THE PATHOPHYSIOLOGY OF CHRONIC RELAPSING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE LEWIS RAT

by G. P. STANLEY and M. P. PENDER

SUMMARY

Electrophysiological studies were performed in Lewis rats with chronic relapsing experimental allergic encephalomyelitis (EAE) induced by inoculation with guinea-pig spinal cord and adjuvants and treatment with low dose cyclosporin A. During clinical episodes there was conduction failure in the central nervous system (CNS), namely the spinal cord dorsal columns, and in the afferent fibres in the peripheral nervous system (PNS). The following observations indicated that the conduction failure was mainly due to demyelination-induced conduction block: (1) rate-dependent conduction block in the CNS and PNS; (2) temporal dispersion due to slowing of PNS conduction; (3) restoration of PNS conduction by cooling; (4) restoration of CNS conduction by ouabain; (5) previously demonstrated histological evidence of primary demyelination in the dorsal columns, dorsal root ganglia and dorsal roots; and (6) the temporal association of restoration of conduction with remyelination. However, it is likely that CNS and PNS axonal degeneration, which occurs in this disease, also contributed to the conduction failure. In clinical remissions there was restoration of conduction in the CNS and PNS which can be explained by ensheathment/remyelination by oligodendrocytes and Schwann cells, respectively. In most rats during clinical episodes the cerebral somatosensory evoked potential was reduced in amplitude and prolonged in latency, which can be accounted for by demyelination and axonal degeneration in the CNS and PNS components of the afferent pathway. In 2 rats with episodes of EAE, however, this potential was markedly increased in amplitude, which might have been due to demyelination-induced conduction block of descending pathways that normally inhibit synaptic transmission in the afferent pathway. In well-established remission there was residual conduction failure in the CNS and PNS which can be mainly accounted for by axonal degeneration.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is an autoimmune demyelinating disease and is widely studied as a possible animal model of the human central nervous system (CNS) demyelinating diseases, particularly multiple sclerosis (Raine, 1984). EAE may have an acute or chronic relapsing course. Acute EAE is monophasic like the human disease, acute disseminated encephalomyelitis. Chronic relapsing EAE has a chronic relapsing course and produces large plaques of CNS demyelination as in multiple sclerosis. The pathophysiology of acute EAE has been studied in detail in the rabbit (Pender and Sears, 1982, 1984, 1985) and in the Lewis rat (Pender, 1986a, b, 1988a, b, 1989; Pender and Sears, 1986; Heininger et al., 1989). These studies have revealed demyelination and nerve conduction abnormalities in the dorsal root ganglia of rabbits (Pender and Sears, 1982, 1984, 1985) and rats with acute EAE induced by inoculation with whole spinal cord (Pender and Sears, 1986) and in the CNS portions of the spinal cord ventral root exit zones in these rats (Pender, 1986a, 1988a). In rats with acute EAE induced by inoculation with myelin basic protein, demyelination and nerve conduction abnormalities were demonstrated in the dorsal roots, ventral roots and ventral root exit zones (Pender, 1986d, 1988b). In rats with acute EAE adoptively transferred with myelin basic protein-specific T lymphocytes, Heininger et al. (1989) demonstrated conduction abnormalities in the spinal cord and spinal roots which they attributed to demyelination. During recovery from acute EAE in the rat there is restoration of conduction in the CNS and peripheral nervous system (PNS) associated with ensheathment/remyelination by oligodendrocytes and Schwann cells, respectively (Pender, 1989).
Visual and auditory evoked potentials have been assessed in guinea-pigs with chronic relapsing EAE (Lidsky et al., 1980; Wisnicwski et al., 1982) but there have been no other studies of the pathophysiology of chronic relapsing EAE. We have therefore undertaken electrophysiological studies on the surgically exposed nervous system to assess function in the CNS and PNS during episodes and remissions of chronic relapsing EAE in the Lewis rat. These studies were performed on the background of a detailed neuropathological assessment which revealed inflammation and demyelination in the CNS, particularly the spinal cord, and in the PNS, specifically the ventral and dorsal roots and dorsal root ganglia, during the first and second episodes, and CNS and PNS remyelination during the first and second remissions (Pender et al., 1990). Brief preliminary reports of these electrophysiological studies have been published in abstract form (Stanley and Pender, 1989; Pender and Stanley, 1989).

**MATERIAL AND METHODS**

**Animals**

Female Lewis rats (JC strain) bred by the Central Animal Breeding House of the University of Queensland were used. They were kept 5 to a cage and with an unrestricted cage supply of rat and mouse cubes and water.

**Induction of chronic relapsing EAE**

Each batch of inoculum was prepared by homogenizing a mixture of 1 g guinea-pig spinal cord, 1 ml 0.9% saline, 1 ml complete Freund's adjuvant (Difco) and 10 mg Mycobacterium tuberculosis H37RA (Difco). Under ketamine/xylazine anaesthesia, rats aged 7-10 wks were injected intradermally with 0.05 ml inoculum into the medial footpad of the right hindfoot. Commencing on the day of inoculation the rats were given subcutaneous injections of cyclosporin A (CyA) (Sandoz) (4 mg/kg) on alternate days until 22 days postinoculation (DPI), as described by Polman et al. (1988). In terminal experiments, electrophysiological studies were performed on rats at different stages of the disease. At the end of the electrophysiological study one of the rats was perfused with fixative for histological studies as previously described (Pender et al., 1990).

**Clinical assessment**

The rats were examined daily from 7 DPI. Tail, hindlimb and forelimb weakness were each graded on a scale of 0 (no weakness) to 4 (complete paralysis) as previously described (Pender, 1986b), and these 3 scores were added together to give a total clinical score (maximal deficit = 12).

**Controls**

Electrophysiological studies were performed on normal control rats 9-13 wks old and on 3 rats treated with CyA as above but not inoculated.

**Electrophysiological studies**

Anaesthesia was induced by the intraperitoneal (i.p.) injection of ketamine (74 mg/kg), xylazine (9 mg/kg) and atropine (36 µg/kg) and maintained with further i.p. injections of half these doses. An adequate depth of anaesthesia was maintained without depressing the corneal reflex. The rats breathed spontaneously through a tracheal cannula. 8 ml of Hartmann's solution (compound sodium lactate BP, Travenol) were given i.p. at the beginning of each experiment, and 1 ml of Haemaccel (polygeline. Behring Ltd) was given i.p. after the laminectomy and cranietomy had been performed.

