Ribonucleic Acid Polymerase Activity in Liver Nuclei from Rats Pretreated with 3-Methylcholanthrene

EDWARD BRESNICK

Department of Pharmacology, Baylor University College of Medicine, Houston, Texas 77025

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SUMMARY

The intraperitoneal administration of 3-methylcholanthrene is attended by an increase in RNA polymerase activity in the liver nuclei. The maximal increase is reached between 6 and 12 hr after administration of the agent; enzyme activity returns to control values by 48 hr. The administration of actinomycin D or cycloheximide prevents the rise in nuclear RNA polymerase activity.

No increase is apparent when the enzyme is assayed at high salt concentrations, i.e., 0.7 M ammonium sulfate. These data suggest that 3-methylcholanthrene may cause the synthesis of or activate a "derepressor" substance that allows for an increased template activity of the liver chromatin in transcription.

INTRODUCTION

The intraperitoneal administration of 3-methylcholanthrene to rats effects a marked increase in the activities of several drug-metabolizing enzymes located within the microsomal fraction of liver (1–7). The liver microsomes isolated from these animals incorporate radioactive amino acids more readily into protein (6, 8). Further, the enhanced activity of the microsomal enzymes following the treatment of the rats with 3-methylcholanthrene is prevented by the administration of puromycin (7, 9), actinomycin D (10), or the amino acid antagonists ethionine and β-2-thienylalanine (11), suggesting the initiation by the polycyclic hydrocarbon of new enzyme synthesis.

Whether 3-methylcholanthrene mediates these increases in enzymic activities by augmenting the rate of synthesis of messenger-RNA is not clear at present (12, 13). The polycyclic hydrocarbon, however, does not alter the polyribosomal pattern of liver (14), the turnover of polyribosomal RNA (14), or the nuclear ribonuclease activity (E. Bresnick and K. Lanclos, unpublished observations). The results reported herein establish that the RNA polymerase activity in liver nuclei is enhanced shortly after the administration of 3-methylcholanthrene to rats. The enhancement in RNA polymerase activity does not appear to represent an increase in enzyme synthesis.

MATERIALS AND METHODS

Male Cheek-Jones (Houston) rats, 50–60 g, were fed ad libitum during the course of the experiments. The rats were injected intraperitoneally with 0.5 ml of Mazola corn oil or with 1.5 mg 3-methylcholanthrene in the corn oil. Periodically, groups of these animals were sacrificed and the livers were removed and rinsed in 0.9% saline. The livers were minced in a cold room and were homogenized to 10% in 2.2 M sucrose containing 3.3 mM calcium chloride; the liver nuclei were isolated by the Chauveau method (15). Generally, each group included the nuclei from 3 livers.

Preparation of "aggregate" RNA polymerase. The nuclei were lysed by the pro-

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cedure of Busch *et al.* (16) in 0.05 M Tris-phosphate buffer, pH 7.4, and the resultant suspension was centrifuged at 10,000 *g* for 10 min. The sediment, representing the "aggregate" enzyme was resuspended in 0.05 M Tris-HCl buffer, pH 7.4. The DNA was extracted from an aliquot of this suspension at 90° after the addition of an equal volume of 10% trichloroacetic acid. The concentration of DNA was then determined by the Burton (17) modification of the diphenylamine method of Dische.

RNA polymerase assay. Enzymic activity was ascertained in a system with a total volume of 0.25 ml consisting of: UTP-2-^{14}C, 24 μC/μmole, 0.1 μC; $MnCl_2$, 1 μmole, or $MgCl_2$, 1.2 μmoles; ATP, GTP, CTP, 0.25 μmole each; Tris buffer, pH 7.4, 40 μmoles; β-mercaptoethanol, 2 μmoles; "aggregate" enzyme; water, to 0.25 ml. Nuclear RNA polymerase activity was simultaneously assayed in the high ionic strength medium of Goldberg (18), i.e., after the addition of a saturated solution of ammonium sulfate. The incubation was conducted at 37° for 10 min. Under these conditions, enzymic activity was directly proportional to time and enzyme concentration. The reaction was terminated upon the addition of 0.1 ml of a solution containing 1 mg bovine serum albumin and 100 μg UTP-^{12}C and 5 ml cold 5% trichloroacetic acid. The precipitate was sedimented after centrifugation at 1000 *g* for 5 min, was washed three times with cold 5% trichloroacetic acid, and finally was dissolved in 1 ml of 70% formic acid. An aliquot of the latter was placed on a stainless steel planchet and dried at 80°; its radioactivity was determined in a end-window, gas-flow counter.

