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DIFFERENCES IN THE MODE OF REGULATION OF ORNITHINE DECARBOXYLASE AND TYROSINE AMINOTRANSFERASE IN H-35 RAT HEPATOMA CELLS SHOWN BY VARYING THE MEDIUM COMPOSITION

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The effects of dibutyryl cyclic AMP, dexamethasone, and asparagine as inducers of ornithine decarboxylase (EC 4.1.1.17) and tyrosine aminotransferase (EC 2.6.1.5) in H-35 rat hepatoma cells were studied; a comparison was made between cells incubated in Eagle's minimal essential medium and cells incubated in Earle's balanced salt solution (salts/glucose solution). The addition of either 1 mM dibutyryl cyclic AMP or 1 μM dexamethasone increased both ornithine decarboxylase and tyrosine aminotransferase activities of H-35 hepatoma cells maintained in Eagle's minimal essential medium, whereas 10 mM asparagine increased the activity of ornithine decarboxylase and had no effect on tyrosine aminotransferase activity. The actions of dibutyryl cyclic AMP and dexamethasone but not asparagine, as inducers of ornithine decarboxylase activity, appeared to be dependent on the incubation medium used; dibutyryl cyclic AMP and dexamethasone were ineffective in increasing ornithine decarboxylase activity of H-35 hepatoma cells maintained in the salts/glucose solution, while asparagine was fully effective. Parallel studies on tyrosine aminotransferase demonstrated that both dibutyryl cyclic AMP and dexamethasone elicited maximal increases of tyrosine aminotransferase activity of H-35 hepatoma cells maintained in the salts/glucose solution. Experiments on the activation of cyclic AMP-dependent protein kinase and the uptake/binding of [^{3}H]dexamethasone to whole cells revealed no significant difference between H-35 hepatoma cells maintained in Eagle's minimal essential medium and cells maintained in the salts/glucose solution. The half-lives of ornithine decarboxylase of cells maintained in minimal essential medium and salts/glucose solution were 45 and 38 min, respectively; the corresponding values for tyrosine aminotransferase were 2.5 and 1.5 h, respectively. It is proposed that the actions of dibutyryl cyclic AMP and dexamethasone as inducers of ornithine decarboxylase may involve the participation of component(s), possibly amino acids, present in the Eagle's minimal essential medium but not in the salts/glucose solution. These components may either mediate or modulate the actions of dibutyryl cyclic AMP and dexamethasone in the induction of ornithine decarboxylase activity.

Introduction

The actions of dibutyryl cyclic AMP and dexamethasone as inducers of ornithine decarboxylase and tyrosine aminotransferase activities have been reported [1–4]. In many of these studies, cells maintained in culture media of varying degrees of complexity were used. It is conceivable that the various components, e.g. amino acids, vitamins, and serum, present in the incubation medium can

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affect the basal phenomenon under investigation. For example, if dibutyryl cyclic AMP has specific effects on the transport of certain amino acids which subsequently cause an increase in enzyme activity, then the effectiveness or non-effectiveness of this cyclic nucleotide as an enzyme inducer may be dependent upon or modified by the composition of the medium used. This is of particular relevance to studies on the regulation of ornithine decarboxylase and tyrosine aminotransferase activities in view of the ability of a large number of agents to induce these two enzymes.

An example of the possible effects of medium composition on enzyme induction is illustrated in a recent study by Chen and Canellakis [5]. In this study, it was demonstrated that while cyclic AMP and fresh serum can induce ornithine decarboxylase activity of N-18 mouse neuroblastoma cells maintained in Dulbecco's modified Eagle medium, these agents are ineffective in inducing ornithine decarboxylase when cells are maintained in a salts/glucose solution. On the other hand, the action of asparagine as an inducer of ornithine decarboxylase is independent of whether the cells are maintained in the Dulbecco's medium or the salts/glucose solution. The results of this study indicate the primodial role of asparagine in the induction of ornithine decarboxylase activity, and that the effect of cyclic AMP on ornithine decarboxylase may be related to a 'facilitation' of ornithine decarboxylase induction by an asparagine-dependent reaction.

In order to determine whether other reactions inducible by cAMP and whether the action of dexamethasone, a glucocorticoid, in the induction of ornithine decarboxylase may be similarly modulated by the presence/absence of asparagine or other components in the incubation medium, we compared and contrasted the induction of ornithine decarboxylase and tyrosine aminotransferase of H-35 rat hepatoma cells. The effects of dibutyryl cyclic AMP, dexamethasone, and asparagine as inducers of these two enzymes were studied using H-35 cells maintained in the Eagle's minimal essential medium and the Earle's balanced salt solution.

