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REGULATION OF RNA POLYMERASE I ACTIVITY BY ORNITHINE DECARBOXYLASE*

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Abstract—Ornithine decarboxylase activity was elevated from 5- to 80-fold in rat liver by the administration of various methylxanthine derivatives, i.e. theophylline, aminophylline, and 3-isobutyl,1-methylxanthine. The increased activity of ornithine decarboxylase in all instances was closely followed (within 0.5 hr) by an increase in the activity of RNA polymerase I. The increment in ornithine decarboxylase activity and the increase in RNA polymerase I activity after all three drugs showed a parallel relationship. That is, theophylline administration resulted in the least stimulation of both ornithine decarboxylase and RNA polymerase I activities, and 3-isobutyl,1-methylxanthine administration produced the greatest stimulation of both enzymes. The enhanced RNA polymerase I activity resulted in a significant increase in the total RNA content of the liver within 24 hr. Administration of inhibitors of protein synthesis and RNA synthesis indicated that attenuation of the increase in ornithine decarboxylase activity was closely paralleled by attenuation of the increase in RNA polymerase I activity. RNA polymerase I activity was not stimulated by putrescine concentrations which were physiological and above. We suggest, therefore, that ornithine decarboxylase may be involved in the regulation of RNA polymerase I activity.

Polyamines are organic cations which are found ubiquitously in living systems [1, 2]. Studies of both mammalian and bacterial cells suggest that the physiological roles of polyamines might be those of growth factors, possibly through their ability to regulate RNA synthesis [3–8]. Several studies indicate a direct correlation between the spermidine concentration in cells and the amount of rRNA that can be accumulated [8–10]. However, significant changes in the accumulation of spermidine and rRNA occur much later than increases in both ornithine decarboxylase (EC 4.1.1.17), the initial enzyme in the polyamine biosynthetic pathway, and RNA polymerase I, a nucleoside triphosphate–RNA nucleotidyltransferase (EC 2.7.7.6), the enzyme responsible for rRNA synthesis. For example, both enzymes are markedly increased in rat liver within 4–6 hr after the administration of 3-isobutyl,1-methylxanthine [11] or Aroclor-1254, a polychlorinated biphenyl [12]. The increase in ornithine decarboxylase consistently occurs prior to the increase in RNA polymerase I activity. This temporal sequence is evident even in a second increment of ornithine decarboxylase activity and RNA polymerase I activity which was detectable 12–16 hr after the administration of Aroclor-1254 [12].

Studies of RNA synthesis in Ehrlich ascites cells and in rat liver have indicated that a short-lived protein is required for a normal level of transcription of the nucleolar genes [13, 14] and thus must be required for the regulation of the activity of RNA polymerase I. Further, in Ehrlich ascites cells, amino acids stimulated the synthesis, or possibly decreased the degradation rate, of this protein(s) [14]. Ornithine decarboxylase is a very labile enzyme with a half-life of 10–20 min [11, 15], is sensitive to amino acids [16, 17] and has been reported to affect the rate of initiation of RNA polymerase I on an endogenous template [18].

In this paper, we report studies of ornithine decarboxylase and RNA polymerase I in rat liver after the administration of various methylxanthine derivatives, compounds which markedly stimulate ornithine decarboxylase activity [19, 20]. Further, using 3-isobutyl,1-methylxanthine as a model inducer of ornithine decarboxylase, we have measured ornithine decarboxylase activity, RNA polymerase I, II, and III activities, polyamine concentrations, and the effects of putrescine itself on RNA polymerase I activity in rat liver at various times after the administration of this phosphodiesterase inhibitor. Moreover, we studied the effects of inhibitors of RNA synthesis and of protein synthesis on these enzyme activities to determine the temporal sequence of expression as well as the possible interrelationships between ornithine decarboxylase activity and RNA polymerase I activity.

