Influence of Turnover Rates on the Responses of Enzymes to Cortisone

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SUMMARY

Cortisone administration over a period of 4 days results in increased enzyme activity of rat liver tryptophan pyrrolase, tyrosine-glutamic transaminase, glutamic-alanine transaminase, and arginase. These enzymes differed greatly in turnover rates: from tyrosine-glutamic transaminase with a half-life of 2.0 hr to arginase with a half-life of 4 days. Tryptophan pyrrolase and tyrosine-glutamic transaminase appear to respond rapidly to cortisone whereas glutamic-alanine transaminase and arginase respond more slowly. Although the time course and magnitude of responses are quite different among the four enzymes, the ratios of rates of synthesis under basal conditions to rates of synthesis in cortisone treated animals are quite similar. Thus the apparent small response of arginase and the slow response of glutamic-alanine transaminase as compared to the rapid and large response of tryptophan pyrrolase and tyrosine-glutamic transaminase are not a reflection of low sensitivity to the effects of cortisone, but of the slower turnover of these enzymes.

INTRODUCTION

A number of studies have demonstrated increases in enzyme levels in animal tissues in response to drug administrations (1), changes in hormonal status (2–6), or changes in diet (7, 8) which indicate that such increases result from *de novo* synthesis of protein. These effects, then, resemble the phenomenon of enzyme induction in bacteria, which results from an increased rate of enzyme synthesis (9). However, problems of interpreting changes in enzyme levels are far more complex in animal tissues than in bacteria because of the existence of a continual degradation and resynthesis of protein, i.e., turnover, that occurs in animal tissues (7, 10–12) but not in growing bacteria (13–15). One such problem of interpretation concerns the question whether an observed enzyme "induction" in an animal tissue results from an increased rate of enzyme synthesis, a decreased rate of enzyme degradation, or both. Thus we have recently demonstrated that the increase of rat liver tryptophan pyrrolase produced by hydrocortisone administration results from an increased rate of enzyme synthesis, whereas that produced by the substrate, L-tryptophan, results from a decreased enzyme degradation (6).

In this paper the problem of specificity of response of an enzyme to an increased rate of synthesis will be discussed. That is, does the agent affect one enzyme, or many enzymes? This problem is complicated by the marked heterogeneity of turnover rates of different enzymes in the same tissue. Thus an enzyme with a rapid rate of turnover will respond rapidly, whereas an enzyme with a slow rate of turnover will respond slowly, to the same increased rate of enzyme synthesis. This problem will be

discussed first as a theoretical formulation, and then this formulation will be applied to the question of the specificity of the cortisone-induced increase in synthesis of certain rat liver enzymes.

Cortisone administration increases the levels of a number of rat liver enzymes. Tryptophan pyrrolase (16) and tyrosine-glutamic transaminase respond rapidly (17) (within 4 hr) and dramatically (5- to 15-fold), whereas the increase in glutamic-alanine transaminase (3, 18) and arginase (19) are much slower and less extensive. These observations have been interpreted as indicating a specific effect of corticosteroids on the synthesis of tryptophan pyrrolase and tyrosine-glutamic transaminase. We have therefore examined the question of the specificity of the corticosteroid-induced response of these enzymes in view of the marked differences in turnover rates, varying from a half-life of 2–3 hr for tyrosine-glutamic transaminase (see Table 2) and tryptophan pyrrolase (6, 20, 21) to 4–5 days for arginase (7). The results indicate that the rate of synthesis of the four enzymes is increased to the same extent, and that the apparent specificity of corticosteroids for tryptophan pyrrolase and tyrosine-glutamic transaminase is a reflection of the rapid rate of turnover of these enzymes.

