CATECHOLAMINE METABOLISM IN THIAMINE-DEFICIENT RATS

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In order to know a possible role of thiamine in the central nervous system, I have, in 1965, started with my coworkers a series of researches using thiamine-deficient rats (1). The study was mainly concentrated on the change of the brain adrenergic mechanism of the deficient rat. In this paper, a relationship between the change of catecholamine (CA) biosynthesis and the appearance of some neural symptoms of the deficiency is described. Furthermore, a possibility is shown to use thiamine-deficient rats as a tool for investigating the interaction among CA metabolism, other hormones and carbohydrate metabolism.

Male Sprague Dawley rats weighing 80-100 g were used throughout the study. Animals were divided into three groups: a thiamine-deficient group, a pair-fed group (fed on a thiamine supplemented diet but its amount was restricted to show a loss of body weight similar to that of the deficient group) and a control group (fed ad libitum on a diet supplemented 3 mg of thiamine HCl per kg of the deficient diet). In the study on glucose intolerance of the deficient rat fed by normal diet were used as the control.

As is well known, in thiamine-deficient rats, a marked bradycardia and circular walk are seen besides neural symptoms which are common in monkey, rat, mouse and pigeon, such as hypomotility, tremor, ataxia, opisthotonus and frequent seizure. Therefore, when the heart rate of the rat fed on thiamine-deficient diet showed less than 70% of the control group they were regarded as acutely deficient and the following experiments were performed. The heart rate was monitored by an electrocardiograph applying neither anesthesia nor detention of the animal.

Changes of tissue CA contents and the contents of other putative neurotransmitters in thiamine-deficient rats (2,6)

When the heart from the rat in thiamine deficiency was perfused by Langendorff's method, its contractile response to tyramine was found to be markedly enhanced though the response to noradrenaline was only slightly increased (2). As tyramine is known to exert its sympathomimetic action through release of CA from its tissue storage site, it was anticipated that there might be an elevated CA accumulation in the heart of thiamine-deficient rats. So, a differential determination of noradrenaline and adrenaline (3,4) was performed and the result showed a marked elevation of the amines in the spleen, the heart atrium and ventricle, and in the brain cortex (2).

As Speeg et al. (5) reported that brain acetylcholine level of the deficient rat is in the normal range, we measured the levels of putative neurotransmitters other than CA and acetylcholine in the deficient rat tissues (6). Serotonin level was significantly increased in the spleen of the deficient rat as seen in the case of CA. However, no change was observed in the tissue level of γ-aminobutyric acid in the deficient animal.

CA turnover in tissues of thiamine-deficient rats (7,8)

It was thought that the raised level of CA in the deficient rat could be due to a decrease in the activities of the degrading process of
the amine. So, monoamine oxidase activities in tissues of the deficient rat were estimated using noradrenaline as substrate (7). And a significant reduction of monoamine oxidase activity in the heart atrium and ventricle and in the spleen of the deficient group was observed. In the brain, the activity remained unchanged. However, when tyramine was used as substrate, a definite reduction of the activity was observed even in the brain cortex (6).

An activity of liver catechol-O-methyl transferase was unchanged in thiamine deficiency (6).

Next, CA concentration in the blood of the deficient rat was measured (8). The amine concentration in the blood of thiamine deficient rats was found to be about one-half of that of either the control or pair-fed group. The reduced blood CA level might be resulted from a decrease of spontaneous release of the amine from tissues in the deficient rat.

The rate of CA biosynthesis in thiamine-deficient rat tissues (9)
The rate of CA biosynthesis was estimated using pheniprazine, a compound having an inhibitory effect on monoamine oxidase activity as well as on CA release from the tissue. An increase in CA level after pheniprazine was less in the brain and heart of the deficient rat than in control and pair-fed animals. This impaired accumulation of CA was restored to nearly the control level by a simultaneous injection of 4 mg/kg of thiamine HCl with pheniprazine, thereby the lowered concentration of blood CA and marked hypotension and bradycardia was also restored to the level of the control rat. Figure 1 shows the relationship between the rate of CA biosynthesis and heart rate in the deficient rat.

However neurological symptoms such as hypomotility, tremor, turning movement and convulsions were not fully overcome by thiamine injection, although they were much improved within 60 min after thiamine.

Effect of drugs on behavior, heart rate and CA levels in thiamine-deficient rats (10)
The results mentioned above suggest a close relationship between CA biosynthesis and the deficient symptoms. So, we examined the changes of behavior and heart rate of the deficient rat after the administration of reserpine, DL-amphetamine and tyramine as well as those of tissue CA levels.

After the injection of reserpine tissue CA content slowly decreased in the deficient rat for 5 hr and during this time neither sedation nor change in the heart rate were observed. DL-Amphetamine caused a greater decrease in tissue CA content and more excitement in the deficient group than in the control group. The release of CA caused by tyramine was similar to that caused by DL-amphetamine. And in the deficient group the release by tyramine was accompanied by a marked increase in heart rate, but no behavioral change were caused by tyramine in any group.

Glucose intolerance in thiamine-deficient rat (11)
It has been reported by several workers that thiamine-deficient rats showed a decreased tolerance to dextrose (12,13) and that the concentration of insulin-like substance in the serum of deficient mice is abnormally low (14). In the next series of the study, the relationship...
between suppressed adrenergic mechanism and glucose intolerance in the deficient rat was examined. Glucose tolerance was measured as the change in the blood glucose level after intraperitoneal administration of 2 g/kg of glucose.

The deficient rat showed a marked glucose intolerance. On the other hand, the hypoglycaemic effect of insulin was similar in the deficient, pair-fed and normal groups.

Next, to investigate a hypoglycaemic action of endogenous insulin in the deficient rat, the effect of tolbutamide, an insulin releaser, was examined. After tolbutamide injection the blood glucose level reached a minimum level in normal and pair-fed rats within 30 to 60 min, while in the deficient rats, the minimum level was only reached after 3 hr.

When tyramine was injected, the basal glucose level was not changed in deficient or normal rats after 3 hr. However, it was found that tyramine restored the impaired glucose tolerance of deficient rats to normal, but not that of alloxan diabetic rats. Furthermore, tyramine did not restore the intolerance of the deficient rats pretreated with alloxan.

These results suggest that the main factor causing glucose intolerance in the deficient rats may be suppressed insulin secretion. Furthermore, it is possible that the effect of tyramine is due to some action in improving insulin secretion or increasing the effectiveness of endogenous insulin. These may be direct actions of tyramine or secondary to the effect of the drug in correcting the impairment of sympathetic nerve function.

Our findings suggest that the CA turnover rate has a close relationship with physical symptoms of thiamine deficiency which seems to be caused by dysfunction of either the central or the peripheral nervous systems.

Furthermore, these results indicate a possibility that thiamine-deficient animals could be highly useful not only for analysis of the role of CA in the central nervous system but also for that of interactions among CA metabolism, other hormones and carbohydrate metabolism.

These works were carried out by co-operation with my co-workers. I would like to express my sincere thanks to them.

REFERENCES

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