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Increase of Hepatic L-Phenylalanine: Pyruvate Aminotransferase by Glucagon in Rats

Possible Role of Adenosine Cyclic 3′,5′-Monophosphate as a Mediator

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SUMMARY

Glucagon caused an approximately 4-fold increase in hepatic L-phenylalanine:pyruvate aminotransferase activity in rats 24 hr after subcutaneous injection. Tetraiodo, mononitro, and monodeamido derivatives of glucagon also increased enzyme activity, the tetraiodoglucagon being more active than glucagon itself. Theophylline, an inhibitor of cyclic AMP phosphodiesterase, enhanced the action of glucagon in increasing phenylalanine aminotransferase. Higher doses of theophylline and of epinephrine, which, like glucagon, can stimulate adenyl cyclase, also increased the activity of this enzyme in both intact and adrenalectomized rats. N^6, O^2-Dibutyryl cyclic AMP likewise increased the enzyme, and theophylline greatly potentiated its action. The results are consistent with a role for cyclic AMP as a mediator in the elevation of hepatic phenylalanine aminotransferase. This enzyme was shown to be distinct from alanine aminotransferase, in that the former enzyme could be elevated by glucagon or dibutyryl cyclic AMP or partially inactivated by freezing, independently of alanine aminotransferase, whereas alanine aminotransferase could be elevated by deprivation of food or inhibited by *p*-chloromercuribenzoate independently of phenylalanine aminotransferase. Liver extracts catalyzed the transamination of phenylalanine with either pyruvate or 2-oxoglutarate as the amino acceptor, but only the phenylalanine:pyruvate transamination was increased by prior 24-hr treatment with glucagon. Phenylalanine aminotransferase activity in heart and kidney was as high as in the liver; brain had lower activity. Glucagon did not affect the enzyme in tissues other than the liver.

INTRODUCTION

Increased hepatic L-phenylalanine:pyruvate aminotransferase in response to glucagon was reported by Civen *et al.* (1) and by Brown and Civen (2). The time course of elevation of this enzyme by glucagon differed markedly from that of another hepatic enzyme, L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5). Whereas the maximum rise in tyrosine aminotransferase occurred after 3 hr, the activity of phenylalanine aminotransferase was highest 24 hr after a single injection of glucagon, and remained elevated for as long as 96 hr. Considerable evidence has accumulated that the induction of tyrosine aminotransferase by glucagon (3, 4) is mediated by increased intracellular cyclic AMP. Other agents besides glucagon that increase intracellular cyclic AMP, such as epinephrine and theophylline, also induce tyrosine aminotransferase in rats (5) and in fetal rat liver in

culture (6). Furthermore, cyclic AMP itself induces the enzyme in cultured liver cells (6, 7) as well as in intact and adrenalectomized rats (8).

No data have been published to indicate whether cyclic AMP may also mediate the effect of glucagon on phenylalanine aminotransferase. Our objective in the studies reported here was to examine further some of the characteristics of the increase in this enzyme caused by glucagon, especially in terms of the structural specificity required in the polypeptide, the specificity of the increase by glucagon of this amino acid transaminase as compared to others, and the possible role of cyclic AMP as a mediator.

MATERIALS AND METHODS

Animals. Male albino rats of the Wistar strain were obtained from Harlan Industries, Cumberland, Ind. They were housed in wire-bottom cages and were maintained on a 12-hr light, 12-hr dark cycle. Purina laboratory chow and water were available ad libitum. The rats weighed about 150 g at the time they were used. They were killed between 8 a.m. and noon by decapitation, and the tissues were immediately removed, frozen on Dry Ice, and stored frozen prior to enzyme assay. Comparative experiments showed that enzyme levels in frozen livers were the same as in fresh livers, despite the fact that once liver extracts are prepared the enzyme loses activity on freezing.

Chemicals. Commercial glucagon and theophylline were obtained from Eli Lilly and Company. Zinc glucagon was used in one experiment. The preparation and characterization of the derivatives and degradation products of glucagon will be described elsewhere. Cyclic AMP was purchased from P-L Biochemicals, and N^6,O^2-dibutyryl cyclic AMP, from Boehringer/Mannheim. L-Epinephrine bitartrate was obtained from Winthrop Laboratories; doses are expressed on the basis of the free base. The doses and routes of administration were based on previous studies (1, 5, 8).

