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THE INFLUENCE OF STEROID–RECEPTOR COMPLEXES ON THE STAGES OF TRANSCRIPTION OF TARGET-TISSUE CHROMATIN

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SUMMARY

Earlier investigations established that complexes formed between 5α-dihydrotestosterone and receptor macromolecules derived from rat ventral prostate subcellular fractions cause changes in the nature and extent of transcription of prostate chromatin by homologous RNA polymerases. The present report describes an investigation into relationships between concentrations of cellular receptor sites and initiation and elongation of RNA chains on prostatic chromatin from rats after castration or after androgen administration to castrated animals. Occupied receptor sites were measured by precipitation with protamine sulphate and replacement of endogenously bound steroid with exogenous [^{3}H]-steroid. Initiation of RNA chains was measured by incorporation of [γ-^{32}P]-purine nucleotides and chain elongation by incorporation of [^{3}H]-UMP under conditions which precluded reinitiation i.e. in the presence of rifamycin AF/013 or under high salt conditions. Castration resulted in a marked depletion of cellular receptors, particularly nuclear sites, and a decrease in initiation sites on chromatin. Testosterone propionate, depending on the time of administration, partially or completely maintained the nuclear content of receptor, but not cytoplasmic receptor. Androgen increased initiation sites above levels observed on prostate chromatin of castrated rats. RNA chain length appeared dependent on androgenic status, but other reasons for this effect are discussed.

INTRODUCTION

The ventral prostate gland of the rat is a steroid-hormone target organ, as shown by its dependence upon an adequate supply of androgens for maintenance of its growth and function [see reviews 1, 2]. The intracellular localisation of the hormonal steroid and fulfilment of its postulated function within the cell are apparently dependent upon the preliminary formation of a cytoplasmic complex between the steroid and receptor proteins [1–3]. Studies in this laboratory [4–7] and elsewhere [8, 9] indicate that complexes formed in rat ventral prostate subcellular fractions between 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) and proteins of selective high affinity profoundly influence the transcriptive processes of the cell. Since changes in template activity can be due to alterations in any one of the many events involved in production of RNA chains [10], it is now necessary to separate the stages of transcription and observe steroid–receptor-induced effects on each stage individually. Moreover, if the steroid hormone–receptor complex is intimately or directly involved at the level of transcription, as previous studies suggest, the number or concentration of receptor sites within the cell should be in some way related to the amount or quality of transcription. This present communication serves as an introduction to a more detailed study of the interrelationship of receptors and transcription. Information is presented concerning the number of cytoplasmic and nuclear 5α-dihydrotestosterone (DHT)-receptor complexes and the initiation and elongation of RNA chains on rat ventral prostate chromatin at various times after castration and administration of androgen to castrated rats.

EXPERIMENTAL

Chemicals

1,2,4,5,6,7(*n*)-[^{3}H]-5α-Dihydrotestosterone (specific radioactivity 107–130 Ci/mmol), [5-^{3}H]-UTP (specific radioactivity 10.1 Ci/mmol). [γ-^{32}P]-ATP (reference date specific radioactivity 15–17 Ci/mmol) and [γ-^{32}P]-GTP (reference date specific radioactivity 13–16 Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks, U.K. The disodium salt of ATP and the trisodium salts of CTP, GTP and UTP were purchased from the Boehringer Corporation (London) Ltd., Ealing, London, U.K. Protamine sulphate (from salmon roe) was obtained from Koch–Light Laboratories, Colnbrook, Bucks, U.K. Crystalline BSA, calf-thymus DNA, dithiothreitol, Tris–base (99% pure) and 5α-dihydrotestosterone were bought from the British Drug Houses, Poole, Dorset, U.K. Other unlabelled steroids and diethylstilboestrol were purchased from either Koch–Light Laboratories, or Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Rifamycin AF/013 was generously provided by Dr. R. Cricchio and Professor L. G. Silvestri of Gruppo Lepetit, Milan, Italy. All other substances were of 'Analar' grade and were dissolved in water distilled from all-glass apparatus.

Animals and tissues

Male Sprague–Dawley rats were bred in the Institute Animal Unit and used when 8–12 weeks old. Animals were castrated by the scrotal route under ether anaesthesia. Orchidectomised animals were left untreated or received daily doses of testosterone propionate (400 μg/100 g body weight, in a max. vol. of 200 μl sesame oil) subcutaneously, beginning immediately, 24 h or 48 h after operation. Ventral prostate tissue was carefully dissected from animals killed when required (see 'Results'), placed in vessels surrounded with crushed ice and used immediately for the preparation of subcellular fractions (see below).

