Effect of Medroxyprogesterone Acetate and Testosterone on Solubilized RNA Polymerases and Chromatin Template Activity in Kidney from Normal and Androgen-Insensitive (Tfm/Y) Mice†

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ABSTRACT: A single in vivo injection of the synthetic progestin, medroxyprogesterone acetate (MPA) (1 mg/25 g of body weight), increased RNA polymerase I and II activities in isolated mouse kidney nuclei. A tenfold higher dose of MPA did not further enhance the polymerase activities. After attaining peak values at about 4 h, both enzyme activities declined to control values. Subsequently, a second peak of activity was observed for RNA polymerase I. No further increase in RNA polymerase II activity was observed at later time points. The levels of solubilized RNA polymerases I, II, and III were also higher in the MPA-treated samples. When used at 10 mg/25 g (but not 1 mg/25 g) of body weight, MPA also stimulated the template capacity of the chromatin transcribed by homologous RNA polymerases I and II. MPA did not alter the activities of RNA polymerases or the chromatin template activity in mice with a defective androgen receptor (Tfm/Y), suggesting that some actions of MPA and testosterone are mediated via a common receptor. The action of this progestin differed from that of testosterone in several respects. First, unlike testosterone (Jänne, O., et al. (1976) *Biochim. Biophys. Acta 418,* 330–343), MPA did not stimulate RNA polymerase II activity in isolated nuclei at later time points. Second, testosterone stimulated chromatin template activity as early as 15 min of administration, attaining a peak value at 1 h whereas MPA exhibited no significant effect on the chromatin activity at time points earlier than 2 h and only when used at a dose ten times as much as that of testosterone. MPA treatment significantly increased the level of extractable RNA polymerase I, whereas no detectable changes in the level of this enzyme were observed, at least within 4 h of treatment with testosterone. The androgen-induced changes in the levels of RNA polymerase were observed only at 4-h time points and confined to polymerases II and III. The results of these studies suggest that MPA and testosterone require an active androgen receptor in order to stimulate transcription in mouse kidney. Even though both steroids exert their actions via the same receptors, the pattern of transcriptional events (whether a change in enzyme or chromatin) induced by MPA differs from that of testosterone.

It is now well accepted that steroid hormones regulate transcriptional processes in the target tissues (for reviews, see Liao, 1975; Mainwaring, 1975; O'Malley et al., 1976; Bardin et al., 1977). In the past few years, studies from this and other laboratories examined the mechanism(s) by which sex steroid hormones mediate the early cellular events (Bardin et al., 1973, 1977; Liao, 1975; Mainwaring, 1975; O'Malley et al., 1976). Several studies dealt with the effect of testosterone on RNA polymerase and chromatin activities in isolated kidney nuclei from normal female and androgen-insensitive Tfm/Y mice (Jacob et al., 1975; Jänne et al., 1976). The results indicated that testosterone stimulated early transcription in kidneys of normal but not in androgen-insensitive (Tfm/Y) mice (Jänne et al., 1976). These observations further indicated that the androgen receptor was required for gene transcription, as Tfm/Y mice have a functional defect in this binding protein (Bullock & Bardin, 1974; Jacob et al., 1975).

Since progestins are known to exhibit androgenic effects (Revesz et al., 1960; Suchowsky & Junkmann, 1961; Mowszowicz et al., 1974; Vomachka et al., 1974), it was pertinent to consider whether androgens and progestins showed a common mechanism of action. Medroxyprogesterone acetate (MPA) has been shown to increase kidney weight and β-glucuronidase activity in female mouse, but not in androgen-insensitive mice, suggesting that the progestin-induced changes in the kidney are mediated by androgen receptors (Mowszowicz et al., 1974; Bullock et al., 1977). In addition, MPA and other progestins can potentiate action of testosterone (Mowszowicz et al., 1974; Bullock et al., 1977; Gupta et al., 1978). It was of considerable interest to show whether MPA can also alter transcription in kidney and, if so, whether such an effect is mediated via the androgen receptor. To answer these questions, we investigated the effect of MPA on the transcriptional machinery in kidney from normal and androgen-insensitive mice. These studies showed that medroxyprogesterone acetate can indeed stimulate RNA polymerase activities in isolated nuclei, increase the levels of extractable RNA polymerases and augment the chromatin template activity in kidney from the normal female mice, and that these changes are not observed in Tfm/Y mice.

