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The immunoreactivity of satellite glia of the spinal ganglia of rats treated with monosodium glutamate

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Abstract

Satellite glia of the peripheral nervous system ganglia provide metabolic protection to the neurons. The aim of this study was to determine the effects of monosodium glutamate administered parenterally to rats on the expression of glial fibrillary acidic protein, S-100β protein and Ki-67 antigen in the satellite glial cells. Adult, 60-day-old male rats received monosodium glutamate at two doses of 2 g/kg b.w. (group 1) and 4 g/kg b.w. (group 2) subcutaneously for 3 consecutive days. Animals in the control group (group C) were treated with corresponding doses of 0.9% sodium chloride. Immediately after euthanasia, spinal ganglia of the lumbar region were dissected. Immunohistochemical peroxidase anti-peroxidase reactions were performed on the sections containing the examined material using antibodies against glial fibrillary acidic protein, S-100β and Ki-67. Next, morphological and morphometric analyses of immunopositive and immunonegative glia were conducted. The data were presented as the mean number of cells with standard deviation. Significant differences were analysed using ANOVA ($P < 0.05$). In all 63-day-old rats, immunopositivity for the examined proteins glia was observed. Increased number of cells expressing glial fibrillary acidic protein was demonstrated in group 2, whereas the number of S-100β-positive glia grew in the groups with the increasing doses of monosodium glutamate. The results indicate the early stage reactivity of glia in response to increased levels of glutamate in the extracellular space. These changes may be of a neuroprotective nature under the conditions of excitotoxicity induced by the action of this excitatory neurotransmitter.

Glial cells, immunohistochemistry, sodium salt of glutamic acid

Spinal ganglia of the peripheral nervous system are structures lying on the course of the nerve fibres that transmit sensory impulses from the periphery of the body to the central nervous system (Hanani 2005). Pseudounipolar neurons and satellite glia are located under a connective capsule which is formed by each spinal ganglion. Glial cells are flattened and have a number of intermeshing processes forming a tight sheath around almost every neuron. These glia control neuronal microenvironment by participating in ion homeostasis and metabolism of, among others, glutamate (Glu) which is one of the major excitatory neurotransmitters of the nervous system. In the sensory ganglia this amino acid is present in 30–70% neurons (Nedergaard et al. 2002; Hanani 2005; Kung et al. 2013). Furthermore, glial sheath is a barrier to different blood compounds which can pass through the fenestrated capillaries present in the connective tissue mesh of the ganglia (Hanani 2005).

Monosodium glutamate (MSG) is a widespread chemical additive that improves food flavour. Experiments conducted on animals have shown that this compound has very low toxicity when applied orally. However, at high doses or when administrated parenterally, monosodium glutamate can be neurotoxic, leading to excitotoxic damage and even death of the nerve structures (Mallick 2007; Ganesan et al. 2013). These changes are accompanied by glial activation which seeks to compensate for changes in the environment immediately after the effect of the damaging agent, e.g. by adjusting the level of ions and

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neurotransmitters (Nörenberg 1994). Within the next hours, early reactivity processes occur due to the modifications of the normal cell characteristics relative to the altered environmental conditions. This phase is manifested by reactive synthesis and increased expression of various proteins, e.g. the glial fibrillary acidic protein (GFAP) and the S-100β protein (Eddleston and Mucke 1993; Stephenson and Byers 1995; Hanani 2005; Ohara et al 2009; Middeldorp and Hol 2011). In the last stage, permanent or long-term morphological and functional cellular changes were observed. During this period, hypertrophy of cellular bodies and processes and also increased glial proliferation were demonstrated (Nörenberg 1994). The phenomenon of excitotoxicity is associated with several diseases of the nervous system, e.g. epilepsy, stroke, ischaemia, Parkinson's disease, or Alzheimer's disease (Yuan et al. 2007; Bezprozvanny and Mattson 2008; Ganesan et al. 2013). Therefore, parenterally administered MSG is often used in animal experimental models of many neurological disorders which allows for a better understanding of their pathomechanisms (Ganesan et al. 2013).

Until now, reactive behaviour of the satellite glia of ganglia in response to the increased concentration of Glu in the extracellular space was never determined. The aim of the study was to investigate the immunoreactivity of GFAP, S-100β and Ki-67 in glial satellite cells after parenteral administration of MSG.

