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Ultrastructural study of cerebellar dentate nucleus astrocytes in chronic experimental model with valproate

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Abstract

The current study focuses on the morphogenesis of changes in the cerebellum dentate nucleus in the course of experimental valproate encephalopathy. Valproate – a broad spectrum antiepileptic and antipsychotic drug – chronically used in rats, intragastrically, once daily at a dose of 200 mg/kg b. w. for 1, 3, 6, 9 and 12 months, induced pronounced ultrastructural changes in the population of glial cells and nerve cells of the dentate nucleus of the cerebellum in the last two phases of the experiment. Astrocytic and neuronal lesions coexisted with a considerable damage to the elements of the blood-brain barrier of the cerebellar structure examined. The changes affected mainly the population of protoplasmic astrocytes lying loosely in a neuropile as well as astrocytes adhering to damaged large multipolar neurons. Focal proliferation of astrocytes was observed. Abnormal astrocytes showed marked swelling expressed by significantly decreased electron density of the cytoplasm that contained almost empty vacuolar structures and by a considerably reduced number of intracellular organelles. It was accompanied by dilation of endoplasmic reticular channels, loss of fibrillopoietic capacity of the cell and features of autophagocytosis. It should be assumed that the essential cause of protoplasmic astroglial damage of the cerebellar dentate nucleus could be associated, apart from the direct effect of valproate and/or its metabolites on these cells, with changes in structural elements of the blood-brain barrier of this CNS region.

Key words: *astrocytes, dentate nucleus of cerebellum, ultrastructure, valproate, rats*

Introduction

Valproate (VPA; valproic acid derivative) is commonly used as the major broad spectrum antiepileptic drug (AED) showing a beneficial anticonvulsant activity against different seizure types – both generalized and partial seizures in adult patients and children. It is also widely used, particularly in the last decade, as a mood stabilizer in bipolar illness – in maniac-depressive patients, in schizoaffective disorders, for therapy of neuropathic pain and for prophylactic treatment of migraine [1,8,9,27].

It should be emphasized here that chronic application of valproate, despite its therapeutic serum concentrations, may include undesired symptoms from the central nervous system (CNS) the so called valproate encephalopathy (v.e.). The essence of this encephalopathy are functional and organic CNS disorders, mainly from the cerebellum

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(ataxia, nystagmus, dysarthria, vertigo), and from the extrapyramidal system [2,3,6,13,15,16,28]. However, the mechanisms of unfavorable action of prolonged valproate administration at therapeutic doses on the CNS are difficult to elucidate.

Despite numerous neurological, neuropharmacological and neurochemical studies, neuropathological observations concerning the effect of long-term valproate administration on the CNS – i.e. morphogenesis of valproate encephalopathy, except for the research performed in our Department, still remain scarce.

Our previous histological studies on the cerebellum and brain stem in rats have shown significant pathological changes in the cerebellar cortex, dentate nucleus and fastigial nucleus of the cerebellum, as well as in the ventral cochlear nucleus and gigantocellular nucleus of the brain stem [18]. Examinations of the cerebellar cortex, including microscopic analysis of the structural elements of the blood-brain barrier, Purkinje cells, synaptic junctions and Bergmann's astroglia were additionally performed basing on the electron transmission microscopy [20,21,23-25].

The aim of the present study was to supplement the existing histological studies on the dentate nucleus of the cerebellum with ultrastructural assessment of this CNS region, which may throw additional light on v.e. morphogenesis.

Material and methods

The experiment used 2 groups of three-month-old male Wistar rats of initial body mass 160-180 g, preselected according to standard pharmacological screening tests. The animals were kept in a well sunlit room at 18-20°C and fed standard granulated rat chow and tap water. All procedures were carried out in strict accordance with the Helsinki Convention guidelines for the care and use of laboratory animals.

