ANDROGEN-INDUCED GENE ACTIVATION IN THE RAT PROSTATE*

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SUMMARY: The effect of androgens on gene activation in the rat prostate has been investigated by examining precursor incorporation into RNA, by DNA-RNA hybridization of RNA transcribed in vitro from prostate chromatin, and by thermal denaturation of prostatic chromatin. The results show a selective synthesis of nuclear RNA and a changed thermal melting profile of prostatic chromatin as a result of testosterone administration. Further, the in vitro synthesized RNA transcribed from prostatic chromatin of androgen-treated rats contained new RNA species that were not transcribed from chromatin of untreated castrated controls. The data provide direct evidence for an activated state of the prostatic chromatin stimulated by androgens.

Gene activation is believed to be the primary action of androgens. Testosterone, or its active metabolite, 5α-dihydrotestosterone, has been shown to stimulate the synthesis of nuclear RNA (1), messenger RNA (2,3) and ribosomal RNA (4-6), as well as RNA polymerase activity (7-9) in the prostate. Testosterone-stimulated increases in RNA polymerase activity and transcriptional template activity of chromatin in kidneys from normal female as compared with androgen-insensitive mice have also been reported by Jänne et al. (10). This androgen-induced gene activation should then be manifested in the transcription of additional or different gene sequences and in a changed state of the chromatin. In the present work, we have determined these two parameters by DNA-RNA hybridization of RNA transcribed from prostatic chromatin and by thermal denaturation of chromatin. The results, reported herewith, indicate that administration of testosterone stimulates a decondensation of the prostatic chromatin which provides for transcription of new RNA species from different DNA sequences.

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MATERIALS AND METHODS: Male Sprague-Dawley rats, weighing 350-400 g, were used in all experiments.

Bilateral orchidectomy was performed via the scrotal route, using ether anethesia. For in vivo labeling of RNA, rats castrated for three days were injected intraperitoneally with 156 µCi of [^{3}H]uridine (28.0 Ci/mmole) and 12.5 µCi of [^{14}C]guanine (54.9 mCi/mmole) 15 min prior to sacrifice. One mg of testosterone in 0.1 ml peanut oil containing 10% ethanol was injected intraperitoneally 15 min, 1 h, 5 h and 12 h prior to sacrifice (6 rats per group). The control rats were injected with 0.1 ml peanut oil only. At sacrifice, the ventral prostates were immediately removed, minced and chilled in cold 0.25 M sucrose containing 0.05 M Tris-HCl, pH 7.4, 0.025 M KCl and 0.005 M $MgCl_2$ (TKM). The prostate nuclei were isolated according to Blobel and Potter (11), collected as pellets after centrifugation at 39,000 rpm in a Spinco SW 39 rotor. The cytoplasmic supernatant from this centrifugation was diluted to 0.25 M sucrose-TKM, and centrifuged at 12,000 x g for 20 min. The supernatant from this centrifugation was then spun at 105,000 x g in a Spinco 40 rotor for 90 min. The pellet is the microsome fraction, and the supernatant, the cytosol. The microsomal pellet was suspended in TKM buffer and re-pelleted at 105,000 x g. RNA was isolated from these cellular fractions and quantitated according to Munro and Fleck (12) using an extinction of 1.0 at 260 nm as a concentration of 32 µg per ml. Radioactivities of 0.5 ml samples were counted by addition of 10 ml of scintillation fluid (300 g naphthalene, 21 g 2,5-diphenyloxazole and 0.9 g 1,4-bis-2(4-methyl-5-phenyloxazolyl)-benzene, made to 3 liters with dioxane) in a Packard scintillation spectrometer.

Chromatin was prepared from prostate nuclei as described elsewhere (13). Thermal denaturation of chromatin (in 2.5 x 10^{-4} M EDTA, pH 8.0) isolated from castrated rats injected with testosterone and from castrated controls was performed in a Gilford Model 2400 spectrophotometer with constant heating rate of 2/3° per min. The derivative dh_{260}/dT plot of the melting profile against temperature, T, was calculated according to Li and Bonner (14), where h_{260} is the percent increase in hyperchromicity at 260_{nm} (25°).