Materials and Methods

Chemicals. Medroxyprogesterone acetate (MPA) was the gift of the Upjohn Co. and testosterone was from Steraloids Inc., Pawling, N.Y. [5,6-^{3}H]UTP (50 Ci/mmol) was obtained from New England Nuclear, Boston, Mass; unlabeled nucleoside triphosphates, α-amanitin, and dithiothreitol were from Calbiochem, San Diego, Calif. Highly polymerized calf thymus DNA was from Worthington Biochemical Corp., Freehold, N.J., and phenylmethanesulfonyl fluoride ($PhCH_2SO_2F$)[1] from Sigma, St. Louis, Mo.

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[1] Abbreviations used: $PhCH_2SO_2F$, phenylmethanesulfonyl fluoride; DTT, dithiothreitol; ACTH, adrenocorticotropic hormone.

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Treatment of Animals. Female adult mice (20–25 g) (C57BL/6J, Jackson Laboratory) were housed in the Animal Facility with constant temperature (22 °C), humidity, and lights on a 12-h dark-light cycle. Androgen-insensitive Tfm/Y mice along with their normal female (+/+) littermates were from a colony maintained at The Milton S. Hershey Medical Center (Bullock & Bardin, 1974).

Treated mice (6–15 in each experimental group) received a single subcutaneous injection of MPA (1 or 10 mg) or testosterone (1 mg) in 0.2 mL of 10% ethanol–sesame oil. Control animals were injected with 0.2 mL of 10% ethanol–sesame oil. Animals were killed by cervical dislocation at 0.25–28 h after treatment and their kidneys removed.

Isolation of Mice Kidney Nuclei and Chromatin. All experimental procedures were carried out at 4 °C. Kidney nuclei were isolated as previously described (Jänne et al., 1976) and suspended in 10 mM Tris-HCl, pH 8, 0.32 M sucrose, 2 mM $MgCl_2$ to a DNA concentration of 0.6 mg/mL. Chromatin was prepared from kidney nuclei as described previously (Jänne et al., 1976) except that the buffer contained 1 mM Tris-HCl (pH 8) rather than 10 mM Tris-HCl (pH 8). The gelatinous chromatin was suspended in 0.4 mM Tris-HCl (pH 8), homogenized gently, and used to measure the template activity within 24 h. The ratio of protein to DNA was 1.8–2.0:1, and the recovery of nuclear DNA was approximately 70%.

Assay of RNA Polymerase Activity in Isolated Kidney Nuclei. Renal nuclear RNA polymerase activity was measured either by the method of Jänne et al. (1976) or in a modified assay mixture containing 140 mM Tris-HCl, pH 8.5, 3.5 mM DTT, 7.5 mM $MgCl_2$, 9.0 mM NaF, 50 mM KCl, 0.6 mM each of ATP, GTP, and CTP, 57 mM [^{3}H]UTP (31 cpm/pmol), in the presence or absence of α-amanitin (4.5 μg/mL) and nuclei equivalent to 20–40 μg of DNA. The assay mixture was incubated at 25 °C for 15 min and the reaction terminated by adding 100 μg of cold UTP. Radioactivity in [^{3}H]RNA was determined by spotting the reaction mixtures on Whatman DE-81 paper as described previously (Austoker et al., 1974; Rose et al., 1976). RNA polymerase activity was proportional to the amount of isolated kidney nuclei and linear for at least 20 min at 25 °C. RNA polymerase I and II were measured by their differential sensitivities to α-amanitin (Jacob et al., 1970; Kedinger et al., 1970; Lindell et al., 1970).

Solubilization and Chromatographic Separation of RNA Polymerases from Mice Kidney. RNA polymerase was extracted from kidneys of hormone-treated or control mice (14 mice/group). Whole kidneys were minced and then homogenized in a glass–Teflon homogenizer (five strokes) in 50 mM Tris-HCl (pH 8.9) (6 mL/g of wet weight of tissue) containing 0.5 mM $PhCH_2SO_2F$ and 40% (v/v) glycerol. The homogenate was filtered through two layers of cheesecloth. Nuclei were broken by 10-s pulses of sonication in Branson (Model W140) sonifier. Complete breakage of the nuclei was monitored under a phase contrast microscope. After dilution with equal volume of the same buffer (without glycerol), the sample was centrifuged at 10 000g for 15 min. The supernatant was placed on ice and the chromatin residue was suspended in $TG_{30}ED$ buffer (50 mM Tris-HCl, pH 7.9, 30% (v/v) glycerol, 0.1 mM EDTA, and 0.5 mM DTT) and then slowly mixed (with constant stirring) with $(NH_4)_2SO_4$, pH 7.9, to final concentration 0.3 M. This viscous chromatin suspension was sonicated for 50 s to release all bound RNA polymerases (Jänne et al., 1975; Rose et al., 1975) and was mixed with the supernatant fraction obtained at the previous centrifugation step. $(NH_4)_2SO_4$ was then added to the final concentration of 60% and the pellet was resuspended in the buffer containing 50 mM Tris-HCl, pH 7.9, 0.1 mM EDTA, 1 mM $MgCl_2$, 0.5 mM DTT, and 50% (v/v) glycerol and dialyzed against the same buffer overnight. After centrifugation at 80 000g for 40 min, the dialysate was subjected to DEAE-Sephadex A25 column chromatography and RNA polymerase activity in each fraction was measured by using calf thymus DNA as template essentially as described earlier (Rose et al., 1976). RNA polymerase activity was linear for 20 min at 30 °C in the presence of saturated amounts of calf thymus DNA.

