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Hypothermia Due to an Ascending Impairment of Shivering in Hyperacute Experimental Allergic Encephalomyelitis in the Lewis Rat

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Abstract

Severe hypothermia and an ascending impairment of shivering are previously undescribed clinical signs in hyperacute experimental allergic encephalomyelitis (EAE) in the Lewis rat. These occurred in hyperacute EAE induced by inoculation with guinea pig spinal cord homogenate and heat-killed *Bordetella pertussis*. Hypothermia was first detected on day 6-7 post-inoculation, within 12-24 h of the onset of neurological signs, and became more severe as the disease progressed. Rectal temperatures ≤ 30°C were common at ambient temperatures of 19-22°C. Shivering was assessed by palpation and by cold tremor electromyography. Shivering was absent in the tail by day 6-7 post-inoculation. The impairment then progressed to affect the hindlimbs, thorax and occasionally the forelimbs. Shivering was absent in hindlimbs with only mild or moderate weakness. Histological studies revealed perivascular inflammation with polymorphonuclear and mononuclear cells, oedema, fibrin deposition, haemorrhage, primary demyelination and axonal degeneration in the spinal cord, dorsal root ganglia and spinal roots. The brainstem was also involved but the cerebral hemispheres, including the hypothalamus, were spared. The close relationship between the severity of hypothermia and the extent of shivering impairment indicates that reduced shivering is an important cause of hypothermia in hyperacute EAE. It is concluded that this impairment of shivering is due not to hypothalamic damage but to lesions elsewhere in the central and peripheral nervous systems.

Keywords: experimental allergic encephalomyelitis; hypothermia; pertussis vaccine; shivering; EAE

Introduction

Mild hypothermia has been observed in rabbits with experimental allergic encephalomyelitis (EAE), an animal model of multiple sclerosis (Pender and Sears 1984), and in rats with whole spinal cord-induced or myelin basic protein-induced acute EAE (Pender, unpublished observations). Hypothermia has also been reported in the terminal stages of myelin basic protein-induced EAE in the guinea pig (Mitsuzawa et al. 1981). Reports of hypothermia in rats with EAE mediated by a T cell line (Fierz and Schaerer 1986), and in man with multiple sclerosis (Sullivan et al. 1987) have suggested hypothalamic lesions as the cause of the hypothermia. In the present study we have used clinical, electrophysiological and histological techniques to (1) document the occurrence of hypothermia in hyperacute EAE in the Lewis rat, (2) investigate the mechanism of the thermoregulatory disturbance and (3) determine the site(s) and nature of the lesions responsible for this disturbance. Brief preliminary communications of this study have been published in abstract form (Hansen and Pender 1987a, b).

MATERIALS AND METHODS

Induction of hyperacute EAE

Male Lewis rats (JC strain) were kept at a room temperature of 21.1 ± 0.7°C (SD) and fed rat and mouse cubes and water ad libitum. An homogenate containing 400 mg guinea pig spinal cord/ml of 0.9% saline was prepared from fresh spinal cord for use on the same day. Under ether anaesthesia, 0.125 ml of the homogenate was injected intradermally into a footpad of each hind foot of rats 10-13 weeks old. A 0.1 ml dose of concentrated *Bordetella pertussis* vaccine (CSL, Melbourne) containing 10^{10} heat-killed organisms was then injected intradermally on the dorsum of each hind foot.

Clinical assessment of inoculated and control animals

Inoculated rats and normal controls were assessed daily from day 6 post-inoculation. Tail, hindlimb and forelimb weakness were each graded on a scale of 0 (no weakness) to 4 (complete paralysis) as previously described (Pender 1986). The total clinical score was derived by adding together the grades of tail, hindlimb and forelimb weakness.

Rectal and tail skin temperatures

Rectal temperature and tail skin temperature were measured in unanaesthetized rats. In two experiments, tail skin temperatures were measured approximately 100 mm from the base of the tail with a surface probe connected to a Digitron thermometer. A length of split plastic tubing was used as a flexible cuff to hold the probe in place. In a third experiment, skin temperature was measured at the base of the tail where the scales commenced. Room temperature was measured with the same probe.

