# A POST-TRANSCRIPTIONAL SITE OF INDUCTION OF TYROSINE AMINOTRANSFERASE BY DEXAMETHASONE IN REUBER H35 HEPATOMA CELLS

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Received 9 February 1981

## 1. Introduction

L-Tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5, TAT) activity can be induced by adenosine 3′:5′-cyclic AMP analogs [1–4] and corticosteroids [2,5,6] in rat liver and several hepatoma cell lines. The mechanism of the induction of TAT by DBcAMP has been extensively investigated [1–4]. Actinomycin D does not inhibit the induction of TAT activity by DBcAMP which indicates a target for DBcAMP at a post-transcriptional level [1,4]. We concluded that DBcAMP induces the TAT synthesis by an increase in the rate of initiation on the TAT mRNA [7]. However, under other experimental conditions evidence has been obtained that the elongation rate of the ribosomes along the TAT mRNA increased after DBcAMP induction [8]. Also, translational experiments in several cell free systems with mRNA isolated from DBcAMP-treated rat liver indicate an increase in the amount of functional TAT mRNA [9,10]. It seems that dependent on the experimental conditions DBcAMP exerts a pleiotropic effect on TAT synthesis at different levels during protein synthesis. Here, we report a hitherto undescribed effect of DBcAMP on TAT synthesis in Reuber H35 hepatoma cells. The induction of TAT synthesis by dexamethasone is completely inhibited by actinomycin D. However, after preinduction with DBcAMP, simultaneous addition of actinomycin D and dexamethasone results in a dexamethasone-dependent increase in TAT synthesis. Apparently, DBcAMP causes a modulation of a regulatory site for TAT synthesis at a post-transcriptional level which is sensitive for dexamethasone in this modulated form.

*Abbreviations:* TAT, L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5), DBcAMP, $N^6$,$O^{2'}$-dibutyryl cyclic adenosine 3′,5′-monophosphate; SDS, sodium dodecylsulphate

## 2. Materials and methods

### 2.1. *Cell culture*

Experiments were done with Reuber H35 cells grown as monolayers in basal Eagle's medium enriched 4-fold with amino acids and vitamins. The medium for growth contained 10% calf serum and 5% fetal calf serum; 20 h before the experiment the medium was replaced by serum-free medium. In the medium leucine was 0.8 mM. The specific activity of L-[$^3$H]leucine during incorporation experiments was 13 Ci/mol.

### 2.2. *Cell harvesting and enzyme determination*

Monolayers were washed twice with ice-cold saline (0.15 M KCl, 1 mM EDTA). After freezing and thawing, the cells were scraped off in 50 mM potassium phosphate buffer (pH 6.5) containing 0.1 mM pyridoxal phosphate, 5 mM 2-oxoglutaric acid, 1 mM EDTA, 0.15 M KCl, 5 mM 2-mercaptoethanol and 0.1% Nonidet P40. Tyrosine aminotransferase activity was determined as in [11]. Protein concentration was determined as in [12].

### 2.3. *Determination of TAT synthesis and degradation*

TAT synthesis was determined by incorporation of [$^3$H]leucine in the enzyme and the TAT degradation by disappearance of [$^3$H]leucine from the enzyme. [$^3$H]Leucine in TAT was determined by direct immunoprecipitation in the cell lysates which were partially purified by incubation at 65°C for 5 min followed by removal of denatured proteins. The TAT-antibody precipitates were dissolved in 2% SDS and 3% dithiothreitol, heated for 30 min at 60°C and analyzed by SDS–polyacrylamide gel electrophoresis performed by a modification of the method in [13]. Radioactivity was eluted from the gel slices overnight at 37°C in a mixture of Lumasolve–$H_2O$–Lipoluma (1:0.2:9).

2.4. *Specificity of the TAT antiserum*

The specificity of the antiserum has been demonstrated by SDS–polyacrylamide gel electrophoresis wherein a single peak of radioactivity of $M_r$ 50 000–55 000 is seen with first precipitates of labeled lysates and only scattered low radioactivity with the second. In addition, only a single precipitation arc is observed with two-dimensional cross-electrophoresis.

