The pain threshold increasing effects by thiamine deficiency in rats

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It is well known that rats maintained on a thiamine deficient diet exhibit significant weight loss, decrease in heart frequency, behavioral disorders such as circling movements, muricide aggression as well as symptoms of polyneuritis. After a series of pharmacological studies on thiamine deficient rats, I reported that the actions of certain drugs affecting the central nervous system were modified by acute thiamine deficiency in animals. I am planning to investigate the effect of analgesics (morphine, pentazocine, aspirin . . . . . .) on thiamine deficient animals, since little is known about changes in the pain threshold following severe thiamine deficiency. In this study, an attempt was made to investigate the changes in the pain threshold of rats as a consequence of thiamine deficiency.

Materials and Methods

Male, 35-day-old Wistar rats were used. They weighed from 65-85 g and were housed individually in mesh cages (17×25×37 cm). The rats were divided into three groups, consisting of a control group, a thiamine deficient group and a pair-fed group. An usual, a complete powdered diet was supplied ad lib. to the control group, while the rats in the experimental group received a thiamine deficient diet ad lib. The pair-fed rats were given a diet, which was identical to that of experimental group in both quantity and composition, except for the addition of thiamine. The actual food intake of the thiamine deficient group was recorded daily during the study, and the diet of the pair-fed group adjusted accordingly. Water was available continuously to all three groups.

The thiamine deficient diet (Funabashi Farm Co., Japan), utilized in this study, consisted of a basic ration, which was composed of 67.6% carbohydrate, 18% protein and 8% lipids, and supplemented with vitamins (except thiamine) and minerals. The control and pair-fed groups were fed a diet identical to the experimental groups, but with the addition of 0.5 mg of thiamine per 100 g of feed.

The food intake and body weight of the rats in each group were determined at 9:30 a.m. Pain threshold was determined by the tail flick analgesia meter (MK-330, Muromachi, Japan) and hot plate (KN-205, Natume, Japan) on certain days of experimental feedings. Tail flick response was used to determine the reflex latency according to the method of D’Amour and Smith. The degree of the heat-source bulb was adjusted so that the untreated normal rats had tail flick latencies of around 5 sec. To avoid tail tissue damages, a cut-off of 20 sec was imposed on animals failing to remove their tail from the light beam.

In the hot plate test the algesimetric method was used, the temperature of the hot plate being set at 49°C for this study. The latency to the licking of the paw was recorded by means of stopwatch. Cut-off
The heart frequency was measured by programmable sphygmomanometer (PS-100, Riken Kaibatsu and Tokai Irika, Japan).

On the 30th day of experimental feeding, pain threshold and heart frequency were tested in the three groups. After that, thiamine deficient rats were given 10 mg/kg thiamine monophosphate disulfide intraperitoneally (i.p.). These rats were still housed in mesh cages, but were provided thiamine added normal diet (Funabashi Farm Co., Japan). 14 days after the injection of thiamine, the three groups were tested for pain threshold and heart frequency.

Statistical significance of the data was estimated using Student’s t-test.

Results and Discussion

The rats in three groups continued to grow during the first 14 days, and then thiamine deficient and pair-fed groups stopped growing and decreased in body weight on the 30th day. During this period of thiamine deficient feeding, only thiamine deficient rats showed marked bradycardia and polyneuritis, as shown in Table 1. The heart frequency of the thiamine deficient rats amounted to 281.4 beats per minute, which was less than 69% of that of the control group.

According to Iwata et al., animals on a thiamine deficient diet, showing a heart frequency of less than 70% of that of control group, are regarded as acutely thiamine deficient. Judging from the decrease in heart frequency and weight loss among thiamine deficient rats in this experiment, it seems they can be considered as exemplifying a state of severe thiamine deficiency.

As shown in Table 2, pain sensitivity is reduced in thiamine deficient rats, the effect being statistically significant as compared with normal and pair-fed groups on the 30th day of experimental feeding when it was measured by hot plate method (P<0.01). However, the pain threshold of

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Body Weight (g)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before injection</td>
<td>14 days after</td>
</tr>
<tr>
<td>Control group</td>
<td>195.8±5.7</td>
<td>240.5±4.6</td>
</tr>
<tr>
<td>Pair-fed group</td>
<td>98.1±6.1a</td>
<td>181.5±1.7a</td>
</tr>
<tr>
<td>Thiamine deficient group</td>
<td>89.5±7.0a</td>
<td>182.5±1.6a</td>
</tr>
</tbody>
</table>

Each value represents the mean±S. E. of 8 rats.

a) P<0.01, when compared to the control group.

Table 2 Effects of thiamine monophosphate disulfide on pain threshold in thiamine deficient rats.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Hot plate method (sec)</th>
<th>Tail flick response (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before injection</td>
<td>14 days after</td>
</tr>
<tr>
<td>Control group</td>
<td>20.8±2.6</td>
<td>25.1±4.3</td>
</tr>
<tr>
<td>Pair-fed group</td>
<td>18.6±2.1</td>
<td>19.6±1.9</td>
</tr>
<tr>
<td>Thiamine deficient group</td>
<td>65.3±8.3a</td>
<td>23.4±2.8</td>
</tr>
</tbody>
</table>

Each value represents the mean±S. E. of 8 rats.

a) P<0.01 when compared with that of rats, 14 days after the injection by hot plate method.

b) P<0.01 when compared with the control and pair-fed groups.
thiamine deficient group remained within normal limits when measured by tail flick analgesia meter in this period.

On the other hand, the thiamine deficient group treated with thiamine 14 days before test, returned to normal growth and heart frequency, as shown in Table 1. In these rats, the pain threshold had significantly decreased within the normal limits of that of 30th day by hot plate method (P<0.01).

However, there were no significant changes among the three groups which were measured by tail flick analgesia meter, 14 days after the i.p. injection of thiamine in this experiment (Table 2).

In this experiment, thiamine deficiency showed an antinociceptive effect in the hot plate test, but failed in the tail flick response. It is suggested that the tail flick response is largely a segmental spinal event, whilst the response in the hot plate test is based on large neuron loops involving the brain stem. It is also well known that dietary-induced thiamine deficiency produces various neurological symptoms in animals consisting of both central and peripheral nervous system dysfunction. Classical Peters' work suggested that thiamine deficiency produced a biochemical lesion in the oxidation of pyruvate, followed by pathological damage. Collins has also found such lesions in the brain stem of animals, maintained on a thiamine deficient diet.

Presumably, thiamine deficiency may affect the large neuron loops involving the brain stem but may not so influence the tail flick response by an action on the spinal reflex pathway beyond the nociceptive afferent.

Whatever the mechanism underlying the pain threshold increasing effect by thiamine deficiency by hot plate method, it is evident the changes of pain threshold can be a consequence of the thiamine deficient feeding since it is reversible.

Finally, attention must be paid to factors that modulate pain threshold, such as anxiety, depression, fatigue as reported, and besides thiamine deficiency in this experiment.

Acknowledgements

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References