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# Comparison of the distribution of $Na^+,K^+$-ATPase and myelin-associated glycoprotein (MAG) in the optic nerve, spinal cord and trigeminal ganglion of shiverer (*shi/shi*) and control (+/+) mice

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$Na^+,K^+$ATPase and myelin-associated glycoprotein (MAG) were studied by immunocytochemistry on paraffin sections of the spinal cord, optic nerve and trigeminal ganglion of adult control (+/+) and CNS myelin-deficient shiverer (*shi/shi*) mice. Immunostaining for $Na^+,K^+$-ATPase outlined the periphery of nerve fibers in the spinal cord white matter, optic nerve and trigeminal ganglion of +/+ and *shi/shi* mice. Immunostaining for $Na^+,K^+$-ATPase appeared somewhat denser in the optic nerve and spinal cord lateral funiculi of *shi/shi* than in +/+ mice. In addition, immunostaining for $Na^+,K^+$-ATPase was demonstrated at the plasmalemma of presumed satellite cells situated at the periphery of ganglion cell bodies in the trigeminal ganglion of both species of mice. Immunostaining for MAG was localized along the periphery of nerve fibers in the spinal cord funiculi (with little immunostaining within gray horns), optic nerve and trigeminal ganglion of both +/+ and *shi/shi* mice. The major differences between *shi/shi* and +/+ mice were that the number of MAG-immunostained nerve fibers was greatly reduced in the spinal cord funiculi and the density of immunostaining was slightly increased in the optic nerve of *shi/shi* mice. The numbers of MAG-immunostained nerve fibers in trigeminal ganglion were similar in both species. Also, the cytoplasm of some oligodendrocyte-like cells was found densely immunostained for MAG in the spinal cord and optic nerve of *shi/shi* mice, but not of +/+ mice. This light microscopic study provides evidence that the defective shiverer gene leads to a decrease in MAG deposition and to aggregations of MAG-like material within perikarya of oligodendrocyte-like cells in regions of the CNS.

## INTRODUCTION

Shiverer (*shi/shi*) mice exhibit a severe deficit in the amount of central nervous system (CNS) myelin and myelin basic protein (MBP) as studied in the cerebrum, spinal cord and optic nerve[2,10]. Additionally, structural abnormalities in CNS myelin, such as lack of major dense lines and the presence of loosely wrapped oligodendroglial membranes, are characteristic of this mutant[6,13,16]. However, myelin in the peripheral nervous system (PNS) of *shi/shi* mice is structurally normal with the preservation of myelin major dense lines even though MBP content is severely reduced[11,17]. It is known that the gene for MBP synthesis is defective in this mutant[8,15]. It is probable that there are also secondary effects on other membrane components due to the lack of myelin assembly and to altered interactions between oligodendroglia and other cell types[20]. The shiverer mutant may be a useful model for elucidating factors important to neurocellular membrane differentiation.

$Na^+,K^+$-ATPase functions in regulating $Na^+$ and $K^+$ ion gradients across membranes. In the brain these gradients are critical for resting membrane potentials, action potentials and postsynaptic potentials[21,23]. In myelinated fibers this enzyme has been localized to the node of Ranvier by immunocytochemistry and electron microscopy[1,18,29]. Therefore, the distribution of $Na^+,K^+$-ATPase in nerve fibers may be related to axonal–oligodendroglial interactions or myelin formation[18].

