INDUCTION OF TYROSINE AMINOTRANSFERASE IN HTC CELLS BY N^6, $O^{2'}$-DIBUTYRYL ADENOSINE 3', 5'-MONOPHOSPHATE

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SUMMARY. The specific activity of tyrosine aminotransferase (TAT) in HTC cells growing in monolayer cultures is increased by N^6, $O^{2'}$-dibutyryl adenosine 3', 5'-monophosphate (DBcAMP). This increase occurs rapidly and becomes maximal in about three hours. Butyrate and the natural 3', 5'-cyclic nucleotides cAMP and cGMP are ineffective. There is no influence of DBcAMP on overall protein synthesis, and the increase in TAT activity is blocked by cycloheximide but not by actinomycin D. Thus, in these hepatoma cells, which were previously considered to lack the responsiveness of liver to cyclic nucleotides, DBcAMP seems to increase specifically the rate of TAT synthesis.

The enzyme TAT (EC 2.6.1.5) can be induced in rat liver by glucocorticoids (1), insulin (2), or glucagon (2). Induction by glucagon is mediated by cAMP and can be mimicked by administering DBcAMP to whole animals (3) or to organ cultures of fetal liver (4). The actions of glucocorticoids and insulin on TAT have been studied extensively in two tissue culture cell lines established from rat hepatomas: HTC cells (5,6) and Reuber H-35 cells (7,8). However, neither of these cell lines has responded to glucagon (free of insulin), presumably because both are deficient in adenylate cyclase and cAMP (7, 9-11), although low levels of glucagon-sensitive cyclase have recently been detected under some conditions (12). Despite the inability of these cells to produce much endogenous cAMP, they might still respond to exogenously added cyclic nucleotides if the appropriate cellular components were present. Indeed, in three strains of Reuber H-35 cells in culture, TAT has been induced by added DBcAMP (10,11,13), but in HTC cells the cyclic nucleotide was without effect (9). The study reported here demonstrates that DBcAMP can induce TAT even in HTC cells, provided that the cyclic nucleotide is present at a sufficient concentration and the cells are grown in the appropriate way.

MATERIALS AND METHODS. Actinomycin D, L-epinephrine, cAMP and DBcAMP were obtained from Calbiochem; glucagon, cycloheximide and cGMP from Sigma Chemical Co.; and hydrocortisone from Mann Research Laboratories. Sera and the powdered culture medium were purchased from Grand Island Biological Co., and the ^{14}C-amino acid mixture from New England Nuclear Corp. A stock culture of HTC cells (14) was obtained from Dr. Gordon M. Tomkins.

The cells were grown in spinner or monolayer cultures in modified Swim's S-77 medium (15) containing 5% bovine and 5% fetal calf sera (heat inactivated at 56° for 30 min). For routine induction experiments, cultures of cells growing exponentially in monolayers (250 ml Falcon plastic flasks) were removed in fresh medium by slow rotation of a sterile magnetic stirring bar. About 10^6 cells were transplanted into each of several 35 X 10 mm plastic tissue culture dishes and incubated at 37° in a humid air atmosphere overnight. Test substances were then added in additional medium to give a final volume of 4 or 5 mls. At the end of the experiment the cells were scraped off the plastic surface, collected by centrifugation, washed in 0.14 M NaCl-0.01 M potassium phosphate buffer at pH 7.6, and frozen. Cells were lysed by thawing in hypotonic buffer and analyzed for TAT activity and protein as described previously (16). One unit of activity corresponds to formation of 1 μmole of product per min at 37°.

RESULTS AND DISCUSSION. Fig. 1 shows the effect of DBcAMP on the specific activity of TAT in HTC cells growing in monolayer cultures. The enzymatic activity increases significantly within 1 hr, becomes maximal in about 3 hrs, and gradually decreases again during more prolonged incubations. The absolute magnitude of the response to DBcAMP is much greater (about 50 versus 10 mU/mg) if TAT is first induced with hydrocortisone, although the percentage increase due to DBcAMP remains about the same as in Fig. 1. The larger absolute response to DBcAMP by the hydrocortisone-induced activity implies that both inducers are affecting the same TAT.

