INFLUENCE OF DIETARY PYRIDOXINE ON GLUTAMIC DECARBOXYLASE ACTIVITY OF BRAIN*

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γ-Aminobutyric acid is present in the free form in large amounts in brain (1-3), where it is formed from glutamic acid by a L-glutamic acid decarboxylase (2, 4, 5). The enzyme requires pyridoxal phosphate as coenzyme (5), which can be synthesized in brain from adenosinetriphosphate and pyridoxal (6). This decarboxylase has a high degree of substrate and coenzyme specificity (6).

The concentration of vitamin $B_6$ was decreased greatly in the tissues of vitamin $B_6$-deficient animals (7) and the activity of the glutamic-aspartic transaminase, a pyridoxal phosphate-requiring system, also was lowered markedly (8, 9). Refeeding of pyridoxine to the deficient animals was found to return the transaminase activity to an almost normal level (9). When a bacterial tyrosine decarboxylase apoenzyme was employed for assay, it was shown that the tissues of rats which received pyridoxine contained several times more codecarboxylase (or pyridoxal phosphate) than did those of animals on a pyridoxine-deficient diet (10). The chief purpose of the present investigation was to determine whether the brain glutamic acid decarboxylase responds to different levels of pyridoxine nutrition in the manner found for transaminase. Additional observations were made on the effects of the feeding of excess pyridoxine to animals on a normal laboratory diet and the administration of desoxypyridoxine, a vitamin $B_6$ antagonist (11), to rats on a pyridoxine-deficient diet.

EXPERIMENTAL

Treatment of Animals—Albino rats of both sexes of the Sprague-Dawley strain were placed on the experimental diets within a week after weaning. The basal ration, essentially that of Halliday and Evans (12), had the following percentage composition: vitamin-free casein (Labco), 30.6; sucrose, 60.3; butter fat, 5.1; Salt Mixture 1 (Merek), 4.0. Each animal received daily 3 drops of corn oil containing 60 U. S. P. units of vitamin A and 15 U. S. P. units of vitamin D. The control animals were given 0.5

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ml. of a solution by stomach tube daily, containing the following amounts of B vitamins: thiamine hydrochloride, 30 γ; riboflavin, 30 γ; niacin, 100 γ; calcium pantothenate, 100 γ; choline chloride, 5 mg.; inositol, 50 γ; p-aminobenzoic acid, 50 γ; pyridoxine hydrochloride, 20 γ. The deficient animals were given the same supplement of B vitamins, with the exception that pyridoxine was omitted. Four animals from the latter group received the complete B vitamin supplement for 12 days, after being kept on the deficient diet for 45 days. One group of animals was fed the vitamin B3-deficient diet and desoxypyridoxine. Assays were also made on brains of rats maintained on Purina laboratory chow, and the results were compared with those obtained from similarly fed animals which were given 1 mg. of pyridoxine hydrochloride by stomach tube daily for 11 days.

The animals were killed by dislocation of the cervical vertebrae and the brains removed rapidly. Homogenates containing 250 mg. of fresh weight of brain per ml. were prepared in ice-cold 0.05 M phosphate buffer, pH 5.9, with a ground glass homogenizer.

Manometric Assay—All determinations were carried out in Warburg flasks in N2 atmosphere at 38° with a final volume of 3.0 ml. The flasks were gassed with purified N2 for 12 minutes during temperature equilibration. Approximately 20 minutes elapsed between the removal of the brains and the beginning of the measurements. The initial pH of the incubation mixtures was between 6.3 and 6.4. The main compartment contained quantities of the enzyme preparations and buffer to make a final volume of 2.0 ml. One side arm contained 0.5 ml. of 0.5 M glutamic acid (pH 7.0 to 7.4), which was tipped in after equilibration. The other side arms contained 0.5 ml. of 1.2 N H2SO4. This was added to stop the reaction and to liberate bound CO2, for which suitable corrections were made. Pyridoxal phosphate (Merck) was added to the main compartments of the flasks when used.

