INDUCTION OF RAT LIVER TYROSINE AMINOTRANSFERASE BY DIBUTYRYL CYCLIC AMP AND ITS INHIBITION BY ACTINOMYCIN D AND α-AMANITIN*

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1. Introduction

It has been shown recently that cyclic adenosine 3′,5′-monophosphate is involved in the control of hepatic enzyme activity [1, 2]. The cyclic nucleotide is thought to be the mediator of the induction of tyrosine aminotransferase (TAT) by glucagon [2] and of serine dehydratase by glucagon and amino acids [1], previously shown to be also good inducers of TAT [3]. The enhancement of TAT activity by dibutyryl cyclic AMP (dbcAMP) has been shown to represent *de novo* enzyme synthesis both *in vivo* [4] and in organ culture [2]. It has also been reported recently that dbcAMP stimulates the incorporation of ^{3}H-uridine into RNA in isolated rat liver nuclei [5].

α-Amanitin [6, 7] is a specific inhibitor of extranucleolar RNA polymerase *in vitro* [8, 9]. *In vivo*, the toxin inhibits the incorporation into whole nuclear RNA [10]. Inhibition of the hydrocortisone induction of rat liver TAT has been obtained with α-amanitin and has been used a evidence for a requirement for new RNA synthesis in the induction process [11].

To gain a better understanding of the mechanisms of TAT induction, it seemed of interest to study the effect of α-amanitin and of actinomycin D on the induction by dbcAMP, and to compare the time courses of enzymatic induction by amino acids, hydrocortisone and dbcAMP.

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2. Materials and methods

N^6,$O^{2'}$-Dibutyryl cyclic AMP was purchased from Schwarz BioResearch. Hydrocortisone was obtained from Mann. Reagents used for the enzyme assays were products of Sigma. α-Amanitin was kindly supplied by Pr.Th. Wieland of the Max-Planck Institute, Heidelberg, and actinomycin D was a generous gift of Dr. W. Dorian of Merck, Sharp and Dohme, Ltd., Canada.

Rats adrenalectomized three to five days before use received 0.9% NaCl as drinking water and were fasted 18 hr before the beginning of the experiments. The amino acid mixture was identical to the one previously used [3] and was given through a stomach tube.

At the times indicated, the rats were sacrificed, the liver perfused with cold saline, removed and homogenized in 4 volumes of ice-cold buffer A (0.020 M Tris-HCl, pH 7.5, 0.1 M KCl, 0.01 M $MgCl_2$, 0.005 M mercaptoethanol) for TAT determination, or in 0.02 M sodium phosphate buffer (pH 7.0) containing 0.15 M KCl for tryptophan pyrrolase (TP) determination. Enzyme activities of the 100,000 *g* supernatant were measured and expressed as described [3]. Protein was measured by the method of Lowry et al. [12]. Statistical analysis was performed as described [3].

3. Results

The time course of stimulation of TAT activity by different inducers can give useful information on the mechanisms involved. Fig. 1 shows that significant in-

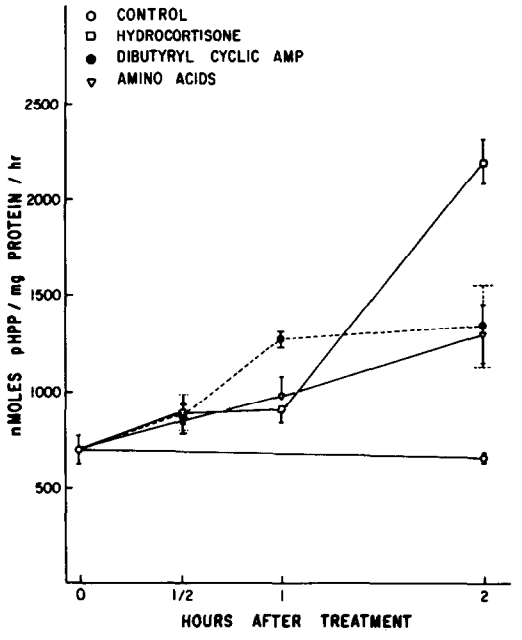

Fig. 1. Time course of induction of TAT activity by hydrocortisone, amino acids and dbcAMP. Adrenalectomized male rats (250 to 300 g) were divided into groups of 4 to 6 animals and received at time zero the following treatments: intraperitoneal (i.p.) injection of hydrocortisone (3 mg/100 g, B.W.) □—□; i.p. injection of dbcAMP (2 mg/100 g, B.W.) ●- - - -●; tube feeding of a mixture of amino acids as described under Materials and methods ▽—▽; and i.p. injection of 0.9% NaCl ○—○. At the indicated times, animals were sacrificed and TAT activity was measured in the 100,000 *g* supernatant. Means and SEM are shown on the graph. pHPP: *p*-hydroxy-phenyl-pyruvate.

