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The Pathophysiology of Myelin Basic Protein-Induced Acute Experimental Allergic Encephalomyelitis in the Lewis Rat

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Abstract

Histological and electrophysiological studies were performed on Lewis rats with acute experimental allergic encephalomyelitis (EAE) induced by inoculation with guinea-pig myelin basic protein (MBP) and Freund's adjuvant. The histological studies showed demyelination in the lumbar, sacral and coccygeal dorsal and ventral spinal roots and to a lesser extent in the spinal cord, including the dorsal root entry and ventral root exit zones. The electrophysiological studies demonstrated reduced conduction velocities between the lumbar ventral roots and sciatic nerve. Conduction block was demonstrated at the ventral root exit zone of the lumbar spinal cord but was less severe than in rats with whole spinal cord-induced acute EAE. Recordings of the M wave and H reflex elicited in a hindfoot muscle by sciatic nerve stimulation showed a normal M wave, indicating normal peripheral nerve motor conduction, but a markedly reduced H reflex. The reduction in the H reflex is accounted for by demyelination-induced nerve conduction block in the dorsal and ventral spinal roots, intramedullary ventral roots and at the dorsal root entry and ventral root exit zones of the spinal cord. Demyelination and nerve conduction abnormalities were well established in the relevant lumbar segments on the day of onset of hindlimb weakness. It is concluded that demyelination in the lumbar ventral roots and to a lesser extent in the lumbar spinal cord, including the ventral root exit zone, is an important cause of hindlimb weakness in myelin basic protein-induced acute EAE in the Lewis rat.

Keywords: conduction abnormalities; demyelination; experimental allergic encephalomyelitis; H reflex; multiple sclerosis; myelin basic protein; peripheral nervous system; spinal roots

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Introduction

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the nervous system induced by inoculation with whole central nervous system (CNS) tissue, myelin basic protein (MBP) or myelin proteolipid protein, and is widely studied as an animal model of multiple sclerosis, a human CNS demyelinating disease of unknown aetiology (Raine 1984). Although CNS demyelination produces neurological signs in multiple sclerosis (Matthews 1985), it has been suggested that the neurological signs of EAE, particularly acute EAE, are due not to demyelination (Panitch and Ciccone 1981; Raine et al. 1981; Simmons et al. 1982; Kerlero de Rosbo et al. 1985) but to other factors such as oedema (Paterson 1976; Simmons et al. 1982; Kerlero de Rosbo et al. 1985; Sedgwick et al. 1987) or an impairment of monoaminergic neurotransmission (White 1984). However, we have recently shown that the neurological signs of whole spinal cord-induced acute EAE in rabbits and rats are accounted for by demyelination in the CNS and peripheral nervous system (PNS) (Pender and Sears 1982, 1984; Pender 1986a, 1987, 1988). Because MBP-EAE has been reported to cause less demyelination than whole white matter-induced EAE (Raine et al. 1981), the present study was undertaken to investigate the pathophysiology of MBP-EAE in the Lewis rat.

Materials and Methods

Induction of EAE

Lewis rats (JC strain) were kept in cages of 5 animals each and fed rat and mouse cubes and water ad libitum. MBP was prepared from guinea-pig spinal cord (the roots having been stripped away) by the method of Deibler et al. (1972). The purity of MBP was ascertained by sodium dodecyl sulphate polyacrylamide gel electrophoresis. MBP in 0.9% saline was emulsified in an equal volume of incomplete Freund's adjuvant containing 4 mg/ml of added *Mycobacterium butyricum*. Under ether anaesthesia, male rats, 8-10 weeks old, received 0.1 ml emulsion in a footpad of each hindfoot. The total dose of MBP was 50 μg per rat. The inoculated animals were examined daily from the 7th day post-inoculation. Histological studies were carried out on 3 male rats with MBP-EAE on the day of onset or day after onset of hindlimb weakness (2-3 days after onset of tail weakness). In terminal experiments, electrophysiological studies were performed on 8 male rats with MBP-EAE, 0-2 days after onset of hindlimb weakness (2-4 days after onset of tail weakness).

Controls

Two and 8 normal male Lewis rats, 10-12 weeks old, served as controls for the histological and electrophysiological studies, respectively. As these studies were performed on the animals with EAE about 2 weeks after inoculation, the control animals were the same age as the animals with EAE at the time of study.

