BIOLOGICAL PROPERTIES OF A NEW THIAMINE DERIVATIVE, CYCLOCARBOTHIAMINE¹

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In recent years, intensive efforts have been made for the synthesis of thiamine derivatives having biological advantages over thiamine (1). The derivatives developed so far are characterized by easier absorption from the intestine and quantitative regeneration of thiamine following absorption. As a result, their administration results in the accumulation of thiamine in the blood and tissues in such a high concentration as is unattainable with thiamine itself.

In the course of our study intended to develop a still better thiamine compound, it was found that one of the new derivatives, which was obtained by the reaction of thiamine with phosgene and for which we propose the name cyclocarbothiamine³ (2), is an excellent substitute for thiamine with regard to the above properties.

This paper deals with the general biological characteristics of this compound.

Cyclocarbothiamine

EXPERIMENTAL

1. Thiamine Activity *in vivo*

Rats.—Male Wistar strain rats, 4 weeks old, were maintained on a thiamine-free diet and rendered thiamine-deficient in the same manner as described previously (3). When the animals were sufficiently depleted of thiamine as revealed by the cessation of growth, three graded doses of thiamine (5, 10 and 20 μ g) or equimolar amounts of cyclocarbothiamine dissolved in 0.2 ml of water were administered daily by a stomach tube and their effects on body weight increase were

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³ The name cycotiamine has provisionally been proposed for this compound by WHO as its International Nonproprietary Name (1966. 9. 6).

compared. The control animals received the same volume of water instead.

Rice-birds—Male rice-birds (*Uroloncha striata* var. *domestica*), weighing approximately 13 g, were maintained on a thiamine-deficient diet, the composition of which had previously been presented (2). The birds received daily 3 μ g of thiamine or an equimolar amount of cyclocarbothiamine dissolved in 0.05 ml of water and their body weights were checked every three days. The control birds were given the same volume of water and observation was made over a period of 20 days.

2. Intestinal Absorption and Deposition in Tissues

Total Thiamine Contents in Rabbit Blood Following Oral Administration of Thiamine and Cyclocarbothiamine—Male rabbits, weighing approximately 2.5 kg, were used after being fasted for 24 hours. Thiamine and cyclocarbothiamine were administered orally as an aqueous solution at three different dose levels equivalent to 5, 10 and 20 mg of thiamine per kg of body weight, respectively. At appropriate time intervals, blood samples were taken by cardiac puncture and total thiamine contents were determined by the method of Fujiwara *et al.* (4, 5). Cocarboxylase was also assayed manometrically according to Uehara *et al.* (6).

Total Thiamine Contents in the Liver and Kidney of Rats Following Oral Administration of Thiamine and Cyclocarbothiamine—Male Wistar strain rats, ranging in body weight from 130 to 150 g, were dosed orally with thiamine, 1 mg, or an equimolar amount of cyclocarbothiamine. One or five hours after the dosage, the animals were killed by decapitation and the total thiamine contents in the liver and kidney were measured.

Urinary Excretion of Thiamine Following Oral Administration of Thiamine and Cyclocarbothiamine to Human Subjects—The two compounds were given orally as an aqueous solution of 50 ml to healthy male subjects, about 25 years of age, in an amount equivalent to 10, 25 and 50 mg of thiamine, respectively. Six-hour urine was collected under the addition of acetic acid and assayed for thiamine contents.

3. Thiamine Levels in the Blood and Urine Following Intravenous Administration of Thiamine and Cyclocarbothiamine to Rabbits

Thiamine or cyclocarbothiamine dissolved in 1.0 ml of physiological saline was injected into a marginal ear vein of male rabbits, about 2.5 kg, in a dose equivalent to 5 mg of thiamine per kg body weight. At the times indicated, blood samples were withdrawn as described before and assayed for total thiamine contents.

Urine was collected at the same intervals by the use of a catheter and analysed similarly after appropriate dilution with distilled water.

4. Action of Thiaminases on Cyclocarbothiamine

Enzyme preparations of thiaminase I and II were the same as described previously (7).