Myelin-associated glycoprotein (MAG), a 100-

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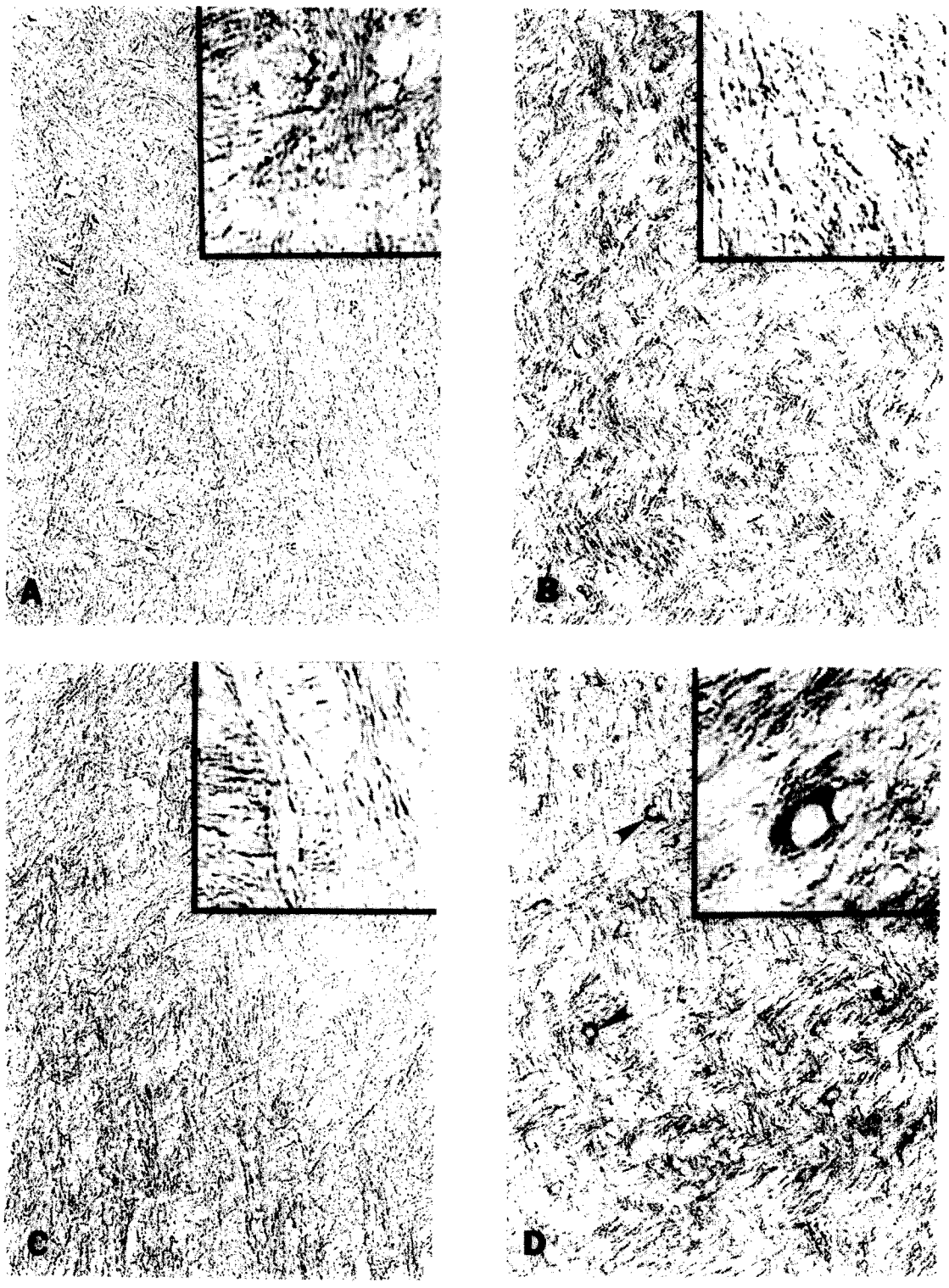


Fig. 1. Immunocytochemical localization of $Na^+,K^+$-ATPase and MAG in the optic nerve (chiasma) of control and shiverer mice. $Na^+,K^+$-ATPase immunostaining outlines nerve fibers in +/+ (A) and *shi/shi* (B) mice, with somewhat increased density in *shi/shi* mice. The inserts of A and B reveal $Na^+,K^+$-ATPase immunostaining at high magnification. Immunostaining for MAG surrounds the axons in the optic nerve of +/+ (C) and *shi/shi* mice and is contained within cells resembling oligodendrocytes in *shi/shi* mice (arrowheads). MAG immunostain surrounding nerve fibers at high magnification is shown in the insert of C. A MAG-immunostained oligodendrocyte and nerve fibers are revealed in the insert to D. Incubation of optic nerve sections with rabbit preimmune serum results in no detectable immunoreactive product in either +/+ (E) or *shi/shi* (F) mice. A–F ×240; inserts (A–D) ×1000.

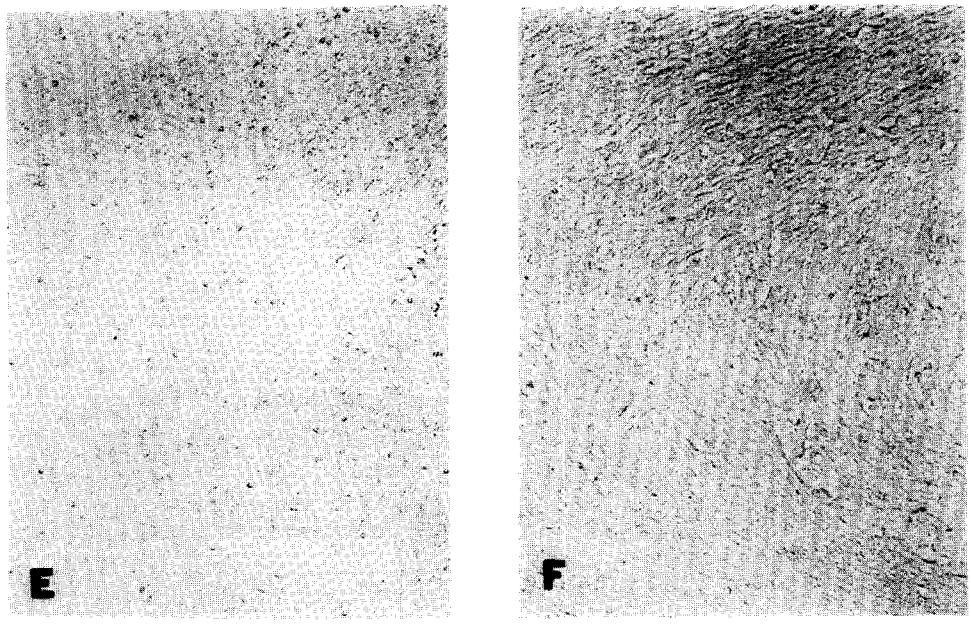


Fig. 1 (continued).

kDa protein, has been localized at the periaxonal region in the PNS (rat trigeminal nerve) and CNS, and lateral loops and Schmidt–Lanterman clefts in the rat PNS[24–27]. Because of this location, it has been suggested that MAG functions in oligodendroglial–axonal interactions[25,27]. However, a study has shown MAG to be localized in compacted CNS myelin rather than in the periaxonal region[28]. Therefore, the distribution of MAG still remains quite controversial[26,28].

