Resistance of Liver Formiminoglutamic Acid Transferase to Experimental Protein Malnutrition in Weanling Rats

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Protein-malnourished infants subsisting on a maize diet usually excrete in the urine excessive quantities of the intermediate metabolite, formiminoglutamic acid (FIGLU), after histidine loading (1–3). The degradation of histidine to glutamic acid in the liver (Fig. 1) requires adequate amounts of folate and normal functioning of the enzymes catalyzing the degradative steps, i.e., histidase, urocanase, and formimino-1-glutamic acid formiminotransferase (FIGLU transferase) (4–7). Impaired degradation of histidine occurs in folate deficiency in humans (8–9); diminished activity of the liver enzymes resulting in impaired degradation has been postulated (10, 11) but not directly demonstrated. In addition, excessive excretion of FIGLU is well documented in vitamin B12 deficiency (12–15). Thus, the impaired histidine degradation in protein-malnourished infants may theoretically be the result of folate or vitamin B12 deficiency, or both, or of changes in liver enzymes. Determination of the role of these various factors must await the direct measurement of liver-enzyme activities of protein-malnourished infants, a study attended by considerable practical difficulties. Relevant data can, however, be obtained from animal studies. The weanling rat given maize provides a model to study the interrelationship of some of these factors. Such animals become protein depleted and show fatty change in the liver and diminution of both the folate and vitamin B12 stores (16). By supplementing the protein-deficient diet with folic acid or vitamin B12, the relative role of these factors can be assessed. Rao et al. (17) reported diminished histidase and urocanase activity in the livers of protein-depleted rats. The present study has extended these observations to include the enzyme FIGLU transferase and the interrelationships between these enzymes and folate and vitamin B12 deficiency.

MATERIAL AND METHODS

Materials

Triphosphopyridine nucleotide, urocanic acid, formiminoglutamic acid (barium salt), and tetrahydrofolic acid were obtained from Sigma, St. Louis, and pteroylglutamic acid (PGA) from Nutritional Biochemicals Corporation, Cleveland. Reduced glutathione and L-histidine monohydrochloride were products of L. Light and Co., Colnbrook, England. Chemicals used for preparation of the buffers for the enzyme assays were all analytical grade (British Drug Houses, Poole, England: E. Merck, Darmstadt, Germany).

Subjects and Diets

Newborn rats, not inbred, of Wistar stock were weaned at 4 weeks and allocated randomly to four dietary regimes as previously described (16), namely 1) stock diet, 2) maize diet, 3) maize diet supplemented with 1 mg PGA twice
weekly by injection, and 2) maize diet supplemented with 100 μg vitamin B₁₂ weekly by injection. The stock diet was known to be adequate for normal growth and nutrition of the rat. The maize diet contained only trace amounts of vitamin B₁₂ and folate (16). Both diets were given ad libitum for a period of 6 weeks, after which some of the rats were sacrificed. The effect of refeeding stock diet to maize-fed rats was studied in the remaining animals.

Procedure

Rats to be studied were transferred to metabolic cages designed to effect separation between urine and feces and to prevent coprophagy. The 24-hour specimens of urine were collected before and after administration by esophageal intubation of a 20-mg load of L-histidine monohydrochloride. The urine specimens were acidified with HCl and stored at −20°C until assayed.

On the day following administration of the histidine load, rats were anesthetized lightly and the abdominal wall opened. Blood was withdrawn to exsanguination from the bifurcation of the abdominal aorta and the livers then thoroughly perfused in situ with cold phosphate buffer (pH 6.0) to the point where macroscopic amounts of blood were no longer present. The livers were then removed and immediately placed on dry ice. Enzyme assays were carried out within 1–2 hr of removing the perfused livers.

Measurement of Urinary Urocanic Acid and Formimino-glutamic Acid

Urocanic acid (UA) and FIGLU in the urine specimens were measured by the combined enzymatic and spectrophotometric method of Chanarin and Bennett (18). The total excretion of UA and FIGLU was measured first, following which UA only was measured after destruction of FIGLU by autoclaving at alkaline pH. The FIGLU concentration was calculated by subtracting the UA concentration from the total excretion of the two histidine derivatives. Results were expressed as micrograms of UA or FIGLU per milliliter of urine.

