Vulnerability of the Dorsal Root Ganglion in Experimental Allergic Encephalomyelitis

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The dorsal (posterior) root ganglion (DRG) is a relatively neglected part of the nervous system from the clinical point of view. This is because clinical examination and neurophysiological studies cannot distinguish lesions of the dorsal root ganglia from lesions of the sensory fibres of the spinal nerve or proximal peripheral nerve or from lesions of the dorsal roots, the dorsal root entry zone or the vicinity of the dorsal horn. Furthermore, clinical assessment unaided by electrophysiological studies may also fail to distinguish between a distal sensory neuropathy and disease localized to the dorsal root ganglia. The situation is complicated by the fact that disease producing degeneration of DRG neurones results in axonal degeneration of the primary sensory neurone fibres in the peripheral nerve, dorsal root and central nervous system (CNS). Thus the DRG is rarely considered as a discrete entity when a neurological diagnosis is being formulated. Nevertheless, there are a number of diseases in man that are known from pathological studies to particularly involve the DRG, such as herpes zoster (Head and Campbell, 1900), diphtheritic neuropathy (Fisher and Adams, 1956), carcinomatous sensory neuropathy (Denny-Brown, 1948; Horwich et al, 1977) and hereditary sensory radicular neuropathy (Denny-Brown, 1951). In experimental animals also, the DRG is especially susceptible to certain diseases, such as experimental allergic encephalomyelitis (EAE) (Waksman and Adams, 1955), experimental allergic neuritis (Waksman and Adams, 1955), diphtheritic neuropathy (Waksman et al, 1957; McDonald, 1963a), methylmercury poisoning (Jacobs et al, 1975), doxorubicin (adriamycin) intoxication (Cho, 1977) and cadmium poisoning (Gabbiani et al, 1967). Electrophysiological studies have shown focal conduction abnormalities in the dorsal root ganglia of cats with diphtheritic neuropathy, a toxic non-inflammatory demyelinating disease (McDonald, 1963b). However, the functional significance of the DRG involvement in inflammatory

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disease has been neglected. Recently, in studies on the pathophysiology of acute EAE in the rabbit, the severe histological involvement of the DRG was confirmed, and focal conduction abnormalities were demonstrated in a high proportion of the large diameter myelinated fibres in the DRG. The results of these studies have been the subjects of two previous papers placing particular emphasis on the functional significance of the peripheral nervous system (PNS) lesions in the context of EAE being the main animal model of multiple sclerosis, a CNS disease (Pender and Sears, 1982; 1984). The localization of functional abnormality in the DRG in EAE has implications for the neurological diseases of man.

Methods

Acute EAE was induced in four- to six-month-old New Zealand white male rabbits by intradermal inoculation in the nuchal region with homogenized rabbit spinal cord and complete Freund's adjuvant. The rabbits were examined and weighed every second day for the first 10 days and daily thereafter. For histological studies, animals were perfused through the left ventricle with 10% formalin or modified Karnovsky fixative. Blocks were taken from the brain, optic nerve, spinal cord, dorsal and ventral roots, dorsal root ganglia, spinal nerves and sciatic nerves. The tissue fixed with formalin was dehydrated with alcohol, cleared in cedar-wood oil, embedded in paraffin, sectioned (sections 7 to 14μm thick) and stained with haematoxylin and eosin. Tissue fixed with Karnovsky fixative was post-fixed with 1% osmium tetroxide, dehydrated with alcohol followed by propylene oxide, embedded in Araldite, sectioned (sections 0.5μm thick) and stained with toluidine blue. Some animals with EAE were perfused after they had been studied electrophysiologically. Histological studies were also performed on normal control rabbits.

