INCREASED CONVERSION OF A PHENYLALANINE LOAD TO TYROSINE IN TETRAIODOGLUCAGON-TREATED RATS

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SUMMARY

The phenylalanine/tyrosine ratios in plasma and liver of rats given a phenylalanine load were lower in rats pretreated with tetraiodoglucagon than in controls. The effect was due to markedly higher tyrosine levels and slightly lower phenylalanine levels in the tetraiodoglucagon-treated rats. The results indicate that hydroxylation of phenylalanine to tyrosine was increased by tetraiodoglucagon pretreatment.

INTRODUCTION

Brand and Harper (1) recently reported that chronic glucagon treatment increased hepatic dihydropteridine reductase in rats. They also showed that conversion of radioactive phenylalanine to radioactive CO_2 was more rapid in glucagon-treated rats than in controls. Brand and Harper suggested that phenylalanine hydroxylation was increased, despite the lack of change in levels of phenylalanine hydroxylase, because glucagon promoted the regeneration of the phenylalanine hydroxylase cofactor, a tetrahydropteridine (2). We report here the effect of glucagon treatment on phenylalanine and tyrosine levels after the administration of a phenylalanine load to rats. Iodinated glucagon (average of four iodine atoms per glucagon molecule) was used because prior studies have shown that the biological activity of glucagon is increased by iodination (3,4).

MATERIALS AND METHODS

Male albino Wistar rats weighing about 150 g were obtained from a local breeder (Harlan Industries, Cumberland, Indiana). The rats were given subcutaneous injections of saline or of tetraiodoglucagon (1 mg/kg) on the morning of days 1, 3, and 4. On day 5, the rats were given L-phenylalanine (500 mg/kg, i.p.). Groups of rats were killed at 30, 60, or 90 min thereafter. Blood was collected into heparinized tubes and centrifuged; the plasma was stored in a freezer (-15°) prior to analysis. Livers were rapidly excised, frozen on dry ice, and stored at -15°. Phenylalanine and tyrosine concentrations in the plasma or in deproteinized liver extracts were measured spectrofluorometrically (5). The results were calculated as mean values with standard errors for groups of 5 rats, and comparisons between groups were made by the Student *t* test.

The tetraiodoglucagon was prepared in the Lilly Research Laboratories by Dr. William W. Bromer and his associates; it was administered as an aqueous (1 mg/ml) suspension. L-Phenylalanine from Nutritional Biochemicals was suspended at a concentration of 125 mg/ml in an acacia solution for injection.

RESULTS

Phenylalanine concentrations in plasma were not significantly altered by tetraiodoglucagon pretreatment, whereas tyrosine levels were slightly but significantly increased (Table 1). After the injection of the phenylalanine load, phenylalanine levels in plasma rose sharply; tyrosine levels were also markedly increased, indicating that much of the phenylalanine was hydroxylated to tyrosine. The peak in plasma phenylalanine levels at 30 min after the load was significantly reduced in the tetraiodoglucagon-pretreated rats. Tyrosine

levels in the tetraiodoglucagon-pretreated rats were almost twice as high as in controls at 30 min after the phenylalanine load, and were also higher at 60 and 90 min than in controls (the difference was statistically significant only at 90 min). Ratios of phenylalanine/tyrosine concentrations were calculated for each rat, and the group means are shown in Table 1. The

Table 1

Plasma concentration of phenylalanine and tyrosine in control or tetraiodoglucagon-pretreated rats after a load of L-phenylalanine

Minutes after L-phenylalanine load	Control	Tetraiodoglucagon	
	Phenylalanine, μg/ml		
0	16.4 ± 1.0	15.8 ± 0.3	
30	224 ± 11	177 ± 7	**
60	86.9 ± 7.8	68.8 ± 7.1	
90	27.1 ± 1.3	23.8 ± 2.7	
	Tyrosine, μg/ml		
0	22.8 ± 1.1	28.5 ± 1.8	*
30	109 ± 10	208 ± 16	***
60	96.2 ± 4.5	124 ± 18	
90	71.7 ± 5.3	113 ± 3	***
	Phenylalanine/tyrosine ratio		
0	0.72 ± .06	0.56 ± .04	
30	2.13 ± .22	0.87 ± .07	***
60	0.91 ± .12	0.58 ± .07	*
90	0.38 ± .02	0.21 ± .02	***

* P<.05, different from control group
** P<.01
*** P<.001

ratio of phenylalanine/tyrosine in plasma was significantly lower in tetraiodoglucagon-pretreated rats than in controls at all three time intervals after the phenylalanine load.

Table 2

Liver concentration of phenylalanine and tyrosine in control or tetraiodoglucagon-pretreated rats after a load of L-phenylalanine

Minutes after L-phenylalanine load	Control	Tetraiodoglucagon
	Phenylalanine, μg/g	
0	145 ± 5	225 ± 27 *
30	305 ± 10	256 ± 14 *
60	242 ± 9	222 ± 16
90	250 ± 16	217 ± 14
	Tyrosine, μg/g	
0	201 ± 14	274 ± 49
30	327 ± 15	411 ± 20 **
60	247 ± 12	313 ± 22 *
90	230 ± 21	205 ± 12
	Phenylalanine/tyrosine ratio	
0	0.74 ± .05	0.87 ± .08
30	0.94 ± .02	0.63 ± .05***
60	0.99 ± .06	0.73 ± .08*
90	1.11 ± .04	1.07 ± .06

* P<.05, different from control group
** P<.01
*** P<.001

The results of amino acid analyses in liver (Table 2) were generally like those in plasma. Whereas phenylalanine levels were somewhat higher at zero time in tetraiodoglucagon-pretreated rats (for which no reason is apparent), the peak in phenylalanine levels at 30 min after the load was significantly less than in controls. Tyrosine levels at 30 and 60 min after the phenylalanine load were significantly higher in tetraiodoglucagon-treated rats than in controls. The ratio of

phenylalanine/tyrosine in liver was also significantly depressed in tetraiodoglucagon-pretreated rats at 30 and 60 min after the phenylalanine load.

DISCUSSION

These results show that tyrosine levels after a phenylalanine load were considerably higher in tetraiodoglucagon-pretreated rats than in controls, particularly in plasma but also in liver. Phenylalanine levels after the load were affected only slightly by tetraiodoglucagon pretreatment, but the peak attained in plasma and in liver was reduced significantly.

Perhaps the best index of phenylalanine to tyrosine conversion is the ratio of phenylalanine/tyrosine. This ratio in plasma was reduced at 30 min after the phenylalanine load to less than half the control value by tetraiodoglucagon pretreatment and to a smaller but still statistically significant extent at 60 and 90 min. The reduction of the phenylalanine/tyrosine ratio in liver was not as marked but nonetheless was statistically significant in tetraiodoglucagon-pretreated rats at 30 and 60 min after the phenylalanine load.

These findings support the suggestion by Brand and Harper (1) that phenylalanine hydroxylation after a phenylalanine load is elevated by glucagon pretreatment in rats. Their observation that glucagon treatment induces hepatic dihydropteridine reductase presumably provides the explanation for the increased conversion of phenylalanine to tyrosine, since the cofactor for phenylalanine hydroxylase is a tetrahydropteridine (2). Our experiments and those of Brand and Harper (1) suggest that at least after a loading amount of phenylalanine the regeneration of this reduced pteridine cofactor can limit the conversion of phenylalanine to tyrosine.

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