MAG of the rat PNS is slightly higher in molecular weight than CNS MAG, although peptide maps of PNS MAG were shown to be almost identical to that of CNS MAG[4]. Brain MAG in 20-day-old myelin-deficient jimpy mice was determined to be about 5% of control brain levels[30]. In contract, a 27% increase in PNS MAG was detected in the PNS myelin-deficient trembler mice[7]. There is only one report of MAG content in adult shiverer mutant mice and this shows reduced MAG in forebrain and hindbrain[20].

The purpose of this study was to determine if severely reduced CNS myelin and MBP affects the distribution of a general membrane component, $Na^+,K^+$-ATPase, and of a myelin component, MAG, in the spinal cord and optic nerve of CNS myelin-deficient shiverer mice. In addition, we wished to compare this distribution with that in the trigeminal ganglion of shiverer mice in which myelin is not reduced or structurally altered despite MBP reduction.

## MATERIALS AND METHODS

### *Mice*

This study used control (+/+) and shiverer (*shi/shi*) male mice at 55–65 days of age. Shiverer mice are easily distinguished from their *shi*/+ or +/+ littermates by exhibiting a shivering motion beginning at 14 days. The control mice were selected from breeding +/+ pairs as determined by several brother/sister matings showing no shivering after several generations. Mice were maintained under NIH standard practices.

### *Antigen and antisera preparation*

The isolation of the mouse brain $Na^+,K^+$-ATPase catalytic subunit and the characterization of the rabbit antisera against this antigen have been reported[22]. Polyvalent rabbit anti-rat MAG antiserum was a kind gift of Dr. R. Quarles (Bethesda, MD) and its characterization has been described[14].

### *Immunocytochemistry*

Six each of +/+ and *shi/shi* mice were anesthetized with sodium pentobarbital (University Hospital Pharmacy, Ann Arbor, MI) (0.1 ml = 5 mg) and fixed by perfusion through the left ventricle with 4%


Fig. 2. Immunocytochemical localization of $Na^+,K^+$-ATPase and MAG in the spinal cord of control and shiverer mice. Immunostaining for $Na^+,K^+$-ATPase surrounds nerve fibers most densely in the spinal cord gray horns of +/+ (A) and *shi/shi* (B) mice. No detectable $Na^+,K^+$-ATPase immunostaining is observed in the posterior (p) or anterior (a) funiculi, while the lateral funiculus (l) of *shi/shi* mice exhibits denser staining than in +/+ mice (A). MAG-immunostained nerve fibers are most prominently localized within the funiculi (a,p,l) of +/+ (C) and *shi/shi* (D) mice, although the number of these stained fibers is significantly reduced in *shi/shi* (D) mice. Also, cells resembling oligodendrocytes are stained for MAG in the spinal cord funiculi of *shi/shi* mice (D) (arrowhead) and also shown in the insert to D. Replacing immune with preimmune serum results in no detectable reaction product in the spinal cord of +/+ (E) or *shi/shi* (F) mice. A–F, ×75; insert (D), ×1000.

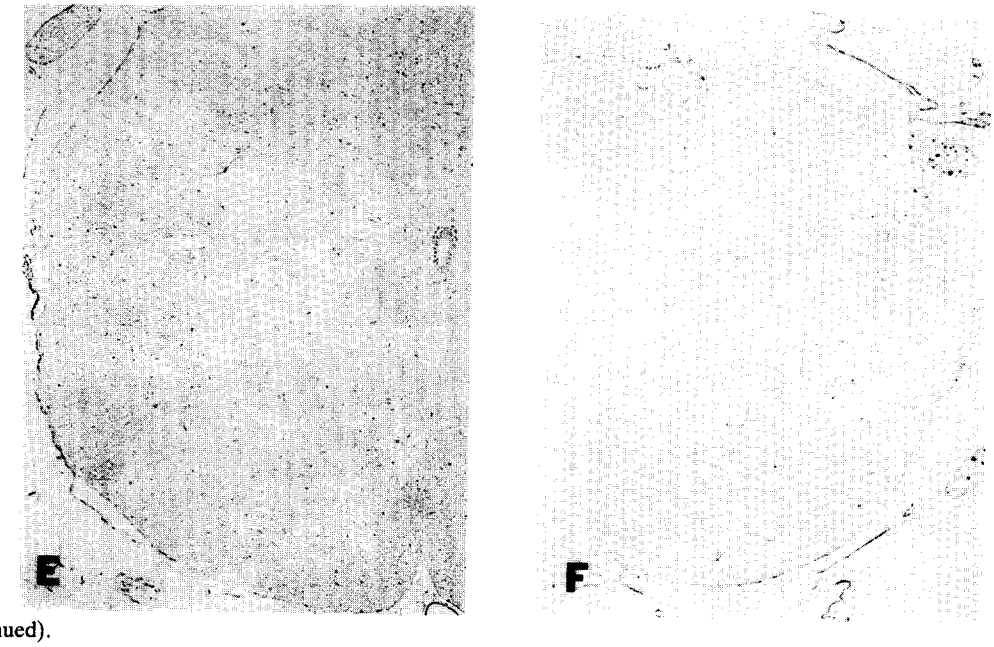


Fig. 2 (continued).

