STIMULATION IN VITRO OF PROSTATIC RIBONUCLEIC ACID POLYMERASE BY 5α-DIHYDROTESTOSTERONE-RECEPTOR COMPLEXES

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SUMMARY In a reconstituted in vitro system, stimulation of RNA polymerase activities by 5α-DHT-receptor complexes prepared from prostatic supernatant and nuclear fractions has been observed. Stimulation of the nucleolar enzyme rather than the nucleoplasmic enzyme was noted. Higher levels of stimulation were observed in the presence of native chromatin as template than when purified exogenous DNA was used. The involvement of chromatin-associated proteins in the system was apparent.

Investigations in many research centres have indicated that protein receptors are essential factors in transmitting the steroid-hormone effect in cells of responsive tissues. Retention of the steroid hormone in the nucleus is dependent upon (a) an interaction with a specific cytoplasmic receptor protein (1-4) then (b) a close association in the nucleus with chromosomalsites composed of DNA and acidic protein (3,5-8). The present investigation is concerned with the effects of 17β-hydroxy-5α-androstan-3-one (5α-DHT) on the prostate and the reconstitution of an in vitro system which will enable 5α-DHT-receptor complexes formed in the prostate to bring about stimulation of the activity of prostatic DNA-dependent RNA polymerase (nucleoside triphosphate-RNA nucleotidyltransferase, EC 2.7.7.6). This involved the purification of the several cellular components from prostate tissue.

MATERIALS AND METHODS

All preparative procedures were performed on ventral prostate tissue excised from 8-12 week old male Sprague-Dawley rats 48 hr after bilateral castration. Nuclei were purified by the method of Mainwaring (9) using 1 mM $MgCl_2$ instead of $CaCl_2$ in all media and 2.2 M sucrose for the final centrifugation. Published procedures were used for the preparation of nucleoplasm and nucleoplasmic RNA polymerase (10), nucleoli, chromatin, nuclear and nucleolar RNA polymerase (11), for the selective removal of chromatin-associated proteins (8,12) and preparation of prostatic DNA (13). Labelling of cytoplasmic receptors with [1α,2α-$^3H_2$]5α-DHT (specific radioactivity 47 Ci/mmol: The Radiochemical Centre, Amersham, Bucks. U.K.) and selective precipitation of receptors was achieved using the method of Mainwaring & Peterken (8) and nuclear [1α,2α-$^3H_2$]5α-DHT-receptor complex was prepared by incubation of equal volumes of purified nuclei (100-150 μg of DNA) and labelled cytosol (0.4 pmol radioactive 5α-DHT per ml of mixture) at 37° for 30 min. The nuclei were spun out, washed extensively and extracted with 0.4 M KCl at 0°C for 30 min. The supernatant from centrifugation for 30 min at 100,000 g contained labelled nuclear receptors. Ionic strength of receptor-complexes could be decreased by passage through columns of Sephadex G-25. Patterns of labelling of cytoplasm and nuclear extract corresponded to those described by others(8). The cytoplasm yielded two steroid-protein complexes, of sedimentation coefficient 8S and 3S, and nuclei yielded one complex, of sedimentation coefficient approx. 4.5S.

RNA polymerase activities were estimated in 500 µl of a medium containing 60 µmol tris-HCl buffer, pH 8.1, 2.5 µmol $MgCl_2$ or 1.5 µmol $MnCl_2$, 15 µmol KCl, 200 nmol dithiothreitol 300 nmol NaF, 300 nmol each of ATP, GTP and CTP, 20 nmol carrier UTP and 125 pmol [U-^{14}C]UTP (specific radioactivity 514 mCi/mmol; The Radiochemical Centre, U.K.). Assays containing $MnCl_2$ also contained $(NH_4)_2SO_4$ (0.4 M). DNA template was added in 50 µl and enzyme in 200 µl medium. In experiments on intact nuclei and nucleoli, DNA template and polymerase were replaced by 250 µl of nuclear (50-100 µg of DNA) or nucleolar (20-50 µg of DNA) suspension. 5α-DHT-receptor complexes were added at a final radioactive 5α-DHT concentration of 250 pM. Control systems contained equal quantities of protein-receptor not equilibrated with 5α-DHT. Neither receptor protein nor 5α-DHT alone affected purified RNA polymerase activity. Enzyme reactions (15 min at 37°C) were terminated by the addition of 2 ml of 10% (w/v) CCl_3 COOH - 1 mM $Na_4P_2O_7$ and acid-insoluble material prepared for measurement of incorporated radioactivity as previously described (14). After correction for controls in the absence of DNA, the incorporation of [^{14}C]UMP into RNA was determined as pmol of [^{14}C]UMP incorporated per unit of DNA.

