Ascending Impairment of Nociception in Rats with Experimental Allergic Encephalomyelitis

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SUMMARY

An ascending impairment of tail nociception is a previously undescribed clinical sign of acute experimental allergic encephalomyelitis (EAE) in the rat. It occurs in EAE induced by inoculation with purified central nervous system (CNS) myelin basic protein (MBP) as well as with whole spinal cord. It is invariably present and consists of an absence of the vocalization response to noxious mechanical stimulation of the tail. This impairment of nociception evolves over 1-3 days, simultaneously with the development of tail weakness, and resolves more rapidly than the tail weakness. Light-microscopic, electron-microscopic and electrophysiological studies indicate that it is due to demyelination-induced conduction block in the small diameter myelinated afferent (A\text{\(\delta\)}) fibres in the sacral and coccygeal dorsal root ganglia, dorsal roots and dorsal root entry zones. Unmyelinated fibres appear to be largely spared.

Key words: Demyelination - Dorsal root - Dorsal root ganglion - Experimental allergic encephalomyelitis - Lewis rat - Myelin basic protein - Nociception – Tail

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is an autoimmune demyelinating disease of the nervous system induced by inoculation with central nervous system (CNS) tissue, or CNS myelin basic protein (MBP), and adjuvants, and is widely studied as a possible animal model of multiple sclerosis (Raine 1984). The clinical features of EAE in the rat, a commonly studied species, are tail weakness and paralysis followed by hindlimb weakness and sometimes paralysis. Forelimb weakness occasionally occurs. Here I report the new clinical observation of impaired tail nociception in Lewis rats with either whole spinal cord-induced or MBP-induced EAE. This is of interest because: (1) it emphasizes the ascending progression of the disease; (2) it is probably due to demyelination-induced conduction block in small diameter myelinated afferent (A\text{\(\delta\)}) fibres in the peripheral nervous system (PNS), with sparing of unmyelinated fibres; (3) its rapid resolution; and (4) its potential use in the study of nociception.
Materials and Methods

Animals

Lewis rats bred by the Animal Breeding Establishment of the John Curtin School of Medical Research (JC strain) were used. The rats were kept in cages of five and were fed rat and mouse cubes and water ad libitum.

Preparation of inoculum and inoculation procedure

(a) Whole spinal cord

The inoculum was a homogenate of equal volumes of a 30% suspension of guinea-pig spinal cord (the spinal roots having been stripped away) in 0.9% saline and a suspension of 4 mg of killed and dried *Mycobacterium butyricum* (Difco)/ml of incomplete Freund's adjuvant (Commonwealth Serum Laboratories, Melbourne, Australia). Under ether anaesthesia rats, 8-10 weeks old, were inoculated with 0.05 ml of inoculum in the footpad of each of the four feet.

(b) myelin basic protein (MBP)

MBP was prepared from guinea-pig spinal cord (the roots having been stripped away) by the method of Deibler et al. (1972). MBP in 0.9% saline was emulsified in an equal volume of incomplete Freund's adjuvant containing 4 mg/ml of added *Mycobacterium butyricum*. Rats, 8-10 weeks old, received 0.1 ml emulsion in a footpad of each hindfoot. The total dose of MBP was 50 µg/rat.

Clinical assessment and management of inoculated animals

The inoculated rats were examined daily, or in some cases every 12 h, from the 7th day post-inoculation. Tail weakness was assessed by holding the animal by the base of the tail and observing tail movement. It was graded as follows: 0 = no weakness; 1 = weakness of distal part of tail only, the distal tail failing to curl around the examiner's finger; 2 = weakness of the whole tail but with the proximal tail still being able to be erected vertically against gravity; 3 = severe weakness with only a flicker of tail movement; 4 = complete flaccid paralysis of the tail. Hindlimb weakness was graded thus: 0 = no weakness; 1 = slight dragging of toes of both hindfeet; 2 = severe dragging of both hindfeet but not of the rest of the hindlimbs; 3 = severe dragging of both hindlimbs, often with both hindlimbs displaced to one side of the body; 4 = total flaccid paralysis of the hindlimbs. The forelimbs were assessed in a similar way to the hindlimbs. Nociception was assessed by determining if vocalization occurred in response to noxious mechanical stimulation of the tail or of the digits of the hindlimbs. The mechanical stimulus consisted of firm pressure between the thumbnail applied to the side of the tail being tested and the index finger pad applied to the opposite side.

Histological studies were carried out on 4 rats (2 male and 2 female) with whole-cord-induced EAE and 2 male rats with MBP-induced EAE, 2-3 days after the onset of neurological signs. In terminal experiments, electrophysiological studies were performed on 3 male Lewis rats with whole-cord-induced EAE and 3 male Lewis rats with MBP-induced EAE, 1-2 days after the onset of neurological signs.