Histological studies

The methods have been described in detail previously (Pender 1985, 1986b). Under ether anaesthesia the rats were perfused through the left ventricle with 0.9 % saline followed by 2.5% (or 2%) glutaraldehyde/2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3-7.4). The brain, optic nerves, spinal cord, dorsal and ventral roots, dorsal root ganglia, spinal nerves, and sciatic and tail nerves were removed and immersed in fixative. The tissues were post-fixed with 2% osmium tetroxide and were embedded in either HistoResin (LKB Bromma) or Epok 812 (Ernest F. Fullam, Inc., Schenectady, NY). HistoResin sections (2 μm) were stained with toluidine blue in phosphate buffer (pH 7.6) or with cresyl violet. Semi-thin (1 µm) Epok 812 sections were stained with toluidine blue for light microscopy, and ultrathin sections were double-stained with uranyl acetate and lead citrate and examined in a Philips 301 electron microscope.

Electrophysiological studies

(a) *Anaesthesia.* Anaesthesia was induced with urethane (25% in 0.9% saline, intraperitoneal (i.p.), 5 ml/kg for controls, 4-5 ml/kg for rats with EAE undergoing laminectomy for ventral root (VR) and ventral root exit zone (VREZ) recordings, and 4 ml/kg for rats with EAE undergoing H reflex studies but not laminectomy). The dose of urethane per kg required to obtain a given depth of anaesthesia was lower in rats with EAE than in normal controls. Pentobarbitone sodium (i.p., 12 mg/kg) supplemented the urethane anaesthesia. The animals breathed spontaneously through a tracheostomy. At the beginning of each experiment 9 ml of Hartmann's solution (compound sodium lactate BP, Travenol) were given i.p.

(b) *M wave and H reflex recordings.* The rat was mounted in a frame, and a metal box, through which water at 37°C was circulated, was placed under the animal. Because of the effects of anaesthetics on synaptic transmission it was important that, for the H reflex studies, an adequate depth of anaesthesia was maintained without depressing the corneal reflex. The left sciatic nerve was exposed in the posterior thigh and immersed in paraffin oil maintained at 37 °C by radiant heat. Under these conditions the rectal temperature was 37-38°C. The freed sciatic nerve was lifted away from the volume conductor and stimulated in continuity with platinum electrodes 3 mm apart (cathode distal) delivering 0.1 msec square-wave voltage pulses at 1.0 Hz. Recordings were made with a 25-gauge needle electrode in the belly of the fourth dorsal interosseus muscle and with a reference 25 gauge needle electrode subcutaneously in the plantar aspect of the distal fourth digit of the left hindfoot.

(c) *VR and VREZ recordings.* A T12-L4 laminectomy was performed and the rat was prepared as previously described (Pender 1986a, 1988). The left sciatic nerve was stimulated in continuity as above except that the polarity of the electrodes was reversed. Volume conductor recordings were made with a 0.5 mm diameter silver ball electrode over each of 2 or 3, in turn, of the left L4, L5 and L6 VRs, 1-3 mm distal to the respective VREZs, and over the rostral parts of these VREZs. A reference electrode was placed on the right paravertebral region at the same level. The L4 and L5 spinal nerves always gave large contributions to the sciatic nerve and the L6 spinal nerve gave a small contribution.

Statistical analysis

Analysis of variance was used to compare the recordings from normal control rats with those from rats with MBP-induced acute EAE, rats with whole spinal cord-induced acute EAE (Pender 1988) and rats recovering from each form of EAE (Pender, unpublished data) to minimize the chance occurrence of statistically significant differences when multiple comparisons were made. For all statistically significant differences reported in the present paper except when otherwise stated, the probability of the respective *F* statistic was <0.05.

Results

Clinical findings

Distal tail weakness commenced 8-13 days after inoculation. The weakness ascended the tail and was accompanied by an ascending impairment of tail nociception as previously reported (Pender 1986b). By 2 days after the onset of tail weakness the tail showed complete flaccid paralysis, and weakness had commenced in the distal hindlimbs. The hindlimb weakness progressed over the following day, sometimes to the stage of complete flaccid paralysis. Forelimb weakness occurred occasionally. Hindlimb weakness lasted about 3 days and then resolved. In a typical animal with tail weakness commencing 10 days after inoculation, the hindlimbs showed full clinical recovery 15 days after inoculation. Motor function in the tail also improved and by 18 days after inoculation there was only mild tail weakness.