RESULTS AND DISCUSSION

Incubation of intact nuclei and nucleoli in the RNA polymerase assay system containing cytoplasmic or nuclear 5α-DHT-receptor complexes resulted in an increased incorporation of [^{14}C]UMP into acid-insoluble material (Table 1). The stimu-

Table 1 Effect of [^{3}H]5α-DHT-Receptor Complexes on RNA Polymerase Activity

Preparation	Exogenous template added	% Increase in incorporation of [^{14}C]UMP in the presence of		
		Cytoplasmic receptors '8S'	'3S'	Nuclear receptor '4.5S'
(a) Nuclei	-	107.0 ± 3.69	115.2 ± 7.29	53.5 ± 1.73
(b) Nucleoli	-	50.5 ± 13.56	77.5 ± 18.69	72.1 ± 2.67
(c) Enzyme solubilised from whole nuclei	Calf thymus DNA	14.4 ± 1.96	10.1 ± 3.01	28.6 ± 2.15
	Prostatic nuclear chromatin	158.2 ± 19.90	91.9 ± 16.02	40.7 ± 4.69
	Prostatic nucleolar chromatin	116.3 ± 13.78	85.8 ± 17.85	58.1 ± 4.86
	Liver chromatin	6.2 ± 3.26	8.1 ± 2.91	3.1 ± 2.64

Enzyme preparations were incubated with 5α-DHT-receptor complexes at a 5α-DHT concentration of 0.25 pmol/ml (based on radioactivity) or with an equal amount (based on protein) of '5α-DHT-free' receptor. Incubations with solubilised enzyme included one of several added templates. Increases in the rate of incorporation of [^{14}C]UMP into acid-insoluble material are expressed as percentages above those values observed in the presence of protein only. Values are the average of at least four determinations ± the standard deviations.

Table 2 Effect of 5α-DHT-protein receptor complexes on prostatic RNA polymerases

Conditions of assay	Template	% increase in enzyme activity in the presence of '8S'	'4.5S'
Nucleolar Enzyme (Form I)	Calf thymus DNA	11.8 ± 2.86	26.5 ± 5.47
	Prostatic nuclear chromatin	96.6 ± 6.12	39.0 ± 1.20
Mg^{2+}/low salt	Prostatic nucleolar chromatin	143.5 ± 10.59	50.4 ± 9.27
	Liver chromatin	4.5 ± 0.81	2.9 ± 1.30
	Calf thymus DNA	0	10.6 ± 0.62
Mn^{2+}/high salt	Prostatic nuclear chromatin	5.7 ± 1.30	10.6 ± 1.58
Nucleoplasmic Enzyme (Form II)	Calf thymus DNA	2.0 ± 1.89	7.8 ± 1.49
Mg^{2+}/low salt	Prostatic nuclear chromatin	12.8 ± 4.73	11.2 ± 7.04
	Liver chromatin	8.0 ± 5.68	0
	Calf thymus DNA	0	0
Mn^{2+}/high salt	Prostatic nuclear chromatin	0	16.3 ± 4.47

Nucleolar and nucleoplasmic RNA polymerases solubilised from the respective subnuclear fractions were incubated with various templates and 5α-DHT-receptor complexes (0.25 pmol radioactive 5α-DHT/ml) in assay mixtures containing either $MgCl_2$ (5 mM) and a low salt concentration (0.03 M KCl) or $MnCl_2$ (3 mM) and at high ionic strength 0.4 M $(NH_4)_2SO_4$. Increases in activity expressed as % increases over values observed in the presence of an equal concentration of 5α-DHT-free receptor protein. Values are the means of 4 or more experiments ± the standard deviation.

lation by both cytoplasmic complexes may suggest the possession of structural similarities (15). The various complexes also stimulated the activity of RNA polymerase solubilised from nuclei (Table 2). The degree of stimulation observed in the presence of purified calf thymus DNA or liver chromatin was slight compared to that observed when prostatic nuclear or nucleolar chromatin were used as template in the system. Nuclear RNA polymerase transcribed native chromatin with approx. 40% of the efficiency with which it transcribed calf thymus DNA. Increases in enzyme activity were not so marked however in the case of the nuclear complex. These results support the concept that specificity of binding of steroid-receptor complexes resides in tissue chromatin(16).

