Interaction of Glucocorticoid Hormones and Cyclic Nucleotides in Induction of Tyrosine Aminotransferase in Cultured Hepatoma Cells*

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Reproducible induction of the enzyme tyrosine aminotransferase by dibutyryl cAMP (Bt,cAMP) in a line of HTC hepatoma cells in suspension culture requires that the cells be preinduced with dexamethasone, a synthetic glucocorticoid which itself induces tyrosine aminotransferase. Concentrations of dexamethasone that do not induce tyrosine aminotransferase fail to support Bt,cAMP induction, removal of the steroid from the medium leads to a loss of the Bt,cAMP effect, and an HTC cell line whose aminotransferase is not steroid-inducible does not respond to the cyclic nucleotide. We show that the further induction of tyrosine aminotransferase by Bt,cAMP in dexamethasone-treated cells is due to an increased rate of enzyme synthesis. The cyclic nucleotide has no effect on aminotransferase synthesis in cells grown in the absence of steroid.

Several lines of evidence suggest that dexamethasone acts at a step beyond the activation of protein kinase by cAMP: (a) basal levels of cAMP are not altered by growth of HTC cells in dexamethasone; (b) accumulation of cAMP from the medium is not enhanced; (c) the glucocorticoid does not induce cAMP dependent protein kinase in HTC cells; and (d) there is no augmentation of cAMP binding to the regulatory protein, nor is there any change in cAMP activation of protein kinase caused by growth in dexamethasone. These results help define a system that should be useful in studying the interaction of cyclic nucleotides and steroid hormones.

The concept that adrenal glucocorticoids allow key metabolic processes to achieve maximal rates was first proposed by Ingle (1). This role of glucocorticoids has subsequently been shown to be important in lipolysis (2, 3), gluconeogenesis (4, 5), glycogen synthesis (6), and glycogenolysis (7). These processes are primarily regulated by peptide and/or catecholamine hormones, presumably through the action of cAMP. Although steroid hormones are thought to act by mechanisms separate from the cAMP-dependent protein kinase system (8), the examples cited above indicate a definite interplay of glucocorticoids and cyclic nucleotides, although the biochemical site of this interaction remains obscure.

Induction by glucocorticoids of the hepatic enzyme tyrosine aminotransferase (L-tyrosine-2-oxoglutarate aminotransferase, EC 2.6.1.5), which is rate-limiting for tyrosine degradation, has served as a valuable model for studying the glucocorticoid action of this class of hormones (9). In addition, cAMP or its dibutyryl derivative induces this enzyme in intact adult liver (10), fetal liver in organ culture (11), and in some hepatic-derived tissue culture lines (12-14). Although an early report suggested that glucocorticoid induction of tyrosine aminotransferase is independent of cAMP (10), the converse is not true. Several studies suggest that cAMP-mediated induction of this enzyme is not maximal unless glucocorticoids are also present. For example, adrenalectomy significantly blunts the inductive effect of cAMP or analogs on rat liver tyrosine aminotransferase (10, 16, 17). Also, combinations of glucocorticoids and dibutyryl cAMP result in a synergistic aminotransferase induction in rat liver (10) and fetal liver organ culture (11). Finally, cortisol has been shown to potentiate the induction of tyrosine aminotransferase by Bt,cAMP in the cultured H35 hepatoma cell system (12). This requirement for glucocorticoids for maximal cAMP-mediated aminotransferase induction has recently been clarified in the well-defined HTC cultured hepatoma cell system. In suspension cultures of HTC cells, significant induction of tyrosine aminotransferase cannot be routinely achieved (12, 14, 15), whereas in monolayer cultures a small effect is observed (13, 18). Restoration of the full responsiveness of HTC cells grown in suspension culture to butyrylated derivatives of cAMP can be accomplished by growing the cells in culture medium containing a glucocorticoid hormone for a period of time prior to addition of the cyclic nucleotide (19). In this paper we provide a detailed description of this phenomenon, which should prove useful in studying the interaction of steroid hormones and cyclic nucleotides.

**EXPERIMENTAL PROCEDURES**

Cell Culture-HTC cells were grown in suspension culture in a modified Swim's S77 (S77S) medium as described previously (14).