Determination of Chromatin Template Activity. RNA polymerases I and II were partially purified from pig kidney nuclei according to a modification of the method described in this laboratory (Jänne et al., 1975) which consists of two successive chromatographic fractionations on DEAE-Sephadex columns rather than one fractionation each on DEAE-Sephadex and phosphocellulose column. At this stage of purification, the enzymes were free of detectable DNase and RNase activities. Chromatin template activity was determined in a reaction mixture containing 45 mM Tris-HCl, pH 8, 1.25 mM $MnCl_2$, 2.5 mM NaF, 6 mM KCl, 6.3 mM mercaptoethanol, 0.6 mM each of ATP, GTP, and CTP, 30 mM (for RNA polymerase I) or 75 mM (for RNA polymerase II) $(NH_4)_2SO_4$, 0.04 mM [^{3}H]UTP (30–40 cpm/pmol), mouse kidney chromatin (10–30 μg of DNA), and 600 units of renal RNA polymerase I, or 1580 units of renal RNA polymerase II. One unit of RNA polymerase was defined as 1 pmol of UMP incorporated in 15 min at 30 °C. The reaction was performed at 30 °C for 15 min and terminated by the addition of unlabeled UTP (100 μg). The pmol of UMP incorporated into RNA was determined by spotting the reaction mixtures on Whatman DE-81 paper as described previously (Rose et al., 1976). DNA was estimated as described by Burton (1956).

Results

Effect of MPA on RNA Polymerases I and II Assayed in Isolated Nuclei. MPA (10 mg/25 g of body weight) was given to female mice. Animals were killed at various time intervals ranging from 15 min to 28 h following hormone administration. In order to eliminate potential diurnal variations in the enzyme activity, injections with the hormone were made in such a manner that animals for each time point were killed at the same time of the day (9 a.m.–10 a.m.) as the untreated control mice. Further, no significant difference in the percent increase in the polymerase activities was observed when oil-injected control was used for each time point. Kidney nuclei were isolated and assayed for RNA polymerase I and II activities. As shown in Figure 1, both RNA polymerase I and II activities rose within 15 min and the increase persisted for at least 4 h. By 12-h time points, the enzyme activities had declined to control levels. Subsequently, RNA polymerase I exhibited a second peak of activity attaining almost 350% of the control value. On the other hand, no further increase in RNA polymerase II activity was observed. This was in contrast to the effect of testosterone on RNA polymerase II activity in isolated nuclei where significant stimulation of this activity was observed at these later time points (Jänne et al., 1976). The effect of MPA on RNA polymerase activity in androgen insensitive (Tfm/Y) mice was next examined. In contrast to normal mice, Tfm/Y mice did not respond to MPA as shown by lack of stimulation of RNA polymerase I or II activity after administration of MPA (Figure 1).

Since MPA is a weak agonist in most androgen bioassay systems, the dose of this steroid used in these studies was ten times that of testosterone used for previous experiments (Jänne et al., 1976) on RNA polymerases. In view of the marked response (Figure 1) it was important to determine whether 1 mg of MPA/25 g of body weight would also stimulate RNA

