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Studies on the Functional Significance of Rat Liver Phenylalanine:Pyruvate Aminotransferase (38313)

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A prominent metabolic anomaly in individuals who are genetically deficient in phenylalanine hydroxylase (phenylketonuria) is excretion in the urine of a large quantity of phenylpyruvic acid. Conversion of phenylalanine to phenylpyruvic acid has long been considered to occur via transamination, but this is not clearly established and, in fact, oxidative deamination (*e.g.*, by an L-amino acid oxidase) has not been ruled out. In the analogous genetic disease of histidine metabolism, histidinemia, blockage of normal histidine degradation results in urinary excretion of a large quantity of imidazolepyruvic acid. Histidine:pyruvate aminotransferase activity has been detected in rat liver (1), and this enzyme has been demonstrated in our laboratory to be induced more than tenfold by glucagon treatment (2). Oral loading of glucagon-treated rats with histidine results in much greater urinary excretion of imidazolepyruvate than it does in untreated animals, indicating that this enzyme is functional in histidine transamination *in vivo* as well as *in vitro* (3). Phenylalanine:pyruvate aminotransferase activity is present in rat liver (1), and is also induced by glucagon (4). This enzyme has been considered important by some investigators (5, 6) in the generation of abnormal metabolites in phenylketonuria. Since rats in which phenylalanine:pyruvate aminotransferase has been induced should excrete increased amounts of phenylpyruvic acid when challenged with a phenylalanine load, we have examined the responses of glucagon-treated rats to phenylalanine administration.

Materials and Methods. Male albino rats of the Holtzman strain, weighing 80–100 g initially, were housed in individual cages in a room maintained at 23° with a 12 hr light—12 hr dark cycle. Food and water were available *ad libitum*. Diets consisted of the following in percentage by weight: vitamin-free casein, 9; corn oil, 5; mineral mixture (7), 5; vitamin mixture (7), 0.5; choline chloride, 0.2; L-methionine, 0.3; autoclaved cornstarch, 40; and glucose monhydrate, 40. All diets were made up as 3% agar gels (7).

Glucagon (Eli Lilly & Co.) was administered by subcutaneous injection, 1.25 mg/kg at 10:00 and 22:00 hr daily for the periods indicated. Control animals were injected with the glucagon diluting solution (Eli Lilly & Co.).

Rats that had been kept without food overnight were administered phenylalanine loads by gastric intubation of a 150 mg/ml suspension. They were then placed in stainless steel metabolism cages and urine was collected in glass containers kept chilled in cracked ice and replaced every 4 hr. Drinking water was available *ad libitum* during the experiments.

Phenylpyruvate was determined by the method of Schepartz (8). Paper radiochromatography of urine samples from rats loaded with ^{14}C-labeled phenylalanine was performed on Whatman #1 paper (6″ × 22″ sheets were slit lengthwise to give 2″ wide sectors). Descending development was for 17 hr in isopropanol: H_2O NH_4OH (8:1:1). After drying, the sectors were cut into 1.0 cm strips, which were counted for radioactivity in 15 ml of the aqueous scintillation fluid of Bruno and Christian (9) to which was added 20 μl of 12 *N* HCl to eliminate phosphorescence of the paper, as recommended by Bosquet and Christian (10). Identification of unknown peaks was made by comparison of their migration with that of authentic samples of amino acids and their derivatives.

At the conclusion of the experiments, animals were killed by decapitation and the excised livers placed in 0.9% NaCl at 4°. Trimmed liver samples were homogenized in 0.25 *M* sucrose and

centrifuged at 105,000*g* for one hour. Resulting supernates were used for enzyme assays, which were performed on the same day. Phenylalanine: pyruvate aminotransferase was assayed by the method of Lin *et al.* (1). Phenylalanine hydroxylase was assayed by the method of Bublitz (11). Ornithine aminotransferase was assayed by the method of Peraino and Pitot (12). Homogenate protein concentration was determined by the procedure of Lowry *et al.* (13).