THEORETICAL CONSIDERATIONS

In view of the presence of continual synthesis and degradation of liver proteins (6, 7, 10–12), any model that describes changes in the levels of enzymes must consider enzyme degradation as well as enzyme synthesis. The consistent finding in rat liver that enzyme activities decay exponentially to basal levels after removal of an inducing agent (3, 5–7, 17, 20–23) indicates that the simplest expression for the rate of enzyme degradation should be first order. Enzyme synthesis has conformed to zero order kinetics (6, 22). The most simplified model for a change in tissue content of an enzyme involving both enzyme synthesis and degradation is:

$$dP/dt = S - kP \qquad (1)$$

where P is the content of enzyme per unit weight, S the rate constant for synthesis, expressed as enzyme units (or weight of enzyme)/time, and k the first order rate constant for enzyme degradation, expressed as time^{-1}. In the experiments to be described, no appreciable change in total liver mass occurred, and hence no expression is required to account for such a change.

At any time that a steady state for the enzyme level exists, i.e., $dP/dt = 0$, then:

$$P = S/k \qquad (2)$$

It is evident that P is determined by the respective values of S and k. The amount of enzyme (P) can be increased by increasing the rate of synthesis, or by decreasing the rate of degradation. The new steady state enzyme level, P', will be determined by the new ratio, S'/k'.

Of particular concern to this paper is the fact that the time course by which P approaches the new level, S'/k', will be an exponential function of the rate of degradation (k') while only a linear function of the rate of synthesis (S').

Consider the time course of the change of P with time where the rate of synthesis is changed from S to S' and rate constant of degradation is changed from k to k':

$$\frac{dP}{dt} = S' - k'P$$

$$\frac{dP}{(S' - k'P)} = dt$$

$$\ln (S' - k'P) = -k't + c$$

At $t = 0$, $P = P_0$, and c, the constant of integration is:

$$c = \ln (S' - k'P_0)$$

Substituting for c:

$$\ln (S' - k'P) - \ln (S' - k'P_0) = -kt$$

$$\frac{S' - k'P}{S' - k'P_0} = e^{-k't}$$

$$P/P_0 = \frac{S'}{k'P_0} - \left(\frac{S'}{k'P_0} - 1\right)e^{-k't} \qquad (3)$$

This equation represents a general solution of Eq. 1.

Figure 1 presents the time courses of a

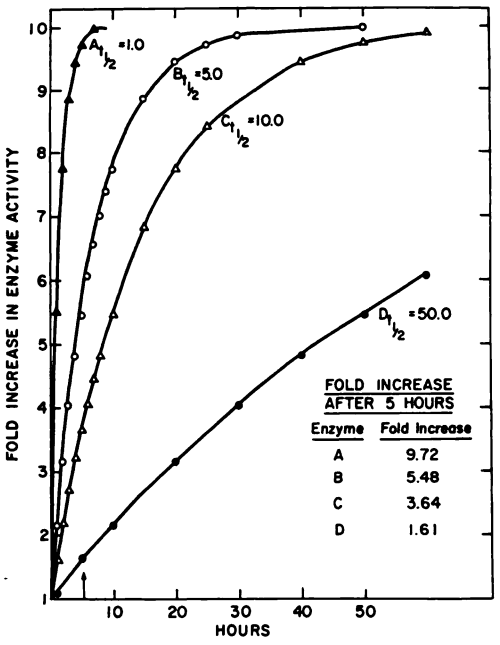

FIG. 1. *The effect of different half-lives on the response of an enzyme to a 10-fold increase in the rate of synthesis*

The arrow is placed at 5 hr; the fold increases at this time shows the wide range of apparent responses to the same increased rate of synthesis.

10-fold increase in rate of enzyme synthesis for four theoretical enzymes which differ only in their rates of degradation (expressed as half-lives).[1] The half-lives range from 1 hr to 50 hr. Each of these curves represents a solution of Eq. 3 in which the rate of synthesis (S) has been increased 10-fold over basal levels ($P_0 = 1$). It is evident that the time course is markedly affected by the value used for k, i.e., the rate constant of enzyme degradation.

Thus in this theoretical example, if enzyme assays were performed at the end of 5 hours, the increase in enzyme A would be 9.72-fold whereas that for enzyme D would be only 1.61 over basal levels. If the assays were performed at 500 hours, this apparent specificity would be lost, since the time courses all converge to the same 10-fold increase in enzyme level.