Controls

One normal 12-week-old male Lewis rat served as a control for the histological studies. Seven normal 10-12-week-old male Lewis rats were used as controls for the electrophysiological studies. As the histological and electrophysiological 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 these studies.
Histological studies

Under ether anaesthesia the rats were perfused through the left ventricle with 0.9% saline, until the effluent was clear, and then with 120ml of 2% glutaraldehyde and 2% formaldehyde in 0.1M sodium cacodylate buffer (pH 7.3-7.4). The brain, spinal cord, dorsal and ventral roots, dorsal root ganglia (DRGs), spinal nerves and the sciatic and tail nerves were removed and immersed in fixative. After dehydration in ascending ethanols and clearing in chloroform, slices of the fixed brain tissue were embedded in paraffin wax, sectioned (4 µm) and stained with haematoxylin and eosin. Tissues from the rest of the nervous system were post-fixed with 2% osmium tetroxide. Most of the osmium-fixed specimens were dehydrated in ascending ethanols, embedded in Histoclear (LKB Bromma), sectioned (2 µm) and stained with toluidine blue in phosphate buffer (pH 7.6) or with cresyl violet, as previously described (Pender 1985). Some were dehydrated in ascending ethanols and after a stage in absolute acetone were embedded in Epok 812 (Ernest F. Fullam, Inc., Schenectady, NY). Semi-thin 1-µm Epok 812 sections for light microscopy were stained with toluidine blue. Ultra-thin Epok 812 sections were double-stained with uranyl acetate and lead citrate and examined in a Philips 301 electron microscope.

Electrophysiological studies

Anaesthesia was induced with urethane (25% in 0.9% saline, intraperitoneal (i.p.), 5ml/kg) and supplemented by pentobarbitone sodium (i.p., 12 ml/kg). 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. A lumbar-sacral-coccygeal laminectomy of variable extent was performed. The rat was mounted in an animal frame, and a metal box, through which water at 37°C was circulated, was placed under the animal. After a laminectomy pool had been formed with the skin flaps, the dura was opened and the nervous tissues were covered with paraffin oil. A controlled radiant heat lamp maintained the laminectomy pool at 37°C. Under these conditions the rectal temperature was 37º-38°C. The filaments of the left sacral or coccygeal dorsal root to be studied were tied together and cut immediately proximal to the tie. The distal cut end was lifted away from the volume conductor into oil and placed on a pair of platinum wire hook electrodes 3mm apart. Short leads connected all recording electrodes to FET source-followers, thence to a preamplifier and thence for display on an oscilloscope and loudspeaker. The band width of the preamplifier was limited to 300-30000 Hz for receptive field analysis and for assessing the response to noxious mechanical stimuli. For the C fibre compound action potential studies it was limited to 5.3-1000 Hz and for the Aδ fibre compound action potential studies, 5.3-10000 Hz. For all recordings, negativity at the active electrode gave an upward deflection on the oscilloscope. Oscilloscope traces were photographed for measurements.

The receptive fields of individual sacral and coccygeal dorsal roots were determined by listening to the loudspeaker while lightly touching the tail skin hairs with a small piece of cotton wool. After the receptive field had been delineated, the noxious mechanical stimulus described above was applied to this territory, and the immediate responses were assessed on the oscilloscope and loudspeaker.

For compound action potential studies on the sacrococcygeal dorsal roots, the left ventral caudal trunk formed by the sacrococcygeal ventral primary rami was exposed, freed just distal to its junction with the respective ventral primary ramus, and stimulated in continuity with a pair of platinum electrodes 2 mm apart. For Aδ fibre conduction studies the stimuli were 0.1 ms square-wave voltage pulses delivered at 1.0 Hz, and for C fibre conduction studies they were 1 ms pulses delivered at 0.1 Hz. At the end of the experiment
the dissection was extended to expose the entire length of the conduction pathway from the ventral caudal trunk to the appropriate dorsal root and to determine the segmental level of the roots. Conduction distance was measured as the length of a thread placed along the conduction pathway.

Results

Clinical findings
The impairment of nociception described below was invariably present in Lewis rats with EAE induced by inoculation with spinal cord, or MBP, and adjuvants. It consisted of an absent vocalization response to noxious mechanical stimulation of the tail. The distal tail was always first involved and this involvement developed simultaneously with distal tail weakness, 8-14 days after inoculation. Over the next 1-2 days the impairment of nociception ascended to affect more proximal regions of the tail. This ascent was simultaneous with ascending progression of tail weakness. Hindlimb weakness usually commenced 1-2 days after the onset of tail weakness. Forelimb weakness, when it occurred, usually began after the onset of hindlimb weakness. By the time the tail was completely paralyzed, nociception was impaired over the greater, and sometimes the whole, length of the tail. The absence of vocalization in the latter cases was not due to weakness of the laryngeal or respiratory muscles, as noxious mechanical stimulation of the hindlimb digits resulted in vocalization. The impairment of tail nociception was usually bilaterally symmetrical. The maximum nociceptive deficit lasted for 1-3 days. Recovery of nociception often commenced while the tail was still totally paralyzed. It occurred in a descending pattern and was usually complete 3-5 days after the onset of impairment, at a time when there was still moderate or severe tail weakness. The ascent of the nociceptive impairment and the descent of recovery were most clearly demonstrated in animals examined every 12 h (Fig. 1).

