MOLECULAR PHARMACOLOGY, 12, 871-878

Adenosine Cyclic 3',5'-Monophosphate-Dependent Protein Kinase and Ornithine Decarboxylase Involvement in the Induction of Cytochrome P-450 and Hepatic Hypertrophy

MAX COSTA, ELIZABETH R. COSTA, CAROL-ANN MANEN,[1] I. GLENN SIPES, AND DIANE HADDOCK RUSSELL[2]

Department of Pharmacology, University of Arizona Medical Center, Tucson, Arizona 85724

(Received March 18, 1976)

(Accepted May 25, 1976)

SUMMARY

COSTA, MAX, COSTA, ELIZABETH R., MANEN, CAROL-ANN, SIPES, I. GLENN & RUSSELL, DIANE HADDOCK (1976) Adenosine cyclic 3',5'-monophosphate-dependent protein kinase and ornithine decarboxylase involvement in the induction of cytochrome P-450 and hepatic hypertrophy. *Mol. Pharmacol.*, 12, 871-878.

The administration of a single dose of Aroclor-1254 (500 mg/kg intraperitoneally) resulted in a biphasic response in rat liver of the activation of cyclic 3',5'-AMP-dependent protein kinase, the induction of ornithine decarboxylase, and the activation of RNA polymerase I. The first cycle of this biphasic response involved activation of cyclic AMP-dependent protein kinase from 2 to 6 hr (177% of control), increased activity of ornithine decarboxylase from 3.5 to 6 hr, and elevation of RNA polymerase I activity from 4 to 6 hr after drug administration. By 8 hr activities of these enzymes had essentially returned to control levels. However, between 10 and 20 hr, cyclic AMP-dependent protein kinase activity again rose to about 170% of control, followed by elevated activities of ornithine decarboxylase and RNA polymerase I. The maximal stimulation of ornithine decarboxylase activity was 5–6-fold in both phases of its response, and RNA polymerase I activity was maximal (150% of control) at 5 and 20 hr. Hepatic cytochrome P-450 concentration was increased within 6 hr after a single dose of Aroclor-1254 and reached a maximal concentration of 4 times the control level by 8 days. The liver weight to body weight ratios in rats given the drug were about 150% of control within 2 days and maintained this level through 15 days. Aminophylline and/or dibutyryl cyclic AMP given together with a single low dose of Aroclor-1254 (50 mg/kg) resulted in a greater increase in cytochrome P-450 concentration than did a low dose of Aroclor-1254 administered alone. These data support the concept that the activation of cyclic AMP-dependent protein kinase, elevation of ornithine decarboxylase activity, and enhanced RNA polymerase I activity form a tight temporal sequence of biochemical events that are related to drug-induced liver hypertrophy and to microsomal enzyme induction.

This work was supported by United States Public Health Service Grants CA-14783 and CA-17094 from the National Cancer Institute, by American Cancer Society Grant IN-110, and by Hoffmann-LaRoche, Inc.

[1] Recipient of PHS Research Fellowship HD-00871 from the National Institute of Child Health and Development.

[2] Recipient of National Cancer Institute Research Career Development Award CA-00072.

INTRODUCTION

The molecular mechanism by which drugs and other foreign compounds induce liver growth and the production of microsomal drug-metabolizing enzymes is unknown, although many specific enzyme changes have been noted following phenobarbital and 3-methylcholanthrene administration (1–5). To date, the specific induction of drug-metabolizing enzymes has not been separable from the process of liver hypertrophy (4, 5). An understanding of the temporal sequence of biochemical events may eventually lead to insight into these two processes.