Materials and Methods

Animals and preliminary examination procedure

The experiment was performed on fifteen 60-day-old male Wistar rats. During the whole period of the experiment, the animals were kept in cages at a room temperature between 20–22 °C and 60% humidity, in a 12h:12h light/dark cycle. The rats had free access to fodder and water, and stress factors were reduced to a minimum. The study was approved by the Second Local Ethics Committee for Experiments on Animals in Lublin (7/2011). Animals were randomly divided into three groups. The two experimental groups received monosodium glutamate (MSG) at doses of 2 g/kg b.w. (group 1) and 4 g/kg b.w. (group 2) subcutaneously for 3 consecutive days. The control group (C) received the corresponding volume of saline. Twenty-four hours after the last injection, the rats were euthanised and the lumbar sensory ganglia (L1–L4) were dissected. The material was fixed in buffered 10% formalin (pH = 7.0, temp. 4 ° C, 12 h) and embedded in paraffin blocks by a routine histological technique. Afterwards, 4 µm thick sections containing the sensory ganglia were obtained using a microtome.

Indirect staining by immunohistochemistry

Indirect peroxidase anti-peroxidase (PAP) immunohistochemical reactions were carried out on deparaffinised and hydrated sections of the ganglia derived from each animal. All antibodies and reagents were obtained from Sigma-Aldrich (St. Louis, Missouri, USA) and were diluted in TBS (Tris Buffered Saline; T5030) pH = 7.6 in accordance with the manufacturer's instructions. In order to retrieve the antigen, each third slide was heated in 0.01 M citrate buffer solution (pH = 6.0) in a microwave oven in three 5-min cycles. Afterwards, all sections were treated with 3% H_2O_2 (30 min at room temperature) and subsequently with normal goat serum for 20 min at room temperature (G9023; 1:10). Next, an incubation of sections at 4 °C for 16 h with the primary antibody (rabbit antibody against glia fibrillary acidic protein, 1:80 (G9269); mouse monoclonal antibody against protein S100β, 1:1000 (S2532); mouse monoclonal antibody against the cell proliferation antigen Ki-67, 1:800 (P6834)) was carried out. The sections were then treated with species specific secondary antibody (goat antibody against rabbit IgG, 1:400 (A9169); goat antibody against mouse IgG, 1:150 (A9917)) at room temperature for 1h and further, a complex of peroxidase anti-peroxidase (rabbit peroxidase anti-peroxidase (PAP) complex, 1:200 (P1291); mouse peroxidase anti-peroxidase (PAP) complex, 1:200 (P3039)). At the end of the procedure a tetrachloride 3,3'diaminobenzidine (30 min at room temperature) was used as chromogen. Subsequently, sections were rinsed in distilled H_2O, counterstained with Mayer's haematoxylin and mounted in DPX (Fluka, Buchs, Schwitzerland). At the same time a specificity controls for the immunohistochemical reactions were performed.

Microscopic and statistical analyses

The sections were examined and photographed under a light microscope Olympus BX-51 with an Olympus Color View IIIu digital camera. Microphotographs with a final magnification of × 400 were archived. Next, 100 immunopositive and immunonegative for each of the three examined proteins derived from the 10 samples from each group of animals satellite glial cells were analysed using Cell ^ D program. The data were presented as the mean number of cells with standard deviation. Significant differences ($P < 0.05$) between the means were shown by one-way ANOVA with *post hoc* Tukey test. All statistical analyses were performed using Statistica 8.0.

Results

In all 63-day-old animals (groups C, 1, and 2) immunopositive reactions for GFAP in glial satellite cells were observed in the spinal ganglia. Numerous glia were immunoreactive for the examined protein (51 ± 6.27) in group 2 in comparison with the mean number of glia present in the spinal ganglia of group C (28.6 ± 8.1) (Plate I, Fig. 1A) and group 1 (30.2 ± 6.66) (Plate I, Fig. 1B). S-100β-positive glia in the examined ganglia were numerous in group 1 (34.8 ± 5.83) and group 2 (43.1 ± 5.17), whereas only average numbers of them were found in control animals (13 ± 4.71). A cytoplasmic expression of S-100β protein in the satellite glial cells was observed in all rats. Additionally, the reaction product was seen in the nuclei of the examined glia in experimental animals (groups 1 and 2) (Plate I, Fig. 1D, Fig. 1E, Fig. 1F). Ki-67-immunopositive satellite glial cells were found in very small numbers in the spinal ganglia of all rats: group C (0.8 ± 1.03), group 1 (1.3 ± 0.95), and group 2 (1.6 ± 0. 7) (Plate I, Fig. 1C).