Enzyme assays. Tissue homogenates were centrifuged for 30 min at 30,000 × *g*, and the supernatant fraction was used for enzyme assays. Phenylalanine and tyrosine aminotransferases were measured by the spectrophotometric method of Lin *et al.* (9). The keto acids formed from 0.1 mM L-phenylalanine or L-tyrosine were measured as the yellow enol-borate complexes in 0.57 M borate buffer, pH 8.1. Alanine aminotransferase was measured by the method of Segal *et al.* (10). The pyruvate formed from 0.33 mM L-alanine (in Tris buffer, pH 8.0) was reduced by excess lactate dehydrogenase, and the rate of NADH oxidation in the latter reaction was measured by the change in absorbance at 340 nm. A Gilford model 2000 absorbance recorder was used for the continuous spectrophotometric measurements. All results are expressed as micromoles of product formed per minute per gram (wet weight) of tissue.

RESULTS

Effect of glucagon and related polypeptides on hepatic phenylalanine aminotransferase. The ability of glucagon and some of its derivatives to elevate hepatic phenylalanine aminotransferase is shown in Table 1. We have confirmed the finding of Civen *et al.* (1) that maximum enzyme levels are reached approximately 24 hr after glucagon treat-

TABLE 1

Increase in hepatic phenylalanine aminotransferase after treatment with glucagon and related polypeptides

Glucagon was injected subcutaneously 24 hr before the rats were killed (0.3 mg/kg). All other compounds were injected at an equimolar dose. Results are expressed as means ± standard errors for the numbers of rats per group indicated in parentheses.

Compound injected	Phenylalanine aminotransferase
	μmoles/min/g
0.9% NaCl	0.94 ± 0.04 (12)
Tetraiodoglucagon	5.33 ± 0.41[a] (5)
Mononitroglucagon	3.33 ± 0.33[a] (5)
Glucagon	3.01 ± 0.41[a] (5)
Monodeamidoglucagon	2.36 ± 0.12[a] (12)
1–21 polypeptide	1.11 ± 0.13 (5)
18–29 and 19–29 polypeptide	0.90 ± 0.05 (12)
1–18 polypeptide	0.79 ± 0.05 (5)

[a] $p < 0.001$, different from 0.9% NaCl-treated group.

TABLE 2

Dose-effect comparison of glucagon and its derivatives in elevation of hepatic phenylalanine aminotransferase

All compounds were injected subcutaneously 24 hr before the rats were killed. The doses shown refer to glucagon; other compounds were injected at doses equimolar to those. Results are expressed as means ± standard errors for five rates per group.

Compound injected	Phenylalanine aminotransferase[a]			
	0.03 mg/kg	0.1 mg/kg	0.3 mg/kg	1.0 mg/kg
	μmoles/min/g			
Glucagon	1.33 ± 0.14	1.91 ± 0.22	2.82 ± 0.16	3.55 ± 0.53
Tetraiodoglucagon	2.80 ± 0.25	3.19 ± 0.20	5.47 ± 0.31	7.47 ± 0.57
Mononitroglucagon	1.30 ± 0.06	1.66 ± 0.19	2.43 ± 0.05	2.99 ± 0.23
Monodeamidoglucagon	1.06 ± 0.10	1.41 ± 0.13	1.99 ± 0.21	2.76 ± 0.34

[a] Rats treated with 0.9% NaCl averaged 0.85 ± 0.07, significantly different ($p < 0.05$) from all values in the table except at the low dose of monodeamidoglucagon.

ment; thus effects were measured at that time. Of the derivatives tested, tetraiodo-, mononitro-, and monodeamidoglucagon were like glucagon in that they increased hepatic phenylalanine aminotransferase levels. The smaller polypeptide fragments of the 29-amino acid hormone did not significantly affect the enzyme. These data are in accord with the hyperglycemic glycogenolytic potency of these preparations, since the smaller polypeptide fragments do not retain the hormonal activity of glucagon whereas the iodo, nitro, and deamido derivatives do.[1] A dose-response comparison of the active compounds is shown in Table 2. At all doses tested, tetraiodoglucagon caused significantly higher enzyme activity than did glucagon; in most cases the difference was about 2-fold. The 0.03 mg/kg dose of the tetraiodo derivative was about as effective as the 0.3 mg/kg dose of glucagon, and the 0.1 mg/kg dose of the tetraiodo compound was only slightly less effective than the 1 mg/kg dose of glucagon, indicating that tetraiodoglucagon was nearly 10 times as active as glucagon in increasing phenylalanine aminotransferase. Moreover, increasing the dose of glucagon from 0.3 to 1.0 mg/kg did not lead to a significant increase in enzyme activity in this experiment (or in several others), whereas increasing the dose of tetraiodoglucagon over that range did result in significantly higher enzyme activity. At the 1 mg/kg dose of the tetraiodo compound, enzyme activity was more than 8 times the control level. The mononitro- and monodeamidoglucagon derivatives appeared to be less effective than glucagon in this experiment, although the differences were statistically significant only at the 0.3 mg/kg dose.