Materials and Methods

The following compounds were purchased: L-asparagine, modified Eagle's minimal essential medium, fetal bovine serum, and Earle's balances salt solution (salts/glucose solution) were from Gibco, Grand Island, N.Y. $N^6,O^{2'}$-Dibutyryl cyclic AMP (dibutyryl cyclic AMP), cyclic AMP, dexamethasone phosphate, L-ornithine, and histone II-AS were from Sigma, St. Louis, MO. 3-Isobutyl-1-methyl xanthine was from Aldrich, Milwaukee, WI. L-[^{14}C]Ornithine monohydrochloride (30–50 mCi/mmol) was from Amersham/Searle, IL. Cyclic [^{3}H]AMP (30–40 Ci/mmol) and [6,7-^{3}H(n)]dexamethasone (40–50 Ci/mmol) were from New England Nuclear, Boston, MA. 8-N_3-cyclic [^{32}P]AMP was from ICN, Irvine, CA. [γ-^{32}P]ATP (0.5–2 Ci/mmol) was prepared by the method of Post and Sen [6]. All other chemicals were of reagent grade.

Cell line. H-35 rat hepatoma cells were grown as monolayer cultures in Eagle's minimal essential medium with 10% fetal calf serum at 37°C in a water-jacketed CO_2 incubator. Upon reaching a stationary phase of growth, cells were subcultured by incubation with a 0.5% Viokase solution in Ca^{2+}-Mg^{2+}-free Earle's balanced salt solution for 3 min at room temperature; the Viokase solution was then aspirated off and cells were flushed off the bottom of the culture flask with a stream of medium. The cell suspension was then diluted 1 : 10 to 1 : 30 and plated in 60 mm tissue culture dishes and incubated as described above.

Induction of enzyme activity. For studying the induction of ornithine decarboxylase and tyrosine aminotransferase activities, cells at an early stationary phase of growth were used. Cells were serum-deprived either in the Eagle's minimal essential medium or in the salts/glucose solution for 12–24 h prior to their use. At the end of this incubation period, cells were rinsed once, refurbished with fresh medium and various test agents were added. At designated time intervals thereafter individual plates of H-35 hepatoma cells were harvested. Harvesting was accomplished by first rinsing the cell monolayer with 5 ml of phosphate-buffered saline (pH 7.4) followed by scraping the cells off the substratum in 0.7 ml of a buffer containing 50 mM Tris-HCl (pH 7.4), 0.1

mM EDTA, 50 μM pyridoxal phosphate, and 5 mM dithiothreitol. Samples were frozen at −20°C for 24 h, and aliquots of the cell extracts were used to assay for ornithine decarboxylase and tyrosine aminotransferase activities.

Ornithine decarboxylase activity was determined by methods previously described [7]. Briefly, cells were broken by two cycles of freeze-thawing. The cell homogenate was centrifuged at 12000 × *g* for 10 min in an Eppendorf microfuge, the supernatant thus obtained was used to assay for ornithine decarboxylase activity. Reactions were carried out at 37°C for 1 h using 0.56 mM L-[^{14}C]ornithine. 1 unit of enzyme activity is defined as 1 nmol of CO_2 evolved per hour.

Tyrosine aminotransferase activity was determined by a modified version [8] of the method described by Diamondstone [9]. The standard assay mixture (final volume 1.0 ml) contained 100 mM potassium phosphate buffer (pH 7.5), 5 mM tyrosine, 10 mM α-ketoglutarate, 0.1 mM pyridoxal phosphate, 1 mM EDTA, and 1 mM dithiothreitol. The reaction was carried out at 37°C for 45 min and terminated by the addition of 0.2 ml of 5 N NaOH. Absorbance at 331 nm was recorded 30 min after the addition of NaOH. *para*-Hydroxyphenylpyruvate was used as the standard. Results are expressed in μg *para*-hydroxyphenylpyruvate formed/min per mg protein. Protein concentration was determined by a modified method of Lowry et al. using bovine serum albumin as the standard [10].

