Delayed Auditory Brainstem Response in Thiamin-Deficient Rats

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Summary We recorded the auditory brainstem responses of rats fed a thiamin-deficient diet. The interpeak latencies between waves I and III, as well as those between waves I and IV, were significantly prolonged from day 24, while the latency of wave I was prolonged on day 26 of the thiamin-deficient diet. These delayed responses were corrected in 2 to 4 days after the initiation of daily intraperitoneal thiamin injections from day 32. The rats that were fed the thiamin-deficient diet, and then sacrificed on day 32, showed a decrease of total thiamin levels in the brain (26% of the level in control rat brains). Based on these results, we emphasize the value of the auditory brainstem response to detect thiamin deficiency.

Key Words thiamin deficiency, auditory brainstem response, cochlear nerve

It is thought that thiamin plays an important role in neuronal function through its effect on energy metabolism as a coenzyme of α-keto acid dehydrogenase and by some other actions of the triphosphorylated form (1,2). Neurological symptoms due to thiamin deficiency in man include sensorimotor polyneuropathy of the symmetrical acrodistal type, ataxia, nystagmus, memory disturbance, and confusion (3,4). The peripheral nerves of thiamin-deficient patients and experimental animals show axonal degeneration (5,6), while degenerative changes have been found in the brainstem in thiamin-deficient rats (7–9). Biochemical studies of the brains of thiamin-deficient animals have revealed abnormalities in the brainstem and parts of the midbrain. The thiamin levels, the activity of thiamin-dependent enzymes, and acetylcholine utilization were all decreased significantly in these regions (10–12). Although hearing disturbance has not been noted in thiamin
deficiency, these biochemical and morphological studies suggested the possibility of disturbance of the auditory brainstem response (ABR) due to thiamin deficiency in man. We performed an experiment which detected an abnormal ABR in thiamin-deficient rats.

MATERIALS AND METHODS

Male Wistar rats (Clea Japan Inc., Tokyo, Japan) which were five weeks old were divided into the following groups.

Thiamin-deficient group. Fourteen rats weighing 106 ± 13 g (mean ± SD) were housed individually and fed a thiamin-deficient diet, the composition of which has been detailed elsewhere (13). The average weight of the rats reached 169 ± 9 g on day 14 of the diet, and decreased to 119 ± 13 g by day 32 when 5 of the 14 rats were killed by decapitation. Their livers and brains were immediately frozen in liquid nitrogen and stored at −80°C until the measurement of thiamin concentrations. The other 9 rats received daily intraperitoneal injections of thiamin (100 μg/kg/day), and their average weight increased by 16 ± 3 g/day after starting thiamin injections.

Control group. Fourteen rats weighing 112 ± 14 g were fed a thiamin-supplemented diet, which was the same as the thiamin-deficient diet except for the thiamin content. Five of them were killed on day 32 and the thiamin concentrations in their livers and brains were determined.

Starvation group. Five rats weighing 104 ± 16 g were fed a thiamin-supplemented diet initially, and then were given nothing but water and received intraperitoneal injections of thiamin (100 μg/kg) every second day from day 21. The average weight of the starved rats decreased from 239 ± 5 g on day 21 to 134 ± 6 g on day 32.

For ABR testing, rats were anesthetized with pentobarbital sodium, and needle electrodes were inserted transcutaneously into the vertex, auricles, and tail. Positivity at the vertex was recorded as an upward deflection. Two thousand responses were amplified, filtered (150 to 3,000 Hz), and averaged with a CA-1000 clinical averager (Nicolet Biomedical, Madison, Wis., U.S.A.). The stimulus was given to the right ear at 80 dB and consisted of clicks (100-μs duration) presented at 13.5 Hz. The temperature in the experimental room was kept at 28°C during testing. The latencies in the recordings of the right side were measured using a cursor with a digital readout. ABR recording was started at seven weeks of age in all groups. Based on our preliminary studies, recording was performed as follows: initially every 4 days and then on alternate days from day 24 in the thiamin-deficient group; every week in the control group; and every four days from day 21 and then on alternate days from day 30 in the starvation group.

Statistical analysis was performed using Student's t-test.

Tissue total thiamin concentrations were determined by the thiochrome reaction (14).

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RESULTS

The total thiamin concentrations in the livers and brains of thiamin-deprived rats were \(0.43 \pm 0.04 \mu g/g\) (wet tissue weight) and \(0.61 \pm 0.07 \mu g/g\), while those for control rats were \(14.24 \pm 2.24 \mu g/g\) and \(2.33 \pm 0.12 \mu g/g\), respectively.

Figure 1 shows typical ABR tracings from a control rat and a thiamin-deficient rat on the 32nd day of the diet. The latencies of wave I and the interpeak latencies (IPLs) between wave I and the other waves (mean \(\pm\) SD) are shown in Fig. 2. In the control group, the latencies of all the waves and their IPLs tended to decrease as the rats grew. Although these decreases were not statistically significant, we compared wave latency or IPL in thiamin-deficient rats with all of the corresponding wave latencies or IPLs recorded on five occasions in the control rats. If the difference between any latency in the thiamin-deficient rats and all of the five corresponding latencies in the control rats was significant, we defined the latency in the thiamin-deficient rats as being significantly different from the control rats.