Fig. 1. Profiles of the neurological signs in a Lewis rat with MBP-induced EAE, showing the onset of and recovery from: impairment in tail nociception (upper panel); tail weakness (middle panel); and hindlimb weakness (lower panel). Observations were made every 12 h from day 7 to day 14 post-inoculation (inclusive) and daily thereafter. See MATERIALS AND METHODS for grading of tail and hindlimb weakness. The tail was 192 mm long.
Histological findings

(a) Light-microscopic observations

The light-microscopic findings in rats with whole-spinal-cord-induced EAE have been previously described in detail (Pender and Sears 1986). In summary, sections of the brainstem and spinal cord showed perivascular mononuclear cell cuffs and infiltrates and perivascular demyelination as well as subpial mononuclear cell infiltrates and demyelination. This involvement increased caudally, and the dorsal root entry and ventral root exit zones were sites of predilection. Perivascular mononuclear cell cuffs and infiltrates and perivascular demyelination were also present in the dorsal and ventral roots and particularly the DRGs. The DRG involvement increased caudally. Both small and large diameter myelinated fibres were involved. The spinal and peripheral nerves showed no or minimal involvement.

(b) Electron-microscopic observations

Electron-microscopic examination of the sacrococcygeal dorsal roots and DRGs from rats with either whole-spinal-cord-induced or MBP-induced EAE demonstrated primary demyelination with sparing of axons (Figs. 3 and 4). Small diameter myelinated fibres (Fig. 3) were affected as well as large ones. In some fibres, macrophages containing myelin debris could be seen within the myelin sheath (Fig. 3). Unmyelinated fibres in the regions of demyelination were usually normal (Figs. 3 and 4). Degenerative changes were occasionally observed in scattered myelinated and unmyelinated axons.
Electrophysiological findings

(a) Receptive fields

The receptive fields of the sacral and coccygeal dorsal roots for light touch in one normal control rat are illustrated diagrammatically in Fig. 5. The findings in 3 other rats were consistent with these observations.

Fig. 3. Transverse section through a coccygeal dorsal root ganglion of a rat with whole-spinal-cord-induced EAE, 2 days after the onset of neurological signs. A small diameter fibre (asterisk) is being demyelinated. A macrophage is present within the myelin sheath and contains myelin debris (arrowhead). A group of normal unmyelinated fibres lies nearby (arrow). Electron micrograph. Bar = 2 µm.

Fig. 4. Electron micrograph of the dorsal root in Fig. 2. A group of normal unmyelinated axons (arrow) lies adjacent to a demyelinated axon (asterisk). Bar = 2 µm.
Fig. 5. Diagram showing the receptive fields of the sacral (S) and coccygeal (Co) dorsal roots for light touch in a normal 12-week-old male Lewis rat. The receptive field of the third coccygeal dorsal root was not determined. The arrow indicates the point at which the tail scales began.

(b) Conduction through the sacrococcygeal dorsal roots and dorsal root ganglia

We have previously described conduction abnormalities in large diameter myelinated fibres in the region of the sacrococcygeal DRGs of rats with whole-spinalcord induced EAE (Pender and Sears 1986). In the present study, unmyelinated C fibre conduction through the region of the sacrococcygeal DRGs appeared to be normal in all 5 ganglia from 3 rats with impaired tail nociception due to whole-spinal-cord-induced EAE (Fig. 6). No conclusions could be made about the integrity of the small diameter myelinated (Aδ) fibre response because the Aδ position in the compound action potential was occupied by the potentials of slowly conducting demyelinated larger fibres.

Conduction in myelinated afferents activated by noxious mechanical stimuli was studied in the sacrococcygeal segments of 3 normal control rats (5 segments) and 3 rats with impaired tail nociception due to MBP-induced EAE (10 segments). In the rats with EAE the responses were reduced in 9 dorsal roots 30-40 mm from the respective ganglia (Fig. 7B). When recordings were made from the same roots 12-20 mm closer to the ganglia the activities were greater than those recorded from the longer lengths but were still decreased compared to normal (Fig. 7C). This indicates that conduction in these fibres is being blocked in the dorsal roots as well as probably in the DRGs. It is likely that at least some of the blocked myelinated fibres were specific nociceptors; however, the stimuli used would also have activated other receptors such as low threshold mechanoreceptors.
Fig. 6. Recordings from the distal cut end of the first coccygeal dorsal root showing the maximum C fibre compound action potential evoked by stimulation of the ventral caudal trunk in a normal control rat (A) and in a rat with impaired tail nociception due to whole-spinal-cord-induced EAE (B). The stimulus commenced at the start of the trace. The conduction distances were similar. Note the different amplitude scales.

