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Transcriptional activation of the rat liver tyrosine aminotransferase gene by cAMP

(glucocorticoid control/mRNA accumulation/nuclear transcription)

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ABSTRACT **The enzyme tyrosine aminotransferase (TyrATase; L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5), which is synthesized in rat liver, is induced by glucocorticoids, insulin, and glucagon or its intracellular mediator cAMP. We have used cloned TyrATase genomic and cDNA sequences to study the mechanism of induction by cAMP. RNA blot analysis shows that cAMP causes a rapid 5-fold increase in TyrATase mRNA concentration in rat liver. Transcription in isolated rat liver nuclei was studied to determine the relative rate of transcription of the TyrATase gene after cAMP administration. We show that the accumulation of TyrATase mRNA after cAMP stimulation is a consequence of transcriptional activation of the TyrATase gene. Combined dexamethasone and cAMP treatment leads to higher TyrATase mRNA concentrations than each inducer alone, which implies that dexamethasone and cAMP act by distinct mechanisms.**

The regulation of tyrosine aminotransferase (TyrATase; L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) activity has served as an excellent model system for the study of hormonal and developmental regulation of gene expression ever since the hormonal regulation of this mammalian enzyme was first shown by Lin and Knox in 1957 (1). Analysis of the effects of glucocorticoids on the synthesis of this enzyme and of tryptophan oxygenase [tryptophan 2,3-dioxygenase; L-tryptophan:oxygen 2,3-oxidoreductase (decyclizing), EC 1.13.11.11], another liver enzyme subject to glucocorticoid control, has helped to lay the groundwork for our understanding of how glucocorticoids act (2, 3).

TyrATase is subject to complex regulation. The activity of this enzyme in rat liver increases severalfold after the administration of glucocorticoids (1, 2), insulin, glucagon, and cAMP (4–6). TyrATase is also regulated during development. In fetal rat liver, enzyme activity is undetectable (for review, see ref. 7). The enzyme activity starts to increase soon after birth. However, premature induction of the enzyme in fetal liver can be caused by the administration of glucagon or cAMP. In contrast, inducibility by glucocorticoids *in vivo* is attained only after birth (7).

Genetic and biochemical analysis of lethal albino mutants of the mouse suggest that a control region located on chromosome 7 is required for expression and induction of TyrATase (8). In these deletion mutants, the activity of several liver-specific enzymes, one of which is TyrATase, is severely diminished and can no longer be induced by glucocorticoids or cAMP. The structural gene for TyrATase, however, is intact, as shown by somatic cell hybridization (8). This was confirmed by Southern hybridization, while the basal level of TyrATase mRNA was shown to be severely decreased in the mutants (unpublished results).

Thus, the expression of TyrATase has been a very suitable model for the study of various aspects of regulation of gene expression in mammalian cells (2, 3). The detailed mechanisms of the regulation of TyrATase gene expression, however, are still unknown.

The increase in TyrATase enzyme activity after glucocorticoid administration is due to an increased rate of synthesis of the enzyme protein (9, 10), which is in turn proportional to an increased amount of functional TyrATase mRNA activity in a cell-free translational system (11–13). Proportionality between relative rate of synthesis of the protein and mRNA content is also seen during the induction of tryptophan oxygenase mRNA by glucocorticoids (14). In the latter case, we have shown that the increase in the concentration of tryptophan oxygenase mRNA after glucocorticoid treatment can be quantitatively explained by an increase in the transcriptional rate of the tryptophan oxygenase gene (15).

Conflicting results have been reported on the mechanism of induction of TyrATase by cAMP. Wicks *et al.* (16) showed that inhibitors of RNA synthesis, which completely prevented glucocorticoid induction, had little effect on induction by cAMP action. Furthermore, cAMP was shown to accelerate the rates of initiation, elongation, and termination of TyrATase synthesis (17–19). It was concluded that the induction by cAMP was exerted at a post-transcriptional level, presumably by a modulation of translation (6). Other groups, however, using *in vitro* translation assays have more recently shown that cAMP administration resulted in a rapid increase in TyrATase mRNA activity in proportion to the increase in enzyme activity (20–22). These observations implicate pre-translational regulation by cAMP. Noguchi *et al.* (23) proposed a model for the regulation in two ways of TyrATase synthesis. Early after the injection of cAMP, TyrATase mRNA is increased in proportion to the increase in enzyme activity. In later phases of the induction, an increased rate of translation of TyrATase mRNA ensues.