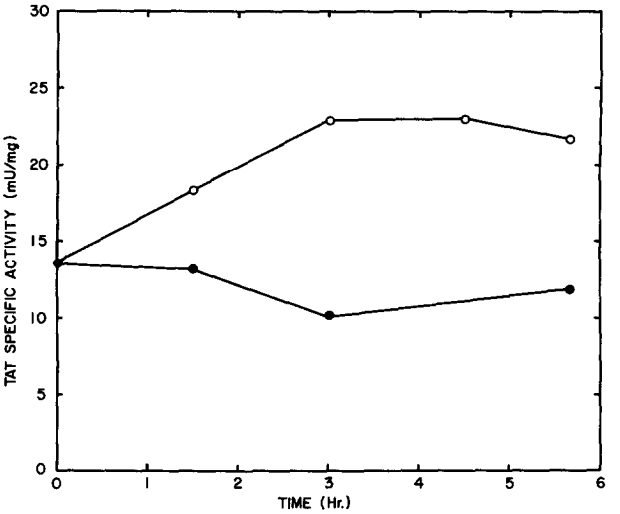

FIGURE 1. Time course of the effect of DBcAMP on TAT activity. Monolayer cultures were incubated for the indicated times in the presence (-o-) or absence (-●-) of 2 mM DBcAMP.

Relatively high concentrations (0.3 to 3 mM) of DBcAMP are needed to produce an increase in TAT activity (Fig. 2). An effect of DBcAMP on TAT has also been observed in some experiments with suspension cultures, but the response is smaller and less reproducible than under the monolayer conditions used here. Thus, it is not surprising that Granner et al. (9) did not observe an effect of DBcAMP since they employed concentrations no greater than 0.1 mM and used only suspension cultures.

The data in Fig. 2 also demonstrate that butyrate, which could arise from breakdown of DBcAMP, has no influence on TAT activity in HTC cells. This indicates that the cyclic nucleotide itself is responsible for the effect. However, unmodified cAMP and also cGMP do not increase the activity of TAT, even when added at concentrations of 10 mM (Fig. 2). A possible reason for the ineffectiveness of cAMP is its rapid degradation by the substantial phosphodiesterase activity in HTC cells (also noted in ref. 12). Added DBcAMP is metabolized much more slowly by the cells and would also be expected to penetrate the cell membrane more readily than cAMP.

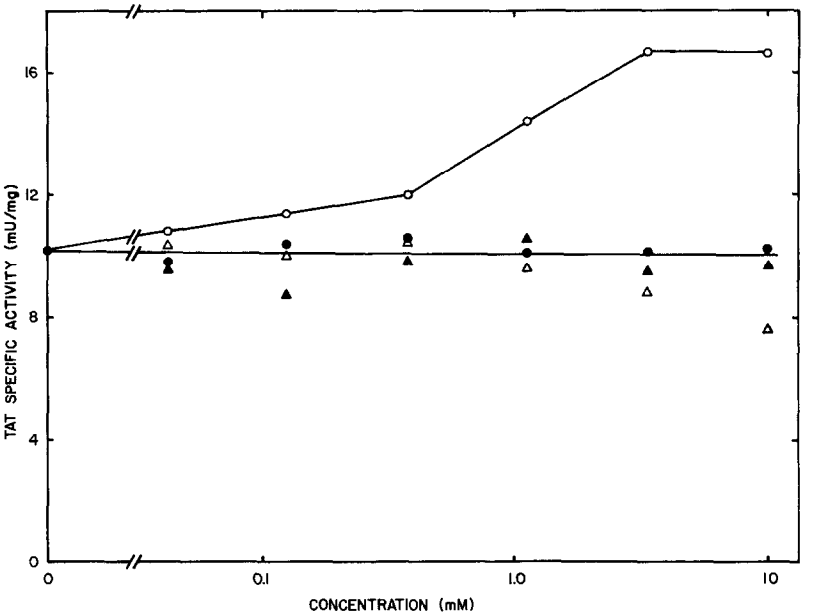

FIGURE 2. Effect of cyclic nucleotide concentration on the activity of TAT. HTC cells were exposed for 3 hrs to the indicated concentrations of DBcAMP (-o-), cAMP (-●-), cGMP (-Δ-), or butyrate (-▲-). Each point is the average of duplicate monolayer cultures.

TABLE I. Effects of various compounds on induction of TAT by DBcAMP

	Compound Added	Final Concentration	TAT Specific Activity [1] (mU/mg)	
			- DBcAMP	+ DBcAMP [2]
A.	None	-	13.0	23.8
	Cycloheximide [3]	2×10^{-4} M	7.0	6.5
	Actinomycin D [3]	0.2 μg/ml	12.9	19.9
B.	None	-	17.0	24.9
	Hydrocortisone	10^{-8} M	18.1 [4]	25.7
	Hydrocortisone	5×10^{-8} M	19.1 [4]	26.0
C.	None	-	11.4	16.6
	Epinephrine	10^{-4} M	11.7	16.1
	Glucagon	2 μg/ml	10.1	15.5
	cAMP	10^{-3} M	11.1	15.5

[1] Determined after incubation for 3 hrs in monolayers with the compounds indicated.

