Arch. Pharm. Res. 3(1), 51~55(1980)

Studies on the Possible Role of Thiamine in the Central Nervous System

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Thiamine, in the form of its diphosphate (TDP), is well known to act as a coenzyme, and during the early stage in the study of thiamine it had been believed that the symptoms of thiamine-deficiency were resulted secondarily from the disturbance of metabolic processes in which TDP participated as a coenzyme. However, the neurological symptoms in thiamine deficiency are now separated from the metabolic disturbances in thiamine deficiency. On the other hand, the specific involvement of phosphorylated thiamine in nerve conduction has been suggested by von Muralt[1], but the nature of this involvement has not been elucidated at a molecular level.

Recently the possible significance of thiamine triphosphate (TTP) in nervous tissue was suggested by the demonstration[2] that TTP is not present in the brain of patients with subacute necrotizing encephalomyelitis, a fatal disease associated with an abnormality in thiamine metabolism. Furthermore, the studies using membrane fragments of rat brain strongly indicated that ion movement across the nerve membrane is associated with dephosphorylation of phosphorylated thiamine.

In 1965, we have started the study on the role of thiamine in central nervous system. We have made two different approaches to this project. One is pharmacological study on adrenergic mechanism in thiamine deficiency, since no one could succeed to characterize the appearance of typical nerve disfunction observed in thiamine-deficient rats. Another approach is a biochemical study of enzymes involved in thiamine metabolism. such as thiamine diphosphatase (TDPase) and thiamine triphosphatase (TTPase). It is important to know the dynamic changes in these thiamine phosphatase activities corresponding to functional changes of the brain. However, little is known about these enzymes especially concerning to those characteristics in the membrane.

CATECHOLAMINE(CA) METABOLISM IN THIAMINE-DEFICIENT RATS

Male Sprague Dawley rats weighing 80-100 g were used throughout the study. Animals were divided into three groups: thiamine-deficient group, 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 control group (fed *ad libitum* on a diet supplemented 3 mg of thiamine HCl/kg of the

deficient diet). Thiamine-deficient rats show a marked bradycardia and circular walk besides neural symptoms such as hypomotility, tremor, ataxia, opisthotonus and frequent seizure which are common in monkey, rat, mouse and pigeon of thiamine deficiency. In this study 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 used for the experiments.

Changes of CA and other Putative Neurotransmitters in the Tissues of Thiamine-deficient Rats.

When the heart of thiamine-deficient rats was perfused by Langendorff's method, its contractile response to tyramine was markedly enhanced while that to noradrenaline was slightly increased. From this finding it was expected that CA content might be elevated in the heart of thiamine-deficient rats, since tyramine is known to mimic its sympathomimetic action through release of CA from its storage site. In fact, marked accumulation of noradrenaline was seen in the spleen, the heart atrium and ventricle, and in the brain cortex. Speeg *et al.*[7] reported that brain ACh level of the deficient rat is in the normal range. In addition, in our study serotonin level was significantly increased only in the spleen of the deficient rats as in the case of CA, whereas no change was observed in the tissue level of GABA in the deficient animals. As like this, among the various candidates CA is only one putative neurotransmitter which its content was changed in thiamine deficient rat.

CA Turnover in Tissues of Thiamine-deficient Rats

It is highly probable that the high level of tissue 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, showing significant reduction of monoamine oxidase activity in the heart atrium and ventricle and in the spleen of the deficient rats. In the brain, the activity of this enzyme remained unchanged if noradrenaline was used as a substrate. However, when tyramine was used as a substrate, a definite reduction of the activity was observed even in the brain cortex. Hepatic catechol-O-methyl transferase activity was unchanged in thiamine deficiency.

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

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. Time-dependent 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 reduced accumulation of CA was restored to nearly the control level by a simultaneous administration 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 rats. However, neurological symptoms such as hypomotility, tremor, turning movement and convulsions were not fully overcome by thiamine administration.

Effect of Drugs on Behavior, Heart Rate and CA Levels in Thiamine-deficient Rats

The results mentioned above suggest that the several symptoms appeared in the deficient rat are possibly derived from the impairment of CA metabolism in the tissues of thiamine deficient rat. So, we examined this possibility using reserpine, amphetamine and tyramine. After the injection of reserpine tissue CA content slowly decreased in the deficient rat for 5 h and during this time neither sedation nor change in the heart rate were observed, while control rats showed a marked sedation and bradycardia. DL-amphetamine caused a greater decrease in tissue CA content and more excitement in the deficient animals 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 was found by tyramine in any group.

Glucose Intolerance in Thiamine-deficient Rats[12]

In the next study, the relationship between suppressed adrenergic mechanism and glucose intolerance in the deficient rats was examined. Glucose tolerance was measured as the change in the blood glucose level after intraperitoneal administration of glucose. The deficient rat showed a marked glucose intolerance. On the other hand, the hypoglycemic effect of insulin was similar in the deficient, pair-fed and normal groups. After tolbutamide injection, to release the endogenous insulin, 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 h. When tyramine was injected, the basal glucose level was not changed in deficient or normal rats after 3h. However, 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 sugget 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. Our findings suggest that the impaired CA turnover rate is related to some physical symptoms of thiamine deficiency which seems to be caused by disfunction of either the central or the peripheral nervous systems.

THIAMINE PHOSPHATASES IN RAT BRAIN MICROSOMES

In biological tissues, four forms of thiamine are present such as free thiamine, thiamine monophosphate (TMP), thiamine diphosphate (TDP) and thiamine triphosphate (TTP).

Thiamine pyrophosphokinase and thiamine pyrophosphate-ATP phosphoryltransferase are the enzymes catalyzing the phosphorylation of thiamine. Thiamine diphosphatse (TDPase) and thiamine triphosphatase (TTPase) are known to dephosphorylate thiamine phosphate esters. Although there are some reports showing the properties of of TDPase and TTPase[13~17], little is known about biochemical and pharmacological characteristics of these enzymes. From this aspect, we performed the biochemical study of TDPase and TTPase using rat brain microsomes.

First we examined the *in vivo* effects of several experimental stages or drugs on these thiamine phosphatase activities, since it is quite necessary to find a factor which can regulate thiamine metabolism. However, these experiments showed that TDPase and TTPase activities are hardly influenced by several conditions[18]. In *in vitro* studies, we found that chlorpromazine caused drastic changes of these phosphatase activities whereas other neuroactive agents, such as no adrenaline, ACh, tyramine and diphenylhydantoin, had no effect on the activities[19]. Chlorpromazine at concentrations of 0.25 to 1.0 mM provoked a diverse effect on these enzyme activities; inhibiting TTPase and activating TDPase.

In the further studies, we examined in more detail the mechanism of the action of chlorpromazine on thiamine phosphatases and the organization of the enzymes in the microsomal fraction, suggesting that the opposite effect of chlorpromazine on thiamine phosphatases are due to a different organization of the enzymes in the membrane[20]. These observations lead us to consider that protein-lipid interaction which induce a conformational change in the membrane might be critical for the regulation of thiamine metabolism in the central nervous system. This hypothesis was further supported by subsequent findings. The various treatments which affect membrane structure, such as alkaline pH treatment, repeated freezing and thawing, phospholipase C, acetone and Triton X-100 caused almost the same effects on thiamine phosphatases in brain microsomes as chlorpromazine[21].

The results described above finally indicate that TDPase and TTPase are localized in a differnet manner in the brain microsomal membrane; TTPase definitely requires membrane phospholipid for the maintenance of the activity whereas TDPase exists in a "latent form" and is influenced by microenvironmental changes within the membrane.

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