paraformaldehyde (Electron Microscopy Sciences, Port Washington, PA) in 0.1 M sodium phosphate, pH 7.4. The optic nerve, spinal cord and trigeminal ganglion were cut into 1–2 mm thick segments and fixed an additional 4 h at room temperature. The tissues were subsequently dehydrated and embedded in paraffin (Paraplast; Monoject Scientific, St. Louis, MO).

Paraffin sections (5 $\mu$m) were mounted on gelatin-coated slides and treated with immunoreagents in the following order[19,20,22]: 20% normal goat serum (Miles Lab., Naperville, IL) for 60 min at room temperature, 1:200 anti-mouse brain $Na^+,K^+$-ATPase or 1:250 anti-rat MAG antisera overnight at 4 °C, and lastly, with 1:250 goat anti-rabbit IgG-horseradish peroxidase (HRP) conjugate (Miles Labs.) overnight at 4 °C. Each antibody incubation was followed by a 60 min rinse in 0.1 M phosphate-buffered saline (PBS), pH 7.4. Immunostaining was completed by treating the sections with a solution containing 0.025% diaminobenzidine (Sigma, St. Louis, MO), 0.01% hydrogen peroxide (Sigma) and 0.05 M Tris-HCl, pH 7.6 (Sigma) for 5 min. Control sections for immunostaining were incubated with preimmune rabbit serum (1:200) instead of antisera.

## RESULTS

Immunoreactive product for $Na^+,K^+$-ATPase outlining nerve fibers in the optic chiasma of *shi/shi* mice appeared somewhat denser and more fasciculated than in +/+ mice (Fig. 1A,B). MAG immunostaining surrounded nerve fibers in the optic nerve of +/+ and *shi/shi* mice, although also slightly denser in *shi/shi* mice (Fig. 1C,D). In addition, MAG-immunoreactive product was localized at the periphery of the cell body and within cytoplasm of cells morphologically resembling oligodendrocytes in the optic nerve of *shi/shi* mice (Fig. 1D). Preimmune serum produced no reaction in both species (Fig. 1E,F).

Immunostaining for $Na^+,K^+$-ATPase was denser in the gray horns than in the white matter of +/+ and *shi/shi* mice spinal cord. Regional variations were evident in both species. Less $Na^+,K^+$-ATPase immunostaining was evident in the posterior and anterior funiculi than in the lateral funiculus of both +/+ and *shi/shi* mice. In comparing *shi/shi* to +/+ mice, denser staining was evident in the lateral funiculus of *shi/shi* mice (Fig. 2A,B). In contrast to results for $Na^+,K^+$-ATPase, immunostaining for MAG, as expected, was predominantly detected in the lateral, posterior and anterior funiculi rather than gray columns of the spinal cord of +/+ mice (Fig. 2C,D), However, the spinal cord funiculi of *shi/shi* mice had many fewer immunostained fibers than did those of +/+ mice, while also displaying oligodendrocyte-like cells immunostaining for MAG as observed in the optic nerve (Fig. 2D). Also, many MAG-stained fibers