[1] W. W. Bromer, manuscript in preparation.

Possible mediation by intracellular cyclic AMP of changes in phenylalanine aminotransferase. Many of the effects of glucagon appear to be mediated by increased intracellular cyclic AMP (11). To see whether the increase in enzyme activity caused by gluca-

TABLE 3

Effect of theophylline on increase in hepatic phenylalanine aminotransferase produced by glucagon

Glucagon was injected subcutaneously at the same time as 0.9% NaCl or theophylline (50 mg/kg) was injected intraperitoneally, 24 hr before the rats were killed. Results are expressed as means ± standard errors for five rats per group.

Dose of glucagon	Phenylalanine aminotransferase	
	0.9% NaCl-treated	Theophylline-treated
mg/kg	*μmoles/min/g*	
0	1.11 ± 0.03	1.41 ± 0.12
0.03	1.90 ± 0.15[a] (+0.79)[b]	3.14 ± 0.22[a] (+1.73)
0.10	2.98 ± 0.33[a] (+1.87)	4.09 ± 0.33[a] (+2.68)
0.30	5.06 ± 0.09[a] (+3.95)	5.68 ± 0.33[a] (+4.27)

[a] $p < 0.01$, different from zero dose of glucagon.

[b] Numbers in parentheses are the differences from the group that received no glucagon.

TABLE 4

Elevation of hepatic phenylalanine aminotransferase by glucagon, epinephrine, and theophylline

All drugs were injected 24 hr before the rats were killed, at the doses shown. Glucagon and epinephrine were injected subcutaneously, and theophylline was injected intraperitoneally. Adrenalectomized rats were used 1 week after adrenalectomy. Results are expressed as means ± standard errors for five rats per group.

Treatment	Dose	Phenylalanine aminotransferase	
		Intact	Adrenalectomized
	mg/kg	*μmoles/min/g*	
Control		1.22 ± 0.06	1.30 ± 0.10
Glucagon	0.3	4.35 ± 0.32[a]	3.55 ± 0.11[a]
Epinephrine	0.2	1.50 ± 0.19	1.35 ± 0.24
	1.0	2.19 ± 0.21[b]	2.46 ± 0.25[b]
	2.0	2.55 ± 0.26[b]	2.46 ± 0.16[a]
Theophylline	25	1.20 ± 0.13	1.11 ± 0.16
	100	4.04 ± 0.82[c]	3.62 ± 0.86[d]
	150	5.04 ± 0.38[a]	

[a] $p < 0.001$, different from control.
[b] $p < 0.005$.
[c] $p < 0.01$.
[d] $p < 0.05$.

gon might be a cyclic AMP-mediated effect, we determined the influence of theophylline on the increased enzyme activity caused by glucagon (Table 3). The dose of theophylline used did not markedly affect enzyme activity. On the other hand, the increase caused by glucagon was much greater in theophylline-treated rats than in controls, as indicated by the parenthetic numbers in the table. Thus there were greater than additive effects of glucagon and theophylline, especially at the lower doses of glucagon. Since theophylline is an inhibitor of cyclic AMP phosphodiesterase and would protect cyclic AMP formed as a result of glucagon stimulation of adenyl cyclase (11), the data are consistent with a role of cyclic AMP in mediating the increased phenylalanine aminotransferase activity occurring after glucagon treatment.

The effects of higher doses of theophylline and of epinephrine, which, like glucagon, can activate hepatic adenyl cyclase (11), are shown in Table 4. Both substances increased hepatic phenylalanine aminotransferase, although epinephrine was not as effective as glucagon at the doses used. The effectiveness of glucagon in adrenalectomized rats was reported earlier by Civen *et al.* (1). Epinephrine and theophylline were also active in adrenalectomized rats. The ability of these agents to increase phenylalanine aminotransferase activity in adrenalectomized rats is different from their effect on another hepatic enzyme, tyrosine aminotransferase. Here glucocorticoids appear to be required in a permissive manner for the induction by epinephrine and for the maximal response to theophylline (5).