Covalent incorporation of 8-N_3-[^{32}P]cAMP. H-35 rat hepatoma cells at an early stationary phase of growth were scraped off their substratum, pelleted, and homogenized in 10 mM Tris-HCl (pH 7.4) buffer containing 1 mM EDTA and 50 μg/ml of phenylmethylsulfonyl fluoride. The cytosol fraction, obtained by centrifuging the cell homogenate at 100000 × *g* for 45 min, was dialyzed against the homogenizing buffer for 16 h at 4°C prior to use. The covalent incorporation of 8-N_3-[^{32}P]cAMP into cytosol proteins was performed as described previously [8]. The standard reaction mixture (final volume 0.1 ml) contained 50 mM 2-(*N*-morpholino)ethane sulfonate (pH 6.2), 10 mM $MgCl_2$, 0.5 mM 3-isobutyl-1-methyl xanthine, 1 μM 8-N_3-[^{32}P]cAMP (spec. act. 4–10 Ci/mmol) and 200 μg cytosol protein. Samples were incubated in the dark at 4°C for 60 min and were then photolyzed for 10 min with a Mineralite UVS-11 handlamp. Samples were then prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to methods previously described [8] and were analyzed for the incorporation of radioactivity into protein bands by autoradiography.

Cyclic [^{3}H]AMP binding. The Millipore filtration method described by Gilman [11] was used to determine the non-covalent binding of cyclic [^{3}H]AMP. The standard reaction mixture used for cyclic [^{3}H]AMP binding was identical to that used for the covalent incorporation of 8-N_3-[^{32}P]cAMP. The amount of cyclic [^{3}H]AMP bound by protein and retained by the Millipore filter was determined by liquid scintillation counting. All results were corrected for nonspecific binding, determined from the amount of radioactivity retained in the presence of 50 μM cyclic AMP.

Histone kinase assay. The catalytic activity of cyclic AMP-dependent protein kinase present in the dialyzed cytosol of H-35 hepatoma cells was determined according to the methods of Witt and Roskoski [12] using histone II-AS as the substrate protein. 1 unit of histone kinase activity is defined as the transfer of 1 μmol of ^{32}P from [γ-^{32}P]ATP to histone per min per mg protein. Under the experimental conditions used, the reaction rate was proportional to time of incubation and amount of protein used.

[^{3}H]Dexamethasone binding. The uptake and binding of [^{3}H]dexamethasone by whole cells was carried out according to methods previously described [13]. H-35 cells at an early stationary phase of growth were serum deprived overnight either in minimal essential medium or in salts/glucose solution. Predetermined concentrations of [^{3}H]dexamethasone (5–50 nM) were added to individual plates of cells and were incubated at 37°C for 45 min. (Control experiments demonstrated that maximal uptake and binding was achieved after a 20 min incubation period at 37°C.) At the end of this incubation period, dishes of cells were placed on ice, the medium was decanted, and cells were washed three times in rapid succession with ice-cold phosphate-buffered saline. Cells were then harvested in 0.5 ml of 1 N NaOH containing 0.1% sodium deoxycholate. Aliquots of cell homogenates were removed for determination of the

amount of [^{3}H]dexamethasone bound. Results of specific binding, i.e. differences in the amount of [^{3}H]dexamethasone bound in the absence and presence of a 100-fold excess of unlabeled dexamethasone, are presented.

Determination of the half-lives ($t_{1/2}$) of ornithine decarboxylase and tyrosine aminotransferase. Dibutyryl cyclic AMP was used to induce ornithine decarboxylase and tyrosine aminotransferase activities of H-35 hepatoma cells maintained in the Eagle's minimal essential medium. After a 4 h inducation period, cells were briefly rinsed and refurbished with either fresh Eagle's minimal essential medium or salts/glucose solution. Cycloheximide, at 50 μg/ml, was added. Samples were harvested at various time intervals after the addition of cycloheximide and enzyme activities assayed according to methods described. $t_{1/2}$ is defined as the time required for decay of half of the induced enzyme activity.

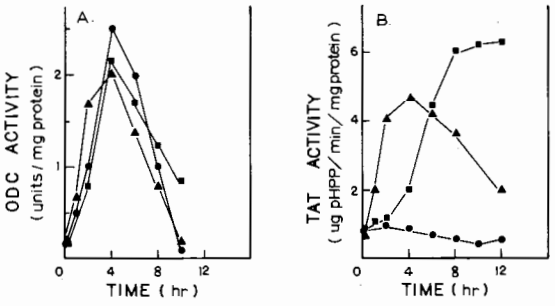

Fig. 1. The kinetics of induction of ornithine decarboxylase (ODC) and tyrosine aminotransferase (TAT) activities by 1 mM dibutyryl cyclic AMP (▲), 1 μM dexamethasone (■), and 10 mM asparagine (●) of H-35 hepatoma cells maintained in the Eagle's minimal essential medium. H-35 hepatoma cells were serum-deprived for 12 h in Eagle's minimal essential medium, at which point cells were rinsed, refed with fresh medium and various test agents were added. Individual plates of H-35 hepatoma cells were harvested at predetermined time intervals and assayed for ornithine decarboxylase and tyrosine aminotransferase activities. Note: the basal activities of ornithine decarboxylase and tyrosine aminotransferase did not vary by more than 5% during the entire incubation period.