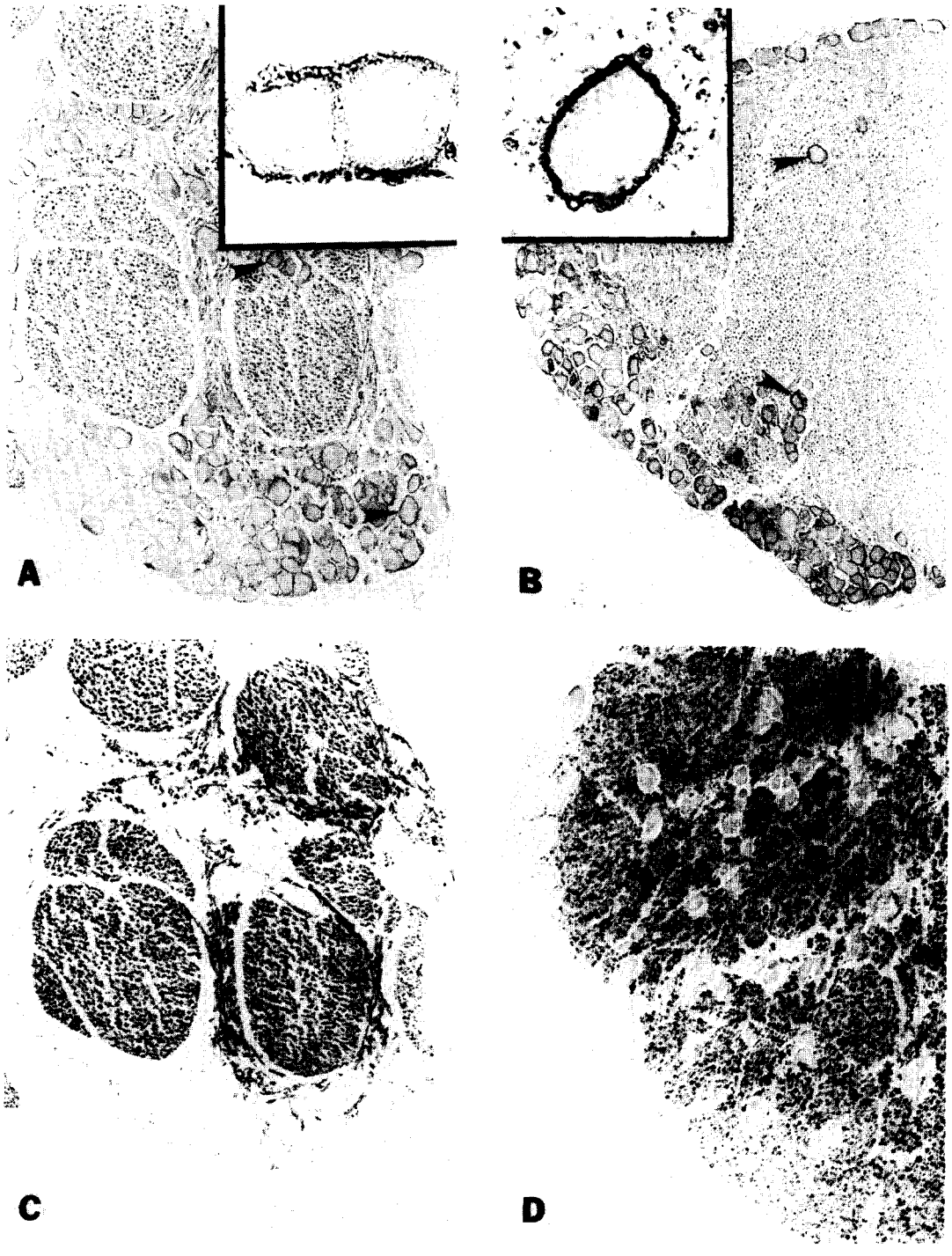


Fig. 3. Immunocytochemical localization of $Na^+,K^+$-ATPase and MAG in the trigeminal ganglion of +/+ and *shi/shi* mice. Immunostaining for $Na^+,K^+$-ATPase is associated with nerve fibers and the periphery of ganglion cell bodies (arrowheads) in the trigeminal ganglion of +/+ (A) and *shi/shi* (B) mice. The inserts of A and B show $Na^+,K^+$-ATPase immunostaining at the periphery of the ganglion cell body at high magnification. This immunostaining is also presumed to be associated with satellite cells which surround the ganglion cell body. MAG was localized at the periphery of nerve fiber in the trigeminal ganglion of both +/+ (C) and *shi/shi* (D) mice. In the trigeminal nerve of *shi/shi* mice the PNS region is clearly distinguished from the CNS segment (asterisk) due to dense MAG immunostaining in the PNS and lack of staining in the CNS region (E). However, $Na^+,K^+$-ATPase immunostaining does not reveal a clear demarcation between the CNS (asterisk) and PNS of the trigeminal nerve of *shi/shi* mice (F). Trigeminal ganglion sections of +/+ (G) and *shi/shi* (H) mice incubated in preimmune serum results in no detectable reaction product. A–D,G,H, ×120; E,F, ×240; inserts (A,B), ×1000.