We have previously suggested that the induction of ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) in response to the activation of cyclic 3′,5′-AMP-dependent protein kinase (ATP-protein phosphotransferase, EC 2.7.1.37) may be a key event in liver growth and may be closely linked to the subsequent induction of drug-metabolizing enzymes (1, 5). Marked increases in the activity and amount of the mixed-function oxygenases require 24 hr or longer after drug administration; however, various studies have been done on a series of earlier events following the administration of phenobarbital and 3-methylcholanthrene. For instance, Blankenship and Bresnick (3) reported that phosphorylation of acidic nuclear proteins increases within a few hours of phenobarbital administration, which suggests that phenobarbital and 3-methylcholanthrene *in vivo* result in early increases in cyclic AMP concentration in the liver and substantial activation of cyclic AMP-dependent protein kinases (5). Furthermore, ornithine decarboxylase, the initial enzyme in the polyamine biosynthetic pathway, which now appears to be induced generally by a cyclic AMP-dependent mechanism, exhibits elevated activity just after the activation of cyclic AMP-dependent protein kinase (2, 6, 7). Studies using inhibitors of protein and RNA synthesis indicate that the induction of ornithine decarboxylase appears to involve synthesis *de novo* (6, 7). An increase in ornithine decarboxylase activity has been detected in all cases of hypertrophy of the liver studied to date (2, 8, 9).

Phenobarbital and 3-methylcholanthrene have been reported also to enhance RNA synthesis, and an increased rate of RNA synthesis appears to be necessary for induction of the microsomal mixed-function oxygenases (10, 11). Recent studies in our laboratory suggest a link between RNA polymerase I (a nucleosidetriphosphate-RNA nucleotidyltransferase, EC 2.7.7.6) activity and ornithine decarboxylase activity, and, in fact, ornithine decarboxylase may directly modulate RNA polymerase I activity in rat liver (12, 13).

Aroclor-1254 appears to be a useful chemical for studying the temporal sequence of biochemical events involved in the induction of mixed-function oxygenases when marked induction after a single administration is desirable. Numerous studies have shown this mixture of polychlorinated biphenyls (industrial chemicals which have a wide spectrum of inducing ability) to enhance the activity of the hepatic mixed-function oxygenase system (14–18). Bickers *et al.* (16) reported that daily injections of Aroclor-1254 (25 mg/kg intraperitoneally) for 6 days resulted in a 2–3-fold increase in hepatic ethylmorphine *N*-demethylase activity and an 11-fold increase in hepatic benzo[*a*]pyrene hydroxylase activity. Czygan *et al.* (17) reported that a single, large dose of Aroclor-1254 (500 mg/kg intraperitoneally) produced a 5-fold increase in the hepatic *N*-demethylation of dimethylnitrosoamine. Aroclor-1254 also induced liver growth, with an increase in microsomal protein cytochrome P-450 (18).

Therefore we have studied the following events in rat liver after the administration of a single injection of Aroclor-1254 (500 mg/kg): (a) activation of cyclic AMP-dependent protein kinase, (b) induction of ornithine decarboxylase, (c) increases in RNA polymerase I activity, (d) induction of cytochrome P-450 protein(s), and (e) increases in the liver to body weight ratios. A single dose of Aroclor-1254 produced biphasic sequences of marked activation of cyclic AMP-dependent protein kinase, induction of ornithine decarboxylase, and ac-

tivation of RNA polymerase I, and ultimately resulted in the induction of an unusual variant of cytochrome P-450. Separation of the above sequence into events contributing specifically to liver hypertrophy and those involved in the induction of the mixed-function oxygenases is not yet possible.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (100–125 g) were maintained on a standard 12-hr light-dark cycle and fed ad libitum. Animals were injected with either Aroclor-1254 (500 mg/kg or 50 mg/kg intraperitoneally, donated by Monsanto Corporation) in sesame oil or with an equivalent volume of sesame oil. Some animals received aminophylline (80 mg/kg intraperitoneally) and/or dibutyryl cyclic AMP (25 mg/kg intraperitoneally) simultaneously with Aroclor-1254 or sesame oil. All animals were killed by cervical dislocation at the same time (1000 hr ± 30 min) to minimize diurnal enzyme changes, and the livers were removed, chilled, and weighed. Determination of protein kinase activation, ornithine decarboxylase activity, and RNA polymerase I activity were all performed on samples from the same liver.