**Dorsal root entry zone (DREL) recordings.** A T12-L6 laminectomy was performed, the animal was mounted on an animal frame, and a metal box, through which water at 37° C was circulated, was placed under the animal. A pool was made with the skin flaps and the dura opened. The left hindlimb was extended and supported in a horizontal position. The left sciatic nerve and gastrocnemius muscle
were exposed in the posterior thigh and a skin pool formed. The sciatic nerve in the midthigh was dissected free with care to avoid damage to its blood supply. The exposed nervous tissues were rinsed in Hartmann's solution, and paraffin oil was added to cover the tissues. A controlled radiant heat lamp maintained the laminectomy and sciatic pools at 37° C. Under these conditions the rectal temperature was 37° C-38° C. The left sciatic nerve was lifted away from the volume conductor and stimulated in continuity with a pair of platinum electrodes 3 mm apart. Stimuli were 0.1 ms square-wave voltage pulses delivered at 1 Hz.

Volume conductor recordings were made over the left L4 DREZ with a 0.5 mm diameter silver ball electrode as the active electrode. The reference electrode was a platinum wire placed on the right paravertebral region at the same level as the active electrode. The recording electrodes were shielded leads connected to FET source-followers and thence to a preamplifier (bandwidth limited to 5.3-10 000 Hz) and then for display on an oscilloscope. For all recordings, negativity at the active electrode gave an upward deflection on the oscilloscope. Oscilloscope traces were photographed for measurements. Conduction velocities were calculated after allowing for a utilization time of 0.1 ms (Blair and Erlanger, 1936). To assess the transmission of high frequency trains of impulses the sciatic nerve was stimulated supramaximally at 10 Hz for 60 s or at 100 Hz for 10 s. The effect of repetitive stimulation was determined by calculating the ratio of the amplitude of the response evoked by the last stimulus of the 10 Hz or 100 Hz train to the amplitude of the response evoked by stimulation at 1 Hz.

At the end of the experiment the dissection was extended to expose the entire length of the conduction pathway from the sciatic nerve to the L4 DREZ. Conduction distance was measured as the length of a thread placed along the conduction pathway. The L4 and L5 spinal nerves always gave larger contributions to the sciatic nerve, and the L3 and L6 spinal nerves gave small contributions.

Dorsal column recordings. Conduction through the dorsal columns was studied by stimulating the exposed left dorsal column 1 mm from the midline with two 0.25 mm diameter platinum wire electrodes (with J-shaped tips, the convexities being placed on the cord) 3 mm apart, with the cathode at the level of the L3 DREZ. Stimuli were 0.02 ms duration square-wave voltage pulses delivered at 1 Hz. The short stimulus duration was chosen to minimize stimulus artefact. The active recording electrode was a 0.5 mm diameter silver ball electrode placed on the left dorsal column at the level of the S4 DREZ. The reference electrode was a 0.2 mm diameter platinum wire electrode placed on the left paravertebral tissues at the same level as the active recording electrode. The distance between the stimulating cathode and the active recording electrode was always 16 mm. A maximal response was obtained in the normal control at a stimulus intensity of about 0.25 V. Cutting the left L3-L6 dorsal roots had no effect on the response. When the right dorsal spinal cord was cut 3 mm caudal to the stimulating cathode or 2 mm rostral to the active recording electrode, the response amplitude was reduced by 50%. Cutting the left dorsal spinal cord at the same level abolished the remaining response. These effects confirmed that the recorded response was transmitted through the dorsal columns.

Cerebral somatosensory evoked potentials (SSEPs). A craniectomy extending from 1 mm anterior to the bregma to 5 mm posterior to the bregma and from 1 mm lateral to the midline to 5 mm lateral to the midline was performed. A pool was made with the skin flaps. The dura was opened and, after rinsing with Hartmann's solution, paraffin oil was added to the pool. The exposed sciatic nerve was stimulated in the same site as for the DREZ recordings, with 0.1 ms square-wave voltage pulses delivered at 0.2 Hz. The active recording electrode was a 0.5 mm diameter silver ball electrode positioned on the exposed cerebral cortex 3 mm lateral and 1 mm posterior to the bregma, as this site was found to yield the maximal response. The reference electrode was a 0.2 mm diameter platinum wire placed on the cranium at the bregma. The bandwidth was limited to 30-10 000 Hz. The signal was fed from the preamplifier to an NL106 a.c.-d.c. amplifier (Digitimer Ltd) and thence to a Digitimer Neurolog NL750 signal averager before display on an oscilloscope; 32 sweeps were averaged.

M wave and H reflex recordings. The left sciatic nerve was stimulated as for the DREZ recordings except that the polarity of the stimulating electrodes was reversed. 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.

Statistical analysis. To compare the recordings from normal control rats with those from rats at each of the different stages of chronic relapsing EAE, parametric analysis of variance was used except for the comparison of the amplitudes of the cerebral somatosensory evoked potentials. For the latter the Kruskal-Wallis test was used because the data in 2 of the groups were not normally distributed.

RESULTS

Clinical findings

The clinical course in this model of chronic relapsing EAE has previously been described in detail (Pender et al., 1990). Tail weakness commenced 11 - 16 DPI. Over the next 2 days the tail usually became completely paralysed and hindlimb weakness developed. Most of the affected animals recovered from this episode and had minimal or no residual deficit by 18-22 DPI. Of those that recovered, 85% had a second episode usually commencing 19-26 DPI. The pattern, severity and temporal profile of the neurological signs in the second episode were similar to those in the first episode. Clinical recovery from the second episode was usually complete 26-34 DPI. Of these rats, 60% had a third episode usually commencing 28 - 34 DPI and had recovered from this episode 33-39 DPI. The clinical profile of a rat studied electrophysiologically in the second episode is shown in fig. 1A. Some of the rats (25%) recovered incompletely or not at all from the first episode and had a chronic persistent or chronic progressive clinical course, although partial exacerbations and remissions often punctuated the course. Neurological signs persisted up to 48 DPI in some of these animals and then resolved.