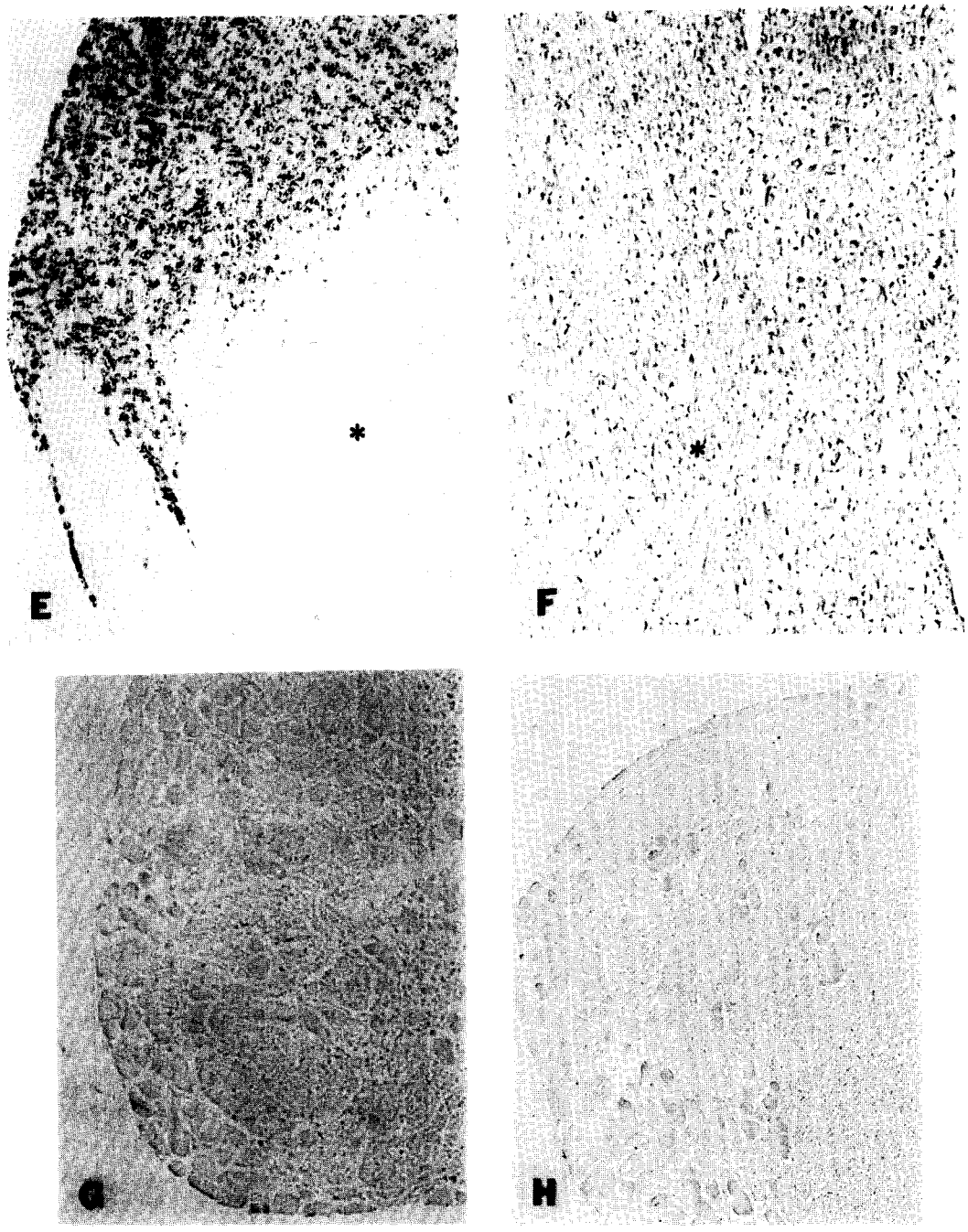


Fig. 3 (continued).

were observed in the gray horns of +/+ but few in *shi/shi* mice (Fig. 2C). Preimmune serum controls were blank (Fig. 2E,F).

$Na^+,K^+$-ATPase immunoreactivity in the trigeminal nerve of +/+ and *shi/shi* mice outlined nerve fibers and was localized at the periphery of ganglion cell bodies and the closely situated satellite cells (Fig. 3A,B). No difference was detected in the distribution of immunostaining for $Na^+,K^+$-ATPase in +/+ and *shi/shi* mice. Immunostaining for MAG in the trigeminal ganglion was localized at the periphery of nerve fibers with equal intensity in +/+ and *shi/shi* mice (Fig. 3C,D). At the zone of transition between CNS and PNS in the trigeminal nerve of *shi/shi* mice, there is dense immunostaining for MAG in the PNS region but little or no staining in the CNS region (Fig. 3E).

However, immunostaining for $Na^+,K^+$-ATPase in the transition zone does not distinguish between the CNS and PNS (Fig. 3F). Preimmune serum produced no reaction product (Fig. 3G,H).

## DISCUSSION

Immunostaining for $Na^+,K^+$-ATPase appeared somewhat denser in the optic nerve and spinal cord lateral funiculus of *shi/shi* than in those of +/+ mice. It is possible that there are variations in $Na^+,K^+$-ATPase content in different CNS myelinated fiber tracts. For example, the lateral funiculus is more densely stained than anterior and posterior funiculi within the same sections in both +/+ and *shi/shi* mice (Fig. 2A,B).

The fact that immunostaining for $Na^+,K^+$-ATPase in optic nerve and spinal cord white matter of *shi/shi* compared to control mice is not reduced (but actually increased in the optic nerve and lateral funiculus) indicates that myelin in these structures does not contribute significantly to this enzyme. The relative increase in $Na^+,K^+$-ATPase immunostaining in the shiverer optic nerve and lateral funiculus might be due to spread of axolemma cation pump insertion beyond nodes of Ranvier or to improved reagent penetration due to the paucity of myelin in shiverer mice. While myelin hindrance is not completely excluded, it is considered improbable since the same effect is not seen in the myelin-deficient posterior and anterior funiculi in the same sections (Fig. 2A,B). Further research to obtain quantitative enzyme activity data in optic nerve and spinal cord white matter is necessary for confirmation.