Electrophysiological studies were carried out under urethane anaesthesia (sometimes supplemented by pentobarbitone) on 25 animals with EAE one to nine days after the onset of neurological signs. In most animals a lumbosacral laminectomy was performed to expose the lumbosacral cord and the left L7 and/or S1 spinal roots, dorsal root ganglia and spinal nerves. The left sciatic nerve was exposed in the posterior thigh. The exposed nervous tissues were immersed in paraffin oil, and the temperatures of the laminectomy and sciatic nerve pools were usually maintained at 37°C. The sciatic nerve was stimulated with 0.1ms duration square-wave voltage pulses at a rate of 1/s, and recordings were made in the volume conductor over the left L7 or S1 spinal nerve or ventral primary ramus (hereafter referred to as the spinal nerve) 3 to 6mm from the midpoint of the DRG, DRG itself and dorsal root entry zone (Figure 1). A reference electrode was placed on nearby bone. For all recordings, negativity at the active electrode gave an upward deflection on the oscilloscope. In five animals with EAE, conduction through the thoracic dorsal root ganglia was studied by stimulating the exposed internal intercostal nerve and recording in the volume conductor over the respective spinal nerve, DRG and dorsal root entry zone.

Electrophysiological studies were also performed on normal control rabbits.
Results

Clinical Findings

Of the 51 inoculated rabbits, 80% developed neurological signs of EAE, the mean day of onset being the 20th day after inoculation (SD, 6.4). The neurological signs consisted of lateral splaying, hypotonia, ataxia and weakness of the limbs, especially the hindlimbs, absent knee jerks and urinary and faecal soiling of the perineal region. These signs were accompanied by weight loss.

Histological Findings

Histological examination of the CNS revealed widespread meningeal and subpial infiltration with mononuclear cells (Figure 2), and perivascular cuffing and para-adventitial infiltration with these cells. Toluidine blue-stained semithin sections showed demyelination confined to the regions of mononuclear cell infiltration in the subpial (Figure 2) and perivascular lesions. Axons were preserved. Similar perivascular inflammatory demyelinating lesions were seen in
the dorsal and ventral roots and particularly the dorsal root ganglia (Figures 3 and 4). Axons were intact and the DRG neuronal cell bodies were usually normal. The dorsal root ganglion was the region of the nervous system most severely affected. Lumbosacral, thoracic and cervical ganglia were all involved. In contrast, the nearby spinal nerve was almost completely spared by the disease (Pender and Sears, 1984). Sciatic nerve sections either were normal or showed minimal involvement.

Electrophysiological Findings

Typical spinal nerve compound action potentials evoked by stimulation of the sciatic nerve in a normal control animal are shown in Figure 5. The responses are triphasic waves (positive, negative, positive), and at all stimulus intensities the amplitude of the negative wave is equal to or greater than the amplitude of the initial positive wave. The initial positive wave is due to passive outward current driven by the approaching impulses, and the negative wave represents active inward current occurring during the rising phase of the action potential under the active recording electrode. The response recorded over the DRG in the normal control was very similar at all stimulus intensities to the corresponding spinal nerve response (Figure 5).
Figure 3. Transverse sections (A) through the S1 dorsal root ganglion of a normal control rabbit and (B) through that of a rabbit with EAE. In the latter there is extensive perivascular cuffing and para-adjunct infiltrate with mononuclear cells around dilated blood vessels (asterisks in two vessels), and there is severe myelin loss in the regions of mononuclear infiltration. Araldite sections stained with toluidine blue. Bars = 50 μm.
Figure 4. Longitudinal sections (A) through the S1 dorsal root ganglion of a normal control rabbit and (B) through that of a rabbit with EAE. In the latter there are demyelinated axons (arrow) in association with mononuclear infiltration; intracellular myelin debris is also seen (arrowhead). Araldite sections stained with toluidine blue. Bars = 25μm.
Figure 5. Compound action potentials recorded, in the volume conductor, over the left S1 spinal nerve (Sp.N.) and dorsal root ganglion (D.R.G.) of a normal control rabbit, in response to stimulation of the left sciatic nerve at: (A) 1.5 times threshold for the spinal nerve response (1.5T); (B) the intensity giving the maximum spinal nerve response (4.2T); (C) an intensity supramaximal for the spinal nerve response (7.3T).