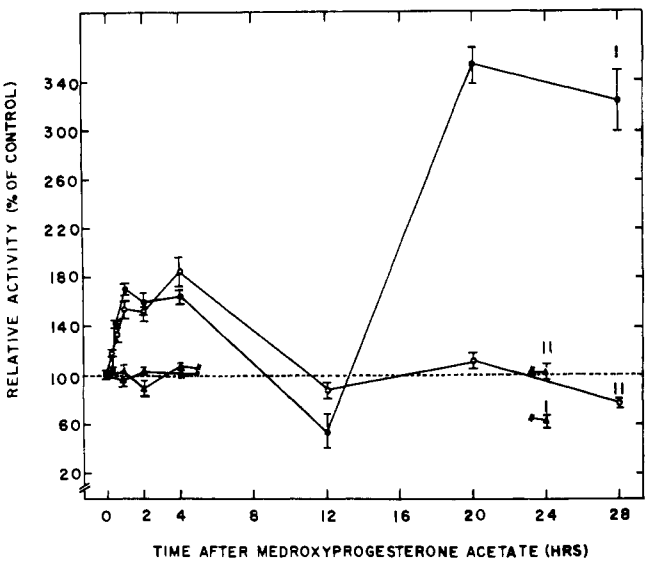

FIGURE 1: Effect of high dose of medroxyprogesterone acetate (MPA) on RNA polymerase activities in isolated kidney nuclei from normal female and androgen-insensitive (Tfm/Y) mice. MPA (10 mg/mouse) was administered subcutaneously to normal or Tfm/Y mice weighing 25 g. At various time intervals, ranging from 30 min to 28 h, after the injection, animals (six for each time point) were killed, nuclei were isolated, and RNA polymerase activities measured as described by Jänne et al. (1976). RNA polymerase I (●—●) and II (○—○) activities from normal female mice kidney and RNA polymerase I (▲—▲) and II (△—△) activities from Tfm/Y mice kidney were expressed as percentage of the controls (=100). Each point represents the mean values of three separate experiments ± SE. The mean values of RNA polymerase I and II activities for untreated normal mice were 112 and 322 pmol of UMP incorporated/mg of DNA, whereas those for untreated Tfm/Y mice were 233 and 305 pmol of UMP incorporated/mg of DNA, respectively.

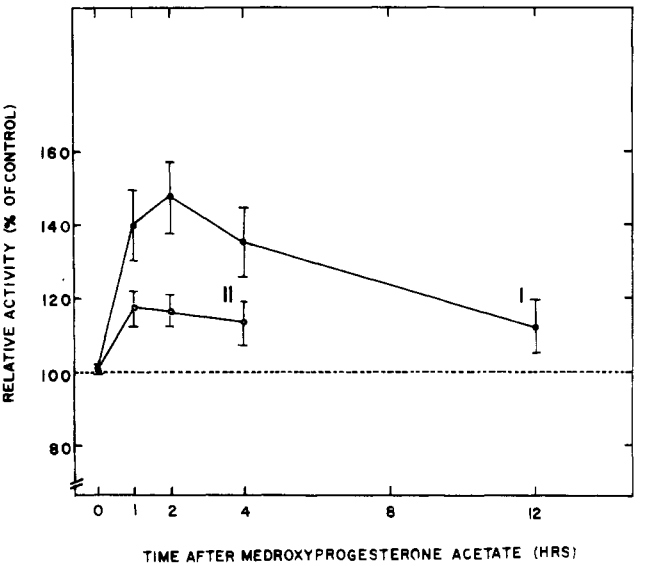

FIGURE 2: Effect of low dose of medroxyprogesterone acetate (MPA) on RNA polymerase activities in isolated kidney nuclei from normal female mice. MPA (1 mg/mouse) was given subcutaneously to normal female mice which were killed at 1, 2, 4, and 12 h after the hormone administration. Six mice were used for each time point. RNA polymerase activities were measured as described in the text. The activities of RNA polymerase I (●—●) and II (○—○) were expressed as percentage of controls (=100). Each point represents the mean values of three independent experiments ± SE. The mean values of RNA polymerase I and II activities for untreated mice were 233 and 305 pmol of UMP incorporated/mg of DNA, respectively.

polymerases. The effect of the lower dose of MPA on RNA polymerase I and II activities in isolated kidney nuclei was investigated at time intervals ranging from 1 to 12 h following steroid administration. As shown in Figure 2, even at this dose, MPA stimulated both RNA polymerase I and II activities. However, the stimulation of RNA polymerase I activity was significantly higher than that of RNA polymerase II. No difference was observed between the different strains of normal (androgen-sensitive) mice in regard to the percent changes in RNA polymerase activities induced by MPA and testosterone. The effect of testosterone or MPA was tissue-specific, because it did not affect the polymerase activities in nontarget tissues such as thymus or liver (data not shown).

Effect of MPA on Solubilized RNA Polymerases from Normal and Androgen-Insensitive Mice. In order to investigate further the effect of MPA on RNA polymerase activities in isolated nuclei, these enzymes were solubilized from whole kidney, within 4 h of administration of 1 mg of MPA/25 g of body weight. There were several reasons for selecting intact tissue rather than nuclei. First, quantitative extraction of RNA polymerases from nuclei required relatively large quantities of tissues. Second, the enzymes extracted from whole tissue were considerably more stable than those solubilized from isolated nuclei. Third, most of RNA polymerase III was lost into the homogenization medium during isolation of nuclei making quantitation of this enzyme by conventional assay procedures difficult (Lin et al., 1976). Finally, the recovery of total polymerases from whole kidney was approximately threefold higher than that from isolated nuclei.