Assessment of shivering

Shivering was assessed by palpation of the muscles of the tail, hindlimbs, thorax, and forelimbs in unanaesthetized animals, or by electromyography of the forelimbs and hindlimbs in lightly anaesthetized rats. All experimental and normal control animals were assessed for shivering on days 1 or 2 and then daily from day 5. Rats were placed singly in plastic mouse cages for 5-10 min and then palpated. Impairment of shivering was graded as follows: 0 = normal shivering palpable at all levels; 1 = no shivering palpable in the tail; 2 = no shivering detected in tail or hindlimbs; 3 = no shivering detected in tail, hindlimbs or thorax; 4 = no shivering palpable in tail, hindlimbs, thorax or forelimbs.

Cold tremor electromyograms (EMGs) were recorded from the biceps brachii and triceps brachii of the left forelimb, and from the tibialis anterior and lateral gastrocnemius of the left hindlimb in controls and experimental rats anaesthetized with ≤30 mg/kg sodium pentobarbitone to abolish voluntary movements. Stuart et al. (1966) have shown that this level of anaesthesia has no effect on shivering. Animals were cooled slightly on an ice-filled cushion to ensure that shivering was induced. For each muscle, the reference electrode was positioned in the tendinous insertion and the active electrode was positioned in the muscle belly. EMGs were measured in 2 normal controls and in 6 rats with hyperacute EAE on day 7 or day 9.

Histological studies

Rats were perfused through the left ventricle with 2% glutaraldehyde, 2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3-7.4) under ether anaesthesia. The brain, spinal cord, dorsal and ventral roots, dorsal root ganglia, spinal nerves and sciatic nerves were removed and placed in fixative. Samples of each tissue were dehydrated and embedded in paraffin, sectioned (4 μm) and stained with haematoxylin and eosin, or with Lendrum's acid picro-Mallory stain for fibrin. For each tissue except the brain, additional samples were postfixed with 2% osmium tetroxide, dehydrated, embedded in HistoResin (LKB, Bromma), sectioned (2 μm) and stained with toluidine blue as previously described (Pender 1985).

To assess the integrity of the blood-brain and blood-nerve barriers, inoculated rats (day 9 post-inoculation) and control rats were injected intravenously with 1 ml of a 2% (w/v) solution

of Evans blue dye in 0.9% saline. One hour later each rat was perfused with 40 ml of 0.9% saline under ether anaesthesia and killed. The brain was removed, cut into coronal sections and quick-frozen in *n*-hexane over dry ice. Cryostat sections (10 μm) were cut, air-dried, fixed with methanol, stained for 1 min with 1% aqueous methyl green, and washed in water. Sections were mounted in 50% aqueous glycerol. Albumin-bound Evans blue, but not the free dye, fluoresces red when excited by light of an appropriate wavelength (Jennische 1985). Sections were examined on a Wild-Leitz Orthoplan fluorescence microscope using an I2 filter block (450-490 nm).

RESULTS

Hypothermia and neurological signs

Mild hypothermia was present within 12-24 h of the onset of neurological signs which commenced 6-7 days after inoculation (Fig. 1). No elevation of rectal temperature occurred before the onset of neurological signs (cf. Mitsuzawa et al. 1981; Fierz and Schaerer 1986). Hypothermia became more severe over the following days. By day 8 post-inoculation, the rectal temperature was 30.3 ± 5.2°C (SD, n = 10) in rats with hyperacute EAE and was 37.8 ± 0.6°C (SD, n = 10) in normal control rats ($P < 0.01$, Mann-Whitney). Within 24 h of death the rectal temperature in diseased rats was 27.7 ± 2.7°C (SD, n = 13). Only one rat whose rectal temperature fell below 34°C recovered.

