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Ultrastructural alterations of the rabbit sciatic nerve, spinal cord and cerebellum, following methionine sulphoximine administration

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Summary. Methionine sulphoximine (MSO) is a centrally acting neurotoxin which inhibits the glutamate metabolism enzymes and has convulsive properties. Small doses of MSO were administered to rabbits, either intravenously (IV) or intracerebroventricularly (ICV), and electron microscopic examination of the cerebellum, the spinal cord and the sciatic nerve was performed on the first day of rabbit hind leg rigid paralysis (myopathy with histological findings resembling myositis), which set in by the 2nd to 4th day after MSO administration. In the cerebellum focal minor alterations were found in the astrocytes (swelling and lucidity, diminution of glycogen granules) and sparsely in the presynaptic terminals (lucidity and clumping), whereas most of the neuron presented a normal appearance. In the spinal cord and in the sciatic nerve a dissociation of the axon from the myelin sheath was evident in a small number of myelinated nerve fibres, along with the appearance of vacuolated spaces. Mitochondrial disorganisation in the axons, as well as glial cell alterations, were also seen. The ultrastructural alterations were non specific, and since they were induced 2 to 4 days after the administration of either minimum doses (IV) or of extremely low doses (ICV) of MSO, they may be attributed to the inordinate increase of metabolism during the period of convulsions.

Key words: Methionine sulphoximine, Rabbit, Sciatic nerve, Spinal cord, Cerebellum, Ultrastructural alterations

Introduction

Methionine sulphoximine (MSO) is a centrally acting neurotoxin with convulsive properties; it has been used for a long time as a tool for the experimental study of epilepsy (Wolf and Elliot, 1962). MSO suppresses the formation of glutamine and glutamate and it has been claimed to reduce the releasable pool of glutamate, aspartate and GABA (De Robertis et al., 1967; De Robertis, 1971; Subbalaeshmi and Murthy, 1984). In a previous paper (Apostolakis et al., 1989) we reported that we used MSO in a series of experiments designed to investigate a possible correlation of the probability of occurrence of a seizure with the power spectrum characteristics of the interictal EEG in the rabbit. During these experiments a serendipitous finding came to our attention: all animals subjected to MSO treatment [either intravenously (IV) or intracerebroventricularly (ICV)] showed severe hind leg gait disturbances which in most animals so treated (12 out of 14 by the time of this report) deteriorated to the point that lower limb rigid paralysis set in. Routine histological examination of the cerebral cortex, spinal cord and sciatic nerve was unremarkable, though histological examination of the cerebellum showed sparse foci of minor degenerative alterations in Purkinje cells, compared to controls. Routine histological examination of the muscle biceps femoralis revealed a picture of myositis, whereas electron microscopy after four days of either IV or ICV administration of MSO, showed localised disorganisation or disruption of the Z bands, as well as swelling of the sarcoplasmic reticulum. These findings were attributed to an inordinate increase in the metabolism during convulsions.

In order to further investigate this hypothesis, i.e. the consequences of the metabolism increase, we undertook an ultrastructural study of the rabbit cerebellum, the sciatic nerve, and the spinal cord after MSO (either IV or ICV) administration, since we have not been able to find in the literature any paper referring to the possible alterations of the above neural structures, following convulsions induced by MSO. Other investigators reported alterations only in the cerebral cortex of rabbits and dogs (Hevor et al., 1985) or rats (De Robertis et al., 1967; Rizzuto and Gonatas, 1974; Gutierrez and Norenberg, 1977), which however had been treated with

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MSO doses per kg of body weight (kg b.w.) strikingly higher than those used in our experiment.

Materials and methods

For the above studies we used eight male NZW rabbits, 2.6-3.5 kg b.w. raised in the Department of Experimental Physiology in all-metal wire floor-type hutches, and given food and water ad libitum. Five of them received MSO treatment; three of them were subjected to IV administration of 6 mg MSO per kg b.w. (total dose not exceeding 20 mg MSO) (DL-Methionine-DL-Sulphoximine, Sigma Chemical Company Lot 69c-0504) from a solution of 2 mg MSO per ml of normal saline (N/S). Two other animals were subjected to ICV administration of 200 µg of MSO (100 µl from the above solution) by a cannula implanted chronically in the right lateral ventricle (coordinates A= 1 mm, L= 2.5 mm, H= +4.5 mm according to the stereotaxic atlas of Sawyer et al., 1954).