2.5. *Materials*

DBcAMP and actinomycin D were purchased from Boehringer (Mannheim), dexamethasone was purchased from Sigma and L-[$^3$H]leucine (126 Ci/mmol) was purchased from the Radiochemical Centre (Amersham).

## 3. Results

TAT activity in Reuber H35 cells is increased after treatment with dexamethasone or DBcAMP (fig.1). The induction of TAT by dexamethasone is completely inhibited by actinomycin D (fig.1B). Actinomycin D does not block the early response to DBcAMP, although over time, inhibition of increasing intensity ensues (fig.1A). The induction capacity in the presence of actinomycin D is decreased as a consequence of the short half-life of TAT and TAT mRNA [14,15].

When the same experiment is carried out with cell cultures which are preincubated with DBcAMP for 1 h, an induction of TAT activity by dexamethasone in the presence of actinomycin D occurs (fig.2A). The kinetics of this induction do not show the usual lagtime of ~1.5 h before the increase in TAT activity after dexamethasone addition (fig.1B). The actinomycin D-insensitive induction was found either in the continuous presence of DBcAMP as well as after removal of DBcAMP. Apparently, the actinomycin D-insensitivity is determined by the pre-induction. The specificity of this pre-induction is further shown in the reversed situation, the influence of pre-induction with dexamethasone on DBcAMP-induced and/or dexamethasone-induced increase in TAT activity. Except for