Cyclic AMP-dependent protein kinase activity. The degree of activation of cyclic AMP-dependent protein kinase was determined by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP (7 Ci/mmole, New England Nuclear) into histones in the absence and presence of exogenous cyclic AMP (10 μM) as described previously (19), except that the reaction was terminated by spotting a 50-μl aliquot of the assay mixture on a Whatman No. 3MM filter. Filters were then washed three times (30 min each time) in 10% trichloracetic acid, dried, and counted in an Omnifluor-toluene mixture (20). The results are expressed as the ratio of ^{32}P incorporation in the absence and the presence of saturating cyclic AMP (−cyclic AMP/+cyclic AMP) (21).

In order to verify the degree of activation of cyclic AMP-dependent protein kinase, Sephadex G-100 chromatography was used to separate the regulatory-catalytic units from the free catalytic units as described previously (19, 21), with the exception that a 1-ml aliquot was applied to the column.

Assay of ornithine decarboxylase activity. Ornithine decarboxylase activity was determined by measuring the liberation of $^{14}CO_2$ from DL-[1-^{14}C]ornithine (5.0 mCi/mmole, New England Nuclear) (9). The buffer used in the assay was 0.05 M sodium-potassium phosphate, pH 7.2, containing 0.1 mM EDTA and 1.0 mM dithiothreitol.

Assay of RNA polymerase I activity. Using a purified nuclear preparation as the enzyme source (22), RNA polymerase activity was determined by measuring the incorporation of [^{3}H]UTP (18.6 Ci/mmole, Schwarz/Mann) into RNA (23). RNA polymerase I activity was separated from RNA polymerase II activity by assaying in the presence and absence of 1.8 μg/ml of α-amanitin (24).

Protein determinations. Microsomal protein concentrations were determined using the biuret procedure (25). All other protein concentrations were measured according to Lowry *et al.* (26), using bovine serum albumin as the standard. Microsomes were isolated by differential centrifugation and resuspended in cold 0.05 M phosphate buffer (pH 7.4) to a final protein concentration of 1 mg/ml. The concentration of cytochrome P-450 was determined according to the procedure of Omura and Sato (27), using the carbon monoxide difference spectra.

RESULTS

Activation of cyclic AMP-dependent protein kinase and increased ornithine decarboxylase and RNA polymerase I activities following Aroclor-1254 administration. The activities of cyclic AMP-dependent protein kinase, ornithine decarboxylase, and RNA polymerase I showed biphasic increases, with maxima at 3–5 hr and 15–22 hr after the administration of Aroclor-1254 (Fig. 1). The increases were sequential in both phases, with initial activation of cyclic AMP-dependent protein kinase followed by increased ornithine decarboxylase and RNA polymerase I activities.

