Brain Research, 367 (1986) pp. 398–401.

Conduction Block Due To Demyelination At The Ventral Root Exit Zone In Experimental Allergic Encephalomyelitis

M. P. Pender

Abstract
Histological and electrophysiological studies were performed in Lewis rats with experimental allergic encephalomyelitis (EAE) to determine the cause of the neurological signs. The ventral root exit zone of the spinal cord was shown to be a major site of demyelination and conduction block. It is concluded that demyelination-induced conduction block in this region is an important cause of hind-limb weakness and paralysis in Lewis rats with EAE.

Keywords
experimental allergic encephalomyelitis; ventral root exit zone; demyelination; conduction block; Lewis rat; hindlimb weakness

Experimental allergic encephalomyelitis (EAE), an autoimmune disease of the nervous system, is widely studied as an animal model of multiple sclerosis (MS), a human central nervous system (CNS) demyelinating disease of unknown actiology[16]. The clinical status is the main guide to the progress of both EAE and MS, and the suppression of or improvement in the neurological signs of EAE by therapeutic agents[1,9] provides the basis for the use of these agents in MS. In MS, CNS demyelination contributes significantly to the development of neurological signs[11]. However, the cause of the neurological signs of EAE (hindlimb ataxia, weakness and paralysis and tail paralysis) in the most widely studied animals, the small rodents, is unknown[18]. It has been suggested that these signs are due not to demyelination but to other factors such as oedema[12,18] or an impairment of serotonergic neurotransmission[4,21]. Further more, peripheral nervous system (PNS) lesions occur in EAE[5,10,15,19] and, in the rabbit, demyelination-induced conduction block in the PNS, specifically the dorsal root ganglion (DRG), contributes significantly to the production of neurological signs[13,14].

It is clearly important to determine whether CNS demyelination contributes to the development of neurological signs in EAE as it does in MS. Therefore, histological and electrophysiological studies have been performed in Lewis rats with EAE to determine the site and nature of the lesions responsible for the neurological signs. It has been recently shown that in these animals the DRG involvement is relatively mild in comparison with that in the rabbit and is of little functional significance and that conduction through the lumbar dorsal root entry zone is normal (Fender, M.P. and Sears, T.A., submitted for publication). I now report that, in these animals, the ventral root exit zone (VREZ) of the spinal cord is a major site of demyelination and conduction block.

Acute EAE was induced in 8-10-week-old Lewis rats by inoculation with 0.2 ml (0.05 ml in the footpad of each foot) of a homogenate of equal volumes of a 30% suspension of guinea pig spinal cord in 0.9% saline and of a suspension of killed *Mycobacterium butyricum* (Difco) (4 mg/ml) in incomplete Freund's adjuvant. Tail weakness commenced 8-14 days after inoculation and was followed by flaccid tail paralysis and hindlimb weakness, and sometimes by complete hindlimb paralysis. Forelimb weakness occurred occasionally. Under ether anaesthesia, rats with hindlimb weakness were perfused through the left ventricle with 2% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3-7.4). The CNS and PNS tissues were postfixed with 2% osmium tetroxide, dehydrated, embedded in either epoxy resin or HistoResin (LKB, Bromma), sectioned (1-2 μm) and stained with toluidine blue. The histological studies showed subpial and perivascular inflammation and demyelination in the CNS, especially the lumbar, sacral and coccygeal segments of the spinal cord, and perivascular inflammation and demyelination in the PNS, predominantly the DRGs. The spinal cord VREZ was a site of predilection for demyelination, which predominantly affected its CNS glial (oligodendrocyte myelinated) part rather than its PNS non-glial (Schwann cell myelinated) part (Fig. 1)

Electrophysiological studies were performed in terminal experiments on 6 male rats with hindlimb weakness due to EAE, 1-4 days after the onset of neurological signs, and in 3

normal 10-12-week-old male rats. Anaesthesia was induced with 25% urethane (5 ml/kg, intraperitoneal (i.p.)) and supplemented with pentobarbitone sodium (12 mg/kg, i.p.). A T12-L4 laminectomy was performed and the left sciatic nerve was exposed in the posterior thigh. Laminectomy and sciatic nerve pools were made and the Jura was opened. The tissues were immersed in liquid paraffin maintained at 37°C by radiant heat. The left L2-L6 dorsal roots were cut close to the cord and displaced laterally. By freeing the two most caudal left denticulate ligaments from the dura and tying them to right paravertebral tendons, the spinal cord was rotated through 90° so that its ventral surface faced laterally to the left (Fig. 2). The left sciatic nerve was stimulated in continuity, and recordings were made in the volume conductor with a 0.5 mm diameter silver ball electrode over one or more, in turn, of the left L4, L5 and L6 ventral roots, 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. At the end of each experiment the dissection was extended to confirm that the L4, L5 and L6 spinal nerves contributed to the sciatic nerve.