Morphometric analyses confirmed the results of microscopic examinations. Significant differences were demonstrated in the number of satellite glial cells expressing GFAP between group 2 and groups C and 1 which were comparable to each other, and in the number of S-100β-positive glial cells between each group of animals (C, 1, 2) (Fig. 2).

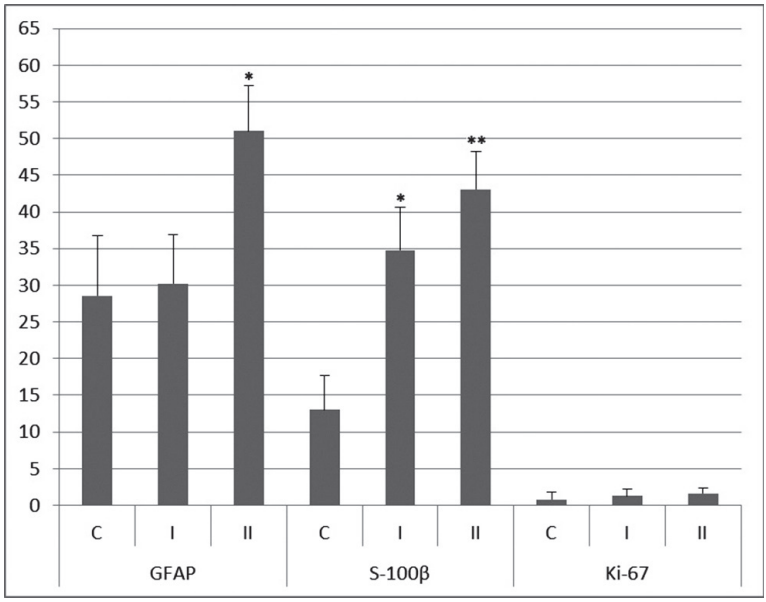

Fig. 2 The mean number of GFAP, S-100β and Ki-67-immunopositive cells of the spinal ganglia of 63-day-old control (C) and experimental (1, 2) rats. Bars represent standard deviation; *, ** significant difference between groups of animals (C, 1, 2) ANOVA ($P < 0.05$).

Discussion

Analyses conducted in this study showed an increase in the number of GFAP and S-100β immunopositive glia of the spinal ganglia of rats after treatment of MSG. The GFAP is a fibrillar protein that affects the shape of the cell and the structural stability of its processes. In addition, it binds to specific glutamate transporters. Hence the increased expression can be a result of the necessity to capture an excessive amount of neurotransmitters from the

extracellular space (Sullivan 2014). The glia are actively involved in the metabolism of Glu due to the presence of both specific for the neurotransmitter membrane transporters as well as glutamine synthetase, an enzyme catalysing the conversion of glutamate to glutamine (Berger and Hediger 2000; Miller et al. 2002). Under physiological conditions, the GFAP is at a very low level in satellite glial cells (Ajima et al. 2001). An increased expression of this protein was observed after nerve damage and axotomy. In adult rats after the excision of a sciatic nerve fragment from the left thigh, six times more neurons surrounded by GFAP-immunopositive glial cells were demonstrated in the dorsal root ganglia in the operated side in comparison with the opposite side (Woodham et al. 1989).

Nucleo-cytoplasmic S-100β protein belongs to the calcium binding proteins from EF-hand family and reacts to changes in the level of calcium in the cell enabling the regulation of many internal and extracellular processes. S-100β influences the activity and metabolism of enzymes, phosphorylation of various proteins and the transcription of genes. Furthermore, this protein acts as a buffer lowering the intracellular concentration of calcium ions (Donato et al. 2009). An increased influx of these ions may be a consequence of the activation of specific receptors for Glu, which initiates a series of calcium-dependent enzymatic reactions. These processes can eventually lead to damage or death of cells (Mattson 2003; Platt 2007). In our study, the increased expression of the S-100β in the satellite glia may result from an increase of the intracellular calcium ion concentration and buffer properties of this protein to protect the cells. In addition, S-100β is actively released into the extracellular space, where it acts autocrinely and paracrinely on other cells. At nanomolar concentrations, it has a trophic effect on neural structures. It modulates Glu uptake by glial cells and also stimulates them to divide, thereby protecting the neurons from excitotoxicity (Selinfreund et al. 1991; Ahlemeyer et al. 2000; Rothermundt et al. 2003; Tramontina et al. 2006; Donato et al. 2009).