Since we have cloned the cDNA (24) as well as the genomic DNA of TyrATase of rat liver (25), it is now possible to quantitate directly the amount of TyrATase mRNA and to determine whether changes in the TyrATase mRNA concentration result from altered transcription of the TyrATase gene. In this paper, we show that TyrATase mRNA accumulates as a consequence of altered transcription after cAMP stimulation.

By analysis of the combined effects of glucocorticoids and cAMP, we give further support to the idea that the two inducers act by independent mechanisms. The multitude of different transcriptional controls makes the TyrATase gene promoter interesting for further study of control of transcription.

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Abbreviations: TyrATase, tyrosine aminotransferase; Bt_2cAMP, N^6,O^2-dibutyryladenosine 3′,5′-cyclic monophosphate.

MATERIALS AND METHODS

Animals. Male Wistar rats (150–200 g) were injected intraperitoneally with N^6,O^2-dibutyryladenosine 3′,5′-cyclic monophosphate (Bt_2cAMP) (5 mg per 100 g of body weight; Boehringer Mannheim) or dexamethasone (10 μg per 100 g of body weight; Sigma) 5–7 days after adrenalectomy. Control rats were adrenalectomized and injected with 0.9% NaCl. The rats were killed at the times indicated in the figure legends. The livers were removed, frozen in liquid nitrogen, and stored at −70°C. Each experimental group consisted of at least three animals. All injections were done between 9 and 10 a.m.

Isolation of Poly(A)$^+$ RNA and RNA Blotting Analysis. Isolation of poly(A)$^+$ RNA, separation on formaldehyde/agarose gels, and blotting to nitrocellulose filters were carried out as described (26). The blots were hybridized to ^{32}P-labeled DNA of pcTAT2 and pcTAT3 (24) and an albumin cDNA clone (27). The intensity of the hybridization signals was quantitated by scanning autoradiograms of different exposure times. Poly(A)$^+$ RNA recovery was normalized by hybridization to the albumin cDNA clone.

***In Vitro* Transcription and Hybridization.** Isolation of nuclei, *in vitro* nuclear transcription, isolation of ^{32}P-labeled RNA, and hybridization to filter-bound DNA were done as described (15). To measure the relative rate of transcription, the following cloned DNAs were immobilized on nitrocellulose filters (Schleicher & Schüll; BA85; diameter, 13 mm) as probes: (*i*) three genomic subclones of the TyrATase gene as shown in Fig. 1 (25) which were demonstrated to be unique in genomic DNA by Southern hybridization analysis (data not shown), 0.5 pmol of each per filter; (*ii*) genomic subclones of the tryptophan oxygenase gene, which represent unique fragments (26), 0.5 pmol of each per filter; (*iii*) pUC8, 1.5 pmol per filter. Hybridization and washing conditions were as described (15). Nonspecific binding to vector DNA was <10 ppm.

RESULTS

Bt_2cAMP Increases the Concentration of TyrATase mRNA. To determine whether control of TyrATase gene expression occurs at the level of transcription or translation, we examined the amount of TyrATase mRNA and the relative rate of transcription of the TyrATase gene in isolated nuclei after cAMP induction. Groups of animals were injected with Bt_2cAMP and killed at the times indicated (Fig. 2). Total rat liver poly(A)$^+$ RNA was isolated to examine the accumulation of TyrATase mRNA from rat livers after Bt_2cAMP administration. RNA was separated on agarose gels, blotted onto nitrocellulose filters, and hybridized with pcTAT2 and pcTAT3 (24) (see Fig. 1) as probes for TyrATase mRNA (Fig. 2*A*). Since the amount of albumin mRNA is not affected by Bt_2cAMP in rat liver (27), we used an albumin cDNA

