Stimulation of Ornithine Decarboxylase Synthesis and Its Control by Polyamines in Regenerating Rat Liver and Cultured Rat Hepatoma Cells*

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Ornithine decarboxylase has been induced in log phase hepatoma cells grown in suspension culture. Induction with N^6,O^2'-dibutyryl cyclic adenosine 3':5'-monophosphate produced a 4-fold increase in enzyme activity by 3 hours which was followed by a return to base levels by 6 hours. Induction with dexamethasone, a potent synthetic glucocorticoid, exhibited a slow steady rate of increase in enzyme activity, reaching a plateau level of approximately 5- to 6-fold stimulation by about 12 hours. Induced cell and regenerating rat liver ornithine decarboxylase were shown to be indistinguishable by titration with antibody monospecific to the latter and by heat stability. L-[1^4C]Leucine incorporation into immunoprecipitable enzyme protein after induction in vitro or partial hepatectomy showed an increase which, when coupled with the increase in enzymatic activity, indicated de novo synthesis of enzyme protein.

Physiological concentrations of the naturally occurring polyamines, spermidine and spermine, abolish cyclic AMP induction whereas they have no effect on dexamethasone induction. Both inductions were abolished by cycloheximide; in contrast, inhibition by actinomycin D was complete for dexamethasone induction and only partial with respect to cyclic AMP induction. The different time pattern of induction seen with cyclic AMP and dexamethasone, the partial inhibition of the cyclic AMP induction seen with actinomycin D, as well as the absence of inhibition of the dexamethasone induction by polyamines, indicate that these inducers might affect different aspects of the control of the same enzyme.

Mounting experimental evidence points to a key role for the enzyme ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) as a key metabolic mediator in rapidly proliferating tissues. In catalyzing the decarboxylation of ornithine and the resultant formation of putrescine, ornithine decarboxylase is the first and rate-limiting enzyme of the metabolic pathway which defines the biosynthesis of the polyamines spermidine and spermine (1-3). Ornithine decarboxylase has been shown to be characteristically induced by hormones that regulate growth, such as growth hormone (4-7) and the glucocorticoids (8, 9). The role of cyclic AMP as mediator of many hormonal responses in hepatic enzyme induction has been documented both in vivo (10-14) and in vitro (15-20), and the specific induction of ornithine decarboxylase by cyclic AMP has also been shown in vivo (9, 21-23) and in baby hamster kidney cells in vitro (24).

The activity of mammalian ornithine decarboxylase is probably regulated by changes in the rate of synthesis of the enzyme protein. Although some reports indicate that ornithine decarboxylase activity is not affected by low molecular weight compounds (3, 25, 26), putrescine, spermidine, and spermine have been shown to be inhibitory (27-32). Such inhibition appears to be quite complex and both transcriptional and post-transcriptional mechanisms may play a role.

In an attempt to evaluate those factors which define ornithine decarboxylase activity, we have studied the induction of ornithine decarboxylase by partial hepatectomy in whole animal experiments or by cyclic AMP and dexamethasone in hepatoma cells in tissue culture. Dexamethasone was selected because glucocorticoids are thought to act without implicating cyclic AMP as a second messenger (33) and hepatoma cells were used because most of the in vivo inductive studies were done with rat liver. Furthermore, hepatoma cells have been used for the study of tyrosine aminotransferase, an enzyme shown to be inducible by the same agents used in this study (17, 34). Antiserum monospecific to regenerating rat liver ornithine decarboxylase (34) was used to analyze both in vivo and in vitro induction of this enzyme. It was shown that, although the time pattern of induction was unique to the particular inducing agent, concomitant protein synthesis was required in both cases and the induced enzyme was indistinguishable from that present in noninduced cells. Immunotitration of L-[1^4C]Leucine-labeled ornithine decarboxylase showed that the increase in precipitable protein paralleled the increase

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in enzyme activity; furthermore, introduction of polyamines at the onset of the in vitro induction selectively blocked the cyclic AMP effect, whereas the dexamethasone induction remained unaffected.