Increased division activity of satellite glial cells was observed after axotomy or inflammation of the sciatic nerve in rats (Hanani 2005). Cutting this nerve leads to damage of neurons, around which there are glia forming a multi-layer sheath in the shape of an onion bulb. The time required for the formation, however, is long. Between 43 and 65 days after axotomy, only 20% of the neurons were surrounded by this characteristic structure (Shinder et al. 1999; Hanani 2005). The proliferation of glia of the dorsal root ganglion was also demonstrated after the treatment of animals with organic compounds of mercury and lead. Satellite glial cells have the ability to uptake these substances in order to protect neurons. Long-term administration of lead acetate in rats leads to increased division activity, as well as hyperplasia of glial cells (Schlaepfer 1969; Schiønning and Danscher 1999). In our study, no increase in the number of Ki-67-immunoreactive nuclei after MSG treatment was observed, which most likely indicates too short duration of the experiment.

Analyses of the immunoreactivity of GFAP, S-100β protein and Ki-67 antigen in satellite glial cells allows us to specify the degree of advanced glial reactivity in response to the damaging factors to the structures of the nervous system. The analysis we carried out for the first time revealed an increase in the number of GFAP and S-100β immunoreactive structures in the dorsal root ganglia, indicating early glial reaction in response to increased levels of glutamate in the extracellular space. These changes are most likely due to the result of the cells' adaptation to altered environmental conditions that may adversely affect the structure and function of neurons. Such a reaction of the satellite glia may confirm its neuroprotective properties. Glial cells are likely to be involved in the course of pathological processes in the spinal ganglia and in the future, the determination of the nature and degree of their activation may thus create new directions for therapies of neurological diseases of the peripheral nervous system.

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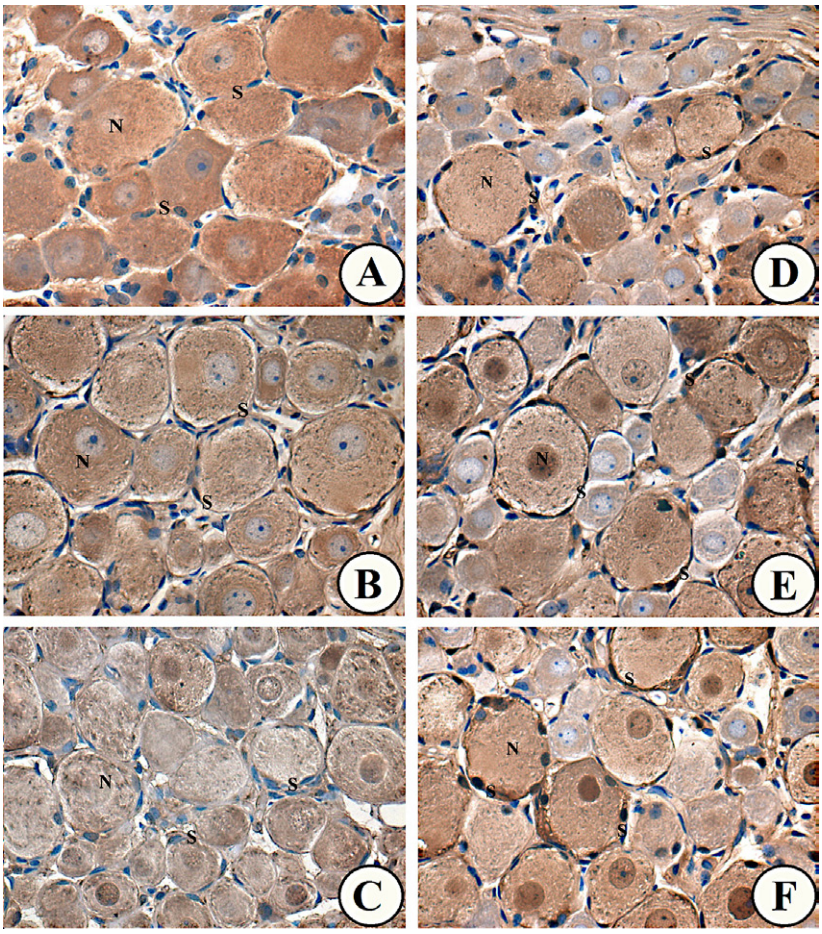

Fig. 1 GFAP (A, B), S100β (D, E, F) and Ki-67 (C) immunoreactive satellite glial cells of the spinal ganglia of 63-day-old control rats (A, D) and those treated with MSG in group 1 (E) and group 2 (B, C, F). (S) - satellite glial cells, (N) - neurons. Magnification approximately × 400