* The abbreviations used are: Bt,cAMP, N\(^{\prime}\)O\(^{\prime}\)-dibutyryl adenosine 3':5'-monophosphate; H35 cells, Reuber hepatoma cells; RLC cells, rat liver culture.
Stock cultures were kept in log growth (2 to 6 x 10^6 cells/ml) by diluting them with fresh medium every other day. Where indicated, cells were removed from the S77 medium by centrifugation and resuspended in "induction medium" (S771) which differs only in that it contains 2% dialyzed bovine serum (v/v) rather than 5% each of fetal calf and bovine sera (v/v). S771 minimizes the changes in basal aminotransferase activity that result when fresh growth medium (serum) is added to the cells. Routine checks for PPLO and bacterial contamination have been negative.

The steroid-resistant HTC cell clone (HTC-M714H) is one of several isolated, in the absence of selective pressure, by serial subclonings of the wild type HTC cell line (20). These cells possess basal levels of aminotransferase which can be precipitated or neutralized by anti-aminotransferase antibody. They also possess glucocorticoid receptors which, by several criteria, appear to be the same as those of wild type cells, but the aminotransferase of these cells is virtually not induced by glucocorticoids (20).

**Enzyme Assays**—The activity of tyrosine aminotransferase was determined by a modified method of Diamandstein (21) and protein concentration was determined by the method of Lowry et al. (22). Results are expressed as specific activity (E,) in terms of picomoles of p-hydroxphenylpyruvate formed/min.

The preparation of cytosol extracts of HTC cells and assays for protein kinase and cAMP binding activities were performed as described in detail previously (23, 24). Briefly, protein kinase activity was determined by measuring the transfer of phosphate from [γ-32P]ATP to purified calf thymus histone H1 in the presence of the indicated concentrations of unlabeled cAMP. Binding activity for cAMP was determined by incubating cytosol extracts with various concentrations of [3H]cAMP (16.3 Ci/mmol, Schwarz Bio-Research), and after 2-h incubation at 4 °C the [3H]cAMP-protein complex was collected on Millipore filters.

**cAMP Measurements**—To quantitate intracellular cAMP levels, HTC cells were removed from the incubation medium by centrifugation and were rapidly washed twice with an ice-cold solution consisting of 0.05 M potassium phosphate, pH 7.6, 0.1 M KCl, and 0.01 M theophylline. Each cell pellet was then suspended in 1 ml of 6% trichloroacetic acid containing [3H]cAMP (approximately 5,000 cpm) as a tracer to monitor recovery. The cells were disrupted by a 20-s burst from a Biosonic III probe sonicator and then subjected to centrifugation at 10,000 rpm for 10 min. The supernatant fraction was saved, and after addition of 200 μl of 1 N HCl, it was extracted three times with ethyl ether. The ether was removed by evaporation, and then the samples were placed on an 8 cm column of Dowex AG50W X8, 100 to 200 mesh, equilibrated in 0.1 N HCl. Contaminating nucleotides were removed by the addition of 4 ml of 0.1 N HCl, then cAMP was eluted with water, concentrated in a Buchler Evapomix, and precipitated by adding 4 ml of 0.1 N HCI, 0.1 mM sodium acetate buffer. Duplicate samples of two to three different volumes were assayed for cAMP by radioimmunoassay (25). Total protein was determined on the trichloroacetic acid precipitate and results, after correction for recovery, are expressed as picomoles of cAMP/mg of protein. Treatment of the extracts with phosphodiesterase abolished all cAMP activity, indicating that the intracellular nucleotide was in fact cAMP, and not [3H]cAMP, which is resistant to phosphodiesterase.

**Rate of Synthesis of Tyrosine Aminotransferase**—The rate of synthesis of aminotransferase was determined by quantifying the radioactivity incorporated into immunoprecipitable aminotransferase (25, 26). Following a 15-min pulse with [3H]leucine, cells were first washed with 0.01 M potassium phosphate buffer, pH 7.6, containing 0.1 M KCl, and 0.2 mM pyridoxal phosphate, 1 mM ethylenediaminetetraacetic acid, and 5 mM L-α-arginine, and then stored frozen at -15 °C. After thawing and sonification, aminotransferase was partially purified by high speed centrifugation, heat precipitation, and DEAE-cellulose chromatography (20). Rabbit anti-aminotransferase antiserum, supplied by Barbara Levinson of the University of California at San Francisco, was added in an amount calculated to completely precipitate the aminotransferase in the extract plus an additional "carrier" aminotransferase. A second, identical immunoprecipitation was done to afford a correction for nonspecific counts bound to the antigen-antibody complex. After correction for the latter, plus the losses in the purification steps, the net counts per min in tyrosine aminotransferase are expressed as the percentage of the counts per min in total protein, thus affording an estimate of the rate of synthesis of this protein.

