MOLECULAR PHARMACOLOGY, 6, 444-447

Kinetics of Induction of Rat Liver Enzymes by Glucocorticoids

JOSEPH L. HAINING

Biochemistry Research Laboratory, Veterans Administration Center, Jackson, Mississippi 39216

(Received February 7, 1970)

SUMMARY

The hypothesis that cortisone increases the synthesis of a group of rat liver enzymes to the same extent, and that their relative rates of accumulation are consequently determined by their respective degradative rate constants, is re-evaluated in terms of the original data and assumptions. The data do not for the most part conform to the changes in enzyme levels predicted by the kinetic model. A fundamental assumption upon which the original conclusions were based is shown to be erroneous; i.e., the initial rate of enzyme accumulation is not a valid estimate of the induced rate of enzyme synthesis. A rearrangement of the equation for enzyme accumulation permits calculation of the induced synthetic rate for any segment of the accumulation curve. Solutions of the equation are consistent with the concept of a lag period in the induction of tryptophan oxygenase (L-tryptophan:oxygen oxidoreductase, EC 1.13.1.12), tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5), and alanine aminotransferase (L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) in adrenalectomized rats. Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) was synthesized at a fairly constant induced rate from the earliest measured point in time, while the synthesis of alanine aminotransferase appeared to rise to a maximum at 1–2 days and then to decline to about half this value at the induced steady-state level. These kinetic analyses provide no support for the original hypothesis, or for the generalization that differences in accumulation rates of cortisone-induced enzymes are simply a function of their normal degradative rate constants.

In 1962, Price and co-workers (1) derived an equation depicting the relationship between synthesis and degradation in controlling the concentration of an intracellular enzyme. The following year, Segal and Kim (2) applied the same equation to a study of the kinetics of induction of rat liver alanine aminotransferase in response to glucocorticoid administration. On the basis of the same theoretical considerations, Schimke *et al.* (3, 4) developed an equation for liver enzyme induction that purported to explain differences in the accumulatory responses of several enzymes to cortisone as being due to differences in their turnover rates. It was concluded by Berlin and Schimke (4) that the synthesis of the several enzymes was increased to the same extent by cortisone. These far-reaching principles have been accepted by at least several groups of workers (5–12) and have been reiterated quite recently (13). However, much of the experimental data offered in support of these concepts actually do not fit the kinetic equation. This has not been pointed out previously.

Figure 1 shows the actual accumulatory responses of several rat liver enzymes to cortisone injection observed by Berlin and Schimke (Fig. 2 of ref. 4) and the corresponding theoretical responses predicted by their Eq. 3 and their kinetic parameters

(Table 2 of ref 4).[1] Tryptophan oxygenase (L-tryptophan:oxygen oxidoreductase, EC 1.13.1.12) actually increased 10-fold during 8 hr of induction, whereas solution of the kinetic equation predicts only a 3.8-fold increase in that time and a total increase of 4-fold at infinite time.[2] Tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) increased 6-fold in 8 hr, while the maximum expected increase from the data of Berlin and Schimke is only about two-thirds of that amount even when the fortuitously high estimate of the induced rate of synthesis (S') is substituted into the equation. Moreover, according to the hypothesis that the time course of change in enzyme level is a function of the magnitude of the degradation rate constant (k') and not a differential stimulation of synthesis by cortisone, tyrosine aminotransferase should accumulate just as rapidly as, if not more so than, tryptophan oxygenase. The data clearly show that tyrosine aminotransferase increases only half as rapidly as tryptophan oxygenase, even though the value of k' is, if anything, greater for the former enzyme. Alanine aminotransferase (L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) actually increased 10-fold in 4 days, but the kinetic equation and the rate constants employed by Berlin and Schimke predict only a 4-fold rise in that length of time. Only the accumulation of arginase (L-arginine amidinohydrolase, EC 3.5.3.1) conformed to the kinetic model to any degree.

Berlin and Schimke (4) assumed that the "initial linear time course of increase in enzyme" during induction is a valid estimate of the induced rate of synthesis. This assumption was supported by similarities of such estimates to values for S' determined by pulse-labeling studies with radioactive amino acids (not, however, during the initial phase of induction). It is inherent in such an assumption that the maximum stimulation of synthesis of all four enzymes occurs during this time interval. It was on the basis of such estimates that these workers concluded that the synthesis of the four enzymes in question was increased to the same extent by cortisone. Careful examination of the hypothetical induction curves of Berlin and Schimke (Fig. 1 of ref. 4) reveals that the initial slope of the accumulation curve is not a good estimate of S' unless the initial time interval is impractically short or k' is exceedingly small. If one estimates S' for their hypothetical enzyme A from the 1-hr level, a value is obtained that is less than two-thirds the value employed to construct the curve. The error in estimating S' from the initial time course decreases with smaller values of k' but increases with time. Thus, for an enzyme such as tryptophan oxygenase that is subject to a coefficient of degradation (k') of 0.3/hr, the accumulation rate measured at the earliest time (4 hr) after increasing the rate of synthesis 4-fold will be only one-half the true value of S'.