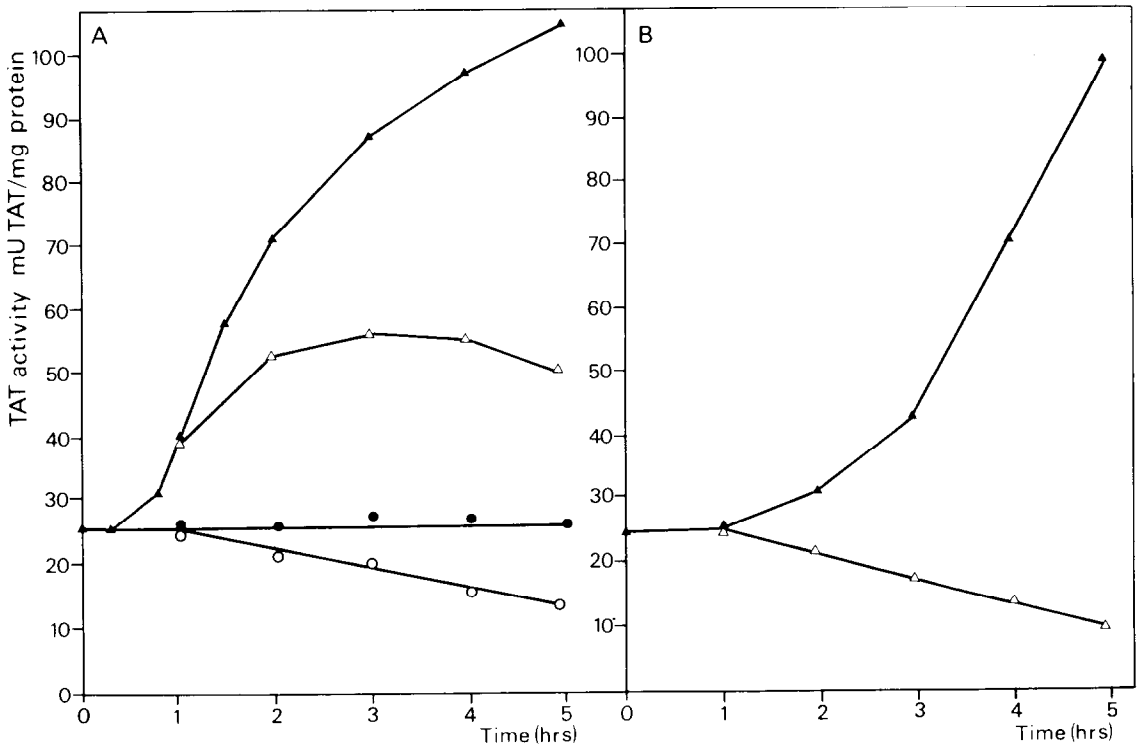


Fig.1. The effect of actinomycin D on the induction of TAT activity by DBcAMP (A) and dexamethasone (B). Cells were grown as in section 2: DBcAMP (1 mM), dexamethasone ($2 \times 10^{-6}$ M) and/or actinomycin D (0.2 μg/ml) were added at zero time. (A) (▲) + DBcAMP; (△) + DBcAMP + actinomycin D; (●) control cells; (○) control cells + actinomycin D. (B) (▲) + dexamethasone; (△) + dexamethasone + actinomycin D.

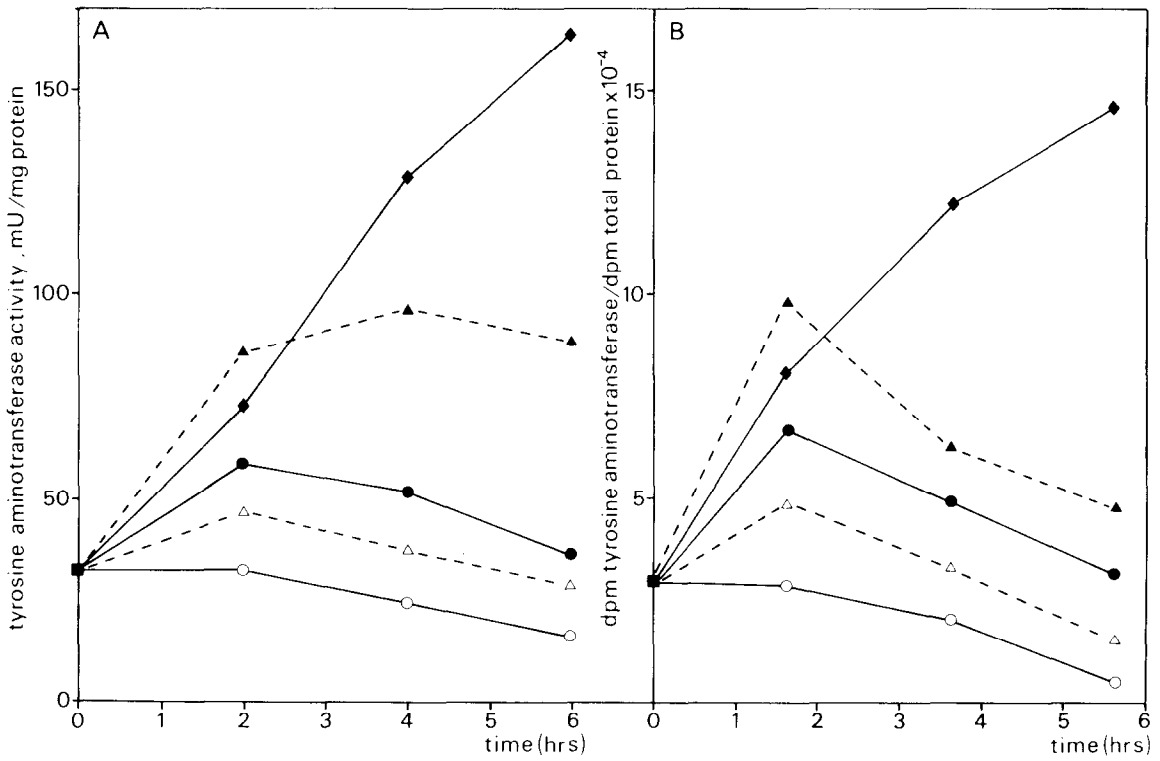


Fig.2. The effect of pre- or co-induction with DBcAMP on the induction of TAT activity (A) and TAT synthesis (B) by dexamethasone in the presence of actinomycin D. Cells were grown as in section 2. DBcAMP was added 1 h before the start of the experiments. At $t = 0$ the cells were washed, fresh serum-free medium was added and the following additions were made: (♦) + dexamethasone; (▲) + dexamethasone + DBcAMP + actinomycin D; (△) + DBcAMP + actinomycin D; (●) + dexamethasone + actinomycin D; (○) + actinomycin D. The final concentrations were: dexamethasone ($2 \times 10^{-6}$ M); DBcAMP (1 mM); actinomycin D (0.2 μg/ml).

