in the CA1 and CA3 sectors – from discrete to total cell disintegration. As the neuronal changes observed in the two sectors of the hippocampal cortex were qualitatively similar, they are described jointly.

Poor or moderate changes were characterized by the occurrence of densely aggregated narrow granular endoplasmic reticular (GER) channels within the cytoplasm of the neurons and slight or moderate mitochondrial swelling (Fig. 1). GER channels were sometimes dilated and showed features of segmental degranulation (Fig. 2). The changes were usually accompanied by dilatation of the Golgi complex (Fig. 2). The cytoplasm of such neurons varied in electron density – from decreased, through normal, to increased (Figs. 1, 2).

Advanced neuronal lesions were seen as a distinctly increased electron density of the cytoplasm and its disintegration. The neuronal perikarya contained large cytoplasmic fragments filled up with elements from a disintegrating GER, i.e. numerous ribosomes and polyribosomes, homogeneous microgranular material of increased electron density, usually close to the cell nucleus and then in more distant parts of the cytoplasm (Figs. 3, 4). In the vicinity, fragments of the cytoplasm were seen, containing distinctly swollen mitochondria – with a substantially increased volume, electron-translucent matrix and residual cristae at the periphery (Figs. 4, 5). The outer mitochondrial membrane was sometimes damaged and organelles underwent disintegration. In some of the degenerated neurons, in the vicinity of markedly swollen mitochondria there were also mitochondria showing a condensed configuration (Fig. 4). All the above changes were frequently accompanied by the dilatation of channels and cisterns of the Golgi complex, i.e. vacuolar lesions within the complex (Fig. 3), and by the appearance of dense bodies (Figs. 3-5). Occasionally, markedly swollen protoplasmic astrocytes were found to adhere to the degenerated neurons (Fig. 3), whose

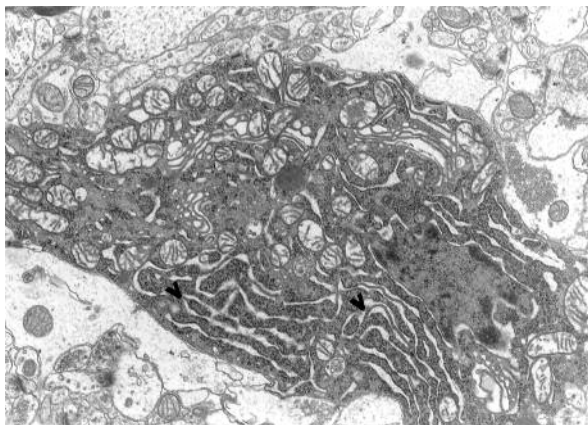


Fig. 1. A damaged, slightly shrunken pyramidal neuron of the hippocampal cortex – numerous densely located GER channels (>) visible in relatively dark cytoplasm; numerous mitochondria with features of poor and moderate swelling. The cells are surrounded by distinctly swollen neuropil elements. FS group. Original magn. $\times 7000$.

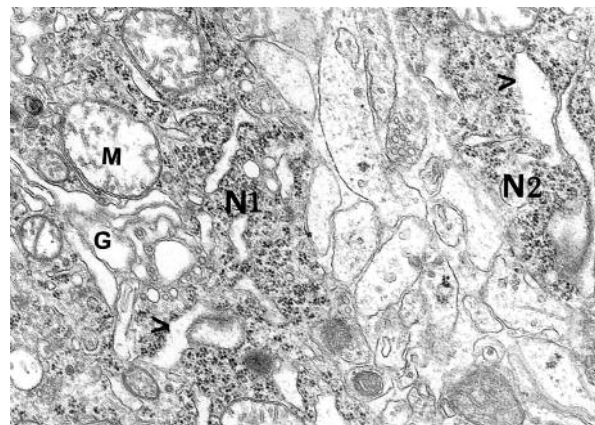


Fig. 2. Fragments of two degenerating perikarya of pyramidal neurons (N1, N2). Relatively dark cytoplasm shows chaotically arranged, shortened, dilated GER channels with features of segmental degranulation (>); moderately swollen mitochondria; dilated Golgi complex (G); focally increased number of free ribosomes. Neuropil elements adhering to the perikarya show features of swelling. FS group. Original magn. $\times 12\,000$.