Fig. 1. Clinical profiles of a rat studied electrophysiologically in the second episode of EAE 26 DPI (A) and of a rat with a chronic persistent course studied electrophysiologically 29 DPI (B).

The clinical profile of a rat which had a chronic persistent course and which was studied electrophysiologically is shown in fig. 1B.

Electrophysiological studies were performed on rats during the first episode (12 - 16 DPI), the first remission (18-20 DPI), the second episode (22-26 DPI), chronic persistent EAE (29-34 DPI) and well-established late (second or third) remission (50-64 DPI). The rats in the chronic persistent EAE group were studied at the usual time of the third episode (29-34 DPI). Two of these rats had a chronic persistent course with a clinical score > 6 from the first episode onwards; the other 3 had 3 episodes but either the first or the second remission was incomplete.
Lumbar DREZ recordings

Conduction through the PNS afferent pathway from the peripheral nerve to the spinal cord was studied in 17 normal controls and in 26 animals with chronic relapsing EAE (Table 1, figs 2, 3). The clinical scores for each group at the time of study also are shown in fig. 3. The normal L4 DREZ response to sciatic nerve stimulation consists of a biphasic wave (positive, negative) representing the afferent volley, and a late slow negative wave, the N wave, which is a field potential due to synaptic currents in the second order dorsal horn neurons excited mainly by low threshold cutaneous afferents (Ponder and Sears, 1986) (fig. 2A).

First and second episodes. During the first and second episodes of chronic relapsing EAE the peak-to-peak amplitude and conduction velocity of the peak of the negativity of the maximal afferent volley potential were significantly reduced without temporal dispersion, and the peak of the maximal N wave was significantly reduced in amplitude and prolonged in latency (Table 1, figs 2, 3). These findings indicate failure of excitation or conduction block of the large diameter afferent fibres.

First and late remissions. During the first remission the afferent volley potential amplitude and velocity and the N wave amplitude and latency were significantly different from those of normal controls (Table 1). However, during the first remission the abnormalities were less severe than in animals studied during the first episode, although the differences between the parameters measured during the first episode and the first remission were statistically significant only for the N wave amplitudes and latencies (Table 1, fig. 3). As the animals studied during the first remission had had clinical episodes similar in severity to those of the animals studied during the first episode, these findings indicate restoration of conduction in some afferent fibres during the first remission.

During late remission the abnormalities of the afferent volley potential amplitude and velocity and of the N wave latency were significantly less severe than in animals studied during the second episode (Table 1, fig. 3). The velocity of the afferent volley and the N wave latency were now normal but the amplitudes of the afferent volley and N wave were significantly lower than in normal controls. As rats studied during late remission had had a similar clinical course to those studied during the second episode, these findings indicate restoration of conduction in many afferent fibres. The return to normal afferent volley conduction velocity indicates that at this stage there was no detectable conduction slowing. The decreased amplitude of the afferent volley without temporal dispersion suggests persistent conduction failure in other fibres.
TABLE 1. LUMBAR DREZ RECORDINGS

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 17)</th>
<th>First episode (n = 7)</th>
<th>First remission (n = 4)</th>
<th>Second episode (n = 6)</th>
<th>Chronic persistent (n = 5)</th>
<th>Late remission (n = 4)</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F = 10.85 P &lt; 0.001</td>
</tr>
<tr>
<td>Maximal afferent volley</td>
<td>1215±275</td>
<td>497±365</td>
<td>561±167</td>
<td>485±411</td>
<td>540±195</td>
<td>736±216</td>
<td>P &lt; 0.025</td>
</tr>
<tr>
<td>Peak-to-peak amplitude (µV)</td>
<td></td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td></td>
<td></td>
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<tr>
<td>Conduction velocity to the peak of negativity (m·s⁻¹)</td>
<td>55.6±4.7</td>
<td>48.1±6.7</td>
<td>49.5±2.6</td>
<td>49.9±1.7</td>
<td>50.0±6.3</td>
<td>57.6±2.3</td>
<td>*P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
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<tr>
<td>Maximal N wave</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Latency of peak (ms)</td>
<td>2.4±0.23</td>
<td>3.0±0.39</td>
<td>2.7±0.15</td>
<td>2.9±0.32</td>
<td>2.8±0.38</td>
<td>2.5±0.08</td>
<td>F = 5.81 P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Amplitude of peak (µV)</td>
<td>1331±294</td>
<td>774±363</td>
<td>1039±393</td>
<td>904±322</td>
<td>759±337</td>
<td>954±254</td>
<td>F = 4.80 P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

* The significance under each mean refers to the comparison with the control mean; n.s. = not significant.
**Fig 2.** Volume conductor recordings of the L4 DREZ maximal afferent volley evoked by sciatic nerve stimulation in a normal control rat (A) and in a rat during the first episode of EAE (B). In these and all subsequent recordings the onset of the stimulus is indicated by a vertical line.

**Chronic persistent EAE.** In rats with chronic persistent EAE the afferent volley potential amplitude and velocity were significantly decreased and the N wave was significantly decreased in amplitude and prolonged in latency compared with normal controls (Table 1, fig. 3). The mean N wave latency of rats with chronic persistent EAE was significantly shorter than that of rats studied.
during the first episode ($P < 0.05$). Temporal dispersion, indicating slowing of conduction, of the afferent volley was observed in 2 rats with chronic persistent EAE. As the degree of temporal dispersion was insufficient to account solely for the reduced amplitude in these 2 rats and as temporal dispersion was absent in the other 3 rats, these findings also indicate failure of excitation or conduction block in large diameter afferents.

**Effects of repetitive stimulation and temperature on DREZ recordings**

As demyelinated fibres have an impaired ability to transmit trains of impulses (McDonald and Sears, 1970) and as conduction in demyelinated fibres is abnormally susceptible to temperature changes (Rasminksky, 1973), the effects of repetitive stimulation and temperature on afferent conduction from the peripheral nerve to the spinal cord were studied. The effects of repetitive supramaximal sciatic nerve stimulation on the peak-to-peak amplitude of the L4 DREZ afferent volley potential were assessed in 7 normal control animals and in 11 animals with chronic relapsing EAE (fig. 4, Table 2). In normal control rats, stimulation at 10 Hz for 60 s had no effect; however, stimulation at 100 Hz for 10 s resulted in a mean amplitude reduction of $24 \pm 12$ (SD) % (fig. 4, Table 2). In rats studied during the first remission and second episode both tests of repetitive stimulation reduced the amplitude more than in normal controls (but $F$ values were not significant) while repetitive stimulation had no greater effect in the rat studied during the first episode and in rats studied during late remission and chronic persistent EAE than in normal controls (fig. 4, Table 2). The effects of repetitive stimulation were fully reversible when stimulation at 1 Hz was resumed after a period of no stimulation. As supramaximal stimulation was used, these findings indicate rate-dependent block rather than a failure of excitation.