**Materials**—Powdered S77 medium was obtained from Grand Island Biological, Inc., and serum was purchased from the St. Louis Serum Co. [3H]cAMP and cAMP assay materials were purchased from Schwarz Bio-Research and unlabeled cyclic nucleotides from the Boehringer Mannheim Corp. All other chemicals were obtained from standard commercial sources. Dexamethasone was a gift from Merck & Co. and purified histone H1 was supplied by Dr. Roger Chalkley of the University of Iowa.

**RESULTS**

**Induction of Tyrosine Aminotransferase by Bt,cAMP in HTC Cells**—Tyrosine aminotransferase cannot be routinely induced in suspension cultures of HTC cells by Bt,cAMP, an observation first reported in 1968 and several times subsequently (12, 14, 15). This behavior is also shown by the HTC subline studied herein. In these cells, grown in suspension culture, there was no consistent induction of basal enzyme by Bt,cAMP, although in a few experiments there was a 20% increase in enzyme activity in cyclic nucleotide-treated cultures (Figs. 1 to 3 and Table I). The addition of 1 mM Bt,cAMP to cultures previously incubated in dexamethasone, however, consistently results in further increase of aminotransferase activity above that achieved with steroid alone (Fig. 1). For example, after incubation for 48 h in steroid-containing medium, addition of the cyclic nucleotide nearly doubles the level achieved by dexamethasone alone; this is an absolute increment 8 to 10 times greater than the enzyme activity found in cells maintained in S77 medium. A previous study showed that this effect, the further induction of tyrosine aminotransferase with cyclic nucleotides, after prior induction by steroids, has a time course similar to aminotransferase induction by Bt,cAMP alone in RLC cells. There is a lag period of 1 to 1.5 h, peak enzyme activity levels are reached between 4 to 6 h,
and levels return to the preinduced level at 8 to 10 h (19). The effect has been seen within 10 h after addition of the steroid, is generally most prominent between Days 2 and 6, and has been seen after 8 days of growth in dexamethasone containing medium. This specificity of the response is indicated by the fact that only concentrations of 0.1 to 1.0 mM N\(^\text{6}\)-monobutyryl-cAMP or Bt\(_{c}\)cAMP are effective. cAMP, cGMP, Bt\(_{c}\)GMP, adenosine, and sodium butyrate do not work (Fig. 1).

**Concentration of Dexamethasone Required for Induction by Bt\(_{c}\)cAMP** — An important question is whether a minimal level of dexamethasone is required for the manifestation of the Bt\(_{c}\)cAMP induction. The results illustrated in Fig. 2 indicate that such is the case. At 2 nM dexamethasone there is no aminotransferase induction and no Bt\(_{c}\)cAMP response (Fig. 2). The lowest effective dexamethasone concentration, 5 nM, permits a small response to Bt\(_{c}\)cAMP, while maximal effects are seen with steroid concentrations of 50 nM or greater. The leftward shift of the induction curve throughout the dexamethasone concentration range of 5 to 50 nM might suggest that the cyclic nucleotide is somehow enhancing entrance of the steroid into the cell, or its subsequent cytoplasmic and/or nuclear binding. The fact that Bt\(_{c}\)cAMP still causes an increase in aminotransferase activity at dexamethasone concentrations 50 times those required for maximal steroid induction makes such a possibility remote. This study also shows that, at each concentration of dexamethasone, the effect is dependent on the concentration of Bt\(_{c}\)cAMP, as 0.1 mM gave values between those of steroid alone and steroid plus 1 mM Bt\(_{c}\)cAMP. Occasionally 0.05 mM Bt\(_{c}\)cAMP has been effective, although usually 0.1 mM is the smallest effective concentration.