EXPERIMENTAL PROCEDURE

Male Sprague–Dawley rats (100–125 g) were injected intraperitoneally with either theophylline (800 μmoles/kg in 0.9% NaCl), aminophylline (200 μmoles/kg in 0.9% NaCl), or 3-isobutyl,1-methylxanthine (40 μmoles/kg in 0.9% NaCl–ethanol, 5:1, v:v). Controls received only the appropriate solvent. For the inhibitor studies, animals were given either cycloheximide (50 mg/kg), cordycepin (30 mg/kg), actinomycin D (50 μg/ml), or actinomycin D

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(2 mg/kg), all in 0.9% NaCl. All animals were sacrificed by cervical dislocation at the indicated intervals, and samples from each liver were assayed for ornithine decarboxylase activity, RNA polymerase activity and polyamine concentrations.

Ornithine decarboxylase assay. Ornithine decarboxylase activity was determined by measuring the release of $^{14}CO_2$ from [^{14}C]ornithine [5] with minor modifications [21]. Freshly obtained tissue was homogenized in 5 vol. of 0.05 M sodium potassium phosphate buffer, pH 7.2, 0.1 mM EDTA and 1.0 mM dithiothreitol. Fifty μl of this 50,000 g supernatant was added to the standard ornithine decarboxylase assay which contained 6.5 μmoles sodium phosphate, pH 7.2, 4 nmoles pyridoxal phosphate and 0.1 μmole D,L-[1-^{14}C]ornithine (5.3 mCi/m-mole, New England Nuclear). One unit of enzyme activity is defined as the amount of enzyme necessary to generate one micromole of $^{14}CO_2$ per min, under the prescribed conditions. Purified cyclic AMP-dependent protein kinase from beef heart activated by 10^{-5} M cyclic AMP, incubated with ornithine decarboxylase, had no effect on its activity, suggesting that cyclic AMP does not regulate ornithine decarboxylase through direct phosphorylation.

RNA polymerase assay. Nuclei were isolated by a modification of the procedures described by Blobel and Potter [22] and Busch *et al.* [23]. The nuclei were checked visually for contamination and adjusted to approximately equal amounts of protein (200 μg/50 μl) before use as the enzyme source in the RNA polymerase assay.

In those experiments utilizing disrupted nuclei, an aliquot of the nuclear preparation was sonicated for 30 sec at 0–2 with an ultrasonic cell disrupter equipped with a 4.5-in. probe (Electro-Mechanics Instrument Corp. Perkasie, PA). The resulting homogenates were checked microscropically for whole nuclei before use in the assay.

The standard RNA polymerase assay mixture contained in a volume of 125 μl: 2.5 μg pyruvate kinase; 7 μmoles Tris–HCl (pH 7.9); 0.2 μmole $MnCl_2$; 1.0 μmole KCl; 0.75 μmole NaF; 0.5 μmole phosphoenol pyruvate; 0.2 μmole 2-mercaptoethanol; 0.075 μmole each of GTP, CTP and ATP; 0.125 μmole of unlabeled UTP; 0.0005 μmole [^{3}H]-UTP (19 Ci/m-mole, Schwarz/Mann); 7.25 μmoles $(NH_4)_2SO_4$; and 50 μl of the nuclear enzyme preparation [24]. One unit of enzyme activity is defined as the micromoles of [^{3}H]UTP per min, incorporated into RNA under the prescribed conditions.

Either 1.8 or 600 μg/ml (final conc) of α-amanitin was added to the standard assay in order to determine the activities of RNA polymerase II and III [25, 26].

RNA polymerase I activity also is not altered by cyclic AMP-dependent protein kinase and cyclic AMP, suggesting that direct phosphorylation is not involved in the regulation of its activity in rat liver after stimulation with phosphodiesterase inhibitors.

Determination of polyamine pool sizes. Polyamine concentrations were determined by means of a Durrum D-500 amino acid analyzer (Durrum Instrument Corp., Sunnyvale, CA) equipped with a 5-mm-pathlength flow cell. A PDP8/M computer, made by Digital Equipment Corp. but standard on the Durrum D-500, controlled the entire assay procedure including sample injection, time of buffer changes, and calculation of the peak areas of the polyamines. The liver samples were homogenized in 4 vol. of cold 5% tricholoroacetic acid, and an aliquot of the supernatant (1–5 mg of tissue) was applied to the column. Sample size and relative peak area of putrescine, spermidine and spermine over the range of 0.05 to 20 nmoles showed a linear relationship. This methodology has been described previously [27, 28].