**EXPERIMENTAL PROCEDURE**

**Cell Culture and Enzyme Induction**—HTC cells (hepatoma tissue culture cells originating from Morris rat hepatoma 7288C) were kindly provided by Dr. Thomas Gelehrter, University of Michigan School of Medicine, and were maintained in tissue culture as previously described (35). Collection of cells and in vitro induction studies were carried out as reported previously (36). Cyclic AMP was dissolved immediately prior to use in the same Tris-Cl/EDTA buffer used in the ornithine decarboxylase assay. An aqueous solution of \(10^{-4}\) M dexamethasone phosphate was kept frozen and was diluted appropriately prior to use.

**Assay and Immunoochemical Procedures**—All assays and immunoochemical procedures were performed as previously described (34, 35). Incorporation of isotopically labeled amino acid into enzyme protein was measured by precipitation of the enzyme with the monospecific antibody (34) as follows: a 12-min pulse of 660 \(\mu\)Ci of DL-[\(^{14}\)C]leucine (29 mCi/mmol) was administered by intraperitoneal injection at given times after partial hepatectomy (20), the 100,000 \(\times g\) supernatant fraction of both normal and regenerating rat liver homogenate was prepared (34) and analyzed for leucine incorporation into total soluble protein and immunorecognizable protein. In an analogous manner, at given time after exposure of HTC cells to inducing agent, radioactive leucine was introduced into the medium at a level of DL-[\(^{14}\)C]leucine, 50 \(\mu\)Ci/5 \(\times\) 10⁶ cells; isotope incorporation was continued for 12 h and then terminated by centrifugation of the spinner culture. Induced and noninduced cells were washed four times with assay buffer at 4\(^\circ\)C, broken by sonication and the supernatant solutions were analyzed for \(T\)-[\(^{14}\)C]leucine incorporation into total soluble protein and enzyme immunorecognizable protein. The level of 6.5 \(\times\) 10⁵ cpm/mg of rat liver protein and 9.0 \(\times\) 10⁵ cpm/mg of HTC cell protein remained constant throughout induction. Correction factors of 5\% for radioactivity precipitated with nonimmune sera and 20\% for nonspecific precipitation (34) were determined and applied routinely.

**RESULTS**

**Enzyme Induction and Inhibitors**—Fig. 1 presents a typical experiment defining the time pattern of cyclic AMP induction of ornithine decarboxylase in log phase HTC cells. Generally, cyclic AMP induced a 4-fold enhancement of enzyme activity which reached a peak at 3 hours and dropped to near base levels by about 6 hours. Spermine at \(10^{-4}\) M or spermidine at \(6 \times 10^{-4}\) M, when added to noninduced cells, always resulted in a depression of enzyme activity below control levels. Even in the continued presence of polyamine the drop in enzyme activity was relieved and slowly returned to normal by 5 to 7 hours. The same pattern of inhibition was observed when cyclic AMP and the polyamine inhibitor were added simultaneously. The pattern of ornithine decarboxylase induction by the glucocorticoid dexamethasone (Fig. 2) was strikingly different from cyclic AMP induction. Dexamethasone at \(2 \times 10^{-4}\) M induced a slow steady increase in enzyme activity over an initial 5-hour period which reached a plateau of 6-fold enhancement of activity by 12 hours; this high level was maintained for a period of time in excess of 25 hours. Concomitant addition of spermine or spermidine at levels which resulted in complete inhibition of cyclic AMP induction had no effect on dexamethasone induction.\(^2\)

Fig. 3 shows the effect of actinomycin D and cycloheximide on cyclic AMP induction of ornithine decarboxylase. In contrast to the degree of inhibition by cycloheximide which was complete, that by actinomycin D was only 70\%. Neither of these inhibitors decreased the level of ornithine decarboxylase in noninduced control cells; whereas, spermine in fact did depress control levels. Both actinomycin D and cycloheximide inhibited the dexamethasone induction.

