PRELIMINARY REPORT

Effect of Thiamine on Serotonin Levels in Magnesium-deficient Animals

By Yoshinori Itokawa, Chikako Tanaka, and Mieko Kimura

To clarify the relationship between thiamine metabolism and peripheral vasodilation symptoms seen in the early stages of dietary magnesium deficiency, various synthetic diets were administered to rats. After a 4-wk period of thiamine excess and magnesium-deficient diet, blood serotonin levels increased significantly. A thiamine-deficient and magnesium-deficient diet revealed no elevation of blood serotonin. Serotonin in stomach and intestine increased in the excess-thiamine, magnesium-deficient group. Blood magnesium concentration decreased markedly in thiamine-supplemented, magnesium-deficient groups but not in the thiamine-deficient magnesium-deficient group. A possible explanation of the mechanism is presented.

PERIPHERAL VASODILATION is the first manifestation of experimental magnesium deficiency in rats and has been described in early work on magnesium deficiency. Although considerable evidence has accumulated suggesting that the metabolisms of thiamine and magnesium are interdependent, little is known about the relationship between the vasodilation in magnesium-deficient animals and thiamine. Because serotonin has a potent effect on vascular systems, the authors investigated the effect on serotonin levels of both excess thiamine and thiamine deficiency in magnesium-deficient rats.

MATERIALS AND METHODS

Thirty male Wistar rats weighing about 100 g were separated into six groups. Table 1 shows composition of the diets fed during experiments. Diets were given ad libitum and averages of daily intake of food, magnesium, and thiamine in each experimental group are shown in Table 2. After 4 wk animals were sacrificed, blood, brain, stomach, and small intestine removed, and portions of these tissues used for determination of serotonin and magnesium. The amount of serotonin was assayed by the method of Bogdanski et al. Magnesium was determined by atomic absorption method after wet oxidation of tissues.

RESULTS

Effects on growth of excess-thiamine or thiamine-deficient rats, with and without magnesium deficiencies, are shown in Fig. 1. In adequate thiamine-sufficient-magnesium and excess-thiamine-sufficient-magnesium rats (groups IV and VI), good growth was observed, while growth in the adequate-thiamine
Table 1. Composition of Diets

<table>
<thead>
<tr>
<th>Group</th>
<th>Thiamine</th>
<th>Magnesium</th>
<th>Deficient</th>
<th>Adequate</th>
<th>Excess</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Thiamine</th>
<th>Magnesium</th>
<th>g/100 g diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>68.9</td>
<td>68.9</td>
</tr>
<tr>
<td>Olive oil</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Salt mixture (Mg-free)*</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Cellulose</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitamin mixture (Thiamine-free)t</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>0</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*Magnesium-free salt mixture contained: NaCl, 5.1%; Na₂HPO₄ • H₂O, 10.1%; K₂HPO₄, 27.8%; CaH₂(PO₄)₂ • H₂O, 15.8%; Fe(C₆H₅O₇) • 5H₂O, 3.4%; Ca(C₃H₅O₃)₂ • 5H₂O, 38.0%.

†Thiamine-free vitamin mixture (100 g) contained: riboflavin, 150 mg; nicotinic acid, 1000 mg; pyridoxine, 100 mg; cyanocobalamin, 1 mg; pantothenic acid, 500 mg; folic acid, 50 mg; ascorbic acid, 3750 mg; Vitamin E, 100 mg; Vitamin A, 250,000 IU; Vitamin D₂, 20,000 IU; sucrose, to 100 g.

and excess-thiamine-magnesium-deficient groups (groups III and V) was slightly depressed. Body weights of thiamine-deficient rats (group II) and thiamine-magnesium-deficient rats (group I) reached a peak in about 15 days followed by a progressive loss. After 3 wk of these dietary regimens, animals fed a thiamine-containing, magnesium-deficient diet (groups III and V) developed erythema in the ear and nose. Thiamine- and magnesium-deficient rats showed no such erythema (group I). After 4 wk, blood serotonin levels increased significantly in excess-thiamine-magnesium-deficient and adequate-thiamine-magnesium deficient groups. The thiamine-deficient, magnesium-deficient group showed no elevation of blood serotonin. In magnesium-supplemented rats, no increase was observed (Fig. 2). Serotonin in the intestine increased in excess-thiamine and adequate-thiamine-magnesium-deficient groups. Stomach serotonin increased in the excess-thiamine-magnesium-deficient group, but brain serotonin remained unchanged (Table 3). Blood magnesium concentr-
tion decreased markedly in thiamine-supplemented, magnesium-deficient groups rather than in the thiamine-magnesium-deficient group (Fig. 3). Magnesium content in brain, stomach, and intestine revealed no significant change. This could be due to the homeostasis. To clarify the mechanism of peripheral vasodilation, 10 mg/kg body weight of serotonin or histamine were injected i.p. into normal Wistar rats. Serotonin-injected rats produced erythema 15–30 min following injection, but rats that had been administered histamine failed to manifest vasodilation symptoms. It can be concluded that serotonin could play a major role in vasodilation in magnesium-deficient rats, but histamine has little effect.