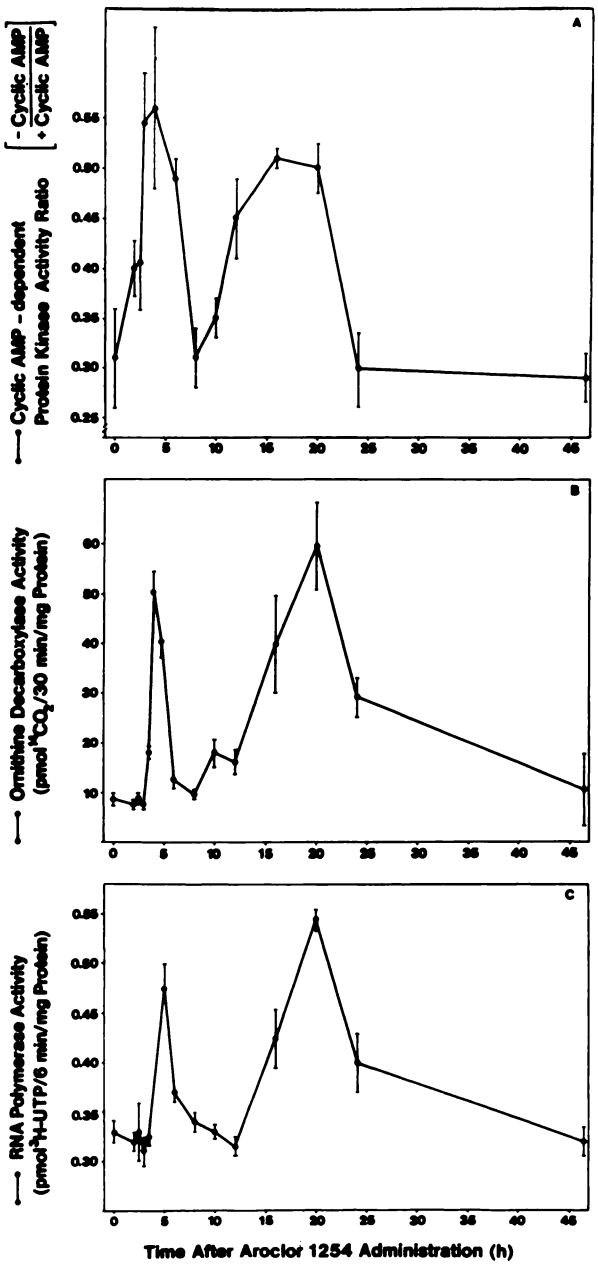

FIG. 1. *Changes in cyclic AMP-dependent protein kinase activity, ornithine decarboxylase activity, and RNA polymerase I activity following a single injection of Aroclor-1254.*

Male Sprague-Dawley rats (100–125 g) received Aroclor-1254 (500 mg/kg intraperitoneally) in sesame oil, were killed at the intervals indicated, and each liver was assayed for cyclic AMP-dependent protein kinase, ornithine decarboxylase, and RNA polymerase I activities. Each point represents the mean ± standard error of duplicate determinations on at least five rats. Total protein kinase activity (+cyclic AMP) changed slightly during the experiment (loss of 10%). This phenomenon has been reported in other systems after prolonged stimulation (28–30). A. Cyclic AMP-dependent protein kinase activity was determined by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP into histones in the absence and presence of exogenous cyclic AMP (−cyclic AMP/+cyclic AMP) (19, 21). B. Ornithine decarboxylase activity was determined by measuring the liberation of $^{14}CO_2$ from DL-[1-^{14}C]ornithine as described by Russell and Snyder (8). C. Using a purified nuclear preparation as the enzyme source (22), RNA polymerase I activity was determined by measuring the incorporation of [^{3}H]UTP into RNA in the presence of α-amanitin (1.8 μg/ml) (13, 23, 24).

Cyclic AMP-dependent protein kinase was significantly activated within 2 hr of drug administration, and maximal activation was detected at 4 hr (177% of control). By 8 hr, the −cyclic AMP/+cyclic AMP ratio had returned to the control value. Ornithine decarboxylase activity was increased significantly 3.5 hr after Aroclor-1254 administration, at which time it was twice the control value. The activity then increased rapidly through 4 hr, when it was 5 times that of control. Six hours after Aroclor-1254 administration, ornithine decarboxylase activity had returned to pretreatment levels. RNA polymerase I activity was 150% of controls at 5 hr and returned to control values by 10 hr.

The second peak of activities occurred over a longer time span than the first peak and was again initiated by the activation of cyclic AMP-dependent protein kinase. The −cyclic AMP/+cyclic AMP ratio was slightly elevated by 10 hr and exhibited a broad peak of activation (170% of control) between 12 and 20 hr. By 24 hr it had returned to control activity. Ornithine decarboxylase activity was twice control values by 10 hr, maximal at 20 hr, and still elevated at 24 hr; it returned to control values by 48 hr. RNA polymerase I activity was still at baseline at 12 hr, increased 40% by 16 hr, and reached maximum activation (170% of control) at 20 hr.

Separation of subunits of cyclic AMP-dependent protein kinase. Figure 2 shows the elution profile of such a separation by Sephadex G-100 chromatography 3.5 hr after the administration of either Aroclor-1254 in sesame oil or sesame oil only. Note the increase in the amount of protein kinase in the catalytic form, fractions 33–45 of Fig. 2B, compared with the control in Fig. 2A. This profile correlates well with the increased activity ratio of cyclic AMP-dependent protein kinase for this time point (Fig. 1A), as the amount of free catalytic subunit is dependent on the increase in intracellular cyclic AMP which binds to the regulatory subunit of the regulatory-catalytic complex, releasing the catalytic subunit (19, 21).