Group I consisted of 30 rats receiving sodium valproate (Vupral, Polfa) once a day in fasting state with an intragastric tube, at the effective dose of 200 mg/kg b.w. for 1, 3, 6, 9 and 12 months (six animals in each time subgroup).

Group II contained 10 control animals matched in respect to age with experimental animals, receiving physiological saline in the same way as the group I rats treated with VPA.

The rats were weighed every two weeks to verify the amount of the antiepileptic.

Serum concentrations of VPA in group I were measured by gas chromatography and ranged between 60 and 135 µg /ml (mean 111.333 ug/ml; SD 21.6131) [20].

The rats of both groups were subjected to behavioral examinations using Lat's test to evaluate the psychomotor and cognitive activity of the animals. Since the sixth month of valproate administration half of the animals demonstrated cerebellar disorders manifested in variously expressed signs of ataxia. 16.7% of rats exhibited severe ataxia, mainly between month 9 and 12 of VPA application [23].

At the end of the experiment, 24h after the termination of the final VPA administration, half of the animals were sacrificed under Nembutal anesthesia (using a dose of 25 m/kg of body weight) by intravital intracardiac perfusion with fixative solution (2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, temp. 20°C), at constant pressure of 80 mmHg. In order to visualize the contents of the blood vessels, the remaining rats were sacrificed by fast decapitation.

After intravital perfusion or fast decapitation of the animals, small tissue blocks (1 mm^3 volume) were taken from the cerebellar dentate nucleus (using a magnifying glass), fixed in 3.6% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2.5 h and washed in the same buffer for 18 h. They were then postfixed in 2% osmium tetroxide in 0.1 M cocodylate buffer (pH 7.4) for 1 h. Subsequently, the material, after dehydration in ethanol and propylene oxide, was embedded in Epon 812. Semithin sections were stained with toluidine blue and examined in the light microscope. Ultrathin sections were double stained with uranyl acetate and lead citrate, and examined using an Opton 900 PC transmission electron microscope (Zeiss, Oberkochen, Germany).

The material obtained from the cerebellar dentate nucleus in the control group was processed using the same techniques as for the valproate-treated animals.

Results

The first ultrastructural changes (slight or moderate swelling) observed in the cerebellar dentate nucleus in the course of the experiment referred to the population of astroglial cells and occurred after 6 months of valproate administration. After 9 and 12 months of the experiment, degenerative changes in these cells were intensified.

Mainly protoplasmic astrocytes, both their perikarya and processes, and less frequently fibrous astrocytes, were affected. Focal proliferation of

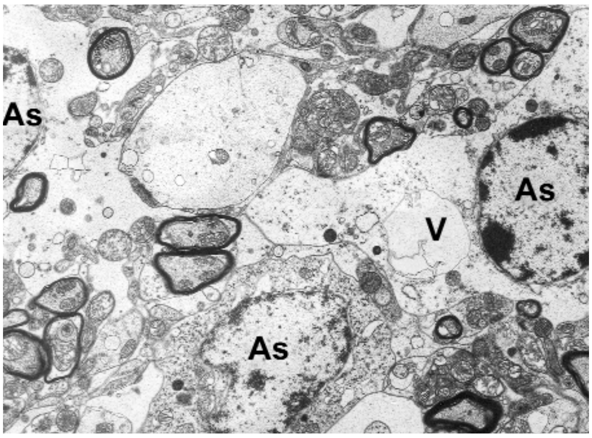

Fig. 1. The picture of swollen protoplasmic astrocytes (As) with residual intracellular organelles; v – a round vacuolar structure within almost electron-translucent cytoplasm of one of them; below quite well preserved protoplasmic astrocytes. Original magn. x 3000