TAT increase due to pre-incubation, no dexamethasone effect was observed when added in the presence of actinomycin D (fig.3A) The DBcAMP induction of TAT activity is independent on pre- or co-induction with dexamethasone.

The actinomycin D-independent increase in TAT activity after dexamethasone addition may be caused by an increased rate of TAT synthesis or an increased TAT stability. Fig.2B,3B show the rates of TAT synthesis under the various conditions. After pre-induction with DBcAMP, dexamethasone is able to increase specifically TAT synthesis in the presence of actinomycin D.

To check if the addition of inducers and/or inhibitor affects the stability of TAT we determined the rate of degradation of the enzyme under the various experimental conditions. The results are presented in table 1. Conditions of induction show the same rates of TAT degradation as control cells. Actinomycin D decreases the rate of degradation. However, the relatively small differences in rates of TAT degradation will have no significant effect on the measurement of the rate of TAT synthesis which is performed during 45 min labeling periods. Apparently, the modulation effect is due to changes in the rate of TAT synthesis.

## 4. Discussion

The mechanism by which steroid hormones regulate the synthesis of TAT in rat liver and hepatoma cells has been extensively investigated. Evidence supporting hormonal control of specific gene transcription is formed by the complete inhibition of TAT synthesis by inhibitors of RNA synthesis [16–19], by investigations on the hormone–receptor complex [20] and by determination of the levels of translatable TAT mRNA after hormone induction in cell-free protein

