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Thiamine Deficiency and Nervous System Function Disturbances

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Summary. Thiamine is important for oxidative metabolism, and B_1 deficiency is thought to give rise to polyneuropathies. A group of male Wistar rats (n = 15) received a vitamin B_1 deficient diet (group-a), and the pair fed control group (n = 20, group-b) received a normal diet with no vitamin deficiency.

A second control group (group-c) was fed unrestrictedly with a standard diet (n = 19). All animals were examined for 25 weeks. The sensory nerve conduction velocity, the compound radicular, spinal and brain stem responses and the SEP were derived for tail and hind paw stimulation. The examination was repeated at 6-week intervals.

There was no difference in nerve conduction between group-a and -b, but for both groups the conduction velocity was significantly slower than in group-c. The SEP latencies were significantly increased in group-a compared with group-b and also with group-c. The spinal and cerebral latencies were delayed in group-a. The diameters of myelinated nerve fibres were decreased in group-a compared with group-b, and in group-b compared with group-c.

The results indicate that a specific polyneuropathy exits as a result of B_1 deficiency, and that the sequelae of the lack of thiamine are pronounced in the CNS.

Key words: Polyneuropathy – Rat SEP – Thiamine – Vitamin B_1

Introduction

Thiamine pyrophosphate, the biologically active form of vitamin B_1, catalyses oxidative decarboxylating reactions as a coenzyme of pyruvate dehydrogenase. It is of crucial importance in the synthesis of acetyl-coenzyme A as well as in connecting the metabolism of carbohydrates, lipids and proteins with the citric acid cycle. Vitamin B_1 is involved in the synthesis of nucleotides and NAD, and in the pentose phosphate pathway as a coenzyme of transketolase. The vitamin is also essential for glucose utilization in the CNS (Hakim and Pappius 1983; Sharp et al. 1982).

The concentration of B_1 is highest in the cerebellum (0.475 nmol/g) (Rindi 1982) and in the rat decreases sucessively from the brain stem to the cortex cerebri (Rindi et al. 1979). In the ischiadic nerve (Rindi 1982) the thiamine concentration is low (0.165 nmol/g) but the turnover is very high (0.58 µg/h). Also the transketolase activity is high in Schwann's cells (Dreyfus 1976). In the light of this data it seems probable that B_1 deficiency leads to various disturbances of neuronal function, and in this context the best known deficiency disease is Wernicke's encephalopathy (Dastur et al. 1976; Smith 1977; Victor 1981; Wernicke 1881). Further, a sensomotor polyneuropathy of the symmetric acrodistal type may develop (Erbslöh and Abel 1970; Kanig 1976; Neundörfer 1980; Rewerts 1973). The first symptoms are "burning feet", cramps in the legs, hypaesthesias concerning vibration and posture sense, touch, pain and temperature (Farmer 1981). Later in the course of the disease, pareses with distal pronounciation, muscle fasciculations, diminution of the stretch reflexes and trophic disturbances of the skin and muscles develop. Even cranial nerves can be affected (Erbslöh and Abel 1970). Polyneuropathies develop during chronic thiamine deficiency of at least 3 months (Farmer 1981). Under experimental conditions symptoms of polyneuropathy with lack of stretch reflexes, loss of muscle strength and pallhypaesthesia of the feet could be provoked under a thiamine deficient diet in man after 110 days (Williams et al. 1943). The slow and incomplete restitution after restoration of vitamin B_1 was estimated as a sign of axonal degeneration. Najjar and Holt (1943) observed no deficiency symptoms in four out of nine thiamine deficient persons. They presumed causative thiamine synthesis by intestinal flora, but the result could also indicate the importance of a genetic disposition. Of the nine persons five became anorectic and four developed symptoms of polyneuropathy with pareses of the legs after an average of 77 days.

The following experiment attempts to investigate the development and distribution pattern of functional nervous disturbances under thiamine deficiency.

Material and Methods

Three groups of male Wistar rats (weight 250–300 g, 12–15 weeks old) were kept in an air-conditioned enviroment under constant day-night-cycles (n = 30, 20 and 19 animals). To avoid coprophagy, wire grids were placed at an adequate height above the cage floor.