RNA was determined spectrophotometrically by a modified Schmidt–Thannhauser procedure [29] and protein was measured by the method of Lowry *et al.* [30] using bovine serum albumin as the standard.

RESULTS

Effect of methylxanthine derivatives on ornithine decarboxylase activity and RNA polymerase activities.

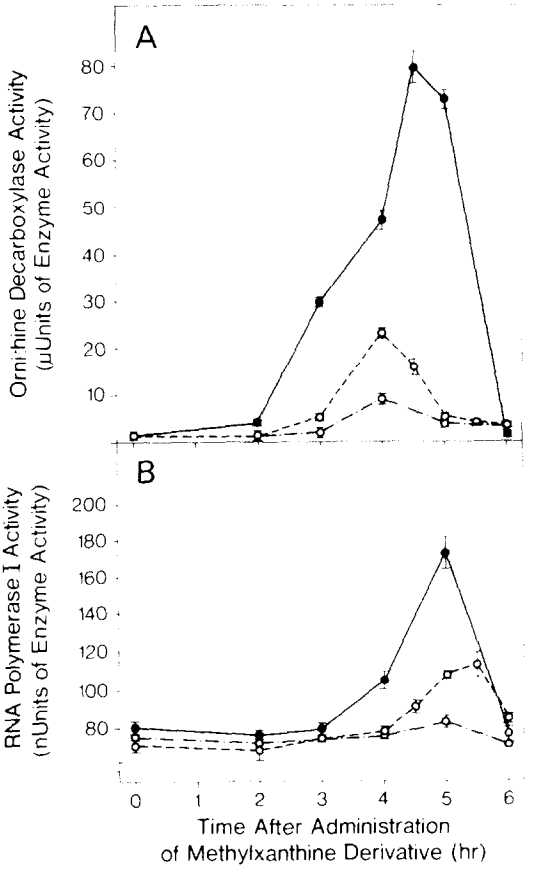

Fig. 1. Changes in ornithine decarboxylase activity (A) and RNA polymerase I activity (B) in the liver after administration of methylxanthine derivatives. Either theophylline (—·—·—) (800 μmoles/kg, i.p. in 0.9% saline), aminophylline (-----) (200 μmoles/kg, i.p. in 0.9% saline), or 3-isobutyl,1-methylxanthine (——) (40 μmoles/kg, i.p. in 0.9% saline–ethanol, 5:1, v:v) were given to rats and the animals killed at the stated intervals. Ornithine decarboxylase activity was determined by measuring the release of $^{14}CO_2$ from D,L-[1-^{14}C]ornithine. Using a purified nuclear preparation as the enzyme source, RNA polymerase I activity was determined by measuring the incorporation of [^{3}H]-UTP into RNA in the presence and absence of 1.8 μg/ml of α-amanitin. Each point represents the mean ± S. E. M. of duplicate determinations on at least ten rats.

Administration of various methylxanthine derivatives resulted in increases in both ornithine decarboxylase activity (Fig. 1A) and RNA polymerase I activity (Fig. 1B). The administration of theophylline produced the smallest increase in ornithine decarboxylase activity (5- to 6-fold) and also the smallest increase in RNA polymerase I activity (10–15 per cent). After the administration of 3-isobutyl,1-methylxanthine, ornithine decarboxylase activity increased to approximately 80-fold of control, and RNA polymerase I activity doubled. Aminophylline administration also resulted in increased ornithine decarboxylase activity (30-fold), and RNA polymerase I activity increased significantly. In all cases, the rise in ornithine decarboxylase activity preceded that of RNA polymerase I activity.