![Fig. 4 Volume conductor recordings of the L4 DREZ afferent volley evoked by supramaximal stimulation of the sciatic nerve at 1 Hz (A) and immediately after a period of stimulation at 100 Hz for 10 s (B) in a normal control rat and in a rat during the second episode of EAE.](image-url)
### TABLE 2. EFFECT OF REPETITIVE STIMULATION ON LUMBAR DREZ RECORDINGS

<table>
<thead>
<tr>
<th>Analysis of variance. Value and significance of $F$</th>
<th>Controls (n = 7)</th>
<th>First episode (n = 1)</th>
<th>First remission (n = 2)</th>
<th>Second episode (n = 3)</th>
<th>Chronic persistent (n = 3)</th>
<th>Late remission (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI</td>
<td>15</td>
<td>20</td>
<td>22-26</td>
<td>30-33</td>
<td>50-63</td>
<td></td>
</tr>
<tr>
<td>Maximal afferent volley</td>
<td></td>
<td></td>
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<tr>
<td>% reduction in peak-to-peak amplitude compared with value at 1 Hz after</td>
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<tr>
<td>10 Hz for 60 s</td>
<td>0</td>
<td>0</td>
<td>13 ± 13</td>
<td>8 ± 8</td>
<td>0</td>
<td>3 ± 4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>*n.s</td>
<td>*$P &lt; 0.001$</td>
<td>*$P &lt; 0.001$</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>100 Hz for 10 s</td>
<td>24 ± 12</td>
<td>16</td>
<td>43 ± 11</td>
<td>55 ±27</td>
<td>20 ± 5</td>
<td>23 ±3</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>n.s</td>
<td>*$P &lt; 0.005$</td>
<td>*$P &lt; 0.001$</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
</tbody>
</table>

* The significance under each mean refers to the comparison with the control mean; n.s. = not significant.
## TABLE 3. EFFECT OF TEMPERATURE ON LUMBAR DREZ RECORDINGS

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 4)</th>
<th>First episode (n = 1)</th>
<th>First remission (n = 1)</th>
<th>Second episode (n = 1)</th>
<th>Chronic persistent (n = 4)</th>
<th>Late remission (n = 2)</th>
<th>Analysis of variance. Value and significance of $F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI</td>
<td>15</td>
<td>20</td>
<td>23</td>
<td>30-34</td>
<td>50-63</td>
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<tr>
<td>Maximal afferent volley</td>
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<tr>
<td>% reduction in peak-to-peak amplitude at 37$^\circ$ C Mean ± SD</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>37$^\circ$ C</td>
<td>100 ± 0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>40$^\circ$ C</td>
<td>$90 \pm 7^{*}$ n.s.</td>
<td>85</td>
<td>87</td>
<td>95</td>
<td>$85 \pm 8^{*}$ n.s.</td>
<td>91 ± 7</td>
<td>$F = 0.43^{*}$ n.s.</td>
</tr>
<tr>
<td></td>
<td>$121 \pm 16$ n.s.</td>
<td>$114 \pm 200$</td>
<td>$129 \pm 29^{*}$ n.s.</td>
<td>$117 \pm 2$ n.s.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37$^\circ$ C</td>
<td>100 ± 0</td>
<td>100</td>
<td>100</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
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</table>

* The significance under each mean refers to the comparison with the control mean; n.s. = not significant; $^{*} n = 3$
The effect of temperature on the peak-to-peak amplitude of the maximal L4 DREZ afferent volley potential was studied in 4 normal controls and in 9 rats with chronic relapsing EAE (Table 3). In 1 rat in the second episode, lowering the laminectomy pool temperature from 37° C to 30° C increased the amplitude by 100% compared with a mean increase of 21 ± 16% in normal controls (fig. 5). After warming to 37° C the amplitude returned to the original value. This indicates that lowering the temperature reversed conduction block in some demyelinated fibres. Increasing the temperature from 37° C to 40° C did not significantly alter the amplitude. In rats in other stages of EAE, increasing or decreasing the temperature had no significant effect compared with normal controls, although in 1 rat with chronic persistent EAE cooling from 37° C to 30° C increased the amplitude by 57%.

**Dorsal column recordings**

To study conduction through afferent fibres in the CNS the spinal cord dorsal column compound action potential was recorded at the S4 level when the dorsal column was stimulated at the L3 level. These recordings were performed in 5 normal controls and in 16 rats with chronic relapsing EAE (Table 4, figs 6, 7). The clinical scores for each

![Dorsal column recordings](image)

Fig. 5 Effects of laminectomy pool temperature on the L4 DREZ maximal afferent volley evoked by sciatic nerve stimulation in a normal control rat and in a rat during the second episode of EAE.
### Table 4. Dorsal Column Recordings

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 5)</th>
<th>First episode (n = 3)</th>
<th>First remission (n = 3)</th>
<th>Second episode (n = 2)</th>
<th>Chronic persistent (n = 5)</th>
<th>Late remission (n = 3)</th>
<th>Analysis of variance Value and significance of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak-to-peak amplitude (µV)</td>
<td>249 ± 100</td>
<td>48 ± 84</td>
<td>180 ± 53</td>
<td>0</td>
<td>43 ± 60</td>
<td>123 ± 64</td>
<td>F = 6.44</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.025</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td>*P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Latency-to-peak of negativity (ms)</td>
<td>0.42 ± 0.02</td>
<td>0.42 *n.s.</td>
<td>0.46 ± 0.09</td>
<td>0</td>
<td>0.50 ± 0.07 *b</td>
<td>0.50 ± 0.15</td>
<td>F = 0.55</td>
</tr>
</tbody>
</table>

* The significance under each mean refers to the comparison with the control mean; n.s. = not significant; *n = 1, as response absent in other 2 rats; *b n = 2, as response absent in other 3 rats.
Fig. 6. Volume conductor recordings of the dorsal column maximal compound action potential in a normal control rat (A) and in rats with EAE during the first episode (B), first remission (C) and chronic persistent course (D). Below. Transverse section through the dorsal columns of the L6 spinal cord of the same rat as for D. Demyelinated axons (arrows) and inflammatory cells (arrowheads) can be seen. HistoResin section stained with cresyl fast violet. Bar = 25 Am.

group at the time of study also are shown in fig. 7. In the normal control the dorsal column compound action potential consisted of a biphasic wave (positive, negative) sometimes followed by a late low amplitude negativity (fig. 6A).