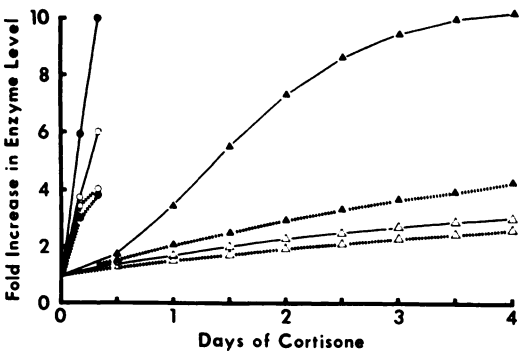

FIG. 1. *Theoretical and actual responses of four liver enzymes to cortisone*

Solid lines represent actual increases in enzyme levels observed by Berlin and Schimke (4); broken lines show responses predicted by their Eq. 3 and the data in their Table 2. The symbols are for tryptophan oxygenase (●), tyrosine aminotransferase (○), alanine aminotransferase (▲), and arginase (△).

[1] In order to obtain an estimate of 1.9 units/hr for the induced rate of synthesis of tyrosine aminotransferase, the enzyme level would have to increase nearly 7-fold in the first 4 hr instead of the less than 4-fold given in Table 1 and Fig. 2 of Berlin and Schimke (4). The data actually yield an estimate of about one-half that given.

[2] Table 1 of Berlin and Schimke (4) shows a 10-fold increase in this enzyme after 4 hr of induction, whereas their Fig. 2 indicates half this increase in 4 hr and a 10-fold elevation only after 8 hr of induction. It is presumed that the former data are in error, since they yield an estimated synthetic rate twice that shown in their Table 2.

TABLE 1

Induced rates of synthesis of four enzymes, calculated from accumulation data and kinetic model

The basal enzyme concentrations given in Table 2 of Berlin and Schimke (4) were multiplied by the "fold" increases given in their Fig. 2. These products were substituted into Eq. 1 (see the text), along with the respective values for k' ($k' = 0.693$/half-life) from Berlin and Schimke, to calculate the induced rates of synthesis (S_c'). Other column heads represent ratio of induced to normal synthetic rate (S_c'/S) and the induced synthetic rate estimated (4) from the initial slope of the accumulation curves (S_e').

Time interval	S_c'	S_c'/S	S_e'	S_c'	S_c'/S	S_e'
hr	Tryptophan oxygenase			Tyrosine aminotransferase		
0–4	0.115	8.2	0.056	2.07	4.6	0.88[a]
4–8	0.166	11.9		3.04	6.8	
	Alanine aminotransferase			Arginase		
0–12	0.29	8.4	0.24	11.7	5.1	9.9
12–24	0.65	18.6		11.8	5.1	
24–36	0.89	25.4		12.3	5.4	
36–48	0.86	24.6		13.1	5.7	
48–60	0.73	20.9		11.0	4.8	
60–72	0.60	17.1		11.5	5.0	
72–84	0.51	14.6		11.9	5.2	
84–96	0.41	11.7		9.6	4.2	

[a] See Footnote 1.

Solving Eq. 3 of Berlin and Schimke (4) for S' permits calculation of this parameter from the accumulation data and the value of k':

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

where P_0 and P are experimental values for initial and subsequent enzyme concentrations, respectively. Testing Eq. 1 against theory (Fig. 1 of ref. 4) reveals that it is applicable to any segment of the accumulation curve; the smaller of any two enzyme concentrations being taken as P_0, the larger as P, and t as the time interval separating them.[3] Table 1 compares the induced rates of synthesis of the four enzymes determined by use of Eq. 1 with those estimated by Berlin and Schimke. The data for tryptophan oxygenase and tyrosine aminotransferase permitted calculation of S' from Eq. 1 for only two segments of the response curves. In both cases the calculated value for S' was not constant, the 0–4-hr value being less than the 4–8-hr figure. The induced synthesis of alanine aminotransferase exhibited a similar lag, appeared to reach a maximum at 1–2 days, and thereafter seemed to decline. Application of Eq. 1 to the data of Segal and Kim (2) for this enzyme presents the same picture. Arginase appeared to be synthesized at a relatively constant rate from the first point in time onward. Except for this enzyme, the maximum values for S' calculated from the kinetic equation exceeded by a considerable margin the corresponding values estimated by Berlin and Schimke (4).