RESULTS

The metal requirement for nuclear "aggregate" RNA polymerase activity was investigated (Table 1) at low and high ionic strength, i.e., in the absence and presence of 0.7 M ammonium sulfate. The enzyme at low ionic strength could utilize either manganese or magnesium ions; the latter was more effective. In the presence of 1.0 and 1.25 μmoles of $MnCl_2$ and $MgCl_2$, respectively, the specific enzymatic activity was 5.2 and 15.5 mμmoles of UTP in-

TABLE 1

Requirements of the RNA polymerase reaction

The complete system included: UTP-2-^{14}C (24 μC/μmole), 0.1 μC; ATP, GTP, CTP, each 0.25 μmole; Tris buffer, pH 7.4, 40 μmoles; β-mercaptoethanol, 2 μmoles; "aggregate" enzyme (see text), DNA content 16.9 μg (A), 19.0 μg (B); water, to 0.25 ml. The assays were conducted in the absence or presence $(NH_4)_2SO_4$, i.e., 0.7 M.

Conditions	Nuclear RNA polymerase (mμmoles UTP incorporated/mg DNA)	
	$-(NH_4)_2SO_4$	$+(NH_4)_2SO_4$
A. Complete system without Mg^{++} or Mn^{++}	<0.5	<0.5
$+MgCl_2$, 0.25 μmole	6.5	ND[a]
0.63 μmole	14.5	ND
1.25 μmoles	15.5	40.3
2.5 μmoles	18.0	ND
$+MnCl_2$, 0.5 μmole	5.0	ND
1.0 μmole	5.2	70.5
2.0 μmoles	2.3	ND
+($MnCl_2$, 1.0 μmole; +$MgCl_2$, 1.25 μmole)	3.3	56.4
B. Complete system without Mg^{++} or Mn^{++}	<0.5	<0.5
$+MnCl_2$, 1.0 μmole	6.4	79.0
$+MnCl_2$, 1.0 μmole; −(ATP, GTP, CTP)	3.0	3.0
$+MnCl_2$, 1.0 μmole; −mercaptoethanol	6.2	74.0
$+MnCl_2$, 1.0 μmole; 1/2 enzyme	3.1	41.0

[a] ND, not determined.

corporated per milligram of DNA. In the presence of both $MnCl_2$ and $MgCl_2$, inhibition was observed. Inhibition of enzyme activity was also apparent at concentrations of $MnCl_2$ greater than 1.0 μmole per 0.25-ml assay system. Enzymic activity was routinely determined with each of these metals.

The addition of ammonium sulfate at 0.7 M greatly enhanced enzymic activity as reported by other investigators (18–20). The increase was more apparent in the presence of $MnCl_2$; the specific activities in the presence of 1.0 μmole $MnCl_2$ and 1.25 μmoles $MgCl_2$ were 70.5 and 40.3 mμmoles UTP incorporated per milligram of DNA.

In the absence of the nucleotides, ATP, GTP, and CTP, little enzymic activity was observed; the omission of β-mercaptoethanol had little effect upon the "aggregate" enzyme.

The effect of ammonium sulfate is further illustrated in Table 2. Maximal activity was recorded with 0.7 M concentration of the salt. Nuclear RNA polymerase activity was also enhanced in the presence of heparin at concentrations from 0 to 40 μg

TABLE 2

Effects of $(NH_4)_2SO_4$ and heparin upon RNA polymerase activity

Male Cheek-Jones rats, 50–60 g, were sacrified, the livers were removed and the liver nuclei were isolated. The RNA polymerase activity was determined. The complete system included: UTP-^{14}C; 0.1 μC; ATP, GTP, CTP, each 0.25 μmole; Tris buffer, pH 7.4, 40 μmoles; β-mercaptoethanol, 2 μmoles; Mn^{++}, 1 μmole; "aggregate" enzyme containing (A) 13 μg DNA, (B) 20.6 μg DNA; water, to 0.25 ml.