Results

Effects of dibutyryl cyclic AMP, dexamethasone, and asparagine as inducers of ornithine decarboxylase and tyrosine aminotransferase activities in H-35 hepatoma cells maintained in Eagle's minimal essential medium

To eliminate possible effects of cell growth on either the basal or the induced activities of ornithine decarboxylase and tyrosine aminotransferase, H-35 hepatoma cells at the early stationary phase of growth were used in all experiments described in this study.

The effects of dibutyryl cyclic AMP, dexamethasone, and asparagine on the ornithine decarboxylase and tyrosine aminotransferase activities of H-35 cells maintained in Eagle's minimal essential medium were studied as a function of time. The results of representative experiments are shown in Figs. 1A and B for ornithine decarboxylase and tyrosine aminotransferase activities, respectively. Basal activity of ornithine decarboxylase of H-35 hepatoma cells at the early stationary phase of growth was in the range of 0.1–0.2 units/mg protein. The addition of 1 mM dibutyryl cyclic AMP, 1 μM dexamethasone, or 10 mM asparagine resulted in a time-dependent increase in ornithine decarboxylase; maximal increases were observed at approx. 4 h after addition of the inducers and represent a 20–25-fold increase over the basal activity. The kinetics as well as the magnitude of increases of ornithine decarboxylase activity produced by dibutyryl cyclic AMP, dexamethasone, and asparagine were similar. Under identical experimental conditions, dibutyryl cyclic AMP and dexamethasone produced, respectively, a 4.5- and a 6-fold increase in tyrosine aminotransfearse activity, while asparagine was without effect. The kinetics of increase in tyrosine aminotransferase activity elicited by dibutyryl cyclic AMP was different from that elicited by dexamethasone. Dibutyryl cyclic AMP produced a transient increase in the tyrosine aminotranferase activity; maximal activity was observed at approx. 4 h after the addition of the inducer. The rate of increase of tyrosine aminotransferase produced by dexamethasone was protracted when compared to that produced by dibutyryl cyclic AMP. The maximal increase was observed at approx. 8 h after the addition of dexamethasone, and remained at that level for time periods up to 37 h, the longest time point studied.

Effects of dibutyryl cyclic AMP, dexamethasone, and asparagine as inducers of ornithine decarboxylase and tyrosine aminotransferase activities of H-35 hepatoma cells maintained in the Earle's balanced salt solution

Confluent H-35 hepatoma cells could be maintained in the salts/glucose solution for more than 48 h with no apparent effects on cell viability, as judged by the trypan blue dye exclusion test, the incorporation of [^{3}H]leucine and [^{3}H]uridine, and plating efficiency (data not shown). The basal activities of ornithine decarboxylase and tyrosine aminotransferase of H-35 cells kept in the salts/glucose solution for the 12–24 h serum-deprivation period were approx. 10–20% lower than that of cells kept in Eagle's minimal essential medium for the same period of time, and remained stable for the next 24 h.

Dibutyryl cyclic AMP (1 mM) and dexamethasone (1 μM), when added to H-35 hepatoma cells maintained in the salts/glucose solution, were without effect on ornithine decarboxylase activity, while asparagine (10 mM) elicited a maximal 15-fold increase in ornithine decarboxylase activity at 4 h after its addition (Fig. 2A) and this level of induced activity was maintained for the remainder of the induction period. The results demonstrated that the induction of ornithine decarboxylase activity of H-35 hepatoma cells maintained in the salts/glucose solution differs from that of cells maintained in Eagle's minimal essential medium in at least two regards: First, the effects of dibutyryl cyclic AMP and dexamethasone as inducers of ornithine decarboxylase are dependent on the incubation medium used; both dibutyryl cyclic AMP and dexamethasone failed to elicit increases of ornithine decarboxylase activity when cells were maintained in the salts/glucose solution without added amino acids, vitamins, and serum. Second, the kinetics of the asparagine-induced increase in ornithine decarboxylase activity of H-35 hepatoma cells maintained in the salts/glucose solution is different from those of cells maintained in Eagle's minimal essential medium.