Histological findings

(a) Light microscopy

In the brainstem and spinal cord there were meningeal and subpial infiltrates and perivascular cuffs and infiltrates of mononuclear cells. Mild demyelination was present in the regions of mononuclear infiltration (Fig. 1). The dorsal root entry and ventral root exit zones were sites of predilection (Fig. 2). In the spinal cord there were also fibres with marked dilatation of the myelin sheath due to expansion of the periaxonal space as described by Brosnan et al. (1988). The cerebellum was only mildly affected and there was minimal cerebral involvement. In the PNS there was prominent perivascular mononuclear infiltration and demyelination in the lumbar, sacral and coccygeal ventral and dorsal roots (Fig. 3A). The dorsal root ganglia were mildly affected and the spinal and peripheral nerves showed no or minimal involvement.

(b) Electron microscopy

Electron microscopy confirmed that there was primary demyelination (Fig. 3B), which involved small diameter myelinated fibres as well as large ones. Axonal degeneration was occasionally seen in the dorsal and ventral roots.

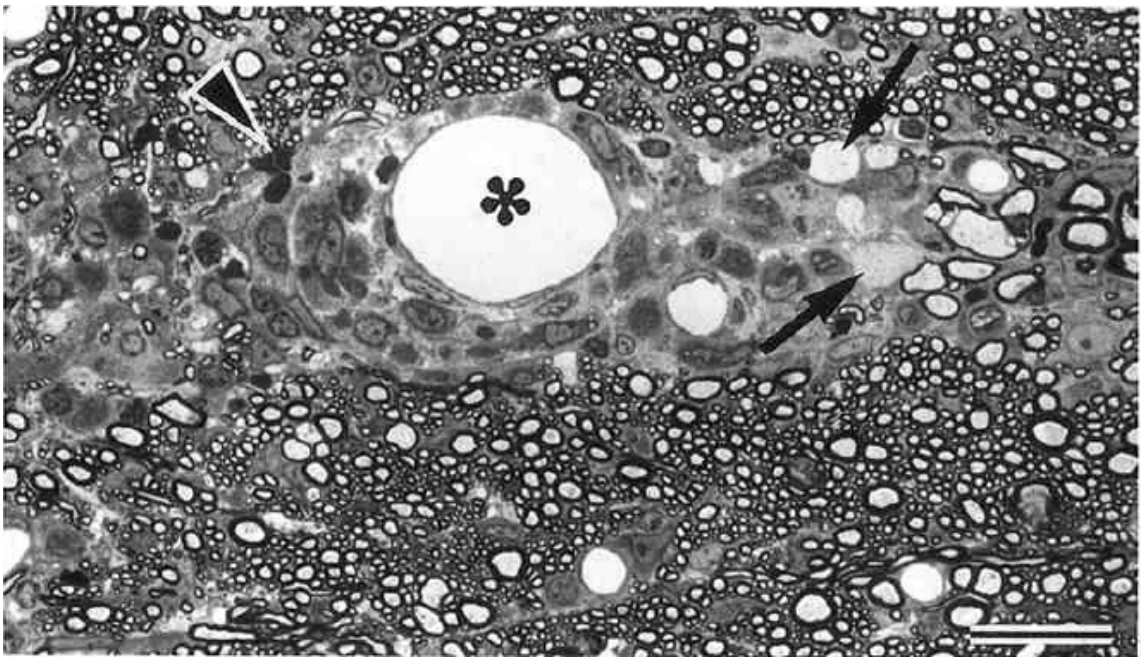

Fig. 1. Transverse section through a ventral column of the L5 spinal cord of a rat with severe hindlimb weakness due to MBP-induced acute EAE, 3 days after onset of tail weakness and 1 day after onset of hindlimb weakness. There is perivascular cuffing with mononuclear cells (asterisk in vessel) and infiltration of these cells into the adjacent neural parenchyma. There is also extravasation of erythrocytes (arrowhead).Some of the fibres destined for the ventral root are demyelinated (arrows). Epok 812 section stained with toluidine blue. Bar = 25 μm