First and second episodes. During the first and second episodes there were statistically significant marked reductions in the peak-to-peak amplitude of the maximal dorsal column compound action potential without temporal dispersion (Table 4, figs 6, 7). This indicates failure of excitation or conduction block in a high proportion of large diameter dorsal column fibres.

First and late remissions. During the first and late remissions the amplitudes of the maximal dorsal column compound action potential were significantly higher than in animals studied during the first and second episodes, respectively, but were still
significantly less than in normal controls (Table 4, figs 6, 7). As the animals studied in the first and late remissions had had clinical courses similar in severity to those of the rats studied during the first and second episodes respectively, these findings indicate restoration of conduction in a high proportion of large diameter dorsal column fibres during the first and late remissions. During late remission the latency of the peak of the negativity of the dorsal column compound action potential was considerably prolonged compared with that in normal controls, although $F$ was not significant (Table 4).

**Chronic persistent EAE.** In rats with chronic persistent EAE the maximal dorsal column compound action potential was markedly reduced in amplitude, without temporal dispersion, and prolonged in latency compared with normal controls (Table 4, figs 6, 7). These findings indicate a failure of excitation or conduction block in a high proportion of large diameter dorsal column fibres. Fig. 6 illustrates the conduction abnormalities and the histological findings in the dorsal column of a rat with chronic persistent EAE that was perfused through the left ventricle with fixative at the end of the electrophysiological studies. Histological examination revealed inflammation, primary demyelination, remyelination and axonal degeneration in the lumbosacral dorsal columns of this rat.

**Effects of repetitive stimulation and ouabain on dorsal column recordings**

The effects of repetitive supramaximal dorsal column stimulation were assessed in 4 normal control rats and in 6 rats with chronic relapsing EAE (Table 5, fig. 8). In normal control rats, stimulation at 100 Hz for 10 s resulted in a mean amplitude reduction of $6 \pm 2\%$. Compared with the effect in normal control rats, such repetitive stimulation had a greater effect in rats studied during the first remission or during chronic persistent EAE (but $F$ was not significant). It had no significant effect in 1 rat during the first episode and in 1 rat during late remission. The effects of repetitive stimulation were fully reversible when stimulation at 1 Hz was resumed after a period of no stimulation.
TABLE 5. EFFECT OF REPETITIVE STIMULATION ON DORSAL COLUMN RECORDINGS

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 4)</th>
<th>First episode (n = 1)</th>
<th>First remission (n = 2)</th>
<th>Second episode</th>
<th>Chronic persistent (n = 2)</th>
<th>Late remission (n = 1)</th>
<th>Analysis of variance. Value and significance of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI</td>
<td></td>
<td></td>
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<tr>
<td>% reduction in peak-to-peak amplitude compared with value at 1 Hz after 100 Hz for 10 s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6 ± 2</td>
<td>0</td>
<td>28 ± 29</td>
<td>—</td>
<td>36 ± 22</td>
<td>15</td>
<td>$F = 1.57$</td>
</tr>
</tbody>
</table>

* The significance under each mean refers to the comparison with the control mean; n.s. = not significant.
It has recently been shown that ouabain, a specific inhibitor of the electrogenic sodium pump, reverses conduction block in single demyelinated fibres by reducing the threshold for transmission (Kaji and Sumner, 1989). We therefore assessed the effect of i.p. ouabain on the dorsal column compound action potential (at 1 Hz stimulation) in 3 rats with chronic persistent EAE. In 1 rat with a markedly reduced dorsal column compound action potential, 0.2 mg (1.5 mg/kg) ouabain i.p. increased the response to 145 µV (58% of the mean in normal controls) 15 min after administration (fig. 9). Administration of a further 0.2 mg ouabain i.p. 95 min after the first dose did not increase the amplitude further. In the other 2 rats the amplitude increased by 42% and 14% 25 min after the i.p. administration of 0.4 mg (2.9 and 2.7 mg/kg, respectively) ouabain. In the latter the response increased by 46% of the baseline value 25 min after a further 0.4 mg ouabain was given i.p., 45 min after the first injection. These findings indicate restoration of conduction in many dorsal column fibres by ouabain.

Cerebral SSEPs

To study conduction along the whole length of the PNS and CNS afferent pathway, the maximal right cerebral cortical somatosensory potential evoked by sciatic nerve stimulation was recorded in 8 normal control rats and in 21 rats with chronic relapsing EAE (Table 6, figs 10, 11, 12). The clinical scores for each group at the time of study also are shown in fig. 12. In the normal control, the averaged cerebral SSEP consisted of a biphasic wave (positive, negative) (fig. 10A).
First and second episodes. During the first and second episodes the mean peak-to-peak amplitude of the maximal cerebral SSEP did not differ significantly from that in normal control rats (Table 6). However, in these episodes the distribution of the amplitudes

![Graph](image1)

Fig. 9. Volume conductor recordings of the dorsal column maximal compound action potential in a rat with chronic persistent EAE before (A) and 15 min after the i.p. administration of 0.2 mg ouabain (B)

![Graph](image2)

Fig. 10. Volume conductor recordings of the averaged maximal right cerebral somatosensory potential evoked by left sciatic nerve stimulation in a normal control rat (A) and in rats with EAE during the first episode (B) and second episode (C). Note that the gain is lower in C.