One rabbit was given IV the same dose (10 ml) of N/S and two rabbits were given ICV 100 µl of N/S (controls); they were sacrificed four days later. The MSO-treated animals were sacrificed two to four days after MSO administration, on the first day of rigid paralysis. All animals were processed for nerve tissue electron microscopic examination, by intracardiac infusion of a phosphate buffer solution (pH 7.3) of 3% glutaraldehyde. Small pieces (approx. 1 mm^3) of sciatic nerve, spinal cord and cerebellum were excised and immersed in the above solution for two hours, at a temperature of 0 °C. After washing with the same buffered solution (pH 7.3) the tissue pieces were postfixed in the same buffer solution of 1% osmium tetroxide for 1.5 hours. After tissue staining in 1% water solution of uranyl acetate and dehydration in a graded series of alcohol, the tissue pieces were embedded in EPON. The ultrathin sections (50-65 nm) were treated with lead citrate and were examined in a JEOL 100 CX TEM, in 80 kv.

Results

The cerebellum, the spinal cord and the sciatic nerve in the control animals presented no alterations ultrastructurally.

Electron microscopic examination of the same structures in the animals which had received the MSO, either IV or ICV, revealed the following findings on the first day of rigid paralysis, i.e. two to four days after the administration of MSO:

A) Cerebellum

Alterations were found in the astrocytes, in a focal pattern; their perivascular end feet were sometimes swollen and lucent, and very rarely they were devoid of any material (Fig. 1). Swelling was located mainly in the glial processes and rarely in the body cells (Fig. 2). Capillaries were normal. No glycogen granules were found in the sections examined. Synaptic terminals were sometimes lucent and presented clumping or a diminution of the number of synaptic vesicles (Fig. 3). A very small number of neurons presented minor alterations focally. However the majority presented a more or less normal appearance. In figure 4 a Purkinje cell with normal appearance is shown.

B) Spinal cord

In the white matter, most neuraxons were normal. Focally there were sparse altered mitochondria and

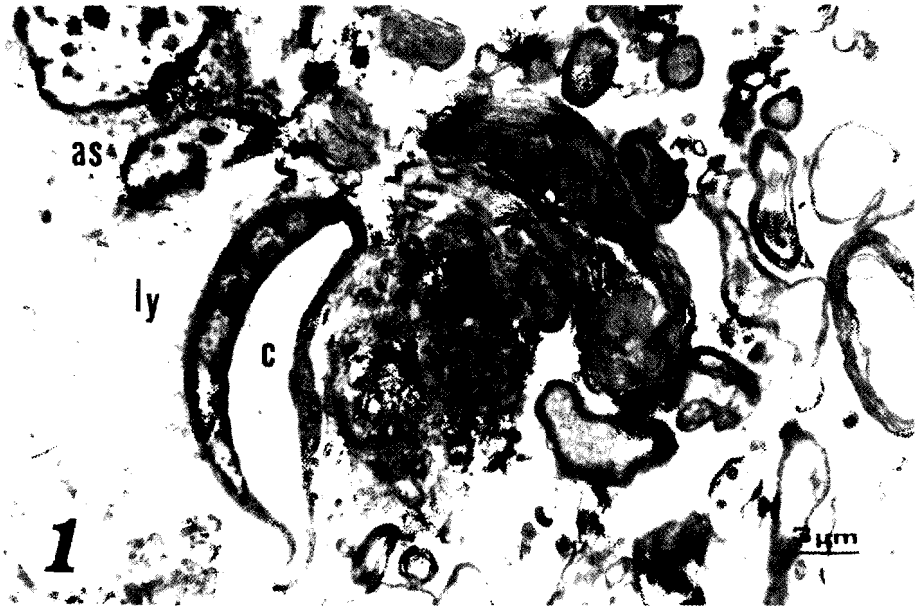

Fig. 1. Cerebellum. Lytic pericapillary area (ly) around the capillary (c), and damaged astrocytes (as). x 2,700

rarely at some sites gross alterations of the myelin sheath were noticed (Fig. 5).