The induction of HTC cell aminotransferase by Bt\(_{c}\)cAMP depends upon the antecedent induction of the enzyme by steroid hormones (Figs. 1 and 2), and the extent of this effect appears to depend on the degree of induction achieved by the hormone (Fig. 2). One might thus expect that the effect of cyclic nucleotides in HTC cells would be lost upon removal of the steroid inducer.

**Effect of Steroid Deinduction** — Previous studies have shown that the increased steady state rate of synthesis of tyrosine aminotransferase achieved after addition of dexamethasone depends upon the continued presence of the inducer (25). Thus deinduction, accomplished by removal of the inducing steroid, results in an exponential decline in the concentration of aminotransferase mRNA, the rate of synthesis of this protein and, ultimately, its specific activity (27). The persistent presence of dexamethasone is also required for sustained expression of the Bt\(_{c}\)cAMP effect (Fig. 3). HTC cells grown in S77S + dexamethasone medium for 4 days had an aminotransferase specific activity of 185 milliunits/mg of protein compared to the basal value of 14. Addition of 0.1 mM Bt\(_{c}\)cAMP (Fig. 3) caused a further increase of 73 milliunits. Deinduction resulted in a rapid decline in aminotransferase activity, and the Bt\(_{c}\)cAMP effect was lost within 12 h after steroid removal from the cells. After the basal level of aminotransferase activity is reached, the cells are again unresponsive to Bt\(_{c}\)cAMP (Fig. 3).

**Lack of Bt\(_{c}\)cAMP Induction in Steroid-resistant Cell Line** — That some steroid-mediated step coincident with induction or aminotransferase is a prerequisite for the effect seen with Bt\(_{c}\)cAMP can be tested in a cell line in which the glucocorticoid is an ineffective inducer. The M714H clone offers this possibility. These cells contain a normal concentration of the cytosol glucocorticoid binding protein which by several criteria, including temperature-dependent translocation to the nucleus in whole cells, behaves in a normal manner. Aminotransferase induction however does not result (Table I). In the M714H clone line, like the wild type HTC cell used here,
Bt,cAMP does not significantly induce aminotransferase. However, unlike the latter, growth in dexamethasone does not result in any induction of the aminotransferase in M714H cells by Bt,cAMP (Table I).

Thus concentrations of dexamethasone which do not themselves induce aminotransferase do not restore the response to Bt,cAMP, removal of the steroid results in a concordant loss of response to the cyclic nucleotide, and finally cells which do not respond to dexamethasone do not respond to the combination of dexamethasone and cyclic nucleotide.

Steroid-mediated Bt,cAMP Induction Is due to Increased Rate of Synthesis of Aminotransferase — The dexamethasone-induced increase of aminotransferase activity in HTC cells is due to a specific increase in the rate of synthesis of the enzyme (25), as is the cAMP-mediated induction in liver (10) and cultured H35 hepatoma cells (28). It thus seemed likely that the increased enzymic activity caused by addition of Bt,cAMP to dexamethasone-treated HTC cells would be due to an increase in the rate of aminotransferase synthesis above that caused by the steroid itself, particularly since we have shown that cycloheximide, an inhibitor of protein synthesis, does not respond to dexamethasone and cyclic nucleotide.

As illustrated in Table II, the steroid-mediated increase in aminotransferase activity in response to Bt,cAMP is accompanied by a similar increase in the relative rate of synthesis of this protein. In each of four such experiments the increase in the rate of synthesis between steroid and steroid + Bt,cAMP-treated cells was directly proportional to the increase in specific activity. The further induction caused by Bt,cAMP thus appears to be due to an enhanced rate of aminotransferase synthesis, and not to the activation of preexisting enzyme protein.

Relationship of Intracellular cAMP Levels to Aminotransferase Induction — Several experiments were performed in an attempt to provide an explanation for the interaction of these two different regulatory molecules. Steroid hormones reportedly augment the increase in cAMP caused by incubation of target tissues with other hormones. Aldosterone potentiates the vasopressin-induced increase of cAMP in toad bladder (29), dexamethasone augments the cAMP increase seen in fibroblasts incubated with catecholamines and prostaglandins (30), and dexamethasone has also been shown to potentiate the prostaglandin-induced increase of cAMP in HTC cells (31).