DISCUSSION

Belanger et al. reported that magnesium-deficient rats showed a reduction of dermal mast cells. These observations suggest that magnesium is required...
Table 3. Serotonin Levels in Brain, Stomach, and Intestine
(Mean ± SE of Five Rats)

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Thiamine Magnesium</th>
<th>Brain (µg/g Wet Tissue)</th>
<th>Stomach (µg/g Wet Tissue)</th>
<th>Intestine (µg/g Wet Tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td>Deficient —</td>
<td>0.44 ± 0.06</td>
<td>3.16 ± 0.49</td>
<td>3.51 ± 0.41</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>Deficient +</td>
<td>0.53 ± 0.33</td>
<td>3.29 ± 0.14</td>
<td>3.68 ± 0.94</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>Adequate —</td>
<td>0.45 ± 0.07</td>
<td>3.37 ± 0.12</td>
<td>4.38 ± 0.96†</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td>Adequate +</td>
<td>0.54 ± 0.09</td>
<td>3.41 ± 0.39</td>
<td>3.41 ± 0.40</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>Excess —</td>
<td>0.54 ± 0.06</td>
<td>6.78 ± 0.89*</td>
<td>6.65 ± 0.46*</td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td>Excess +</td>
<td>0.52 ± 0.05</td>
<td>2.59 ± 0.16</td>
<td>2.70 ± 0.57</td>
</tr>
</tbody>
</table>

†Difference between III and IV is statistically significant (0.01 < p < 0.05).
*Difference between V and VI is statistically significant (p < 0.01).

for the maintenance of mast cells and magnesium deprivation may presumably liberate serotonin and histamine. It is noteworthy that thiamine- and magnesium-deficient rats showed no elevation of blood serotonin and that the decrease of blood magnesium was lower than in the thiamine-supplemented, magnesium-deficient groups. Because thiamine-dependent enzymes (pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and transketolase) require magnesium it is possible that excess thiamine promotes magnesium deficiency and liberates serotonin into the blood stream from the mast cells.\textsuperscript{10,12} Thiamine deficiency does not deplete magnesium, and mast cells remain intact in the thiamine- and magnesium-deficient groups.
EFFECT OF THIAMINE ON SEROTONIN LEVELS

REFERENCES

lum, E. V.: Studies on magnesium deficiency
in animals. I. Symptomatology resulting
from magnesium deprivation. J. Biol. Chem.
96:519, 1932.

2. Zieve, L., and Hill, E.: Vitamin unre­
1963.

3. —, Doizaki, W. M., and Stenroos, L.
E.: Effect of magnesium deficiency on
growth response to thiamine of thiamine­
1968.

4. —: Influence of magnesium deficiency
on the utilization of thiamine. Ann. N. Y.

5. Itokawa, Y., Inoue, K., and Natori, Y.:
Relationship between thiamine and mag­
nesium in animal bodies. I. Effect of mag­
nesium deficiency on growth, levels of
magnesium, calcium and thiamine in tissues.

6. Erspamer, V.: 5-Hydroxytryptamine and
related indolealkylamines. In Eichler, O.
and Farah, A. (Eds.): Handbook of Experi­
mental Pharmacology, Vol. XIX. Berlin,

7. Bogdanski, D. F., Pletscher, A., Brodie,
B. B., and Udenfriend, S.: Identification and
Exp. Ther. 117:82, 1956.

8. Alcock, N. W., and MacIntyre, I.: Methods for estimating magnesium in bio­
logical materials. In Glick, D. (Ed.): Methods of Biochemical Analysis. New

Jackerow, A.: Behavior of the dermal mast
cells in magnesium-deficient rats. Science
126:29, 1957.

ism of action of transketolase. II. The sub­
strate-enzyme intermediate. J. Biol. Chem.

11. Koike, M.: Mammalian α-keto acid
dehydrogenase complexes. J. Vitamin.
(Kyoto) 14:86, 1968.

12. Lenn, T. C., Pettit, F. H., and Reed,
L. J.: α-Keto acid dehydrogenase complexes.
X. Regulation of the activity of the pyruvate
dehydrogenase complex from beef kidney
mitochondria by phosphorylation and de­
phosphorylation. Proc. Nat. Acad. Sci. USA