The animals of group-a were fed unrestricted amounts of a vitamin B_1 deficient diet (Altromin C 1021 R, 0.05 mg thiamine HCl/kg). The concentration of the remaining vitamins, minerals and trace elements in the feed was sufficient. The

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Table 1. Average values of the neurophysiological parameters, measured at 6-week intervals repeatedly (examinations 1 to 5); Thiamine deficient group-a and pair fed group-b

		Weight (g)		NCV tail (m/s)		Latency tail L5 (ms)		Hind paw stimulus									
								Latency L5 (ms)		Latency L1 (ms)		Latency CII (ms)		SEP N1 (ms)		Spinal latency (CII–L1) (ms)		Cerebral latency (N1–CII) (ms)	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
1	$\bar{x}$	310.7	305.1	42.5	41.2	5.6	5.4	3.2	3.1	4.1	4.0	6.8	6.9	11.8	11.6	2.71	2.88	5.01	4.72
	SD	10.0	8.1	2.26	2.21	0.23	0.30	0.11	0.15	0.16	0.23	0.25	0.22	0.44	0.36	0.281	0.289	0.453	0.381
	t			1.71		2.02		2.04		1.38		0.99		1.51		1.75		2.00	
	P			—		= 0.05		< 0.05		—		—		—		—		= 0.05	
2	$\bar{x}$	241.1	307.7	43.6	42.0	5.7	5.3	3.6	3.2	4.5	4.1	7.5	7.0	13.8	12.5	3.03	2.86	6.27	5.48
	SD	13.1	8.1	3.50	3.52	0.28	0.33	0.20	0.19	0.21	0.17	0.36	0.21	1.23	0.46	0.250	0.192	1.029	0.509
	t			1.35		3.48		5.27		5.75		5.30		4.03		2.19		2.73	
	P			—		< 0.01		< 0.001		< 0.001		< 0.001		< 0.001		< 0.05		< 0.05	
3	$\bar{x}$	326.1	325.0	43.5	42.8	5.4	5.2	3.3	3.1	4.2	3.9	7.2	6.7	12.5	11.7	3.01	2.82	5.31	4.93
	SD	20.6	9.0	2.23	2.39	0.25	0.22	0.14	0.17	0.18	0.19	0.26	0.22	0.90	0.54	0.271	0.170	0.792	0.422
	t			0.93		1.60		3.82		4.66		5.63		3.27		2.39		1.71	
	P			—		—		< 0.001		< 0.001		< 0.001		< 0.01		< 0.05		—	
4	$\bar{x}$	324.0	330.9	42.5	43.2	5.4	5.3	3.3	3.0	4.2	3.9	7.0	6.7	11.9	11.4	2.87	2.77	4.88	4.71
	SD	35.0	9.3	2.26	3.53	0.35	0.26	0.10	0.17	0.17	0.19	0.25	0.23	0.69	0.52	0.202	0.235	0.622	0.475
	t			0.76		0.93		6.16		3.61		3.88		2.30		1.35		0.91	
	P			—		—		< 0.001		< 0.001		< 0.001		< 0.05		—		—	
5	$\bar{x}$	310.7	325.3	42.9	42.4	5.5	5.3	3.4	3.1	4.3	4.0	7.4	6.8	13.2	11.5	3.05	2.79	5.85	4.72
	SD	25.8	11.0	2.60	3.27	0.12	0.28	0.13	0.21	0.19	0.17	0.23	0.25	0.44	0.51	0.173	0.192	0.484	0.402
	t			0.51		2.13		6.12		5.45		7.44		10.77		4.20		7.34	
	P			—		< 0.05		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	

Group-a thiamine deficiency (n = 15); group-b pair fed control (n = 20)

Table 2. Average values of the neurophysiological parameters, normal fed control group