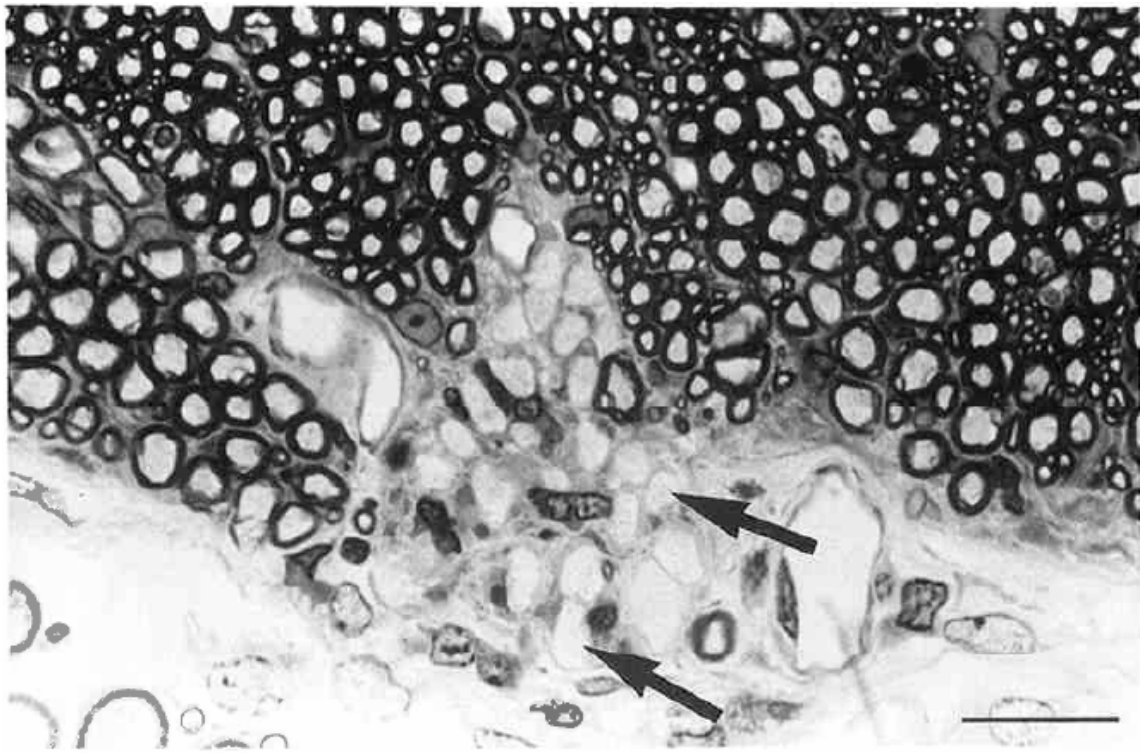

Fig. 2. Transverse section through a ventral root exit zone of the L4 spinal cord segment of a rat with severe hindlimb weakness due to MBP-induced acute EAE, 2 days after onset of tail weakness and on day of onset of hindlimb weakness. Demyelinated axons (arrows) can be seen in the CNS part of the ventral root exit zone. HistoResin (LKB Bromma) section stained with cresyl violet. Bar = 25 μm.