In a previous communication (5) conditions for assay of the glutamic decarboxylase activity of brain acetone powder were described in which the reaction was of zero order and the activity was proportional to the quantity of powder employed. This was achieved in the presence of excess substrate and pyridoxal phosphate. The results in Fig. 1 show that similar results can be attained with homogenates of fresh brain. Assays performed in this manner give an idea of the maximal potentialities of a tissue, with respect to the reaction which the enzyme catalyzes, and may be considered to be a measure of the total quantity of apoenzyme present. However, in the case of a possible coenzyme deficiency the measurement of greatest interest is that which gives an estimate of the actual activity of the tissue without added coenzyme, since it is entirely possible that the quantity of apoenzyme can remain unchanged. In Fig. 2 are shown the
results obtained in the absence of added pyridoxal phosphate with aliquots of the same homogenate that was employed to give the results in Fig. 1. When no coenzyme was added, the rate was considerably slower and showed a steady decline during the experiment. At all time intervals, however, the activity was proportional to the quantity of tissue used. It was desirable to know the reaction velocity at zero time, when inactivation had not yet taken place. Of numerous relations tested, the method for graphical representation of the experimental data which most closely approximated straight lines and which, therefore, gave the most accurate evaluation of the initial rate was found when the reciprocal of the time was plotted against the reciprocal of the CO₂ liberated. The slope of the line was equal to the initial reaction rate, as shown in the formulation employed for calculating the initial rates in the case of the glutamic acid decarboxylase of carrots (13).

In Table I are presented the initial rates calculated from the data shown in Fig. 2 and the maximal velocities obtained in the presence of excess pyridoxal phosphate (Fig. 1). The initial velocities were proportional to the amount of tissue used and were approximately 92 per cent of the maximal velocity. The values for initial velocities must be considered minimal, since some inactivation may have taken place during the homogenization and equilibration. The above results were obtained with mouse brain. Rat brain gave entirely comparable results, with the exception that the initial reaction velocities of the brains of normal rats were approxi-
mately 80 per cent of the maximal velocity when measured under the same conditions.

In all of the assays to be described subsequently determinations were made on homogenates of individual rat brains, corresponding to 500 mg. of fresh brain per flask with and without added pyridoxal phosphate. The values obtained in the presence of pyridoxal phosphate gave an indication of the total quantity of apoenzyme, while those obtained in the absence of added coenzyme allowed an estimate of the relative degree of saturation of apoenzyme with coenzyme.

**Table I**

Comparison of Initial and Maximal Velocities for Different Quantities of Homogenate

Rates expressed as microliters of CO₂ per hour. Initial velocities determined by graphical method from curves obtained with no added pyridoxal phosphate. Maximal velocities obtained in the presence of pyridoxal phosphate (500 γ per flask).

<table>
<thead>
<tr>
<th>Quantity of tissue</th>
<th>Initial velocity</th>
<th>Maximal velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µl. CO₂</td>
<td>µl. CO₂ per 100 mg.</td>
</tr>
<tr>
<td>250</td>
<td>160</td>
<td>64</td>
</tr>
<tr>
<td>375</td>
<td>240</td>
<td>64</td>
</tr>
<tr>
<td>500</td>
<td>324</td>
<td>65</td>
</tr>
</tbody>
</table>

**Results**

The average time-activity curves for the brains of different dietary groups obtained in the absence of added pyridoxal phosphate are shown in Figs. 3 to 5. Initial rates calculated from the average curves agreed well with the means of the initial rates calculated from the individual determinations (see Table II). Since the activity was linear with time when the measurements were made in the presence of excess pyridoxal phosphate, the curves for these determinations are not shown.

The data for the animals fed the stock laboratory diet (Group 1, Table II) showed that there were no significant differences either in the initial rates or the maximal rates in the brains of animals ranging in weight from 49 to 198 gm. No sex differences were observed. The initial rate was approximately 80 per cent of the maximal rate. In the case of the animals fed the experimental diet with the vitamin B₆-containing B vitamin supplement (Group 2), the mean values for the initial and maximal rates were closely similar to those found for the animals fed the chow diet. This demonstrates that the decarboxylase activity was unaffected by keeping the rats for a prolonged period on the complete experimental diet.

