EFFECTS OF THIAMINE DEFICIENCY AND TREATMENT WITH THE ANTAGONISTS, OXYTHIAMINE AND PYRITHIAMINE, ON THE LEVELS AND DISTRIBUTION OF THIAMINE DERIVATIVES IN RAT BRAIN

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(Received May 30, 1973)

Total thiamine, free thiamine, thiamine diphosphate (TDP) plus thiamine triphosphate (TTP) and total hydroxyethylthiamine (HET) levels were measured in brains of control, deficient, and oxythiamine (OTH) and pyrithiamine (PTH)-treated rats. The brain levels of TDP + TTP decreased to 39% and 12% of the control thiamine levels in deficient and PTH-treated rats respectively. The brain HET and TDP + TTP levels of the OTH-treated rats were not significantly different from the controls. The HET levels were decreased significantly ($\alpha < 0.005$) by the fourth day of treatment and decreased steadily to 1/7th of the control values in the terminal stages only in the PTH-treated rats. A significant drop in the HET levels from the control levels was interpreted to mean that the pyruvate utilization was significantly impaired in the brain.

It is known that, in pyrithiamine (PTH)-treated rats, PTH accumulates in the brain (16, 23) and that thiamine is displaced more rapidly from the brain than in the thiamine-deficient rat (6–8). This latter fact may explain why the PTH-treated rat usually experiences convulsions in the terminal stages, while thiamine-deficient rats seldom do (7, 11, 12, 19). However, the levels of thiamine and its derivatives and the corresponding level of PTH in the brains of PTH-treated rats during the treatment period prior to and during the terminal convulsive stage have not been determined.

In this study, the levels of thiamine and its derivatives, as well as of PTH in the brains of PTH-treated rats were determined, using improved techniques, at weekly intervals of treatment up to the terminal convulsive stage. The brain levels of thiamine and its derivatives in thiamine-deficient, oxythiamine (OTH)-

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1 This study was supported by Grant No. AM-02448 from the National Institute of Arthritis and Metabolic Diseases, NIH. This work was in partial fulfillment of the requirements for the Ph. D. by D. S. Murdock.
treated and control rats were also determined for the same periods of time as the PTH-treated rats for comparative purposes. Although the levels of the thiamine\(^2\) derivatives in the brain of normal (22) and thiamine-deficient (9) rats as well as the TDP and OTH levels in the brain of OTH-treated rats (24) have been reported, the thiamine-deficient, OTH-treated and control rats are included in this study so that the levels of the various forms of thiamine in the brain of each group of the treated rats can all be directly compared under similar experimental conditions. Such a comprehensive study would circumvent any differences in the determinations of thiamine and PTH levels that have been reported by different investigators, which might be due to: 1) different assay techniques, 2) different ages, weights and strains of rats, 3) different diets, and 4) different levels and routes of administration of thiamine and antagonists.

**METHODS**

Male Sprague-Dawley rats, weighing initially between 110–140 g, were fed a high-carbohydrate, thiamine-deficient diet and were treated with OTH and PTH antagonists according to the procedure of GUBLER (11). For the analysis of thiamine, hydroxyethylthiamine, (HET)\(^3\) and PTH, the methods reported by MURDOCK and GUBLER (18) were used. Two sets (Sets I and II) of the control, thiamine-deficient and OTH- and PTH-treated rats were prepared and analyzed, with 5–10 rats per treatment. In Set I, the free thiamine, TDP + TTP, and total thiamine\(^4\) analyses were performed and, in Set II, the HET\(^5\), TDP + TTP and total thiamine levels were determined. The PTH analysis in the PTH rats was included in both Sets I and II.

**RESULTS AND DISCUSSIONS**

*Average weight gain*

The average weight gain of the controls, the weight gain and subsequent weight loss of the thiamine-deficient, OTH- and PTH-treated rats were similar to previous reports (1, 5, 11, 19), (Figs. 1 and 2). The average weight gain of the control rats in Set I and Set II for the first 21 days of treatment was 6 and 5 grams per day, respectively.

