Eur Arch Psychiatr Neurol Sci (1984) 234 : 102-105

European Archives of Psychiatry and Neurological Sciences

The Influence of Vitamin B_6 Deficiency on Somatosensory Stimulus Conduction in the Rat

Detlef Claus, Bernhard Neundörfer, and Krystyna Warecka

Klinik für Neurologie (Direktor: Prof. Dr. B. Neundörfer) der Medizinischen Hochschule Lübeck, Ratzeburger Allee 160, D-2400 Lübeck, Federal Republic of Germany

Summary. Whilst 22 male Wistar rats were fed on a pyridoxine-deficient diet for 26 weeks, 22 controls received a normal diet. The vitamin B_6 deficient animals lost no weight but they developed symptoms of rat pellagra.

The sensory nerve conduction velocity, the compound radicular, spinal and brain stem responses and the SEP were derived following tail and hind paw stimulation. The examination was repeated at 6 week intervals.

A disturbed central stimulus conduction was indicated by the delayed SEP and intracerebral conduction times. An impairment of neurotransmitter metabolism may be of importance in this case.

Considering related data the results implicate the importance of vitamin B_6 substitution in the case of CNS disturbances due to malnutrition, e.g., chronic alcoholism. The nerve conduction velocity decreases subsequently. A disturbance of myelin function is indicated in adult rats under conditions of pyridoxine deficiency.

Key words: Nerve conduction velocity – Polyneuropathy – Pyridoxine deficiency – Rat pellagra – SEP

Introduction

The vitamin B_6 group pyridoxine, pyridoxal and pyridoxamine, along with its biologically effective phosphorylated derivatives are significant in the synthesis of neurotransmitters (GABA, dopamine, noradrenaline, serotonin) as well as in protein and lipid metabolism (Bersin 1966; Dakshinamurti 1982). Apart from haematological and mucosal changes, fits caused by disturbed GABA synthesis (Bonjour 1980) and disorders of the myelination of nerves appear as a result of a vitamin deficiency. The disorders of the myelination of nerves are due to a diminished sphingolipid synthesis. This has been demonstrated using young rats which had been kept in a state of malnutrition for a period of time (Dakshinamurti 1982).

Vitamin B_6 deficiency is quite common. Lumeng and Li (1974) observed that out of a group of 66 alcoholics without liver damage, 53% had a reduced pyridoxal-5-phosphate serum concentration. Davis and Smith (1974) were able to confirm this by establishing that 50% of chronic alcoholics had a vitamin B_6 deficiency. This B_6 deficiency has also been found amongst older people, between 44% and 57% of people over 60 years of age according to Chen and Fan-Chiang (1981). Although this vitamin is thoroughly reabsorbed through passive diffusion (Hines 1978; Lang 1979; Rose 1980), the cause of its deficiency is still unclear. In the case of alcoholism, a disturbance in phosphorylation produced by acetaldehyde (Lumeng and Li 1974; Majumdar et al. 1981) may be significant. In addition to this, it is certain that malnutrition (Bonjour 1980) coupled with a disordered alcohol-induced liver accumulation is a relevant factor (Majumdar et al. 1982; Sorrell et al. 1974).

The frequency and the distribution pattern of illnesses due to B_6 deficiency in the neurological field can be evaluated in different ways. A causal significance for the development of the mainly sensory, distal accentuated symmetrical type of polyneuropathies is probable (Farmer 1981; Neundörfer 1980; Vilter et al. 1953). In this way experimental scientists were able to discover demyelination (Swank and Adams 1948), and axon degenerations were also seen to be present (Erbslöh and Abel 1970). It cannot, however, be said that the B_6 group has a general pathogenetic significance for polyneuropathies. Láhoda, in 1976, assumed that pyridoxine deficiency was, to a certain extent, accountable for polyneuropathies as a result of changes in methionine metabolism. In later experiments, however, Levin et al. (1981) discovered that in spite of a vitamin dose, no therapeutic effect could be attained in diabetic polyneuropathies.