Alpha-amanitin-sensitive activity (RNA polymerase II) doubled within 4.5 hr of the administration of 3-isobutyl,1-methylxanthine and remained elevated at 5 hr (Table 1). RNA polymerase I activity continued to increase through 5 hr when it was double the control value (Table 1). Both enzymes returned to control levels (82.3 ± 2.1 nUnits/mg of protein for RNA polymerase I and 80.9 ± 3.5 nUnits/mg of protein for RNA polymerase II) within 6 hr after the administration of 3-isobutyl,1-methylxanthine.

Increasing the concentration of α-amanitin to 600 μg/ml, a concentration which inhibits forms II and III of RNA polymerase [26], indicated that there was a slight decrease in RNA polymerase III activity 5 hr after the administration of 3-isobutyl,1-methylxanthine as compared to the control (9.0 nUnits/mg of protein after 3-isobutyl,1-methylxanthine). We concluded, therefore, that the rise in α-amanitin-insensitive RNA polymerase activity described herein is due almost totally to a change in RNA polymerase I activity.

Effects of various inhibitors of RNA and protein synthesis on ornithine decarboxylase activity and activities of RNA polymerases. To assess the effects of cycloheximide on ornithine decarboxylase activity and on RNA polymerase activities, cycloheximide was given to rats which had received 3-isobutyl,1-methylxanthine 4 or 4.5 hr previously (Table 1). A similar inhibitory pattern was observed for ornithine decarboxylase activity and RNA polymerase I activity. Cycloheximide for 30 min resulted in an inhibition of ornithine decarboxylase activity of 78 per cent and an inhibition in RNA polymerase I activity of 45 per cent (Table 1). RNA polymerase II activity was not inhibited by cycloheximide administration.

When cordycepin was given simultaneously with 3-isobutyl,1-methylxanthine and both ornithine decarboxylase and RNA polymerase I activities were assayed 5.5 hr later, both enzymes were inhibited by about 14 per cent (Table 1). RNA polymerase II activity, however, was inhibited 47 per cent. This inhibition pattern would be consistent with the reported specific inhibition of RNA polymerase II activity by cordycepin and a subsequent effect on the amount of messenger RNA available for ornithine decarboxylase synthesis.

Actinomycin D (2 mg/kg) given simultaneously with 3-isobutyl,1-methylxanthine totally inhibited the increased activity usually demonstrated by ornithine decarboxylase and RNA polymerases I and II (Table 1). A lower concentration of actinomycin D (50 μg/kg), a concentration reported to selectively inhibit rRNA synthesis by forming a complex with rDNA [31], resulted in a slight inhibition of ornithine decarboxylase activity (16 per cent), an inhibition of RNA polymerase I activity of 83 per cent, and an inhibition of RNA polymerase II of 36 per cent (Table 1), data compatible with decreased template activity.

Increase in total RNA in response to administration of 3-isobutyl,1-methylxanthine. The increased RNA polymerase I and II activities resulted in an increased amount of RNA in the liver within 24 hr (Table 2). After a single injection of 3-isobutyl,1-methylxanthine, total RNA in the livers of treated rats increased 12 per cent compared to controls.

Concentration of polyamines in the liver after administration of 3-isobutyl,1-methylxanthine. Examination of the concentrations of the polyamines in the liver after the administration of 3-isobutyl,1-methylxanthine showed that the putrescine concentration increased 10-fold within 3 hr of stimulation (Table 3). This increase followed closely the elevation of ornithine decarboxylase activity and preceded the increase in RNA polymerase I activity. The concentration of putrescine continued to increase to a level 20- to 25-fold that of the control within 5 hr, and remained elevated at 6 hr, whereas ornithine decarboxylase and RNA polymerase I activities had returned to control levels by then. The concentration of spermidine was relatively constant until 6 hr post-stimulation, at which time it dropped to 40 per cent of the control value (Table 3). The spermine concentration was constant throughout the time period studied.