For these and all other recordings, negativity at the active electrode is represented by an upward deflection. The 200μV calibration bar applies to B and C.
In the animals with EAE the spinal nerve recordings were normal in threshold, peak-to-peak amplitude, configuration and conduction velocities of the initial positive and negative waves (Figure 6). In particular, the amplitude of the negative wave was equal to or greater than the amplitude of the initial positive wave. In contrast, the recordings over the DRG in the animals with EAE were markedly abnormal (Figure 6). At all stimulus intensities there was a reduction in the amplitude of the negative wave and in the ratio of the amplitude of the negative wave to the amplitude of the initial positive wave. The mean of this ratio at the stimulus intensity giving the maximum spinal nerve response was 0.5 (range 0.2 to 1.0) compared with 1.5 (range 1.0 to 1.8) in the normal control rabbits (Pender and Sears, 1984). This reduction in the negativity indicates conduction block in a high proportion of the large diameter myelinated afferents in the dorsal root ganglia of the rabbits with EAE. Conduction block was also found in the thoracic dorsal root ganglia. The effect of the conduction block in the lumbosacral and thoracic dorsal root ganglia was to severely reduce the afferent volley arriving at the dorsal root entry zone (Figure 7). In some animals with EAE, slowing of conduction through the DRG in large diameter afferents was also demonstrated (Pender and Sears, 1984).

Discussion

It has been demonstrated that conduction block occurs in a high proportion of the large diameter fibres in the dorsal root ganglia of rabbits with EAE. The resulting severe functional peripheral deafferentation accounts for the lateral splaying of the limbs, hypotonia, ataxia and areflexia in these animals. Furthermore, this deafferentation is likely to mask the expression of any CNS lesions that in the absence of PNS lesions might produce these signs. The cause of the weakness is unclear. It has previously been shown that conduction from the peripheral nerve to muscle and from the peripheral nerve through the distal half of the ventral root is normal in rabbits with EAE, lesions of these sites thus being excluded as the cause of the weakness (Pender and Sears, 1984). It was concluded that lesions involving the CNS and PNS myelin in the vicinity of the ventral root exit zone were the most likely cause of the weakness, although such lesions were not evaluated electrophysiologically. However, it remains possible that the DRG lesions themselves are responsible, as peripheral deafferentation per se is known to cause weakness (Mott and Sherrington, 1895; Nathan and Sears, 1960).

The vulnerability of the rabbit DRG to autoimmune demyelinating disease and to diphtheritic neuropathy was attributed by Waksman (1961) to the deficiency that he and others before him had observed in the blood-nerve barrier of the DRG. More recently Jacobs et al (1976) have shown that, in the rat, intravenously administered horseradish peroxidase readily enters the DRG and the trigeminal ganglion but not the peripheral nerve, and they attributed this to the presence of open junctions between, and fenestrae within, the ganglionic endothelial cells. They suggested that the known susceptibility of the rat DRG to methylmercury poisoning (Jacobs et al, 1975) was due to deficiency of the blood-
Figure 6. Compound action potentials recorded, in the volume conductor, over the left S1 spinal nerve (Sp.N.) and dorsal root ganglion (D.R.G.) of a rabbit with EAE, in response to stimulation of the left sciatic nerve at: (A) 1.5 times threshold for the spinal nerve response (1.5T); (B) 2T; (C) the intensity giving the maximum spinal nerve response (3.2T); (D) an intensity supramaximal for the spinal nerve response (9.6T).

The 50μV calibration bar applies to A and B, the 200μV calibration bar to C and D.
nerve barrier at this site. The dorsal root ganglia of other mammalian species have also been shown to have a reduced blood-nerve barrier to proteins (Olsson, 1971). It should be noted, however, that in the guinea-pig the blood-nerve barrier of the peripheral nerve as well as of the DRG is deficient, and this accounts for the much greater involvement of the peripheral nerve by EAE, experimental allergic neuritis and diphtheritic neuropathy in guinea-pigs than in rabbits (Waksman, 1961). It has recently been suggested that there may be a second factor contributing to the vulnerability of the DRG to demyelinating disease, namely the peculiar anatomy of the DRG neurone, which has a stem process bifurcating into a central and a peripheral process (Pender and Sears, 1984). It was postulated that this anatomical arrangement may render the branch point particularly susceptible to the functional consequences of demyelination in its vicinity.