Centrifuges and rotors

All high-speed centrifugation procedures were carried out in a Beckman L2-65B or L5-65B ultracentrifuge using either the SW50.1 (6 × 5 ml) swinging-bucket rotor (r_{av} 8,35 cm.) or the SW60 (6 × 4 ml) swinging-bucket rotor (r_{av} 9.17 cm.).

Preparation of subcellular fractions

Methods for the preparation of soluble supernatants, nuclei and chromatin devoid of endogenous RNA polymerase activity have been previously described [4].

Measurement of receptor sites

(a) Cytoplasmic sites. Those receptor sites present in soluble supernatant (cytosol) fractions which were unoccupied by endogenous steroid were estimated by incubation (2 h at 0–4°C) of aliquots (100 μl) of cytosol (7–12 mg protein/ml) with equal vol. of [^{3}H]-DHT (2–25 nmol/l) with and without a 100-fold higher concentration of unlabelled DHT. Free and some non-specifically bound steroid were removed by mixing with 400 μl of charcoal suspension (2% (w/v) Norit A, 0.2% (w/v) Dextran T-70) for 15 min. Charcoal was sedimented at 800 *g* for 10 min, and radioactivity present in the supernatant was assessed by liquid scintillation counting. Total (occupied and unoccupied) sites were assessed by a method dependent upon prior precipitation of receptor sites by protamine sulphate. Protamine sulphate (100 μl of 1 mg/ml solution) was added to equal vol. of cytosol and precipitates were washed 5 times with 1 ml of buffer. Precipitates were then dispersed in [^{3}H]-steroid solution (see above) at various temperatures (usually 15°C, see 'Results') for various periods of time (usually 16 h, see 'Results'). After incubation, precipitates were resedimented, washed 6 times in ice-cold buffer, extracted twice with ethanol (1 ml), the combined extracts evaporated in scintillation vials, and radioactivity assessed as above.

(b) Nuclear sites. The number of receptor sites present in purified nuclei [4, 7] was estimated using an exchange assay. Protamine sulphate (100 μl of 1 mg/ml solution) was added to equal vol. of nuclear suspension (30–100 μg of DNA). Resulting precipitates were washed, incubated with [^{3}H]-steroid, washed and extracted as described above for total cytoplasmic sites.

Purification of rat ventral prostate RNA polymerase B

RNA polymerase B was solubilised from rat ventral prostate nuclei and purified in a similar manner to published procedures [11–14], involving sonication at high ionic strength, selective precipitation of contaminating nucleic acid with protamine sulphate, fractionation with ammonium sulphate, and chromatography on columns of DEAE-cellulose and phosphocellulose.

Estimation of RNA polymerase activity

Routine assessment of RNA polymerase B activity was carried out in a reaction mixture (500 μl) containing Tris–HCl buffer (120 mmol/l), pH 7.9, KCl (30 mmol/l), dithiothreitol (400 μmol/l), NaF (600 μmol/l), $MnCl_2$ (3 mmol/l), ATP, GTP and CTP (all 600 μmol/l), [^{3}H]-UTP (0.5 μCi), UTP (40 μmol/l) and glycerol (10% (v/v)). DNA template (10–25 μg) and RNA polymerase (4–20 μg protein) were added in 100 μl and 50 μl respectively. Certain assays contained $(NH_4)_2SO_4$ (40 mmol/l) and α-amanitin (40 ng/assay). Incubations (15 min at 37°C) were terminated and acid-insoluble material processed and assessed for incorporated radioactivity as described previously [4, 6].

Certain modifications were necessary to study separately RNA chain initiation and elongation. This was done in either of two ways. (a) By use of rifamycin AF/013. The final concentrations of reactants was as described above. RNA polyermase was preincubated (15 min at 37°C) with template in the absence of nucleoside triphosphates, which were then added together with rifamycin AF/013 (50 μg/assay). As well as [^{3}H]-UTP, this mixture contained either [γ-^{32}P]-ATP or [γ-^{32}P]-GTP (0.5 μCi in each case). Incubation was continued for a further 15 min, then terminated and material processed as above. (b) By initiation in low salt followed by elongation in high salt. RNA polymerase was preincubated (15 min at 37°C) in a mixture (270 μl) containing the same concentrations of substances as in the routine assay, including labelled nucleoside triphosphates, but with $MnCl_2$ at 1 mmol/l and excluding CTP. The initiation reaction was stopped and reinitiation prevented by addition of 85 μl of $(NH_4)_2SO_4$ (1.6 mol/l) and chain propagation begun by addition of $MnCl_2$ (final concentration 3 mmol/l) and CTP. Incubation and processing was carried out as for the routine assay. All assays performed under any of these regimes were corrected for zero-time controls, controls in the absence of DNA, and incorporation of radioactivity in the absence of enzymes.