A recent immunocytochemical and immunoblot study showed reduced MAG in the cerebrum, cerebellum and medulla of *shi/shi* mice[20]. Finding a greatly reduced number of MAG-immunostained nerve fibers in the spinal cord of *shi/shi* mice is consistent with this previous study of MAG (Fig. 2C,D). There is no obvious explanation for detecting an increased density of immunostaining for MAG in the optic nerve of *shi/shi* mice. Regional diversity in regulation of expression of myelin components is implicated by this finding. Additional research into the type and quantity of MAG, numbers of oligodendrocytes and type and amount of myelin wrappings in the optic nerve are needed. No such quantitative information is presently available.

The satellite cells surrounding the trigeminal ganglion cell bodies are thought to function in regulating the metabolism and fluid transport of the ganglion cells[3,12]. These functions are supported by our demonstration of the presence of $Na^+,K^+$-ATPase at the periphery of these cells (Fig. 3A,B).

In the PNS (trigeminal ganglion) of *shi/shi* mice, MAG and $Na^+,K^+$-ATPase are typically normal in distribution and relative concentation, which indicates that these two proteins are not affected by the shiverer gene, although MBP is significantly reduced[11]. Thus, the deficit in MBP does not necessarily lead to reduced MAG if myelin is assembled as in the PNS. In the CNS, however, the assembly of myelin depends on MBP. The relative absence of MBP or of myelin assembly may influence the processing and metabolism of other myelin components such as MAG.

The accumulation of MAG immunostaining in oligodendrocyte-like cells of *shi/shi* mice is consistent with evidence that MAG synthesis is normal or potentially normal in *shi/shi* mice[5]. A block in intracellular transport into cell extensions or an inhibitory feedback mechanism resulting from reduced myelin assembly may cause a build-up of immunoreactive MAG in the oligodendrocyte perikarya. Further research will be required to elucidate the specific intracellular site and mechanism of MAG accumulation in oligodendrocytes in the CNS of *shi/shi* mice. These data may increase our understanding of normal processing and transport of myelin membrane components. In CNS myelin-deficient mice, immunostaining for MAG was detected in cytoplasmic organelles (endoplasmic reticulum) of oligodendrocytes[9].

The major findings in this study are that the immunocytochemical distribution and staining density of $Na^+,K^+$-ATPase and MAG in the trigeminal ganglion but not spinal cord or optic nerve are similar in *shi/shi* and +/+ mice. $Na^+,K^+$-ATPase immunoreactive staining is somewhat increased in the optic nerve and lateral funiculus of the shiverer mutant as compared to the control mice. There are regional variations in $Na^+,K^+$-ATPase staining density among the funiculi in both species. The number of MAG-immunostained nerve fibers is significantly reduced throughout the spinal cord whereas MAG immunostaining is slightly increased in the optic nerve of *shi/*

*shi* as compared to +/+ mice. Finally, MAG immunostaining in cytoplasm of oligodendrocyte-like cells found only in *shi/shi* mice may indicate a block of MAG transport in the CNS of *shi/shi* mice.