The thiamine-deficient rats gained weight at the same rate as the controls for the first 6 and 13 days in Set I and Set II experiments respectively, after which

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\(^2\) The thiamine derivatives include free thiamine, thiamine monophosphate (TMP), and thiamine diphosphate (TDP) and thiamine triphosphate (TTP) in separate assays.

\(^3\) HET is an intermediate compound formed when pyruvate is oxidized to AcCoA and CO\(_2\) by pyruvate dehydrogenase.

\(^4\) The total thiamine assay includes free thiamine and the mono-, di- and triphosphate esters of thiamine.

\(^5\) HET is determined in a separate assay from the total thiamine determination.
time they gained at a subnormal rate for 4 days, then rapidly lost weight at the average rate of 5 to 6 g per day until they were sacrificed. The OTH-treated rats used in the Set I and Set II experiments did not gain more than 20 g above their starting weights during the entire treatment period of 27 and 25 days, respectively. The PTH-treated rats used in Set I and Set II grew at the same rate as the controls for 7 and 9 days, respectively, then lost on the average 3 and 7 g per day in Set I and Set II, respectively.

**Total thiamine levels**

The control rats (Tables 1 and 2) showed a 25% decrease ($\alpha<0.005$) of brain total thiamine during the first week of the treatment period which resulted from the drastic decrease in the thiamine intake with the switch in the diet from Purina Lab Chow (16 µg thiamine/g) to the synthetic dietary regimen. On this regimen, control rats received only 10 µg of thiamine/100 g body weight per day as compared to a consumption of over 200 µg of thiamine per day while on the commercial chow. Thus, the decrease in the brain thiamine encountered in the first week of treatment in all the groups includes this 25% decrease from day zero levels.
Fig. 2. Average weight gain of control (●—●), deficient (○—○), OTH- (△—△) and PTH-treated (□—□) rats (Set II).

Table 1. Brain levels of total thiamine, free thiamine, TMP, and TDP + TTP

| Treatment day | No. Rats | Treatment | \% of Total Th | Th-deficient^a |
|---------------|----------|-----------|----------------|----------------|
| Total Th levels | 2 5 | 8.2 ± 0.34 | 100 | 5.9 ± 0.39^b |
| | 7 5 | 6.2 ± 0.13 | 100 | 3.7 ± 0.34^b |
| | 14 5 | 5.9 ± 0.12 | 100 | 2.3 ± 0.09^b |
| | 21 5 | 6.1 ± 0.23 | 100 | 1.6 ± 0.10^b |
| | 27 5 | 5.8 ± 0.20 | 100 | 1.41 ± 0.05^b |
| TDP + TTP | 7 5 | 4.6 ± 0.24 | 74 | 3.1 ± 0.24^b |
| | 14 5 | 4.7 ± 0.13 | 80 | 1.8 ± 0.18^b |
| | 21 5 | 5.1 ± 0.13 | 84 | 1.6 ± 0.11^b |
| | 27 5 | 4.3 ± 0.17 | 74 | 1.7 ± 0.09^b |
| Free Th levels | 2 5 | 0.50 ± 0.02 | 6 | 0.33 ± 0.02 |
| | 7 5 | 0.10 ± 0.09 | 1.6 | 0.10 ± 0.09 |
| | 14 5 | 0.10 ± 0.09 | 1.7 | 0.04 ± 0.09 |
| | 21 5 | 0.10 ± 0.05 | 1.6 | — |
| | 27 5 | 0.10 ± 0.09 | 1.2 | 0 |
| TMP levels | 7 5 | 1.4 | 22.6 | 0.6 |
| | 14 5 | 0.1 | 1.7 | 0.5 |
| | 21 5 | 0.9 | 1.5 | 0.04 |
| | 27 5 | 1.5 | 26.0 | 0 |

^a Means expressed as nanomoles/g wet tissue ± standard error.

^b Significant difference at α < 0.005 in a two-tailed t-test.
The total thiamine and TDP + TTP levels in the control and OTH-treated groups are not significantly different ($\alpha > 0.05$) when compared within Set I or within Set II. The total thiamine and TDP + TTP levels in the control and OTH-treated group in Set II, however, are significantly lower ($\alpha < 0.005$) than those in Set I. The reason for this is not apparent.