It is possible to calculate, by using Eq. 3, the time at which the enzyme has undergone one-half its total change in amount:[2]

$$\text{Steady state level at infinite time, } P' = \frac{S'}{k'}$$

$$\text{Initial enzyme level, } P_0, = 1$$

$$\text{One-half of the total change} = \frac{1}{2}\left(\frac{S'}{k'} + 1\right)$$

This expression is substituted for the left-hand side of Eq. 3.

$$\frac{1}{2}\left(\frac{S'}{k'} + 1\right) = \frac{S'}{k'} - \left(\frac{S'}{k'} - 1\right)e^{-k't}$$

$$\frac{1}{2} = e^{-k't}$$

$$t = \frac{\ln 2}{k'}$$

Thus the time taken to increase to one-half of the final increase at the steady state is equal to the half-life ($t_{1/2}$) of the enzyme, as defined by footnote 1. Therefore it is possible experimentally to estimate the half-life of any enzyme by following its time course of increase to a new steady state level.

The theoretical model described above is highly oversimplified. This model is presented only to facilitate an understanding of some of the processes involved in changing enzyme levels in animal tissues. Thus the steady state is an idealized condition that in all probability does not actually exist. Rather it is more likely that all enzymes are constantly responding to changes in hormonal, dietary, and other physiological conditions (26). Multiple factors, largely unknown, are obviously involved in controlling the rates of both enzyme synthesis and enzyme degradation.

[1] A term often used to express the rate of degradation is the half-life ($t_{1/2}$). This is defined by the following relationship:

$$t_{1/2} = \frac{\ln 2}{k}$$

where ln 2 is the natural logarithm of 2, and k is rate constant for degradation. For a more complete discussion of the concept of half-life see Reiner (24) and Tarver (25).

[2] We are indebted to D. B. Goldstein and A. Goldstein for pointing out this interesting relationship.

EXPERIMENTAL PROCEDURE

Treatment of animals. Adrenalectomized male Osborne-Mendel rats each weighing 120–140 g were used in all experiments. Completeness of adrenalectomy was checked both at autopsy and by the failure of animals to survive without oral saline. Animals were maintained on a Purina chow diet with 0.85% saline as drinking water. Cortisone acetate[3] was administered in a dose of 10 mg intramuscularly every 8 hours for a period of 4 days. Less frequent dosage (e.g., 25 mg once per day) resulted in marked fluctuations of enzyme levels of tryptophan pyrrolase and tyrosine-glutamic transaminase.

Assays. Three animals were killed every 12 hr by decapitation; the livers were rapidly removed and stored at −20° until assay. Freezing did not affect the enzyme levels. At the time of assay livers were homogenized in three volumes of 0.15 M KCl containing 0.005 M NaOH. Tryptophan pyrrolase was assayed by the method of Feigelson and Greengard (27). Tyrosine-glutamic transaminase was assayed according to Rosen *et al.* (28). Glutamic-alanine transaminase was assayed by the method of Segal *et al.* (29). Arginase was assayed according to Greenberg (30) as modified by Schimke (31). Control livers were taken from adrenalectomized, uninjected animals. Enzyme activity is expressed in terms of a unit, defined as that amount of enzyme which results in the formation of 1 μmole of product per minute.

RESULTS

Table 1 shows the activities of tryptophan pyrrolase, tyrosine-glutamic transaminase, glutamic-alanine transaminase, and arginase in control animals, and in animals 4 hr after the administration of 10 mg of cortisone acetate. It would appear from these data that glutamic-alanine transaminase and arginase are relatively insensitive to cortisone and that cortisone specifically increases tryptophan pyrrolase and tyrosine-glutamic transaminase activities.

[3] Cortone, Merck, Sharpe & Dohme.

TABLE 1

Activity of liver enzymes after cortisone treatment

The activities of the four enzymes were measured at zero time and 4 hr after an intramuscular injection of 10 mg cortisone acetate. Similar results were obtained after an intraperitoneal injection of 5 mg hydrocortisone 21-phosphate. Each value is the mean of three animals.