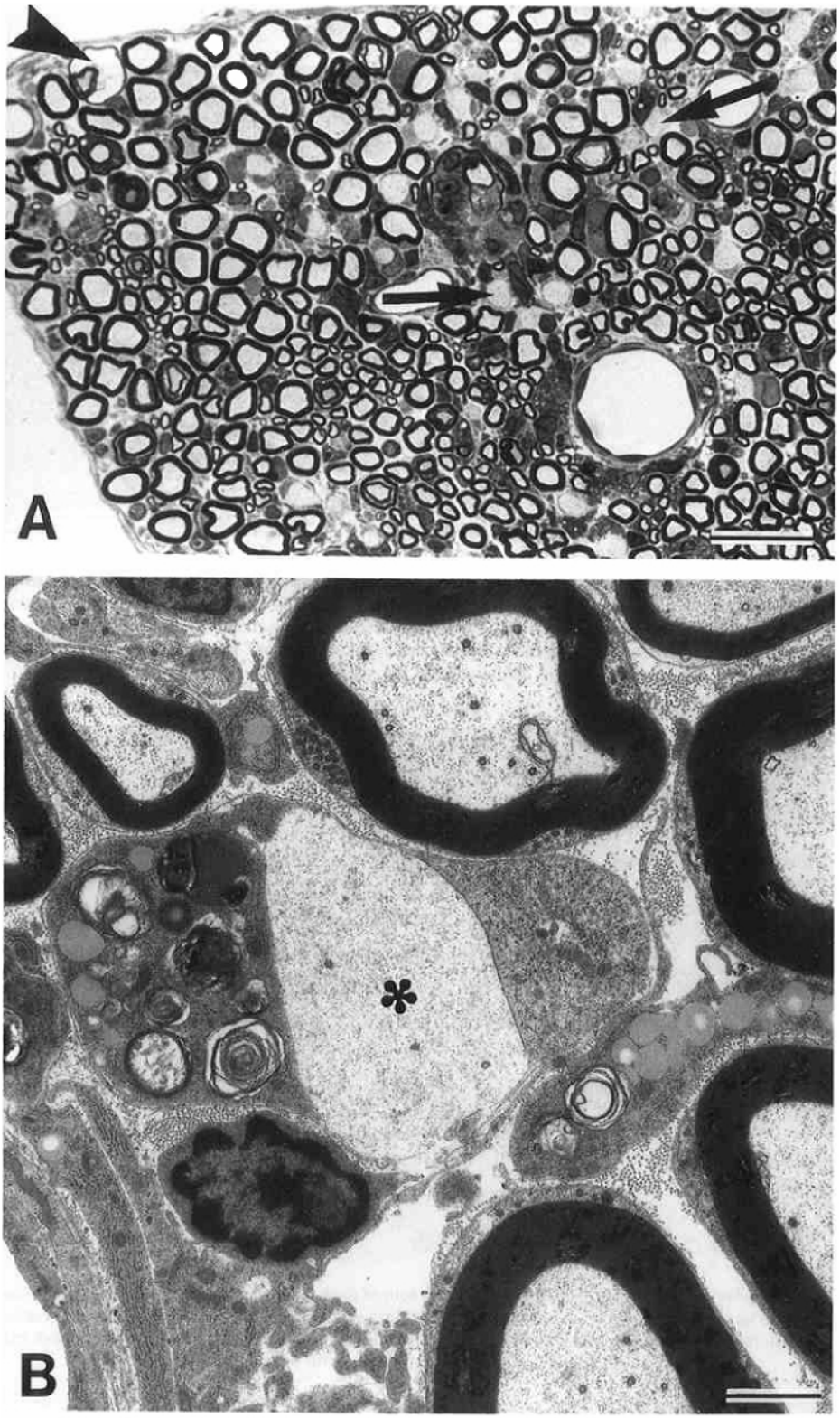

Fig. 3. (A) Transverse section through a L6 ventral root of a rat with severe hindlimb weakness due to MBP-induced acute EAE, 3 days after onset of tail weakness and 1 day after onset of hindlimb weakness. There are many demyelinated axons (arrows). Splitting and wide distension of one myelin sheath is present (arrowhead). Epok 812 section stained with toluidine blue. Bar = 25 μm. (B) Electron micrograph of same root showing demyelinated axon (asterisk) associated with a macrophage containing myelin debris. Bar = 2 μm.

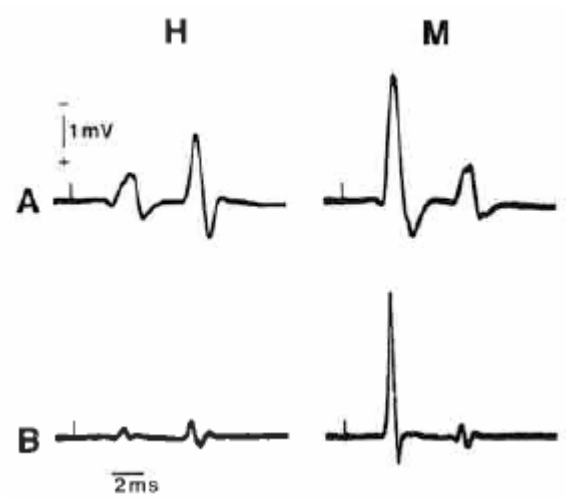

Fig. 4. Maximal H reflex (H) and maximal M wave (M) elicited in the left fourth dorsal interosseus muscle by sciatic nerve stimulation in a normal control rat (A) and in a rat on the day of onset of hindlimb weakness due to MBP-induced acute EAE (B). The M wave has a shorter latency than the H reflex. The difference in width of the maximal M wave in A and B is due to a difference in distal limb temperature. However, the sciatic pool and rectal temperature, on which the M-H latency depends, were 37°C in both A and B.