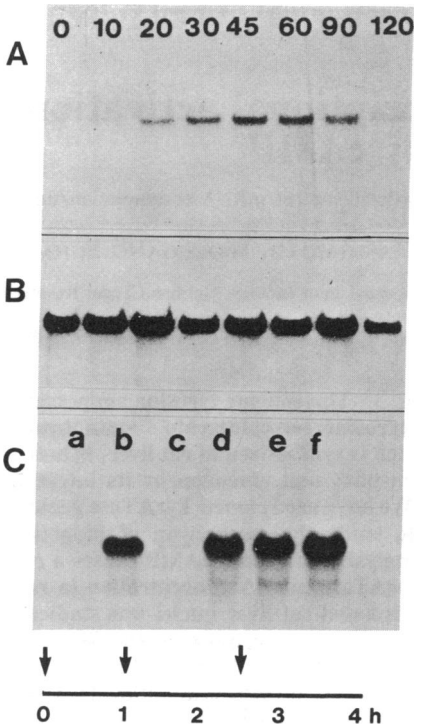

FIG. 2. Effect of Bt_2cAMP on level of mRNAs for TyrATase. (*A* and *B*) Poly(A)$^+$ RNA was prepared from animals at the times indicated (in min) after administration of a single dose (5 mg per 100 g of body weight) of Bt_2cAMP. Five micrograms of poly(A)$^+$ RNA was electrophoresed, blotted, and hybridized to ^{32}P-labeled DNA of the TyrATase (*A*) or albumin (*B*) cDNA clones. Specific activity of the probes was ≈10^8 dpm per μg of cDNA. Only parts of the autoradiograms are shown, because no other bands were seen. (*C*) Effect of multiple doses of Bt_2cAMP on level of TyrATase mRNA. Bt_2cAMP (5 mg per 100 g of body weight) was injected at time 0, and additional doses of 3 mg per 100 g of body weight were given at 1 hr and 2.5 hr after the first injection to maintain a high level of Bt_2cAMP, as indicated by arrows. The animals were killed 1 hr (lane b), 2 hr (lane d), 3 hr (lane e), and 4 hr (lane f) after the first injection. Lane a, saline control; lane c, 2 hr after a single injection of Bt_2cAMP at time 0. Analysis of the amount of TyrATase mRNA was carried out as described above.