Cytochrome P-450 concentration and hepatic enlargement. Aroclor-1254 induced a CO-binding pigment that is an unusual variant of cytochrome P-450. The absorption maximum for the induced cytochrome was 449 nm instead of the usual 450 nm. Other investigators have reported a similar peak at 449 nm (18). The concentration of the CO-binding pigment, referred to as cytochrome P-450, was elevated 6 hr after drug administration and continued to increase up to day 8. By day 15, the amount of cytochrome P-450 had begun to decrease (Table 1).

A single dose of Aroclor-1254 also caused marked hypertrophy of the liver. Within 1 day of administration, liver weight had increased almost 10% (Table 2). The liver weight to body weight ratios continued to

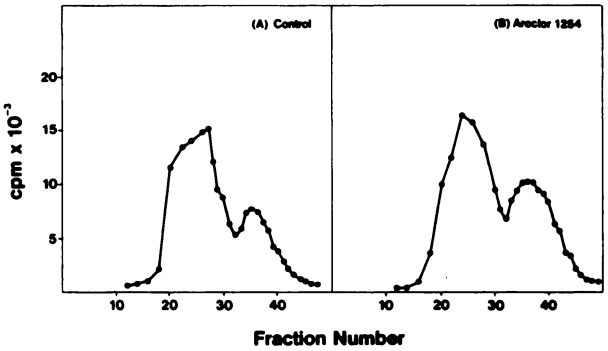

FIG. 2. *Sephadex G-100 chromatography of rat liver cyclic AMP-dependent protein kinase 3.5 hr after administration of either Aroclor-1254 (500 mg/kg intraperitoneally) in sesame oil (B) or sesame oil only (A)*

The fractions were assayed in the presence of exogenous cyclic AMP (16). The regulatory-catalytic form of cyclic AMP-dependent protein kinase is found in fractions 18–30, and the free catalytic form, in fractions 33–45 (19, 21).

TABLE 1

Effect of Aroclor-1254 administration on microsomal concentration of cytochrome P-450

Male Sprague-Dawley rats (100–125 g) received a single injection of Aroclor-1254 (500 mg/kg intraperitoneally in sesame oil) or sesame oil only (0.25 ml intraperitoneally) and were killed at the times shown. Microsomal P-450 concentrations were determined according to Omura and Sato (27). Each value represents the mean ± standard error for four animals.

Time	Control P-450	Aroclor-1254-treated P-450
days	*nmoles/mg microsomal protein*	
0.25	0.759 ± 0.12	0.989 ± 0.07[a]
0.75	0.747 ± 0.01	2.026 ± 0.17[b]
1	0.737 ± 0.11	2.417 ± 0.22[b]
2	0.784 ± 0.14	2.810 ± 0.30[b]
4	0.806 ± 0.18	3.190 ± 0.26[b]
8	0.770 ± 0.16	3.420 ± 0.46[b]
15	0.790 ± 0.16	3.150 ± 0.40[b]

[a] Data differ significantly from controls ($p < 0.05$).

[b] Data differ significantly from controls ($p < 0.005$).

TABLE 2

Changes in ratio of rat liver weight to body weight following Aroclor-1254 administration

Male Sprague-Dawley rats (100–125 g) received a single dose of Aroclor-1254 (500 mg/kg intraperitoneally in sesame oil). Controls received an equivalent amount of sesame oil. Animals were weighed and killed at the intervals indicated. The livers were perfused with cold 0.9% NaCl, excised, chilled on ice, blotted dry, and weighed. Each point represents the mean ± standard error of at least four animals.