Based on the suggestive results that increased intracellular cyclic AMP might mediate the increase in hepatic phenylalanine aminotransferase, we attempted to examine the effects of cyclic AMP on the enzyme. The dibutyryl derivative was used to facilitate accumulation of intracellular cyclic AMP (Table 5). The 50 and 100 mg/kg doses of dibutyryl cyclic AMP alone significantly increased enzyme activity. In theophylline-treated rats, even the lowest dose tested (20 mg/kg) gave a maximal increase in the enzyme activity. The doses of dibutyryl cyclic

TABLE 5

Elevation of hepatic phenylalanine aminotransferase by dibutyryl cyclic AMP, and effect of theophylline

Dibutyryl cyclic AMP was injected along with either 0.9% NaCl or theophylline (50 mg/kg). All injections were made intraperitoneally 24 hr before the rats were killed. Results are expressed as means ± standard errors for five rats per group.

Dose of dibutyryl cyclic AMP	Phenylalanine aminotransferase	
	0.9% NaCl-treated	Theophylline-treated
mg/kg	*μmoles/min/g*	
0	1.34 ± 0.09	1.31 ± 0.16
20	2.80 ± 0.76[a]	6.50 ± 1.44[b]
50	4.78 ± 1.33[a]	7.39 ± 0.52[b]
100	4.72 ± 1.29[a]	5.02 ± 0.82[b]

[a] $p < 0.05$, different from zero dose of dibutyryl cyclic AMP.
[b] $p < 0.01$.

TABLE 6

Lack of effect of insulin on elevation of hepatic phenylalanine aminotransferase by glucagon and dibutyryl cyclic AMP

Glucagon (0.3 mg/kg, subcutaneously), dibutyryl cyclic AMP (50 mg/kg, intraperitoneally), and insulin (10 units/kg, intraperitoneally) were injected as indicated 24 hr before the rats were killed. Results are expressed as means ± standard errors for the numbers of rats per group indicated in parentheses.

Treatment	Phenylalanine aminotransferase	
	Control	Insulin-treated
	μmoles/min/g	
0.9% NaCl	0.98 ± 0.11 (5)	1.14 ± 0.07 (5)
Glucagon	2.79 ± 0.42[a] (5)	3.39 ± 0.31[a] (5)
Dibutyryl cyclic AMP	3.72 ± 0.73[a] (5)	3.78 ± 1.30[a] (3)

[a] $p < 0.001$, different from 0.9% NaCl-treated group.

AMP shown in Table 5 are similar to those reported by Wicks *et al.* (8) to induce hepatic tyrosine aminotransferase in rats.

In view of the report by Wicks (7) that insulin antagonized the induction of another hepatic enzyme, phosphoenolpyruvate carboxykinase, by glucagon, we determined the effect of insulin on the increase in phenylalanine aminotransferase caused by glucagon and dibutyryl cyclic AMP (Table 6). The dose of insulin was that used by Wicks *et al.* (8). Both glucagon and dibutyryl cyclic AMP were as active in insulin-treated rats as in controls.

Cyclic AMP added to hepatic phenylalanine aminotransferase preparations *in vitro* at a concentration of 0.1 mM did not increase enzyme activity.

Specificity of changes in phenylalanine aminotransferase. Because the induction pattern of phenylalanine aminotransferase was similar to that of hepatic glutamate-pyruvate transaminase (L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) when the latter enzyme was induced by hydrocortisone, Civen *et al.* (1) had suggested that the two enzyme activities might be due to the same protein. We therefore compared the activities of these enzymes. Increases in phenylalanine aminotransferase after glucagon or dibutyryl cyclic AMP injection were not accompanied by increases in alanine aminotransferase activity (Table 7). Fasting for 48 hr more than doubled alanine aminotransferase activity without affecting phenylalanine aminotransferase activity. Alanine aminotransferase activity was inhibited by concentrations of *p*-chloromercuribenzoate that had essentially no effect on phenylalanine aminotransferase activity (Fig. 1).

The transamination of phenylalanine with another amino acceptor, 2-oxoglutarate, is also catalyzed in rat liver, but the activity with 2-oxoglutarate is not increased by glucagon treatment, which does increase phenylalanine aminotransferase activity (Table 8). On the other hand, at least part of the phenylalanine:2-oxoglutarate transamination may be catalyzed by tyrosine aminotransferase, an enzyme that is induced by glucagon over a much shorter time span than phenylalanine aminotransferase. Experiment 2 in Table 8 shows that the transamination of both phenylalanine and tyro-

TABLE 7

Independent changes in hepatic phenylalanine and alanine aminotransferases

Glucagon (0.3 mg/kg, subcutaneously) or dibutyryl cyclic AMP (50 mg/kg, intraperitoneally) was injected 24 hr before the rats were killed. Results are expressed as means ± standard errors for five rats per group.