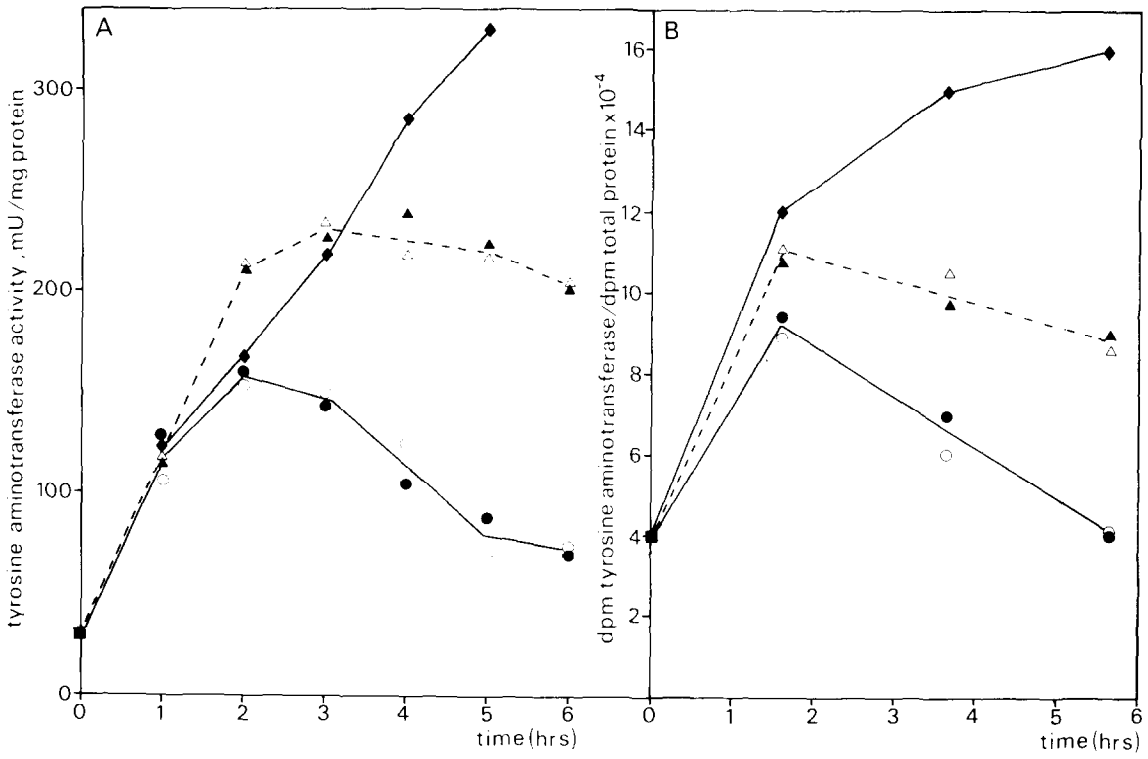


Fig.3. The effect of pre- or co-induction with dexamethasone on the induction of TAT activity (A) and TAT synthesis (B) by DBcAMP in the presence of actinomycin D. Cells were grown as in section 2. Dexamethasone was added 1 h before the start of the experiment. At $t = 0$ the cells were washed thoroughly and the following additions were made: (♦) + dexamethasone; (▲) + dexamethasone + DBcAMP + actinomycin D; (△) + DBcAMP + actinomycin D; (●) + dexamethasone + actinomycin D; (○) + actinomycin D. The final concentrations were: dexamethasone ($2 \times 10^{-6}$ M); DBcAMP (1 mM); actinomycin D (0.2 (μg/ml).

Table 1
Degradation of TAT under different induction conditions

| Addition at $t = 0$ | TAT degraded from 2.5–6 h |
|---|---|
| None | 59% |
| Dexamethasone + actinomycin D | 69% |
| Actinomycin D | 43% |
| DBcAMP + dexamethasone + actinomycin D | 70% |
| DBcAMP + actinomycin D | 59% |

Cells were grown as in section 2. At $t = 0$, after 1 h preinduction with DBcAMP (1 mM), the cultures were washed, fresh serum-free medium was added and the cells were supplemented with either DBcAMP (1 mM), dexamethasone ($2 \times 10^{-6}$ M), actinomycin D (0.2 μg/ml) or combinations of these compounds. At $t = 1.5$ h [$^3$H]leucine was added. At $t = 2.5$ h the medium was removed, the cells were washed and the inducer(s) and/or inhibitor were added again in fresh serum-free medium. At $t = 6$ h the cells were harvested and the [$^3$H]leucine incorporation in TAT was determined. Data are expressed as radioactivity present in TAT at $t = 6$ h as percentage of the radioactivity present at $t = 2.5$ h

synthesizing systems [21–23]. The conclusion is that steroid hormones induce the TAT synthesis by increasing the rate of transcription of the TAT gene.

Here, we describe an extragenomic effect of the synthetic corticosteroid dexamethasone on the TAT synthesis in a minimal deviation hepatoma cell line. This effect is dependent on pre- or co-induction with DBcAMP and is not inhibited by actinomycin D. Apparently, this is a new level on which DBcAMP can regulate the TAT synthesis. Until now the known levels of regulation of TAT activity by analogs of cAMP are transcription [9,10] and translational events occuring during elongation [8] and initiation [7]. Nothing is known about the mechanism of the DBcAMP-dependent induction of TAT synthesis by dexamethasone. Indications that it is a post-transcriptional event are formed by the actinomycin D-independency of the effect and the lack of lagtime in the increase of TAT activity after dexamethasone addition.

In [24,25], addition of another hormone that induces TAT, cortisol, caused extragenomic effects in rat liver such as immediate phosphorylation of cyto-

plasmic proteins and activation of protein phosphokinase and phosphatase activities. This indicates that hormonal effects do not have to be mediated by genomic events.

Apparently, the regulation of the TAT activity is a very complex phenomenon. Probably the liver and hepatoma cells have the possibility to regulate the TAT activity via several specific mechanisms and it depends on the metabolic or culture conditions which mechanism is used. This variation in regulatory mechanisms offers the cell the possibility to regulate the TAT activity fast and over a wide range without extreme variations in the rates of transcription or translation.

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