		Weight	NCV tail	Latency tail L5	Hind paw stimulus					
					Latency				Spinal latency (CII-L1)	Cerebral latency (N1-CII)
					L5	L1	CII	SEP N1		
1	$\bar{x}$	287.3	39.3	5.8	3.3	4.1	7.0	12.2	2.91	5.17
	SD	6.7	2.79	0.31	0.16	0.26	0.20	0.58	0.194	0.636
2	$\bar{x}$	357.9	46.1	5.2	3.2	4.1	7.1	12.2	2.96	5.11
	SD	22.7	4.01	0.26	0.13	0.22	0.15	0.61	0.214	0.530
3	$\bar{x}$	374.2	45.0	5.2	3.2	4.2	7.0	12.1	2.87	5.05
	SD	25.2	4.73	0.23	0.18	0.31	0.21	0.82	0.214	0.718
4	$\bar{x}$	401.2	45.5	5.2	3.2	4.1	7.0	11.8	2.96	4.77
	SD	24.7	3.73	0.18	0.13	0.17	0.20	0.55	0.230	0.489
5	$\bar{x}$	410.5	48.0	5.1	3.2	4.1	7.0	11.9	2.93	4.87
	SD	25.7	5.22	0.28	0.15	0.24	0.23	0.51	0.216	0.455

Group-c normal fed controls (n = 19)

control analysis of the special diet (Institute of Agricultural Examinations and Research, Kiel, FRG) showed less than 50 µg thiamine HCl per kg solid food (12.6 µg/1000 Kcal).

The animals of group-b were given a standard feed (Altromin 1323 R) in a restricted amount (pair fed), so that the animals weights were comparable to group-a. The control animals of group-c were given a standard feed (Altromin 1320 R) in unrestricted amounts.

After being fed for 7 days, the animals underwent neurophysiological examinations which were repeated at 6-week intervals for a period of 25 weeks. These studies followed a previously described method (Claus and Neundörfer 1983). The animals were anaesthetized using 40–50 mg/kg pentobarbital sodium (Nembutal) i.p. and put onto a paraffin bath at a constant temperature of 37° ± 1°C (Ebara et al. 1981; Ono et al. 1981). The platinum needle electrodes were inserted transcutaneously for neurophysiological examinations.

With the aid of the initial responses, the mixed conduction velocity of fast conducting nerve fibres of a ventral tail nerve and the radicular L5 latency were measured for stimulation at the tip of the tail. After stimulating the right hind paw, the peak of the presynaptic compound potential was measured above L5 vertebra. The following responses could also be measured after hind paw stimulation: the third component of the compound response attained above L1 which is a postsynaptic spinal potential (Wall and Devor 1981); the postsynaptic CII response of the brain stem potentials—derived cranially to C1 vertebra (Claus and Neundörfer 1983); and the first negativity N1 of the cortical SEP primary complex—derived from the contralateral convexity of the skull. The spinal latency was calculated by subtracting the latencies of L1 from CII, and the cerebral latency by subtracting CII from N1.

After the last examination the animals were sacrificed. Arterial blood was used to measure the thiamine saturation status by the transketolase activation test (thiamine pyrophosphate (TPP) effect) (Delaney et al. 1966; Inokuchi et al. 1981; McLaren et al. 1980; Somogyi et al. 1980).

The more distal segment (North and Sinclair 1856) of the right ischiadic nerve was prepared and fixed by immediate immersion in 2.5% glutaraldehyde and cacodylate buffer. The semi-thin sections were stained with osmic acid. Microphotographs, enlargement 1870x, were made of three regions of each nerve (total area 7500 µm^2). The smallest diameters of all myelinated fibres were measured using the MOP AMO3-apparatus (Carl Zeiss R) on these photographs.

A close approximation to the normal distribution was confirmed by the Kolmogorow-Smirnow-Approximation Test. The results were examined using the t-Test for independent random samples and non-homogeneous variances. A two-way analysis for significance was evaluated.

Results

Only 15 of the 30 animals in group-a survived the thiamine deficient diet for 25 weeks. It was necessary to add 20% of a normal standard diet to the B_1 deficient feed for two periods of 1 week each because of the weight loss after the second examination. All animals were underweight compared with group-c (average weight at the last examination 410.5 ± 25.7 g) (Table 1 and 2). The animals of group-a ate 47 g (≙ 186 Kcal) food per kg animal-weight per day on average. The average value was 44 g (≙ 136 Kcal) in group-b and 55 g (≙ 171 Kcal) in group-c. Therefore, the intake of calories was highest in group-a.