Electrophysiological findings

(a) M wave and H reflex studies

In normal control rats a M wave and a longer latency H reflex were recorded from the fourth dorsal interosseus muscle of the hindfoot when the ipsilateral sciatic nerve was stimulated (Fig. 4A). The M wave is due to direct activation of motor fibres in the sciatic nerve while the H reflex is a monosynaptic reflex mediated by the L5 dorsal root and the L5 and L6 ventral roots (Pender 1988). In rats with hindlimb weakness due to MBP-EAE, the M wave was normal in amplitude and latency indicating normal peripheral nerve motor conduction (Fig. 4B, Table 1). However, the H reflex was markedly reduced in amplitude, without temporal dispersion, indicating interruption of the monosynaptic reflex arc (Fig. 4B, Table 1). The ratio of the peak-to-peak amplitude of the maximal H reflex to that of the maximal M wave serves as a reliable indicator of the integrity of the monosynaptic reflex arc. The mean ratio in rats with hindlimb weakness due to MBP-EAE was significantly less than in normal controls (Table 1). The difference between the latency to the onset of the H reflex and the latency to the onset of the M wave is the time for transmission through the monosynaptic reflex arc from the sciatic nerve back to the sciatic nerve at 37°C. The mean time in rats with hindlimb weakness due to MBP-EAE was longer than that in normal controls (Table 1).

TABLE 1

Maximal M wave and maximal H reflex in fourth dorsal interosseus muscle in normal control rats and in rats with hindlimb weakness due to MBP-induced acute EAE

	Controls (n = 5) mean ± SD	EAE (n = 5) mean ± SD	Statistical significance of difference between groups (ANOVA)
M amp[a] (mV)	4.6 ± 1.1	5.7 ± 1.3	NS[b]
H amp/M amp	0.50 ± 0.09	0.19 ± 0.11	$P < 0.001$
M latency to onset (msec)	2.23 ± 0.34	2.42 ± 0.24	NS
M-H latency[c] (msec)	4.62 ± 0.28	5.12 ± 0.52	$P < 0.05$[d]

[a] Peak-to-peak amplitude.
[b] Not significant ($P > 0.05$).
c Difference between latency to onset of maximal M wave and latency to onset of maximal H reflex.
[d] But P of $F > 0.05$.

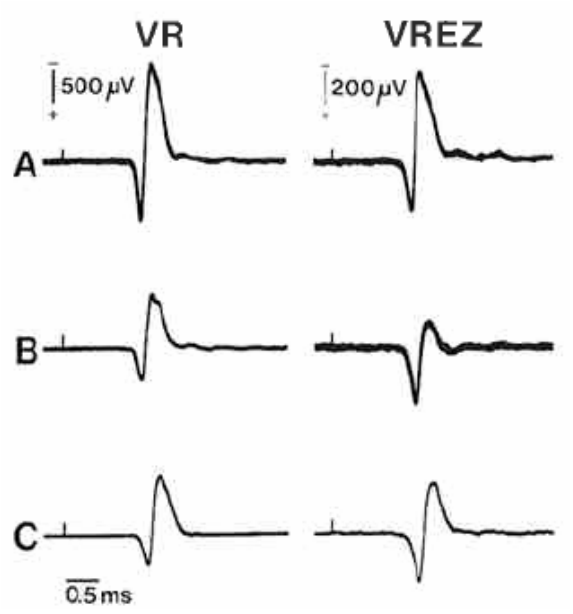

Fig. 5. Volume conductor recordings of the maximal L4 ventral root (VR) and L4 ventral root exit zone (VREZ) compound action potentials evoked by sciatic nerve stimulation in a normal control rat (A), in a rat with severe hindlimb weakness due to whole spinal cord-induced EAE (B) and in a rat with severe hindlimb weakness due to MBP-induced EAE (C).

TABLE 2

L4 and L5 ventral root (VR) conduction velocities in normal control rats and in rats with hindlimb weakness due to MBP-induced EAE

	Controls (n = 3) range (mean ± SD)	EAE (n = 2) values	Statistical significance of difference between groups (ANOVA)
CV[a] peak positivity L4 VR (m/sec)	68.4-70.0 (69.3 ± 0.8)	63.8 61.2	$P < 0.025$
CV peak negativity L4 VR (m/sec)	59.9-61.0 (60.5 ± 0.6)	54.0 51.5	$P < 0.005$
CV peak positivity L5 VR (m/sec)	67.6-72.3 (69.8 ± 2.4)	62.5 65.2	$P < 0.01$
CV peak first negativity L5 VR (m/sec)	58.1-60.9 (59.6 ± 1.4)	50.9 53.4	$P < 0.01$

[a] Conduction velocity at 37 °C.