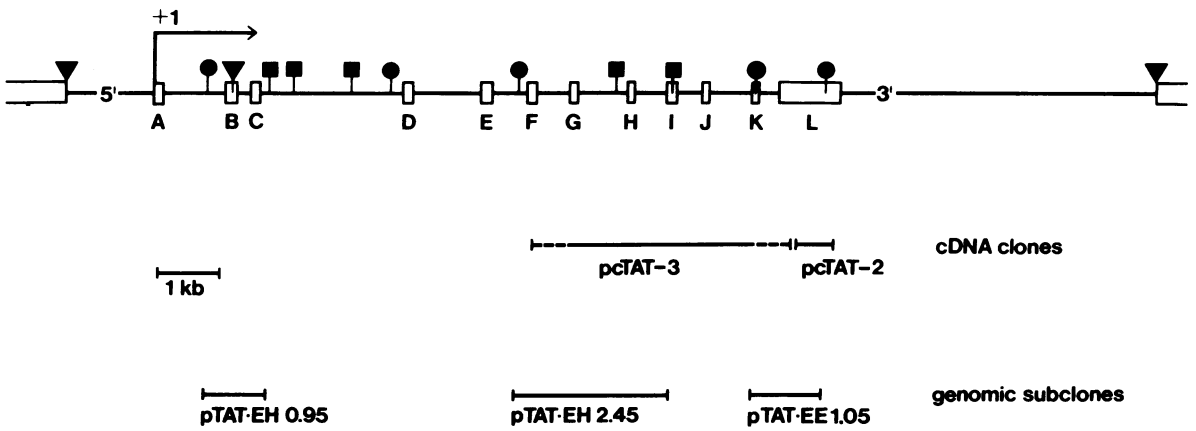

FIG. 1. Diagram of the λrTAT1 recombinant phage, the TyrATase cDNA clones and genomic subclones. Restriction map indicating *Eco*RI (●), *Hin*dIII (■), and *Sal* I (▼) sites. Start site and direction of transcription are indicated by arrow; exons are shown by open boxes. Position of the two cDNA clones pcTAT2 and pcTAT3 is indicated; position of pcTAT3 is approximate, as indicated by a broken line. Position of genomic clones pTAT·EH 0.95, pTAT·EH 2.45, and pTAT·EE 1.05 carrying single copy fragments is depicted on bottom line. kb, Kilobase.

clone to control for variable amounts of mRNA in the different poly(A)$^+$ RNA preparations (Fig. 2*B*). Hybridization signals for each mRNA were quantitated by scanning autoradiograms of different exposure times to verify the time course of induction (see, for example, Fig. 4). An increase in TyrATase mRNA was discernible 20 min after the administration of Bt_2cAMP. Accumulation was maximal between 45 and 90 min, at a level ≈5-fold higher than the basal level. By 2 hr, the mRNA concentration had returned to baseline. The level of albumin mRNA did not change significantly during this period (Fig. 2*B*). The same is true for the amount of tryptophan oxygenase mRNA (data not shown).

The amount of TyrATase mRNA remains high when an inducing level of Bt_2cAMP is maintained by repeated injections (Fig. 2*C*, lanes d–f), which is in contrast to previous observations of a dual effect of Bt_2cAMP on the synthesis of TyrATase in rat liver (23). Using a cell-free translational assay to analyze the concentration of TyrATase mRNA, an increase in TyrATase mRNA activity proportional to the increase in enzyme activity was seen early after the injection of Bt_2cAMP. However, after multiple injections of Bt_2cAMP, a delayed effect on TyrATase synthesis was observed. The rate of synthesis of the enzyme remained at an increased level even though TyrATase mRNA as detected by translation had returned to the basal level (23). This finding was explained to be due to a delayed but sustained increase in translational efficiency of TyrATase mRNA. In contrast to these results, our data show that TyrATase mRNA concentration remains at an induced level after multiple injections of Bt_2cAMP. Except for differences in strains used and methods of mRNA analysis used, no obvious explanation is available for the discrepancy between our results and those obtained by Noguchi *et al.* (23). A similar observation has recently been made using primary hepatocytes (unpublished results). The TyrATase mRNA level remained at the induced level as long as the inducing cAMP derivative was present.

Bt_2cAMP Increases the Rate of Transcription of the TyrATase Gene. To determine whether the accumulation of TyrATase mRNA after cAMP stimulation is due to stabilization of the mRNA or is a consequence of enhanced transcription of the TyrATase gene, we measured the rate of transcription of this gene in isolated nuclei. Since reinitiation does not occur when isolated nuclei are transcribed *in vitro*, the amount of nucleotide incorporation into RNA in such an assay is an accurate indication of the rate of transcription initiation on a particular gene *in vivo*. Liver nuclei were isolated and the endogenous RNA polymerases were allowed to synthesize RNA in the presence of [^{32}P]UTP. The labeled transcripts were isolated and hybridized to immobilized DNA of subclones containing unique fragments of the TyrATase gene (Fig. 1). The tryptophan oxygenase gene, which is not affected by cAMP (see above), served as a reference gene. To establish a sensitive and accurate transcription assay, different amounts of ^{32}P-labeled RNA from induced and uninduced animals were hybridized against the cloned unique DNA fragments (see *Materials and Methods* and Fig. 1). The data of Fig. 3 show that the hybridized radioactivity is proportional to the RNA input. The relative rate of transcription of the TyrATase gene 20 min after cAMP administration is ≈4-fold higher when compared to the saline control. The rate of transcription of the tryptophan oxygenase gene is not significantly affected by cAMP treatment.