In the thiamin-deficient group, the latencies of waves III and IV and the IPLs between waves I and III (I-III IPL) and between waves I and IV (I-IV IPL) were significantly greater than those in the control group on day 24. The latency of wave I also became significantly greater on day 26. The latency of wave I and the I-III and I-IV IPLs in the thiamin-deficient group on day 32 were respectively \(1.45 \pm 0.06\) ms (mean \(\pm\) SD), \(1.71 \pm 0.05\) ms, and \(2.72 \pm 0.09\) ms. Those for the control group were \(1.35 \pm 0.02\) ms, \(1.58 \pm 0.05\) ms, and \(2.50 \pm 0.06\) ms, respectively, on day 28. The differences between the two groups disappeared 2 or 4 days after the initiation of thiamin injection. The I-II IPL in the thiamin-deficient group did not show any significant difference from that in the control group.

![Fig. 1. Typical auditory brainstem response tracings of a control rat at 9 weeks of age (A) and of a thiamin-deficient rat on day 32 of the thiamin-deficient diet (B). The Roman numerals indicate the number of the waves.](image-url)
Fig. 2. Latency of wave I and interpeak latencies between waves I and II, I and III, and I and IV in ABR recordings of thiamin-deprived rats (closed circles), control rats (open circles), and starved rats (open triangles). Values are indicated as the means±SD. The results of statistical analysis (see text) are shown as follows: *p<0.05, **p<0.01.

Wave latencies and IPLs in the starvation group did not show any significant prolongation compared to those in the control group after 11 days of starvation; 2 of the 5 rats died on day 12 of starvation.

DISCUSSION

We found that thiamin-deprived rats exhibited abnormal ABR while starved rats did not. It seems possible that such abnormal responses are related to the decrease of thiamin derivatives in the auditory organs and brainstem, although thiamin content of every region was not examined. However, we showed that the
total thiamin concentration decreased significantly in the brains of thiamin-deprived rats, and Pincus and Grove (10) have reported that total and phosphorylated thiamin levels decreased more markedly in pons, midbrain, and cerebellum than in the cortex in thiamin-deficient rats.

In the ABR recording, waves I to IV are generated by auditory system structures located between the cochlear nerve and the inferior colliculus (15), with wave II being related most strongly to the activity of the cochlear nuclei (16,17). Therefore, prolongation of the I-III and I-IV IPLs suggests impairment of the central part of the auditory system beyond the cochlear nuclei. Such a disturbance is quite feasible in light of previous reports documenting degenerative changes in the central nervous systems of thiamin-deprived animals (7–9). In these animals, lesions were located in the pons, medulla, cerebellum, the floor of the fourth ventricle, and the lateral vestibular nucleus. Functional disturbance of the central nervous system was also indicated by a study that showed that the sensory evoked potential latencies were significantly increased in thiamin-deprived rats compared with pair-fed controls (3). It is interesting that no prolongation of the I-II IPL was demonstrated in this study, since it may imply that the cochlear nucleus (which is adjacent to the vestibular nucleus) is relatively resistant to thiamin deficiency.

It is generally thought that wave I is derived from the activity of the eighth cranial nerve (16,17), so that prolongation of the latency of wave I in thiamin-deprived rats suggests impairment of this cranial nerve. Although pathological changes of the eighth nerve in thiamin deprivation have not yet been examined, the changes of peripheral nerves are well known in thiamin-deficient animals (18) and in humans (6). The most prominent feature of such changes is axonal degeneration, which is more remarkable in myelinated fibers than in unmyelinated ones. The bipolar cell bodies and the axons of the cochlear nerve are invested by myelin (19), and it is possible that changes similar to those observed in peripheral nerves occurred in the cochlear nerves of thiamin-deprived rats. However, any such changes must be mild, since the delay of the ABR disappeared within several days after the initiation of thiamin injections.

Since a thiamin-deficient diet causes appetite loss and then undernourishment, it is true that pair-fed controls are necessary to estimate the actual effects of thiamin deficiency itself. However, in experiments on thiamin deprivation, it is difficult to accurately measure the food intake of each rat, and overestimation of the intake may cause considerable differences between the thiamin-deficient diet group and the pair-fed controls (4,12). Although we used the starved controls as an extreme form of pair-fed control, the contribution of factors other than thiamin deficiency to ABR abnormalities remains to be examined.

Claus et al. (3) stated that the peripheral nerve changes in rats fed a thiamin-deficient diet were a consequence of undernourishment, since the sensory nerve conduction velocity was slower in both rats fed a thiamin-deficient diet and pair-fed controls than in normal controls. However, we found that the latency of wave I was not increased in starved rats compared with control rats. It remains to be
clarified whether the cochlear nerve is different from ordinary peripheral nerves with regard to its sensitivity to undernourishment or not.

It is noteworthy that the I-III and I-IV IPLs started to increase from a relatively early stage of thiamin deficiency. This finding suggests that ABR testing is potentially a sensitive tool to detect thiamin deficiency, although its specificity remains to be investigated.

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