Tyrosine-α-Ketoglutarate Transaminase: Induction by Epinephrine and Adenosine-3′,5′-Cyclic Phosphate

Abstract. *Epinephrine and the N^6-$O^{2'}$-dibutyryl analog of adenosine-3′,5′-cyclic phosphate both are effective inducers of tyrosine-α-ketoglutarate transaminase in explants of fetal rat liver maintained in organ culture. Combinations of these inducers with each other and with hydrocortisone, another inducer, yielded results which suggest that cyclic adenylic acid is an intermediate in the induction by epinephrine and that the mechanism by which it induces is different from that by which hydrocortisone operates.*

Insulin and glucagon bring about an induction of rat liver tyrosine-α-ketoglutarate transaminase [L-tyrosine: 2-oxoglutarate aminotransferase (E.C. 2.6.1.5)] in vivo (*1*), in the isolated perfused liver (*2*), and in organ cultures of fetal rat liver (*3*). Induction of this enzyme by hydrocortisone is more extensive than that obtained with the protein hormones and has been demonstrated to operate by a separate mechanism (*1*). Available data support the conclusion that induction by glucagon and insulin occurs by the same mechanism, but this has been difficult to reconcile with the well-known physiological antagonism exhibited by these two hormones. Since glucagon stimulates the formation of adenosine-3′,5′-cyclic phosphate (cyclic AMP) in rat liver (*4*), as does epinephrine (*4*), it was of interest to test the latter two compounds as potential inducers of tyrosine transaminase.

Fetal rat liver maintained in organ culture was used for these studies because this system is not complicated by the secretion of other hormones that occur in vivo (*1*). The viability of fetal liver explants over a 2-day period in culture and details of the preparation of the cultures have been reported (*3*).

Explants of liver from fetal rats at term were first incubated in the absence of inducers for 42 hours at 37°C in Eagle minimum essential medium containing Hanks balanced salt solution, streptomycin, and penicillin in a humidified incubator with a gas phase of 98 percent air and 2 percent CO_2. Occasional adjustment of the *p*H was necessary, and dilute NaOH was used to keep the *p*H at 7.4 ± 0.2.

Various additions were made to the culture medium at the end of this initial incubation period, with Hanks solution used as a vehicle. Five hours later the explants were removed, blotted, and homogenized in 1 ml of 0.15*M* KCl containing 0.001*M* ethylenediaminetetraacetate. Samples of the homogenate were assayed for tyrosine transaminase activity (*5*) and for protein (*6*). Incorporation of ^{3}H-leucine into protein was measured by the filter-paper-disc method (*7*).

Epinephrine at concentrations of $10^{-6}M$ or higher produced a two- to threefold increase in the tyrosine transaminase activity of the explants (Fig. 1A, Table 1). There were smaller effects at 10^{-7} and $10^{-8}M$. The extent of induction with optimum amounts of epinephrine in this system is essentially the same as that observed with insulin and glucagon but less than that seen with hydrocortisone (*3*). As Litwack and Wojciechowski have reported (*8*), the addition of epinephrine at concentrations from 10^{-6} to $10^{-4}M$ to tissue homogenates was without significant effect on transaminase activity assayed 30 minutes later. The effects of epinephrine and hydrocortisone on transaminase activity with optimum concentrations of each hormone were more than additive, an indication that each induces by a separate mechanism.

That both epinephrine and glucagon induce tyrosine transaminase suggests that cyclic AMP should also be an effective inducer. The cyclic nucleotide did in fact lead to three- to fourfold increases in transaminase activity but only with concentrations of 3 m*M* or higher and only in the presence of 1 m*M* theophylline, an inhibitor of the phosphodiesterase that inactivates cyclic AMP (Table 1) (*4*). Theophylline itself led to an increase in transaminase activity suggesting that endogenous cyclic AMP is active as an inducer (Table 1). Because of the very high concentrations of cyclic AMP required to produce a response, the N^6-$O^{2'}$-dibutyryl analog of cyclic AMP was tested as an inducer. It has been suggested that this more lipid-soluble analog penetrates cells more readily and it is more resistant to enzymatic degradation than cyclic AMP (*4*). The dibutyryl analog proved to be an effective inducer of the transaminase at considerably lower concentrations than cyclic AMP (Table 1). At $7 \times 10^{-5}M$ a two- to threefold increase in enzyme activity was achieved with the analog alone.