This effect, ascribed to a decrease in the activity of phosphodiesterase, has been postulated to be involved in the permissive effects of steroid hormones (31). If such a mechanism were operative, one might hypothesize that steroid hormones permit intracellular HTC cell cAMP levels to reach an "aminotransferase induction threshold" unachievable in cells incubated with Bt,cAMP in regular medium. Although studies performed on cells grown in the absence of steroids suggested this was not a likely explanation for Bt,cAMP induction or the lack thereof (14), the experiment described in Table III directly tested this hypothesis. Growth of HTC cells in S77S + dexamethasone medium for 1 or 4 days caused no alteration in the intracellular concentration of cAMP, suggesting that phosphodiesterase activity in not decreased enough to cause an elevation of basal cAMP levels. This is in contrast to a previous report in which HTC cells grown in 10 μM dexamethasone for 72 h, with 1 mM theophylline added for the last 10 min, had an intracellular cAMP concentration of 1.5 pmol/mg of protein compared to 0.4 pmol/mg in control cells (31). More importantly, the intracellular cAMP levels achieved by incubating HTC cells, grown in either S77S or S77S + steroid, with 0.1 mM Bt,cAMP for 15 or 30 min were, if anything, higher in the control cells (Table III). Other studies conducted for as long as 120 min showed similar findings, thus the cyclic nucleotide "threshold" postulate is not applicable. It is also clear that the lesion in HTC cells must lie beyond the ability to accumulate cAMP.

Table I

| Cell type          | Tyrosine aminotransferase specific activity |
|--------------------|-------------------------------------------|
|                    | S77                     | S77 + dexamethasone |
| Control            | 3.9 ± 0.2               | 4.7 ± 0.3           |
| Bt,cAMP            | 2.0 ± 0.2               | 2.3 ± 0.2           |
| Control + Bt,cAMP  | 30.5 ± 1.4              | 49.7 ± 1.2          |
| Bt,cAMP            | 2.4 ± 0.3               |                    |

Table II

| Culture conditions | Total amino-transferase | Tyrosine aminotransferase |
|--------------------|-------------------------|---------------------------|
|                    | Total protein | cpm | cpm × 10⁴ | %     |
| S77S medium        | 708          | 1.28 | 0.055     | 27    |
| S77S ± 1 mM Bt,cAMP| 636          | 1.20 | 0.053     | 25    |
| S77S + 5 μM dexamethasone | 9469         | 1.70 | 0.306     | 199   |
| S77S + 5 μM dexamethasone + 1 mM Bt,cAMP | 3205           | 1.31 | 0.245     | 175   |
TABLE III

Effects of dexamethasone on cAMP content of HTC cells before and after addition of 0.1 mM Bt,cAMP

| Incubation medium | Incubating time | Intracellular cAMP concentration (pmol/mg protein) |
|-------------------|-----------------|-----------------------------------------------|
| S77S + dexamethasone | 0.90 | 15 min | 31.3 |
|                   | 0.85 | 30 min | 42.7 |
|                   | 16.8 | 15 min | 30 min | 14.0 |
| Incubated 4 days  | S77S + dexamethasone | 1.18 | 15 min | 35.5 |
|                   | 36.1 | 30 min | 36.1 |

Fig. 4. DEAE-Sephadex chromatography of cytosol protein kinase from HTC cells grown in the presence or absence of dexamethasone (DEX). HTC cells were grown in S77S ± dexamethasone for 4 days. The procedures used to prepare cytosol extracts from these cells and liver, and for the chromatography were described previously (14). Protein kinase assays were performed on aliquots from each fraction in the presence of 1 μM cAMP. The symbols indicate the protein kinase activity from liver, HTC cells grown in S77S, and in S77S + 5 μM dexamethasone.