[2] Present at 2 mM in A and 1 mM in B and C.

[3] Added 15 min before DBcAMP.

[4] Little induction is observed with steroid alone because of the short duration of exposure (5,14).

Studies with inhibitors indicate that DBcAMP acts by affecting the rate of synthesis of TAT. As shown in Table IA, cycloheximide completely prevents the increase in activity. In the presence of this inhibitor of protein synthesis the specific activity of TAT drops, due to degradation of the enzyme (5,14), at the same rate whether or not DBcAMP is present. On the other hand, actinomycin D, at a concentration which inhibits RNA synthesis extensively in these cells (18), has only a small effect on the increase in TAT activity produced by DBcAMP (Table IA). In liver (4) and in Reuber H-35 cells (10,11), actinomycin D is also less effective than cycloheximide in blocking the action of DBcAMP, although the precise conditions of the experiments and the quantitative results vary from study to study.

That the effect of DBcAMP on TAT is not due to a general increase in pro-

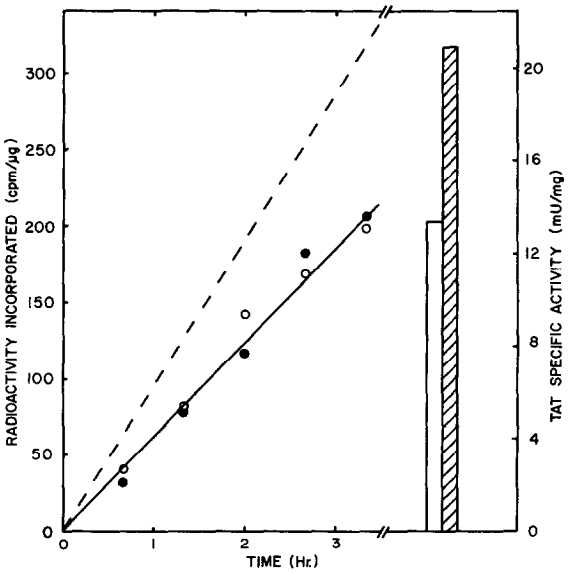

FIGURE 3. Effect of DBcAMP on amino acid incorporation into total cellular protein. HTC cells in monolayers were exposed for the times shown to 0.50 µci of ^{14}C-amino acids/ml, either in the presence (-o-) or absence (-●-) of 2 mM DBcAMP. Cellular proteins which were insoluble in cold 5% CCl_3COOH after treatment (15 min, 90°) with hot 5% CCl_3COOH were analyzed for radioactivity by scintillation counting (16) and for protein by the method of Lowry et al. (17). The bar graph on the right indicates the enzymatic specific activity of TAT in this experiment after 3.3 hrs with (shaded bar) or without (open bar) DBcAMP. The dashed line is the expected rate of amino acid incorporation if the effect on TAT were due to a general increase in protein synthesis.

tein synthesis is demonstrated by the data in Fig. 3. The rate of amino acid incorporation into cellular proteins is the same in the presence and absence of the cyclic nucleotide. Uptake of radioactive amino acids into the intracellular pool of small molecules is also unaffected by DBcAMP (data not shown). These results and the experiment with inhibitors indicate that DBcAMP induces an increased rate of TAT synthesis by a specific mechanism which does not affect many other proteins and which may not require RNA synthesis.

The maximal extent of induction of TAT by DBcAMP in HTC cells has ranged from about 50 to 100% in different experiments. This is a somewhat smaller effect than found in liver (3,4) or Reuber H-35 cells (10,11), and a variety of attempts have been made to enhance the response. No increase is produced by various agents which alter membrane permeability. Theophylline, which is often used to inhibit cyclic nucleotide breakdown by phosphodiesterase, is also not very useful because it interferes with protein and RNA synthesis in these cells. For one strain (H-4-II-E) of Reuber H-35 cells, Sahib et al. (10) have reported that a low concentration of hydrocortisone is needed for DBcAMP to induce TAT. However, as shown in Table IB, similar low hydrocortisone concentrations do not alter the response of HTC cells to DBcAMP. Since, in monolayer cultures of HTC cells, Makman (12) has detected adenylate cyclase which responds to glucagon and epinephrine, these hormones were tested in the present study. As indicated in Table IC, neither hormone induces TAT or increases the response to DBcAMP under the conditions used here. Thus, none of the approaches tested enhances the induction observed with DBcAMP, suggesting that some cellular component involved in the action of the cyclic nucleotide may be limiting (see ref. 19).

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