Treatment	Phenylalanine aminotransferase	Alanine aminotransferase
	μmoles/min/g	
Control	1.34 ± 0.09	9.15 ± 0.77
Glucagon	4.23 ± 0.22[a]	8.62 ± 0.94
Dibutyryl cyclic AMP	4.78 ± 1.33[b]	11.12 ± 0.90
Control after freezing	0.36 ± 0.04[a]	8.71 ± 0.63
Control	1.13 ± 0.13	9.22 ± 0.49
48-hr fasting	1.26 ± 0.21	19.94 ± 3.00[c]

[a] $p < 0.001$, different from control.
[b] $p < 0.05$.
[c] $p < 0.01$.

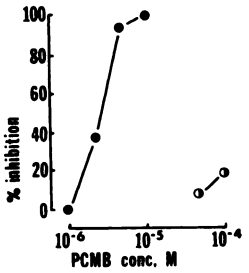

FIG. 1. *Inhibition by p-chloromercuribenzoate (PCMB) of hepatic alanine aminotransferase (●) and phenylalanine aminotransferase (◑) activity in vitro*

sine with 2-oxoglutarate was increased by prior treatment for 3 hr with zinc glucagon.

Table 9 shows the activity of other tissues in catalyzing the phenylalanine:pyruvate transamination reaction before and after glucagon treatment. The heart and kidney contained as much enzyme activity as liver but were not affected by glucagon. Nor was the brain enzyme activity, which was markedly lower than in the other tissues. Thus, in glucagon-treated rats, hepatic phenylalanine aminotransferase activity was greater than in any of the other tissues examined.

DISCUSSION

There is now considerable evidence that the induction of hepatic tyrosine aminotransferase may be mediated by cyclic AMP. *In vivo*, the enzyme is induced not only by agents that increase intracellular cyclic AMP, i.e., glucagon, epinephrine, and theophylline, but also by dibutyryl cyclic AMP (3–8). Mediation of these actions by cyclic AMP is indicated by the induction of tyrosine aminotransferase in fetal liver cells in organ culture by cyclic AMP, dibutyryl cyclic AMP, and agents that activate adenyl cyclase through a *beta*-adrenergic stimulatory effect (7). By analogy, and because a number of other biological effects of glucagon seem to be mediated by cyclic AMP, one might speculate that the effect of glucagon on hepatic phenylalanine aminotransferase involves cyclic AMP as a mediator. Although the results in this paper do not prove that hypothesis, they do lend support to it.

First, those glucagon derivatives we tested that retained hyperglycemic activity also retained the ability to elevate phenylalanine aminotransferase, whereas shorter polypeptides without hyperglycemic activity did not increase this enzyme activity. Inasmuch as cyclic AMP is known to be involved in the hyperglycemic activity of glucagon (11), this finding is consistent with mediation by cyclic AMP in the effect on the enzyme. Second, the ability of theophylline to enhance the elevation of hepatic phenylalanine aminotransferase caused by glucagon is suggestive evidence that the effect is mediated by cyclic AMP, since theophylline inhibits cyclic nucleotide phosphodiesterase. The ability of other agents that elevated intracellular cyclic AMP (epinephrine, higher doses of

TABLE 8

Specificity of increased hepatic transaminase activity after glucagon treatment

Results are as means ± standard errors for five rats per group. Glucagon and zinc glucagon were injected subcutaneously at the doses indicated.

Treatment	Rate of transamination		
	Phenylalanine: pyruvate	Phenylalanine: 2-oxoglutarate	Tyrosine: 2-oxoglutarate
		μmoles/min/g	
Experiment 1			
Control	1.02 ± 0.09	3.16 ± 0.40	
Glucagon, 0.3 mg/kg, 24 hr	3.94 ± 0.28[a]	3.37 ± 0.05	
Experiment 2			
Control		2.70 ± 0.26	0.71 ± 0.12
Zinc glucagon, 2 mg/kg, 3 hr		5.99 ± 0.10[a]	2.54 ± 0.10[a]

[a] $p < 0.001$, different from control group.

TABLE 9

Effect of glucagon on phenylalanine aminotransferase activity in rat liver, heart, kidney, and brain

Glucagon (0.3 mg/kg, subcutaneously) was injected 24 hr before the rats were killed. Results are expressed as means ± standard errors for five rats per group.