Chemical analyses

Standard procedures were employed for the determination of DNA [15], using calf-thymus DNA as standard, and protein [16] using crystalline BSA as standard.

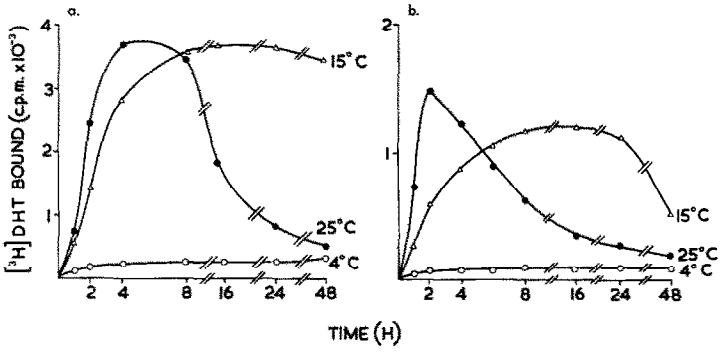

Fig. 1. Effect of time and temperature on the [^{3}H]-DHT exchange assay for rat ventral prostate receptor. Aliquots (100 μl) of (a) cytosol (7–12 mg protein/ml) or (b) nuclei (30–100 μg DNA) were mixed with equal vol. of protamine sulphate solution (1 mg/ml), and resulting precipitates were sedimented and washed. Precipitates were incubated with [^{3}H]-DHT (20 nmol/l) alone, or with [^{3}H]-DHT plus 100-fold excess of unlabelled DHT at various temperatures. At indicated times, incubations were stopped and the amount of specifically bound [^{3}H]-DHT determined as described in 'Experimental'. Each value represents the mean of three determinations.

RESULTS

Estimation of receptor sites in cytoplasmic and nuclear preparations

Difficulties have been experienced in attempting to demonstrate rat ventral prostate DHT-receptor binding by conventional Dextran-coated charcoal techniques [see 17], but certain success has been reported [18–21]. Using the protocol described above (see 'Experimental'), it was possible to show specific accessible binding sites for exogenous [^{3}H]-DHT, including a high-affinity saturable component with a dissociation constant (K_D) of 1–5 nmol/l. Full information will be published elsewhere. However, measurement of [^{3}H]-DHT replacement of endogenous DHT occupying receptor sites requires elevated temperature and prolonged incubation time. Thermolability of receptor sites precludes such an assay being performed with receptor free in solution. Precipitation of receptor by protamine sulphate [17] conferred greater stability on the receptor and allowed an exchange assay to be performed.

As shown in Fig. 1a, negligible exchange occurred at 0–4°C, while at higher temperatures (15°C and 25°C) the exchange process was more rapid. At 15°C, a plateau was reached after 12 h extending to 24 h of incubation. Increased temperature (25°C) increased the exchange rate, reaching a maximum after 4 h. Further elevation of the temperature to 30°C or 37°C (data not shown) resulted in rapid degradation of receptor making these temperatures unsuitable for the assay. For convenience and because of better reproducibility the assay was routinely employed at 15°C for 16 h. These conditions combined maximum replacement with [^{3}H]-steroid with minimal loss of binding capacity. Specific binding of [^{3}H]-DHT under these conditions, determined by bound radioactivity in the absence and presence of a 100-fold higher concentration of unlabelled DHT, was saturable, and in the cytosol preparations used in these studies, had an apparent K_D of 2–10 nmol/l.