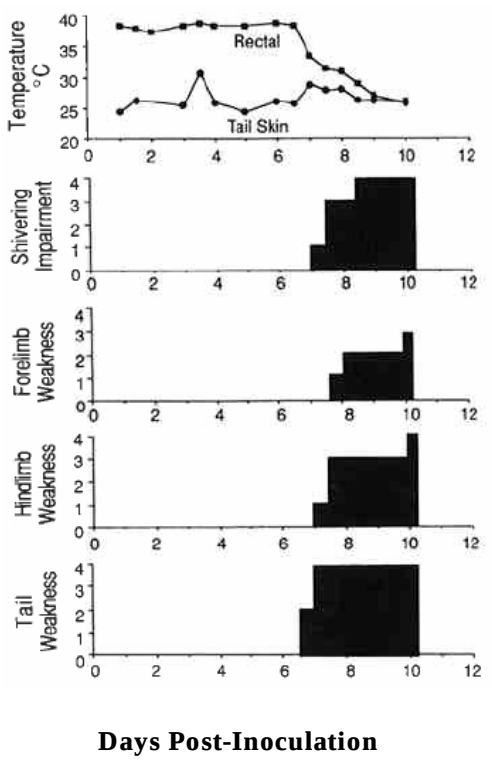

Fig. 1. Profiles of the clinical signs in a Lewis rat with hyperacute EAE, showing the onset and progression of: tail weakness (bottom panel); hindlimb weakness (2nd from bottom panel); forelimb weakness (3rd from bottom panel); impairment of shivering (2nd from top panel); and the rectal and tail skin temperatures (top panel). Observations were made every 12 h from day 6 to day 9 post-inoculation, and every 24 h from day 9 to day 11. This rat was dead on day 11. See Materials and Methods Section for grading of weakness and shivering impairment.

An ascending weakness of the tail commenced 6-7 days post-inoculation, and hindlimb weakness usually began within the next 24 h (Fig. 1). Between days 7-11 post-inoculation, all rats developed hindlimb weakness and 11 of 13 rats developed forelimb weakness. Only 2 of

13 rats showed complete paralysis of the hindlimbs and no rats had complete forelimb paralysis. Rectal temperatures fell as the total clinical score increased during the course of the disease (Fig. 2). The day of death ranged from day 7 to day 12 post-inoculation (mean = 9.5 ± 1.7 SD, n = 12). One rat recovered.

Impairment of shivering

An ascending impairment of shivering was seen in rats with hyperacute EAE. Shivering could be palpated in the tails, hindlimbs, thoraces and forelimbs of normal rats at room temperatures of 19-22°C. Shivering could not be detected by palpation of the tails of rats with hyperacute EAE by day 6-7 post-inoculation when rectal temperatures were subnormal. There was a close relationship between the severity of hypothermia and the ascent of the impairment of shivering (Figs. 1 and 3). In 4 of 13 rats the shivering impairment ascended to affect the forelimbs. Shivering was never absent or reduced at a given body level before the onset of weakness in that area, but shivering was absent in limbs that were not completely paralyzed. Shivering was absent in the hindlimbs of one rat which had only mild (grade 1) hindlimb weakness and in 11 of 13 rats with moderate (grade 2) hindlimb weakness. Forelimb shivering was preserved in 7 of 8 rats with grade 1 or 2 forelimb weakness and was absent in 4 of 6 rats with severe (grade 3) forelimb weakness.

Cold tremor EMG confirmed the impairment of shivering in hyperacute EAE and was consistent with assessments made by palpation. In normal rats, a tremor in response to cold was recorded from antagonistic muscle groups in both the forelimbs and hindlimbs. Representative EMG recordings are shown in Fig. 4. In hyperacute EAE, hindlimb tremor was absent in all 6 rats studied (rectal temperature = 32.2 ± 2.7°C, SD). Forelimb tremor was unaffected in 4 of these rats (rectal temperature = 33.5 ± 1.1°C, SD) and reduced or absent in the other 2 (rectal temperature = 29.8 ± 3.9°C, SD). In limbs where shivering was absent, a forceful pinch of the foot still produced a motor response detectable on the EMG.