Swank and Adams (1948) observed that B_6 deficient pigs showed gait disturbances of the hind legs after 2–4 weeks which finally developed into a complete form of lameness. Histologically, they saw distally accentuated severe degeneration of the myelin sheath, and axon destruction, predominantly in large calibred nerve fibres. Within the area of the dorsal roots individual fibres were affected and chromatolysis was frequently found in the spinal ganglia. Within the area of the spinal cord only sporadic degeneration of the dorsal columns appeared; there were no distinct lesions. Anterior horn cells, ventral nerve roots, medulla oblongata, cerebellum, basal ganglia and cerebral cortex remained unchanged. However, Wintrobe et al. (1943) observed a loss of myelin sheaths in the ascending tracts in the medulla oblongata in B_6 deficient pigs. Yet in connection with a B_6 deficiency, there were no degenerative changes to be seen in the supratentorial cerebral structures. Even though the CNS concentration of the vitamin is regulated by an active transport system (Spector 1978), functional disorders arising from deficiency are probable; this is due to the importance of

Offprint requests to: D. Claus Neurologische Universitätsklinik, Schwabachanlage 6, D-8520 Erlangen, Federal Republic of Germany

the vitamin in the synthesis of neurotransmitters and lipoproteins. These functional disorders are to be found in the laboratory-induced fits of animals (Coleman and Schlesinger 1965; Swank and Adams 1948; Wintrobe et al. 1953), and in extended latencies of visually evoked potentials in rats (Stewart et al. 1973). They are also found in individuals (especially in childhood) when epileptic fits are seen in relation to malnutrition or to treatment with antivitamins, e.g. isoniazid. Similarly, these functional disorders can be found in "rum fits" (Farmer 1981; Lerner et al. 1958), tiredness and lethargy (Vilter et al. 1953), or psychotic reactions (Erbslöh and Abel 1970; Kunze 1981).

Since experiments have shown a deficiency of mainly afferent nervous structures in which the proximal spread remained unclear, it seemed worthwhile examining the disturbances of sensory conduction under vitamin B_6 deficiency over an extended period of time. The question also arose whether even in adult rats disorders of the myelin sheath would appear and, if so, whether it would result in a delay in conduction velocity.

Materials and Methods

Over a period of 26 weeks 22 male Wistar rats (12–15 weeks old and weighing 250–300 g) were fed unrestricted amounts of a vitamin B_6 deficient diet (Altromin No. C1023, ca. 0.05 mg vitamin B_6 in 100 mg solid feed). The concentration of the remaining vitamins, minerals and trace elements in the feed was sufficient. To avoid coprophagy, wire grids were placed at an adequate height above the cage floor. The similarly aged control animals were given a standard feed (Altromin No. 1320) in unrestricted amounts and were kept under the same conditions as the others in climatized rooms. The control analysis of the special diet (Institute for Agricultural Examinations and Research Kiel) showed less than 0.1 mg pyridoxal-HCL/kg special diet.

After being fed for 14 days, the animals underwent neurophysiological examinations. These studies followed a previously described method (Claus and Neundörfer 1983) and took place every 6 weeks with a break in this procedure occurring only once. Due to initial responses, the conduction of fast conducting sensory nerve fibres on the tail were measured. After supramaximal stimulus to the tail, the early component was measured as a presynaptic response of fast conducting sensory fibres above L-5. After stimulating the right hind paw, the late component of the presynaptic compound potential (this represents the conduction of slower sensory nerve fibres, Wall and Devor 1981) was measured at the same point. The following responses could also be measured after hind paw stimulus: the third component of the compound response attained above L-1—as a postsynaptic potential of spinal afferent systems (Wall and Devor 1981); the postsynaptic C_{II} response of the brain stem potentials—cranial to C-1 (Claus and Neundörfer 1983); and the first negative sensory evoked potential (SEP) component N_1 derived from the contralateral convexity. Due to the fact that a vitamin B_6 deficiency is difficult to produce without antivitamins, the blood of 14 arbitrarily chosen animals was examined at the end of the experiment. The GOT activation test was used. The activation quotient was increased by an average of 2.37 ± 0.33 (control animals $\bar{x} = 1.59 \pm 0.29$, $n = 13$), proving vitamin deficiency. The results were then examined using the t-test for independent random samples and nonhomogeneous variances in which a two-way analysis for significance was evaluated.