In the presence of theophylline up to fivefold induction was achieved at $7 \times 10^{-5}M$ dibutyryl cyclic AMP (Fig. 1B, Table 1). In the absence of theophylline concentrations of the analog up to five times higher were required for maximum induction. The optimum concentration of theophylline for potentiating the effects of $7 \times 10^{-5}M$ dibutyryl cyclic AMP was determined to be 1 m*M* in a separate experiment. Consequently, theophylline was added at 1 m*M* in those experiments involving dibutyryl cyclic AMP. The addition of butyrate, a possible contaminant or metabolite of the analog at $10^{-3}M$ had little or no effect on transaminase activity, as was the case with AMP, adenosine diphosphate, or adenosine triphosphate at the same concentrations. The addition of dibutyryl cyclic AMP at $7 \times 10^{-5}M$ to homogenates was without significant

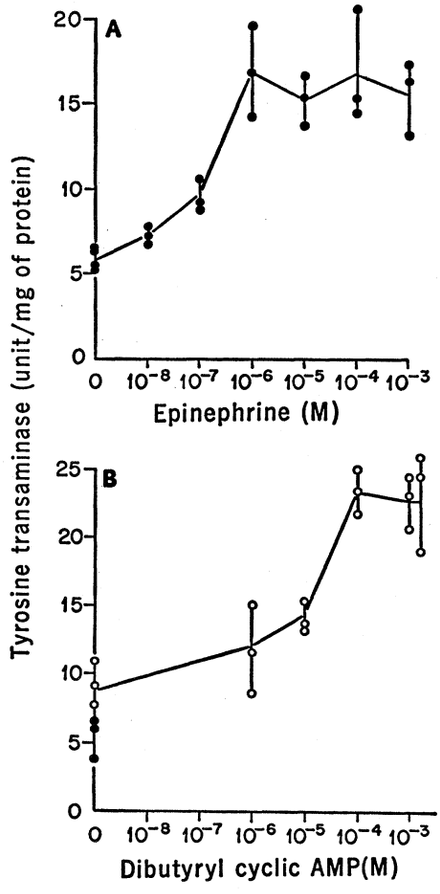

Fig. 1. Concentration dependence of tyrosine transaminase induction by epinephrine and dibutyryl cyclic AMP. Each point represents a single culture dish. In each case, additions were made to 42-hour explants of liver from term fetuses, and 5 hours later the tissues were homogenized and assayed for transaminase activity. (A) Epinephrine; (B) N^6-$O^{2'}$-dibutyryl adenosine-3′,5′-cycle phosphate in the presence of theophylline ($10^{-3}M$); ●, controls without added theophylline.

Table 1. Effects of various additions on tyrosine transaminase activity in organ cultures of fetal rat liver. Data are the mean ± standard error, with the number of observations in parentheses. Five hours after additions were made, explants were homogenized and assays performed.

Addition	Final concentration (M)	Tyrosine transaminase (unit/mg of protein)
None		5.3 ± 0.2 (14)
Epinephrine	5×10^{-5}	15.4 ± .6 (9)
Hydrocortisone	10^{-6}	22.7 ± 1.1 (16)
Epinephrine + hydrocortisone	5×10^{-5} 10^{-6}	45.3 ± 1.9 (3)
Cyclic AMP + theophylline	3×10^{-3} 10^{-3}	19.8 ± 2.1 (2)
Theophylline	10^{-3}	9.2 ± 0.9 (3)
Dibutyryl cyclic AMP	7×10^{-5}	14.2 ± 2.3 (3)
Dibutyryl cyclic AMP + theophylline	7×10^{-5} 10^{-3}	27.5 ± 2.6 (5)
Dibutyryl cyclic AMP + theophylline + hydrocortisone	7×10^{-5} 10^{-3} 10^{-6}	47.7 ± 6.9 (4)
Dibutyryl cyclic AMP + theophylline + epinephrine	7×10^{-5} 10^{-3} 5×10^{-5}	18.9 ± 1.5 (2)
Dichloroisoproterenol	10^{-3}	8.8 ± 0.8 (2)
Dichloroisoproterenol + epinephrine	10^{-3} 5×10^{-5}	7.5 ± .5 (3)
Dichloroisoproterenol + hydrocortisone	10^{-3} 10^{-6}	27.3 ± 1.6 (3)

effect on the activity of tyrosine transaminase.