In the grey matter, nerve cells were mostly intact, with normal ribosomes, Nissl bodies, Golgi apparatus, lysosomes, mitochondria, microfibrils and microtubules (Fig. 6). Glial cells focally showed a slight swelling of the rough endoplasmic reticulum and a few altered mitochondria (Fig. 7).

C) Sciatic nerve

Most nerve fibres were normal. A few myelinated nerve fibres presented a dissociation of the axon from the myelin sheath, and some vacuolar spaces. Some mitochondria with disrupted internal cristae were also observed in nerve fibres (unmyelinated and myelinated) and in gial cells as well (Figs. 8, 9).

Discussion

As we reported in a previous paper (Apostolakis et al., 1989), routine histological examination of the spinal cord, sciatic nerve and cerebellum in rabbits treated with the same dose of MSO (either IV or ICV), as in the present study, revealed only a focal and partial degeneration of cerebellar Purkinje cells. In this study

Fig. 2. Cerebellum. Lytic area (ly) in the proximity of the nucleus (N) of a glial cell presenting swollen mitochondria (m). x 6,000

Fig. 3. Cerebellum. Lucent synaptic terminals (st). x 17,400

electron microscopic examination of the same structures, showed minor focal alterations in the spinal cord and sciatic nerve. These were mainly degeneration of myelin sheath and alterations in the mitochondria, slight disorganisation of neuraxons, as well as glial cell alterations focally.

The ultrastructural alterations found in the cerebellum glial cells, as well as in the presynaptic terminals, are in agreement with our findings in a previous preliminary study (Manthos et al., 1990), and with the alterations reported by other investigators in the cerebral cortex of rabbits and dogs (Harris, 1964) or rats (De Robertis and Sellinger, 1967; Rizzuto and Gonatas, 1974; Gutierrez and Norenberg, 1977), which had been treated with MSO doses per kg b.w. higher than those used in our experiments. More specifically, in the experiments of Harris (1964) rabbits were given 500 mg of MSO per kg b.w. and cortical alterations were seen at the onset of seizure activity, consisting of swollen cytoplasmic processes of glial cells with swollen mitochondria, qualitatively similar to those seen in our experiments in the cerebellum. De Robertis et al. (1967) found that in the beginning of the MSO-induced convulsions in rats, given 400-800 mg MSO per kg b.w. intraperitoneally, synaptic terminals were swollen and synaptic vesicles were depleted, while later glial swelling occurred in the

Fig. 4. Cerebellum. Purkinje cell with normal appearance. A small myelinoid degeneration is shown by the arrow (md). x 3,600

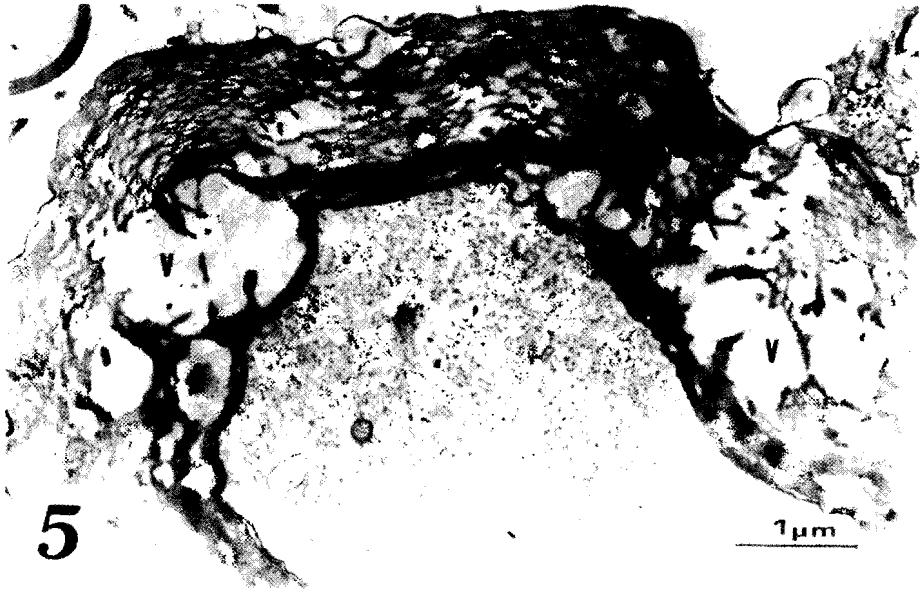

Fig. 5. Spinal cord, white matter. Neural axon with myelin alterations having the form of myelin sheath separation and vacuolation (v). x 20,000