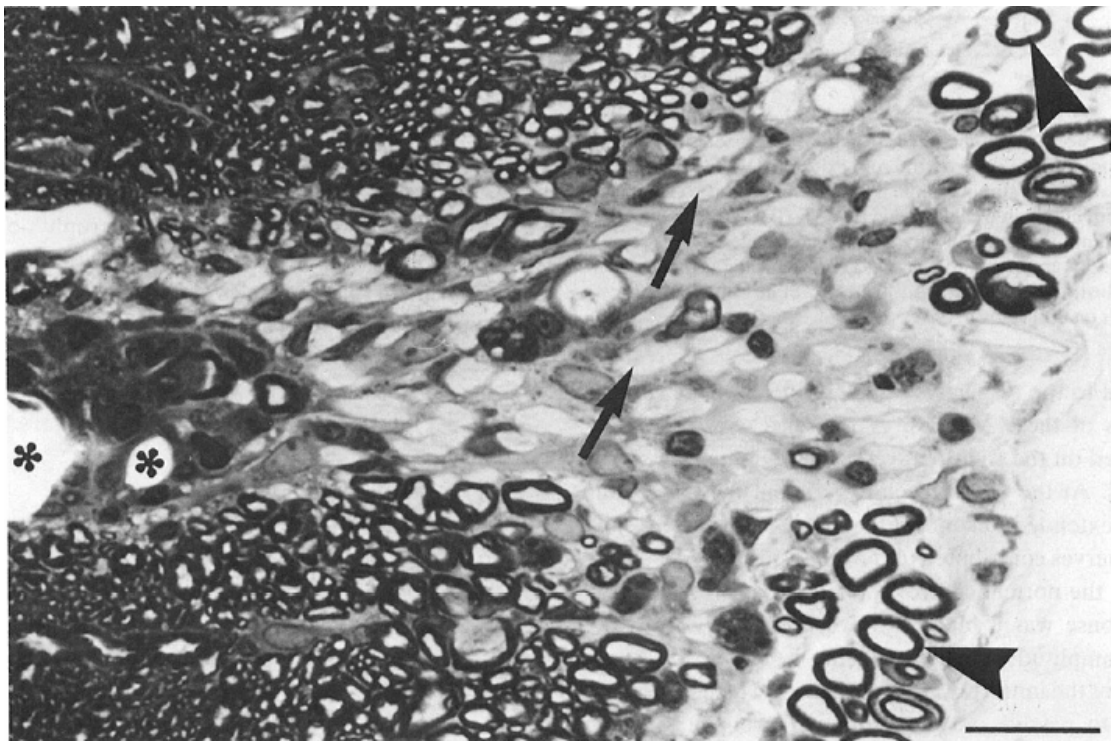

Fig. 1. Transverse section through a L5 ventral root exit zone (VREZ) of the spinal cord of a rat with EAE, two days after the onset of neurological signs. Demyelinated axons (arrows) are present in the CNS part of the VREZ. There is perivascular cuffing and infiltration with mononuclear cells (asterisks in vessels). Normal ventral root myelinated fibres can be seen to the right (arrowheads). HistoResin section, cresyl violet stain. Bar = 25μm.

In the normal control animals the L4 ventral root response was a biphasic wave (positive, negative), the amplitude of the negativity being greater than that of the initial positivity (Fig. 2A). The positivity is due to passive outward current driven by the approaching impulses, and the negativity is due to active inward current occurring during the rising phase of the action potential under the active recording electrode. The L4 VREZ response is very similar(Fig. 2A). In the rats with EAE, the L4 ventral root response was normal, but there was a marked reduction in the amplitude of the negativity, without temporal dispersion, in the L4 VREZ response, indicating conduction block in a high proportion of the large diameter myelinated fibres in the region of the VREZ. (Fig. 2B). The ratio of the amplitude of the negativity to that of the initial positivity in the maximum L4 VREZ, response was 0.6 ± 0.1 (± S.D.; n = 4) in the rats with EAE, compared to 2.1 ± 0.2 (n = 3) in normal control rats ($P < 0.001$, Student's *t*-test). Conduction block was also present at the L5 and L6 VREZs (Fig. 3) and was demonstrated at all of the 14 lumbar VREZs studied in the 6 animals with EAE. The demyelination at the VREZ accounts for the conduction block.