Results. The specific activity of hepatic phenylalanine: pyruvate aminotransferase in rats treated with glucagon had risen by 8 days to a maximum of eight times the control value (Fig. 1).

Urinary excretion of phenylpyruvic acid by control rats or by those treated with glucagon for 3 or 8 days was determined at 4 hr intervals for 12 hr after they had been force-fed a 450 mg phenylalanine load. The somewhat unexpected result of this experiment was that although phenylalanine:pyruvate aminotransferase activity (expressed as units of activity per 100 *g* body wt) of rats treated with glucagon for 8 days was nearly 12 times that of controls, their urinary phenylpyruvate excretion was less than one-half that of control animals having much lower aminotransferase activity. During the 8–12 hr period, excretion of phenylpyruvate by control animals was more than 8 times that of animals treated with glucagon for 8 days (Table I). Induction of phenylalanine:pyruvate aminotransferase not only failed to elicit greater urinary excretion of phenylpyruvate, but was in fact associated with a significant decrease in urinary excretion of this metabolite (Fig. 2).

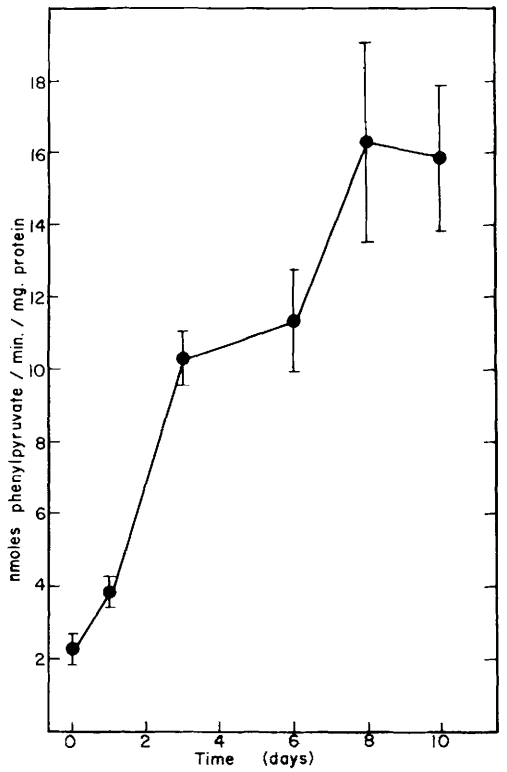

FIG. 1. Kinetics of liver phenylalanine: pyruvate aminotransferase induction by glucagon. Rats were injected with 1.25 mg/kg of glucagon at 12 hr intervals for the periods indicated and killed 12 hr after the last injection. Values given are means of at least three animals ± SEM.

In order to further assess the effect of the glucagon treatment on urinary excretion of phenylalanine metabolites, an experiment similar to the previous one was performed, using carboxyl-labeled L-phenylalanine (0.147 μCi/mmole) for the phenylalanine load, and analyzing for the amount of radioactivity in the excreted metabolites by paper chromatography. As indicated in Fig. 3, the excretion patterns of ^{14}C-labeled metabolites were qualitatively the same for both the glucagon-treated and control groups, only phenylalanine and its transamination products being excreted. However, the group receiving glucagon for 8 days displayed lower urinary excretion of phenylalanine, phenylpyruvate and phenyllactate than the control group.