**Comparison of Ornithine Decarboxylase from Induced and

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\(^1\)The abbreviation used is: HTC, hepatoma tissue culture.

\(^2\)Preliminary experiments with ornithine decarboxylase induction by cyclic AMP enclosed in phospholipid vesicles (37) indicate that the polyamines are incorporated into the cell and exert their effects intracellularly.

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**FIG. 1.** Ornithine decarboxylase induction by dibutyryl cyclic AMP and the effects of spermidine and spermine on the cyclic AMP induction of ornithine decarboxylase in HTC cells. HTC cells were handled as described under "Experimental Procedure." The polyamines were added at the time of addition of cyclic AMP. The results are expressed as percentage of control (control = 100\%) of ornithine decarboxylase activity. Logarithmically growing HTC cells induced by \(10^{-4}\) M dibutyryl cyclic AMP (O—O) or \(10^{-4}\) M dibutyryl cyclic AMP (■—■), log phase HTC cells and \(10^{-4}\) M spermidine (O—O), cyclic AMP-induced log phase HTC cells and \(10^{-4}\) M spermine or \(6 \times 10^{-4}\) M spermidine (×—×).

**FIG. 2.** Ornithine decarboxylase induction by dexamethasone phosphate and the effects of spermidine and spermine on the dexamethasone induction of ornithine decarboxylase in HTC cells. HTC cells were handled as described under "Experimental Procedure." The polyamines were added at the time of addition of dexamethasone. Results are expressed as percentage of control (control = 100\%) of ornithine decarboxylase activity. Logarithmically growing HTC cells induced by \(2 \times 10^{-4}\) M dexamethasone phosphate (■—■), dexamethasone-induced log phase HTC cells and \(10^{-4}\) M spermidine (■—■), cyclic AMP-induced log phase HTC cells and \(6 \times 10^{-4}\) M spermidine (×—×).
Noninduced Cells—These studies employed monospecific antisera against enzyme from regenerating rat liver (34). Crude cell extracts from HTC cells were prepared from cultures induced with $10^{-4}$ M dibutyryl cyclic AMP or $2 \times 10^{-6}$ M dexamethasone phosphate. These extracts were analyzed by immunoprecipitation using the Ouchterlony double-diffusion technique and immunoelectrophoresis. In all cases a single precipitin line was observed, and the precipitin line was identical for the noninduced as well as the cyclic AMP- and dexamethasone-induced HTC cells.

In order to establish that the induced enzyme was identical with the noninduced one, we studied the ratio of their catalytic to antigenic activities. Increasing amounts of enzyme from noninduced as well as cyclic AMP- or dexamethasone-induced HTC cells were added to a constant amount of specific antiserum. It is apparent from Fig. 4 that all preparations of enzyme gave the same equivalence point indicating that the ratio of catalytic to antigenic activity was the same for all preparations. The possibility that the increase in enzyme activity in the induced cells might be due to transformation of a cross-reacting enzymatically inactive precursor form of ornithine decarboxylase is eliminated. These studies do not, however, preclude the possibility of the presence of a non-cross-reacting enzymatically inactive form of ornithine decarboxylase.

The temperature sensitivity of enzyme derived from noninduced and cyclic AMP- or dexamethasone-induced cells is described in Fig. 5. The heat inactivation pattern is similar to that of enzyme from regenerating rat liver. In all cases there is a labilization of ornithine decarboxylase when exposed to temperatures above 50° for 5 min, with only 10% of the initial activity remaining at 70°.