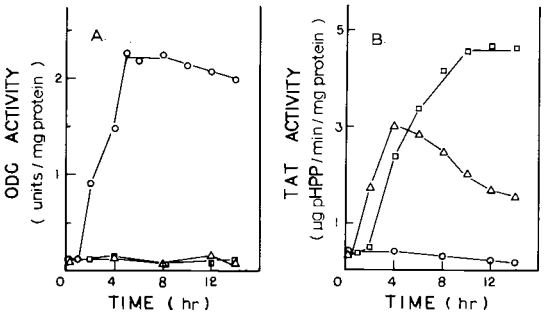

Fig. 2. The kinetics of induction of ornithine decarboxylase and tyrosine aminotransferase activities by 1 mM dibutyryl cyclic AMP (△), 1 μM dexamethasone (□), and 10 mM asparagine (○) of H-35 hepatoma cells maintained in Earle's balanced salt solution. Cells were serum-deprived in the salts/glucose solution for 12 h. The experimental conditions used were identical to those described in the legend of Fig. 1. Note: basal activities of ornithine decarboxylase and tyrosine aminotransferase did not vary by more than 5% during the entire 14 h incubation period.

The results obtained on the regulation of ornithine decarboxylase activity of H-35 hepatoma cells maintained in the salts/glucose solution are to be contrasted with those obtained for tyrosine aminotransferase activity. Dibutyryl cyclic AMP and dexamethasone produced maximal increases in tyrosine aminotransferase activity of H-35 hepatoma cells maintained in the salts/glucose solution (Fig. 2B); the kinetics and magnitude of increase were comparable to those of cells maintained in the Eagle's minimal essential medium (Fig. 1B). Asparagine was not an inducer of tyrosine aminotransferase activity.

In order to examine if the inability of dibutyryl cyclic AMP and dexamethasone to induce ornithine decarboxylase activity of H-35 hepatoma cells maintained in the salts/glucose solution may be attributable to decreased potencies in their intracellular action, we examined the effects of increasing concentrations of the inducers on activities of ornithine decarboxylase and tyrosine aminotransferase. The results of a representative experiment using increasing concentrations of dibutyryl cyclic AMP are shown in Fig. 3; comparison was made between cells maintained in the Eagle's minimal essential medium and the salts/glucose solution. The concentration of dibutyryl cyclic AMP needed to produce half-maximal increases of ornithine decarboxylase and tyrosine aminotransferase activities of H-35 hepatoma cells maintained in the minimal essential medium were approx. 0.2 and 0.8 mM, respectively. When cells were maintained in the

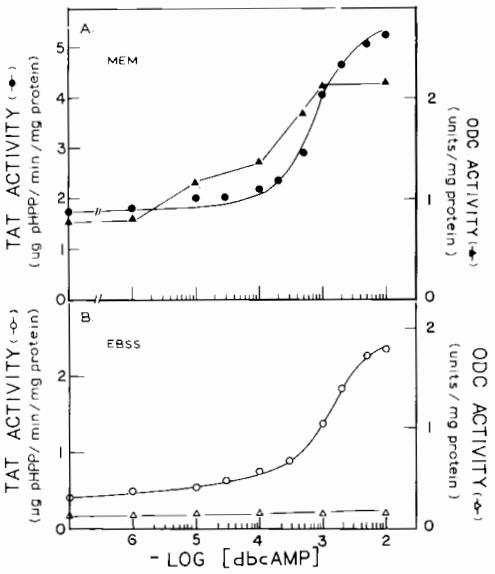

Fig. 3. Effect of increasing concentrations of dibutyryl cyclic AMP on ornithine decarboxylase (▲, △) and tyrosine aminotransferase (●, ○) activities of H-35 hepatoma cells maintained in Eagle's minimal essential medium (Panel A: MEM, filled symbols) and in Earle's balanced salt solution (Panel B: EBSS, open symbols). Various concentrations of dibutyryl cyclic AMP were added to 60 mm dishes of H-35 hepatoma cells and were incubated for 4 h at 37°C.

salts/glucose solution, 1 mM dibutyryl cyclic AMP gave a half-maximal increase in tyrosine aminotransferase activity. More important, however, was the observation that dibutyryl cyclic AMP had no effect on the ornithine decarboxylase activity of H-35 hepatoma cells maintained in the salts/glucose solution over the entire concentration range of dibutyryl cyclic AMP (1 μM–10 mM) tested.

Qualitatively similar results were obtained with dexamethasone. Dexamethasone, from 0.1 nM to 10 μM, had no effect on ornithine decarboxylase activity when H-35 hepatoma cells were maintained in the salts/glucose solution. Assays of tyrosine aminotransferase of these samples revealed that dexamethasone gave a concentration-dependent increase in the tyrosine aminotransferase activity, with a half-maximal increase observed at 5 nM of dexamethasone (data not shown).

