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Central Nervous System Dysmyelination Induced by Pre- and Postnatal Treatment of Rats with Anti-Galactocerebroside Sera

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Myelin membrane has a very high lipid:protein ratio not found in other membranes. Among lipids, galactocerebrosides (GC) are considered the best marker of myelin as their concentration closely parallels myelination. In the present study rats were treated with anti-GC sera during different periods of central nervous system (CNS) myelination. Electronmicroscopic studies revealed marked dysmyelination in the brain of rats treated with anti-GC during active myelination, when the maximum deposition of GC has been reported. The brain stem was the site of predilection. It may be suggested that the CNS myelinogenesis become affected by improper GC supply during active myelination.

Key words: central nervous system, dysmyelination, anti-galactocerebroside serum.

Introduction

Myelin multilamellar membrane that surrounds axons is the most lipid-rich one of any known membrane. The distinguishing feature of myelin lipids is the high content of two glycolipids — galactocerebroside (galactosyl ceramide) and sulfatide. Glycosphingolipids are lipid haptens and specific antibodies can be raised to galactocerebroside and sulfatide. Immunohistochemical studies using these antibodies have shown that GC is a specific marker for oligodendroglia in CNS cultures [18] and is restricted to these cells and myelin in tissue sections [9, 27]. During myelination the accumulation of GC is directly proportional to the amount of myelin that has been deposited [14]. In the rat the maximum rate of myelination as measured both by myelin content and GC content of brain occurs at 20 days [16].

Numerous biochemical studies have shown that the accumulation of myelin and of myelin specific lipids was depressed by undernourishment [2, 3, 7, 8, 11, 13, 23, 24]. An inhibition of CNS myelin development in vivo was demonstrated by implantation of anti-GC hybridoma cells [19].

In the present study rats were treated with anti-GC sera during different developmental and myelination periods to test the vulnerability of brain myelinogenesis due to improper GC supply. Portions of this work have been presented in abstract form [29].

Material and Methods

The asynchronousness in the course of myelination and the difference of the onset of myelination in different species necessitates the morphological characterization of these periods for every animal species. The myelination periods in the rat brain have been precisely determined by us previously [28] as follows: premyelination period - up to the 10th postnatal day; period of active myelination (17-22 postnatal days); period of completed myelination (after the 60th postnatal day).

Sprague-Dawley rats were treated with anti-GC sera (general gift from Dr P. Dupouey) during the following developmental and myelination periods: prenatal period - pregnant rats (gestinational days 18-20) — group A (n=12), premyelination period (5- and 9-day-old rats) — group B (n=12), period of active myelination (17-22-day-old rats) — group C (n=12). Group D — (n=12) represented the group of the control, untreated rats.

Under ketamine/xylazine anesthesia 36 rats of the all experimental groups (group A, group B and group C) and 12 control animals (group D) were perfused through the left ventricule at the age of 75 postnatal day (period of completed myelination) with 0.9% saline followed by 2.5% glutaraldehyde/formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3-7.4). The brain was removed and immersed in fixative. The forebrain, cerebellum and brain stem were postfixed with 2% osmium tetraoxide and embedded in Epon. Ultrathin sections were stained with uranylacetate and lead citrate and examined on Jeol 100CX electron microscope.

Results

Electron microscopy revealed normal myelination in the forebrain, cerebellum and brain stem of the rats treated with anti-GC sera during the prenatal and premyelination period (group A and group B). No abnormality of the myelin mem-

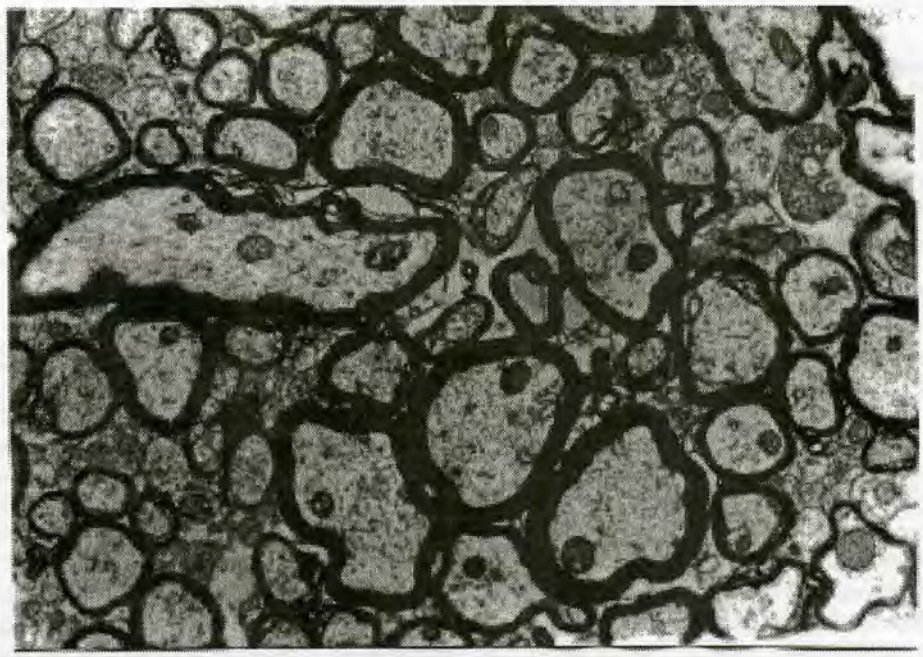

Fig. 1. Brain stem of 75-day-old rat, treated with anti-GC sera during the premyelination period (group B). No abnormality of the myelin membranes is evident (× 20 000)