Fig. 7. Recordings from the distal cut end of the combined left S₃ and S₄ dorsal roots in a normal control rat (A) and in a rat with impaired tail nociception due to MBP-induced EAE (B and C). Background activity and activity evoked by noxious mechanical stimulation are shown. The conduction distance along the roots is 12 mm shorter in C than in B.
Discussion

An impaired response to noxious mechanical stimuli has not been previously reported in rats with EAE. We have, however, observed it in the limbs of rabbits with EAE and suggested that this might be due to demyelination-induced conduction block in the small diameter myelinated afferent (Aδ) fibres in the DRGs (Pender 1983; Pender and Sears 1984). The present study demonstrates that small diameter myelinated fibres in the sacrococcygeal DRGs, dorsal roots and dorsal root entry zones are frequently demyelinated in rats with whole-spinal-cord- or MBP-induced EAE and that there is a reduced dorsal root response to noxious mechanical stimulation of the tail. A significant proportion of cutaneous small diameter myelinated afferent (Aδ) fibres respond specifically to noxious mechanical stimulation of the skin (Burgess and Perl 1967; Perl 1968). Unmyelinated afferent (C) fibres also respond to noxious mechanical stimulation of the skin (Iggo 1960). As the electron-microscopic and electrophysiological studies indicate that unmyelinated afferent fibres are largely spared, the impaired mechanical nociception of rats with EAE is most likely due to demyelination-induced conduction block in the Aδ fibres in the sacrococcygeal DRGs, dorsal roots and dorsal root entry zones. It appears that vocalization induced by noxious mechanical stimuli is dependent on Aδ mechanical nociceptors but not on mechanical or polymodal C nociceptors. The effect of heat, which activates certain Aδ and C nociceptors (Chéry-Croze 1983), could not be assessed because the normal response of tail withdrawal was prevented by the tail paralysis.

The prominent involvement of the PNS when animals are inoculated with whole CNS tissue or purified CNS MBP is explained by the fact that the P1 MBP from the PNS is similar, if not identical, to CNS MBP (Brostoff and Eylar 1972; Greenfield et al. 1973). The ascending progression of the impairment of nociception is accounted for by the caudally increasing length of the dorsal roots. In a study on male albino rats, Waibl (1973) has shown that the lengths of the dorsal roots increase progressively from the first thoracic root (2 mm) to the third coccygeal root (59 mm). Thus the probability of many lesions in an entire dorsal root increases progressively in a caudal direction. Therefore the probability of a high proportion of dorsal root fibres undergoing demyelination-induced conduction block increases caudally. Simmons et al. (1982) have used a similar anatomically-based argument to account for the ascending weakness in rats with EAE but they attributed the weakness to oedema rather than demyelination. An additional contributing factor to the ascending nature of the nociceptive loss, at least in whole-spinal-cord-induced EAE, is the caudally increasing histological involvement of the DRGs (see Pender and Sears 1986). This pattern of DRG involvement was not observed in MBP-induced EAE in the present study. The invariability of the nociceptive loss may reflect a particular susceptibility of small diameter myelinated fibres to demyelination or the consequences thereof. Smaller fibres may be more readily demyelinated than larger ones (Jacobs 1967; Brown et al. 1980; Saida et al. 1983) and/or more likely to undergo conduction block after limited demyelination (Lafontaine et al. 1982). In the present study there was no obvious difference in the proportions of small and large diameter fibres demyelinated, but quantitative studies would be necessary to establish this.

The rapid recovery of nociception is most likely due to the restoration of conduction in demyelinated AO fibres by the development of electrical excitability in the demyelinated internodal axolemma, as described by Bostock and Sears (1978) and Smith et al. (1982) in rat ventral roots demyelinated by diphtheria toxin or lysophosphatidyl choline. The more rapid improvement in nociception than in tail movement may be due to the earlier onset of this process in small fibres (see Bostock and Sears 1978). Repair of structurally minor yet
functionally significant damage to the myelin sheath, for example loosening of the paranodal axoglial junction (Hirano et al. 1978), may also contribute to the rapid recovery of nociception. The laying down of new myelin lamellae is also a possibility, although the earliest reported onset of remyelination is 7 days after the induction of demyelination (Smith et al. 1982).

Acknowledgement

I am grateful to Ms Ailsa Rolinson for excellent technical assistance.

References