Isotopic Leucine Incorporation into Enzyme Protein—Incorporation of L-$^{14}$C]leucine into specific enzyme protein was measured following induction with either cyclic AMP or dexamethasone. In addition, the incorporation of radioactivity was examined when actinomycin D, cycloheximide, or spermine were added at the onset of the experiment; the inhibitors were added both to the induced and to the noninduced cells. If the inducers affected an increase in the rate of synthesis of ornithine decarboxylase, the ratio of L-$^{14}$C]leucine counts obtained with the noninduced enzyme over those of the induced enzyme ought to reflect the change, if any, in the rate of enzyme protein synthesis due to induction. If the experimentally observed ratio were found to approximate one, any increase in enzyme molecules would have to derive from diminished enzyme degradation and/or from transformation of a non-cross-reacting enzyme precursor molecule into its active form. Incorporation of radioactivity was examined at 4, 8, and 16 hours following partial hepatectomy, at 3 hours after the addition of cyclic AMP, and at 12 hours after the addition of dexamethasone. The time points of 4 and 16 hours were selected because they represent the biphasic rise of enzyme activity observed following partial hepatectomy. Approximately the same time points were used for studying the incorporation of radioactivity into noninduced cells, as well as into cells induced in the presence of the various inhibitors. The results in Table I are expressed as counts per min of L-$^{14}$C]leucine-labeled enzyme which were precipitated by the specific antiserum. It can be seen that both in response to cyclic AMP and to dexamethasone the rates of ornithine decarboxylase synthesis are considerably increased as compared with the controls.

![Fig. 3. The effect of actinomycin D, cycloheximide, and spermine on cyclic AMP induction and of actinomycin D and cycloheximide on dexamethasone induction of ornithine decarboxylase in HTC cells. HTC cells were handled as described under "Experimental Procedure." All inhibitors were added at the time of addition of the inducing agent. The results are expressed as percentage of control (control = 100%) of ornithine decarboxylase activity. Logarithmically growing HTC cells induced with $10^{-4}$ M dibutyryl cyclic AMP (O—O), cyclic AMP-induced log phase HTC cells, and $5 \mu$g/ml of actinomycin D (O—O), cyclic AMP-induced log phase HTC cells and $25 \mu$g/ml of cycloheximide (O—O), cyclic AMP-induced log phase HTC cells and $10^{-6}$ M spermine (1—1), dexamethasone (2 $\times$ 10$^{-6}$ M) induced log phase HTC cells (A—A), and dexamethasone-induced log phase HTC cells and $5 \mu$g/ml of actinomycin D or $25 \mu$g/ml of cycloheximide (Δ—Δ).](http://www.jbc.org/)

![Fig. 4. Immunotitration of rabbit anti-ornithine decarboxylase serum with ornithine decarboxylase from noninduced and induced cells. Extracts containing ornithine decarboxylase were derived from noninduced log phase HTC cells, and log phase HTC cells induced either with cyclic AMP or dexamethasone. Various amounts of ornithine decarboxylase were added to 2.0 mg of antiserum and the volume was adjusted to 2.0 ml with assay buffer. Preparation of HTC cell extracts and the conditions of the incubation are described under "Experimental Procedure." The figure shows precipitated ornithine decarboxylase activity in the supernatant over ornithine decarboxylase activity originally added. Logarithmically growing HTC cells (O—O), log phase HTC cells induced with $2 \times 10^{-4}$ M dexamethasone (O—O), log phase HTC cells induced with $10^{-4}$ M cyclic AMP (Δ—Δ), and regenerating rat liver (□—□).](http://www.jbc.org/)
Incorporation of L-[^14]C]leucine into specific enzyme protein at various times following partial hepatectomy (Fig. 6) was measured; assays of enzyme activity are also recorded. The increase in enzyme activity paralleled the increase in immunoprecipitable radioactivity in each instance.

**DISCUSSION**

The present study has shown that ornithine decarboxylase can be induced by dibutyryl cyclic AMP and dexamethasone in an established hepatoma line in culture. Both of these types of induction are quite similar to the induction of tyrosine aminotransferase seen with dibutyryl cyclic AMP (17) and dexamethasone (35) in HTC cells. The induced enzyme has now been characterized by immunoprecipitation and heat stability and shown to be identical with ornithine decarboxylase from noninduced HTC cells as well as from regenerating rat liver. The two types of induction have been shown to differ considerably in their time course and in responsiveness to actinomycin D and to polyamines. We conclude that cyclic AMP and dexamethasone affect different aspects of the control of the same form of ornithine decarboxylase. Our data further suggest that the inhibition seen with the polyamines is not due to a simple feedback mechanism. A similar conclusion has also been reached for polyamine inhibition of ornithine decarboxylase.