Effect of various concentrations of putrescine on RNA polymerase I activity. In an effort to assess whether ornithine decarboxylase itself or its product, putrescine, might affect RNA polymerase I activity, the nuclei were incubated in various concentrations of putrescine, including physiological concentrations, for 10 min before the assay was started. There was no increase in RNA polymerase I activity in response to pre-incubation with putrescine in intact or sonicated nuclear preparations (Table 4). Also, isolating the nuclei with concentrations of putrescine from 0.01 to 20 mM had no effect on RNA polymerase I activity in either intact or sonicated preparations.

DISCUSSION

There appears to be a direct correlation between increased rRNA synthesis and increased RNA polymerase I activity [32–35]. Therefore, the mechanism(s) involved in the regulation of RNA polymerase I activity are of extreme interest in the control of hypertrophy. Amino acid deprivation or cycloheximide treatment rapidly decreases RNA polymerase I activity *in vivo* and in cells in culture [13, 14, 36–38]. Reciprocally, enzyme activity increases *in vivo* within a few hours after partial hepatectomy [39] or the administration of a variety of drugs and/or hormones [11, 12, 31, 34, 40] and in cells in culture within minutes after supplementation of the media with amino acids [14]. Theoretically, the activity of RNA polymerase I could be altered either by changing the amount or the activity of the enzyme, or by alterations in the availability of the DNA template. Studies assaying RNA polymerase I activity after hor-

Table 1. Effect of inhibitors on ornithine decarboxylase activity and RNA polymerase activity after stimulation by the administration of 3-isobutyl,1-methylxanthine (MIX)*

Time after injection of MIX (hr)	Inhibitor and time after injection	Ornithine decarboxylase activity (μUnits enzyme activity/ mg protein)	Per cent inhibition	RNA polymerase I activity (nUnits enzyme activity/ mg protein)	Per cent inhibition	RNA polymerase II activity (nUnits enzyme activity/ mg protein)	Per cent inhibition
0.9% Saline/ ethanol only	None	1.1 ± 0.7		82.3 ± 3.3		73.0 ± 8.5	
4	None	47.0 ± 2.3		105.7 ± 5.7		103.7 ± 10.2	
4.5	None	89.7 ± 3.3		143.3 ± 5.3		144.7 ± 13.0	
5	None	73.3 ± 1.7		173.7 ± 10.0		150.0 ± 13.5	
	Cycloheximide						
4.5	30 min	20.0 ± 1.9	78	78.3 ± 7.2	45	143.0 ± 14.0	0
5	30 min	17.7 ± 1.3	76	100.0 ± 5.5	42	151.0 ± 13.4	0
	Cordycepin						
4.5	4.5 hr	76.9 ± 5.5	14	125.3 ± 9.9	13	76.0 ± 8.4	47
	Actinomycin D						
4	4 hr (50 μg/kg)	39.3 ± 3.7	16	18.0 ± 0.2	83	65.4 ± 7.8	37
4	4 hr (2 mg/kg)	0.9 ± 0.1	98	2.0 ± 0.2	98	1.3 ± 0.1	99

* Rats received 3-isobutyl,1-methylxanthine (50 μmoles/kg, i.p. in 0.9% saline–ethanol, 5:1, v:v). Cycloheximide (50 mg/kg, i.p.), cordycepin (30 mg/kg, i.p.), or actinomycin D (50 μg/kg or 2 mg/kg, i.p.) in 0.9% saline were given at the times indicated. The animals were killed at the times indicated and the livers removed and assayed for enzyme activity. Ornithine decarboxylase activity was determined by measuring the release of $^{14}CO_2$ from D,L-[1-^{14}C]ornithine. Using a purified nuclear preparation as the enzyme source, RNA polymerase activity was assayed by measuring the incorporation of [^{3}H]UTP into RNA. RNA polymerase I and II activities were distinguished by assaying in the presence and absence of 1.8 μg/ml of α-amanitin. Each point represents the mean ± S. E. M. of duplicate determinations of at least five rats.

