Effect of Dietary Amino Acids on Hepatic Drug Metabolism in the Rat

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A great deal of interest has been shown in recent years in establishing the relationship between nutrient deficiencies and hepatic drug metabolism *in vitro*. The effect of protein deficiency on drug metabolism has been reported by various workers (KATO *et al.* 1962, KATO *et al.* 1968, MCLEAN and MCLEAN 1966, WEATHERHOLTZ and WEBB 1971, MGBODILE and CAMPBELL 1972).

The studies described herein were initiated to investigate drug metabolism in livers of rats fed diets containing varying levels of amino acids. Two diets were used: the diet containing an unsupplemented gluten was regarded as low in some amino acids, primarily, lysine, whereas the other diet containing gluten supplemented with five amino acids was considered sufficient in most, if not all, of the essential amino acids for rat growth.

Materials and Methods

Animals and diet. Male, weanling Sprague-Dawley derived rats were divided into two dietary groups. Group 1 was fed for 10 days a diet containing (in percent): wheat gluten, 25; sucrose, 64.8; corn oil, 4; Jones-Foster Salt Mixture, 4; and Vitamin Diet Fortification Mixture, 2.2. Group 2 was pair-fed a similar diet supplemented with the following amino acids (in percent): L-lysine HCl, 1.25; L-histidine HCl, 0.25; DL-methionine, 0.25; DL-threonine, 0.25 and DL-tryptophan, 0.06. The amounts of amino acids used were based on the studies of TAMPHAICHITR and BROQUIST (1973).

Preparation of tissue samples. After 10 days of feeding, the animals were killed by decapitation. The livers were quickly excised and 9000 x *g* supernatant fractions were prepared according to the method described by MGBODILE and CAMPBELL (1972). An aliquot of the 9000 x *g* supernatant was centrifuged at 105,000 x *g* to obtain microsomes. Both the protein of 9000 x *g* supernatant and microsomal protein were assayed by the method of LOWRY *et al.* (1951).

Enzyme assays. The reaction mixtures for ethylmorphine and aniline metabolism consisted of the following components: 0.5 ml of 9000 x *g* liver supernatant (1 g liver/3 ml Tris buffer), 6 µmoles of ethylmorphine HCl or aniline HCl, 30 µmoles of glucose-6-phosphate, 15 µmoles of $MgCl_2$ and sufficient 0.15 M Tris buffer,

pH 7.4, to make a final volume of 3 ml. The mixtures were incubated aerobically with shaking at 37° C in a Dubnoff metabolic shaker for 10 or 15 minutes. The N-demethylation of ethylmorphine was determined by measuring the liberated formaldehyde according to the method of NASH (1953) while the p-hydroxylation of aniline was determined by estimating the amount of p-aminophenol formed (GUARINO *et al.* 1969).

The activity of cytochrome c reductase was determined by the method of OMURA and TAKESUE (1970). One tenth ml of a microsomal suspension (12-15 mg protein/ml) was added to a cuvette containing 3 µmoles of KCN, 0.06 µmoles of oxidized heart cytochrome c, and sufficient 0.15 M Tris buffer, pH 7.4, making a total volume of 3 ml. The mixture was then placed in a single beam spectrophotometer equipped with a Gilford cuvette selector and a Honeywell recorder. After adjusting the wave-length at 550 nm, the recorder was started with a chart speed of 4 inches per minute and then 25 µl of 0.01 M NADPH was added to the cuvette. The reductase activity was calculated from the initial linear phase of the reaction using an extinction coefficient of 21 mM^{-1} cm^{-1} (MASSEY 1959) for the difference in absorbance between reduced and oxidized cytochrome c.

Results and Discussion

The effect of amino acid supplementation on body weight, liver weight, and liver proteins are presented in Table 1. Rats fed the supplemented gluten gained twice as much weight as those fed an equivalent amount of unsupplemented gluten diet. Liver weights were not affected by varying the amino acid composition of the diets. The protein of 9000 x *g* liver supernatants as well as microsomal protein was significantly higher in the group fed the diet supplemented with amino acids.

Table 2 shows the rate of the *in vitro* metabolism of ethylmorphine and aniline. Rats fed unsupplemented gluten had reduced ability to metabolize these 2 substrates. Supplementing gluten with 5 essential amino acids elicited a significant increase in the metabolism of both ethylmorphine and aniline. Thus, the enhancing effect of amino acids on drug metabolism is clearly indicated. The increase in drug metabolism after supplementing the diet with amino acids indicates further that it is nutrient (amino acids) deficiency and not an inhibitor possibly present in gluten that is responsible for the lowered rate of ethylmorphine and aniline metabolism in animals fed unsupplemented gluten diet.

