Short report

Lumbar and cortical somatosensory evoked potentials in rats with vitamin E deficiency

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SUMMARY Somatosensory evoked potentials (SEPs) from lumbar and cortical areas and electromyographic activity (EMG) were recorded in 40-42 week vitamin E deficient rats and in age matched controls. A significant increase in the latency ($p < 0.001$) of the cortical SEP and a significant reduction in the lumbar to cortical conduction velocity ($p < 0.001$) were observed in vitamin E deficient rats compared with controls. No significant differences were obtained in the latency of the lumbar SEP or in the peripheral conduction velocity from the ankle to lumbar region. All the vitamin E deficient rats had abnormal EMG findings (fibrillation potentials, positive sharp waves and polyphasic activity), whereas none of the controls showed any of these signs of dysfunction.

A severe deficiency of vitamin E (alpha-tocopherol) results in a progressive neuromuscular disease both in man¹ and experimental animals.²⁻⁴ Although in animals a primary myopathy is recognised² as well as changes in the nervous system,^{3,4} in man the latter predominate.¹ In the rat a necrotising myopathy has been described² but this is less obtrusive in man.⁵ In muscle from both animal and man autofluorescent bodies are present⁵⁻⁸ and there is evidence of both denervation and primary muscle disease. EMG studies in man suggestive of denervation have been reported.^{9,10} The lesions in the nervous system of both man and experimental animals (for example rat and monkey) with vitamin E deficiency appear to be similar, with degeneration of axons in the posterior columns and the gracile and cuneate nuclei, and a selective loss of large calibre myelinated axons in the spinal cord and peripheral nerves.^{3,4,11}

Electrophysiological studies in man have been carried out in several fat malabsorptive states

associated with vitamin E deficiency. These show that somatosensory evoked potentials can often be abnormal^{8,12-15} and more rarely studies of peripheral nerves have demonstrated signs of dysfunction.^{10,12,14} A common finding is that despite EMG evidence of denervation, motor nerve conduction velocities are usually within the normal range.^{9,10}

We have studied the lumbar and cortical SEPs and EMG in rats maintained on a vitamin E deficient diet for between 40 and 42 weeks from weaning and in age matched controls in order to investigate more fully the extent and degree of abnormalities in neuromuscular activity.

Materials and methods

Animals

Male weanling Wistar rats were obtained from Charles Rivers Ltd, UK. Half were placed on a vitamin E deficient diet (vitamin free casein-dextrose diet, Machlin/Draper—HLR #814, supplied by Dyets Pennsylvania, USA) and the remainder (controls) received the same diet to which alpha tocopherol acetate (100 mg/kg) had been added. The vitamin E (alpha-tocopherol) status, including that of nervous tissue, was closely monitored during the course of the deficiency and is reported elsewhere.¹⁶ After 40-42 weeks of the study, 12 rats from the vitamin E deficient group were randomly

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selected and their electrophysiological recordings compared with eight control rats.

Electrophysiology

The rats were anaesthetised with sodium phenobarbitone (60 mg/kg). Rectal temperatures were maintained between 35°C and 37°C, by warming the animals when necessary with an infra red lamp. All recordings were made within 15 min from onset of anaesthesia, when neither a corneal reflex nor a response to painful stimuli could be elicited. The right tibial nerve was stimulated electrically with a needle electrode inserted at the ankle, posterior to the medial malleolus. A 20–25 V stimulus was given such that a moderate paw twitch was obtained. The stimulus frequency was 2–5 Hz. Lumbar SEPs were recorded from a needle electrode inserted between the 5th and 6th lumbar vertebral spines (L5/L6), referred to one inserted 2 cm proximally between the 2nd and 3rd lumbar vertebral spines (L2/L3). Cortical SEPs were recorded from a needle electrode inserted subcutaneously over the bregma and advanced laterally in the direction of the left eye referred to an electrode inserted between the 1st and 2nd cervical vertebrae (C1/C2). Distances between the stimulating and recording electrodes were measured with calipers. Typically 16–10 ms sweeps were averaged for lumbar potentials, and 256–30 ms sweeps for cortical potentials. All recordings were made using a Medelec MS6 electrophysiological recorder set to a recording band pass of 8 Hz to 8 KHz. Averaged waveforms were stored on floppy disc and printed onto photographic paper using a Scopix 100 video imager supplied by courtesy of Agfa-Gavaert Limited.

EMG activity was recorded using a coaxial needle electrode inserted into the gastrocnemius muscle of the right hind limb. Four well separated sites in this muscle were always sampled acoustically and visually and permanent paper records of activity were stored. Other muscles in the lower limb and occasionally in the fore limb were also tested.

Results are expressed throughout as mean and 1 SD and the significance of difference between mean values was calculated by Student's *t* test.

Results

After 40 weeks on the vitamin E deficient diet, all rats showed signs of weakness, an ataxic gait, muscle wasting in the hind limbs and had a significantly reduced ($p < 0.001$) body weight (469.4, SD 86 g; $n = 16$), whereas the controls had a normal gait, good muscle bulk and the expected body weight for their age (681.3 SD 83.3 g; $n = 12$).