The maximum value of the transketolase activation test was estimated in 25 control animals (mean value + 2 SD); it was 1.24. In comparison with this value no case of thiamine deficiency was found in group-b or -c. The activation quotient was increased in 8 of the 11 controled animals of group-a, proving a vitamin deficiency (average value in group-a = 1.73 ± 0.7).

The thiamine deficient animals lost weight. After 4 to 8 weeks they showed less spontaneous activity, only as much as was necessary to get food. The muscles atrophied and their tone decreased. The gait became unstable, giddy and atactic, and the animals lost hair and their coats became unkempt.

Compared with group-b the nerve conduction velocity (NCV) in group-a remained unchanged (Fig. 1). But after

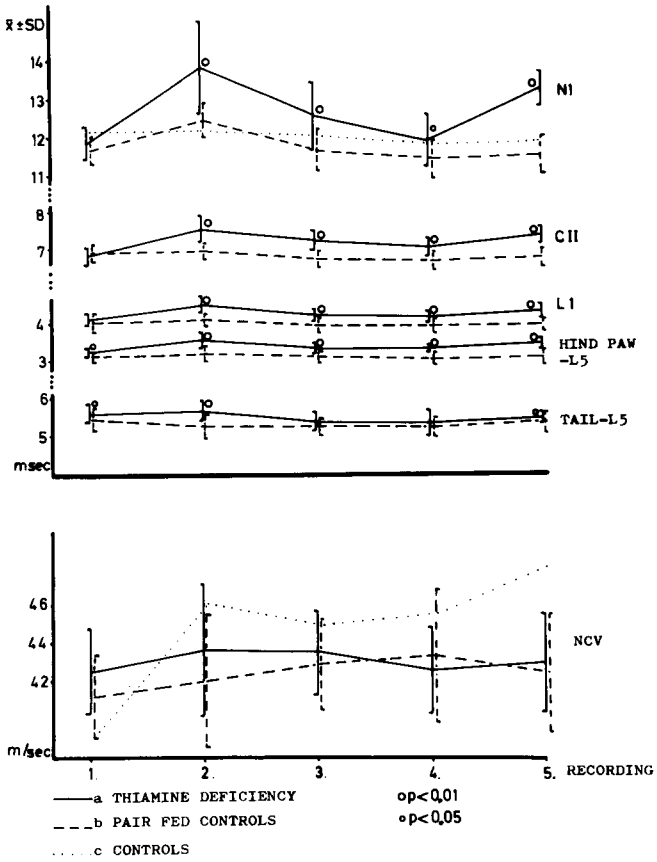

Fig. 1. Tail NCV (m/s) and latencies (ms) for L5, L1, cervical and scull derivation. Recordings after 1-week of thiamine deficiency and repeated at 6-week time intervals

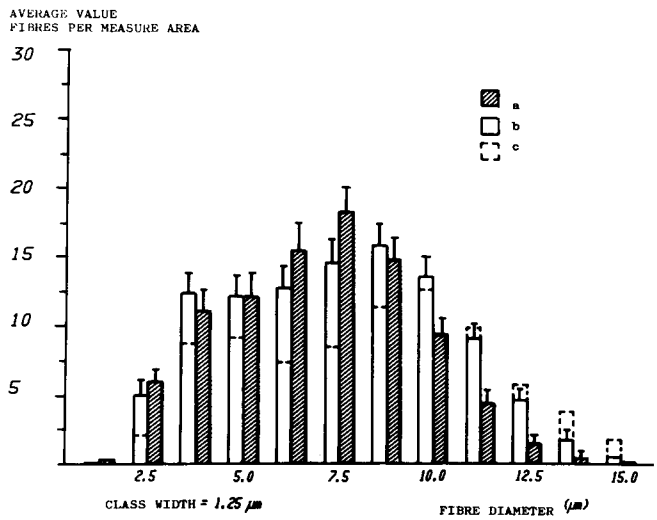

Fig. 2. Distribution of myelinated fibre diameters. Average values for the ischiadic nerves +95% confidence intervals (groups a, b, c)

6 weeks a radicular, spinal and cerebral stimulus delay with significant latency delays was clearly. Compared with group-c the latencies of L5, L1, CII and N1 also increased significantly. However, also compared with group-c the tail NCV in group-a and group-b was significantly slower ($P < 0.01$) at the first, fourth and fifth examinations.