To determine how quickly the transcription of the TyrATase gene changes, a time course of induction of transcription by Bt_2cAMP was established, and changes in the rate of transcription were compared to alterations in TyrATase mRNA concentration (Fig. 4). Nuclei and poly(A)$^+$ RNA were prepared from the same livers. The transcriptional rate changes rapidly: after 10 min a new plateau has been reached, and by 2 hr the rate of transcription has returned to ≈1/5th that level. A lag between the increase in the relative rate of TyrATase gene transcription and the accumulation of the TyrATase mRNA is clearly visible.

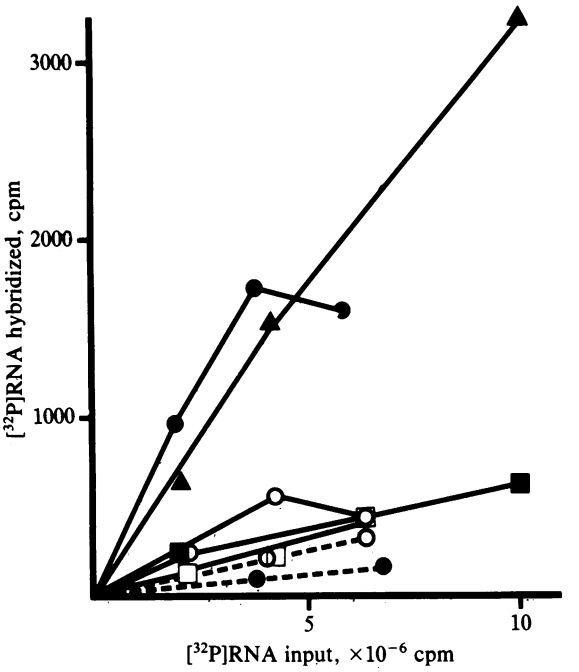

FIG. 3. Relative rates of transcription of the TyrATase and tryptophan oxygenase genes. Rats treated with either Bt_2cAMP (5 mg per 100 g of body weight) or saline alone, were killed after 20 min (●, ○), 60 min (▲), or 120 min (■, □), and liver nuclei were isolated for *in vitro* transcription. Two or three different inputs of [^{32}P]RNA were hybridized to nitrocellulose filters containing TyrATase or tryptophan oxygenase genomic subclones. After treatment with RNase A and T1, RNase-resistant radioactivity bound to filters was counted and corrected for scintillation background and radioactivity hybridized to pUC8 DNA alone. Hybridization to TyrATase subclones is indicated by solid lines and to tryptophan oxygenase subclones by broken lines. Filled symbols represent cAMP values; open symbols are saline controls.

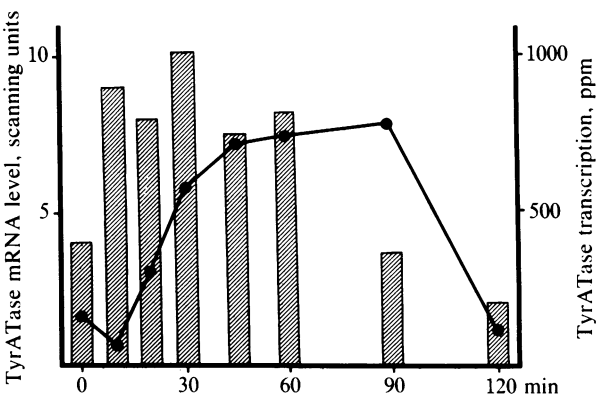

FIG. 4. Time course of TyrATase mRNA accumulation and transcription rate after a single dose of Bt_2cAMP (5 mg per 100 g of body weight). An RNA blot as shown in Fig. 2*A* was probed with ^{32}P-labeled pcTAT2 and pcTAT3, and the autoradiogram was scanned densitometrically. The amount of TyrATase mRNA is shown by full circles, after correction for the amount of albumin mRNA as an internal standard. Rate of transcription of TAT gene was measured in hepatic nuclei derived from the same pool of animals as used for the preparation of the poly(A)$^+$ RNA. Incorporation is corrected for TAT gene length and for a hybridization efficiency of 40% (15) and is given as ppm (hatched bars).

