ANIMAL EXPERIMENTS ON THIAMINE AVITAMINOSIS AND CEREBRAL FUNCTION¹

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Summary Before industrial production of thiamine became possible, among many beriberi patients some showed symptoms of encephalopathy, the cerebral form of the disease. In this animal experiment, thiamine-deficient rats showed failure or blocking of the operant behavior in the maze box, pole climbing box and shuttle box, indicating orientation disturbance and defective memory. This Wernicke's syndrome-like sign dramatically disappeared by treatment with thiamine. Potentiated narcosis with thiopental or alcohol induced in thiamine-deficient rats and mice was readily reversible by thiamine administration. These phenomena are associated with thiamine content of the brain and are found long before histopathological changes in the brain of deficient animals. It is easily surmised that thiamine deficiency in the brain may block the brain metabolism and subsequently cause changes in any chemical substances in the brain, refracting on biophysical phenomenon, such as EEG. However, in the present study, generally speaking, no meaningful results concerning these points were obtained.

Before the chemical structure of thiamine was clarified and its synthesis achieved in 1935 by Williams, vitamin B1 avitaminosis, or beriberi, was a common disease in Asia, including Japan, that shows clinical signs of paralysis and edema based on polyneuritis. Besides this common type of the disease, beriberi patients with encephalopathy or the cerebral form of beriberi were reported by KURE (1), KANAI (2), MATSU-URA (3), and TSUCHIYA (4) in Japan at the beginning of this century; Korsakoff's syndrome or Wernicke's syndrome were said to be a characteristic symptom. These clinical reports may suggest the influence of vitamin B1 deficiency on cerebral function. Today there are very few beriberi patients

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in Japan, but vitamin B1 deficiency can be induced in experimental animals in order to reproduce the encephalopathic signs observed in patients. We have attempted to use experimental animals in this way to clarify the hitherto obscure correlation between vitamin B1 and cerebral function.

MATERIAL AND METHODS

Wistar strain male rats initially weighing approximately 100 g and DS strain male mice weighing about 18 g were used. They were fed on a normal stock diet (normal group) or a synthetic diet (thiamine-deficient group) and housed in wire-mesh-bottom cages in air conditioned rooms. The thiamine-deficient diet consisted of 30% sucrose, 35% washed rice powder, 20% vitamin free casein, 10% soy-bean oil and 5% salt mixture. The diets were supplemented with 5 g cod liver oil, 5 mg riboflavin, 5 mg pyridoxine, 25 mg ascorbic acid, 10 mg nicotinamide, 50 mg calcium pantothenate and 2.5 mg per kg of the diet folic acid. The pair feeding group (control group) were given the same amount of the above thiamine-deficient diet as consumed by the deficient group on the previous day, and administered 200 μ g of thiamine per rat and 20 μ g per mouse orally. To assay the cerebral dysfunction, the operant behavior in LASHLEY's maze (5), in the pole climbing box of COOK and WEIDLEY (6), and the shuttle box of MOWRER and MILLER (7) were observed. Young rats, weighing approximately 100 g initially, were trained to respond to the conditioned stimulus for about 2 to 4 weeks on a normal diet. After establishing conditioned response the animals were divided into 2 groups, each consisting of 6 to 10 animals; *i.e.* thiamine-deficient group and the control group. Each group was maintained on their proper diets described above. In one examination each animal was made to perform the operant behavior 10 to 20 times and the number of habit loss or avoidance loss was employed as criteria for the cerebral dysfunction.

Quantitative determination of thiamine in tissues (8) and catecholamines (9), indolamine (10) and acetylcholine (11) of the cerebrum were carried out by chemical methods.

RESULTS

1. *Symptoms of thiamine deficiency in rats*

As has been stated by all investigators who have studied thiamine deficiency in rats, significant anorexia was observed especially after 2 weeks in parallel with development of avitaminosis. Food intake of deficient rats decreased to approximately 1/2 and 1/10 or less at 2 weeks and 4 weeks, respectively. Body weight of animals increased during the first 2 weeks and then decreased linearly until it reached approximately 2/3 of the initial value at 5 weeks. Thus when the changes in body weight were plotted on graph paper with the vertical axis showing the body weight and horizontal axis showing passage of time, the line showing the

increase during the first two weeks and line showing the decrease over the next two weeks made roughly an isosceles triangle. Body weight change of the control group showed a similar figure to that of the deficient group. This fact indicated that body weight decrease in thiamine deficiency is mainly due to calory deficiency on account of anorexia.