A conceivable explanation for this observation may be that in addition to inducing phenylalanine:pyruvate aminotransferase, glucagon also induces the enzymes responsible for normal degradation of phenylalanine. To investigate this possibility, livers from rats treated with glucagon for 10 days and from untreated controls were assayed for phenylalanine hydroxylase activity. Hepatic ornithine aminotransferase activity was also measured to determine the effectiveness of the hormonal treatment, since this enzyme is known to be induced by glucagon under these conditions (14). The results presented in Table II indicate that glucagon treatment sufficient to induce ornithine aminotransferase more than twofold did not produce a significant effect on the specific activity of hepatic phenylalanine hydroxylase, although when the activities were expressed per 100 *g* of body wt an apparent increase in enzyme content

TABLE I. Effect of Glucagon on Kinetics of Urinary Phenylpyruvate Excretion.[a]

Collection period (time after loading)	Phenylpyruvate excreted (mg) Control	Glucagon-treated (8 days)
0–4 hr	7.3 ± 2.5[b]	8.3 ± 1.6
4–8 hr	21.9 ± 3.1	8.3 ± 3.1*
8–12 hr	21.2 ± 0.8	2.5 ± 1.5**
Total	50.4 ± 1.5	19.0 ± 5.7***

[a] These are the same animals described in Fig. 2.

[b] Means of four rats ± SEM.

* Significantly different ($P < 0.025$) from corresponding control.

** Significantly different ($P < 0.001$) from corresponding control.

*** Significantly different ($P < 0.005$) from corresponding control.

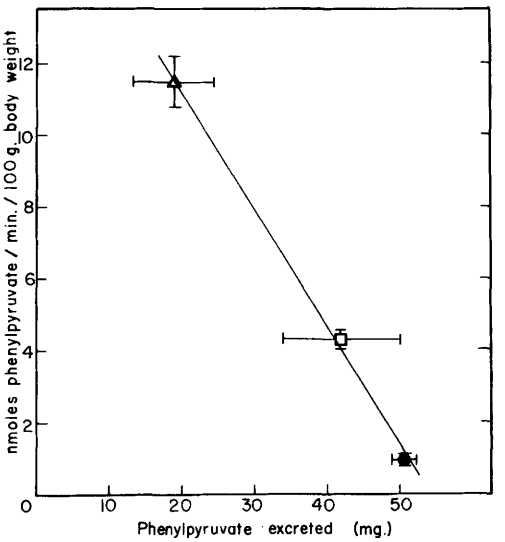

FIG. 2. Relationship between urinary phenylpyruvate excretion and liver phenylalanine-pyruvate aminotransferase activity in glucagon-treated and control rats. Untreated controls (●) and animals receiving glucagon for 3 days (□) or 8 days (△) were loaded orally with 450 mg of L-phenylalanine and urines collected for 12 hr. Values given are means of four animals ± SEM.

was observed, due primarily to the decreased body weight relative to liver weight which results from prolonged glucagon administration.

Discussion. It appears reasonable to conclude from the observations reported here that, in the rat, hepatic phenylalanine:pyruvate aminotransferase does not play a significant role in phenylpyruvic acid formation *in vivo*. Although the capacity for hepatic phenylalanine transamination of rats receiving extended glucagon treatment was theoretically increased through induction of this enzyme, the expected increase in phenylpyruvic acid excretion was not observed (Fig. 2). Fuller *et al.* (15) have made similar observations using a much smaller phenylalanine load and a shorter period of glucagon treatment; these investigators, however, did not observe the inverse relationship between aminotransferase activity and phenylpyruvate excretion reported here. Fellman *et al.* (16) have presented evidence suggesting an extra-hepatic site for aromatic amino acid transamination in phenylketonuria and tyrosinemia.

The possibility has been considered that failure of phenylalanine:pyruvate aminotransferase induction to increase phenylpyruvate production was due to increased catabolism of phenylalanine through conversion to tyrosine. Although the results presented in Table II indicate that phenylalanine hydroxylase is not inducible by glucagon, the capacity of the hormone-treated animals for phenylalanine metabolism might be enhanced to some extent by virtue of

TABLE II. Effect of Glucagon Treatment on Phenylalanine Hydroxylase and Ornithine Aminotransferase Activity.