Enzymes were extracted from whole kidneys (3 g derived from 14 mice) and subjected to DEAE-Sephadex column chromatography as described previously (Rose et al., 1976; Lin et al., 1976). Polymerase I, II, and III were eluted at 0.12 M, 0.2 M, and 0.3 M $(NH_4)_2SO_4$, respectively, and were identified by their differential sensitivities to α-amanitin. Enzyme activity in peak I was insensitive to α-amanitin, whereas activities under peaks II and III were sensitive to 1 and 130 μg/mL of α-amanitin, respectively. A typical DEAE-Sephadex column profile is shown in Figure 3. The levels of all three RNA polymerases were elevated after treatment of normal mice with MPA, whereas the levels of the enzymes remained unaltered in the Tfm/Y mice under these conditions. Fractions corresponding to I, II, and III were then pooled and assayed under optimal salt conditions for each enzyme. As shown in Table I, the activities of RNA polymerases I, II, and III increased 115, 44, and 29%, respectively, after MPA treatment. No increase in the activity of these enzymes was observed in Tfm/Y mice in response to MPA (Figure 3, Table I) implying that the stimulatory effect of this steroid was mediated via the androgen receptor.

Effect of Testosterone on Extracted RNA Polymerases from Normal and Androgen-Insensitive Mice. The activities of RNA polymerase I, II, and III from kidney were also determined at 4 h after testosterone administration and the results are shown in Figure 4 and Table I. As shown in a typical DEAE-Sephadex column profile, both RNA polymerases II and III were elevated after hormone administration, but there was no detectable change in RNA polymerase I. When fractions corresponding to each of the three peaks were pooled and assayed under optimal salt conditions, the activities of polymerases II and III were 135% and 132%, respectively, of that of the control samples (Table I). However, the level of RNA polymerase I remained unaltered even under the optimal assay conditions (Table I). It should be noted that there was no change in the levels of any of the RNA polymerases at earlier time points (e.g., 2 h; data not shown).

The activities of extractable RNA polymerases were next

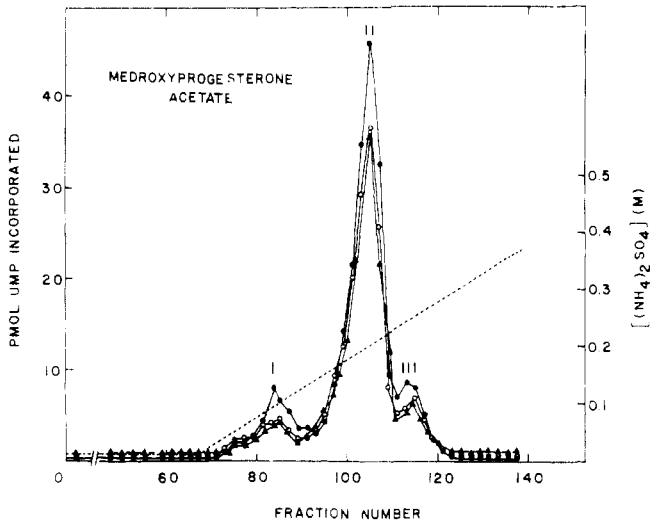

FIGURE 3: DEAE-Sephadex chromatography of kidney RNA polymerases from control or medroxyprogesterone acetate (MPA) treated (1 mg/25 g of body weight) normal and androgen insensitive mice. RNA polymerases were extracted from 3 g of kidney derived from MPA-treated (●--●) or oil-treated normal (○--○) mice or MPA-treated Tfm/Y mice (▲--▲). The mice were killed at 4 h after the treatments, as described in the text. Enzyme extracts containing the same amount of protein (100-125 mg) from each group of animals were simultaneously applied to three 2.5 × 10 cm DEAE-Sephadex (A25) columns previously equilibrated with 50 mM Tris-HCl (pH 7.0) containing 25% (v/v) glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA, and 0.5 mM dithiothreitol. The column was washed with 2 bed volumes of the same buffer containing 0.01 M $(NH_4)_2SO_4$ and fractionated with 3 bed volumes of a linear gradient of 0.01-0.6 M $(NH_4)_2SO_4$. Fractions of 3.3 mL were collected in tubes containing bovine serum albumin (2 mg) and aliquots (40 μL) were taken for RNA polymerase assay as described previously (Rose et al., 1976) with inclusion of 1 mM spermine. Enzyme activity was expressed as pmol of UMP incorporated.

measured in androgen-insensitive (Tfm/Y) mice before and after treatment with testosterone. As expected, none of the three polymerases were affected in the Tfm/Y animals (Figure 4, Table I). These findings are consistent with previous studies indicating a lack of androgen effect on RNA synthesis in isolated nuclei of Tfm/Y mice (Jänne et al., 1976).