2. Thiamine content in organs of thiamine-deficient rats

In rats reared on the thiamine-deficient diet, thiamine content in the organs except for the brain decreased markedly within a week according to the course of deficiency. However in the brain the thiamine content was maintained for a relatively long time and decreased to approximately 2/3, 1/2 and 1/3 of the normal value (approximately 3 $\mu\text{g/g}$) at respectively 2, 3 and 4 weeks during the deficiency period. When thiamine level of the brain decreased to below 1/3 of the normal level at 5 to 6 weeks of the deficiency period piloerection, ataxia, tremor and convulsions were observed in the rats.

3. Specific blocking of operant behavior in thiamine-deficient rats

a) *Habit loss in the enclosed alley maze.* No difference was found in starting time between the deficient and control groups. However, the former took longer in running time than the latter because of grope error on the way after 20 days of thiamine deficiency as shown in Fig. 1, while no meaningful alteration in the latter was found during the 28 day examination period. The lost habit in the deficiency group was completely recovered by treatment with thiamine.

b) *Avoidance loss in the pole climbing box.* The conditioned response (CR), or avoidance response, and the unconditioned response (UR), or escape response,

Fig. 1. A comparison of running time in the LASHLEY's maze between rats maintained for 28 days on a thiamine-deficient diet and rats pair-fed on the same diet supplemented with thiamine to show cerebral dysfunction caused by the deficiency. From the 28th day of the experiment thiamine 1 mg/rat was administered orally for 3 days to observe recovery of dysfunction. See text for details of the diets. a: Thiamine 1 mg/rat, p.o. for 3 days. * $P < 0.05$, ** $P < 0.01$.

were employed for respectively ascertaining psychotic and nonpsychotic effects of the thiamine deficiency. After 7 to 16 days of deficiency the percentage of CR loss began to increase progressively with the passing of deficiency time (Fig. 2).

Fig. 2. Performance in the pole climbing box of rats maintained for 36 days on a thiamine-deficient diet, showing inhibition of avoidance and escape responses due to cerebral dysfunction. From the 36th day of the experiment thiamine 1 mg/kg was orally administered for 6 days to the deficient-diet-fed rats to observe recovery. See text for details of the diets. Avoidance loss (●—●) and escape loss (■—■) in thiamine-deficient rats. Avoidance loss (○—○) and escape loss (□—□) in pair feeding rats.

UR loss appeared from approximately the fourth week of the deficiency. These lost response were recovered dramatically when thiamine was administered for a few days. When the animals were sustained at a relatively slight degree of deficiency after 2 weeks by feeding thiamine 3 to 10 μ g daily, an amount sufficient to maintain the body weight at the 2 week period level of deficiency, CR loss increased only gradually and it took another 4 weeks to show 50% CR loss.

The CR of animals was temporarily blocked by an electroshock (35 mA, 0.2 sec) in both ear auricles. Return to the previous CR value in deficient animals was delayed according to degree of thiamine deficiency, that is 6 to 7 hr at 3 weeks of deficiency compared with 2 to 3 hr in the control group.

c) *Avoidance loss in shuttle box.* The CR block in the thiamine-deficient rats was observed to commence at 2 to 3 weeks of deficiency and proceed to approximately 30 to 50% avoidance loss at 4 weeks (Fig. 3). No meaningful CR block was seen in the control group during 40 days of the experiment. This CR block recovered completely by thiamine administration or by feeding with the normal diet. No other vitamins except thiamine, *i.e.* vitamin B2 (1 mg/kg) or vitamin B6 (1 mg/kg), caused a recovery effect on the CR block.