The fact that the OTH-treated rat does not gain weight during treatment cannot be correlated with the level of thiamine derivatives in the brain during the same period. The blood brain barrier allows only a small amount of OTH to accumulate in the brain (24) and the daily thiamine plus OTH injections provide an adequate amount of thiamine to the brain tissue. Thus the OTH-treated rats served as another "control" group (also GURTNER (12)). The free thiamine levels in the brains of OTH-treated rats were higher than in the control rats on the second day of treatment, but by the seventh day, and until the experiment was terminated, the level of free thiamine in the brains of the controls and OTH-treated rats was not significantly different. The TMP levels in the brain of the control and OTH-treated rats did not vary in a regular fashion. This is not surprising since it was a small difference between two larger numbers.

The rate at which total thiamine was depleted from the brains of thiamine-deficient rats in the studies of DECARO et al. (9) and McCANDLESS and SCHENKER (15) was slower than was determined in this study, although the terminal levels in control, thiamine-deficient, OTH- and PTH-treated rats (Set I).

| % of Total Th | OTHa | % of Total Th | PTHa | % of Total Th |
|---------------|------|---------------|------|---------------|
| 100           | 8.8 ±0.23 | 100           | 5.8 ±0.36b | 100           |
| 100           | 5.8 ±0.28 | 100           | 1.8 ±0.25b | 100           |
| 100           | 6.7 ±0.06 | 100           | 0.7 ±0.09b | 100           |
| 100           | —      | —             | day 0.7 ±0.12b | 100           |
| 100           | 5.8 ±0.25 | 100           | 19   | —             |
| 83            | 5.2 ±0.18 | 90            | 1.6 ±0.21b | 89            |
| 78            | 5.5 ±0.23 | 82.0          | 0.5 ±0.04b | 72            |
| 100           | —      | —             | day 0.4 ±0.12b | 57            |
| 121           | 5.0 ±0.31 | 87.0          | 19   | —             |
| 6             | 0.71 ±0.06 | 8.1           | —    | —             |
| 3             | 0.14 ±0.03 | 2.4           | 0.1 ±0.01 | 2.8           |
| 2             | 0.14 ±0.02 | 2.1           | 0    | —             |
| 0             | —      | —             | day 0    | 5.7           |
| 0             | 0.1 ±0.01 | 1.2           | 19   | —             |
| 16            | 0.5    | 8.6           | 0.1   | 5.6           |
| 21            | 1.1    | 16.4          | 0.2   | 28.5          |
| 2.5           | —      | —             | 0.2   | 28.5          |
| 0             | 0.7    | 12.0          | —    | —             |
as determined by the above authors were identical to those determined in the present study (Fig. 3). DeCaro et al. (9) and McCandless and Schenker (15) used basic K$_3$Fe(CN)$_6$ as the oxidant for thiamine and thus their analysis of the total thiamine levels inadvertently included HET.

The brain total thiamine levels in the thiamine-deficient rats in Sets I and II were significantly ($\alpha<0.005$) lower than the brain levels of the control rats of the respective set by the second and ninth days of treatment in Sets I and II, respectively. The brain total thiamine levels in the thiamine-deficient rats then decreased to minimal levels of 24% (Set I) and 36% (Set II) of the levels in the corresponding control rats by treatment days 27 and 36 in Set I and Set II, respectively.

The total thiamine levels in the brains of the PTH-treated rats decreased rapidly in both the Set I and Set II analyses and there was no significant difference between the total thiamine levels in the two sets of analyses. By treatment days 2 and 4 in Sets I and II, respectively, the total thiamine levels were 30% lower ($\alpha<0.005$) than the corresponding control levels, and decreased on the average to

Table 2. Brain levels of total thiamine, TDP + TTP, and HET in control, PTH-treated, and thiamine-treated rats.