(b) VR and VREZ recordings

To determine if the lumbar VREZ was a major site of nerve conduction block in MBP-EAE as it is in whole spinal cord-induced EAE (Pender 1986a, 1988), volume conductor recordings were made over the lumbar VRs and VREZs when the sciatic nerve was stimulated. Recordings were made over 2 of the L4, L5 and L6 segments in each of 3 rats with hindlimb weakness due to MBP-EAE. In the normal rat the L4 VR response is a biphasic wave (positive, negative) with the negativity having a greater amplitude than the positivity (Pender 1986a, 1988) (Fig. 5A). Of 4 rats with hindlimb weakness due to whole spinal cord-induced EAE the L4 VR response was normal in amplitude and configuration in 3 (Pender 1986a, 1988) and reduced in peak-to-peak amplitude and temporally dispersed in the other (Fig. 5B). Such a reduction in peak-to-peak amplitude was also seen in the L4 VR response in MBPEAE (Fig. 5C). In rats with MBP-EAE the conduction velocities of the L4 and L5 VR peak positivities and negativities were reduced compared to normal controls (Table 2). Although only 4 roots were studied in 3 animals with MBP-EAE, analysis of variance showed statistically significant differences from normal controls, and the conduction velocities of the L4 and first L5 VR peak negativities were more than 4 standard deviations below the normal mean (Table 2). These findings indicate conduction slowing and/or block in fibres between the sciatic nerve and ventral root.

The L4 VREZ response in the normal rat is similar to the L4 VR response (Fig. 5A). In all 4 rats with hindlimb weakness due to whole spinal cord-induced EAE, the L4 VREZ response had a markedly reduced negativity, without temporal dispersion,

indicating conduction block in many fibres at the VREZ (Pender 1986a, 1988) (Fig. 5B). In rats with a similar degree of hindlimb weakness due to MBP-EAE the proportion of fibres blocked at the L4 VREZ was smaller (Fig. 5C). The ratio of the amplitude of the negativity to that of the initial positivity in the maximal L4 VREZ compound action potential, which serves as a reliable indicator of conduction block, was 1.0 in each of two rats with MBP-EAE compared to a mean of 0.6 ± 0.1 (n = 4) in whole spinal cord-induced EAE and a mean of 2.1 ± 0.2 (n = 3) in normal controls (Pender 1986a, 1988). There were statistically significant differences in the mean ratio between each of the following pairs of groups: MBP-EAE and controls ($P < 0.001$); MBP-EAE and whole spinal cord-induced EAE ($P < 0.05$); whole spinal cord-induced EAE and controls ($P < 0.001$). Furthermore, in contrast to rats with whole spinal cord-induced EAE where conduction block was demonstrated in all 10 L5 and L6 VREZs studied (Pender 1986a, 1988), conduction block was not demonstrated at any of 4 L5 and L6 VREZs in rats with a similar degree of hindlimb weakness due to MBP-EAE.

Discussion

The marked reduction in the amplitude, without temporal dispersion, of the H reflex in rats with MBP-EAE indicates either (1) conduction block in fibres mediating the reflex or (2) impairment of synaptic transmission between the Ia afferent terminals and motor neurones. The reduction in ventral root motor conduction velocity, the conduction block at the VREZ and the evidence from a previous study (Pender 1986b) of nerve conduction block in the dorsal roots indicate that axonal conduction block is the cause. The prolonged time of transmission through the monosynaptic reflex arc, as shown by the prolonged M-H latency, is explained by conduction slowing and/or block of the fastest fibres mediating this reflex. The demonstrated demyelination in the dorsal roots, dorsal root entry zone, intramedullary ventral roots (Fig. 1), VREZ and ventral root is likely to be responsible for these nerve conduction abnormalities. This conclusion is strongly supported by the recent finding that early clinical recovery is accompanied by restoration of the H reflex, investment of PNS and CNS demyelinated axons by debris-free Schwann cells and oligodendrocytes respectively and early remyelination (Pender, unpublished data). Such investment by Schwann cells has been shown to be associated with the restoration of nerve conduction by the development of electrical excitability in internodal membrane demyelinated by lysophosphatidyl choline (Smith et al. 1982).