Treatment	Body weight	Liver weight	Phenylalanine hydroxylase activity[b] units/mg protein	Phenylalanine hydroxylase activity[b] units/100 g weight	Ornithine aminotransferase activity[c] units/mg protein	Ornithine aminotransferase activity[c] units/100 g weight
Control	202 ± 3[a]	8.6 ± 0.2	13.0 ± 1.8	8100 ± 800	2.17 ± 0.01	1380 ± 50
Glucagon (10 days)	186 ± 5[d]	10.6 ± 0.3**	16.8 ± 0.7	13800 ± 800***	4.77 ± 0.33**	3830 ± 170**

[a] Means of five rats ± SEM.

[b] Units expressed as nmoles tyrosine formed/minute.

[c] Units expressed as nmoles pyrroline 5-carboxylic acid formed/minute.

[d] Statistical significance from corresponding controls indicated as in Table I.

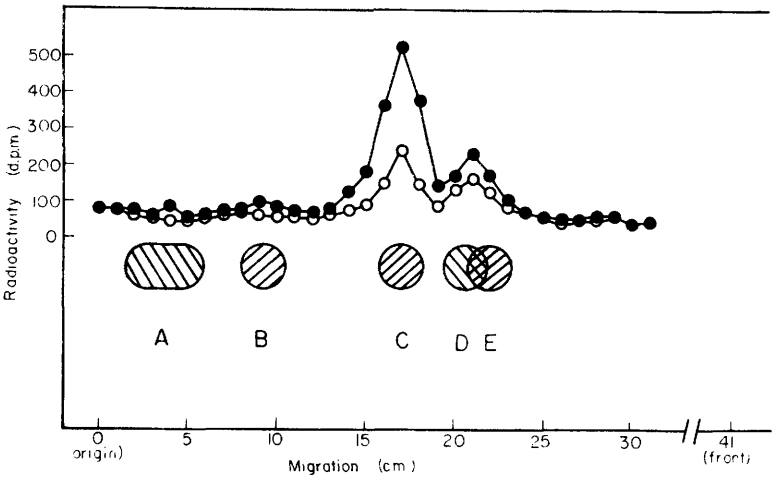

FIG. 3. Paper chromatography of pooled urine collections from 10-day glucagon-treated (O——O) and control (●——●) rats loaded with 750 mg of L-[^{14}C-1] phenylalanine (0.147 μCi/mmole). Urine collection was for 12 hr. Samples were pooled from seven rats in each group. Reference compounds: A, *p*-hydroxyphenylpyruvate; B, tyrosine; C, phenylalanine; D, phenylpyruvate; E, phenyllactate.

their relatively higher hydroxylase activity per unit of body mass. The apparently lower phenylalanine excretion (Fig. 3) also indicates that phenylalanine is disposed of more efficiently by glucagon-treated rats. Other observations (17) indicate that glucagon treatment does promote phenylalanine oxidation *in vivo*. Despite this, increased phenylpyruvate production by the hormone-treated rats might be anticipated, if hepatic phenylalanine amino transferase is important in the conversion of phenylalanine to phenylpyruvate, since Morris *et al.* (3) have demonstrated in similar studies a positive correlation between rat liver histidine: pyruvate aminotransferase activity and urinary imidazolepyruvate excretion under conditions in which the enzymes of normal histidine degradation are induced and the rate of histidine oxidation to CO_2 *in vivo* is greatly increased.

Summary. Glucagon treatment for 10 days induced rat liver phenylalanine:pyruvate aminotransferase approximately eightfold but did not result in increased urinary phenylpyruvate excretion by rats administered a phenylalanine load after they had been injected with an amount of glucagon sufficient to induce the enzyme substantially. In fact, an inverse correlation was observed between phenylpyruvate excretion and aminotransferase activity. Hepatic phenylalanine hydroxylase was not induced in rats treated for 10 days with glucagon. It is suggested that hepatic phenylalanine:pyruvate aminotransferase does not function as such *in vivo*.

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