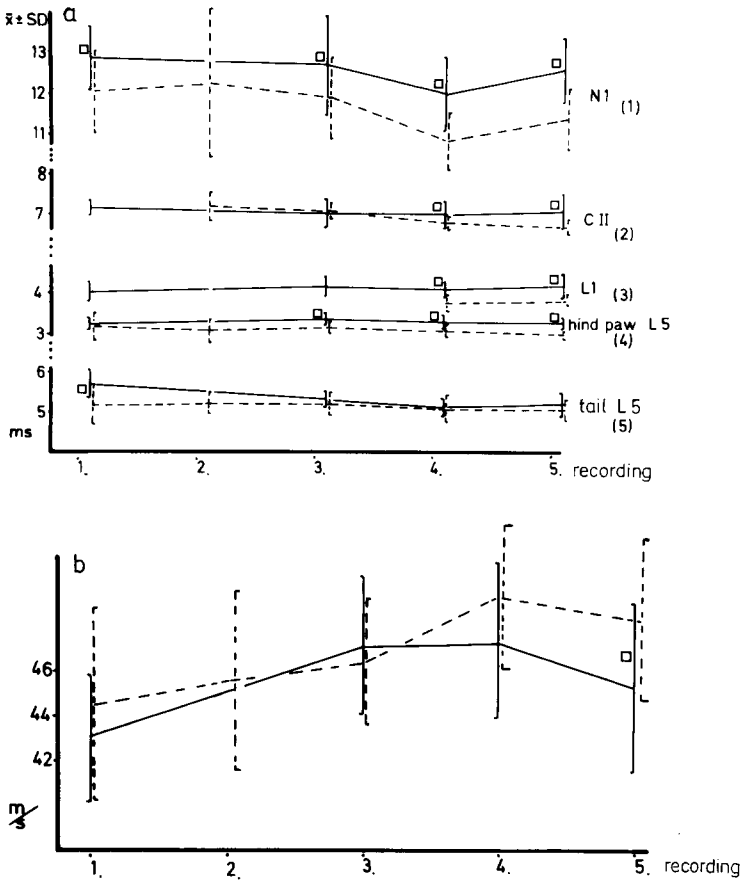

Fig. 1. a Average values of the somatosensory compound answers for L-5, L-1, cervical and scull derivation. Examinations after 2 weeks of pyridoxine deficiency and repeated 6 weeks time intervals. **b** Sensory conduction velocity on the tail, average values. (——) B_6 deficiency ($n = 22$); controls ($n = 22$); □ $P < 0.05$

Results

The rats were fed the vitamin B_6 deficient diet after the phase of the active myelination had been completed (i.e. within the first 45 days of their lives (Dakshinamurti 1982)). After only 2 weeks of feeding, the latency of the N_1 response (Fig. 1 a *(1)*, Table 1) had been significantly extended. Whereas the latency of the L-5 response was not particularly delayed after tail stimulus (Fig. 1 a *(5)*), the decrease of the sensory nerve conduction velocity (which is significant in the last derivation ($P = 0.01$)) indicated a diminished stimulus conduction of fast fibres in the peripheral nerve (Fig. 1 b). During the course of the investigation significantly delayed L-5 and L-1 responses also occurred in pyridoxine deficiency following hind paw stimulus (Fig. 1 a *(3, 4)*); delayed C_{II} responses also occurred (Fig. 1 a *(2)*).

During the period of observation the animals' coats became unkempt and they developed a perioral skin change which is described as "rat pellagra" (Erbslöh and Abel 1970). The gait became slow and straddling, whereas the fine motor movements of the front paws remained unaffected. At no time did the gait disorder progress to lameness. Furthermore, the body weights, which in parts differed slightly, cannot explain the observed differences in the neurophysiological measurments (Claus and Neundörfer 1983). It is also out of the question that the differing lengths of the tail and of the limbs had any falsifying influence on the results.

Table 1. Average values and standard deviations for the first derivation (2 weeks of B_6 deficiency) and the following investigations (6 week intervals)

		Weight (g)		Sens. conduction tail (ms)		Latency tail L-5 (ms)		Hind paw stimulus							
								Latency L-5 (ms)		Latency L-1 (ms)		C_{II} (ms)		N_1 (ms)	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b
1	$\bar{x}$	289.7	303.4	44.5	43.0	5.1	5.8	3.2	3.3		4.0		7.2	12.0	12.9
	SD	18.6	20.7	4.2	2.8	0.5	0.3	0.4	0.2		0.3		0.2	1.0	0.8
	t				1.34		4.98		0.89						3.25
	P						<0.01.								<0.01.
2	$\bar{x}$	321.5		45.6		5.3		3.1				7.2		12.3	
	SD	24.7		3.9		0.3		0.3				0.4		1.8	
	t														
	P														
3	$\bar{x}$	361.0	360.2	46.2	47.1	5.3	5.4	3.2	3.4		4.2	7.1	7.1	12.0	12.8
	SD	23.5	26.6	2.8	3.1	0.3	0.2	0.2	0.1		0.2	0.2	0.4	1.0	1.2
	t				1.07		0.93		4.52				0.59		2.32
	P								<0.01						<0.05
4	$\bar{x}$	375.7	379.8	49.1	47.3	5.2	5.2	3.1	3.3	3.8	4.2	6.8	7.0	10.9	12.0
	SD	24.4	27.5	3.1	3.4	0.3	0.2	0.1	0.2	0.2	0.2	0.2	0.4	0.7	0.9
	t				1.90		0.41		4.46		6.17		2.23		4.69
	P								<0.01		<0.01		<0.05		<0.01
5	$\bar{x}$	383.5	391.3	48.1	45.3	5.1	5.3	3.0	3.3	3.9	4.3	6.7	7.2	11.4	12.7
	SD	25.4	26.9	3.5	3.7	0.3	0.3	0.1	0.1	0.2	0.3	0.2	0.4	0.7	0.8
	t				2.65		1.70		7.81		4.88		4.45		5.25
	P				= 0.01				<0.01		<0.01		<0.01		<0.01