Time	Control	Aroclor-1254
days	*g liver weight/g body weight*	
1	0.047 ± 0.003	0.053 ± 0.002[a]
2	0.041 ± 0.002	0.060 ± 0.006[b]
4	0.043 ± 0.002	0.075 ± 0.004[b]
8	0.048 ± 0.005	0.077 ± 0.006[b]
15	0.050 ± 0.002	0.084 ± 0.005[b]

[a] Data differ significantly from controls ($p < 0.05$).

[b] Data differ significantly from controls ($p < 0.005$).

increase until the end of the study (day 15).

Facilitation of drug induction by dibutyryl cyclic AMP with a low dose of Aroclor-1254. A large dose of Aroclor-1254 (500 mg/kg) given with aminophylline and dibutyryl cyclic AMP did not increase the hepatic cytochrome P-450 levels above that seen with Aroclor-1254 (500 mg/kg) alone. The cytochrome P-450 concentrations were 1.868 ± 0.21 nmoles/mg of microsomal protein for Aroclor-1254 (500 mg/kg) alone and 1.745 ± 0.34 nmoles/mg of microsomal protein for Aroclor-1254 (500 mg/kg) given with aminophylline (80 mg/kg).

Administration of aminophylline and dibutyryl cyclic AMP, either alone or together, decreased the microsomal cytochrome P-450 concentration below the control levels (Table 3). Administration of a low dose of Aroclor-1254 (50 mg/kg) alone produced only a slight increase in the concentration of cytochrome P-450 at 24 hr, but Aroclor-1254 (50 mg/kg) given with either aminophylline or dibutyryl cyclic AMP doubled the P-450 concentration. This elevation appeared only when the low dose of Aroclor-1254 was given with these agents.

DISCUSSION

These data add further credence to a previous study which reported that cyclic AMP and cyclic AMP-dependent protein kinase were involved in the induction of hepatic drug-metabolizing enzymes. The administration of either 3-methylcholanthrene or phenobarbital in single doses, while causing only slight increases in cyclic AMP, did elevate significantly the activity ratio of cyclic AMP-dependent protein kinase (5). Thus the activation of cyclic AMP-dependent protein kinase appears to be a more reliable index of a cyclic AMP-mediated event than the actual concentration of cyclic AMP. It was for this reason that, in this study, we measured the activity ratio of cyclic AMP-dependent protein kinase and not the absolute changes in cyclic AMP. Although we were not able to show an increase in cyclic AMP after the administration of Aroclor-1254 (data not shown), it was possible to facilitate the induction of mixed-function oxygenases by the administration of dibutyryl cyclic AMP along with a low dose of Aroclor-1254. This is in agreement with a report by Yamasaki *et al.* (31) that the addition of dibutyryl cyclic AMP and/or amino-

TABLE 3

Effects of phosphodiesterase inhibitors and analogues of cyclic AMP on microsomal cytochrome P-450 concentration

Male Sprague-Dawley rats (100–125 g) received an intraperitoneal injection of either aminophylline (80 mg/kg in 0.9% NaCl), 0.9% NaCl (0.5 ml), Aroclor-1254 (low dose, 50 mg/kg in sesame oil; high dose, 500 mg/kg in sesame oil), sesame oil (0.25 ml), dibutyryl cyclic AMP (25 mg/kg in 0.9% NaCl), or some combination as indicated, and were killed 24 hr later. Livers were perfused with chilled 0.9% NaCl prior to excision. The microsomal cytochrome P-450 concentrations were measured according to Omura and Sato (27). Each value represents the mean ± standard error for determinations on 4–12 animals.

Treatment	Cytochrome P-450
	nmoles/mg microsomal protein
Sesame oil (N = 6)	0.661 ± 0.06
0.9% NaCl (N = 4)	0.712 ± 0.04
Aminophylline (N = 6)	0.566 ± 0.09[a]
Dibutyryl cyclic AMP (N = 4)	0.542 ± 0.10[a]
Aminophylline and dibutyryl cyclic AMP (N = 6)	0.540 ± 0.08[a]
Aroclor-1254, low dose (N = 12)	0.754 ± 0.28
Aroclor-1254, high dose (N = 4)	2.417 ± 0.22[b]
Aroclor-1254 and aminophylline (N = 7)	1.324 ± 0.33[c]
Aroclor-1254 and dibutyryl cyclic AMP (N = 7)	1.382 ± 0.27[c]
Aroclor-1254, aminophylline, and dibutyryl cyclic AMP (N = 6)	1.018 ± 0.21[a]

[a] Data differ significantly from controls ($p < 0.05$).

