THIAMINE AND NERVE MEMBRANE

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In 1947, von Muralt proposed a dual role for thiamine in nervous tissue, one being the well-known coenzyme function in intermediary metabolism and the other a possible role in nervous excitation. Recently, considerable evidence has accumulated which demonstrates the second function of the vitamin. Such evidence includes: (1) Electrical stimulation of a variety of preparations of nervous tissue causes a release of the vitamin (1-3). (2) Anti-metabolites of thiamine have profound effects on the electrical activity of nerve preparation in vitro (4-6). (3) The action potential of ultraviolet-irradiated nerves can be restored by the addition of thiamine (7). (4) The poly-neuritis that is associated with a state of thiamine deficiency does not always correlate with an inhibition of enzymes in nervous tissues which require cocarboxylase as coenzyme (8-10). (5) Thiamine triphosphate which is considered to have no cocarboxylase activity (11), is lacking in the brain of patients with subacute necrotizing encephalomyelopathy and may be closely related to the etiology of this fatal disease (12-14). In light of these findings, it is probable that thiamine functions in the conduction process and is involved in certain neural diseases. In an attempt to determine this action of thiamine, the following investigations were done.

1. Thiamine-deficient neuropathy in pigeons

In pigeons fed on a diet of polished rice, convulsion occurs and a significant decrease of thiamine and calcium in the central nervous system rather than the peripheral nervous system was observed. In subcellular fractions of telencephalon in these pigeons, thiamine and calcium levels decreased in the membrane-myelin and synaptosomal fractions. There was no marked difference between convulsive and normal pigeons regarding activities of cocarboxylase-dependent enzyme in the brain. When radioactive thiamine was injected into the pigeon during a convulsion, radioactivity was the most prominent in the telencephalon and in subcellular fractions, in membrane-myelin and synaptosomal fractions of the telencephalon immediately after recovery from the convulsion. When calcium was added to the polished rice, convulsion never occurred. Thiamine and calcium concentrations in membrane-myelin and synaptosomal fractions of telencephalon of pigeons fed polished rice with the addition of calcium were significantly higher than in the convulsive pigeons.

Fifty percent of the effective dose of thiamine to these convulsive pigeons was 48.3 µg/kg of body weight according to the method of Litchfield and Wilcoxon (15). The author tested various drugs other than thiamine for recovery from convulsion and it was clarified that the following two substances were temporarily effective. These are diphenyl hydantoin, an anticonvulsant agent, (100 mg/kg) and calcium chloride (50 mg/kg). However, after 5-10 hr, the convulsion recurred.

As polished rice is deficient both in thiamine and calcium, it is postulated that thiamine and calcium deficiency caused such a nervous disease in the pigeons. Calcium may play a role in binding thiamine and membrane structures.

2. Release of thiamine from intact nerve preparation

Large bullfrogs were injected with radio-labeled thiamine and spinal cords were subsequently
removed and perfused. The perfusate was monitored for the efflux of labeled thiamine using a flow cell mounted in a liquid scintillation spectrometer (Fig. 1). With this technique it was shown that neuroactive drugs added to the perfusion fluid promoted the release of labeled thiamine. Thus, acetylcholine, tetrodotoxin and potassium chloride all had a releasing action whereas agents such as choline and sodium chloride had no effect. This apparent relationship between ion movements and thiamine adds a further dimension to that in the nerve preparations, the bulk of the vitamin was in the form of the di- or triphosphate, whereas the released material consisted of free thiamine and thiamine monophosphate.

3. Release of thiamine from broken cell preparation

Rats were injected with $^{35}$S-thiamine, and subsequently, brain, spinal cord and sciatic nerves were removed and homogenized. When particulate fractions of these tissues which contained radioactive thiamine were incubated with a variety of neuroactive drugs, thiamine was released into the medium. After separation of the particulate preparation into myelin, membrane, synaptosomes and mitochondria, it was found that thiamine was released essentially only from the membrane fragments.

4. Thiamine binding protein in rat brain

To brain homogenate with water, one-fifth volume of 0.3 N Ba(OH)$_2$ and 5% ZnSO$_4$ were added, protein was precipitated by centrifugation and phosphorylated and free thiamine in supernatant and precipitate were determined. As the results shown in Fig. 2, about 90% of total thiamine was precipitated with protein and almost all of precipitated thiamine was found to be in the phosphorylated form. When brain homogenate was incubated at 37°C, protein binding thiamine which is precipitated by the addition of barium and zinc, decreased with the incubation time. When phosphatase was added to the incubation medium, thiamine binding protein decreased more rapidly. While, the addition of fluoride, an inhibitor of phosphatase, prevented the decrease in protein binding thiamine (Fig. 3). This fact suggested that the autocleavage of protein-thiamine binding can be attributed to the effect of phosphatase in brain homogenate and thiamine in brain may be bound to protein at the site of phosphate esters.

5. Model of thiamine in sodium channel

Figure 4 shows the model of the current...
Fig. 4. Model of the situation of thiamine in sodium channel.

view of the situation of thiamine in excitable membrane.

Thiamine pyrophosphate or triphosphate occupies a site in the membrane that is either a sodium channel or very close to it in binding with calcium and protein perhaps at the site of phosphate ester. The action of tetrodotoxin may be explained by assuming that the poison displaces thiamine and calcium and occupies the site so that sodium ion cannot enter. With the other drugs it can be inferred that they displace thiamine also but do not occupy the fixed site so that the early inward current of sodium ion is observed.

REFERENCES