**Fig. 5.** Heat inactivation of ornithine decarboxylase (ODC) derived from logarithmically growing HTC cells, and from log phase HTC cells induced either with dexamethasone or cyclic AMP. Duplicate aliquots of either preparation of ornithine decarboxylase were incubated at 37° for 5 min and then at the temperatures indicated for an additional 5 min. Crude HTC extracts were prepared by sonication as described under "Experimental Procedure." All samples were made up to 1.0 ml with assay medium; after heat treatment the samples were chilled for 30 min at 4° brought up to a total volume of 2.0 ml with assay medium and assayed directly for ornithine decarboxylase activity. Residual ornithine decarboxylase activity is expressed as a percentage of the activity in control samples which had been incubated for 5 min at 37°.

Logarithmically growing HTC cells (C—C), log phase HTC cells induced with 2.0 x 10^-5 MI dexamethasone phosphate (C—□), log phase HTC cells inducted with 1.0 x 10^-4 M dibutyryl cyclic AMP (A—A), and regenerating rat liver (C—□).

**Fig. 6.** Immunological precipitation of ornithine decarboxylase obtained at different times following partial hepatectomy. Labeled leucine was injected 12 min before killing at each time point and the labeled ornithine decarboxylase was precipitated with specific antiserum. Aliquots of 100,000 x g supernatant fluid were taken for ornithine decarboxylase assay at 0, 4, 8, and 16 h following partial hepatectomy. Identical aliquots, obtained at the same time points, were made to contain 700 cpm by addition of unlabeled liver extracts and were precipitated with 2.0 mg of anti-ornithine decarboxylase serum. The conditions of the incubation were those described in Fig. 1. Endogenous ornithine decarboxylase activity (C—C); ornithine decarboxylase present in precipitate as measured with labeled enzyme (C—□).

**Table I**

<table>
<thead>
<tr>
<th>Inhibitors added at onset of induction</th>
<th>HTC cell L-[^14]C]leucine-labeled immunoprecipitable ornithine decarboxylase</th>
<th>1.0 x 10^-3 M dibutyryl cyclic AMP</th>
<th>2.0 x 10^-5 M dexamethasone phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm</td>
<td>%</td>
<td>cpm</td>
</tr>
<tr>
<td>No inhibitor</td>
<td>276</td>
<td>0.06</td>
<td>1848</td>
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<tr>
<td>Cycloheximide 25 µg/ml</td>
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<td>0.08</td>
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<tr>
<td>Actinomycin D, 5 µg/ml</td>
<td>569</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Spermine, 10^-4 M</td>
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<td>a</td>
<td></td>
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</table>

*Could not be measured*
Biosynthesis of Ornithine Decarboxylase

Our results with L-[14C]leucine incorporation indicate that both cyclic AMP and dexamethasone increase the rate of ornithine decarboxylase biosynthesis. Furthermore, spermine is seen to inhibit ornithine decarboxylase biosynthesis only with respect to induction by cyclic AMP. The fact that spermine drops even the basal enzyme activity, while cycloheximide does not, points to a possible unique role for spermine.

There is a striking parallel between the biphasic pattern of ornithine decarboxylase activity seen in vivo (38-40), the one seen in vitro (31, 41-43) and the patterns of induction reported here for HTC cells. It is tempting to speculate that the biphasic response might be due to the physiological action of cyclic AMP (early rise) and the glucocorticoids (late rise). In vivo induction of hepatic ornithine decarboxylase in response to growth hormone (4-7), glucagon (44), insulin (23), or cyclic AMP (9, 21) showed a peak in enzyme activity at 4 hours with return to basal levels by 8 hours. Furthermore, exposure of the animal to stress witnessed a rise in cyclic AMP levels in the adrenal medulla followed by an increase in ornithine decarboxylase activity at 4 hours (45). These findings provide support for the notion that cyclic AMP might be responsible for the first rise in enzyme activity.