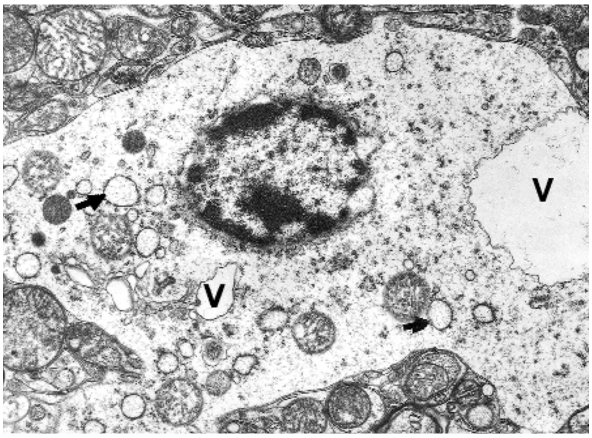

Fig. 2. Markedly swollen protoplasmic astrocyte, enclosed by neuropile elements and containing cistern-like, almost empty vacuolar structures (v) and numerous markedly dilated granular endoplasmic reticular channels (->). The cytoplasm of astrocyte shows significantly decreased electron density. Intracellular organelles accumulated in the perinuclear area. Original magn. x 7000

astrocytes was sometimes observed (Fig. 1). Swelling was the predominant feature. Markedly swollen astroglial cells were often surrounded by the elements of loosened neuropile (Figs. 1-3). The cytoplasm of such astrocytes showed considerably reduced electron density and contained vacuolar, oval or round spaces enclosed by elements of the smooth endoplasmic reticulum, which were almost empty or with delicate microfibrillary contents (Figs 1-3). Granular endoplasmic reticulum channels were dilated (Fig. 2). Some areas of the cytoplasm contained only few, often residual cellular organelles (Figs. 1, 3) or were completely empty. Abnormal organelles were relatively frequently grouped in the perinuclear area (Figs 2).

The cellular nuclei were enlarged and had a small amount of heterochromatin irregularly distributed under the nuclear capsule.

Altered astrocytes contained a markedly reduced, often residual, number of gliofilaments, suggesting fibrillopoietic failure. Abnormal, irregularly distributed and often disintegrating bundles of gliofilaments were sometimes observed in the perinuclear area (Fig. 3).

Quite frequently astrocytes showed morphological features of autophagocytosis, which was manifested in the presence of electron dense phagocytic vacuoles and loosely lying lipid drops in the cytoplasm (Figs. 3, 4).

Markedly swollen processes of the cells frequently adhered to the perikarya of large multipolar neurons, also showing degenerative changes that varied in intensity (Figs 4, 6).

Neuronal changes were usually expressed by marked swelling of mitochondria accompanied by dilation and segmental degranulation of granular endoplasmic reticulum as well as dilation of channels and cisterns of Golgi apparatus (Figs 4-6). Such neurons sometimes had an increased amount of lipofuscin granules (Fig. 5).

In the vicinity of the altered neurons, damage to the blood-brain barrier was observed resulting in a markedly reduced vascular lumen (Fig. 5) and nervous tissue ischemia. 'Dark ischemic neurons' characterized by condensed cytoplasm filled with damaged, disintegrated organelles and deposits of lipofuscin were quite frequently observed. Completely disintegrating large multipolar neurons and activated microglial cells in their vicinity were also found.

Discussion

Most pronounced morphological changes in the astroglia of the cerebellar dentate nucleus in the course of chronic administration of valproate – a broad spectrum antiepileptic and antipsychotic drug – were observed after 9 and 12 months of the experiment. They mainly referred to the population of protoplasmic astrocytes lying loosely in neuropile