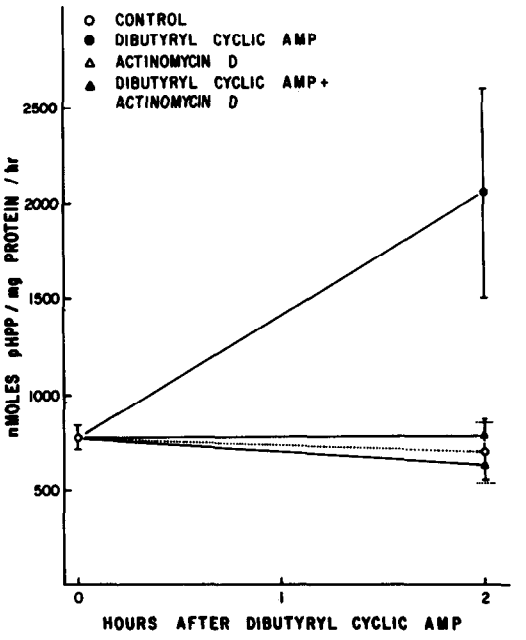

Fig. 2. Effect of actinomycin D on basal and dbcAMP-induced TAT activity. Adrenalectomized male rats (250 to 300 g) were divided into 5 groups of 5 to 7 animals. The animals of one control group were killed at time zero while the others received the following intraperitoneal injections; NaCl 0.9% at time zero - ○—○; dbcAMP (2 mg/100 g, B.W.) at time zero ●—●; actinomycin D (0.1 mg/100 g, B.W.) 30 min before time zero △—△; actinomycin D (0.1 mg/100 g, B.W.) 30 min before the injection of dbcAMP at time zero ▲—▲. Two hours after the last injection, the animals were sacrificed and liver TAT activity was measured in the 100,000 *g* supernatant, as described under Materials and methods. Means and SEM are shown on the graph.

crease of enzymatic activity is first measured 2 hr after hydrocortisone injection as observed previously in rat liver, both *in vivo* [13], and *in vitro* [14]. Feeding a mixture of amino acids leads to a significant ($P < 0.05$) stimulation of TAT activity 1 hr after treatment and a progressive rise of activity during the following hour (fig. 1). A significant ($P < 0.01$) increase of TAT activity is observed 1 hr after injection of dbcAMP and the level remains unchanged throughout the next hour (fig. 1). It should be noticed that the extent of stimulation reached 2 hr after treatment with either amino acids or dbcAMP is about 60% of that obtained after hydrocortisone. We could not detect any stimulation of tryptophan pyrrolase (TP) activity after treatment with dbcAMP. Fig. 2 shows that actinomycin D (0.1 mg/100 g, B.W.) injected 30 min before dbcAMP completely inhibits the marked rise of TAT activity produced by dbcAMP. Such treatment is without any significant effect on the basal level of enzymatic activity, in agreement with previous reports [3, 13]. In fig. 3, it can be seen that α-amanitin (0.1 mg/100 g, B.W.), given 1 hr before the cyclic nucleotide, leads to a complete inhibition of the induction process. TAT activity measured 3 hr after the injection of α-amanitin was depressed ($P < 0.05$) to 62% of the control level.

4. Discussion

The different magnitude and time courses of stimulation of TAT activity in rat liver by hydrocortisone, amino acids and dbcAMP suggest at least two different mechanisms of action, one for hydrocortisone and at least one for dbcAMP and amino acids. It has been suggested that a mixture of amino acids [1] and tryptophan alone [15] stimulate respectively serine dehydratase and TAT activities with cyclic AMP acting as a mediator. We should expect, then, that the action of amino acids and of cyclic AMP would be identical on different hepatic enzymes. However, a mixture of amino acids and tryptophan alone enhance both TAT and TP activities in rat liver [3], while dbcAMP stimulates only TAT activity without affecting the level of TP activity [4]. These data indicate that amino acids and dbcAMP control TAT activity by different mechanisms. Coupled with the evidence already obtained for hydrocortisone, insulin and cyclic AMP [2, 16], amino acids would be a fourth mechanism controlling TAT activity in rat liver.