In order to eliminate the possibility that the differences in the activities of control and hormone-treated animals were due to the presence of inhibitors and stimulators, respectively, known aliquots of the enzymes from the control and hormone-treated samples were mixed and assayed for individual RNA polymerases I, II, and III. The enzyme activity after mixing was equal to the anticipated values (data not shown). This implies that the testosterone and MPA increase either the activity and/or the number of RNA polymerase(s) molecules.

Effect of MPA and Testosterone on Chromatin Template Activity. The effect of MPA on chromatin template activity was then investigated. Chromatin was isolated as described under Materials and Methods and was transcribed using renal RNA polymerases I and II partially purified from pig kidney (Jänne et al., 1975). The chromatin activity was determined at 1, 2, and 4 h following administration of 1 and 10 mg of MPA. RNA polymerases I and II were used at 30 mM and 75 mM $(NH_4)_2SO_4$, respectively, for transcribing the chromatin from control and hormone-treated kidneys (Table II). MPA (1 mg/25 g of body weight) did not alter chromatin activity up to 4 h. When the dose of MPA was increased to 10 mg, renal chromatin activity increased by 2 h (140% and 131% with polymerases I and II) and remained elevated for up to 4 h. By contrast, 1 mg of testosterone increased chromatin activities

TABLE I: Effect of MPA and Testosterone on the Levels of RNA Polymerases I, II, and III Solubilized from Mouse Kidney Nuclei.[a]

strain	treatment	RNA polymerase act. % of control I	II	III
C57BL/6J	MPA	215 ± 10	144 ± 4	129 ± 4
Tfm/y	MPA	92 ± 6	93 ± 6	101 ± 1
C57BL/6J	testosterone	99 ± 10	135 ± 13	132 ± 2
Tfm/y	testosterone	80 ± 3	102 ± 3	94 ± 4

[a] Fractions containing RNA polymerases I, II, and III were pooled from each DEAE-Sephadex chromatography. After dialyzing overnight against buffer containing 50 mM Tris-HCl, pH 7.9, 0.1 mM EDTA, 0.5 mM DTT, and 25% (v/v) glycerol, fractions were analyzed for RNA polymerase activity by using 30 mM $(NH_4)_2SO_4$ for RNA polymerase I and 80 mM $(NH_4)_2SO_4$ for RNA polymerases II and III in the presence of excess of calf thymus DNA as described by Rose et al. (1976). MPA or testosterone was given at a dose of 1 mg/25 g of body weight. The results are the average of at least three determinations and expressed as percent control with standard errors.

transcribed with either RNA polymerases I or II by 1 h. Thereafter, both activities declined to 110 and 123% of controls by 4 h (Table II).

Discussion

Medroxyprogesterone acetate is a synthetic progestin that simulates some of the actions of testosterone and other androgens on mouse kidney and fetal reproductive tract (Revesz et al., 1960; Suchowsky & Junkmann, 1961; Mowszowicz et al., 1974; Vomachka et al., 1974; Bullock et al., 1977; Gupta et al., 1978). In view of these androgenic actions of MPA, it was of interest to determine whether the early effects of this steroid on the transcription were similar to those of testosterone.

The present studies demonstrated that a single dose of MPA (1 or 10 mg) or testosterone (1 mg), given subcutaneously, increases the activities of RNA polymerases I, II, and III in the kidney of female mice. Testosterone and MPA also enhance the chromatin template activity within 1 or 2 h of treatment, when homologous RNA polymerase I or II is used for transcription. On the other hand, Tfm/Y mice do not respond to treatment with MPA or testosterone. These observations together with the finding that [^{3}H]MPA binds to the androgen receptor in mouse kidney (Bullock et al., 1977) are consistent with the hypothesis that both MPA and testosterone enhance the early transcriptional events via a common receptor.