[b] Data differ significantly from controls ($p < 0.001$).

[c] Data differ significantly from controls ($p < 0.01$).

phylline to isolated baby hamster kidney cells also resulted in induction of aryl hydrocarbon hydroxylase. These experiments support cyclic AMP mediation of drug induction.

The evidence that relates the induction of ornithine decarboxylase to the activation of cyclic AMP-dependent protein kinase is now substantial (1, 5–7, 32, 33), and the findings reported in this study, which include a tight temporal sequence for increments in protein kinase and ornithine decarboxylase and also biphasic coupling, are more convincing than previous studies (1, 5–7, 32, 33). We have demonstrated biphasic increases not only in protein kinase and ornithine decarboxylase but also in RNA polymerase I activity, the enzyme responsible for ribosomal RNA synthesis. We feel that this early chain of biochemical events (i.e., elevation of protein kinase activity, induction of ornithine decarboxylase, and acceleration of ribosomal RNA synthesis) is necessary for the liver hypertrophy which occurs after the administration of various drugs and foreign compounds and which has been well documented by many investigators (4, 15, 34).

To date it has not been possible to separate hypertrophy of the liver per se from the induction of drug-metabolizing enzymes (4). This may mean that new ribosomal RNA synthesis is necessary for drug induction processes. There are some data to substantiate this hypothesis. For example, after either phenobarbital or 3-methylcholanthrene administration, enhancement of RNA synthesis appears to be necessary for the induction of microsomal mixed-function oxygenases (10, 11). We have recently obtained some direct evidence to suggest that ornithine decarboxylase may regulate the activity of RNA polymerase I, the enzyme responsible for ribosomal RNA synthesis. RNA polymerase I activity can be increased by the addition of a partially purified ornithine decarboxylase preparation to the assay. The amount of the increase was directly related to the units of ornithine decarboxylase activity added to the assay (14). These data, as well as the close temporal relationship between increased ornithine decarboxylase activity and increased RNA polymerase I activity in many systems that have been studied, suggest that ornithine decarboxylase may be the labile protein that modulates the level of RNA polymerase I activity (12, 13).

We are not able to postulate at this time the mechanism or mechanisms by which foreign compounds affect the cyclic AMP-dependent protein kinase activity ratio. Preliminary evidence indicated that 3-methylcholanthrene directly activated

adenylate cyclase, since the activity of this enzyme was stimulated in liver homogenates isolated from rats treated with a polycyclic hydrocarbon. However, no such stimulation of adenylate cyclase was detectable after the administration of phenobarbital. Both drugs resulted in a marked stimulation of cyclic AMP-dependent protein kinase. It is possible that drugs could have a direct effect on a cyclic AMP-dependent protein kinase, altering its binding potential for cyclic AMP, although this has not been proven. The wide variation in stimulated hepatic processes elicited by various classes of chemicals suggests that drug induction is indeed a complicated process, with one of the major consistent patterns being liver enlargement. Further studies are necessary to understand mechanistically how drugs affect cyclic AMP-dependent protein kinase and the precise mechanism by which this activation, as well as other early enzyme inductions, may affect the induction of drug-metabolizing enzymes.