The inhibition by actinomycin D (fig. 2) of the stimulatory effect of the cyclic nucleotide suggests that RNA synthesis is required for the induction process. A similar inhibitory effect of actinomycin D on the dbcAMP-induced stimulation of TAT activity [2] and on the effect of glucagon [17] which acts through cyclic AMP [18] has been reported. Since actinomycin D has been reported to affect enzyme degradation [19] and to exert other effects besides inhibition of DNA-dependent RNA synthesis [20, 21], the stringent requirement of RNA synthesis for the induction process, already suggested by our results obtained with actinomycin D, is strengthened by our similar findings with α-amanitin, another inhibitor of RNA synthesis [10]. As for the induction by hydrocortisone [11], these data suggest that new RNA synthesis is an essential link in the stimulation of TAT activity by dbcAMP. The absence of any detectable effect of dbcAMP on total RNA synthesis [2] does not exclude the possibility of a specific stimulation by the cyclic nucleotide of a small RNA fraction responsible for the induction process.

A post-transcriptional site of action has been suggested for the effect of dbcAMP on rat liver TAT [16] and adenohypophyseal protein synthesis [22]. Assuming that α-amanitin does not enhance TAT degradation and that TAT messenger RNA (mRNA) is stable under those conditions [13, 19], one would expect an increase of TAT activity after treatment with dbcAMP in the presence of α-amanitin, the stimulation resulting from increased translation of the preformed mRNA. This type of response has been observed with insulin and actinomycin D [23]. However, no important stimulation of TAT activity by dbcAMP in the presence of either α-amanitin or actinomycin D is observed under our experimental conditions.

If α-amanitin does not interfere with TAT degradation, a half-life of approximately 3 hr can be calculated for the enzyme activity in the presence of the toxin alone (fig. 3). This value agrees with previous

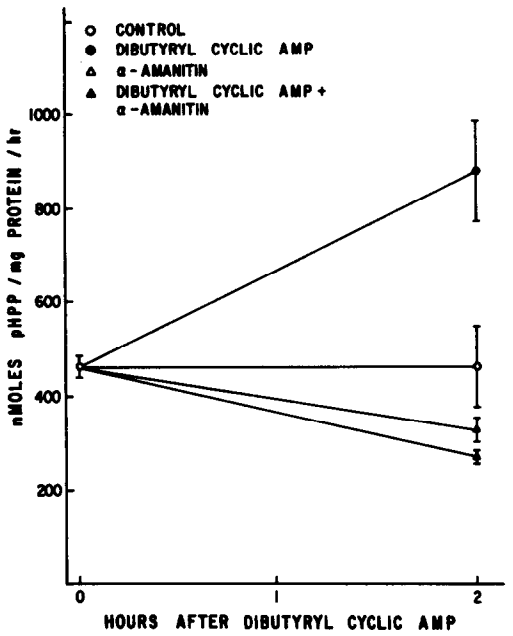

Fig. 3. Effect of α-amanitin on basal and dbcAMP-induced TAT activity. Adrenalectomized male rats (90 to 140 g) were divided into 5 groups of 6 to 7 animals. The animals of one control group were killed at time zero while the others received the following intraperitoneal injections: NaCl 0.9% at time zero ○—○; dbcAMP (2 mg/100 g, B.W.) at time zero ●—●; α-amanitin (0.1 mg/100 g, B.W.) 1 hr before time zero △—△; α-amanitin (0.1 mg/100 g, B.W.) 1 hr before the injection of dbcAMP at time zero ▲—▲. 2 hr after the last injection, the animals were sacrificed and liver TAT activity was measured in the 100.000 *g* supernatant, as described under Materials and methods. Means and SEM are shown on the graph.

measurements [16, 24] although higher [25] and lower [26] values have been reported. Assuming that no side effects of the toxin are involved, the half-life of TAT mRNA would be less than 1 hr. With measurements based on the ability of the tissue to synthesize TAT after treatment with actinomycin D, a value between 2 to 3 hr for the half-life of TAT template has been obtained [19].

If, on one hand, TAT mRNA is stable, if its concentration cannot be enhanced by dbcAMP [16] and if RNA synthesis is required for the induction by cyclic AMP, one must postulate the existence of an unstable "RNA-protein" intermediate playing an essential role in the mediation of the effect of cyclic AMP at the translational level such as proposed for glucocorticoid action [27]. If, on the other hand, the half-life of the TAT template is short, the population of TAT mRNA could be reduced to such a low level in the presence of α-amanitin that a stimulation at the translational level by dbcAMP could not be detected. Alternatively, a direct stimulation of TAT mRNA synthesis would also explain the present findings. Until precise measurements of TAT mRNA synthesis and degradation are made, one must leave open the possibility that cyclic AMP could act at more than one level in the regulation of TAT activity.

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