Enzyme	Enzyme activity (units/g wet wt liver) 0 hr	4 hr	Fold increase
Tryptophan pyrrolase	0.05	0.48	9.6
Tyrosine-glutamic transaminase	2.2	7.3	3.3
Glutamic-alanine transaminase	4.2	5.8	1.4
Arginase	330	360	1.1

Figure 2 indicates that contrary to the results of Table 1, glutamic-alanine transaminase and arginase do indeed respond to cortisone acetate if the administrations are continued for longer periods of time. Glutamic-alanine transaminase activity is increased only 3-fold over control levels by the end of 1 day of treatment, but is increased to 10-fold by the fourth day. The initial lag in increase in glutamic-alanine transaminase activity during the first 36 hr was not observed in other experiments. Arginase activity responds even more slowly, and increases only 3-fold during the 4-day experimental period. Tryptophan pyrrolase and tyrosine-glutamic transaminase are not plotted for longer than 8 hr because subsequent enzyme levels fluctuate, especially after the first 24 hr of cortisone administration.

Table 2 presents the calculated rates of enzyme synthesis of the four enzymes under basal conditions and as stimulated by the administration of cortisone. The rate of synthesis of each enzyme under basal conditions was estimated from Eq. 2 in which P, the steady state level of enzymes, and k, the rate of degradation were known (k is derived from the half-life by the expression $t_{1/2} = \ln 2/k$). The rate of synthesis of each enzyme during cortisone administra-

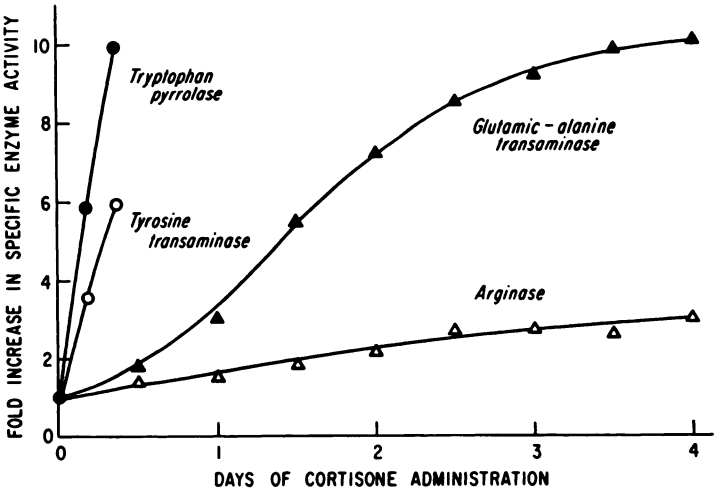

FIG. 2. *Time course of the increase in tryptophan pyrrolase, tyrosine-glutamic transaminase, glutamic-alanine transaminase, and arginase with cortisone administration*

Animals received 10 mg cortisone acetate every 8 hr intramuscularly. Each value is the mean of 3 animals.

tion was estimated from the initial linear portion of the time course of increase in enzyme as obtained experimentally in Fig. 2. Such estimations were based on the assumption that the initial increase in enzyme activity represented primarily the increased rate of synthesis, rather than an effect produced by enzyme degradation. This would appear valid, since the absolute rate of enzyme degradation, i.e., units degraded/time, will be a function of the enzyme concentration, i.e., rate = kP, and hence any effect of enzyme degradation will not be apparent until there has been significant increase in new enzyme.

It is evident from Table 2 that the ratios

TABLE 2

Comparison of rates of enzyme synthesis under basal conditions and during cortisone treatment

Enzyme	Basal		Enzyme synthesized		Ratio cortisone: normal
	Half-life (hr)	Enzyme activity (units[a])	Normal (units[a]/hr)	Cortisone (units[a]/hr)	
Tryptophan pyrrolase	2.5[b]	0.05	0.014	0.056	4.0
Tyrosine-glutamic transaminase	2.0[c]	1.3	0.45	1.9	4.2
Glutamic-alanine transaminase	84[d]	4.2	0.034	0.24	7.0
Arginase	96[e]	33	2.3	9.9	4.3

[a] A unit here refers to specific activity: unit per gram wet liver.