Analysis of the cyclic AMP and glucocorticoid receptor proteins of H-35 hepatoma cells maintained in the Eagle's minimal essential medium and in the Earle's balanced salt solution

The inability of dibutyryl cyclic AMP and dexamethasone to increase ornithine decarboxylase activity of H-35 hepatoma cells maintained in the salts/glucose solution under conditions where these agents were fully effective in increasing tyrosine aminotransferase activity raises the following question: Given two responses to cyclic AMP in a single cells, does each proceed through independent causal chains that (a) involve different types of cyclic AMP-dependent protein kinase and glucocorticoid receptor; or (b) branch at a

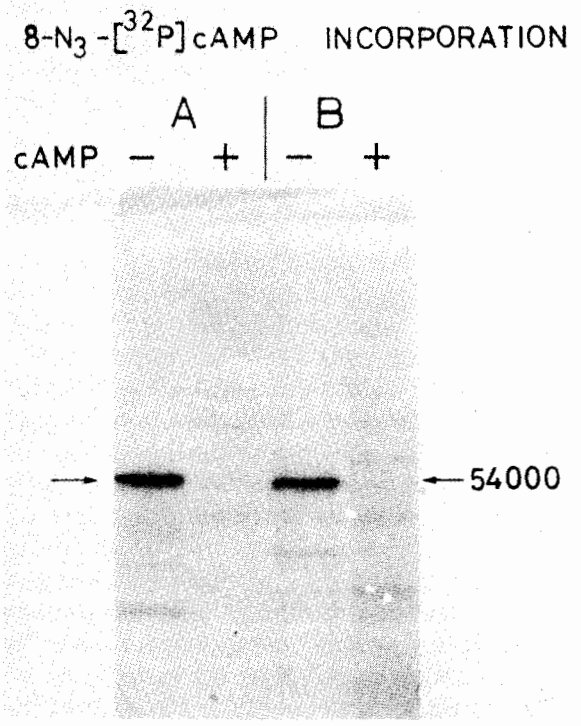

Fig. 4. Autoradiograph illustrating the specific incorporation of 8-N_3-[^{32}P]cAMP into cytosol proteins of H-35 hepatoma cells maintained in (A) Eagle's minimal essential medium and (B) Earle's balanced salt solution for 24 h. The photoactivated incorporation of 8-N_3-[^{32}P]cAMP into cytosol proteins of H-35 hepatoma cells was done under standard conditions using 1 μM 8-N_3-[^{32}P]cAMP in the absence and presence of 50 μM cyclic AMP. Each channel contains 200 μg protein. The arrows identify the position on the gel of the 54000-dalton regulatory subunit of the type II cAMP-dependent protein kinase.

TABLE I

COMPARISON OF THE CYCLIC AMP-DEPENDENT PROTEIN KINASE ACTIVITY OF H-35 HEPATOMA CELLS MAINTAINED IN EAGLE's MINIMAL ESSENTIAL MEDIUM AND IN EARLE's BALANCED SALT SOLUTION

Cells were serum-deprived either in minimal essential medium or in salts/glucose solution for 24 h, cells were harvested and a 100000 × *g* supernatant fraction (cytosol) prepared. The identity and amount of regulatory subunit of cyclic AMP-dependent protein kinase were studied by the covalent incorporation of 8-N_3-[^{32}P]cAMP as well as the reversible binding of [^{3}H]cAMP. The catalytic activity of cyclic AMP-dependent protein kinase was studied by assaying for the cyclic AMP-dependent histone kinase activity. All assays were carried out under standard conditions. Results represent means of three independent experiments ± standard errors.

Source of cytosol	Regulatory subunit		Catalytic subunit (units/mg protein)
	8-N_3-[^{32}P]cAMP incorporated into R_{II} [a] (pmol/mg protein)	cyclic [^{3}H]AMP bound (pmol/mg protein)	
H-35 cells maintained in Eagle's minimal essential medium	2.01 ± 0.31	2.15 ± 0.44	317 ± 65
H-35 cells maintained in salts/glucose solution	1.89 ± 0.28	2.08 ± 0.35	290 ± 49

[a] The 54000-dalton regulatory subunit of the type II cyclic AMP-dependent protein kinase.

point after receptor binding and activation, and that the use of the salts/glucose solution obliterates the effect of cyclic AMP and dexamethasone in activating the pathway leading to ornithine decarboxylase induction. To this end, we have carried out experiments both to identify and to quantitate the amount and activation of cyclic AMP-dependent protein kinase in extracts of H-35 hepatoma cells maintained in the Eagle's minimal essential medium and in the salts/glucose solution. Experiments were also carried out to examine the binding of dexamethasone to its receptor protein, again comparison was made between cells maintained in Eagle's minimal essential medium and in the salts/glucose solution.