Table 2. Increase in total RNA in rat liver after the administration of 3-isobutyl,1-methylxanthine*

	RNA (mg/g wet wt)	Per cent increase
Control	7.70 ± 0.17	
Experimental	8.72 ± 0.30†	12

* Rats received 3-isobutyl,1-methylxanthine (50 μmoles/kg, i.p. in 0.9% saline–ethanol, 5:1, v:v); controls were given an equivalent amount of solvent. After 24 hr, the animals were sacrificed, the RNA was isolated, and the amount determined by the procedure of Munro and Fleck [29]. The data represent the mean ± S. E. M. of triplicate determinations of the livers of six separate rats for each group.

† Data differ from control value ($P < 0.005$).

mone treatment on an exogenous template indicate that the regulation of enzyme activity is independent of template availability [38–40]. Moreover, if an unspecific poly d (A-T) template is used to assay RNA polymerase I activity after cycloheximide treatment [33], or amino acid deprivation [41], it is seen that neither the amount nor the activity of the enzyme has been changed.

On the basis of the experiments utilizing the artificial poly d (A-T) template, it has been postulated that RNA polymerase I exists in two functional states, one of which is attached to and actively transcribes the template (engaged enzyme) while the other is not (free enzyme) [41, 42]. The difference between these two forms seems to be a labile protein that interacts with the free enzyme and allows it to initiate transcription [33, 39, 41]. We propose that this labile enzyme is ornithine decarboxylase. Not only does ornithine decarboxylase have the characteristics described for this labile protein (sensitivity to amino acids [16, 17] and rapid turnover [15]) but also when the half-lives of both ornithine decarboxylase and RNA polymerase I were estimated in methylxanthine-stimulated rat liver after cycloheximide treatment, both enzymes declined with a half-life of 15 min. In the first 15 min after treatment, the amount of ornithine decarboxylase decreased by half and there was no change in RNA polymerase I activity. Thereafter, they declined in parallel [11]. Utilizing immunochemical techniques, it has been demonstrated that changes in ornithine decarboxylase activity in certain instances are a direct result of changes in the amount of ornithine decarboxylase protein [43]. If it is assumed that ornithine decarboxylase has a direct effect on RNA polymerase I activity, the decrease in the amount of ornithine decarboxylase protein would explain the similar half-lives of the two enzymes. That is, ornithine decarboxylase activity declines first and has to decrease to a certain level before it affects RNA polymerase I activity; once this point is reached, the activities of the two enzymes decline together. A physical interaction between ornithine decarboxylase and RNA polymerase I was demonstrated by the use of RNA polymerase I coupled to activated Sepharose to purify ornithine decarboxylase over 2000-fold [18]. The addition of this purified ornithine decarboxylase preparation to nuclei increased the initiation rate of RNA polymerase I as determined by the incorporation of $^{32}P/^{14}C$-labeled ATP [18].

Table 3. Polyamine concentrations in rat liver at various times after the administration of 3-isobutyl,1-methylxanthine*

Time (hr)	Putrescine	Spermidine (nmoles/mg wet wt)	Spermine
0	0.03 ± 0.003	2.84 ± 0.04	1.53 ± 0.29
2	0.02 ± 0.003	2.20 ± 0.16	1.42 ± 0.04
3	0.25 ± 0.028†	2.74 ± 0.26	1.84 ± 0.30
4	0.39 ± 0.050†	2.89 ± 0.53	2.12 ± 0.23
5	0.55 ± 0.050†	2.73 ± 0.05	1.62 ± 0.44
6	0.52 ± 0.036†	1.68 ± 0.06‡	1.33 ± 0.29

* Rats received 3-isobutyl,1-methylxanthine (40 μmoles/kg, i.p. in 0.5 ml of 0.9% saline–ethanol, 5:1, v:v); controls received 0.5 ml saline–ethanol. The animals were sacrificed at the times indicated and each liver was assayed for polyamine concentrations by analysis on a Durrum D-500 amino acid analyzer. Each point represents the mean ± S. E. M. of duplicate determinations of at least five separate livers.

† Data differ from control ($P < 0.005$).

‡ Data differ from control ($P < 0.025$).