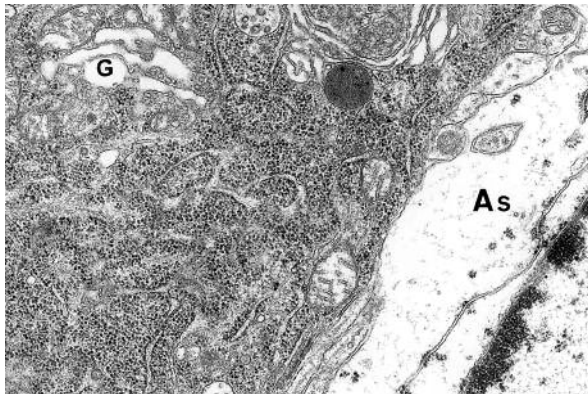


Fig. 3. Fragment of a perikaryon with a considerably increased electron density of the cytoplasm filled with a dark homogeneous microgranular material, probably from disintegrated free ribosomes. Distinctly dilated channels and cisterns of the Golgi complex (G) and dense body are visible. A markedly swollen protoplasmic astrocyte (As) adheres to the neuron. FS group. Original magn. $\times 12\ 000$.

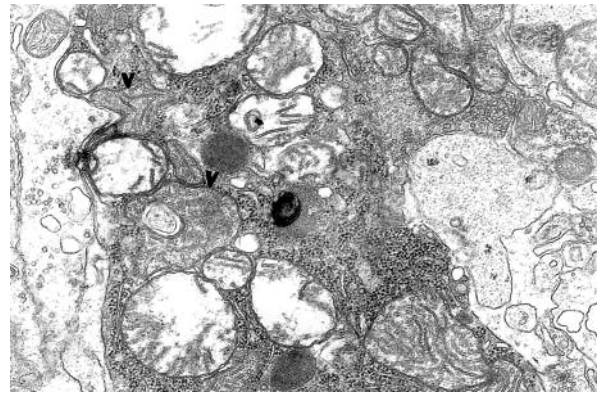


Fig. 4. Fragment of a distinctly shrunken dark perikaryon of the pyramidal neuron with mitochondria that are markedly swollen and showing features of condensation (>); the cytoplasm is filled with a very dark homogeneous microgranular material; dense bodies are visible. The surrounding neuropil elements are distinctly changed. FS group. Original magn. $\times 12\ 000$.

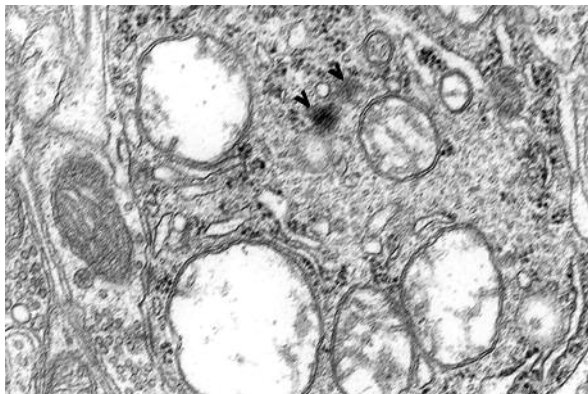


Fig. 5. Fragment of a damaged perikaryon of the pyramidal neuron shows markedly swollen mitochondria – with electron-translucent matrix and residual cristae at the periphery. In the vicinity, fine forming dense bodies (>). FS group. Original magn. $\times 20\ 000$.

ultrastructure has been presented in our previous paper [11].

The most substantially damaged pyramidal neurons in rats exposed to hyperthermic stress showed distinct features of aponecrosis. The aponecrotic cells were usually shrunken, very dark, nearly black (so-called “dark neurons”), with the cytoplasm showing features of marked disintegration (Figs. 6, 7).

In the vicinity of dark disintegrating perikarya, separated fragments of cell bodies, i.e. apoptotic bodies, were found (Fig. 6).

The current ultrastructural study, like all the previous histological observations [15], demonstrated marked neuronal loss. However, pyramidal neurons showing relatively well preserved ultrastructure in the close vicinity of dark neurons were sometimes visible.

The neuronal changes described above were accompanied by substantial damage to the hippocampal cortex neuropil elements that surrounded the perikarya of the pyramidal neurons (Figs. 1, 2, 4, 6, 7), especially to axodendritic synaptic endings. We could also see pronounced damage to the structural components of the blood-brain barrier of this CNS structure, which has been reported previously [10].

FS + TPM group

The ultrastructure of the pyramidal neurons in the CA1 and CA3 sectors of the hippocampal cortex in the animals which after experimentally induced febrile seizures received topiramate was qualitatively similar to that observed in the previous study group (Fig. 8). However, advanced perikaryal lesions, including aponecrosis (so-called “dark neurons”) were relatively less common.