The regulatory subunit of cyclic AMP-dependent protein kinase was studied by either the covalent incorporation of 8-N_3-[^{32}P]cAMP or the reversible binding of cyclic [^{3}H]AMP. The catalytic subunit activity of cyclic AMP-dependent protein kinase was studied by the incorporation of ^{32}P from [γ-^{32}P]ATP into histone II-AS.

Results in Fig. 4 and Table I illustrate that the pattern and amount of 8-N_3-[^{32}P]cAMP incorporated into cytosol proteins obtained from H-35 hepatoma cells incubated in the Eagle's minimal essential medium for 24 h were not significantly different from those of cells incubated in the salts/glucose solution for an identical period of time. In both cases, 8-N_3-[^{32}P]cAMP was specifically incorporated into a 54000-dalton protein, which has previously been identified as the regulatory subunit of a type II cyclic AMP-dependent protein kinase [8]. The amount of 8-N_3-[^{32}P]cAMP covalently incorporated was similar to the amount of cyclic [^{3}H]AMP reversibly bound (Table I). Assays of the catalytic subunit of cyclic AMP-dependent protein kinase demonstrated the presence of similar activity in extracts obtained from H-35 cells maintained in Eagle's minimal essential medium or the salts/glucose solution. These results suggest that there is little or no difference in the amount or activation of cyclic AMP-dependent protein kinase present in extracts of cells maintained in the Eagle's minimal essential medium when compared to that of cells maintained in the salts/glucose solution.

Experiments on the uptake and binding of [^{3}H]dexamethasone by whole cells revealed no differences either in the affinity (K_d) or the capacity (R_T) for binding between H-35 hepatoma cells maintained in Eagle's minimal essential medium

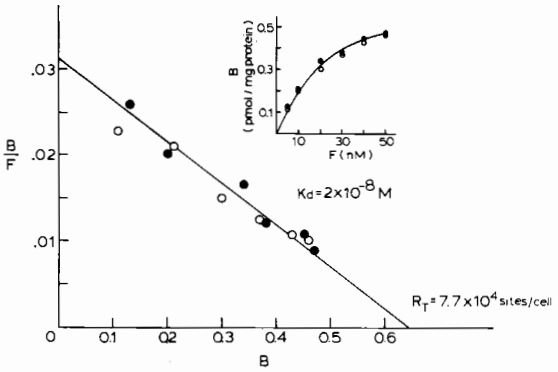

Fig. 5. Scatchard analysis of specific uptake/binding of [^{3}H]dexamethasone by H-35 hepatoma cells maintained in Eagle's minimal essential medium and in Earle's balanced salt solution. H-35 hepatoma cells were serum deprived either in Eagle's minimal essential medium (●) or in the salts/glucose solution (○) for 12 h, at which point cells were refed with fresh media and various concentrations of [^{3}H]dexamethasone were added. Cells were incubated with [^{3}H]dexamethasone for 30 min at 37°C. The amount of [^{3}H]dexamethasone specifically bound, i.e. that which is displaced by a 100-fold excess of unlabeled dexamethasone, is presented. (Note: the amount of nonspecific binding represents approx. 20–30% of the total radioactivity bound.) The number of binding sites per cell (R_T) was calculated from the maximal amount of [^{3}H]dexamethasone bound per mg protein (expressed in pmol/mg) and that there are $5.2 \cdot 10^6$ cells per mg protein. Inset: the amount of [^{3}H]dexamethasone bound (B) plotted as a function of the concentration of [^{3}H]dexamethasone added (F).

and in the salts/glucose solution. In both cases, the amount of [^{3}H]dexamethasone bound was approx. $7.7 \cdot 10^4$ sites/cell with an apparent affinity of about 20 nM.

Half-lives of ornithine decarboxylase and tyrosine aminotransferase of H-35 hepatoma cells maintained in Eagle's minimal essential medium and in Earle's balanced salt solution

Previous studies have demonstrated that the half-lives of both ornithine decarboxylase and tyrosine aminotransferase may be affected by the culture conditions used [14,15]. In view of this consideration and the lack of increase of ornithine decarboxylase activity in response to stimulation by either dibutyryl cyclic AMP or dexamethasone when cells were maintained in the salts/glucose solution, we studied and compared the $t_{1/2}$ of ornithine decarboxylase and tyrosine aminotransferase of cells maintained in the Eagle's minimal essential medium and in the salts/glucose solution.