The diminution of the *in vitro* metabolic rates per 100 g body weight may be accounted for by a reduction in microsomal enzyme protein and in part by a decrease in specific activity (activity per mg microsomal protein) of the enzymes. The reduction in specific activity could not be related to the effect of

diet on NADPH-cytochrome c reductase, one of the important components of the hepatic microsomal electron transport system for drug hydroxylations. NADPH-cytochrome c reductase activity (per 100 g body weight) was not lowered by feeding unsupplemented gluten diet (Table 2). On a per mg microsomal protein basis, there was even a significant increase in the reductase activity. Some authors (KATO *et al.* 1968, MGBODILE *et al.* 1973) found that the feeding of protein-deficient diets produced a decrease in the reductase activity whereas ANTHONY (1973) reported no alteration of the enzyme activity in protein-calorie malnourished rats.

TABLE 1

Weight gain, food intake, liver weight and liver proteins of growing rats

Parameter	Dietary Group: Gluten	Dietary Group: Gluten + a.a.[1]	P<0.05[2]
Weight gain, g[3]	19.83 ± 1.47	38.83 ± 2.49	S
Food intake, g/10 days[3]	80.67 ± 3.16	79.19 ± 2.86	NS
Liver weight, g[3]	3.46 ± 0.12	3.61 ± 0.15	NS
Protein of 9000 x *g* supernatant, mg/g[4]	59.3 ± 1.32	75.7 ± 2.68	S
Microsomal protein, mg/g[4]	8.73 ± 0.20	11.21 ± 0.52	S

[1]The amino acids (a.a.) are given in the text.

[2]By Student *t*-test. S is significant while NS is not significant.

[3]Mean ± SE of twelve animals.

[4]Each value (mean ± SE) represents 6 experiments, each with 2 rats whose livers were pooled together for the analysis.

The relative effects of feeding unsupplemented gluten and gluten supplemented with the "limiting" amino acids on cytochrome P-450, cytochrome b_5 and phosphatidylcholine have not been determined. It is possible that the overall changes in drug metabolism seen upon amino acid supplementation of diets could be associated with alteration of these components of the microsomal electron transport system.

Summary

The effect of dietary amino acids on hepatic drug metabolism

TABLE 2

Effect of amino acid supplementation of gluten on EM metabolism, AN metabolism and NADPH-cytochrome c reductase[1]

Parameter	Dietary group: Gluten	Dietary group: Gluten + a.a.[2]	P<0.05[3]
EM metabolism, per 100 g body weight[4]	6.76 ± 0.37	10.92 ± 0.43	S
EM metabolism, mg microsomal protein[5]	154.8 ± 6.6	240.1 ± 21.3	S
AN metabolism,[6] per 100 g body weight	208.3 ± 10.3	244.1 ± 12.6	S
AN metabolism, mg microsomal protein	4.77 ± 0.18	5.30 ± 0.21	S
Cytochrome c reductase, per 100 g body weight[7]	2.95 ± 0.11	2.68 ± 0.14	NS
Cytochrome c reductase, mg microsomal protein[8]	67.82 ± 3.11	57.85 ± 2.12	S

[1]Each value (Mean ± SE) represents six experiments, each with 2 rats whose livers were pooled for the enzyme assay. EM and AN metabolism was determined using 9000 x *g* liver supernatants. Cytochrome c reductase was assayed using microsomes.

[2]The amino acids (a.a.) used are given in the text. This group was pair-fed to the unsupplemented gluten group on an individual basis.

[3]By Student *t*-test. S is significant while NS is not significant.

[4]In μmole/10 min.

[5]In nmole/10 min.

[6]In nmole/15 min.

[7]In μmole cytochrome c reduced/min.

[8]In nmole cytochrome c reduced/min.

in rat liver preparations was studied. Supplementation of gluten with the essential amino acids, such as lysine, histidine, methionine, threonine and tryptophan produced an increase in body weight, protein content of 9000 x *g* liver supernatant, microsomal protein and in the metabolism of ethylmorphine and aniline by 9000 x *g* liver supernatants. Liver weight and

cytochrome c reductase activity in liver microsomes were not increased by feeding the gluten diet supplemented with the above amino acids.

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