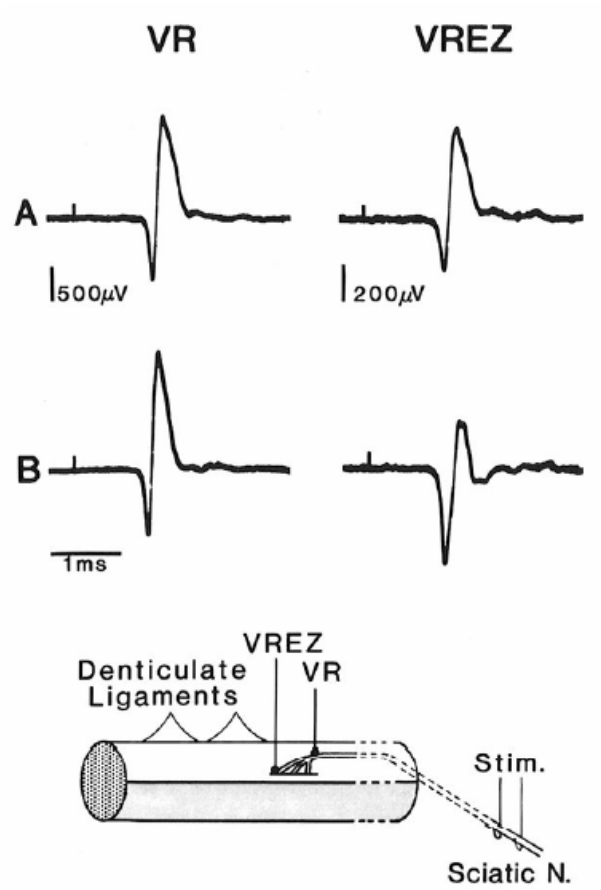

Fig. 2. Volume conductor recordings of the maximum L4 ventral root (VR) and L4 ventral root exit zone (VREZ) responses to sciatic nerve stimulation in a normal control rat (A) and in a rat with hindlimb weakness due to EAE (B). For these and the recordings in Fig. 3, negativity at the active electrode is represented by an upward deflection. The recording arrangements are illustrated diagrammatically below. Stim., stimulating electrodes on the sciatic nerve.

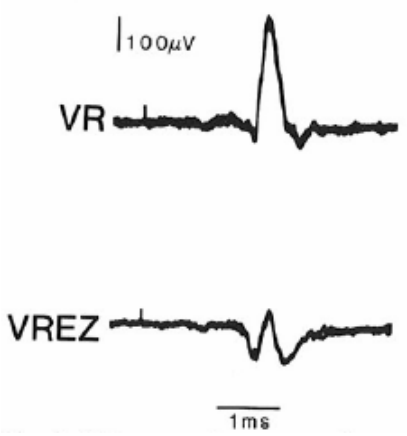

Fig. 3. Volume conductor recordings of the maximum L6 ventral root (VR) and L6 ventral root exit zone (VREZ) responses to sciatic nerve stimulation in a rat with hindlimb weakness due to EAE.

Conduction block at the VREZ. could explain the reported failure to elicit reflex contraction of the anterior tibial muscle by posterior tibial nerve stimulation in rats with EAE[2]. On the other hand, White[20] was able to elicit lumbar monosynaptic responses in rats with hindlimb paralysis due to EAE; however, as the response amplitudes were relatively low in normal and EAE rats and inter-animal variability was high, the sensitivity of this method for detecting conduction block is likely to be low. Pender and Sears[14] have recently

suggested that demyelination at the VREZ may account for limb weakness in rabbits with EAE but did not assess this region electrophysiologically. It is often stated that the recovery of function in EAE and in some episodes of MS is too rapid to be accounted for by remyelination and thus indicates that demyelination is not responsible for the neurological signs[6,17]. However, such rapid recovery could be due to the development of continuous conduction in demyelinated nerve fibres[3] or to repair of structurally minor, yet functionally significant, damage to the myelin sheath, for example loosening of the paranodal axoglial junction[7].

It is concluded that demyelination-induced conduction block in the region of the VREZ is an important cause of hindlimb weakness and paralysis in Lewis rats with EAE. While this demyelination is predominantly in the CNS, as in the case of MS, its distribution differs from that of the demyelination responsible for the limb weakness in MS. In the latter the weakness is of the upper motor neurone type owing to involvement of descending motor pathways[8].

This work was supported by the National Multiple Sclerosis Society of Australia. I thank Mr. Cameron McCrae and Ms. Ailsa Rolinson for excellent technical assistance.

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