Dibutyryl cyclic AMP was added to H-35 hepatoma cells incubated in Eagle's minimal essential medium to induce both ornithine decarboxylase and tyrosine aminotransferase activities. After a 4 h induction period, cells were rinsed and refed with either fresh minimal essential medium or the salts/glucose solution, Cycloheximide (50 μg/ml) was then added. The time needed after the addition of cycloheximide for a 50% decrease of the induced enzyme activity was used as an index of the $t_{1/2}$ of the enzyme. The $t_{1/2}$ values of ornithine decarboxylase of H-35 hepatoma cells incubated in the Eagle's minimal essential medium and in the salts/glucose solution were, respectively, 45 and 38 min. The corresponding $t_{1/2}$ values for tyrosine aminotransferase were 2.5 and 1.5 hours. While there are a number of pitfalls associated with the use of cycloheximide to determine $t_{1/2}$ of proteins [16,17], these results qualitatively suggest that the difference in half-lives of ornithine decarboxylase may not be sufficient to account for the marked difference in the inducibility of this enzyme under different experimental conditions.

Discussion

The results of this study indicate that asparagine may have a primodial role in the induction of ornithine decarboxylase activity of H-35 hepatoma cells, while the effects of cyclic AMP and dexamethasone are dependent on the incubation medium used. In a previous study [5], it was suggested that the action of cyclic AMP in the induction of ornithine decarboxylase may be mediated by asparagine or other amino acids; our present findings are in agreement with this hypothesis.

The results obtained on the regulation of ornithine decarboxylase are to be contrasted to that obtained for tyrosine aminotransferase; the effects of cyclic AMP and dexamethasone as inducers of tyrosine aminotransferase activity are direct and do not appear to be modulated or mediated by asparagine or other components normally present in the Eagle's minimal essential medium.

The possibility of decreased receptor binding as the basis for the inactivity of dibutyryl cyclic AMP and dexamethasone in the induction of ornithine decarboxylase activity of H-35 hepatoma cells maintained in the salts/glucose solution seemed unlikely. No significant difference, either in the affinity or the capacity, of the binding of cyclic AMP and dexamethasone to their respective receptor proteins was detected. The identification of a single type, the type II, cyclic AMP-dependent protein kinase [8] and a single component of [^{3}H]dexamethasone binding in the Scatchard analysis raises the possibility that the biochemical pathways, elicited either by cyclic AMP or dexamethasone, leading to increases of ornithine decarboxylase and tyrosine aminotranserase activities may branch at a point after receptor binding and activation.

In studying the induction of ornithine decarboxylase and tyrosine aminotransferase activities in cells maintained in the salts/glucose solution, one important consideration is the possibility of enhanced degradation of ornithine decarboxylase and tyrosine aminotransferase. In both bacterial and mammalian cell systems, the degradative rate for total cell protein is affected by the nutritional environment. In this connection, we have carried out experiments to determine the $t_{1/2}$ of ornithine decarboxylase and tyrosine aminotransferase of H-35 hepatoma cells maintained in Eagle's minimal essential medium and in the salts/glucose solution. We observed a 15 and a 40% decrease, respectively, in the $t_{1/2}$ values of ornithine decarboxylase and tyrosine aminotransferase of H-35 hepatoma cells maintained in the salts/glucose solution when compared to those of cells maintained in the Eagle's minimal essential medium. However, it seems unlikely that the decreased $t_{1/2}$ of cellular protein (i.e. enhanced degradation) is the primary cause for the inactivity of dibutyryl cyclic AMP or dexamethasone in the induction of ornithine decarboxylase when cells are maintained in the salts/glucose solution. Thus, (1) while both ornithine decarboxylase and tyrosine aminotransferase were degraded at an enhanced rate in cells maintained in the salts/glucose solution (and the magnitude of this enhanced degradation was apparently greater for tyrosine aminotransferase than for ornithine decarboxylase), the addition of dibutyryl cyclic AMP and dexamethasone increased tyrosine aminotransferase activity but was without effect on ornithine decarboxylase activity; (2) the action of asparagine as an inducer of ornithine decarboxylase was similar whether Eagle's minimal essential medium or the salts/glucose solution was used as the incubation medium.

It may be noted that the use of a simple salts/glucose solution as the incubation medium in enzyme induction studies may offer a means of studying the mode of action of various enzyme inducers, and to define the possible roles (none, supportive, modulatory, or mediatory) of the various medium components in enzyme induction.

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