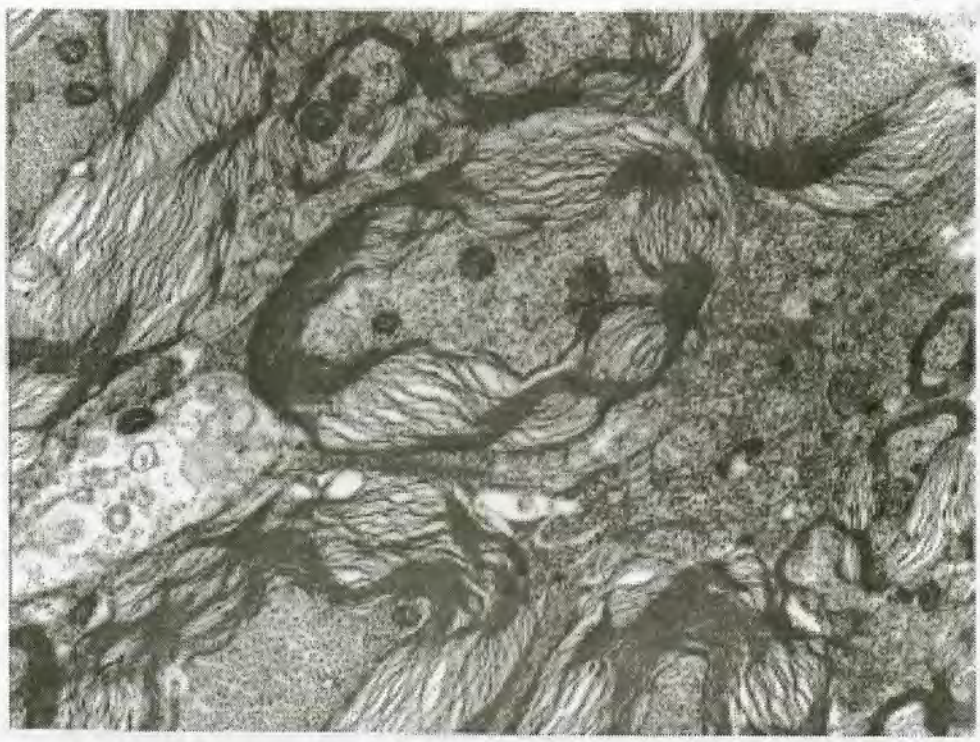

Fig. 2. Brain stem of 75-day-old rat, treated with anti-GC sera during the period of active myelination (group C). The myelin sheaths exibit prominent ultrastructural abnormalities (× 42 000)

branes was found (Fig. 1). The myelinated fibres resembled those in the forebrain, cerebellum and brain stem of the control animals (group D).

Marked defective myelination (dysmyelination) was observed in the brain of the rats, treated with anti-GC sera during the period of active myelination (group C). The brain stem was the site of predilection. The myelin sheaths exibited prominent ultrastructural abnormalities (Fig. 2). Rare axons are enveloped by normal compact myelin membrane.

Discussion

This study shows, for the first time, that a treatment of rats with anti-GC sera during the period of active myelination induces marked dysmyelination in the brain stem.

Galactocerebrosides and sulfatides account for 26.5% of CNS myelin lipids [1]. Many lines of evidence have revealed an important role of these galactolipids in myelin function and stability. Addition of anti-GC serum to the medium of CNS myelinated cultures was followed by a number of morphological myelin changes including intramyelin swelling, which resulted in complete demyelination [4,20]. Implantation of hybridoma cells that secrete a monoclonal anti-GC into the dorsal columns of < or = 9-day-old rat spinal cord caused a failure of development of dorsal column myelin in the vicinity of the implant [19]. Recent studies have found an increased frequency of uncompacted myelin sheaths as well as unmyelinated axons in double mutant mice lacking galactolipids and the proteolipid protein [5].

Although the sequence of myelination periods is similar for all mammals, the time of birth in relation to these periods is various in different species. In the rat the first myelin can be seen in hindbrain at about 10 postnatal days. The period of active myelination is one of the most dramatic in nervous system development [17]. At 15 days of age in the rat, about 4 mg myelin can be isolated from one brain. This amount increases sixfold during the next 15 days [16]. The accretion of GC became explosive during the period of active myelination. It could be suggested that because of the binding of anti-GC to newly synthetized GC at that time, the normal supply of myelin membranes with GC has been disturbed.

In our study the brain stem was the site of predilection of dysmyelination. These results are in agreement with the findings of Nonaka and Kishimoto [15] and Martinez [13]. Nonaka and Kishimoto [15] demonstrated higher concentration of myelin lipids in the brain stem than in the cerebellum during the whole maturation period in the rat brain. Martinez [13] found that galactolipids were much higher in the immature human brain stem than in immature cerebellum or cerebrum. The mature brain stem (8-year-old child) still had a higher concentration of lipids than the rest of the brain.

As mentioned above, myelin sheaths and myelination can be altered by diet [21, 25, 26]. Wender et al.[22] have shown that rats maintained on a fatty acid deficient diet during the last period of intrauterine and during their whole postnatal life developed a myelin deficient in all lipid constituents. The study carried out on the brain of undernourished humans supported previous lipid studies performed on malnourished animals [2,3,12] and humans [10] in that myelinogenesis seems to be preferentially affected by undernutrition. It has been reported by Martinez [13] that children born with a good nutritional status and subjected to undernutrition for a short period of time could exhibit a decrease in myelin lipids. On the other hand, immunohistochemical investigations have revealed accelerated myelinogenesis induced by dietary lipids in rats [5].

In conclusion, this study reveals, for the first time, dysmyelination in the brain stem of rats treated with anti-GC sera during the period of active myelination, when the maximum deposition of GC has been reported. The myelin sheaths exibited prominent ultrastructural abnormalities. It may be suggested that CNS myelinogenesis could be affected by improrer galactocerebroside supply during the period of active myelination.

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