TPM + FS group

In rats given TPM prior to febrile seizures, neuronal degeneration in the hippocampal CA1 and CA3 areas was less severe, affecting a lot fewer cells as compared to the FS group. Pyramidal neurons with features of aponecrosis were rare. The antiepileptic drug had a distinct beneficial neuroprotective effect, efficiently lightening the neuronal damage, reducing cell aponecrosis and enhancing cell viability. Neuronal loss in the areas of the hippocampal cortex studied was far less frequent than in the FS group.

The perikarya were quite frequently seen to have only slight submicroscopic changes and did not differ much as compared to the control group (Fig. 9).

In this group, we also observed a distinct beneficial effect of topiramate on the structural BBB components, which has been reported previously [10].

Discussion

The ultrastructure of the hippocampal pyramidal neurons in CA1 and CA3 subfields observed in the current study in warm water induced rat FS (i.e. in

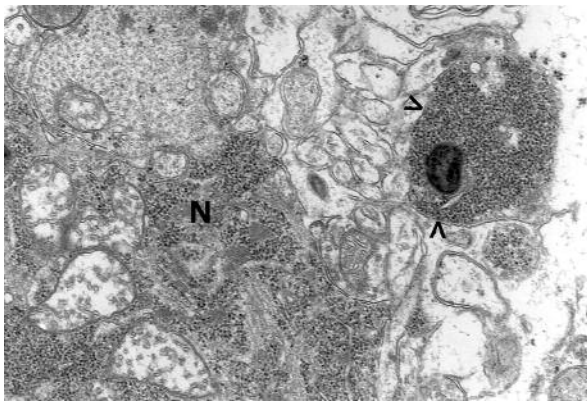


Fig. 6. A very dark, almost black apoptotic body (>), separated from an aponecrotically changed neuronal perikaryon (N). The apoptotic body is surrounded by substantially swollen and disintegrated neuropil elements. FS group. Original magn. $\times 12\ 000$.

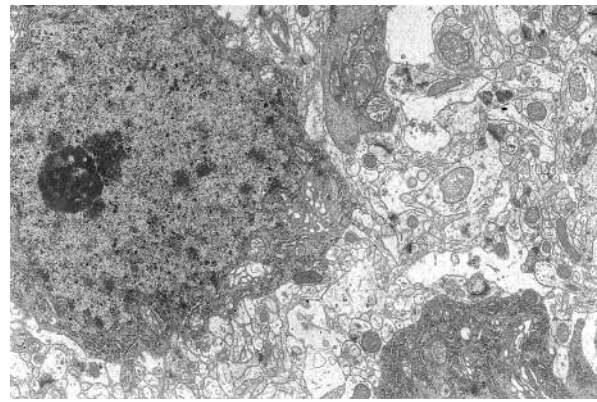


Fig. 7. A dark, aponecrotically changed pyramidal neuron; in its vicinity, a fragment of a similar neuron. The surrounding neuropil elements are markedly swollen in places. FS group. Original magn. $\times 4400$.

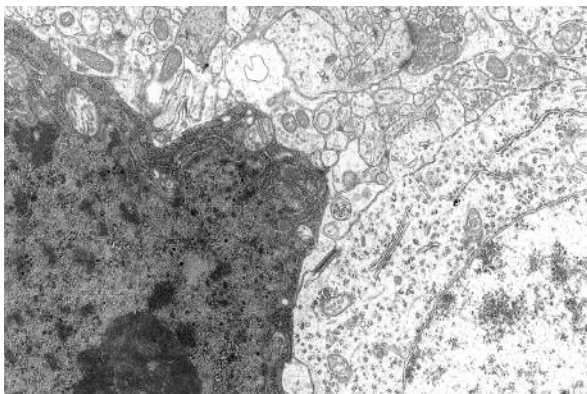


Fig. 8. Two large fragments of the perikarya of the pyramidal neuron of the hippocampal cortex – one shows features of aponecrosis ("dark neuron"), the ultrastructure of the other is almost unchanged. FS + TPM group. Original magn. $\times 4400$.

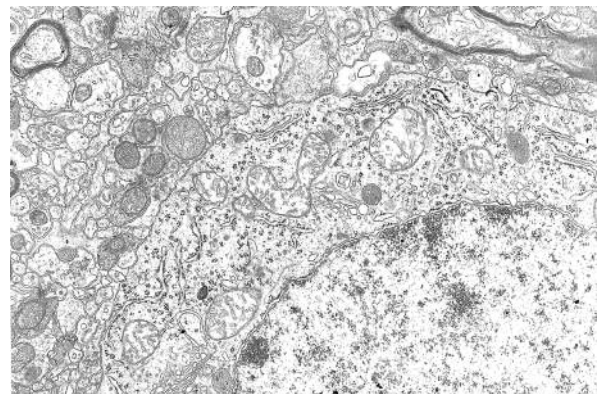


Fig. 9. A large fragment of a relatively well-preserved perikaryon of the pyramidal neuron. Some cellular mitochondria are slightly swollen. TPM + FS group. Original magn. $\times 7000$.