The Combination of cAMP and Dexamethasone Is More Effective Than Each Inducer Alone. It has previously been shown (5, 16) that the effects of cAMP and glucocorticoids on TyrATase enzyme activity are additive, suggesting that they affect different steps in the synthesis of TyrATase. We have therefore analyzed the combined action of Bt_2cAMP and dexamethasone at the level of TyrATase mRNA. To demonstrate that a combination of Bt_2cAMP and dexamethasone is more effective than either one alone, we had to determine the plateau of TyrATase mRNA concentration after dexamethasone treatment. Dexamethasone leads to an ≈6-fold increase in the concentration of TyrATase mRNA (Fig. 5*C*). Two hours after the injection of dexamethasone, a plateau is reached, after which no significant changes occur for up to 4 hr. We chose a period of 2.5 hr for dexamethasone action, after which the effects of addition of Bt_2cAMP for 45 min were analyzed, because the TyrATase mRNA concentration has attained its new plateau 45 min after cAMP treatment. Fig. 5 shows that the mRNA concentration increases above the dexamethasone-induced level after cAMP stimulation for 45 min (compare lane e with d in Fig. 5*A*). Whereas dexamethasone and Bt_2cAMP treatment lead to a 7- and 5-fold increase, respectively, in TyrATase mRNA concentration (Fig. 5*A*, lanes b and c), the combination of both inducers results in an ≈13-fold higher level of the mRNA, as evidenced from scanning the autogram of Fig. 5*A* and correcting for poly(A)$^+$ RNA recovery by hybridization to the albumin cDNA clone (Fig. 5*B*). The increased response of TyrATase mRNA concentration to a combination of cAMP and dexamethasone implies that the inducers act by distinct mechanisms.

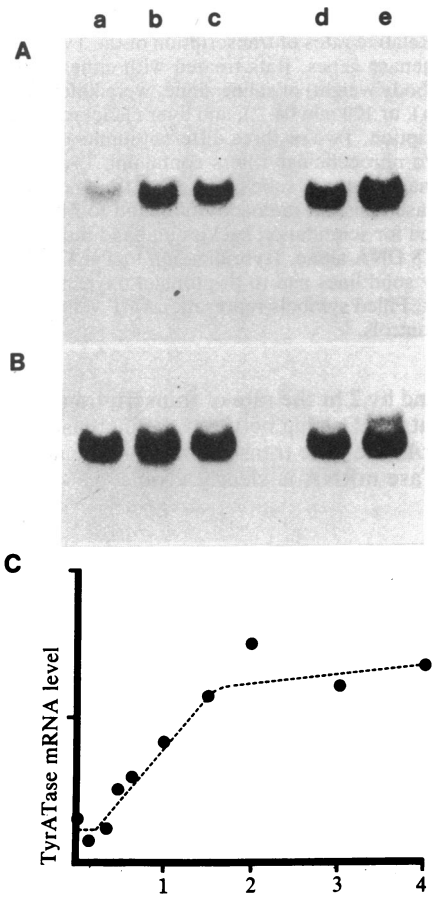

FIG. 5. Combined effect of dexamethasone and Bt_2cAMP on TyrATase mRNA; 150 min after application of dexamethasone at 10 μg per 100 g of body weight (lane b), Bt_2cAMP (5 mg per 100 g of body weight) was given, and rats were killed 45 min (lane e) after the i.p. injection of Bt_2cAMP. Lane a, saline control; lane c, Bt_2cAMP for 45 min; lane d, dexamethasone for 195 min. Poly(A)$^+$ RNA was analyzed as described in Fig. 2 using pcTAT2 and pcTAT3 (*A*). The same filter was rehybridized with albumin cDNA clone (*B*) without removal of labeled TyrATase probe. (*C*) Time course (in hr) of TyrATase mRNA accumulation after a single dose of dexamethasone. TyrATase mRNA level is indicated in relative units after scanning the autoradiogram.