| Treatment | Treatment day | No. Rats | Con | % of Total Th | Treatment day | No. rats | Deficienta |
|-----------|---------------|----------|-----|---------------|---------------|----------|------------|
| Total     | 0             | 10       | 5.6±0.17 | 100            | —             | —        | —          |
| Th        | 4             | 8        | 5.0±0.09 | 100            | 4             | 10       | 4.9±0.12   |
|           | 9             | 8        | 4.2±0.13 | 100            | 9             | 9        | 3.2±0.11e  |
|           | 18            | 4        | 4.1±0.26 | 100            | 18            | 9        | 3.2±0.16b  |
|           | 25            | 10       | 3.8±0.17 | 100            | 25            | 10       | 1.8±0.16e  |
|           | 33            | 3        | 3.6±0.16 | 100            | 32            | 3        | 1.3±0.25e  |
|           | 36            | 7        | 4.4±0.18 | 100            | 33            | 4        | 1.7±0.21e  |
| TDP+ TTP  | 0             | 10       | 4.8±0.24 | 86             | —             | —        | —          |
|           | 4             | 8        | 4.2±0.12 | 84             | 4             | 10       | 4.1±0.12   |
|           | 9             | 7        | 3.9±0.28 | 93             | 9             | 9        | 3.5±0.18   |
|           | 18            | 5        | 2.9±0.20 | 71             | 18            | 8        | 2.5±0.13   |
|           | 25            | 10       | 3.1±0.14 | 82             | 25            | 10       | 1.5±0.14e  |
|           | 32            | 3        | 3.3±0.17 | 92             | 32            | 4        | 1.2±0.14e  |
|           | 36            | 7        | 3.4±0.11 | 77             | 33            | 5        | 1.4±0.25e  |
| HETd      | 0             | 10       | 0.8±0.04 | 24.3           | —             | —        | —          |
|           | 4             | 8        | 1.2±0.22 | 24.0           | 4             | 10       | 1.0±0.09   |
|           | 9             | 8        | 0.4±0.02 | 9.5            | 9             | 9        | 0.40±0.02  |
|           | 18            | 4        | 0.8±0.08 | 19.6           | 18            | 8        | 0.93±0.11  |
|           | 25            | 9        | 0.8±0.07 | 21.0           | 25            | 10       | 0.45±0.09  |
|           | 32            | 3        | 1.6±0.17 | 44.4           | 32            | 3        | 0.61±0.03  |
|           | 36            | 7        | 0.7±0.05 | 15.9           | 33            | 4        | 0.52±0.12  |

a Mean expressed as nanomoles/g wet tissue ± standard error.

b Significant difference with $\alpha<0.05$ in a two-tailed t-test.

c Significant difference with $\alpha<0.005$ in a two-tailed t-test.
25% of the respective control levels by treatment day 10. The terminal PTH-treated rats showed a brain total thiamine level of 14% of the control values. The levels of total thiamine in the brains of the PTH-treated rats in this study after 6 days of treatment were about the same as in the brains of rats which had received 2.5 and 6 mg of PTH intraperitoneally (i.p.) daily for 6 days (DeCARO et al., 6, 7). Also the total thiamine levels in the brains of terminal rats that received (i.p.) 6 mg of PTH per day for 9 days (7) was almost twice the brain total thiamine levels reported in the present study at the corresponding time of treatment. The above authors did not determine the PTH content of the brain tissue. The thiochrome method used by DeCARO et al. (6, 7) was not adequate for the accurate determination of thiamine in the presence of high levels of PTH. Therefore, the levels of thiamine reported above by DeCARO et al. are probably high.

It has been determined that PTH (18) and HET (17, 18) do not interfere with the thiamine analysis with 1% HgCl₂ used in this study, since neither is oxidized to fluorescent compounds by this oxidation procedure.