DISCUSSION

The important role products of the adrenal cortex play in regulating carbohydrate metabolism (35, 36) was well established by the time the so-called glucocorticoid hormones were specifically identified in the late 1940's. Shortly thereafter it was recognized that such hormones are involved in a variety of seemingly unrelated processes, leading luger to postulate that the glucocorticoids are general hormones which allow tissues to sustain homeostasis by permitting maximal rates of critical metabolic processes (1). The classical examples of this requirement for glucocorticoids are gluconeogenesis, glycogenolysis, and glycogenesis, processes on which the steroid has little or no effect (4–7). Steroids themselves affect lipolysis and inhibition of glucose entry into cells and in addition greatly magnify the effect of other hormones on these processes (37–39). The hormones which primarily regulate these processes, epinephrine, glucagon, and growth hormone, all are thought to act by increasing intracellular cAMP concentrations and/or activity of the nucleotide. In addition, other cAMP-mediated hormone effects, such as the action of catecholamines on the cardiovascular system (40) and on the reaction of skin to cold (41, 42) as well as amino acid transport in liver cells (43), appear to depend on glucocorticoids. The mechanism of the apparent interaction of the steroid and peptide hormones (presumably acting through cAMP) has not been explained in any of these cases (44).

The experiments which suggest that glucocorticoids and cyclic nucleotides interact in regulating tyrosine aminotransferase activity were cited in the introduction. The availability of a tissue culture cell line in which consistently reproducible cAMP effects require that steroid be present allows for a detailed analysis of this interplay. One can approach this problem either from the manner by which cAMP could influence...
binding assays were performed as described under "Experimental procedures." The results in A illustrate the amount of \([^3H]cAMP\) bound/100 mg of cytosol protein (ordinate) as a function of the amount of \([^3H]cAMP\) added (abscissa). B illustrates protein kinase stimulation, expressed as the percentage increase over the control value (no \(cAMP\) added), as a function of \(cAMP\) added (abscissa). The same cytosol extracts were used for both the binding and protein kinase determinations, and the results represent the average values of two different experiments. The symbols indicate HTC cells grown in S77S (○) and in S77S + 5 \(\mu M\) dexamethasone (●).

**Fig. 5.** Effect of dexamethasone on \(cAMP\) binding activity and protein kinase stimulation in HTC cells. HTC cells were maintained in log growth for 4 days by diluting them every 48 h with S77S or S77 containing 5 \(\mu M\) dexamethasone. Cytosol extracts were prepared as described previously (14) and protein kinase and \(cAMP\) binding assays were performed as described under "Experimental Procedures." The results in A illustrate the amount of \([^3H]cAMP\) bound/100 mg of cytosol protein (ordinate) as a function of the amount of \([^3H]cAMP\) added (abscissa). B illustrates protein kinase stimulation, expressed as the percentage increase over the control value (no \(cAMP\) added), as a function of \(cAMP\) added (abscissa). The same cytosol extracts were used for both the binding and protein kinase determinations, and the results represent the average values of two different experiments. The symbols indicate HTC cells grown in S77S (○) and in S77S + 5 \(\mu M\) dexamethasone (●).

ence any of the steps known to be involved in glucocorticoid action or vice versa.

Several experiments suggest that steroid induction is required in order to consistently produce an effect on Bt\(_2\)cAMP on tyrosine aminotransferase in HTC cells grown in suspension culture. (a) Concentrations of steroid which do not induce aminotransferase fail to restore responsiveness to Bt\(_2\)cAMP (Fig. 1). (b) The maximal response to Bt\(_2\)cAMP is obtained when the steroid induction nears its maximal level (Fig. 2). Since the nucleotide effect is seen even with concentrations of steroid 50 times greater than required to elicit maximal induction, it is unlikely that Bt\(_2\)cAMP is enhancing any of the early steps of steroid hormone action such as cytoplasmic and/or nuclear binding. A study by Rousseau et al., which appeared while this manuscript was in preparation, offers direct evidence for the lack of such an effect (45).

When aminotransferase levels return to the basal level after removal of the glucocorticoid the cyclic nucleotide is again ineffective (Fig. 3).