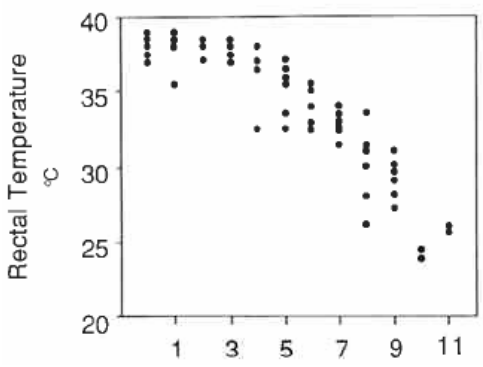

Fig. 2. The relationship between rectal temperature and the total clinical score for 13 rats with hyperacute EAE, from day 0 to day 12 post-inoculation. Only one rat survived to day 12. The total clinical score was derived by adding together the grades of tail, hindlimb and forelimb weakness. See Materials and Methods Section for grading of weakness and shivering impairment. One point on the diagram often represents more than one observation. The number of rats for each total clinical score was as follows: 0, n = 61; 1, n = 4; 2, n = 4; 3, n = 6; 4, n = 5; 5, n = 7; 6, n = 10; 7, n = 6; 8, n = 6; 9, n = 9; 10, n = 2; 11, n = 2. No rat received the maximum score of 12.

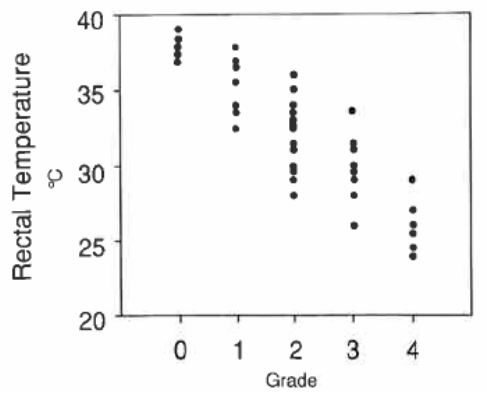

Fig. 3. The relationship between rectal temperature and the grade of shivering impairment for 13 rats with hyperacute EAE, from day 0 to day 12 post-inoculation. Only one rat survived to day 12. See Materials and Methods Section for grading of shivering impairment. One point on the diagram often represents more than one observation. The number of observations for each grade of shivering impairment was as follows: grade 0, $n = 72$ (13/13 rats); grade 1, $n = 15$ (10/13 rats); grade 2, $n = 19$ (13/13 rats); grade 3, $n = 8$ (5/13 rats); grade 4, $n = 6$ (4/13 rats).

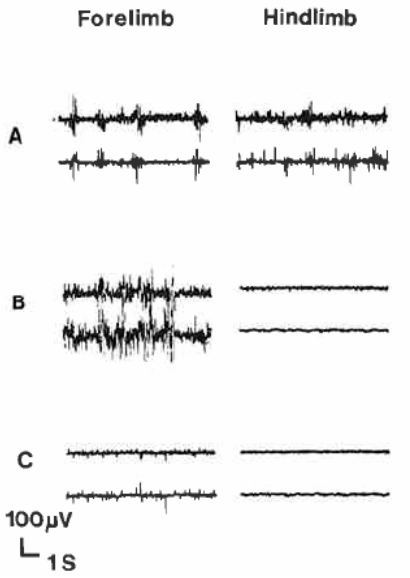

Fig. 4. Cold tremor electromyograms recorded from a normal rat (A) and 2 rats with hyperacute EAE 9 days post-inoculation (B, C) under light pentobarbitone anaesthesia and mild cooling. For all forelimb recordings (left column) the upper trace is from the biceps brachii and the lower trace is from the triceps brachii. In the hindlimb (right column), the upper trace is from the tibialis anterior and the lower trace is from the lateral gastrocnemius. Shivering was absent in the hindlimbs of rat B, but preserved in the forelimbs, as assessed by palpation at an ambient temperature of 22.8°C. The rectal temperature was 33.5°C. Shivering was absent in the hindlimbs of rat C and barely detectable in the forelimbs, as assessed by palpation at an ambient temperature of 22.8°C. The rectal temperature was 27°C.