was bimodal (fig. 11). In 1 rat during the first episode and in 1 during the second episode the amplitude of the maximal cerebral SSEP was 4 and 5 SDs, respectively, above the normal mean (figs 10C, 11). In the other 6 animals the maximal cerebral SSEP amplitude was lower than the normal mean (figs 10B, 11); in 2 animals in the first episode it was 2 SDs below the normal mean and in 1 animal in the second episode it was absent. The latency to the peak of the positivity of the maximal cerebral SSEP was significantly prolonged in rats during the first and second episodes (Table 6, figs 10, 12). It was prolonged for both the high amplitude and low amplitude responses.
### TABLE 6. CEREBRAL SSEPs

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 8)</th>
<th>First episode (n = 4)</th>
<th>First remission (n = 4)</th>
<th>Second episode (n = 4)</th>
<th>Chronic persistent (n = 5)</th>
<th>Late remission (n = 4)</th>
<th>Kruskal-Wallis test and analysis of variance. Value and significance of K and F</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Peak-to-peak amplitude (µV)</td>
<td>782 ± 207</td>
<td>707 ± 637</td>
<td>412 ± 168</td>
<td>757 ± 763</td>
<td>255 ± 170</td>
<td>256 ± 167</td>
<td>K = 12.6</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>n.s.</td>
<td>*n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Latency-to-peak of positivity (ms)</td>
<td>13.8 ± 1.1</td>
<td>15.6 ± 2.1</td>
<td>14.6 ± 1.6</td>
<td>17.8 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.0 ± 5.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.0 ± 2.5</td>
<td>F = 5.99</td>
</tr>
<tr>
<td></td>
<td>*n.s.</td>
<td>*n.s.</td>
<td>*n.s.</td>
<td>*n.s.</td>
<td>n.s.</td>
<td>*n.s.</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

<sup>a</sup> The significance under each mean refers to the comparison with the control mean; n.s. = not significant;<sup>b</sup> n = 3, as absent in other rat;<sup>b</sup> n = 4, as absent in other rat.
First and late remissions. In the first and late remissions the mean cerebral SSEP amplitudes were reduced, but not significantly, compared with those in rats during the first and second episodes, respectively, and were significantly reduced compared with that in normal controls (Table 6). The latencies were shorter in the first and late remissions than in the first and second episodes, respectively, but the differences were not significant (Table 6, fig. 12). Compared with that in normal controls the mean latency was not significantly prolonged during the first remission and was significantly prolonged during late remission.

Chronic persistent EAE. In rats with chronic persistent EAE the cerebral SSEP was significantly decreased in amplitude and prolonged in latency compared with that in normal controls (Table 6, fig. 12). The latency was particularly prolonged. In one rat the cerebral SSEP was absent.

M wave and H reflex studies

To assess transmission from peripheral nerve to muscle and through the monosynaptic reflex pathway, the M wave and H reflex were studied in 9 normal control rats and 22 rats with chronic relapsing EAE (Table 7, fig. 13). In normal control rats an M wave and a longer latency H reflex were recorded from the fourth dorsal interosseous muscle of the hindfoot when the ipsilateral sciatic nerve was stimulated (fig. 13A). 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 L5 and L6 ventral roots (Pender, 1988a). As the amplitude of the H reflex was greater after a period of no stimulation for several seconds, the maximal H reflex was usually recorded as the response to the first stimulus after a 5 s period of no stimulation. 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 M-H latency is the difference between the latency to the onset of the H reflex and the latency to the onset of the M wave and is the time for transmission through the monosynaptic reflex arc from the sciatic nerve back to the sciatic nerve at 37°C.

Fig. 11. Amplitudes of the averaged maximal right cerebral somatosensory potential evoked by left sciatic nerve stimulation in the chronic relapsing EAE.
First and second episodes. During the first and second episodes the peak-to-peak amplitude of the maximal M wave and the latency to the onset of the M wave were normal, indicating normal peripheral nerve motor conduction (Table 7, fig. 13B). However, the mean H/M ratios were significantly reduced in both the first and second episodes compared with that in normal controls (Table 7, fig. 13B), although $F$ was not significant. The mean M-H latency was prolonged in the first and second episodes but the difference from the normal mean reached statistical significance in the first episode only and $F$ was not significant (Table 7). These findings indicate interruption of the monosynaptic reflex arc.

First and late remissions. In the first and late remissions the mean H/M ratios were higher than those in the first and second episodes, respectively, but only the difference between late remission and the second episode was significant (Table 7, fig. 13). The mean M-H latency during the first remission was shorter than that during the first episode but the difference was not statistically significant; this latency was also not significantly different from the normal mean. As the animals studied in the first and late remissions had had clinical courses similar in severity to those of the rats studied in the first and second episodes, respectively, these findings are consistent with restoration of conduction in the monosynaptic reflex pathway.

Chronic persistent EAE. In rats with chronic persistent EAE the mean H/M ratio was reduced compared with that in normal controls but the difference was not statistically significant (Table 7). The H/M ratio was normal in 3 rats with chronic persistent EAE but the H reflex was absent in the other.

Cyclosporin A (CyA) controls

Three rats which had been treated with CyA but not inoculated were studied electrophysiologically 26, 27 and 29 days after initiation of CyA treatment. Recordings of the M wave, H reflex, lumbar DREZ response, dorsal column compound action potential and cerebral SSEP were normal in these animals, indicating that, in the dose given, CyA itself did not affect transmission in the PNS or CNS.
### TABLE 7. M WAVE AND H REFLEX RECORDINGS