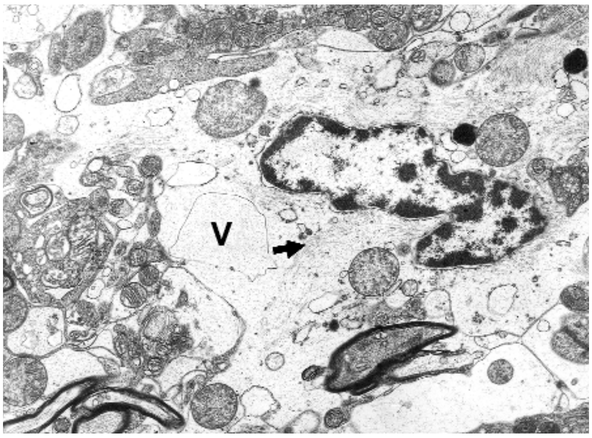

Fig. 3. The picture of markedly swollen fibrous astrocyte enclosed by neuropile elements and containing few dispersed organelles, almost empty vacuolar structure (v), loosely lying lipid droplets, gliofilaments (->) mainly in the perinuclear area; irregularly shaped cellular nucleus characteristic of fibrous astrocyte. Original magn. x 4400

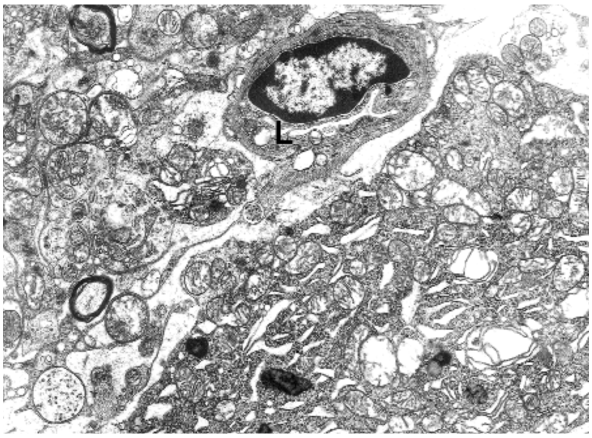

Fig. 5. Fragment of the large multipolar cell containing swollen mitochondria and Golgi apparatus, markedly dilated granular endoplasmic reticulum and dispersed lipofuscin granules. Above, in close vicinity of the neuron – a capillary with almost obliterated lumen (L) and alterations in endothelial mitochondria. Original magn. x 4400

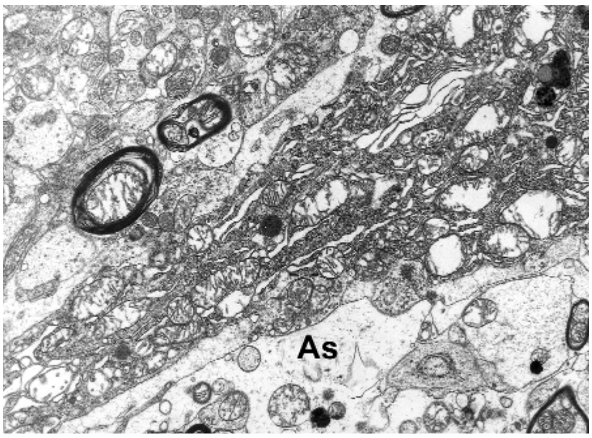

Fig. 4. Fragment of markedly swollen astrocyte (As) showing phagocytic properties adheres to altered neuron; some neuronal mitochondria markedly swollen. Original magn. x 4400

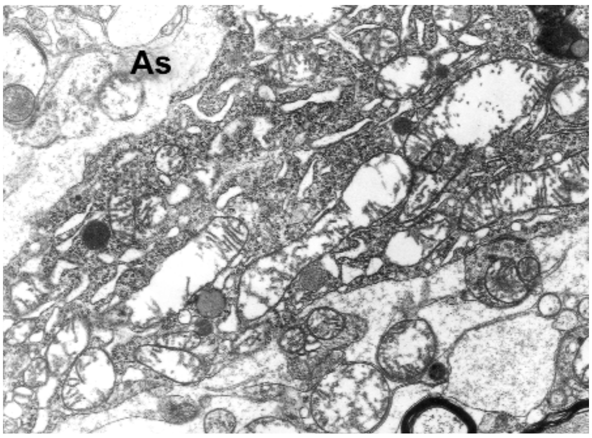

Fig. 6. Fragment of the neuron containing markedly swollen mitochondria and enclosed by swollen astrocytic processes (As). Original magn. x 7000