[a] Controls ($n = 22$)
[b] Vitamin B_6 deficient ($n = 22$)

Discussion

During experiments with rats, it was possible to observe objectively the development of a disturbed sensory stimulus conduction due to B_6 deficiency. Whilst the weight curves of both the control and test groups did not differ (Table 1), the rats in the test group developed symptoms of "rat pellagra" (Erbslöh and Abel 1970). The fits which Swank and Adams (1948) had noted in B_6 deficient pigs and the audiogenically-induced fits in B_6 deficient mice noted by Coleman and Schlesinger (1965) were not observed during our experiment. The animals were not, however, continuously observed and night observations were not made. Though the rats had grown out of the phase of active myelination by the beginning of the experiment (in which phase Dakshinamurti (1982) was able to prove disorders of the myelin sheath due to pyridoxal deficiency), the delayed sensory nerve conduction in the tail nerve indicated a functional disorder of the myelin sheath of peripheral nerves. This became significant during the course of the experiment. Also affected were the sensory conduction functions which are represented in the second component of the compound potential measured at L-5 after hind paw stimulus (Fig. 1 a *(4)*). The neurophysiological findings confirmed the myelin degeneration which was observed in pigs by Swank and Adams (1948). A vitamin B_6 deficient polyneuropathy similar to that found in laboratory-induced sensory neuropathy in humans (Vilter 1953) can be regarded as verified.

A delayed spinal response at L-1 and delayed supraspinal brain stem responses (Fig. 1 a *(3,2)*) can be explained as an epiphenomenon of the peripheral nerve lesions with consecutively delayed postsynaptic stimulus conduction. This is supported by Swank and Adams (1948) who were unable to find degenerative changes in the spinal cord in pigs. The gait instability which progressed during the investigation period does not necessarily have to indicate a lesion of the dorsal column; it could be explained as a symptom of a disordered perception in sensory polyneuropathy. This however does not sufficiently explain the prolonged N_1 latency of the SEP which had been significantly extended before a C_{II} delay at the 1% level (Table 1). This is proved by the intracerebral conduction (the difference of the latencies of N_1 and C_{II}) which is significantly delayed from the third derivation onwards ($P<0.01$; t 2.71, 4.40 and 3.18). The findings show an intracranial disturbance in stimulus conduction. This has also been observed in vitamin B_6 deficient rats by means of a delayed visual evoked potential (Stewart et al. 1973). In this way the pathogenetic significance of vitamin B_6 deficiency in CNS defects is emphasized. Dastur et al. (1976) observed that alcoholics with CNS defects had significantly decreased blood and CSF B_6 concentrations as opposed to alcoholics with entirely peripheral neurological symptoms. The early occurence of the conduction delay 2 weeks after the experiment's commencement may indicate a functional disorder, perhaps due to a disturbance of the metabolism of the neurotransmitters.

Because there were no morphological changes in vitamin B_6 deficient pigs, Swank and Adams (1948) also concluded that a functional CNS disturbance had been the cause of the observed fits. However, under similar experimental conditions Wintrobe et al. (1943) observed myelin degeneration in the medulla oblongata of pigs, a morphological finding which could also explain a delayed stimulus conduction in the lemniscal system. The mutual findings enable us to conclude that within CNS disorders caused by chronic alcoholism not only is thiamine substitution indicated but also a therapeutic dose of vitamin B_6 should be taken.