For ornithine decarboxylase to be involved in the regulation of RNA polymerase I activity, there would have to be a consistent relationship between increased ornithine decarboxylase activity and increased RNA polymerase I activity. There is now considerable evidence derived from studies *in vivo* using such diverse stimuli as the industrial chemical Aroclor-1254 [12], a polychlorinated biphenyl, the methylxanthine derivatives [11], and phenobarbital (unpublished data, Manen, Sipes and Russell), to indicate that this is so. In all cases, the increase in ornithine decarboxylase activity precedes the increase in RNA polymerase I activity. Moreover, the administration of 3-isobutyl,1-methylxanthine not only resulted in considerable increases in the activities of ornithine decarboxylase and RNA polymerase I but also a significant increase in the total RNA within 24 hr, indicating that the increased activity of RNA polymerase I is translated into the synthesis of RNA. The magnitude of the increases in both ornithine decarboxylase and RNA polymerase I activities after the administration of 3-isobutyl,1-methylxanthine makes this an ideal system to study the relationship between ornithine decarboxylase and RNA polymerase I.

Table 4. Effect of putrescine concentration on RNA polymerase I activity of intact and sonicated nuclear preparations*

Putrescine conc	RNA polymerase I activity (nUnits enzyme activity/mg protein)	
	Intact	Sonicated
0	82.3 ± 4.0	84.1 ± 6.3
0.1 μM	84.2 ± 4.0	83.3 ± 3.4
1 μM	83.0 ± 6.7	78.5 ± 1.1
10 μM	73.4 ± 5.8	81.6 ± 1.9
100 μM	80.1 ± 4.0	86.6 ± 2.4
1 mM	80.6 ± 5.9	75.4 ± 3.1
10 mM	73.4 ± 5.5	82.2 ± 0.8

* RNA polymerase I activity was assayed as described Experimental Procedure. The nuclei were preincubated for 10 min with the indicated concentration of putrescine. Each point represents the mean ± S. E. M. for five determinations.

The studies reported herein utilizing inhibitors of RNA synthesis and protein synthesis in methylxanthine-stimulated rat liver corroborated a tight relationship between the induction of ornithine decarboxylase and the modulation of RNA polymerase I activity. Cycloheximide attenuation of the increase in ornithine decarboxylase resulted in a similar attenuation of RNA polymerase I activity. The activity of RNA polymerase II was not affected by cycloheximide. Cordycepin interferes with the synthesis of poly(A) chains needed for functional mRNA [44]. The administration of this antibiotic, therefore, should cause a decrease in the putative ornithine decarboxylase message, and the resulting decrease in ornithine decarboxylase should appear as an attenuation of RNA polymerase I activity; and indeed, when cordycepin was given simultaneously with 3-isobutyl,1-methylxanthine, this is what occurred (see Table 1). A low dose of actinomycin D, which selectively inhibits rRNA synthesis [31], almost totally inhibited RNA polymerase I activity, as determined on the endogenous template, with only a slight inhibition of ornithine decarboxylase activity and of RNA polymerase II activity.

Because the putrescine concentration rises rapidly in response to increased activity of ornithine decarboxylase, we felt it was possible putrescine might be involved in the regulation of RNA polymerase I activity. Incubation of nuclear preparations with a wide range of putrescine concentrations, including those concentrations that are physiological, did not affect RNA polymerase I activity. Further, the putrescine concentration remained elevated in the liver 6 hr after the administration of 3-isobutyl,1-methylxanthine at a time when RNA polymerase I activity had returned to control value. The half-life of putrescine in rat liver is about 2 hr [45]; therefore, it seems unlikely that putrescine is involved directly in the regulation of RNA polymerase I activity.

On the basis of the close temporal relationship between increased ornithine decarboxylase activity and increased RNA polymerase I activity, inhibitor studies indicating that *de novo* ornithine decarboxylase synthesis is required for increased RNA polymerase I activity, the similar short half-lives of the two enzymes, and the ability of ornithine decarboxylase to increase the initiation rate of RNA polymerase I, we propose that ornithine decarboxylase may modulate RNA polymerase I activity.

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