In the above experiment with the shuttle box, L-DOPA (100 mg/kg *i.p.*),

Fig. 3. Inhibition of avoidance response in the shuttle box due to cerebral dysfunction caused by thiamine deficiency. The rats were maintained on a thiamine-deficient diet for 72 days, thiamine 1 mg/rat was administered orally on the 40th and 41st day, and from the 72nd day the animals were fed a normal diet to show recovery. See text for details of the diets. ●—●, thiamine deficient rats; ○—○, pair feeding rats.

iproniazid (10 mg/kg s.c.), tyramine (10 mg/kg s.c.) and methamphetamine (1 mg/kg s.c.) were tested if they had any effect on the CR block in the deficient rats. It was found that tyramine and methamphetamine have only a temporary effect on recovery in rats with a light degree CR block (Fig. 4).

Fig. 4. The influence of tyramine and methamphetamine on the loss of avoidance response in the shuttle box seen in rats maintained on a thiamine-deficient diet. ↓: Tyramine 10 mg/kg, s.c., methamphetamine 1 mg/kg, s.c. and saline 10 ml/kg, s.c. ↓↓: Thiamine 1 mg/kg, p.o.

d) *CR and spontaneous motor activity in thiamine-deficient rats.* The response delay or failure of the rats in the above 3 types of operant behavior might be due to incapacitation of motor function. The Animex and Rotarod were used to investigate motor function and it was proven that at least until 4 weeks the thiamine-deficient rats were equally as active as the control animals.

4. Potentiated narcosis in thiamine-deficient rats and mice

Thiopental-Na was administered intravenously (20–35 mg/kg) to the thiamine-deficient rats and mice. Duration time of narcotic effect was investigated by the Grindt's method. Prolongation of the duration time was seen at a relatively early stage of deficiency, *i.e.* before 2 weeks, and was promoted parallel with development of deficiency. For instance, in 3 week deficient mice 35 mg/kg of thiopental-Na induced narcosis for approximately 20 min while in the control mice the narcosis period was only 5 min. This potentiated narcosis was also observed by an intravenous injection of diluted alcohol to thiamine-deficient mice. The potentiated narcosis seen in thiamine-deficient rats and mice recovered completely by thiamine administration for a few days (Fig. 5).

Fig. 5. Potentiation of narcosis by thiamine deficiency seen in rats maintained on a thiamine-deficient diet for 28 days. Thiopental-Na 20 mg/kg was administered intravenously and duration of narcotic effect measured according to Grindt's method. See text for details of the diets. Intravenous injection of thiopental-Na (20 mg/kg, 1 ml/200 g). a: Thiamine 1 mg/rat, p.o. for 3 days. *** $P < 0.01$.

5. Electroencephalography in the thiamine-deficient rats

Bipolar electrodes were chronically implanted into the left hippocampus, left amygdala and frontal sigmoid gyrus of rats. The above implanted areas were confirmed histologically in sacrificed rats after the experiment.

EEG patterns of the awake, slow wave sleep and fast wave sleep from the above three areas in thiamine-deficient rats showed qualitatively no difference from those of control rats. However, in the awake-sleep rhythm of deficient rats investigated during day time, the total quantity of awaking stage decreased so much that the sleeping stage increased contrarily at the 2nd or 3rd week of deficiency.

6. Chemical transmitters in the brain of thiamine-deficient rats

The transmitters in the brain, *i.e.* adrenaline, noradrenaline, serotonin and

acetylcholine, were measured by chemical assay along the time course of thiamine deficiency. Adrenaline in the brain showed a tendency to decrease at days 14, 24 and 34, noradrenaline showed no significant change and serotonin was found to decrease to a slight degree at day 14. Acetylcholine has been thought to decrease in the brain of thiamine-deficient animals, however our results showed no meaningful difference between the deficient and control groups.

7. *Histological changes in the brain of thiamine-deficient rats*

Five groups, a total of 43 rats, were the subject of this experiment. Three groups were animals in different stages of thiamine deficiency; 5 rats fed the deficient diet for 20 days, 11 rats for 28 days and 10 rats for 33 days. The fourth group of 6 rats had been submitted to thiamine deficiency for three times, that is they were fed the thiamine-deficient diet for a total of 82 days but once on the 37th day and once on the 60th day had been orally administered single doses of 1 mg of thiamine, thus allowing recovery of thiamine deficiency for brief periods. The fifth group consisted of control animals from the pair feeding groups, 5 which had been reared for 33 days and 6 for 82 days. These animals had been prepared specially for the histological examination and were not used in any of the above described experiments.