(d) Addition of dexamethasone to a variant line of HTC cells, in which the steroid itself causes no induction of aminotransferase, does not permit any induction of this enzyme by Bt\(_2\)cAMP (Table I). These results thus differ from the observation that noninducing concentrations of dexamethasone are effective in making H35 cultured hepatoma cells sensitive to cyclic nucleotides (46), or that HTC cells grown in monolayer culture respond to cyclic nucleotide in the absence of steroid (13, 18). Suspension cultures of HTC cells seem to respond differently; during the past 2 years we have rarely seen aminotransferase induction by Bt\(_2\)cAMP in the absence of prior induction by dexamethasone (14, 15) (Figs. 1 to 3). This could be due to some fundamental difference in the way attached and suspended HTC cells respond to steroid hormone or it could be due to some difference in the culture media used by different laboratories, such as the concentration of steroids in the serum employed. It is important to resolve this point for the mechanistic implications are quite different. Cyclic AMP is thought to enhance the translation of protein from pre-existing mRNA (47). This model predicts that the cyclic nucleotide will affect a similar percentage increase of aminotransferase in both basal and steroid-induced cells. The steroid would thus not be rate-limiting but would simply increase the amount of translatable aminotransferase mRNA (48). An absolute requirement of steroid treatment for a cyclic nucleotide effect could not be explained by this model. In this case the steroid would have to effect some process which then allows Bt\(_2\)cAMP to act, and induction would occur only when this has been achieved.

Steroid-mediated aminotransferase induction requires cyclic adenine nucleotides since only Bt\(_2\)cAMP or N°-monobutyryl-cAMP are effective (Fig. 1). cAMP or its dibutyryl derivative do not work, and possible metabolites of Bt\(_2\)cAMP such as 5′-AMP, adenosine, and butyrate are also ineffective. We therefore looked for an effect of dexamethasone on several of the steps in the sequence of cAMP action. First, dexamethasone did not alter basal CAMP levels in HTC cells (Tables III), thus if this hormone decreases phosphodiesterase activity in these cells, this has not been suggested (31), this has no effect on steady state CAMP levels. Likewise, such treatment does not allow cells to accumulate more CAMP from the incubation medium, hence the concept that the glucocorticoid allows intracellular CAMP to reach an "induction threshold" does not appear to be a likely explanation for this interaction. The observation that glucocorticoids fail to increase CAMP entry into liver-derived cells has been noted by others (12, 16, 46), as has the observation that the effect of steroids occurs at a step beyond CAMP (4, 7). Further support for this notion is given by the observation that when H1C cells are made sensitive to cyclic nucleotides, the effective concentration range for these compounds is the same as in RLC cells, a line that is normally responsive (14).

Since cAMP is thought to act in eukaryotic cells by stimulating protein kinases, an effect of the glucocorticoid on this complex seemed possible, particularly since HTC cells lack the major cAMP-dependent protein kinase of liver (23, 33). Munder and Turkington suggested a precedent for such an effect by showing that prolactin increased the activities of both the regulatory and catalytic subunits of protein kinase (34). The experiments reported here do not support such an effect of
Glucocorticoid-Cyclic AMP Interaction

Glucocorticoids on HTC cell protein kinase. There is no suggestion of an induction of protein kinase (Fig. 4), and we were unable to demonstrate any change in the kinetics of binding of cAMP to the receptor protein or in the activation of kinase by cAMP (Fig. 5). Furthermore, Rousseau et al. have also recently shown that glucocorticoid hormones do not alter the basal or cyclic AMP-stimulated activity of hepatic protein kinase, nor do they effect its subcellular distribution (45). In addition, studies using HTC cells this group has shown that dexamethasone: (a) does not alter the ratio of basal to cAMP-stimulated protein kinase activity, an estimation of the in vivo activation of this enzyme; (b) does not alter the half-life of protein kinase; (c) does not result in a translocation of the kinase; and (d) does not inhibit the heat-stable protein inhibitor of protein kinase. The accumulated data from these two laboratories therefore suggests that the effect of the glucocorticoids is distal to the activation of cytoplasmic protein kinase, assuming this is involved in aminotransferase induction. Recently, a protein which can be phosphorylated has been found in a number of steroid hormone target tissues (49). This is thus a possible site for the biochemical interaction of these compounds. Other possible sites of cAMP action, such as ribosomal protein phosphorylation (50) or an effect independent of protein kinase, have not been excluded however.

The interaction of glucocorticoids with hormones that act through cAMP appears to be an important biological phenomenon. The general features of the mechanism of action of steroid hormones and cyclic nucleotides have been elucidated, and the regulation of tyrosine aminotransferase synthesis has also been studied in detail. The convergence of studies of these three processes in a cultured cell line that is amenable to experimental manipulation should provide an excellent opportunity for studying the general role of glucocorticoid hormones that was postulated 25 years ago.

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