[b] As determined by following a decay curve from high levels induced with cortisone (20); by decay of normal levels after administration of puromycin (21), and by isotope decay under basal conditions (6).

[c] Determined by achieving high levels with two hydrocortisone injections at 0 and 4 hr, and then sacrificing 3 animals every 2 hr during the decay (6–12 hr after second injection). The value thus obtained was 2.0 hr.

[d] As determined by following a decay curve from high levels induced with cortisone (3).

[e] As determined by following specific activity decay of prelabeled arginase (7).

for the rate of enzyme synthesis during cortisone treatment to that under basal conditions were very similar for all four enzymes, varying from 4.0 for tryptophan pyrrolase to 7.0 for glutamic-alanine transaminase. Thus in spite of marked differences in the time course and apparent magnitude of response to cortisone, the extent of stimulation of enzyme synthesis was similar for all four enzymes. Furthermore the validity of the assumptions made in estimating the rates of enzyme synthesis from the time course is substantiated by the similarities of the calculated rates in Table 2 to those obtained by isotopic amino acid incorporation studies indicating that glucocorticoids increase the rate of enzyme synthesis 5- to 6-fold for tryptophan pyrrolase (6), 4- to 5-fold for tyrosine-glutamic transaminase (2),[4] and 4-fold for glutamic-alanine transaminase (3).

DISCUSSION

Glucocorticoids increase the activities of a number of rat liver enzymes to different extents and at different rates (33). In view of the results presented in this paper, it is tempting to suggest that glucocorticoids affect the synthesis of a large number of enzymes to the same extent, and that the differences in extents and rates of increase reflect differing rates of turnover. It is equally evident, however, that some specificity of the effects of corticoids does exist, because the synthesis of all liver proteins is not increased to the same extent: the incorporation of radioactivity into total liver protein is stimulated at most 30–40% by cortisone administration (3, 6, 34), in contrast to 400–500% stimulation of synthesis of certain enzymes.

The mechanism for the effect of glucocorticoid-mediated increase in enzyme synthesis is at present unknown. It would, however, appear that the mechanism is common to a number of enzymes involved in gluconeogenesis (35). At present no positive information is available to indicate at what level this mechanism may be operating. In any case, the finding of a hormone affecting the synthesis of a group of enzymes to the same extent may be considered as operationally similar to the coordinate induction of enzymes that occurs in bacteria (9).

The results presented in this study illustrate the importance of the rate of turnover of an enzyme in determining the time course and magnitude of response to an increased rate of enzyme synthesis such as produced by cortisone administration. The effect of differing rates of turnover can perhaps be most graphically portrayed by the example shown in Fig. 3: Let there be two enzymes, A and B, which are both synthesized at the same rate, i.e., 1 unit/hr/g tissue. If the half-life of A is 0.693 hr, then at the steady state, the level will be 1 unit, i.e., 1 unit of enzyme is synthesized, and 1 unit is degraded, every hour. If the half-life of B is 69.3 hr, then at the steady state the level of B will be 100 units. It is already evident that the total content of an enzyme need not be a reflection of its rate of synthesis. Now if the rate of synthesis of both A and B are increased 10-fold, then at the end of 1 hr A will have increased from 1 unit to 6.7 units, i.e., 6.7-fold. On the other hand, an increase in the rate of synthesis of B by the same factor will raise its level in 1 hr to only 109 units, only a 9% increase. From this example and the experimental results presented it is evident that claims of specificity for a response to an increased rate of synthesis may be totally invalid unless the turnover rate of the specific enzyme in question is also considered.

The significance of differences in turnover rates in affecting the time course of response to an increased rate of enzyme synthesis is not limited to that produced by corticosteroids, but would also be applicable to any physiological condition that causes an increase in the rate of liver protein synthesis. The stimulation of protein synthesis produced by feeding rats a high-

[4] Although not stated explicitly in Kenney's paper (2), this value can be derived from Fig. 1c. Since no more isotope is presumed to be present after the pulse at zero time, the rate of decay of label in the enzyme will reflect the rate of replacement by synthesis from unlabeled amino acids (32). The slope of the curve for induced animals is five times the slope for noninduced animals.