Skin temperature of the tail

Skin temperatures of the tail were measured to determine whether inappropriate vasodilatation of the tail contributed to the hypothermia. In one experiment, tail temperatures in rats with hyperacute EAE rose above the mean normal tail temperature (25.7 ± 1.8 °C, SD) for several time points, after the rectal temperature had dropped below normal (Fig. 1). Tail temperatures were significantly higher in 5 rats with hyperacute EAE than in 5 normal controls on day 7 and

day 8 ($P < 0.01$, Mann-Whitney). In a second experiment, 6 of 8 rats showed no increase in tail temperature after the rectal temperature had begun to fall. Two rats had elevated tail skin temperatures at one observation after the onset of hypothermia, but skin temperatures of this group of rats with hyperacute EAE were never significantly higher than those of normal controls. When results from both experiments were combined, there was no significant difference in the tail skin temperatures of diseased rats and normal controls at any time. In a third experiment, skin temperatures were measured at the base of the tail and there was no significant difference between diseased and normal rats at any time.

Histological findings

Polymorphonuclear and mononuclear cell infiltration, oedema, primary demyelination and mild haemorrhage were present at all levels of the spinal cord, and in the spinal roots and dorsal root ganglia of rats with hyperacute EAE. The lumbar and sacral levels were more severely affected than more rostral levels of the spinal cord. Polymorphonuclear cells were dispersed throughout the parenchyma of the spinal cord, and also were found in perivascular cuffs. Demyelination was particularly apparent in the subpial area of the cord, and at the dorsal root entry and ventral root exit zones (Fig. 5). Axonal degeneration was found in dorsal and ventral roots and in the subpial area of the spinal cord. The sciatic nerves were normal. Polymorphonuclear and mononuclear cell infiltration were present in the brainstem, especially along the ventrolateral wall of the fourth ventricle. The cerebellum and the cerebrum were normal. In particular no abnormalities were found in the hypothalamus.

Fibrin deposition (days 9-11 post-inoculation) and extravasation of administered Evans blue dye (day 9) were used as indicators of oedema and noncellular exudate. Fibrin deposition was detected in the meninges, the subpial area of the spinal cord, and in some spinal roots and dorsal root ganglia, but not in the brainstem, hypothalamus or other regions of the brain. Evans blue fluorescence was not seen in the parenchyma of the brain or brainstem, but was present in an occasional vessel in the meninges on the ventral aspect of the brain.

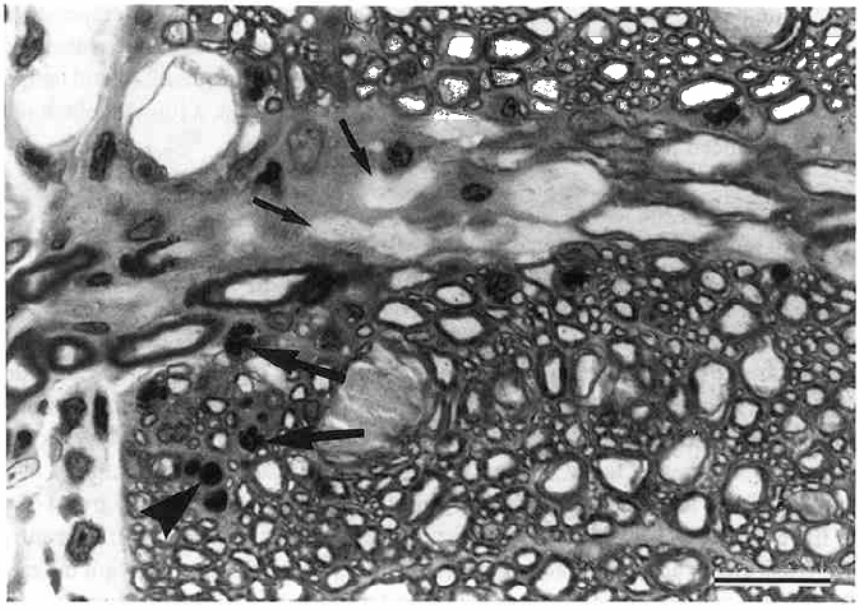

Fig. 5. A transverse section through the C6 spinal cord of a rat with hyperacute EAE, 9 days post-inoculation. Demyclinated axons (small arrows) can be seen in the ventral root exit zone. Polymorphonuclear leukocytes (large arrows) and mononuclear leukocytes are present. Extravasated erythrocytes (arrowhead) can also be seen. HistoResin section stained with toluidine blue. Bar = 25 μm.