brain cortex. Rizzuto and Gonatas (1974) found that in rats injected intraperitoneally with 400-600 mg per kg b.w. there was always a clear relationship between severity, frequency and duration of the seizures, and structural changes in the cerebral cortex. These changes are similar to those observed in cerebellum astrocytes in our experiments. They consisted of swelling of presynaptic endings as well as of glial cells and their processes, without changes in the mitochondria.

The findings reported by the above investigators (Harris, 1964; De Robertis et al., 1967; Rizzuto and Gonatas, 1974; Gutierrez and Norenberg, 1977) were seen at the onset of the seizures, or two hours after the beginning of the convulsions, and were induced by exceedingly greater doses of MSO in comparison with the dose that we used. Moreover the alterations found in our experiments occurred two to four days following MSO administration (at the end of seizure activity, first day of paralysis). The alterations in astrocytes found in our experiments were mainly degenerative, in contrast to the mainly proliferative changes found by Gutierrez and Norenberg (1977) in the cerebral cortex of rats, during the preictal period following MSO administration (150 mg per kg b.w. intraperitoneally). These investigators

Fig. 6. Spinal cord, grey matter. Neural cell (nc) with normal appearance. A slightly vacuolated area is shown in v_1 and v_2. x 15,000

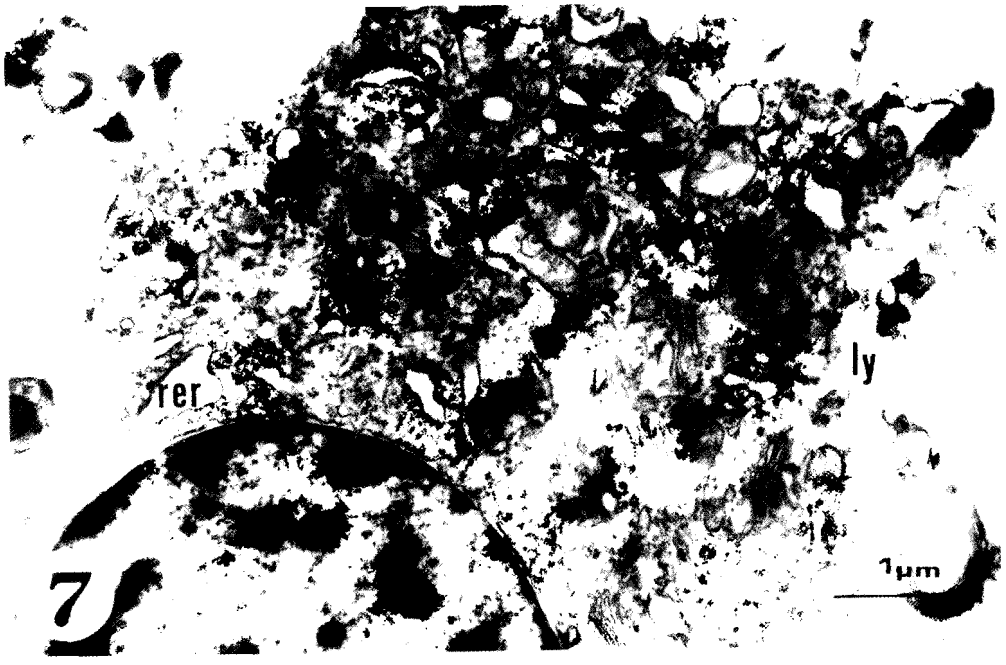

Fig. 7. Spinal cord, grey matter. Glial cell presenting swollen rough endoplasmic reticulum (rer) and lytic areas (ly). x 14,400

stated that the findings resemble those seen in experimental ammonia encephalopathy. The fact that MSO produces blood and brain ammonia elevation (Warren and Schenker, 1964; Gutierrez and Norenberg, 1975; Raabe and Onstad, 1982), combined with the involvement of astrocytes in brain ammonia metabolism (Meijer et al., 1990) could explain the findings of Gutierrez and Norenberg (1977) in the preictal period. In