Additions	Nuclear RNA polymerase activity (mμmoles UTP incorporated/mg DNA)
A. None	6.2
$(NH_4)_2SO_4$, 0.11 *M*	21.0
0.23 *M*	25.8
0.46 *M*	38.6
0.7 *M*	54.0
1.2 *M*	54.0
B. None	5.8
Heparin, 20 μg	11.0
40 μg	18.3
100 μg	10.9

TABLE 3

RNA polymerase activity in liver nuclei after the administration of 3-methylcholanthrene

Male Cheek-Jones rats, 50–60 g, were injected with 0.5 ml Mazola corn oil or with 3-methylcholanthrene (3-MC), 1.5 mg per rat, in the corn oil. i.p.; the animals were periodically sacrificed thereafter. The livers were removed, and pooled in groups of 3, the nuclei were concentrated, and the "aggregate" enzyme was assayed as described in the text. The enzyme activity in the animals receiving the corn oil sacrificed at 3, 12, 24, and 48 hours did not substantially differ and is presented as a single group, i.e., 0 time. The assay was conducted in the low ionic strength medium in the presence of Mg^{++} or Mn^{++} (see table) or in the high ionic strength medium in the presence of Mn^{++} (0.7 M $(NH_4)_2SO_4$).

Time after 3-MC administration (hr)		Nuclear RNA polymerase (mμmoles UTP incorporated/mg DNA)		
		$+Mg^{++}$	$+Mn^{++}$	$+(NH_4)_2SO_4 + Mn^{++}$
0	(6)[a]	13.6 ± 0.6[b]	6.8 ± 0.3	69.1 ± 4.5
3	(5)	15.4 ± 1.0	7.1 ± 0.6	59.8 ± 5.0
6	(6)	21.4 ± 1.4	11.5 ± 0.8	62.6 ± 6.8
12	(5)	18.0 ± 1.5	11.0 ± 0.9	76.1 ± 11.5
24	(5)	18.1 ± 1.4	8.3 ± 0.8	66.1 ± 6.3
48	(6)	14.5 ± 0.9	6.3 ± 0.3	69.0 ± 10.0

[a] Number of determinations is given in parentheses.

[b] Mean ± standard error.

per 0.25-ml assay system. Above the latter, inhibition was apparent.

The effects of the administration of 3-methylcholanthrene upon the "aggregate" enzyme activity are depicted in Table 3. The specific activities have been determined in the presence of $MgCl_2$, $MnCl_2$, and $(NH_4)_2SO_4$-$MnCl_2$. By 6 hr after the administration of the polycyclic hydrocarbon, enzymic activity was elevated. The increase however, was recorded only when the enzyme was assayed in the low ionic strength media, i.e., $MgCl_2$ or $MnCl_2$. The maximal activity was reached between 6 and 12 hours after administration of the drug; control values were reached by 48 hours.

The properties of the "aggregate" enzyme concentrated from 3-methylcholanthrene-treated rats were similar to the control enzyme. Maximal specific activity was observed in the presence of Mg^{++} and required the presence of ATP, GTP, and CTP. The addition of 20 μg heparin increased the activity of the former enzyme by about 2-fold.

The enhancement in RNA polymerase activity after treatment of the rats with 3-methylcholanthrene was abolished when actinomycin D or cycloheximide were administered (Table 4). When actinomycin D was given 10 min before and 2.5 hr after 3-methylcholanthrene, no increase above control values was observed. When actinomycin D was administered at 2 or 4 hr after 3-methylcholanthrene, slight enhancement of enzymic activity was apparent. Cycloheximide when administered at 10 min before and 2.5 hr after 3-methylcholanthrene prevented the enhancement in the activity of the "aggregate" enzyme.