All animals were infused with a 10% formalin solution intravenously under nembutal narcosis. Tissues of the central nervous system were embedded in paraffin and the sections stained by the Krüver-Barrera method besides hematoxylin-eosin. Freezing sections were subjected to Fink-Heimer's staining for the purpose of investigating degenerated fibers.

Microscopic observation revealed that histological change was generally seen as axonal degeneration, especially in the vestibular, cochlear nuclei and olivary superior nucleus. In the dorsal cochlear nucleus at the joint site of the VIIIth cerebral nerve, degenerated axon granules are seen in the axon and axon endings of the cochlear nerve by Fink-Heimer staining (Fig. 6). However, in the superior olivary nucleus, axonal degeneration is confined only to axon endings. These findings might indicate the centripetal degeneration of the cochlear nerve. Further to these findings, hemorrhage in the Virchow-Robin space and vacuolizations were found independently. These histological changes were mostly seen in the pons and medulla oblongata and not in the cerebrum and cerebellum. Furthermore, it is noteworthy that histological changes were most marked in rats that had been repeatedly subjected to thiamine deficiency, followed by the 33-day-deficient group. No changes were seen in the 20-day and 28-day deficiency groups.

DISCUSSION

It is well known that energy metabolism in the brain is mainly based on glucose, and carbohydrate metabolism is hindered by thiamine deficiency which subsequently decreases ATP synthesis. It is not difficult to surmise a decrease in

Fig. 6. Granules of the degenerated axons and hemorrhages in the Virchow-Robin space found in the dorsal cochlear nucleus of the thiamine-deficient rats. Fink-Heimer staining. (Original magnification $\times 50$)

acetylcholine synthesis also because of the Co-A insufficiency following thiamine deficiency. Thus the fact that thiamine deficiency in the brain might induce cerebral dysfunction is not strange from a theoretical viewpoint.

In this study potentiated narcosis by barbiturate or alcohol in thiamine-deficient rats and mice which disappears after thiamine treatment for a few days is thought to be one of the proofs that the brain is in a state of latent or sub-conscious hypofunction. This phenomenon may be used as a biological test of thiamine deficiency. The hypofunction observed could be reconfirmed by behavioral change in operant behavior. The loss of habit in LASHLEY's maze and loss of discriminated avoidance in the pole climbing and shuttle box were significantly increased with the degree of thiamine deficiency. The loss of habit or avoidance disappeared by thiamine treatment. Among drugs tested for a preventive effect against operant behavioral block in thiamine-deficient rats, tyramine and methamphetamine were found to have some activity. However, they were only effective when the degree of deficiency was slight and the efficacy vanished when the degree of deficiency became more developed. From the known pharmacological action of these two drugs, it may be supposed that liberation of synaptic adrenergic agents from the storage site is hindered in thiamine-deficient rats. Quantitative determination of chemical transmitters, so far as studied on adrenaline, noradrenaline, serotonin and acetylcholine, in the brain of thiamine-deficient rats showed no significant change, thus indorsing the above assumption.

Histological studies of thiamine-deficient rats revealed degeneration as a main change in the nuclei of the pons and medulla oblongata with hemorrhage and vacuolization independently from the degenerative change. It is particularly noteworthy that the changes were not found in the cerebrum or cerebellum, but only in the pons and medulla oblongata of rats that had suffered from thiamine deficiency for over 33 days. These histological findings well agree with those reported for humans by Japanese workers (12-16) about 65 years ago. As the operant behavioral block was found far earlier than histological changes in the course of thiamine deficiency, it may be concluded that psychotic dysfunction is due to biochemical changes readily reversible by thiamine administration and nonpsychotic or somatic dysfunction is due to organic changes in the spinal nuclei when unconditional response is blocked.

In the EEG from the hippocampus, amygdalla and the frontal sigmoid gyrus the patterns of arousal, slow wave sleep and fast wave sleep were not altered qualitatively during thiamine deficiency. However, the total sleeping stage period, as measured by EEG pattern, seemed to have been increased while that of arousal decreased.

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