Thiaminase I—The reaction mixture contained the following components in a final volume of 10 ml: 4 μ g substrate, 133 μ moles phosphate buffer, pH 6.0, 10 μ moles aniline and 2 ml of 20-fold diluted culture supernatant of *Bacillus thiaminolyticus* Matsukawa et Misawa.

The mixture was incubated at 37°. At the indicated time intervals, an aliquot

of 1.0 ml was removed and added to 1.0 ml of 0.1 *N* NaOH, which was allowed to stand at room temperature for 10 minutes. During this treatment, cyclocarbothiamine was quantitatively converted to thiamine. Three ml of 25 % KCl in 0.1 *N* HCl was then added and thiamine was measured by the thiochrome method (4). When thiamine was used as the substrate, the alkaline treatment was omitted.

Thiaminase II — The composition of the reaction mixture in 10 ml was as follows: 4 μ g substrate, 133 μ moles phosphate buffer, pH 7.0, and 2 ml of 10-fold diluted culture supernatant of *Bacillus aneurinolyticus* Kimura et Aoyama.

Subsequent procedure was the same as described above for thiaminase I.

RESULTS

1. Thiamine Activity in Rats and Rice-birds

As is shown in Fig. 1, thiamine activity of cyclocarbothiamine was in no way different from that of thiamine, as judged by the growth-promoting effect on thiamine-deficient rats. Similarly, the two compounds prevented the development of thiamine-deficiency in rice-birds equally well (Fig. 2).

These facts indicate that cyclocarbothiamine is completely transformed into thiamine in the body, which will be demonstrated in full detail in the following paper.

FIG. 1 Growth-promoting Effect of Thiamine and Cyclocarbothiamine on Thiamine-deficient Rats

Each curve represents the average of eight animals. The arrow indicates the onset of administration.

Dose: 5, 10 and 20 μ g equivalent of thiamine per day. ●, cyclocarbothiamine; ×, thiamine; ○, control.

FIG. 2 Preventive Effect of Thiamine and Cyclocarbothiamine on the Thiamine-deficiency of Rice-birds

Each curve is the average of five birds. The cross (×) indicates death.

Dose: 3 μ g equivalent of thiamine per day. ○, cyclocarbothiamine; ×, thiamine; △, control.

2. Intestinal Absorption and Deposition in Tissues of Cyclocarbothiamine

Total thiamine contents in the blood after oral administration of cyclocarbothiamine increased remarkably in proportion to the dosage, which presents a striking contrast to the corresponding effect of thiamine (Fig. 3). The same was true

FIG. 3 Total Thiamine Contents in the Blood Following Oral Administration of Thiamine and Cyclocarbothiamine to Rabbits

Each curve is the average of seven animals. Dose: 5 (Δ), 10 ($\times$) and 20 ($\circ$) mg equivalent of thiamine per kg body weight. —, cyclocarbothiamine; ---, thiamine.

FIG. 5 Total Thiamine Contents in the Liver and Kidney Following Oral Administration of Thiamine and Cyclocarbothiamine to Rats

Each value is the average of five animals and the vertical line indicates the standard deviation of the mean.

Dose: 1 mg equivalent of thiamine per animal. □, cyclocarbothiamine; ▨, thiamine.

FIG. 4 Cocarboxylase Levels in the Blood Following Oral Administration of Thiamine and Cyclocarbothiamine to Rabbits

Each value is the average of seven animals and the vertical line represents the standard deviation of the mean.

Dose: 10 mg equivalent of thiamine per kg body weight.

□, cyclocarbothiamine; ▨, thiamine.

FIG. 6 Urinary Excretion of Thiamine Following Oral Administration of Thiamine and Cyclocarbothiamine to Human Subjects

Each value is the average of five subjects and the vertical line represents the standard deviation of the mean. Determination was done on six-hour urine.

Dose: 10, 25 and 50 mg equivalent of thiamine per subject.

□, cyclocarbothiamine; ▨, thiamine.

of blood cocarboxylase and total thiamine levels in the liver and kidney (Figs. 4,5).