Following electrical stimulation at the ankle, a consistent response was invariably recorded over the lumbar region in both groups of rats (fig. a). This response had a characteristic triphasic morphology with a prominent negative peak (R response).¹⁷

Conduction velocities were calculated using the onset latency of the first lumbar component (R response) and the distance between the stimulating electrode and the recording electrode at L5/L6. There were no significant differences in either latencies or

conduction velocities between the vitamin E deficient and control groups (see table).

The cortical SEP (fig. b) had an initial small positive deflection which could not always be identified reliably, followed by a small negative peak at 13 ms in controls and a larger positive peak at 15 ms which was always the most prominent component of the complex. The cortical SEPs were generally broader and less well defined in the vitamin E deficient as compared with the control rats.

Conduction velocities for the central nervous system pathway were obtained by calculating the transmission time from the first negative peak of the lumbar response (R response) to the first negative peak of the cortical response (N13) and subtracting 2 ms to take account of synaptic delays at the gracile nucleus and the thalamus. This net transmission time was divided by the distance between the recording electrodes over L5 and the somatosensory cortical area. The mean latencies of the vitamin E deficient group were significantly longer ($p < 0.001$) and the conduction velocities significantly slower ($p < 0.001$) than those of the control group (see table).

In the EMG studies, spontaneous fibrillation potentials were generally found at all four sampled sites of the gastrocnemius muscle of all the vitamin E deficient rats. Positive sharp waves and polyphasic

Fig (a) Lumbar SEP recorded in a vitamin E deficient rat, showing the characteristic prominent negative peak (R) which was invariably of normal latency. (b) Delayed cortical SEP from a vitamin E deficient rat. Dotted lines indicate the mean latencies for the control animals for the initial negative (N13) and positive (P15) peaks. The arrowheads identify the delayed peaks.

Table Somatosensory responses—Latencies and conduction velocities

		Lumbar evoked potentials		Cortical evoked potentials		
		Latency (ms) R	Peripheral conduction velocity (ms^{-1})	Latency (ms)		Central conduction velocity (ms^{-1})
				N13	P15	
E Deficient (n = 12)*	Mean	1.84	44.08	15.15	17.30	14.04
	1 SD	0.11	2.44	0.63	0.79	1.13
Controls (n = 8)	Mean	1.91	45.10	13.65	15.35	16.71
	1 SD	0.09	2.33	0.47	0.79	0.96
p Value		NS	NS	<0.001	<0.001	<0.001

NS = Not significant

* = Three of the 12 E deficient rats had severely degraded cortical responses in which the components could not be reliably identified.

groups were also commonly present. No abnormal spontaneous activity was found in any control rat.

Discussion

The reduced growth and poor health in the vitamin E deficient rats observed in this study are similar to that found by others using the same diet (J S Nelson, personal communication). No other abnormalities were noted in the deficient rats until around 28 weeks when some began to show hair loss, abnormal gait and muscle wasting which was particularly evident in the hind limbs.

The electrophysiological studies were performed after 40–42 weeks on the diet, by which time all the vitamin E deficient rats were ataxic and had muscle wasting and weakness in the hind limbs. The results demonstrate signs of muscle dysfunction, probably due to denervation, and slowed conduction in the somatosensory pathway within the central nervous system. There was no indication of slowing in the peripheral pathway in the lower limbs.

Abnormal SEPs in vitamin E deficient rats do not appear to have been reported previously. In the present study, latencies of cortical responses were consistently between 1.5 and 2.0 ms later in the physically smaller experimentally deficient group; similarly, conduction velocities from the lumbar region to somatosensory cortex were also significantly less in the deficient rats. Barbiturate anaesthesia and the decrease in body temperature which can accompany it, may also produce an increase in SEP latency,^{18,19} but both these factors were carefully controlled and systematic bias avoided.

The abnormalities in central conduction are consistent with neuropathological evidence from other studies. Nelson *et al*³ reported that in man, monkey and rat, vitamin E deficiency leads to axonal degeneration which is most severe in the posterior columns. Loss of large calibre myelinated sensory axons in

peripheral nerve also occurs. The results are also consistent with electrophysiological recordings obtained in man.^{10,14,15} Satya-Murti *et al*¹⁵ found abnormal cortical SEPs in the presence of normal peripheral sensory nerve action potentials in patients with a severe and prolonged deficiency and who had neurological signs. It is of interest that Bradley *et al*²⁰ recorded normal latencies in vitamin E deficient rats for brainstem SEPs elicited by tibial nerve stimulation, despite the presence of prominent degenerative changes in the gracile nuclei. This is paralleled in observations in man by the finding of Willison *et al*² who reported delayed cortical SEPs with a normal latency for brainstem somatosensory responses in a patient with cystic fibrosis and vitamin E deficiency. There may, therefore, be additional pathological changes in the more rostral parts of the somatosensory pathway not so far demonstrated, or the abnormal latency of cortical potentials may be related to delayed transmission at brainstem level.

Wichman *et al*¹⁰ described abnormal EMG findings, compatible with denervation in their patients. The occurrence of myopathic changes has also been suggested.¹² Our studies together with those of Machlin *et al*² suggest that both these processes also occur in the vitamin E deficient rat. Further studies are therefore required to delineate the precise effects of vitamin E deficiency on nerve, muscle and the neuromuscular junction.

These studies show that electrophysiological recordings, in particular the measurement of cortical SEPs, provide a sensitive method for monitoring the neural effects of vitamin E deficiency in serial longitudinal studies in the rat.

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