as well as those adhering to the damaged large multipolar neurons. Damage to fibrous astrocytes was less frequent. Focal proliferation of astrocytic cells was sometimes seen. Astrocytic and neuronal changes were accompanied by a significant damage to the structural elements of the blood-brain barrier of this CNS region. Abnormal astrocytes exhibited considerable swelling, which was manifested in markedly decreased electron density of the cytoplasm containing almost translucent vacuolar structures and in a reduced number of intracellular organelles. Dilation of granular endoplamic reticulum channels, loss of fibrillopoietic ability of the cell and features of autophagocytosis were also observed. The submicroscopic changes in astroglia and capillaries of the cerebellar dentate nucleus found in the present study corresponded to those observed in earlier preliminary studies [19].

It is worthy of noting that we have previously observed morphological changes, similar in quantity but more intensified in respect of phagocytosis, in protoplasmic astrocytes in the same experimental model with VPA in the cerebellar cortex [20,25], and in other CNS structures – i.e. in the cortex of the hippocampal gyrus and in the neocortex of the temporal lobe [22]. Moreover, it should be noticed that the cerebellar cortex astrocytes, i.e. Bergmann's astroglia, which filled up the losses after disintegrated Purkinje cells, were subjected to greater proliferation than the astroglia in the dentate nucleus and in other CNS structures we examined [20,25].

It should be mentioned that the changes found in the present study in the population of astrocytes, like in the case of the hippocampal cortex and neocortex [22], were nonspecific. Similar, though varying in intensity astrocytic abnormalities have been noted in various CNS regions in other experimental models, e.g. in the ischemic model [10,29].

According to some authors, chronic therapy of various AEDs can cause functional disorders and organic lesions in some anatomical structures of the CNS, especially within the cerebellum and the hippocampal gyrus, already affected by epilepsy [16,17,26]. Predilective susceptibility of the cerebellum to the toxic effects of long-term AED therapy may be explained by significantly reduced cerebellum/global cerebral metabolism of glucose [17].

It is believed that the population of astrocytes is the primary site of ammonia detoxification in the brain, is directly involved in the metabolism of glutamate-glutamine and GABA and causes capture of glutamate excess from the synaptic cleft [7,11,12]. It should be considered that all morphological changes found in astrocytic cells in the present study indicate attenuation or loss of their ammonia detoxification properties. This may lead to an increase in glutamine concentration in the extracellular space of the CNS.

Similar findings, though based on *in vitro* observations, have been reported by Collins et al. [4], who evaluated the effects of valproate on glutamate and glutamine metabolism in primary cultures of rat brain astrocytes.

The results of our *in vivo* ultrastructural study using the experimental model of valproate encephalopathy are in line with the findings presented by Fennrich et al. [5] and Nilsson et al. [11] based, like those of Collins et al. [4, on observations of *in vitro* VPA actions in organotypic cultures of rat hippocampus. It should be noted that it was Fennrich et al. [5], analyzing potential neurotoxic effects of therapeutically administered valproate, who found that even low valproate doses damaged the population of astrocytes. We think that it is a very valuable finding, demanding great caution in chronic valproate therapy, which however needs to be confirmed in other research centers.

In our opinion, morphogenesis of the changes observed in the population of astrocytes of the cerebellar dentate nucleus observed in the current study could be associated, like in the case of the hippocampal cortex [22], apart from the direct effect of VPA and/or its metabolites on these cells, with damage to the blood-brain barrier of the nucleus, i.e. – vasogenic factor – leading to ischemia of this CNS region. A simultaneous morphological response from damaged large multipolar neurons of the dentate nucleus may also be very important.

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