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DISCUSSION

We have demonstrated that severe hypothermia occurs in rats with hyperacute EAE, and is accompanied by an ascending impairment of shivering. Although hypothalamic lesions have been proposed as the cause of hypothermia in EAE mediated by T cell lines (Fierz and Schaerer 1986) and in multiple sclerosis (Sullivan et al. 1987), we found no cellular infiltration or oedema in the hypothalamus. These results indicate that some mechanism other than hypothalamic damage was responsible for the hypothermia.

The close relationship between the severity of hypothermia and the extent of shivering impairment indicates that the impairment of shivering plays a major role in causing hypothermia in hyperacute EAE. Interruption of shivering pathways in the central and peripheral nervous systems by primary demyelination and axonal degeneration could account for the impairment of shivering. The demonstrated lesions in the dorsal root ganglia, dorsal roots and dorsal root entry zones of the spinal cord would reduce the afferent proprioceptive input to the spinal cord and thereby reduce the intensity and regularity of shivering (see Stuart et al. 1966; Thauer 1970). Although these lesions would also affect thermosensory input, Heath and Crabtree (1987) have shown that cutaneous deafferentation has little, if any, effect on shivering in the rat and does not substantially interfere with the ability to regulate body temperature.

The lesions of efferent fibres in the intramedullary ventral roots, ventral root exit zones of the spinal cord and extramedullary ventral roots would also reduce shivering. Pender (1988) has shown that demyelination at the ventral root exit zone leads to conduction block in α-, *β*- and γ-fibres in rats with acute whole spinal cord-induced EAE. Alpha-motor activity is essential for shivering. Indeed it could be argued that weakness of the lower motor neurone type could be the sole cause of the shivering impairment in hyperacute EAE. However, there was no clear relationship between the degree of weakness and degree of shivering impairment in a given limb. For example, shivering was absent in the hindlimbs of most rats with only mild or moderate hindlimb weakness but was preserved in the forelimbs of most with mild or moderate forelimb weakness. Thus weakness of the lower motor neurone type appears not to be the only cause of the shivering impairment. Lesions of γ-motor fibres may also impair shivering as there is evidence that these fibres are important in the generation of cold tremor (Schafer and Schafer 1973; Sato 1981).

Lesions of ascending and descending shivering pathways in the brainstem and spinal cord could also contribute to the shivering impairment. The intensity of cold tremor is reduced in spinalized dogs and rabbits (Simon et al. 1966; Kosaka and Simon 1968). The lesions of the floor of the fourth ventricle could affect the thermosensitive and thermoregulatory medullary region identified by Lipton (1973) and Lipton et al. (1974) and might thus interfere with shivering.

In addition to the impairment of shivering, other factors may contribute to the development of hypothermia in hyperacute EAE. Reduced sympathetic outflow, as occurs in rats with acute EAE (Baum et al. 1972), may also occur in hyperacute EAE and interfere with non-shivering thermogenesis, a sympathetically mediated function (Hsieh et al. 1957). However, we have not examined this possibility. Vasoconstriction and behavioural strategies are important means of heat conservation for thermoregulation. At temperatures below thermoneutrality (30°C for rats (Heath and Crabtree 1987)) blood vessels in the skin are constricted to minimize radiant heat loss. In the rat, 20% of total heat production can be lost through vasodilatation of the tail at ambient temperatures of 27-30°C (Rand et al. 1965). We found evidence of vasodilatation, as measured by tail skin temperature, after the onset of hypothermia in one experiment and not the other. Inappropriate vasodilatation does not seem to be essential for the development of hypothermia in hyperacute EAE. Rats with hyperacute EAE also displayed postures that result in heat dissipation (Roberts and Mooney 1974). Lack of behavioural heat conservation may have contributed to the severity of the hypothermia.

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