Morphometric analysis of the cerebellar cortex capillaries in the course of experimental valproate encephalopathy and after chronic exposure to sodium valproate using transmission electron microscopy

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Morphometric analysis of the cerebellar cortex capillary cross-section area performed in experimental valproate encephalopathy using transmission electron microscopy showed that prolongation of VPA application resulted in more enhanced lumen narrowing manifested in gradual reduction in the mean value of the coefficient examined. After 6, 9 and 12 months of experiment this value was statistically different from that obtained in control subgroups, being respectively lower by approximately 22%, 48% and 65%. One month after terminating of chronic administration this value was close to the one found after 12 months of the study. Three months after the drug withdrawal the coefficient was higher by approximately 44% compared to the one after 12 months, which seemed to indicate an increase in capillary lumen patency. The morphometric analysis of the cerebellar cortex capillary cross-section area performed in the present study objectifies the results of qualitative ultrastructural investigations concerning the microcirculation of this CNS structure.

key words: morphometric analysis, capillaries, cerebellar cortex, valproate, ultrastructure

INTRODUCTION

Sodium valproate (VPA) — the sodium salt of valproic acid is one of the most commonly used first-line, wide-spectrum antiepileptic drugs, acting on the GABA-ergic system, especially effectively in the therapy of primary generalised seizures and myoclonic seizures [2, 5, 7, 19, 20]. However, long-term application of valproate, despite its therapeutic serum concentration, may induce undesired toxic symptoms from the CNS, mainly from the cerebellum and extrapyramidal system, defined as “valproate encephalopathy” [1, 3, 4, 6, 14].

Up to the present, our own electron-microscopic studies of chosen CNS structures (e.g. cerebellar cortex, cerebellar dentate nucleus, cortex of hippocampal gyrus, temporal lobe neocortex) in experimental valproate encephalopathy have indicated that chronic application of VPA leads to significant damage to morphological elements of the blood-brain barrier, thus causing a pronounced narrowing of the capillary lumen, including obliteration and subsequent reduction in tissue blood supply [8, 9, 11, 15, 18]. It also causes marked neuronal and neuroglial changes, especially concerning Purkinje cells and Bergmann’s astrocytes [11, 13, 16, 17].
The aim of the research was a morphometric analysis of the cerebellar cortex capillaries in the course of valproate encephalopathy and after VPA withdrawal to objectify the ultrastructural picture of this microcirculation.

MATERIAL AND METHODS

The experiment was conducted on 3 groups of three-month-old male Wistar rats of initial body mass 160–180 g: group I (30 animals) receiving VPA (Vupral, Polfa) once a day with an intragastric tube in a dose of 200 mg/kg b.w. for 1, 3, 6, 9 and 12 months; group II (12 animals), in which the 12-month-exposure to VPA was followed by the drug’s withdrawal for 1 and 3 months; group III — control animals (28 rats) matched in respect to age with experimental animals, receiving physiologically saline in the same way as the group I rats treated with VPA.

Serum concentrations of VPA in group I were measured by gas chromatography and ranged between 60 and 135 μg/ml (mean 111.333 μg/ml; SD 21.6131) [12, 13].

At the end of the experiment half of the animals were sacrificed under Nembutal anaesthesia by intravital intracardiac perfusion with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at constant pressure of 80 mmHg. In order to visualise the content of the blood vessels, the remaining rats were sacrificed by fast decapitation. The material was taken from the cerebellar cortex of hemispheres and vermis, routinely processed for ultrastructural examinations and analysed using an Opton 900 electron microscope (details in the previous papers [13, 15]).

Quantitative analysis of capillaries was performed using an automatic picture analyser “Imager-512” (IMAL) conjugated with an electron microscope (Opton 900 PC). Final magnification of the picture obtained on a black and white monitor (resolving power $512 \times 512$ pixel) was approximately $12,000$. Coefficient values ($S$) expressing the ratio of the capillary lumen cross-section area ($a$) to the capillary cross-section area ($A$) were analysed ($S = a/A$). Depending on the distribution of the analysed feature, the results were subjected to statistical analysis using Student-t test or Wilcoxon’s test for unrelated samples. The critical value was at $p < 0.05$ [11].