| %Th | Treatment day | No. Rats | OTH | Tot. TH % | Treatment day | No. Rats | PTH | Tot. TH % |
|-----|---------------|----------|-----|-----------|---------------|----------|-----|----------|
| 100 | 4             | 10       | 4.7 ±0.20 | 100         | 4           | 10       | 2.1 ±0.12² | 100     |
| 100 | 9             | 10       | 4.6 ±0.19 | 100         | 9           | 10       | 1.0 ±0.06² | 100     |
| 100 | 18            | 7        | 3.8 ±0.22 | 100         | 17          | 6        | 0.39 ±0.08² | 100     |
| 100 | 25            | 8        | 3.7 ±0.22 | 100         | 21          | 10       | 0.28 ±0.01² | 100     |
| 100 | —             | —        | —        | —          | —           | —        | —        | —        |
| 83.8 | 4          | 10       | 3.6 ±0.28 | 76.6        | 4           | 10       | 2.0 ±0.22² | 94      |
| 109.0 | 9         | 7        | 4.2 ±0.03 | 91.3        | 9           | 8        | 1.1 ±0.05² | 110     |
| 78.2 | 18           | 8        | 3.1 ±0.07 | 81.6        | 17          | 6        | 0.47 ±0.03² | 125     |
| 83.4 | 25           | 8        | 3.3 ±0.21 | 89.3        | 21          | 10       | 0.34 ±0.02² | 121     |
| 92.3 | —             | —        | —        | —          | —           | —        | —        | —        |
| 82.5 | —             | —        | —        | —          | —           | —        | —        | —        |
| 22.4 | 4             | 9        | 0.99 ±0.08 | 21         | 4           | 9        | 0.57 ±0.07² | 27.2   |
| 12.5 | 9             | 10       | 0.45 ±0.03 | 10         | 9           | 10       | 0.16 ±0.01² | 16.0   |
| 28.1 | 18            | 7        | 0.72 ±0.10 | 18.9        | 17          | 6        | 0.24 ±0.12² | 61.5   |
| 25.0 | 25            | 8        | 0.69 ±0.09 | 18.7        | 21          | 9        | 0.10 ±0.03² | 35.8   |
| 46.9 | —             | —        | —        | —          | —           | —        | —        | —        |
| 30.6 | —             | —        | —        | —          | —           | —        | —        | —        |

The total thiamine determination includes the TTP, TDP, TMP and free thiamine, but does not include HET.
Fig. 3. Levels of total thiamine in the brains of thiamine-deficient rats according to this study, Set II, (○—○), DeCaro et al. (9) (■—■) and McCandless and Schenker (15), (▲—▲).

**TDP + TTP levels**

The quantity represented by TDP + TTP in all the groups of rats varied in direct proportion to the total thiamine levels and constituted around 85% of the total thiamine levels.

There was no significant difference between the levels of TDP + TTP in the brains of the thiamine-deficient rats in Set I and in Set II (Tables I and II). However, due to the higher levels of total thiamine in the control rats in Set I, the rate of depletion of TDP + TTP in the brains of the thiamine-deficient rats of Set I is greater than in Set II. By the second week of treatment, the TDP + TTP levels in the thiamine-deficient rats in Set I had decreased to 38% of the control values, decreasing to a minimum of 31% on the 21st day of treatment, then increasing slightly to 40% of the control levels in the terminal rats sacrificed on the 27th day of treatment.

The TDP + TTP levels in the brains of the PTH-treated rats decreased to 30% of the corresponding control values by the first assay period in Sets I and II.
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(day 2 and 4 respectively) and continued to decrease to a minimum average value of 13% of the control values after two weeks of treatment.

The TMP and free thiamine decreased rapidly in the brains of thiamine-deficient and PTH-treated rats and by the 21st day of treatment no free thiamine could be detected.

Hydroxyethyl thiamine and pyrithiamine levels

The HET levels in the brains of the control, thiamine-deficient and OTH-treated rats were not significantly different ($\alpha > 0.05$), (Tables 1 and 2). The brain HET levels of the PTH-treated rats on treatment day four were 40% lower than in the control rats and rapidly decreased further to less than 17% of the control levels by the terminal treatment period. Thiamine does not interfere with the HET analysis ($17, 18$). On the other hand, PTH contributes significantly to the HET fluorescence spectra, and causes the HET determination in the PTH-treated rat brain to be high ($18$). However, since the level of PTH in the brain reaches a maximum and is then stable after one week of treatment (Table 3), the continuous decrease in the HET levels in the brains of PTH-treated rats during the remaining two weeks of treatment is felt to be meaningful. The brain levels of HET in the control, thiamine-deficient and OTH-treated rats varied around the value of 0.7 nanomoles/g wet tissue during the entire treatment period. The HET levels in the brain of normal rats has been reported as 1.4 nanomoles/g wet tissue ($17$).