The amount of large nerve fibres was reduced morphometrically (Fig. 2), while medium sized fibres were more frequent than in group-b. In comparison to group-c a clear reduction of the fibre diameter spectrum was displayed in group-a and in group-b.

Discussion

A reduction of spontaneous activity, hypotonia and atrophy of muscles, hair loss and weight reduction have been described repeatedly in thiamine deficient rats (Gibson et al. 1982; Hakim and Pappius 1983; Juntunen et al. 1979; Kalm et al. 1952; Kark et al. 1975; Rewerts 1973; Sharp et al. 1982). Sometimes serious CNS disturbances with no symptoms of polyneuropathy have been seen in rats fed on a B_1 deficient diet (Kalm et al. 1952; Magun 1953). A number of researchers (DeCaro 1962; Kunze and Muskat 1969; Rewerts 1973) have observed a significantly reduced motor NCV in rats with chronic thiamine deficiency. Other authors (Bischoff et al. 1975; Kunze and Muskat 1969) have noted denervation potentials using electromyography. Juntunen et al. (1979) found no motor conduction delay as a sign of myelin disturbance after a 36-week thiamine deficient diet.

In our examination no difference in NCV was found between the 15 animals with proven thiamine deficiency (group-a) and the undernourished but not B_1 deficient animals in group-b, which confirms the results of Juntunen et al. (1979). But on the other hand a clear increase in latency developed for the radicular L5 response and the spinal L1 potential, and the spinal and cerebral volley conduction later became disturbed. The result indicates a CNS emphasis for disturbances associated with B_1 deficiency (Magun 1953; Kalm et al. 1952).

A clear NCV delay (Kunze and Muskat 1969; Rewerts 1973) as a sign of myelin disturbance of the peripheral nerves was not be confirmed. This difference could be explained by a possible concomittant deficit of different vitamins in the older studies. Therefore, the NCV was significantly reduced ($P < 0.01$) in the B_1 deficient group-a and also in the undernourished, but not vitamin deficient, group-b compared with the normally fed group-c.

Damage to the myelin sheath in the peripheral nerves could also be explained as a consequence of undernourishment which also appears concomittantly with thiamine deficiency. However, a disturbance of the central nervous volley conduction is provable, as a specific consequence of B_1 deficiency, in the comparison with group-b as well as group-c (Fig. 1).

The results of the histological examinations of peripheral nerves of thiamine deficient rats were different. Light microscopy showed axonal and myelin degeneration with distal pronounciation (North and Sinclair 1956; Prineas 1970) and "dying back" neuropathy (Juntunen et al. 1979; Kark et al. 1975; Smith 1977). Bischoff and collegues (Bischoff et al. 1975), using electron microscopy, described a primary axonal degeneration, mainly of the large fibres, and a secondary myelin loss. Kalm et al. (1952) saw no pathological changes in the ischiadic nerves of four rats after a 65-day period of B_1 deficiency. The morphometry of the present examination shows a reduction of fibre diameters in cases of B_1 deficiency and also in undernourishment (Fig. 2). Magun wrote (1953) that isocaloric fed rats show the same histological changes in the peripheral nerves as B_1 deficient animals. Therefore, this change could be because of a non-specific undernourishment. However, the frequency of large fibres is clearly reduced in B_1

deficiency (group-a), compared with pair fed controls (group-b). This result indicates that symptoms of polyneuropathy can be due to causes other than the disturbed metabolism associated with thiamine hypovitaminosis. Obviously a specific thiamine deficiency polyneuropathy exists which mainly assails large fibres. In comparison with the pair fed group-b, the unchanged NCV seems to confirm a primary axonal lesion (Bischoff et al. 1975; Collins et al., 1964). The NCV is delayed in the undernourishment and the B_1 defient groups (a and b) compared with group-c. This disturbance seems to be because of a secondary myelin impairment as a result of a reduced energy metabolism. The disturbances of neuronal function as a result of B_1 deficiency in animal experiments are pronounced in the CNS. This pronounciation is related to the thiamine concentration pattern in the nervous system of the rat (Rindi et al. 1979).

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