When pyridoxine was omitted from the vitamin supplement (Group 3),
there was a highly significant decrease in the initial rate to about 50 per cent of that found in the controls (Table II, Fig. 4). The decrease was no greater after 72 days on the diet than after 49 days. The mean value for the maximal rate in Group 3 was slightly higher than that found either in the control group or in that receiving the stock diet. The initial rate was only approximately 40 per cent of the maximal rate, a value one-half of that found for Groups 1 and 2. This result shows clearly that in pyridoxine deficiency there is a decrease in the degree of saturation of the apoenzyme with coenzyme, but no decrease in the content of apoenzyme.

**Fig. 3**

Decarboxylase activity of brains of rats fed the stock diet and the stock diet supplemented with vitamin B₆ measured in the absence of added pyridoxal phosphate. The numbers in parentheses represent the number of animals employed in each group. The vertical lines show the range of the values at each point.

**Fig. 4**

Influence of pyridoxine deficiency and the refeeding of vitamin B₆ on glutamic decarboxylase activity of brain.

Administration for 12 days of the vitamin B₆-containing B vitamin supplement to rats previously made deficient in pyridoxine for 45 days (Group 4) resulted in a return of the values for the initial rate and the degree of saturation of the apoenzyme to normal levels. It is thus apparent that the coenzyme deficiency is readily reversible. These findings parallel those reported for glutamic-aspartic transaminase (9).

The animals in Group 5 were placed on the pyridoxine-deficient diet for 5 days. From the 6th day on all animals were given 5 mg. of desoxypyridoxine daily by stomach tube. The first two animals of this series were sacrificed 10 days later. The remaining three rats received 7.5 mg. of the antagonist for 6 more days prior to the determination of the decarboxylase activity. The duration of the experiment was shorter than for simple
**Fig. 5.** Influence of desoxypyridoxine on the glutamic decarboxylase activity of brain.

**Table II**

Influence of Dietary Pyridoxine on Glutamic Acid Decarboxylase Activity in Brain

The initial rates were estimated from determinations performed in the absence of added pyridoxal phosphate, while the maximal rates were estimated from determinations made with 500 μg of pyridoxal phosphate added. The average weight of all of the animals in Groups 2 to 5 at the start of the experimental diets was 55 gm. All rates were expressed as microliters of CO₂ per hour per 500 mg. of fresh weight of brain.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of animals</th>
<th>Diet</th>
<th>Weight range</th>
<th>Initial rate (μl. CO₂ per hr.)</th>
<th>Maximal rate (μl. CO₂ per hr.)</th>
<th>(A) (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Chow</td>
<td>49-198</td>
<td>218 (216)*</td>
<td>286</td>
<td>0.77</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Deficient + vitamin B₆, 49-60 days</td>
<td>110-218</td>
<td>222 (217)*</td>
<td>291</td>
<td>0.76</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Deficient, 49-72 days</td>
<td>52-88</td>
<td>119 (112)*</td>
<td>322</td>
<td>0.38</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Deficient, 45 days; refeed vitamin B₆, 12 days</td>
<td>102-156</td>
<td>244 (246)*</td>
<td>294</td>
<td>0.84</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>Deficient + desoxypyridoxine, 10 days†</td>
<td>70-75</td>
<td>264 (252)*</td>
<td>360</td>
<td>0.75</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Deficient + desoxypyridoxine, 16 days‡</td>
<td>73-89</td>
<td>153 (161)*</td>
<td>278</td>
<td>0.55</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>Chow + vitamin B₆, 11 days</td>
<td>167-219</td>
<td>182 (192)*</td>
<td>241</td>
<td>0.76</td>
</tr>
</tbody>
</table>

