EFFECT OF THIAMINE DEFICIENCY, PYRITHIAMINE AND OXYTHIAMINE ON PYRUVATE METABOLISM IN RAT LIVER AND BRAIN IN VIVO

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Summary Rats were fed either a thiamine-deficient diet or diets containing pyrithiamine or oxythiamine. When symptoms of thiamine deficiency appeared, the animals were injected intraperitoneally with [2-14C] pyruvate six to twelve minutes prior to sacrifice. Free glutamic and aspartic acids were isolated from liver and brain and degraded. The results indicate that, in thiamine-deficient or oxythiamine-treated rats, pyruvate metabolism in liver and brain is similar to that in normal animals. In contrast, pyrithiamine drastically decreases the oxidative decarboxylation of pyruvate by rat liver.

Since the classical work of PETERS (1) using pigeons, thiamine deficiency has often been described as a well defined biochemical lesion. Lack of thiamine is supposed to diminish the organism’s ability to decarboxylate pyruvate, and this impairment of pyruvate oxidation is thought by many to account for the physiological changes, particularly polyneuritis, observed in thiamine deficiency.

However, the results of a large number of diverse experiments (2–16) have led to serious questioning of the concept that thiamine deficiency results in a gross change in the ability of animal organs to decarboxylate pyruvate. The parameters measured include rates of pyruvate oxidation in vivo (2, 12) and in vitro (3–8), concentrations of blood pyruvate (6, 8) and brain thiamine (13, 14) and 14C labeling patterns in glutamic acid after administration of pyruvate-14C (15, 16). In many investigations, thiamine deficiency was induced by administration of the antimeabolites oxythiamine and pyrithiamine as well as by thiamine deprivation. Although some treatments have resulted in decreased rates of pyruvate oxidation in vitro (3, 6), these do not necessarily correlate with increases in blood pyruvate and lactate concentrations (6), indicating that there is no clear evidence that the
gross ability of any tissue to oxidize pyruvate is diminished, *in vivo*.

Some of the evidence suggests that the decrease in transketolase activity may be a more important parameter during thiamine deficiency than a decrease in pyruvate oxidase activity (9, 10, 12).

The labeling patterns observed in glutamic acid of rat tissue after the administration of pyruvate-2-14C or its precursors (15-21) have been used to indicate an appreciable amount of pyruvate converted to acetate and to oxalacetate. As discussed previously (15-18), labeling in carbon 5 reflects acetate formation or decarboxylation pathway, whereas labeling in carbon atoms 2 and 3 reflects the carboxylation to form a dicarboxylic acid. If thiamine deficiency grossly alters pyruvate metabolism, one would expect to find less labeling in carbon 5 of glutamate in the deficient animal than in the normal animal when [2-14C] pyruvate or its precursors is used. However, when we performed such studies with rats and chicks (15, 16), the labeling in carbon 5 of glutamate in either liver (15) or brain (16) was invariably equal to or higher than that observed in normal animals. Although the lack of knowledge of pool size variation makes interpretation somewhat difficult (15), the finding that liver glutamate from thiamine-deficient rats had more than ten times as much [14C] in carbon 5 as did liver glutamate from fasted rats given [2-14C] pyruvate. This appears to be strong evidence against the idea that the thiamine-deficient animal has a greatly diminished ability to decarboxylate pyruvate.

Because our previous work was done primarily with thiamine-deprived animals and since thiamine deficiency created by treatment with oxythiamine or pyrithiamine is often different from that obtained by thiamine deprivation (22, 23), we have repeated and extended some of our earlier studies using pyrithiamine- and oxythiamine-fed rats.

After intraperitoneal injection of [2-14C] pyruvate, the free glutamic and aspartic acids were isolated from liver and brain, degraded, and assayed for radioactivity. The data indicate that none of the treatments used grossly affected the decarboxylation of pyruvate in rat brain and only the pyrithiamine treatment drastically inhibited pyruvate oxidation in rat liver.

**MATERIALS AND METHODS**

Male albino rats weighing 150–180 g were purchased from the Holtzman Rat Company, Madison, Wisconsin; all were caged individually. The animals were fed a basal thiamine-deficient diet obtained from Nutritional Biochemical Corporation for four days. They were then given a basal thiamine-deficient diet plus supplements as follows:

1. Rats nos. 268, 271, 276, 289, 290, 294, 360 and 361 received *ad libitum* 22 mg of thiamine/kg basal diet.
2. Rats nos. 270, 273, 291, and 292 (pair fed control) received the same diet as in 1 in the amounts equal to the consumption of those receiving basal diet, with no supplement.
3. Rats nos. 269, 272, 274 and 288 (thiamine-deficient) received the basal diet with no supplement.

4. Rats nos. 280, 281 and 283 (pyrithiamine) received 6.25 mg of pyrithiamine and 1.25 mg of thiamine/kg basal diet.

5. Rats nos. 305 and 306 (oxythiamine) received 250 mg of oxythiamine and 1.25 mg of thiamine/kg basal diet.

Pyrithiamine hydrobromide and oxythiamine chloride were obtained from Calbiochem and Mann Research Laboratory, U.S.A., respectively.