PTH accumulates in the brain as the treatment progresses, so that by treatment day seven, according to the results from Table 3, the PTH levels approach a maximum concentration of 5.0 nanomoles/g wet tissue. These levels are close to the concentration of total thiamine in the brains of control rats. Neither HET

| Day of treatment | No. Rats | Mean ± Standard error |
|------------------|----------|----------------------|
| 2                | 5        | 1.5 ± 0.17           |
| 7                | 5        | 5.3 ± 0.44           |
| 14               | 5        | 4.8 ± 0.54           |
| 19               | 5        | 5.1 ± 0.36           |

| Day of treatment | No. Rats | Mean ± Standard error |
|------------------|----------|----------------------|
| 4                | 7        | 4.6 ± 0.27           |
| 9                | 7        | 5.0 ± 0.16           |
| 17               | 2        | 5.1 ± 0.38           |
| 21               | 8        | 5.5 ± 0.39           |

* Means expressed as nanomoles/g wet tissue ± standard error.
nor thiamine contributed significantly to the PTH analyses in this study (18).

**Weight gain and thiamine levels**

According to Tables 1 and 2, the brain levels of TDP + TTP in the thiamine-deficient rats were 3.2 and 3.0 nanomoles/g wet tissue, respectively, when the rats reached their peak weight (Figs. 1 and 2). This concentration in the brain seems to be a critical value since the rats decrease in weight rapidly as the TDP + TTP decreases below these levels.

The PTH-treated rats reach their peak weight about the same time as the thiamine-deficient group, yet the brain total thiamine levels in the former group are 1/2 to 1/5 the levels of the latter group at this stage of treatment. Perhaps the rate at which thiamine is lost from other organs of the body in the thiamine-deficient and the PTH-treated rats help determine the rate at which the treated rats lose weight. Another explanation might be that PTH-treatment may not deplete thiamine at the same rate from all areas of the brain. However, thiamine has been reported to be depleted at a uniform rate in all areas of the brain in the thiamine-deficient rat (10).

**Mechanism of action**

The more rapid rate and extent of depletion of thiamine from the brain of PTH-treated rats as compared to thiamine-deficient rats causes the former group of rats to reach a terminal condition one to two weeks sooner than in the latter group. None of the thiamine-deficient rats experienced convulsions. In the terminal thiamine-deficient and PTH-treated rats, the brain TDP + TTP levels averaged 1.5 and 0.5 nanomoles/g wet tissue, respectively (Tables 1 and 2). It is significant that there is a striking difference in the total thiamine and TDP + TTP levels in the brains of terminal thiamine-deficient and PTH-treated rats and only the latter group experiences convulsions. It would be extremely interesting to determine the brain levels of total thiamine and TDP + TTP as well as the HET levels in terminal, convulsive thiamine-deficient rats.

The reason for the depletion of thiamine in the brains of the PTH-treated rats was not due to an insufficient level of daily thiamine intake, as in the case of the thiamine-deficient rats, since 10 μg of thiamine is injected subcutaneously daily along with the 50 μg of PTH per 100 g of body weight. The thiamine levels in the heart and liver of thiamine-deficient rats are much less than in PTH-treated rats (MURDOCK and GUBLER to be published, and 6, 8, 9). Hence, there is a much larger supply of total tissue thiamine for the brain to draw upon in the PTH-treated rats.

It is interesting that PTH accumulates rapidly in the brain with treatment and levels off at about 5.0 nanomoles/g wet tissue in the first week of treatment and remains at about this level for the next two or three weeks. The daily intake of thiamine in the PTH-treated rat tends to displace some PTH from the brain (16).
and hence appears to result in keeping PTH from accumulating in the brain to higher levels.