RESULTS

The results of the morphometric analysis of the cerebellar cortex capillaries in experimental and control animals are presented in Tables 1–3.

The mean values of the $a/A$ coefficient were as follows: after 3 months of the experiment $S_3 = 0.533$ (SD-0.03) (in control subgroup $S_{k3} = 0.587$ (SD-0.027)); after 6 months $S_6 = 0.462$ (SD-0.061) ($S_{k6} = 0.586$ (SD-0.036)); after 9 months $S_9 = 0.307$ (SD-0.084) ($S_{k9} = 0.585$ (SD-0.04)); after 12 months $S_{12} = 0.209$ (SD-0.101) ($S_{k12} = 0.583$ (SD-0.038)).

One month after termination of the 12-month-administration of VPA the mean $a/A$ ratio was $S_{12+1} = 0.218$ (SD-0.078) ($S_{k12+1} = 0.582$ (SD-0.037)), while after 3 months it was $S_{12+3} = 0.372$ (SD-0.114) ($S_{k12+3} = 0.580$ (SD-0.032)).

Table 1. The capillary lumen cross-section area ($a$) of the cerebellar cortex in rats: of control group, in group with VPA (Group I) and in group after terminating of VPA application (Group II)

<table>
<thead>
<tr>
<th>Exp. group</th>
<th>Period of exp. (months)</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Med.</th>
<th>Min</th>
<th>Max</th>
<th>SE</th>
<th>Var. coeff.</th>
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<td></td>
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<tr>
<td>Control group</td>
<td>3</td>
<td>4</td>
<td>15.312</td>
<td>3.23988</td>
<td>15.325</td>
<td>11.87</td>
<td>18.73</td>
<td>1.6199</td>
<td>21.158</td>
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<td>4</td>
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<td>13.32</td>
<td>19.15</td>
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<td>17.60</td>
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<td>2.89821</td>
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DISCUSSION

Electron microscopic exponents of the prolonged influence of VPA upon the blood-brain barrier of the cerebellar cortex and after termination of chronic application of this antiepileptic drug in the same experimental model were presented in our earlier publications [8–11, 15].

The first very slight ultrastructural lesions of capillaries in the cerebellar cortex were observed after 3 months of the experiment. They were more pronounced in later stages of the study and after 9 and 12 months of VPA application signs of severe damage were observed. The most prominent morphological lesions in the capillary wall included considerable swelling of endothelial cells, leading to reduced size of vessel lumen, with lumen occlusion, damage to endothelial mitochondria (distinct swelling; degeneration of matrix and cristae) and GERL.
system (Golgi-endoplasmic reticulum-lysosome) in particular, injury to tight intercellular junctions; changes in the basement lamina. Damage to the vessel wall was accompanied by marked changes in glial cells, mainly perivascular processes of astrocytes [8, 9, 11, 15]. One month after the end of chronic VPA administration the ultrastructure of capillaries and astrocytic processes surrounding the capillaries did not differ significantly from the one observed directly after 12 months of study. Three months after the end of the treatment with valproate, the wall of many capillaries, in the molecular layer as well in the granular layer of the cerebellar cortex, showed features of minor damage, which indicated the process of repair [8, 10, 11].

Morphometric analysis of the cerebellar cortex capillary cross-section area showed that the prolongation of VPA intensification resulted in more enhanced lumen narrowing manifested in a gradual reduction in the mean value of the coefficient examined. After 6, 9 and 12 months of experiment this value was statistically significantly different from that obtained in control subgroups, being respectively lower by approximately 22%, 44% and 65%.

One month after terminating of chronic VPA administration the value was close to the one found after 12 months of the experiment. Three months after the drug withdrawal the coefficient was higher by approximately 44% compared to $S_{12}$, which seemed to indicate an increase in capillary lumen patency and the improvement of the morphological condition of the blood-brain barrier [11].

CONCLUSION

The morphometric analysis of the cerebellar cortex capillary cross-section area performed in the present study objectifies the results of qualitative ultrastructural investigations concerning the microcirculation of this CNS structure.

REFERENCES