Rats fed pyrithiamine showed polyneuritic symptoms in 16 days, whereas those on oxythiamine did not suffer from polyneuritis but did lose weight; some of these animals died. Those on thiamine-deficient diets showed polyneuritic symptoms after 36 days. Rats were weighed on alternate days.

When the animals exhibited severe symptoms of deficiency they were injected intraperitoneally with [2-14C] sodium pyruvate. Six or twelve minutes later the rats were decapitated and livers and brains were removed immediately, frozen in liquid nitrogen and weighed. Sodium pyruvate-[2-14C] (specific activity 7–9 mCi/mmole) was obtained from Nuclear Chicago. The purity of these labeled compounds was checked as described previously (23, 24). Data concerning animal size, the dose in μCi and duration of the experiment after administration of isotope are included in Tables 1 and 2. Free glutamic and aspartic acids were isolated from perchloric acid filtrates of liver and brain, assayed for [14C] and degraded as described previously (17, 23, 25).

RESULTS AND DISCUSSION

Growth data are presented in Fig. 1. For the first few days all animals gained weight. Those on pyrithiamine and oxythiamine then lost weight rapidly, two animals died on the twentieth day. Without exception, the rats on pyrithiamine developed extreme polyneuritic symptoms. Similar effects of thiamine inhibitors have been reported by others (22, 26).

The [14CO₂] excretion data (Tables 1 and 2) do not suggest any gross change in the rate of [14CO₂] expiration. However, these are short term experiments and the data are subject to quite wide experimental variation. In general, they support the results of others who found little change in overall formation of [14CO₂] from labeled pyruvate (2, 12).

The labeling patterns in liver free glutamic and aspartic acids are summarized in Tables 1 and 2. It is clear that only treatment with pyrithiamine caused a low incorporation of radioactivity into carbon 5 of liver glutamate. The rather variable results with the controls sacrificed six minutes after administration of isotope are not readily explained. However, the decrease in labeling of carbon 5 was never as low as with the pyrithiamine-treated animals. In fact, the results with pyrithiamine-treated rats (Tables 1 and 2) are the only ones ever obtained in which the
labeling in carbon 5 of liver glutamate, following administration of [2-\(^{14}\)C] pyruvate, is as low as that consistently observed with the fasted rat (15, 23, 27). This labeling pattern is reflected in carboxyl carbons of aspartate also. Thus, one is faced with the rather paradoxical situation in pyrithiamine-augmented thiamine deficiency in which the change in blood pyruvate is rather minimal (6), the change in liver glutamate labeling pattern is dramatic.

![Fig. 1. Effect of different diets on the growth of rats.](image)

The picture is further complicated when one looks at the brain data (Table 3). Here we must use the sum of the labeling in carbons 4 and 5 to get a measure of the decarboxylation of pyruvate in brain because, as discussed previously (20), much of the injected pyruvate is first converted to blood glucose by liver (27). During this conversion, randomization of isotope occurs (20), hence the increased labeling in carbon 4 of brain glutamate. None of the treatments used had any appreciable effect on the distribution of the label in brain glutamate between the sum of carbons 4 and 5 (pyruvate conversion to acyl-CoA) and of carbons 2 and 3 (carboxylation of pyruvate).

Comment should be made concerning the randomization in carbon 4 of brain glutamate (Table 3). It will be noted that except for the normal rats, randomization is quite high, indicating considerable prior conversion of pyruvate-[2-\(^{14}\)C] to blood glucose-1, 2, 5, 6-[\(^{14}\)C] (20). The results with the thiamine-deficient animals (rats 318, 319) show a much higher randomization than that reported by WARNOCK and BURKHALTER (28). These authors interpreted the low randomization in brain glutamate in thiamine-deficient rats given [2-\(^{14}\)C] pyruvate as being indicative of a decrease in the blood brain barrier for pyruvate during thiamine deficiency. The results reported here are not contradictory to those of WARNOCK and BURKHALTER (28) because in our experiments the labeled pyruvate was administered intraperitoneally whereas it was given intravenously in their studies. An intraperitoneal
Table 1. Labeling in liver free glutamic and aspartic acid of rats under different nutritional states 6 min after intraperitoneal administration of [2-\textsuperscript{14}C] pyruvate.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Nutritional state</th>
<th>Amount of radioactivity</th>
<th>Dose excreted as [\textsuperscript{14}CO\textsubscript{2}]</th>
<th>Distribution of radioactivity in glutamate</th>
<th>Distribution of radioactivity in aspartate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>((\mu\text{Ci}))</td>
<td>(%)</td>
<td>Total radioactivity</td>
<td>% of total radioactivity in carbon atoms</td>
</tr>
<tr>
<td>268</td>
<td>Thiamine-sufficient diet \textit{ad libitum}</td>
<td>15</td>
<td>2</td>
<td>4.3</td>
<td>4</td>
</tr>
<tr>
<td>271</td>
<td>Thiamine-sufficient diet \textit{ad libitum}</td>
<td>13</td>
<td>4</td>
<td>3.7</td>
<td>6</td>
</tr>
<tr>
<td>289</td>
<td>Thiamine-sufficient diet \textit{ad libitum}</td>
<td>15</td>
<td>4</td>
<td>11.1</td>
<td>6</td>
</tr>
<tr>
<td>360</td>
<td>Thiamine-sufficient diet \textit{ad libitum}</td>
<td>8</td>
<td>3</td>
<td>5.4</td>
<td>—</td>
</tr>
<tr>
<td>361</td>
<td>Thiamine-sufficient diet \textit{ad libitum}</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>270</td>
<td>Thiamine-sufficient diet pair-fed</td>
<td>10</td>
<td>3</td>
<td>9.1</td>
<td>8</td>
</tr>
<tr>
<td>273</td>
<td>Thiamine-sufficient diet pair-fed</td>
<td>10</td>
<td>3</td>
<td>9.2</td>
<td>6</td>
</tr>
<tr>
<td>269</td>
<td>Thiamine-deficient diet</td>
<td>10</td>
<td>2</td>
<td>11.3</td>
<td>6</td>
</tr>
<tr>
<td>272</td>
<td>Thiamine-deficient diet</td>
<td>10</td>
<td>1</td>
<td>1.4</td>
<td>9</td>
</tr>
<tr>
<td>280</td>
<td>Pyrithiamine-treated</td>
<td>10</td>
<td>1</td>
<td>14.3</td>
<td>5</td>
</tr>
</tbody>
</table>
### Table 2: Labeling in liver free glutamic and aspartic acid of rats under different nutritional states 12 min after intraperitoneal administration of [2-14C]pyruvate.