Several possible causes of these deviations from theory suggest themselves. A lag between the time of hormone administration and the onset of increased synthesis (14, 15) would account for an actual increase in S' with time and would also contribute to the error in estimating this parameter from the initial slope of the accumulation curve. Changes in k' during induction would explain other differences between the actual accumulation curves and those predicted by the kinetic equation, as well as the discrepancies between the solutions of Eq. 1 and the pulse-labeling results. Until recently it was thought that k' for alanine aminotransferase increases during induction with gluco-

[3] While this paper was being considered for publication, a similar equation was published by Russell and Snyder (5).

corticoids (2, 15–17). However, Kim (18) has lately claimed that this is not the case. In view of the different responses of basal and induced levels of tyrosine aminotransferase to puromycin, Grossman and Mavrides (11) reported that k' for this enzyme is increased during induction with hydrocortisone, but Kenney (19) showed that inhibitors of protein synthesis also block degradation of tyrosine aminotransferase in the noninduced state. The alternative possibility, that the value of k' is lowered during the course of induction, perhaps by substrate stabilization resulting from a influx of amino acids, appears to be ruled out by some of these as well as other observations. Schimke *et al.* (3) concluded from kinetic studies that hydrocortisone increases the rate of synthesis of tryptophan oxygenase without altering its rate constant of degradation. Garren and coworkers (20) reached the same conclusion on the basis of enzyme regression rates following administration of puromycin during induction. Segal and Kim (17) stated that there is very good evidence against an increase in the tryptophan concentration in the liver during corticoid induction and that alanine aminotransferase is not "induced" by its substrate. Kenney and Albritton (21) found that tyrosine actually depressed tyrosine aminotransferase when administered to adrenalectomized rats, and that changes in the rate constant of degradation have little or no role in the hydrocortisone-induced increase of this enzyme in adrenalectomized animals (14; see also ref. 22). In any event, in order to obtain from Eq. 1 and the accumulation data the values for S' estimated by Berlin and Schimke, k' for each enzyme would have to be practically zero. In such a case k' would, of course, have essentially no effect on the rate of enzyme accumulation.

REFERENCES

1. V. E. Price, W. R. Sterling, V. A. Tarantola, R. W. Hartley, Jr., and M. Rechcigl, Jr., *J. Biol. Chem.* **237**, 3468 (1962).
2. H. L. Segal and Y. S. Kim, *Proc. Nat. Acad. Sci. U. S. A.* **50**, 912 (1963).
3. R. T. Schimke, E. W. Sweeney and C. M. Berlin, *Biochem. Biophys. Res. Commun.* **15**, 214 (1964).
4. C. M. Berlin and R. T. Schimke, *Mol. Pharmacol.* **1**, 149 (1965).
5. D. H. Russell and S. H. Snyder, *Mol. Pharmacol.* **5**, 253 (1969).
6. M. Rechcigl, Jr., *Enzymol. Acta Biocatal.* **34**, 23 (1968).
7. R. W. Swick, A. K. Rexroth and J. L. Stange, *J. Biol. Chem.* **243**, 3581 (1968).
8. I. M. Arias and A. De Leon, *Mol. Pharmacol.* **3**, 216 (1967).
9. G. Litwack, *Top. Med. Chem.* **1**, 3 (1967).
10. A. L. Tarentino, D. A. Richert and W. W. Westerfeld, *Biochim. Biophys. Acta* **124**, 295 (1966).
11. A. Grossman and C. Mavrides, *J. Biol. Chem.* **242**, 1398 (1967).
12. F. T. Kenney, D. L. Greenman, W. D. Wicks and W. L. Albritton, *Advan. Enzyme Regul.* **3**, 1 (1965).
13. R. Bernhard, *Sci. Res.* 30 (Nov. 10, 1969).
14. F. T. Kenney, *J. Biol. Chem.* **237**, 3495 (1962).
15. Y. S. Kim, *Mol. Pharmacol.* **4**, 168 (1968).
16. H. L. Segal, Y. S. Kim and S. Hopper, *Advan. Enzyme Regul.* **3**, 29 (1965).
17. H. L. Segal and Y. S. Kim, *J. Cell. Comp. Physiol.* **66**, 11 (1965).
18. Y. S. Kim, *Mol. Pharmacol.* **5**, 105 (1969).
19. F. T. Kenney, *Science* **156**, 525 (1967).
20. L. D. Garren, R. R. Howell, G. M. Tomkins and R. M. Crocco, *Proc. Nat. Acad. Sci. U. S. A.* **52**, 1121 (1964).
21. F. T. Kenney and W. L. Albritton, *Proc. Nat. Acad. Sci. U. S. A.* **54**, 1693 (1965).
22. E. C. C. Lin and W. E. Knox, *Biochim. Biophys. Acta* **26**, 85 (1957).