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 9)</th>
<th>First episode (n = 5)</th>
<th>First remission (n = 5)</th>
<th>Second episode (n = 4)</th>
<th>Chronic persistent (n = 4)</th>
<th>Late remission (n = 4)</th>
<th>Analysis of variance</th>
<th>Value and significance of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI</td>
<td></td>
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<tr>
<td></td>
<td>12–16</td>
<td>18–20</td>
<td>22–26</td>
<td>29–34</td>
<td></td>
<td>50–64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M peak-to-peak amplitude (mV) Mean ± SD</td>
<td>4.7 ± 0.83</td>
<td>4.2 ± 1.26</td>
<td>4.8 ± 0.99</td>
<td>4.4 ± 0.43</td>
<td>5.8 ± 3.2</td>
<td>4.7 ± 2.1</td>
<td>F = 0.49</td>
<td>n.s.</td>
</tr>
<tr>
<td>H/M ratio</td>
<td>0.46 ± 0.05</td>
<td>0.34 ± 0.14</td>
<td>0.39 ± 0.20</td>
<td>0.32 ± 0.20</td>
<td>0.37 ± 0.26</td>
<td>0.55 ± 0.09</td>
<td>F = 1.37</td>
<td>n.s.</td>
</tr>
<tr>
<td>M latency to onset (ms)</td>
<td>2.5 ± 0.24</td>
<td>2.6 ± 0.14</td>
<td>2.6 ± 0.31</td>
<td>2.6 ± 0.05</td>
<td>2.7 ± 0.12</td>
<td>2.8 ± 0.22</td>
<td>F = 1.21</td>
<td>n.s.</td>
</tr>
<tr>
<td>M-H latency (ms)</td>
<td>4.6 ± 0.28</td>
<td>5.2 ± 1.1</td>
<td>4.8 ± 0.25</td>
<td>4.9 ± 0.46</td>
<td>4.6 ± 0.40</td>
<td>4.5 ± 0.30</td>
<td>F = 1.13</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* The significance under each mean refers to the comparison with the control mean; n.s. = not significant; ^ n = 3, as H reflex absent in 1 rat.
DISCUSSION

The present study has demonstrated a failure of excitation or conduction block in the PNS afferent pathway and in the spinal cord dorsal columns of rats with clinical episodes of chronic relapsing EAE. A failure of excitation is a feature of axonal degeneration while conduction block is typical of primary demyelination. The following observations indicate that the conduction failure was mainly due to demyelination-induced conduction block: (1) the occurrence of rate-dependent block in the PNS and CNS; (2) the restoration of PNS conduction by cooling; (3) the restoration of CNS conduction by the administration of ouabain; (4) the presence of temporal dispersion indicating conduction slowing due to demyelination in the PNS in chronic persistent EAE; (5) histological evidence of marked primary demyelination in the dorsal root ganglia, dorsal roots and dorsal columns (Pender et al., 1990); and (6) the temporal association of restoration of conduction with remyelination (see below). However, failure of excitation due to axonal degeneration is also likely to contribute to the conduction failure, as axonal degeneration was observed in the PNS and in the dorsal columns (Pender et al., 1990).

The reduction in amplitude and prolongation of latency of the cerebral SSEP during clinical episodes of chronic relapsing EAE are likely to be due to conduction abnormalities in both the PNS and CNS. As the sciatic nerve-L4 DREZ pathway constitutes part of the afferent pathway from the sciatic nerve to the cerebral cortex, the contribution of the PNS lesions to the SSEP abnormalities is obvious. In the spinal cord the dorsal and dorsolateral columns are the primary pathways mediating the SSEP (Cohen et al., 1981; York, 1985). While fibres generating the dorsal column compound action potential (from the S4 and coccygeal segments) in the present study did not contribute to the sciatic nerve-cerebral cortex afferent pathway, the marked conduction abnormalities in the lumbosacral dorsal columns indicate the likelihood of similar conduction abnormalities due to the demyelination and axonal degeneration we have observed in the thoracic and cervical spinal cord dorsal columns (Pender et al., 1990). Lesions in the dorsolateral column of the spinal cord and in the brainstem and cerebral white matter are also likely to contribute to the SSEP abnormalities. The absence of cerebral SSEPs in 2 rats in which DREZ responses were present, although reduced in amplitude, is evidence of the contribution of CNS lesions to the SSEP abnormalities. The increased amplitude of the cerebral SSEP observed in 2 rats may be due to demyelination-induced conduction
block of descending CNS pathways that inhibit synaptic transmission through the afferent pathway (see McIntyre et al., 1989).

The interruption of the lumbar monosynaptic reflex arc can be explained mainly by conduction block due to the demonstrated demyelination in the dorsal root ganglion, dorsal root, dorsal root entry zone, L5 and L6 spinal cord segments, ventral root exit zones and ventral roots (Pender et al., 1990). Lesions of descending pathways in the brainstem and spinal cord may also have affected the H reflex by increasing or decreasing excitability of the motor neuron pool.

**CNS conduction abnormalities**

This study demonstrates conduction block in CNS tracts in EAE by direct recordings from surgically exposed tracts. Direct stimulation of, and recording from, the exposed dorsal columns allowed conduction to be assessed in pathways that were restricted to the CNS and that did not have any intervening synapses. Conduction block in the dorsal columns was found in the first episode, as early as 14-15 DPI, and also in the later stages of chronic relapsing EAE. Pender (1986a, 1988a) demonstrated conduction block in the CNS part of the ventral root exit zone of the spinal cord in whole spinal cord-induced acute EAE in the Lewis rat; however, this region is a CNS-PNS transitional zone along the lower motor neuron pathway and is not a long fibre tract of the CNS. Others have used somatosensory, visual or auditory evoked potentials to assess CNS neurotransmission in acute and chronic relapsing EAE (Lumsden et al., 1975; Lidsky et al., 1980; Hayreh et al., 1981; Wiśniewski et al., 1982; Bilbool et al., 1983; Heininger et al., 1989); however, these recordings assessed transmission through pathways involving one or more synapses. Recordings of responses relayed through synapses are difficult to interpret because it is unknown whether the abnormalities are the direct effect of impaired axonal transmission or whether they are due to altered synaptic transmission. In the case of the cerebral SSEPs, lesions may have opposite effects on synaptic transmission, according to whether the lesions affect facilitatory or inhibitory pathways. In the only previous electrophysiological studies on chronic relapsing EAE, visual and auditory evoked potentials were recorded from guinea-pigs (Lidsky et al., 1980; Wiśniewski et al., 1982). Lidsky et al. (1980) found that the visual evoked potentials were reduced in amplitude and prolonged in latency during clinical episodes.

The finding of marked conduction abnormalities due to demyelination in the spinal cord during the first episode of EAE as well as during later episodes indicates that CNS demyelination is an important cause of neurological dysfunction in the first and later episodes of chronic relapsing EAE, as it is in whole spinal cord-induced acute EAE in the Lewis rat although the distribution of CNS lesions may vary (Pender, 1986a, 1988a). This supports the concept that acute and chronic relapsing EAE are essentially part of the same disease process, as indicated by the conversion of acute EAE to chronic relapsing EAE by treatment with low dose cyclosporin A (Polman et al., 1988; Pender et al., 1990).