* Calculated from average curves shown in Figs. 3 to 5.
† Animals on vitamin B₆-deficient diet for 5 days prior to daily administration of 5 mg. of desoxypyridoxine.
‡ Same as first two animals in this group except that 7.5 mg. of desoxypyridoxine were given daily for 6 additional days.
vitamin B₆ deficiency, because symptoms of the deficiency appear sooner in the animals given the antagonist. In the two animals fed the antagonist and killed after 10 days the initial rates and maximal rates were at the upper levels of the normal range, the ratios of the rates showing a normal value. In the brains of the rats fed increased amounts of the antagonist for an additional 6 days there was a reduction of the initial rates to about 60 per cent of the control value, and the ratios of the initial to maximal rates lay between those found for the controls and those for the rats with a simple vitamin B₆ deficiency. The maximal rates were essentially the same as those in the control animals. From these results it would not seem likely that the acceleration of the deficiency symptoms by desoxypyridoxine is related to an increased rate of depletion of the glutamic acid decarboxylase activity of brain. It is interesting in this connection that the contents of vitamin B₆ and pyridoxal phosphate in the livers of rats receiving desoxypyridoxine for 2 weeks while on a vitamin B₆-deficient diet were lower than those of the controls but higher than those of deficient animals receiving no antagonist (11).

The feeding of 1 mg. of pyridoxine hydrochloride daily for 11 days to rats on the chow diet (Group 6) produced small decreases in the mean values for both the initial and maximal rates, the ratio of the two remaining at the normal level. This shows that the administration of the vitamin in more than 50-fold excess over that needed to maintain a normal rate of growth produced no change in the degree of saturation of the apoenzyme as measured and calculated by the present methods. However, measurements made on rat muscle showed that an increased supply of pyridoxine, beyond that required by growth, yielded a higher level of codecarboxylase (10).

DISCUSSION

The results of the present investigation show that the recently discovered glutamic acid decarboxylase of brain, which requires pyridoxal phosphate as a coenzyme, responds to changes in pyridoxine nutrition in the same manner as does glutamic-aspartic transaminase (9). It has been demonstrated clearly in the present study that the maximal potential activity (or apoenzyme content) of the brain is not changed in pyridoxine deficiency, but that the degree of saturation of the enzyme with coenzyme is notably decreased. Refeeding pyridoxine hydrochloride to deficient animals at a level of 20 γ per day quickly restored the activity to normal.

It is not possible at the present time to assign a specific rôle in the complex syndrome of pyridoxine deficiency to the decreased glutamic acid decarboxylase activity in brain. However, since brain possesses a uniquely high content of this enzyme among the various tissues tested, it would appear possible that some of the symptoms indicating involvement of the
central nervous system in vitamin B₆-deficient animals (14–18) might be related to the functioning of this enzyme system. Symptoms of a nervous nature were relieved in nutritionally deficient patients by the feeding of pyridoxine when other B vitamins failed to help (19).

SUMMARY

1. A manometric method was described for the assay of the glutamic acid decarboxylase activity of brain. The values obtained in the presence of excess pyridoxal phosphate gave an estimate of the total quantity of apoenzyme, while those obtained in the absence of added coenzyme allowed an estimate of the relative degree of saturation of apoenzyme with coenzyme.

2. The glutamic acid decarboxylase activity of brains of rats maintained on the experimental diet with a vitamin B₆-containing supplement of the B vitamins for a prolonged period was closely similar to that found in animals fed the stock diet.

3. When pyridoxine was omitted from the vitamin supplement, there was a decrease of approximately 50 per cent in the degree of saturation of the apoenzyme with coenzyme, but the content of apoenzyme was normal. Refeeding pyridoxine to rats previously made deficient resulted in a return of the activity to a normal level.

4. The feeding of excess pyridoxine to animals on the stock diet did not result in an increase in activity.

5. From results obtained with rats which were given desoxypyridoxine, while being fed a deficient diet, it does not seem likely that the acceleration of the deficiency symptoms by the antagonist is related to an increased rate of depletion of the glutamic acid decarboxylase activity of brain.

BIBLIOGRAPHY

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