REFERENCES

1. Costa, M., Byus, C. V., Sipes, I. G., Brodie, B. B. & Russell, D. H. (1975) *Fed. Proc.*, **34**, 263.
2. Russell, D. H. (1971) *Biochem. Pharmacol.*, **20**, 3481–3491.
3. Blankenship, J. & Bresnick, E. (1974) *Biochim. Biophys. Acta*, **340**, 218–227.
4. Argyris, T. S. (1974) in *Control of Proliferation in Animal Cells* (Clarkson, B. & Baserga, R., eds.), pp. 49–66, Cold Spring Harbor Laboratory, New York.
5. Byus, C. V., Costa, M., Sipes, I. G., Brodie, B. B. & Russell, D. H. (1976) *Proc. Natl. Acad. Sci. U. S. A.*, **73**, 1241–1245.
6. Byus, C. V. & Russell, D. H. (1974) *Life Sci.*, **15**, 1991–1997.
7. Byus, C. V. & Russell, D. H. (1975) *Science*, **187**, 650–652.
8. Russell, D. H. & Snyder, S. H. (1968) *Proc. Natl. Acad. Sci. U. S. A.*, **60**, 1420–1427.
9. Russell, D. H. & Snyder, S. H. (1969) *Endocrinology*, **84**, 223–228.
10. Jacob, S. T., Scharf, M. G. & Vesell, E. S. (1971) *Proc. Natl. Acad. Sci. U. S. A.*, **71**, 704–707.
11. Gelboin, H. V., Wortham, J. S. & Wilson, R. G. (1967) *Nature*, **214**, 281–283.
12. Manen, C.-A. & Russell, D. H. (1975) *Fed. Proc.*, **34**, 586.
13. Manen, C.-A. & Russell, D. H. (1975) *Life Sci.*, **17**, 1769–1776.
14. Alvares, A. P., Bickers, D. R. & Kappas, A. (1973) *Proc. Natl. Acad. Sci. U. S. A.*, **70**, 1321–1325.
15. Chen, T. S. & DuBois, J. P. (1973) *Toxicol. Appl. Pharmacol.*, **26**, 504–512.
16. Bickers, D. R., Kappas, A. & Alvares, A. P. (1974) *J. Pharmacol. Exp. Ther.*, **188**, 300–309.
17. Czygan, P., Greim, H., Garro, A. J., Hatterer, R., Schaffner, F., Popper, H., Rosenthal, O. & Cooper, D. Y. (1973) *Cancer Res.*, **33**, 2982–2986.
18. Alvares, A. P. & Kappas, A. (1975) *FEBS Lett.*, **50**, 172–174.
19. Costa, M., Manen, C.-A. & Russell, D. H. (1975) *Biochem. Biophys. Res. Commun.*, **65**, 75–81.
20. Reimann, E. M., Walsh, D. A. & Krebs, E. G. (1971) *J. Biol. Chem.*, **246**, 1986–1995.
21. Soderling, T. R., Corbin, J. D. & Park, C. R. (1973) *J. Biol. Chem.*, **248**, 1822–1824.
22. Blobel, G. & Potter, V. R. (1966) *Science*, **154**, 1662–1665.
23. Roeder, R. G. & Rutter, W. J. (1969) *Nature*, **224**, 234–237.
24. Lindell, T., Weinberg, F., Morris, P. W., Roeder, R. G. & Rutter, W. J. (1970) *Science*, **170**, 447–449.
25. Albro, P. W. (1975) *Anal. Biochem.*, **64**, 485–493.
26. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265–275.
27. Omura, T. & Sato, R. (1964) *J. Biol. Chem.*, **239**, 2470–2478.
28. Guidotti, A., Kurosawa, A. & Costa, E. (1976) *Fed. Proc.*, **35**, 296.
29. Castagna, M., Palmer, W. K. & Walsh, D. A. (1975) *Eur. J. Biochem.*, **55**, 193–199.
30. Jungman, R. A., Hiestrand, P. C. & Schweppe, J. S. (1974) *Endocrinology*, **94**, 168–183.
31. Yamasaki, H., Huberman, E. & Sacks, L. (1975) *J. Biol. Chem.*, **250**, 7766–7770.
32. Byus, C. V. & Russell, D. H. (1976) *Biochem. Pharmacol.*, **25**, 1595–1600.
33. Byus, C. V. & Russell, D. H. (1975) *Fed. Proc.*, **34**, 265.
34. Hutterer, F., Dressler, K., Greim, H., Czygan, P., Schaffner, F. & Popper, H. (1975) *Adv. Exp. Med. Biol.*, **58**, 117–126.