DISCUSSION

The experiments described show that Bt_2cAMP increases the concentration of TyrATase mRNA (Fig. 2*A*) and that the increased level is maintained as long as a high Bt_2cAMP level is maintained by repeated injections, at least up to 4 hr (Fig. 2*C*). These results confirm and extend conclusions of previous studies on the effect of cAMP on TyrATase mRNA using *in vitro* cell-free systems (20–22) and disagree with conclusions postulating a post-transcriptional site of action of cAMP (16–19). Furthermore, no evidence for dual action of cAMP as proposed by Granner and his colleagues (23) could be obtained.

The induction of TyrATase mRNA is accompanied by an increase in the transcriptional rate of the TyrATase gene. This effect on TyrATase gene transcription was expected, because several studies have demonstrated that inhibitors of mRNA synthesis and processing interfere with the cAMP-induced synthesis of TyrATase and TyrATase mRNA (20–22, 28). The data allow the conclusion that the accumulated level of TyrATase mRNA after cAMP administration is largely due to a specific increase in the rate of transcription. An effect of cAMP on stabilization of the TyrATase mRNA cannot, however, be completely ruled out. Measurements of the stability of the TyrATase mRNA *in vivo* by pulse–chase experiments are required to determine whether changes in the stability of the TyrATase mRNA contribute to the cAMP-induced mRNA accumulation (29).

The TyrATase mRNA concentration and the transcriptional rate of the TyrATase gene change rapidly after cAMP administration. An increase in TyrATase gene transcription can be detected within 10 min and reaches a maximal level 30 min after Bt_2cAMP administration. This rapid increase in TyrATase gene transcription may help to explain the relatively high zero time value compared to the 120-min time point if one considers the stress inadvertently imposed on the animals by their handling. An increase in TyrATase enzyme activity in normal and adrenalectomized rats has been observed after application of stress (30). Analysis of the TyrATase gene transcription after cAMP administration to hepatocytes in primary culture may avoid these complications. The rapid effect of Bt_2cAMP on TyrATase gene transcription implies that this represents a direct effect and argues against the involvement of a protein intermediate that has to be synthesized. Similar findings have also been made in studies on control of expression of phospho*enol*pyruvate carboxykinase, which is also induced by cAMP in rat liver. It has recently been shown that the inducer acts at the level of mRNA production (31, 32). The increase in mRNA concentration is a consequence of an altered transcriptional rate of the phospho*enol*pyruvate carboxykinase gene (33). Effects of cAMP on gene-specific transcription in higher eukaryotic systems have also been shown to underlie induction of prolactin synthesis in cells from the anterior pituitary (34, 35).

The greater effects on TyrATase mRNA concentration after combined treatment with dexamethasone and Bt_2cAMP than with each inducer alone suggest that both inducers act differently. This is supported by the differences in the time course of induction of TyrATase mRNA, which is more rapid with Bt_2cAMP than with dexamethasone (Figs. 4 and 5*C*).

The maximum response of the cyclic nucleotide occurs at a time when the response to dexamethasone is 25% of maximum. This difference in mode of action is expected from earlier work, which showed that progesterone, a competitive inhibitor of glucocorticoid action, blocks the effect of dexamethasone on TyrATase synthesis but does not interfere with the response to cAMP (36).

It is not clear by what mechanism synthesis of TyrATase and phospho*enol*pyruvate carboxykinase are controlled by cAMP. In particular, it is not understood what role if any the cAMP-dependent protein kinase plays in this process. Activation of cAMP-dependent protein kinases have been suggested to mediate the effect of cAMP on TyrATase gene expression (37). Introduction of the cloned TyrATase gene into cAMP-sensitive cells may allow us to dissect the steps required for cAMP induction of TyrATase gene expression.

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