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REFERENCES

1 Ariyasu, R.G., Nichol, J.A. and Ellisman, M.H., Localization of sodium/potassium adenosine triphosphatase in multiple cell types of the murine nervous system with antibodies raised against the enzyme from kidney, *J. Neurosci.*, 5 (1985) 2581–2596.
2 Bird, T.D., Farrell, D.F. and Sumi, S.M., Brain lipid composition of the shiverer mouse (genetic defect in myelin development), *J. Neurochem.*, 31 (1978) 387–391.
3 Carpenter, M.B. and Satin, J., *Human Neuroanatomy, 8th edn.*, Williams and Wilkins, Baltimore, 1983, pp. 181–182.
4 Figlewicz, D.A., Quarles, R.H., Johnson, D. Barbarash, G.R. and Sternberger, N.H., Biochemical demonstration of the myelin-associated glycoprotein in the peripheral nervous system, *J. Neurochem.*, 37 (1981) 749–758.
5 Frail, D.E. and Braun, P.E., Abnormal expression of the myelin-associated glycoprotein in the central nervous system of dysmyelinating mutant mice, *J. Neurochem.*, 45 (1985) 1071–1075.
6 Inoue, Y., Nakamura, R., Mikoshiba, K. and Tsukada, Y., Fine structure of the central myelin sheath in the myelin deficient mutant shiverer mouse, with special reference to the pattern of myelin formation by oligodendroglia, *Brain Research,* 219 (1981) 85–94.
7 Inuzuka, T., Quarles, R.H., Heath, J. and Trapp, B.D., Myelin-associated glycoprotein and other proteins in trembler mice, *J. Neurochem.*, 44 (1985) 793–797.
8 Kimura, M., Inoko, H., Katsuki, M., Ando, A., Sato, T., Hirose, T., Mikoshiba, K., Tsukada, Y. and Watanabe, I., Molecular genetic analysis of myelin-deficient mice: shiverer mutant mice show deletion in gene(s) coding for myelin basic protein, *J. Neurochem.*, 44 (1985) 692–696.
9 Matthieu, J.M. and Omlin, F.X., Myelin-associated glycoprotein in the CNS of myelin-deficient (MLD) mutant mice. An immunochemical and immunocytochemical study, *Trans. Am. Soc. Neurochem.*, 16 (1985) 139.
10 Mikoshiba, K., Aoki, E. and Tsukada, Y., 2:3′-Cyclic nucleotide 3′-phosphohydrolase activity in the central nervous system of a myelin deficient mutant (shiverer), *Brain Research,* 192 (1980) 195–204.
11 Mikoshiba, K., Kohsaka, S., Takamatsa, K. and Tsukada, Y., Neurochemical and morphological studies on the myelin of peripheral nervous system from shiverer mutant mice: absence of basic proteins common to central nervous system, *Brain Research,* 204 (1981) 455–460.
12 Pineda, A., Maxwell, D.S. and Kruger, L., The fine structure of neurons and satellite cells in the trigeminal ganglion of cat and monkey, *Am. J. Anat.*, 121 (1967) 461–488.
13 Privat, A., Jacque, C., Bourre, J.M., Dupouey, P. and Baumann, N., Absence of the major dense line in myelin of the mutant mouse 'shiverer', *Neurosci. Lett.*, 12 (1979) 107–112.
14 Quarles, R.H., Johnson, D., Brady, R.O. and Sternberger, N.H., Preparation and characterization of antisera to the myelin-associated glycoprotein, *Neurochem. Res.*, 6 (1981) 1115–1127.
15 Roach, A. Takahashi, N., Pravtcheva, D., Ruddle, F. and Hood, L., Chromosomal mapping of mouse myelin basic protein gene and structure and transcription of the partially deleted gene in shiverer mutant mice, *Cell,* 42 (1985) 149–155.
16 Rosenbluth, J., Central myelin in the mouse mutant shiverer, *J. Comp. Neurol.*, 194 (1980) 639–648.
17 Rosenbluth, J., Peripheral myelin in the mouse mutant shiverer, *J. Comp. Neurol.*, 193 (1980) 729–739.
18 Schwartz, M., Ernst, S.A., Siegel, G.J. and Agranoff, B.W., Immunocytochemical localization of $(Na^+ + K^+)$-ATPase in the goldfish optic nerve, *J. Neurochem.*, 36 (1981) 107–115.
19 Sheedlo, H.J., Desmond, T.J. and Siegel, G.J., $(Na^+ + K^+)$-ATPase and MAG localization in the CNS and PNS of shiverer mice, *Trans. Am. Soc. Neurochem.*, 17 (1986) 278.
20 Sheedlo, H.J. and Siegel, G.J., Myelin-associated glycoprotein (MAG) in the CNS of adult shiverer (*shi/shi*) mice, *J. Neurosci. Res.*, 16 (1986).
21 Siegel, G.J., Stahl, W.L. and Swanson, P.D. In G.J. Siegel, R.W. Albers, B.W. Agranoff and R. Katzman (Eds.), *Ion Transport in Basic Neurochemistry,* Little, Brown and Co., Boston, 1981, pp. 107–143.
22 Siegel, G.J., Holm, C., Schreiber, J.H., Desmond, T. and Ernst, S.A., Purification of mouse brain $(Na^+,K^+)$-ATPase catalytic unit, characterization of antiserum, and immunocytochemical localization in cerebellum, choroid plexus, and kidney, *J. Histochem. Cytochem.*, 32 (1984) 1309–1318.
23 Stahl, W.L., $(Na^+,K^+)$-ATPase: function, structure, and conformation, *Ann. Neurol.*, 16 (1984) 5121–5127.
24 Sternberger, N.H., Quarles, R.H., Itoyama, Y. and Webster, H. deF., Myelin-associated glycoprotein demonstrated immunocytochemically in myelin and myelin-forming cells of developing rat, *Proc. Natl. Acad. Sci. U.S.A.*, 76 (1979) 1510–1514.
25 Trapp, B.D. and Quarles, R.H., Presence of the myelin-associated glycoprotein correlates with alteration in the periodicity of peripheral myelin, *J. Bell Ciol.*, 92 (1982) 877–882.
26 Trapp, B.D. and Quarles, R.H., Immunocytochemical localization of the myelin-associated glycoprotein: fact or artifact?. *J. Neuroimmunol.*, 6 (1984) 231–249.
27 Trapp, B.D., Quarles, R.H. and Suzuki, K., Immunocytochemical studies of quaking mice support a role for the myelin-associated glycoprotein in forming and maintaining the periaxonal space and periaxonal cytoplasmic collar of myelinating Schwann cells, *J. Cell Biol.*, 99 (1984) 594–606.
28 Webster, H. deF.,Palkovits, C.G., Stoner, G.L., Favilla, J.T., Frail, D.E. and Braun, P.E., Myelin-associated glyco-

protein: electron microscopic immunocytochemical localization in compact developing and adult central nervous system myelin, *J. Neurochem.*, 41 (1982) 1469–1479.
29 Wood, J.G., Jean, D.H., Whitaker, J.N., McLaughlin, B.J. and Albers, R.W., Immunocytochemical localization of the sodium, potassium activated ATPase in knifefish brain, *J. Neurocytol.*, 6 (1977) 571–581.
30 Yanagisawa, K. and Quarles, R.H., Jimpy mice: quantitation of myelin-associated glycoprotein and other proteins, *J. Neurochem.*, 47 (1986) 322–325