References

Bersin Th (1966) Biochemie der Vitamine. Akademische Verlagsgesellschaft, Frankfurt

Bonjour JP (1980) Vitamins and alcoholism, III. Vitamin B_6. Int J Vitam Nutr Res 50:215–230

Chen LH, Fan-Chiang WL (1981) Biochemical evaluation of riboflavin and vitamin B_6 status of institutionalized and noninstitutionalized elderly in central Kentucky. Int J Vitam Nutr Res 51:232–238

Claus D, Neundörfer B (1983) Zur Untersuchung der peripheren und zentralen Nervenleitung bei der Ratte. Z EEG-EMG 14:160–163

Coleman DL, Schlesinger K (1965) Effects of pyridoxine deficiency on audiogenic seizure susceptibility in inbred mice. Proc Soc Exp Biol Med 119:264–266

Dakshinamurti K (1982) Neurobiology of pyridoxine. In: Draper HH (ed) Advances in nutritional research, vol 4, chapter 6. Plenum Press, New York London, pp

Dastur DK, Santhadevi N, Quadros EV, Avari FC (1976) The B-vitamins in malnutrition with alcoholism. Br J Nutr 36:143–159

Davis RE, Smith BK (1974) Pyridoxal and folate deficiency in alcoholics. Med J Aust 2:357–360

Erbslöh F, Abel M (1970) Deficiency neuropathies. In: Vinken PJ, Bruyn GW (eds) Handbook of clinical neurology, vol 7, chapter 23. North-Holland Publ Comp, Amsterdam New York Oxford, pp

Farmer WT (1981) Neurologic complications of vitamin and mineral disorders. In: Baker AB, Baker LH (eds) Clinical neurology, chapter 42. Harper and Row Publ, Philadelphia, pp

Hines C (1978) Absorption and malabsorption. Arch Intern Med 138:619–621

Kunze K (1981) Polyneuropathien bei Mangelernährung und Alkoholabusus. In: Hopf HCh, Poeck K, Schliack H (Hrsg) Neurologie in Praxis und Klinik, Bd II. Thieme, Stuttgart New York, S 247–254

Láhoda F (1976) Störungen des Methioninstoffwechsels bei Polyneuropathien. Münch Med Wochenschr 118:1661–1662

Lang K (1979) Biochemie der Ernährung, 4. Aufl. Steinkopff, Darmstadt

Lerner AM, DeCarli LM, Davidson ChS (1958) Association of pyridoxine deficiency and convulsions in alcoholics. Proc Soc Exp Biol Med 98:841–843

Levin ER, Hanscom ThA, Fisher M, Lauvstad WA, Lui A, Ryan A, Glockner D, Levin SR (1981) The influence of pyridoxine in diabetic peripheral neuropathy. Diabetes Care 4:606–609

Lumeng L, Ting-Kai Li (1974) Vitamin B_6 metabolism in chronic alcohol abuse. J Clin Invest 53:693–704

Majumdar SK, Shaw GK, Thomson AD (1981) Vitamin utilisation status in chronic alcoholics. Int J Vitam Nutr Res 51:54–58

Majumdar SK, Shaw GK, Gorman PO, Aps EJ, Offerman EL, Thomson AD (1982) Blood vitamin status B_1, B_2, B_6, folic acid and B_{12}) in patients with alcoholic liver disease. Int J Vitam Nutr Res 52:266–271

Neundörfer B (1980) Neurologische Störungen bei Hyper- und Hypovitaminosen. Nervenarzt 51:207–216

Rose RC (1980) Water soluble vitamin absorption in intestine. Ann Rev Physiol 42:157–171

Sorell MF, Baker H, Barak AJ, Frank O (1974) Release by ethanol of vitamins into rat liver perfusates. Am J Clin Nutr 27:743–745

Spector R (1978) Vitamin B_6 transport in the central nervous system: in vitro studies. J Neurochem 30:889–897

Stewart CN, Coursin DB, Bhagavan HN (1973) Cortical evoked responses in pyridoxine-deficient rats. J Nutr 103:462–467

Swank RL, Adams RD (1948) Pyridoxine and pantothenic acid deficiency in swine. J Neuropathol Exp Neurol 7:274–286

Vilter RW, Mueller JF, Glazer HS, Jarrold Th, Abraham J, Thompson C (1953) The effect of vitamin B_6 deficiency induced by desoxypyridoxine in human beings. J Lab Clin Med 42:335–357

Wall PD, Devor M (1981) The effect of peripheral nerve injury on dorsal root potentials and transmission of afferent signals into the spinal cord. Brain Res 209:95–111

Wintrope MM, Follis RH, Miller MH, Stein HJ, Alcayaga R, Humphreys S, Suksta A, Cartwright GE (1943) Pyridoxine deficiency in swine. Bull Johns Hopkins Hosp 72:1–25

Received January 30, 1984