MBP-EAE differs from whole spinal cord-induced EAE in that the nerve conduction block is less severe at the VREZ. Nevertheless, the present study indicates that there is sufficient demyelination in the lumbar ventral roots and to a lesser extent in the lower motor neurone pathway in the lumbar spinal cord, including the VREZs, to account for the hindlimb weakness in MBP-EAE. However, as conduction through descending motor pathways in the brainstem and spinal cord was not studied, it is not possible to exclude a contribution to the hindlimb weakness by demyelination of these pathways. Demyelination and nerve conduction abnormalities were well established in the relevant lumbar segments on the day of onset of hindlimb weakness. As the γ-loop is necessary for maximal skeletal muscle contraction (Hagbarth et al. 1986), demyelination

of the smaller diameter motor (β and γ) fibres as well as of the larger (α) fibres is likely to cause hindlimb weakness. Demyelination of the lumbar dorsal roots would also interrupt the γ loop and may therefore also contribute to the hindlimb weakness. The tail weakness in MBP-EAE is readily accounted for by demyelination of the sacrococcygeal ventral roots (and perhaps dorsal roots) and of the sacrococcygeal spinal cord segments. It should be noted that the same neurological signs of tail paralysis and hindlimb paralysis and similar nerve conduction abnormalities in the spinal roots occur in rats with experimental allergic neuritis, a disease confined to the PNS (Izumo et al. 1985; Heininger et al. 1986).

The present findings of CNS and PNS demyelination in rats with MBP-EAE confirm previous reports (McFarlin et al. 1974; Dal Canto et al. 1977). The failure of other studies to detect demyelination in MBP-EAE in Lewis rats may be due to the use of inadequate histological techniques and to incomplete examination of the nervous system, particularly the PNS and the lumbar, sacral and coccygeal segments of the spinal cord. Demyelination was less severe in the spinal cord and more prominent in the spinal roots in rats with MBP-induced EAE than in rats with whole spinal cord-induced EAE (Pender, unpublished data). However, in the spinal cords of rats with MBP-EAE there was another myelin abnormality, namely marked dilatation of the myelin sheath due to expansion of the periaxonal space. Such axon-myelin dissociation has recently been described by Brosnan et al. (1988) in rats with MBPEAE and might also cause nerve conduction abnormalities. The involvement of the PNS when animals are inoculated with purified CNS MBP is explained by the fact that the P_1 protein from the PNS is similar, if not identical, to CNS MBP (Brostoff and Eylar 1972; Greenfield et al. 1973). The distribution of demyelination in MBP-EAE is likely to be determined by the concentration of the relevant MBP epitope, the association of this epitope with class II (Ia) major histocompatibility complex antigens, and the blood-tissue barrier in the different regions of the CNS and PNS. The preferential involvement of the spinal roots with sparing of the peripheral nerves in the rat may be due to the reduced blood-nerve barrier in the roots (Olsson 1968) and the almost 3-fold higher concentration of P_1 in the rat spinal root than in rat peripheral nerve (Greenfield et al. 1973). In guinea-pigs, sensitization to MBP would be expected to cause more peripheral nerve involvement than in the rat because of the reduced blood-nerve barrier (Waksman 1961) and the much higher concentration of P_1 in the peripheral nerve (Greenfield et al. 1973). Demyelination of the peripheral nerves and other regions of the PNS may account for the occurrence of neurological signs in the absence of CNS demyelination in guinea-pigs with bovine MBP-induced EAE (Raine et al. 1981).

MBP may also be a target antigen in the human demyelinating diseases. Sensitization to MBP occurs in patients developing neurological complications after inoculation with rabies vaccine containing sheep brain (Hemachudha et al. 1987a) and could thus account for the PNS involvement seen in such cases (Appelbaum et al. 1953; Swamy et al. 1984; Hemachudha et al. 1987b). As sensitization to MBP also occurs in post-infectious encephalomyelitis (Lisak et al. 1974; Johnson et al. 1984), PNS involvement would also be expected and could possibly account for many of the neurological signs in this disease. Although multiple sclerosis may sometimes involve the PNS, the neurological signs of multiple sclerosis are generally regarded as being due

to CNS demyelination (Matthews 1985). If MBP is the target antigen in multiple sclerosis, the relevant epitope must be recognised in the CNS but not in the PNS.

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