Fig. 8. Sciatic nerve. Neural axon is dissociated from the myelin sheath (arrows). x 10,000

Fig. 9. Sciatic nerve. Minor disarrangement of the myelin sheath in a neuraxon and some swollen mitochondria (m) in the glial cell. x 9,500

contrast, the minor degenerative alterations of astrocytes in our experiments could be the result of metabolic hyperactivity, since they have been demonstrated two to four days after MSO administration (at the end of seizure activity, first day of paralysis). On the other hand, it is also known that ammonia is detectable in blood and brain one hour after administration of MSO, and persists for at least 24 hours (Gutierrez and Norenberg, 1975).

The overall differences between the bibliographic findings and the results of this work, are: a) the striking difference in the MSO dose (very high in the literature, extremely low in this study); b) differences in the type of histological findings, c) difference in the time of the histological examination, suggesting in the literature a direct pharmacological effect of MSO and in our experiments an indirect action of MSO, considering on one hand the delayed examination and on the other the presence of the same alterations in the ICV-treated animals which received an extremely low dose of MSO but which resulted in the same convulsive activity and hind leg rigid paralysis.

The degenerative nature of the alterations seen in our experiments is supported by the fact that the acute ischaemic alterations of neocortical, hippocampal and cerebellar neurons observed after prolonged epileptic seizures in experimental animals (Meldrum and Brierley, 1973; Corsellis and Meldrum, 1976; Blennow et al., 1978) are morphologically and topographically similar in experimental models of epilepsy (Levy et al., 1975) and in experimental models of ischaemia-hypoxia (Meldrum et al., 1982).

It has been found by neurochemical methods (Folbergrova et al., 1969; Folbergrova, 1973; Hevor et al., 1985; Swanson et al., 1989) that in astrocytes, glycogen begins to rise before the convulsions start, increases then to a high level and remains high for up to 72 hours after MSO administration. Ultrastructural studies (Phelps, 1975; Delorme and Hevor, 1985) also showed glycogen granule accumulation in astrocytes 24 or 48 hours after MSO administration in rats; this rise has been attributed to neoglycogenesis (Delorme and Hevor, 1985) or to slowed glycogenolysis (Swanson et al., 1989). Nevertheless, our findings suggest that structural damage of glial elements at a later stage after MSO administration (4th day) could be a factor responsible for the absence of glycogen granules.

In the spinal cord and the sciatic nerve the appearance of vacuolated spaces between myelin sheaths is associated with the findings of Harris (1964) who reported the presence in the cerebral cortex of dogs of distended spaces inside the myelin sheaths of axons, also separating the inner myelin lamella from the axon, following MSO-induced convulsions. The above vacuolated spaces and the disorganized mitochondria, which have been found in the present study, suggest a degenerative process. This can be attributed rather to the inordinate increase of metabolism during the convulsions, than to direct toxic action of MSO, since the findings are also present in the animals which received the MSO by ICV administration. For future research in this direction another set of experiments could be undertaken using another convulsant, to investigate whether the same effects could be reproduced.

In conclusion, there is suggestive evidence that the ultrastructural alterations of the cerebellum, the spinal cord and the sciatic nerve of rabbits, presenting hind leg myopathy after IV or ICV MSO administration, are non-specific and may be attributed to the inordinate increase in metabolism during the period of convulsions. In a previous paper (Apostolakis et al., 1989) we also suggest that the ultrastructural and light microscopic alterations of biceps femoralis muscle of rabbits, which had received MSO, either IV or ICV, are probably due to the inordinate increase in metabolism during the period of convulsions. The two facts combined may be of great importance, since they could contribute to the interpretation of the sequence of events, among them exhaustion and lower nephron-nephrosis due to myoglobinuria (Engel, 1989), which may be encountered in fatal cases of status epilepticus.

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