DISCUSSION

The activity of the "aggregate" liver nuclear RNA polymerase is enhanced within 6 hr after the intraperitoneal administration of 3-methylcholanthrene to rats. An increased enzymic activity could be the resultant of several factors: (a) an actual increase in enzyme synthesis; (b) the presence of activators or diminished production of inhibitors; (c) the presence within the chromatin–RNA polymerase complex of a template with improved transcribing ability; (d) the alteration of the configuration of RNA polymerase so that the latter is more active.

The presence of soluble activators does not account for all the experimental data. In "mixed" experiments, i.e., experiments in which "aggregate" enzyme from untreated and 3-methylcholanthrene-treated

TABLE 4

Effect of actinomycin D and cycloheximide upon nuclear RNA polymerase activity after administration of 3-methylcholanthrene

Male rats, 50–60 g, were injected i.p., with 3-methylcholanthrene (3-MC), 1.5 mg in corn oil at 0 time. At times thereafter, animals were injected i.p., with either actinomycin D (Act D), 100 μg, or cycloheximide, 2.0 mg. The animals were sacrificed at 6 hr after the administration of 3-MC. The RNA polymerase activity was determined in the presence of Mn^{++} or Mg^{++}, as described in the text, at low ionic strength. The control values are presented in Table 3.

Treatment		Nuclear RNA polymerase (mμmoles UTP incorporated/mg DNA)	
		$+Mg^{++}$	$+Mn^{++}$
3-MC	(6)[a]	21.4 ± 1.4[b]	11.5 ± 0.8
3-MC; Act D, at −10 min and +2.5 hr	(4)	15.7 ± 1.3	5.7 ± 1.0
3-MC; Act D at +2 hr	(3)	15.7 ± 1.5	7.9 ± 1.0
3-MC; Act D at +4 hr	(3)	16.8 ± 1.1	8.9 ± 0.9
3-MC; cycloheximide at −10 min and +2.5 hr	(4)	11.6 ± 1.1	5.6 ± 0.6

[a] Number of determinations is given in parentheses.

[b] Mean ± standard error.

rats were mixed, the summation of enzymic activities was observed. The first of these possibilities also may be eliminated since no increase in enzyme activity is apparent in the high ionic strength medium, i.e., 0.7 M ammonium sulfate. The ammonium sulfate may act by removing "blockers" from the DNA template, thus allowing for the observed stimulation in enzymic activity, or simply by inhibiting nuclear ribonuclease activity. In unpublished studies (E. Bresnick and K. Lanclos), we have not been able to demonstrate any effect of pretreatment of rats with 3-methylcholanthrene upon the activity of the latter in liver. These data suggest that treatment of the rats *in vivo* with 3-methylcholanthrene may bring about an increase in the template activity of the liver chromatin without altering the total amount of nuclear RNA polymerase. One must also recall that the effect of the polycyclic hydrocarbon was blocked by protein-synthesis inhibitors. Perhaps, the polycyclic hydrocarbon causes the synthesis of a protein which acts as a "derepresser" of the chromatin, i.e., removes a genetic regulator from the chromatin. A similar explanation has been offered for the action of estrogens upon rat uterine RNA polymerase activity by Gorski and colleagues (19). Dahmus and Bonner (20) have also reported the isolation of chromatin from the liver of hydrocortisone-treated adrenalectomized rats that possessed greater template activity in transcription than chromatin isolated in a similar manner from control rats. Further, the removal of proteins from the chromatin abolished this difference in template activity.

The manner in which 3-methylcholanthrene elicits this effect is not known. One might speculate that the binding of the polycyclic hydrocarbon to a particular protein might endow the latter with "derepresser" properties or that the production of a "derepresser" protein might be enhanced by 3-methylcholanthrene. We are presently attempting to show the binding of 3-methylcholanthrene to a specific cytoplasmic or nuclear protein in liver. We are also isolating chromatin from the livers of control and 3-methylcholanthrene-treated rats with the view of establishing their efficacy as templates in an *Escherichia coli* RNA polymerase system. These experiments should help to decide whether the effect of 3-methylcholanthrene is on the template or upon the RNA polymerase itself.

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