Although we have observed a late rise in ornithine decarboxylase activity in response to dexamethasone in vitro, no such increase has been observed when glucocorticoids alone were administered in vivo (8, 9, 14, 46, 47). It is possible that the simultaneous and early presence of cyclic AMP and glucocorticoids is necessary for the late in vivo increase in ornithine decarboxylase activity. This possibility might be supported by the fact that cortisol and growth hormone exhibited a synergistic effect in inducing ornithine decarboxylase activity in both hypophysectomized and intact rats (8) and the observation that intact adrenals are required for optimum induction due to growth hormone (4). In addition, it might be relevant to point out that although $10^{-8}$ M dibutyryl cyclic AMP failed to induce tyrosine aminotransferase in cultured hepatoma cells, concurrent addition of $10^{-8}$ M hydrocortisone resulted in a delayed increase in enzyme activity (15).

We have shown that ornithine decarboxylase is synthesized during both cyclic AMP and dexamethasone induction and at the specific time intervals of 4, 8, and 16 hours following partial hepatectomy; however, although enzyme protein is synthesized during both the early and late rise in ornithine decarboxylase activity, the particular RNA functional in either induction is synthesized during the early period only (40, 48). When actinomycin D was added at the beginning of our experiments only partial inhibition of the early cyclic AMP induction of ornithine decarboxylase activity and enzyme biosynthesis in HTC cells was observed. There have been reports of a similar partial inhibition of the early rise in ornithine decarboxylase activity due to growth hormone (6) or cyclic AMP (40) in liver, insulin in cultured mammary tissue (23), dilution of HTC cells (49), addition of amino acids to cultured lymphocytes (50), and cyclic AMP in cultured baby hamster kidney cells (24); furthermore, actinomycin D failed to inhibit the rise in ornithine decarboxylase activity observed in the early period of lymphocyte transformation (51). Theses data provide suggestive evidence that cyclic AMP might be acting at a post-transcriptional level in contrast to that of dexamethasone. This notion is in agreement with recent studies showing that cyclic AMP acts at a translational or post-transcriptional site in its induction of phosphoenolpyruvate carboxykinase (52) as well as of tyrosine aminotransferase (16, 53).

We also report that only the early rise in ornithine decarboxylase activity was specifically inhibited by polyamines. Relevant to our results is the observation (32) that putrescine administration to rats inhibited that increase in ornithine decarboxylase activity observed during the initial period following partial hepatectomy. These findings suggest that the endogenous polyamine levels when elevated during the initial part of the biphasic response may themselves cause the subsequent decline in ornithine decarboxylase activity. Independently, Clark suggested (31) that accumulation of polyamines might inhibit translation and thus bring about the decrease in enzyme activity which is evidenced after the first peak in activated lymphocytes in vitro; in addition, he suggested that since ornithine decarboxylase activity declined more rapidly after putrescine administration than after treatment with actinomycin D, the polyamine effect was not directly associated with RNA synthesis. Thus, accumulated evidence points to a possible coordinated, however disjunct, role of cyclic AMP and the glucocorticoids.

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REFERENCES

*While this manuscript was in press a post-transcriptional site of action for cyclic AMP was suggested in studies of certain factors regulating ornithine decarboxylase activity during development of mouse mammary epithelium (54). This suggestion corroborates that made for HTC cells during the 1974 Laurentian Conference (55).
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50. Kay, J. E., Lindsay, V. J., and Cooke, A. (1972) FEBS Lett. 21, 129-139
52. Gunn, J. M., Tilghman, S. M., Hanson, R. W., Reshef, L., and Bailard, F. J. (1975) Biochemistry 14, 2350-2357
Stimulation of ornithine decarboxylase synthesis and its control by polyamines in regenerating rat liver and cultured rat hepatoma cells.

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