The RNA transcribed from prostatic chromatin in vitro was synthesized using RNA polymerase (fraction VI) purified from Micrococcus luteus according to the method of Nakamoto et al. (15). The reaction mixture, scaled up 40-fold, was in 10 ml and contained the following: 50 µmoles of Tris-HCl, pH 7.5, 25 µmoles $MnCl_2$, 20 µmoles spermidine phosphate, 10 µmoles each (0.125 mCi) of tritium-labeled ATP, CTP, GTP and UTP, prostatic chromatin equivalent to 1.0 mg of DNA, and 1,000 units of RNA polymerase. The reaction mixture was incubated at 30°C for 30 min and then an additional 500 units of the enzyme were added and incubation was continued for another 30 min. At the end of incubation, sodium dodecylsulfate and NaCl were added to the mixture to final concentrations of 0.5% and 0.14 M, respectively. The in vitro synthesized RNA was extracted with phenol and purified as described elsewhere (16).

DNA-RNA hybridization was carried out according to Gillespie and Spiegelman (17). Annealing of synthesized RNA was performed by the procedure of Tan and Miyagi (18). One mg of alkali-denatured prostatic DNA in 6 x saline-citrate (1 x saline-citrate is 0.15 M NaCl and 0.015 M sodium citrate) was immobilized on nitrocellulose membrane filter (25 mm). A reaction mixture of 1 ml of [^{3}H]RNA in 30% formamide solution and 2 x saline-citrate was incubated with two DNA filters and two blank filters at 37°C for 24 h in screw cap vials. At the end of incubation, one DNA filter with a saturating amount of RNA and one blank filter were transferred to increasing amounts of another [^{3}H] RNA preparation. These filters were incubated as before. Both sets of filters were washed with 2 x saline-citrate, incubated with pancreatic RNase, dried and counted for radioactivity.

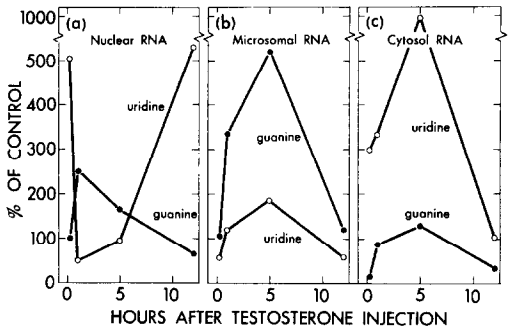

Fig. 1. Incorporation in vivo of [^{14}C]guanine and [^{3}H]uridine into (a) nuclear RNA, (b) microsomal RNA, and (c) cytosol RNA of prostates from castrated rats receiving a single injection of testosterone. One mg of testosterone in peanut oil was injected into castrated rats at the time intervals indicated. The radioactively-labeled guanine and uridine were injected intraperitoneally 15 min prior to sacrifice. The isolation of nuclei, microsomes and cytosol and quantitation of RNA were as described in the text. The incorporation of radioactive labels into RNAs is expressed as the percent change in specific activity, defined as pmoles of the radioactive precursors per mg RNA, as compared with that of the untreated castrated controls.

RESULTS AND DISCUSSION: Fig. 1 shows a time course study of incorporation in vivo of [^{14}C]guanine and [^{3}H]uridine into the nuclear, microsomal and cytosol RNA of prostates from castrated rats following a single injection of testosterone. The uracil-rich nuclear RNA synthesis, detectable 15 min after the hormone administration, was almost shut off at the first hour. During the 1-5 hr period, the RNA synthesis in the prostatic nuclei was predominantly guanine-rich RNA which decreased steadily after the first hour. Concomitantly, guanine-rich microsomal RNA synthesis showed a proportional increase. Subsequent nuclear RNA synthesis was reverted to a uracil-rich type, parallel to a reduction in microsomal RNA synthesis during the same period. The result suggests a differential stimulation of nuclear RNAs and a sequential transfer of ribosomal RNA from nucleus to cytoplasm. This interpretation is consistent with the data of Liao and coworkers (4,7) which show a turnover of ribosomes and the synthesis of primarily ribosomal RNA after testosterone administration.