the FS group) varied greatly, ranging from discrete lesions to total cell disintegration. In the first place, the abnormalities included mitochondrial swelling, dilated GER and Golgi complex and ribosome disaggregation from endoplasmic reticula. Pyramidal neurons frequently exhibited severe structural defects, with features of aponecrosis manifested as dark, shrunken, ischaemic, disintegrating cells (“dark neurons”), with accompanying extensive neuronal loss. The ultrastructure of the pyramidal neurons was similar to that described by other authors in various models of experimental hyperthermic stress [3,5,16,17,21,24-26]. The pattern of the changes indicates substantial metabolic disorders in the neuron, mainly affecting the oxidation-reduction transformations and protein synthesis, and leading to its death. We believe that the appearance of the so-called dark aponecrotic neurons, especially in the FS group in the CA1 and CA3 hippocampal sectors, was caused by extensive ischaemia and oedema of the nervous tissue, which was confirmed by our earlier ultrastructural study concerning the blood-brain barrier components of the gyrus hippocampal cortex in the same experimental hyperthermia-induced convulsions [10]. Then we observed distinct damage to BBB components manifested by significantly delimited capillary patency, the lumen almost completely occluded by injured endothelial lining and a substantial increase in BBB permeability [10]. The significant role of the vascular factor in the generation of neurodegenerative lesions defined as ischaemic has been confirmed by studies of hyperthermic stress conducted by other authors [13,18,22]. According to Sharma *et al.* [18], heat stress is instrumental in opening of the BBB, either directly or indirectly leading to vasogenic oedema formation, a feature crucial in molecular and cellular changes in the brain inducing cell and tissue damage. The authors suggest that hyperthermia inducing BBB damage is the major cause of neurodegenerative changes [18].

The current submicroscopic investigations indicate a neuroprotective effect of topiramate in the experimental model of FS. This refers particularly to the TPM + FS group, where this antiepileptic was administered prior to febrile seizure induction – as prevention against these seizures. However, it had a far less beneficial effect in the group in which the drug was given after experimentally induced febrile seizures. In the TPM + FS group, as compared to the non-treated FS group, we found efficiently lightened

neuronal damage and basically reduced cell aponecrosis in the CA1 and CA3 subfields of the hippocampus. The number of pyramidal neurons with swollen mitochondria and lesions in the granular endoplasmic reticulum and Golgi complex was much lower than in the FS group. Aponecrotic neurons manifesting as dark neurons were rarely observed.

The current study is the first to document the effect of topiramate on the ultrastructure of CNS neurons in an experimental model of febrile seizures. A similarly distinct beneficial effect of this drug on neurons has also been observed in other experimental models of CNS damage, e.g. in the pilocarpine rat model of chronic epilepsy [7,8], and in an *in vitro* study of primary dopaminergic neuronal cell cultures exposed to 6-OHDA toxicity [6].

It should be added that contrary to other antiepileptic drugs, such as phenytoin, valproate and phenobarbital, therapeutic doses of topiramate do not exhibit neurotoxic properties in the developing rat brain [4].

Kudin *et al.* [7,8] have provided interesting data that may help elucidate the mechanisms of the neuroprotective action of TPM, with a focus on the role of mitochondria.

The authors, studying the effect of topiramate on the function of mitochondria in a pilocarpine experimental model of chronic epilepsy and *in vitro* investigations on isolated rat brain mitochondria, found that mitochondrial function is a key determinant of both excitability and viability of hippocampal pyramidal neurons [7,8]. They demonstrated seizure-dependent changes in mitochondrial oxidative phosphorylation in the epileptic rat hippocampus and suggested that seizure activity inhibits the expression of mitochondrial-encoded enzymes of oxidative phosphorylation. This could be induced during diverse forms of pathological neuronal activity and may severely impair viability of hippocampal pyramidal neurons [7]. The authors report that TPM treatment increases the activity of mitochondrial respiratory chain complex I in the CA1 and CA3 pyramidal subfields and causes a decrease in seizure frequency in chronic epileptic rats. According to them, the neuroprotective action of topiramate appears to be directly related to its inhibitory effect on the mitochondrial permeability transition pore [8].

Our ultrastructural observations of the beneficial effect of TPM on the hippocampal pyramidal neurons in an experimental model of FS, together with

our earlier morphometric analysis of this cell population [15], may in future serve as comparative material for similar investigations.

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