Tissue	Phenylalanine aminotransferase	
	Control	Glucagon
	μmoles/min/g	
Liver	0.90 ± 0.02	2.04 ± 0.25[a]
Heart	0.98 ± 0.04	0.95 ± 0.04
Kidney	1.07 ± 0.04	1.00 ± 0.04
Brain	0.25 ± 0.01	0.22 ± 0.01

[a] $p < 0.005$, different from control.

theophylline, and exogenous cyclic AMP) to increase hepatic phenylalanine aminotransferase activity is also consistent with a role of cyclic AMP in mediating the effect of glucagon on that enzyme. On the other hand, one must consider that the effects of epinephrine, theophylline, and possibly even dibutyryl cyclic AMP in rats may be indirect. For instance, might they act by stimulating the release of glucagon from the pancreas? Theophylline has been reported to increase glucagon secretion (12). Leclerq-Meyer *et al.* (13) have recently reported that epinephrine stimulated glucagon secretion, although others (12, 14) have not observed that effect of epinephrine. It has not been shown that *endogenous* glucagon exerts an effect on phenylalanine aminotransferase. Perhaps the pharmacological effect we have produced with glucagon is not physiologically relevant.

Insulin counters the actions of glucagon in some cases, perhaps through an effect on cyclic AMP; indeed, it has been suggested that insulin antagonizes the actions of cyclic AMP itself (7, 15, 16). For example, Wicks (7) has shown that the induction of phosphoenolpyruvate carboxykinase by either glucagon or cyclic AMP was inhibited by insulin. Our findings show that insulin did not antagonize the increase in phenylalanine aminotransferase activity caused by either glucagon or dibutyryl cyclic AMP; in that respect our results resemble those of Ureta *et al.* (17), who found that insulin did not antagonize the action of cyclic AMP in inhibiting glucokinase induction.

The suggestion by Civen *et al* (1) that the same protein might be responsible for the enzyme activities of phenylalanine aminotransferase and glutamate-pyruvate transaminase appears not to be valid. Our data in Table 7 and Fig. 1 show that each enzyme may be manipulated upward or downward independently of the other. That is, the increase in phenylalanine aminotransferase caused by glucagon or dibutyryl cyclic AMP is not accompanied by changes in alanine aminotransferase, nor does the decrease in phenylalanine aminotransferase caused by freezing lead to any alteration in alanine aminotransferase. Conversely, neither the increase in alanine aminotransferase caused by fasting nor the inhibition by *p*-chloromercuribenzoate is associated with effects on phenylalanine aminotransferase. These results constitute evidence that the two represent separate enzymes.

Although rat liver can catalyze the transamination of phenylalanine with 2-oxoglutarate at an even greater rate than with pyruvate, different enzymes appear to be responsible. The activity with 2-oxoglutarate as the amino acceptor was not altered by prior 24-hr treatment with glucagon. In glucagon-treated rats, in contrast to untreated rats, the transamination of phenylalanine with pyruvate proceeded at a faster rate than with 2-oxoglutarate. The transamination of phenylalanine with 2-oxoglutarate is catalyzed by hepatic tyrosine aminotransferase (18). Prior 3-hr treatment with zinc glucagon, which caused a 3–4-fold increase in tyrosine aminotransferase activity, caused a 2–3-fold increase in phenylalanine:2-oxoglutarate transamination. The fact that the magnitude of the increase in the two activities was not identical raises the possibility that phenylalanine:2-oxoglutarate transamination may be catalyzed by more than one enzyme.

Whether or not glucagon, dibutyryl cyclie AMP, epinephrine, and theophylline act directly on liver to increase phenylalanine aminotransferase synthesis, as seems to be the case with tyrosine aminotransferase, needs

to be elucidated. It is doubtful that any of the agents persists for more than a fraction of the 24-hr interval after its injection until enzyme activity is measured. Apparently they affect a system that continues to operate after the agents themselves have been destroyed. We have confirmed the observation of Civen *et al.* (1) that phenylalanine aminotransferase activity remains elevated for several days after a single injection of glucagon, but do not yet have information on the duration of enzyme elevation after treatment with dibutyryl cyclic AMP, epinephrine, or theophylline. Sudilovsky *et al.* (19) recently showed that hepatic cyclic AMP levels returned close to normal values within 4 hr after glucagon had been given at a larger dose than we used. Thus there is a wide temporal dissociation between cyclic AMP concentration and phenylalanine aminotransferase levels.

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