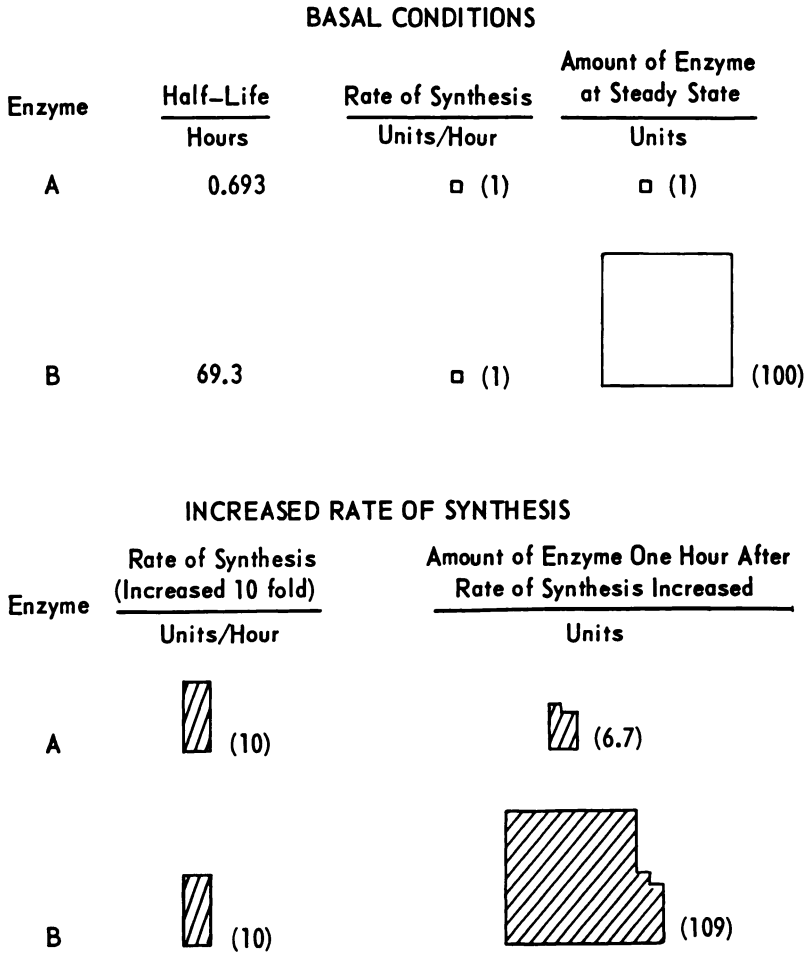

FIG. 3. *Role of rate of turnover in determining the response of two hypothetical enzymes to the same increased rate of synthesis*

protein diet (26, 31) or amino acids after a period of protein depletion (7, 36, 37) results in increases in specific enzymes and total liver protein at different rates and to different extents. As with cortisone treatment, tryptophan pyrrolase and tyrosine-glutamic transaminase respond to increases in dietary protein rapidly, whereas glutamic-alanine transaminase and arginase respond more slowly (C. M. Berlin, unpublished results). Thus the differences in response to such dietary changes may also in part be a reflection of differences in turnover rates.

The marked heterogeneity of turnover rates of total liver protein and of various enzymes is striking. Although the biochemical basis for such heterogeneity is unknown, certain suggestions may be made regarding its physiological importance. Thus it is suggested that enzymes whose levels are rate limiting for a specific biochemical reaction *in vivo* (e.g., tryptophan pyrrolase) will have a rapid rate of turnover. Such enzymes would respond rapidly, both increasing and decreasing, in response to environmental alterations such as changes in diet, substrate levels, or hormonal levels. On the other hand, enzymes that are in constant usage, or whose physiological activity is controlled by feedback inhibition or by the availability of substrate (e.g., arginase),

would not be required to have the ability to fluctuate rapidly, and hence would be those enzymes with slower rates of turnover.

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