The degree of stimulation of subnuclear forms of prostatic RNA polymerase depended not only on the DNA template provided but also on the intranuclear source of the enzyme and the ionic conditions employed (Table 2). Ionic conditions of assay are known to influence the type of RNA synthesized (17,18). Nucleolar RNA polymerase was preferentially stimulated by 5α-DHT-receptor complexes, especially with Mg^{2+} as activating cation. The nucleoplasmic enzyme, which transcribed prostatic chromatin much more efficiently (40% of DNA) than did the nucleolar enzyme (15% of DNA) was stimulated only slightly in the presence of Mg^{2+} and not at all by cytoplasmic 8S complex in the presence of Mn^{2+}/ $(NH_4)_2SO_4$. The nuclear complex did however stimulate nucleoplasmic enzyme in the presence of prostatic chromatin. These results further suggest that the major RNA product of steroid-

Table 3 Effect of cytoplasmic 5α-DHT-protein receptor complexes on nuclear RNA polymerase in the presence of selectively altered templates

Template	pmol [^{14}C]UMP incorporated in the presence of "control receptor fraction" '8S'	'3S'	pmol [^{14}C]UMP incorporated in the presence of receptor complex '8S'	'3S'	% age increase in [^{14}C]UMP incorporation in the presence of receptor complex '8S'	'3S'
Calf thymus DNA	1.12	1.22	1.29	1.30	15	7
Liver chromatin	0.28	0.26	0.30	0.28	6	8
Prostatic nuclear chromatin	0.46	0.56	1.17	0.93	154	66
Histone-deficient prostatic chromatin	0.63	0.72	1.54	1.08	144	50
Histone and nonhistone-deficient prostatic chromatin	0.71	0.76	1.62	1.13	128	49
Prostatic DNA	0.89	0.75	0.97	0.86	9	15

RNA polymerase solubilised from prostatic nuclei was incubated in an assay medium containing cytoplasmic 5α-DHT-receptor complexes (0.25 pmol of radioactive 5α-DHT per ml). The template for RNA synthesis varied in composition - calf thymus DNA, liver chromatin, prostatic chromatin intact, deficient of histones, deficient of histone and acidic proteins, and prostatic DNA. Full details are given in the experimental section. The enzyme activity in the presence of the cytoplasmic complexes or an equal quantity of steroid-deficient receptor is expressed as pmol of [^{14}C]UMP incorporated/μg of template DNA and the percentage increase in activity in the presence of 5α-DHT-receptor complex is specified in each case.

hormone stimulation is of a nucleolar or ribosomal type and that the production of nucleoplasmic or messenger RNA is a secondary effect. A system in which chromatin initiation sites recognized by nucleolar enzyme are available for transcription in a normal androgenic environment and unavailable after castration may be hypothesised.

A further insight into the role of chromatin in the system is shown in Table 3. Stimulation of RNA polymerase by cytoplasmic complexes was low in the presence of calf thymus DNA and liver chromatin, and high in the presence of prostatic chromatin. Histone removal increased transcription but not the degree of stimulation by the complexes. Removal of the major part of the nonhistone-protein again increased transcription but stimulation remained at the same level. 10% of the chromatin-associated protein remained at this stage. With protein-free prostatic DNA as template, transcription was once again higher, but the levels of stimulation brought about by 5α-DHT-receptor complexes were low. This suggests that the stimulation of RNA polymerase activity by the complexes was influenced by nonhistone protein as was the binding of these complexes to DNA (8). Unlike nonhistone protein (20, 21), histones do not influence tissue-specificity of chromatin (19). The lack of any effect resulting from the removal of most of the nonhistone protein confirms that the major portion of this fraction is not concerned with tissue-specificity (22, 23) and that this property is conferred by proteins tightly bound to DNA (24) and only released by phenol treatment (8).

Our results, therefore, indicate that stimulation of RNA polymerase activity by 5α-DHT-receptor complexes is mainly confined to the nucleolar form of the enzyme in Mg^{2+} - low salt conditions and is under the control of acidic chromatin-associated proteins. It is still possible, however, that further controlling factors which exist in vivo will have to be introduced into the system.

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