Even though both MPA and testosterone exert effects via the androgen receptor, there are clear differences between the two hormones with regard to their effects on chromatin and RNA polymerases: (a) the increase in RNA polymerase II activity in isolated nuclei observed hours after testosterone administration (Jänne et al., 1976) is not seen with MPA; (b) testosterone stimulates chromatin template activity as early as 15 min after administration attaining a peak value at 1 h (Jänne et al., 1976), whereas MPA does not exhibit significant effect on chromatin activity until 2 h; (c) a much larger dose of MPA than testosterone is required to elicit chromatin activation; (d) at an early time point (4 h) MPA increases the activities of all three extracted RNA polymerases as opposed to testosterone which stimulates only polymerases II and III. It therefore seems likely that the stimulation of RNA polymerase I activity in isolated nuclei at a low dose of MPA is due to the effect on the enzyme rather than on the template. On the

TABLE II: Effect of MPA or Testosterone on Chromatin Template Activity Using Kidney RNA Polymerases I and II for Transcription.[a]

time (h)	treatment (dose/25 g of body weight)	template act. pmol of UMP incorp/mg of DNA RNA polymerase I	RNA polymerase II	% of control RNA polymerase I	RNA polymerase II
1	control	620 ± 89	2410 ± 177	100	100
	MPA (1 mg)	568 ± 45	2065 ± 103	91	86
	MPA (10 mg)	530 ± 34	2156 ± 99	85	89
	testosterone (1 mg)	849 ± 71	3312 ± 227	137	137
2	control	383 ± 34	2806 ± 106	100	100
	MPA (1 mg)	328 ± 31	2525 ± 76	86	90
	MPA (10 mg)	538 ± 39	3662 ± 153	140	131
	testosterone (1 mg)	468 ± 49	3364 ± 234	122	120
4	control	461 ± 26	4826 ± 138	100	100
	MPA (1 mg)	400 ± 50	4170 ± 191	87	86
	MPA (10 mg)	574 ± 28	6275 ± 135	125	130
	testosterone (1 mg)	511 ± 38	5960 ± 286	110	123

[a] Adult female mice (10 weeks old, ten in each group) were administered with a single dose of either 1 mg of MPA, 10 mg of MPA, or 1 mg of testosterone. At indicated times, animals were killed and kidney chromatin isolated. Chromatin activity was measured in the presence of saturated amounts of mammalian RNA polymerase as described in Materials and Methods. The incorporation of UMP into RNA was linear and proportional to the amount of chromatin at 30 °C for 15 min. The amount of mammalian RNA polymerases I and II used in each corresponded to 599 and 1590 pmol of UMP incorporated into RNA with excess of DNA as the template. Results are the average of at least three determinations ± SE.

other hand, the testosterone-induced stimulation of RNA polymerase I activity (as measured in isolated nuclei) at 1-2 h (Jänne et al., 1976) does not appear to result from increase in the enzyme activity per se (Figure 4). The early stimulation of RNA synthesis in the kidney by androgens must, therefore, be attributed to chromatin activation (Jänne et al., 1976).

It should be pointed out that α-amanitin sensitive RNA polymerase II activity in isolated nuclei is stimulated to the same extent when the enzyme assay is performed either at 50 mM or 200 mM $(NH_4)_2SO_4$. Since low ionic conditions appear to retain the structural integrity of the nuclei during incubation, 50 mM $(NH_4)_2SO_4$ was used in these studies. The lack of stimulation of RNA polymerase II activity assayed under high salt conditions in uterine nuclei after estradiol administration (Bouton et al., 1977; Weil et al., 1977) could be due to susceptibility of these nuclei to lysis in high ionic strength media, which may have masked the effect of the hormone on transcription in the nuclei. Use of very high salt concentrations (500 mM) may also explain the lack of effect of testosterone on RNA polymerase II activity reported by other investigators (Avdalovic & Kochakian, 1969).

To date, most studies on the hormone-induced changes on RNA polymerases have focused on class I and II polymerases. The reason for not investigating effects on RNA polymerase III has been primarily due to the inability to detect this enzyme in most assay systems. To our knowledge investigation where a hormone has been shown to increase the level of extractable RNA polymerase III is in regard to the effect of ACTH on adrenal polymerases (Fuhrman & Gill, 1975). In these studies, the elevation of RNA polymerase III was specific to RNA polymerase IIIA. Since the enzyme was extracted from whole kidney tissue, the polymerase III fraction probably contains a mixture of IIIA and IIIB. Consequently, it has not been possible to differentiate the effect of MPA or testosterone on IIIA from that on IIIB. Attempts to enhance the polymerase III activity by using poly[d(A-T)] as the template (Weinmann et al., 1975) have failed in our hands. The stimulation of this enzyme by ACTH or sex steroid hormones is interesting in view of the fact that these enzymes are involved in the synthesis of low molecular weight RNA species including tRNA and 5S