The vulnerability of the dorsal root ganglion to disease in experimental animals leads to the question of its vulnerability in man. It is known that the dorsal root ganglia and immediately adjacent parts of the dorsal and ventral roots and spinal nerves are the sites of predilection for diphtheritic neuropathy, a toxic demyelinating disease, in man (Fisher and Adams, 1956). The involvement of structures in the immediate vicinity of the DRG is likely to be due to diffusion, into these structures, of diphtheria toxin that has entered the DRG from the blood. With regard to inflammatory demyelinating disease, the dorsal root ganglia and spinal roots are definitely involved in the Guillain-Barré syndrome (Asbury et al, 1969), but it is unclear whether the region of the DRG is more vul-
nerable than the peripheral nerve. This question needs to be answered. Focal involvement of the DRG and immediately adjacent parts of the dorsal and ventral roots and spinal nerves may account for the observations that some patients with diphtheritic neuropathy and some patients with the Guillain-Barré syndrome have neurological signs but normal peripheral nerve conduction studies (Kurdi and Abdul-Kader, 1979; McLeod et al, 1976). The possibility of involvement of the DRG region in association with acute disseminated encephalomyelitis should also be seriously considered. The form of acute disseminated encephalomyelitis that is the closest human analogue of EAE is a complication of rabies vaccination, which can also be complicated by PNS disease, including the Guillain-Barré syndrome (McIntyre and Krouse, 1949; Appelbaum et al, 1953; Swamy et al, 1984). It is again unclear, however, whether the DRG is a site of predilection for such PNS involvement. In patients with other forms of acute disseminated encephalomyelitis (Miller et al, 1956) many of the neurological signs, such as hypotonia, weakness, ataxia, sensory loss and areflexia, could also be due to involvement of the DRG region as well as other parts of the PNS. With regard to multiple sclerosis, Lumsden (1970) concluded that the PNS was not involved, even though he found leptomeningeal lymphocytic and lipophagic infiltrates in the spinal nerve roots and ganglia. There have been several reports, however, of PNS involvement in otherwise typical cases of multiple sclerosis (Hopf, 1965; Calder and Pollock, 1976; Schoene et al, 1976). Further investigation into this matter is warranted. Clinicians managing patients with multiple sclerosis should ask the question: could some of the clinical features and abnormalities of the somatosensory evoked potentials be due to involvement of the PNS, especially the region of the dorsal root ganglion?

The vulnerability of the human DRG should be considered not only in demyelinating diseases, but also in diseases primarily affecting the neurone (either the cell body or the axon) such as herpes zoster (Head and Campbell, 1900) and carcinomatous sensory neuropathy (Denny-Brown, 1948; Horwich et al, 1977). The region of the dorsal root ganglion in man may in fact be vulnerable to a wide range of inflammatory diseases and circulating toxins.

**Summary**

The dorsal (posterior) root ganglion is a relatively neglected part of the nervous system from the clinical point of view. In recent studies on the pathophysiology of experimental allergic encephalomyelitis (EAE), the main animal model of multiple sclerosis, the DRG of the rabbit was shown to be the site of extensive inflammation and demyelination and of focal conduction block in a high proportion of the large diameter afferents. The resulting severe functional peripheral deafferentation accounts for the postural disturbance, hypotonia, ataxia and areflexia in rabbits with EAE. The vulnerability of the DRG is due to a deficient blood-nerve barrier and possibly also to a susceptibility of the branch point of the ganglion neurone to demyelination-induced conduction block. These and other studies in experimental animals suggest that in man the DRG may be a
preferential (but neglected) site of focal structural and functional abnormalities in inflammatory and also other neurological diseases.

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