These data suggest that the intestinal absorption of cyclocarbothiamine proceeds in large quantities, whereas that of thiamine is severely restricted. A considerable amount of thiamine detected in urine after oral dosage also supports this view (Fig. 6).

As will be shown later, cyclocarbothiamine is rapidly converted into thiamine after absorption and not a trace of the compound is detected unaltered in blood sample taken 30 minutes after oral dosage. Therefore, the total thiamine measured here does not include cyclocarbothiamine itself.

3. Retention of Thiamine in the Body Following Intravenous Administration of Cyclocarbothiamine

Rabbits, when loaded with thiamine intravenously, excreted a fairly large portion of it into urine in a comparatively short period. In the case of cyclocarbothiamine, its urinary output tended to be delayed (Fig. 7).

This, taken together with the time course of total thiamine levels in the blood (Fig. 8), proves that cyclocarbothiamine is retained in the body for a longer period presumably because of its stronger affinity for the blood and tissues.

FIG. 7 Urinary Excretion of Thiamine Following Intravenous Administration of Thiamine and Cyclocarbothiamine to Rabbits. Each value is the average of six animals. Dose: 5 mg equivalent of thiamine per kg body weight.

FIG. 8 Total Thiamine Contents in the Blood Following Intravenous Administration of Thiamine and Cyclocarbothiamine to Rabbits

Each curve is the average of three animals. Dose: 5 mg equivalent of thiamine per kg body weight.

○, cyclocarbothiamine; ●, thiamine.

4. Stability of Cyclocarbothiamine to Thiaminases

As is evident in Fig. 9, cyclocarbothiamine survived the action of both types of thiaminase perfectly, while under identical conditions, about 80% of thiamine was decomposed.

FIG. 9. Action of Thiaminase on Thiamine and Cyclocarbothiamine

×, thiamine; ○, cyclocarbothiamine.

Experimental conditions are given in the text.

DISCUSSION

It has been repeatedly demonstrated that the intestinal absorption of thiamine proceeds only to a limited extent and its oral administration in a large dose results in the excretion of its major part in feces. Therefore, efficient deposition of thiamine in the tissues through oral route cannot be effected with thiamine itself.

Allithiamine is a typical example which has overcome this difficulty by modifying the chemical structure of thiamine (8, 9). Several similar compounds have been developed so far and their biological advantages may be summarized as follows.

(a) Oral administration brings about far higher thiamine levels in the blood as well as in the tissues.

(b) Parenteral administration effects better accumulation of thiamine in various parts of the body.

(c) These modified thiamines are not destroyed by thiaminases produced by certain types of intestinal bacteria.

As is seen from the experimental findings presented in this paper, cyclocarbothiamine may be regarded as a new compound which satisfies the above requirements. Furthermore, the following feature can be pointed out as an additional advantage of this compound.

In the process of its enzymatic transformation into thiamine, cyclocarbothiamine splits off its carbonyl segment which is imparted by phosgene as carbon dioxide, no unphysiological substances being produced. This is definitely a desirable property in view of the fact that this class of compounds have come to be used in increasingly large doses for clinical purposes.

Pharmacological aspects of this compound will be reported separately.

SUMMARY

Cyclocarbothiamine, a new thiamine derivative obtained by the reaction of thiamine with phosgene, was found to have a number of advantages over thiamine in its biological properties.

1. Its thiamine activity was indistinguishable from that of thiamine when examined *in vivo* with rats and rice-birds.

2. Oral administration of cyclocarbothiamine resulted in far higher thiamine contents in the rabbit blood as well as in the liver and kidney of rats. Marked increase was observed also in blood cocarboxylase levels of rabbits.

These data indicate that, as against thiamine, cyclocarbothiamine is absorbed from the intestine in large quantities.

3. Intravenously injected cyclocarbothiamine similarly brought about higher blood thiamine levels in rabbits than did thiamine and its urinary excretion was delayed.

4. Unlike thiamine, cyclocarbothiamine was not decomposed by either thiaminase I of *Bacillus thiaminolyticus* or thiaminase II of *Bacillus aneurinolyticus*.

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