<table>
<thead>
<tr>
<th>Nutritional state</th>
<th>Dose exerted as [CO₂]-radioactivity (mM)</th>
<th>% of radioactivity in carbon atoms</th>
<th>Total radioactivity (μCi/mmol)</th>
<th>% of total radioactivity in aspartic acid (μCi/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat No.</td>
<td>(μCi)</td>
<td>(%)</td>
<td>(μCi)</td>
<td>(%)</td>
</tr>
<tr>
<td>276 Thiamine-sufficient diet ad libitum</td>
<td>18</td>
<td>6.3</td>
<td>11</td>
<td>72.5</td>
</tr>
<tr>
<td>290 Thiamine-sufficient diet ad libitum</td>
<td>15</td>
<td>11</td>
<td>13</td>
<td>45</td>
</tr>
<tr>
<td>294 Thiamine-sufficient diet ad libitum</td>
<td>10</td>
<td>4</td>
<td>8.5</td>
<td>14</td>
</tr>
<tr>
<td>275 Thiamine-sufficient diet ad libitum</td>
<td>10</td>
<td>4</td>
<td>4.7</td>
<td>11</td>
</tr>
<tr>
<td>291 Thiamine-sufficient diet ad libitum</td>
<td>10</td>
<td>3</td>
<td>5.6</td>
<td>14</td>
</tr>
<tr>
<td>292 Thiamine-sufficient diet ad libitum</td>
<td>10</td>
<td>3</td>
<td>5.6</td>
<td>11</td>
</tr>
<tr>
<td>274 Thiamine-deficient diet ad libitum</td>
<td>10</td>
<td>3</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>288 Thiamine-deficient diet ad libitum</td>
<td>10</td>
<td>4</td>
<td>14.0</td>
<td>8</td>
</tr>
<tr>
<td>281 Thiamine-deficient diet ad libitum</td>
<td>10</td>
<td>4</td>
<td>7.6</td>
<td>9</td>
</tr>
<tr>
<td>305 Thiamine-treated</td>
<td>10</td>
<td>3</td>
<td>5.6</td>
<td>11</td>
</tr>
<tr>
<td>306 Thiamine-treated</td>
<td>10</td>
<td>3</td>
<td>8.0</td>
<td>8</td>
</tr>
</tbody>
</table>

Note: The values represent the distribution of radioactivity in glutamic acid under different nutritional states.
injection makes the isotopic compound more readily available to liver than does an intravenous injection.

If the labeling pattern in the free glutamate of an organ, following administration of [2-¹⁴C] pyruvate or its precursors, is a valid measurement of the proportion of pyruvate which is being oxidatively decarboxylated and if the specific activities of the isolated glutamic acids do not vary greatly (they do not, Tables 1 to 3), the data presented here suggest that none of the treatments grossly inhibited pyruvate decarboxylation in brain and only the pyrithiamine treatment affected its decarboxylation by liver. It is further suggested that, either the biochemical lesion in various forms of thiamine deficiency is not in the pyruvate oxidase system, or that this lesion is in a limited portion of the pyruvate oxidase system of an organ or organs. Thus, we are left with the situation that to date except for rat liver after pyrithiamine treatment (herein reported), there is no convincing evidence that any type of thiamine deficiency causes a gross change in the oxidation of pyruvate by the whole animal or its tissues in vivo despite thiamine deficiency produces a complicated series of biochemical derangements (29).

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REFERENCES