The cytosol RNA synthesis was predominantly of the uracil-rich type, but manifested differences in the ratios of uridine and guanine incorporation

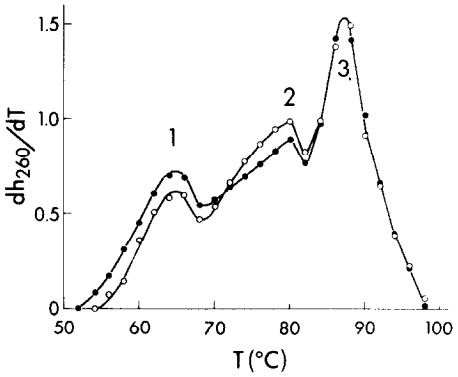

Fig. 2. Derivative melting profiles of prostatic chromatins prepared from 12-h-testosterone injected (●—●) and untreated (○—○) castrated rats.

throughout the 12 hr period after testosterone injection. This probably reflects the processed nuclear RNAs transported to the cytoplasm.

Fig. 2 shows the derivative melting profiles of chromatins from castrated rats injected with testosterone and the castrated control. Three melting bands may be resolved at melting temperatures of 56°, 78° and 86°. Band 1 is mainly contributed by non-histone proteins and bands 2 and 3, by histones (14). The areas under band 3 for both androgen-stimulated and control chromatins are identical; whereas the androgen-treated chromatin exhibits a less histone-bound band 2 area but an increased band 1 area relative to the castrated control. This indicates that testosterone injection induces a change in the state of prostatic chromatin in which contribution to DNA stabilization due to histones is reduced and that due to non-histone proteins increased. Since histone binding represents the more stabilized segment of chromatin, this suggests a less condensed chromatin induced by testosterone administration, in accordance with a gene-activated state of chromatin.

To ascertain that the new RNA synthesis in, and the changed state in chromatin from, prostates of testosterone-injected rats reflect gene activation, DNA-RNA double saturation hybridization of RNAs transcribed from chromatins of testosterone-treated and control castrates was carried out. As

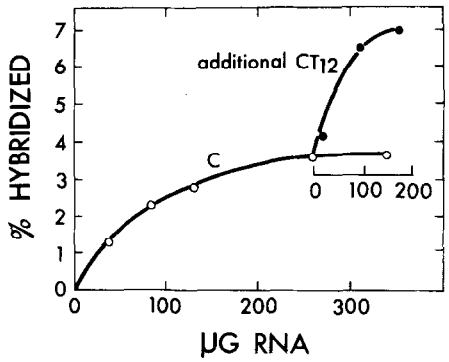

Fig. 3. Double-saturation DNA-RNA hybridization of RNAs synthesized from the prostatic chromatin of castrated rats injected with testosterone and chromatin of untreated castrated rats. The prostatic DNA was annealed to saturation with [^{3}H]RNA transcribed from castrate chromatin (C). This RNA-saturated DNA was then hybridized with increasing amounts of [^{3}H]RNA transcribed from the prostatic chromatin of castrated rats injected with testosterone for 12 hr (CT_{12}).

seen in Fig. 3, the RNA synthesized in vitro from prostatic chromatin isolated from untreated castrated rats was annealed to prostatic DNA to 3.7% DNA-RNA hybrid formation. When the RNA-saturated DNA was further hybridized with RNA transcribed from the prostatic chromatin isolated from castrated rats injected with testosterone and sacrificed 12 hr later, the RNA transcribed from the testosterone-treated chromatin gave an additional 3.3% DNA-RNA hybrid formation. The data show that the RNA transcribed from the chromatin of castrated rats injected with testosterone is qualitatively different from that synthesized from the chromatin of the untreated castrated rats and represents RNA transcribed from DNA sequences which have been made available by testosterone injection. Administration of testosterone to castrated rats, therefore, results in the induction of activity in different genes. These results thus indicate an activation of genome sequences in the prostate following testosterone administration, resulting in the selective synthesis of new RNA species.

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