Assay of Liver Enzymes

Perfused rat livers were homogenized in 2 vol of cold potassium phosphate buffer (pH 6.0 for histidase and urocanase assay, and pH 7.2 for FIGLU transferase assay) for 1 min. When necessary, livers from individual animals were pooled to provide sufficient material for assay. The homogenates were centrifuged at 0°C at 38,000 rpm for 30 min and the supernatants decanted and filtered. Enzyme assays were carried out on the filtrates. The FIGLU transferase was measured by the method of Tabor (20), one unit of activity being defined as 1 μmole substrate changed per minute. Histidase and urocanase activities were measured by the method of Tabor and Mehler (19). One unit of enzyme was defined as that amount that caused an increase (for histidase activity producing urocanic acid) or decrease (for urocanase activity degrading urocanic acid) in optical density at 277 mμ of 0.001/min at 25°C. Total liver homogenate protein was measured by the Biuret method (21) and specific activities of the enzymes were expressed as milliunits per milligram of liver protein homogenate where 1 unit represented 1 μmole substrate changed per minute.

RESULTS

The mean values cited are from groups of at least 10 animals.

Urinary FIGLU

Rats fed the stock diet for 6 weeks failed to excrete detectable amounts of FIGLU in the urine (Table 1). One-third of the weanling rats tested excreted trace amounts of FIGLU, not exceeding 4 μg/ml. All but one of 12 rats given maize excreted excessive quantities of FIGLU. This FIGLU excretion was prevented by supplementation of the maize diet with vitamin B₁₂ in 11 of 13
Urocanic Acid

Liver Urocanase

The urocanase activity in the livers of rats fed the stock diet for 6 weeks averaged 0.75 mU/mg (Fig. 2). Rats given the maize diet for 6 weeks showed markedly reduced activities (mean, 0.20 mU/mg). When the maize diet was supplemented with PGA or vitamin B₁₂, the fall in liver urocanase activity was less marked but remained severe, ranging from 0.27 to 0.32 mU/mg. Refeeding the maize-fed rats with the stock diet resulted in rapid elimination of the UA excretion after histidine loading (Table 1).

Liver FIGLU Transferase

The FIGLU transferase activity in the livers of rats given maize averaged 62 mU/mg (range, 50–75), which was similar to that of age-matched rats fed the stock diet for 6 weeks (mean, 66 mU/mg; range, 51–76) (Fig. 2).

Urinary Urocanic Acid

Weanling rats fed the stock diet for periods of 6–12 weeks all failed to excrete detectable amounts of UA either before or after histidine loading. Rats given maize did not excrete UA unless administered a histidine load. This UA excretion was prevented by supplementation of the maize diet with either PGA or vitamin B₁₂. Refeeding the maize-fed rats with stock diet resulted in rapid elimination of the UA excretion after histidine loading (Table 1).

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Without Histidine Load</th>
<th>After Histidine Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock diet</td>
<td>Urocanic acid, µg/ml</td>
<td>FIGLU, µg/ml</td>
</tr>
<tr>
<td>Maize</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maize plus PGA</td>
<td>± 6.0</td>
<td>± 37.3</td>
</tr>
<tr>
<td>Maize plus vitamin B₁₂</td>
<td>± 9.1</td>
<td>± 27.2</td>
</tr>
<tr>
<td>Maize plus vitamin B₁₂</td>
<td>± 1.1</td>
<td>± 24.9</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± se of groups of at least 10 animals.
Protein Malnutrition

concentrations. In the rat with deficiency of both folate and vitamin B₁₂, fall in serum vitamin B₁₂ concentration reflects the low liver vitamin B₁₂ stores, but serum *Lactobacillus casei* folate levels are high. The serum folate level falls when the vitamin B₁₂ deficiency is corrected (16). These changes in serum *L. casei* folate concentration in B₁₂-deficient rats have been confirmed by Vitale and Hegsted (22). Supplementation of the maize diet with folic acid maintains the folate stores, but does not prevent the depletion of liver vitamin B₁₂ concentration. Supplementation with only vitamin B₁₂ prevents the depletion of both the folate and vitamin B₁₂ content of the liver.

Weanling rats given maize consistently excreted excessive quantities of FIGLU, even without histidine loading. This defective degradation of FIGLU to glutamic acid was not the result of inadequate liver FIGLU transferase activity, which was similar to that of control rats. However, it has been reported that liver FIGLU transferase activity can be increased by administering vitamin B₁₂ to young male rats given a B₁₂-deficient diet (22).