It has been established that free PTH inhibits the following reaction *in vitro*:

\[
\text{ATP} \quad \text{Thiamine} \rightarrow \text{TDP}
\]

[JOHNSON and GUBLER (14); PETERSON (20)]

PTH also inhibits the active transport of thiamine into the animal cell (25, 26). The conversion of free thiamine to the diphosphate is closely associated with the active transport process. The PTH + thiamine injection schedule used in this study resulted in a 50% depletion (\(a<0.005\)) of total thiamine from the brain by treatment day three. From treatment day four to nine, the total thiamine levels were decreased another 50%. The rate of loss after this period diminished. The rate at which PTH accumulates in the brain equals approximately the rate at which thiamine is displaced from the brain. One cannot tell from the data presented in this analysis how much thiamine is entering the brain after the maximum levels of PTH are reached in this tissue. Obviously since the total thiamine levels decrease in the brain tissue, the rate of entry of thiamine into the cell is much less than the rate at which the thiamine is displaced from its active sites within the cell, dephosphorylated and excreted out of the cell. The free thiamine and TMP decrease to very low levels in PTH-treated rats, showing that the TDP and TTP breakdown products do not accumulate in the brain (Table 1). The mechanisms which control the rate of entry of thiamine into the cell, the rate of dephosphorylation of the thiamine phosphates and the rate at which the TMP or free thiamine leave the cell has not been defined but seems to operate up to the time of death of the rat according to the results of this study. The TDP + TTP levels in the brain remain at around 85% of the total thiamine in all the groups.

The \(K_i\) of free PTH is about equal to the \(Km\) of free thiamine, *i.e.* 0.2 \(\mu\)M, toward thiamine pyrophosphokinase isolated from rat brain (14). The PTH diphosphate ester has a \(K_i\) of 78 \(\mu\)M for yeast pyruvate decarboxylase (27), a value about three times the \(Km\) of TDP for this enzyme. Therefore, the initial effect of PTH on the brain tissue is probably due to the inhibition of the thiamine pyrophosphokinase activity. Thiamine entering the tissue that cannot be phosphorylated to the diphosphate ester would leave the cell. As the total PTH/(TDP + TTP) ratio increased from about 4:1 on treatment day seven to about 10:1 by treatment day fourteen, the PTH diphosphate [(16) and unpublished work in this laboratory] which is probably formed in the tissue may effectively compete with TDP for the active site on the TDP-requiring enzymes.

The continuously decreasing levels of HET observed in the brains of PTH-treated rats can be coordinated with the reduced pyruvate dehydrogenase activity in rats (2, 11) and in mice (13). No studies have been reported dealing with the preterminal effect of PTH on the pyruvate dehydrogenase activity in the brain.
Note also that the pyruvate dehydrogenase activity in the brain of OTH-treated rats is normal (3, 11) which fact correlates well with the brain HET levels in the OTH-treated rats (Table 2).

Investigators have shown that in rats treated with OTH, the TDP level in the liver and brain (12, 24) and heart (12) is not significantly affected. The pyruvate dehydrogenase activity in the brain of thiamine-deficient rats is decreased 25, 30 and 72% from the control rats according to GUBLER (11), MCCANDLESS and SCHENKER (15) and REINAUER et al. (21), respectively. The first two reports tend to support the somewhat decreased levels of HET observed in this study in the thiamine-deficient rat brains (Table 1 and Fig. 3). The 24% decrease of brain HET in the terminal thiamine-deficient rats as compared to the control group is not significant in the student t-test at the 95% level of confidence.

Another interpretation of a lowering of the HET levels would be that HET is used up via the citric acid cycle at a faster rate than in the control rat. The acetylCoA could be converted to cholesterol and/or fatty acids at an increased rate or the decrease in the HET levels might be due to a rapid breakdown of the HET molecule with no concurrent formation of acetylCoA. The first hypothesis does not seem likely, at least in the PTH-treated rat, since the α-ketoglutarate dehydrogenase activity decreases in the brains of the PTH-treated rats during treatment (11). The decrease in the transketolase activity of the brains of thiamine-deficient rats (4, 15) would not favor increased synthesis of fatty acids or cholesterol since both processes required NADPH. Breakdown of the HET moiety with no concurrent acetylCoA formation would be a wasteful process and is therefore an unlikely route.

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