The degree of induction achieved with theophylline and dibutyryl cyclic AMP together is slightly greater than that elicited by hydrocortisone (Table 1). Optimum concentrations of both dibutyryl cyclic AMP (plus theophylline) and hydrocortisone resulted in a slightly greater than additive increase in the activity of tyrosine transaminase. The effects of epinephrine and the cyclic nucleotide together were not additive, as would be predicted if they operated by the same mechanism. The apparent inhibition of the effect of dibutyryl cyclic AMP by epinephrine is not yet understood.

More evidence that epinephrine exerts its effect on tyrosine transaminase by way of cyclic AMP was provided by studies with dichloroisoproterenol, which completely blocks the effects of epinephrine on adenyl cyclase in liver (*4*). The addition of this inhibitor alone produced a small increase in transaminase activity, but induction by epinephrine was completely blocked (Table 1). In contrast, no effect of this agent was observed on the induction by hydrocortisone. The small elevation in enzyme activity with the blocking agent alone is consistent with the report that it increases the concentration of cyclic AMP slightly (*4*). These results underscore the point once again that hydrocortisone and epinephrine induce the transaminase by different mechanisms.

So far, all hormonal modifications of tyrosine transaminase have involved changes in the rate of enzyme synthesis (*1*, *9*). As an indirect test of this point in the present case, the effect of cycloheximide on induction of the transaminase by epinephrine and dibutyryl cyclic AMP (plus theophylline) was determined (Table 2). Cycloheximide, at a concentration that inhibits protein synthesis by about 90 percent, abolished induction by the hormone and the cyclic nucleotide. These results lend support to the conclusion that enhanced *de novo* synthesis of transaminase protein underlies the action of these agents. There was no effect of epinephrine on general protein synthesis, but a slight increase did occur with dibutyryl cyclic AMP. This effect may have significance in view of a report that this nucleotide stimulates the release of nascent protein from the polysomes of rat liver (*10*).

My results are consistent with the conclusion that epinephrine induces tyrosine transaminase by a mechanism involving cyclic AMP, and that this mechanism is different from that by which hydrocortisone increases the synthesis of this enzyme. Since glucagon also increases the concentration of cyclic AMP in liver, it seems reasonable to suppose that induction by this hormone should proceed via cyclic AMP as an intermediate. However, the induction response to insulin is virtually identical to that with glucagon both in vivo (*1*) and in the isolated perfused liver (*2*), and yet insulin lowers the concentration of cyclic AMP in the liver (*11*). This apparent discrepancy points out the need for further experimentation to resolve the mechanisms operating to control the synthesis of tyrosine transaminase.

Table 2. Effects of cycloheximide on induction of transaminase and ^{3}H-leucine incorporation. Data are the mean ± standard error, with the number of observations in parentheses. Cycloheximide was added together with the inducers to 42-hour explants of fetal rat liver. Four hours later, 2 μc of ^{3}H-leucine (5067 μc/μmole) were added; and 60 minutes later, the explants were homogenized. Theophylline ($10^{-3}M$) was added to those dishes receiving dibutyryl cyclic AMP.

Addition	Final concentration (M)	Tyrosine transaminase (unit/mg of protein)	^{3}H-leucine incorporation (cpm/mg of protein)
None		3.9 ± 0.2 (3)	639 ± 46 (3)
Epinephrine	5×10^{-5}	10.6 ± .3 (2)	647 ± 3 (2)
Dibutyryl cyclic AMP	7×10^{-5}	20.6 ± .2 (2)	744 ± 49 (2)
Cycloheximide	3×10^{-5}	2.8 ± .3 (2)	54 ± 5 (2)
Epinephrine + cycloheximide	5×10^{-5} 3×10^{-5}	2.7 ± .3 (2)	48 ± 8 (2)
Dibutyryl cyclic AMP + cycloheximide	7×10^{-5} 3×10^{-5}	3.7 ± .4 (2)	86 ± 16 (2)

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References and Notes

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