Other factors known to produce excessive FIGLU excretion are folate deficiency and vitamin B₁₂ deficiency. Administration of PGA to rats given maize prevented FIGLU excretion in some; administration of vitamin B₁₂ had a much more consistent effect in preventing FIGLU excretion. Supplementation with PGA preserves the folate but not the vitamin B₁₂ concentration in the liver of rats given maize; supplementation with vitamin B₁₂ preserves both the vitamin B₁₂ and the folate concentration of the liver (16). The results suggest, therefore, that the excessive FIGLU excretion by rats fed maize is the result of the combined folate and vitamin B₁₂ deficiency produced by this diet, and that adequate liver concentrations of both vitamins are necessary to prevent FIGLU excretion.

The excretion of FIGLU by weanling rats fed a vitamin B₁₂-deficient diet has been
demonstrated by McGeer et al. (21). Following administration of a urocanic acid load, rats fed a $B_{12}$-deficient diet showed an 18-fold increase over normal in FIGLU excretion, while those fed a folate-deficient diet showed a 17-fold increase.

The amount of FIGLU that can be degraded to glutamic acid by the rat is limited. The differences in FIGLU excretion by maize-fed rats compared to stock-fed rats were obscured following the administration of a histidine load as small as 20 mg. Histidine loading also obscured the preventative effect of PGA and vitamin $B_{12}$ supplementation on FIGLU excretion by maize-fed rats. The exhibition of a histidine load to the maize-fed rat obscures the physiologic mechanism of FIGLU degradation; the results underline the caution required in interpretation of the significance of FIGLU excretion following histidine loading in protein-depleted animals.

Rats given maize showed a moderate fall in liver histidase activity with return to normal on refeeding. This fall is unrelated to folate or vitamin $B_{12}$ nutrition, since administration of these vitamins failed both to prevent the fall in activity during protein depletion or to enhance the regeneration of activity following refeeding. These results are in keeping with the observations of Baldridge (23), who reported normal liver histidase activity in rats fed a folate-deficient (but protein-replete) diet, and of Rao et al. (17) who found diminished liver histidase activity in rats fed a maize diet supplemented with PGA. The fall in liver histidase activity in rats given maize is thus related to the effects of protein depletion per se, or to the deficiency of nutritional factors other than folate or vitamin $B_{12}$.

The decrease in liver urocanase activity in rats given maize occurred despite supplementation with PGA or vitamin $B_{12}$. Fall in hepatic urocanase activity was thus the result of protein depletion rather than deficiency of folate (or vitamin $B_{12}$). This observation is compatible with that of Rac et al. (17), who found significantly lower liver urocanase activities in PGA-supplemented maize-fed rats, when compared to control animals.

While liver urocanase activity is dependent on adequate protein nutrition, diminished enzyme levels have also been reported in folate deficiency in the absence of protein depletion (23). In the present study, the fall in urocanase activity in protein-deficient rats was slightly less marked when the maize diet was supplemented with PGA (or vitamin $B_{12}$), and regeneration of activity on refeeding was enhanced by these vitamins. This effect of folate on liver urocanase activity is probably not a direct one, but rather the result of negative feedback inhibition produced by high tissue FIGLU levels in folate deficiency.

Although liver urocanase activity was reduced, rats given maize did not excrete detectable amounts of UA unless administered a histidine load. Supplementation with PGA prevented the excretion of UA after histidine loading, in spite of failure to prevent the fall in hepatic urocanase activity. This suggests that the UA excretion was the result of folate deficiency superimposed on the effect of diminished liver urocanase activity. Vitamin $B_{12}$ supplementation had a similar effect in preventing UA excretion; this may be mediated by the ability of vitamin $B_{12}$ to maintain normal folate stores.

**SUMMARY**

Histidase, urocanase, and formimino-glutamic acid transferase, three enzymes necessary for the degradation of histidine to glutamic acid, have been assayed in the livers of weanling rats given a maize diet. The enzymes showed different sensitivity to the effect of the maize diet. Histidase activity was moderately reduced, urocanase markedly diminished, whereas the specific activity of FIGLU transferase remained...
similar to that of control animals. Enzyme activity rapidly returned to normal following refeeding with stock diet.

Although rats given maize showed normal FIGLU transferase activity in the liver, excessive FIGLU excretion occurred even in the absence of histidine loading. This excessive FIGLU excretion did not occur when the liver concentrations of both folate and vitamin B₁₂ were maintained.

The reduction in histidase and urocanase activities was not related to folate or vitamin B₁₂ deficiency per se. The diminution of urocanase activity was associated with excessive urocanic aciduria only when the pathway was stressed by administration of a histidine load, and this urocanic aciduria could be prevented by PGA supplementation, despite persistently diminished liver urocanase activity.

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REFERENCES


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