**PNS conduction abnormalities**

This study demonstrates conduction abnormalities in the PNS in chronic relapsing EAE. The conduction abnormalities in the afferent pathway from the peripheral nerve to the spinal cord are explained mainly by demyelination in the dorsal root ganglia and dorsal roots, and are similar in nature to those previously described in rabbits with acute EAE (Pender and Sears, 1982, 1984, 1985) and in Lewis rats with whole spinal cord-induced acute EAE (Pender and Sears, 1986), although less severe than in the former and more severe than in the latter. The greater severity of these abnormalities in the first episode of chronic relapsing EAE than in acute EAE in the Lewis rat may be due to the different sexes of the animals studied, to differences in the amount of spinal cord tissue and adjuvants in the inocula, to the administration of cyclosporin A or to a combination of these factors.

Our histological studies have revealed that in rats with clinically active disease studied 29 DPI, there is little active PNS demyelination in contrast to prominent active CNS demyelination (Pender et al., 1990). This difference in disease activity in the PNS and CNS in later stages may be reflected in some of the electrophysiological findings. Rats with chronic persistent EAE (29-34 DPI) had similar afferent
voltage conduction velocities and N wave latencies but considerably prolonged cerebral SSEP latencies compared with rats in the second episode (22-26 DPI). This suggests progression of CNS disease but not PNS disease in the later stages of chronic relapsing EAE. In rats with chronic persistent EAE (30-33 DPI) there was no evidence of rate-dependent block in the PNS but prominent rate-dependent block in the CNS. Rate-dependent block indicates insecure high frequency transmission in fibres that are able to conduct signals at lower frequencies. Such fibres are likely to be either in the process of being demyelinated or in the early stages of remyelination. Demyelination of the PNS has been reported in other models of chronic EAE but the functional significance of these lesions has not been assessed (Raine et al., 1969; Madrid and Winiewski, 1978; Lassmann et al., 1980a; Brown et al., 1982).

**Restoration of conduction during remission**

During the first and late clinical remissions there was evidence of restoration of conduction in the PNS and in the CNS. This can be explained by the observed remyelination by Schwann cells and oligodendrocytes, respectively (Pender et al., 1990). There is evidence from studies on noninflammatory models of PNS demyelination that nerve conduction may be restored in demyelinated fibres in the early stages of repair before the formation of compact myelin lamellae (Bostock and Sears, 1978; Smith and Hall, 1980; Smith et al., 1982). In the lysophosphatidyl choline model, restoration of conduction occurred when demyelinated fibres became closely associated with debris-free Schwann cells (Smith and Hall, 1980; Smith et al., 1982). As the rats studied electrophysiologically during the first remission had only recently recovered from the first episode, some of the PNS and CNS fibres in which conduction had been restored may have still been demyelinated but invested by Schwann cells and oligodendrocytes, respectively, as has been suggested to occur during early recovery from acute EAE (Pender, 1989; Pender et al., 1989). The rate-dependent block observed in the PNS and CNS during the first remission may have been occurring in such invested demyelinated fibres. In the rats studied electrophysiologically during late remission, only a small proportion, if any, of the fibres in which conduction had been restored were still demyelinated, as these rats were studied in late second or third remission at which stage there were very few demyelinated fibres and remyelination was well established in the PNS and CNS (Pender et al., 1990).

Conduction slowing would be expected during the early stages of restoration of conduction by remyelination. The only definite evidence of conduction slowing was temporal dispersion of the afferent volley potential in chronic persistent EAE. However, it is likely that conduction slowing contributed to the reduced afferent volley conduction velocities and the prolonged N wave, dorsal column compound action potential and cerebral SSEP latencies during the first remission and at all subsequent stages of disease. During late remission the afferent volley conduction velocity and the N wave latency returned to normal, which is consistent with the histological findings of well-established PNS remyelination.

**Residual conduction abnormalities during late remission**

While there was evidence of restoration of conduction in the PNS and CNS in late remission, the recovery was incomplete. There was a persistent significant reduction in the amplitude of the dorsal root entry zone afferent volley potential with normal conduction velocity and without temporal dispersion. The dorsal column compound action potential was persistently reduced in amplitude, without temporal dispersion, and prolonged in latency. These findings indicate persistent conduction failure in the PNS and CNS during late remission. This can be explained by the observed axonal degeneration in the PNS and particularly in the CNS (Pender et al., 1990). Slowing of conduction in thinly remyelinated CNS fibres may have contributed to the prolonged latency of the dorsal column compound action potential although axonal degeneration of the fastest fibres could also account for this. Axonal degeneration in the PNS and CNS and conduction slowing in thinly remyelinated CNS fibres may explain the persistently reduced amplitude and prolonged latency of the cerebral SSEP during late remission. Axonal damage and degeneration are well-recognized features of hyperacute EAE (Lampert, 1967; Hansen and Pender, 1989), acute EAE (Lampert and Kies, 1967; Pender, 1989) and chronic relapsing EAE (Lassmann et al., 1980h; Brown et al., 1982; Pender et al., 1990).
CONCLUSION

In conclusion the present study has demonstrated conduction failure in both the CNS and PNS during the early and later stages of chronic relapsing EAE. Conduction was restored in some CNS and PNS fibres during remission but conduction abnormalities persisted in animals that were in late remission and had no neurological signs. The reversible conduction abnormalities are explained by demyelination followed by remyelination in the CNS and PNS. The persistent conduction failure in late remission is mainly due to axonal degeneration. These findings may have implications for the human demyelinating disease, multiple sclerosis.

ACKNOWLEDGEMENTS

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REFERENCES


LUMSDEN CE, HOWARD L, APARICIO SR (1975) Anti-synaptic antibody in allergic encephalomyelitis. I. Neurophysiological studies, in guinea pigs, on the exposed cerebral cortex and peripheral nerves, following immunological challenges with myelin and synaptosomes. Brain Research, Amsterdam, 93, 267-282.


RAINE CS, WISNIEWSKI H, PRINEAS J (1969) An ultrastructural study of experimental demyelination and remyelination. II. Chronic experimental allergic encephalomyelitis in the peripheral nervous system. Laboratory Investigation, 21, 316-327.