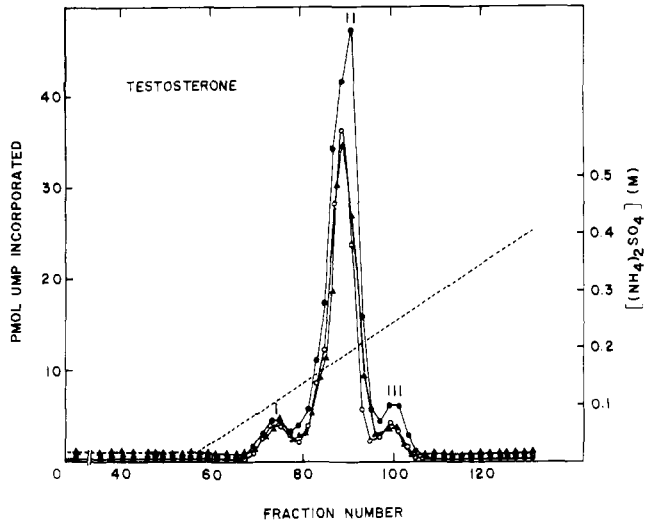

FIGURE 4: DEAE-Sephadex chromatography of kidney RNA polymerase isolated from untreated or testosterone-treated normal and androgen-insensitive mice. RNA polymerases were extracted from 3 g of kidney derived from testosterone (1 mg/mouse) (●—●) or oil-treated (○—○) normal female mice or testosterone-treated Tfm/Y mice (▲—▲). The mice were killed at 4-h time points. Enzyme extraction, column chromatographic fractionation, and enzyme assay were as described in the legend to Figure 3.

RNA (Weinmann and Roeder, 1974). Indeed, ACTH has been shown to increase the synthesis of these RNAs in adrenal gland (Fuhrman & Gill, 1976). It thus seems possible that MPA also augments the synthesis of one or more of these RNA species in mouse kidney and that this stimulation is brought about by enhancing the RNA polymerase III activity. Similarly, the increased levels of RNA polymerase I and II should lead to enhanced synthesis of rRNA and mRNA. It is conceivable that the stimulation of mRNA synthesis corresponds to specific mRNA species such as the mRNAs for β-glucuronidase, an enzyme which is induced several fold by MPA in normal but not in Tfm/Y mice (Mowszowicz et al., 1974; Bullock et al., 1977).

The increased levels of RNA polymerases could not be due to higher recovery of these enzymes after steroid treatment since: (1) there is no enzyme activity in the residue remaining after enzyme extraction; (2) the activities of RNA polymerases are not altered by hormone administration to Tfm/Y mice; (3) the percent stimulation of enzyme activities is similar when they are measured in isolated nuclei, in solubilized extracts, or after partial purification. Finally, the presence of stimulating factors (e.g., RNase inhibitors) in the steroid-treated mice is excluded by mixing aliquots of each RNA polymerase fraction (pooled samples) from control and hormone-treated mice and assaying them under standard assay conditions. The enzyme activity in such a mixture of enzymes is equal to the anticipated value. In a typical experiment, 30 μL each of the pooled RNA polymerase I fractions from control and MPA-treated animals corresponded to 2.5 and 1.2 units (pmol of UMP incorporated) of enzyme, respectively; mixing 30 μL of the control enzyme with 30 μL of the hormone-treated enzyme resulted in 3.6 units of enzyme activity. Based on these considerations, the results of the present study suggest that MPA and testosterone increase the levels of RNA polymerase. It is not possible to ascertain whether the changes are due to an increase in new polymerase molecules or to activation of catalytically inactive molecules.

Since our studies have emphasized changes at early time points, no attempts have been made to extract RNA polymerases at later time intervals. The significantly higher RNA polymerase I activity observed in isolated kidney nuclei at 20–28-h time points (Figure 1) after MPA treatment may indeed be due to correspondingly increased levels of extractable RNA polymerase. Further, the effect of MPA is not confined to chromatin-bound population of RNA polymerase; the levels of RNA polymerases I and II extracted from the bound fraction after 4 h of MPA treatment were 126% and 115% of the control, respectively.

Finally, it should be borne in mind that, although these studies suggest a role of androgen receptor in the action of progestins, MPA might exert a positive effect on the transcriptional process by other mechanisms. The final proof for the specific role of the androgen receptor in the biological effects of progestin will rest on the studies on the direct interaction of purified hormone–receptor complexes with the transcriptional machinery.

Note Added in Proof

Recent studies in our laboratory have shown that testosterone increased the number of initiation sites on the kidney chromatin from 1900 to 2400 with homologous RNA polymerase I and from 13 300 to 15 700 with homologous RNA polymerase II. Such an increase was not observed in the Tfm/Y mice.

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