The number of receptor sites in prostate nuclei was measured by a similar method. Originally, [^{3}H]-steroid was exchanged directly with endogenous steroid in purified nuclear preparations. This method was successful in that salt-extraction of nuclei previously incubated with [^{3}H]-steroid yielded a [^{3}H]-DHT-receptor complex of sedimentation coefficient 3.5–4.5 S when analysed on sucrose-density gradients (Fig. 2). The peak of radioactivity was depressed if incubations also contained unlabelled DHT, indicating low capacity for steroid and was characteristic

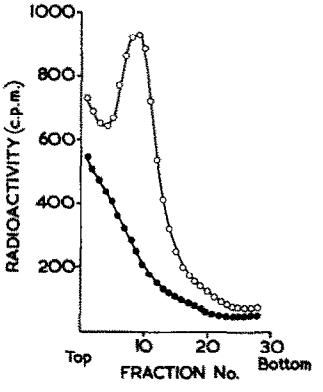

Fig. 2. Sedimentation analysis of nuclear [^{3}H]-DHT-receptor complex. Nuclei were prepared from the ventral prostate glands of normal intact rats and aliquots (25–50 μg DNA) were incubated under exchange conditions (without protamine sulphate precipitation) with [^{3}H]-DHT (20 nmol/l) (○) or with [^{3}H]-DHT plus 100-fold excess of unlabelled DHT (●). Nuclei were sedimented and washed 5 times with ice-cold buffer, and extracted with KCl (0.4 mol/l) for 30 min at 0–4°C with continual stirring. Debris was sedimented at 100,000 g_{av} for 30 min, and samples (400 μl) of extract were layered over linear 5 ml sucrose gradients (5–20% (w/v)) containing an uniform concentration of KCl (0.5 mol/l). Gradients were centrifuged and fractionated as described previously [4, 5] and the sedimentation coefficient of nuclear receptor protein based on the position of radioactivity peaks calculated with reference to sedimentation markers.

of specific binding [4, 5]. However, as with cytoplasmic sites, nuclear receptor was extremely susceptible to thermal degradation, resulting in rapid loss of binding. At the lowest temperature used (15°C) it was not possible to observe receptor levels similar to those obtained *in vivo* using animals of similar age and hormonal status, or reproducibility regarding optimal time and temperature for the assay. Therefore, precipitation of nuclear receptor prior to incubation was necessary. Under these conditions (Fig. 1b), exchange was minimal at 0–4°C, rapid but to an inconsistent level at 25°C and higher temperatures, but amenable to assay at 15°C. Saturation analysis of DHT binding by nuclei showed that the amount of [^{3}H]-steroid specifically retained was a saturable function (Fig. 3a), representing a single class of binding sites with an apparent K_D in the region 4–5 nmol/l (Fig. 3b). These results show the presence of low-capacity, high-affinity binding sites for DHT in rat ventral prostate nuclei.

Nuclear binding sites in rat ventral prostate show stereospecificity in that oestradiol, diethylstilboesterol, progesterone and cortisol did not compete with [^{3}H]-DHT for these sites, whereas unlabelled DHT significantly depressed the amount of bound radioactivity (Table 1). As expected if DHT-binding sites are due to specific receptor, they occur only in androgen target tissues (Table 2). Ventral prostate and seminal vesicle nuclei contain significant numbers of binding sites while rat liver, spleen and skeletal muscle do not.

Effects of androgen deprivation and restoration on cellular receptor sites

The concentration of intracellular DHT-binding sites in rat ventral prostate was studied in the intact rat, at 24 h, 48 h, and 72 h after castration, and in the glands of castrated animals which were left untreated for 72 h, or received daily doses of testosterone propionate beginning immediately or 24 h or 48 h after orchidectomy.

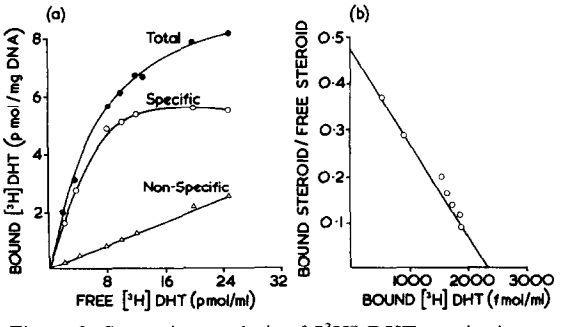

Figure 3. Saturation analysis of [^{3}H]-DHT uptake in rat ventral prostate nuclei. Nuclei prepared from prostates of normal intact rats were incubated with different concentrations of [^{3}H]-DHT in the absence (●) and presence (△) of a 100-fold higher concentration of DHT, under optimal conditions for exchange (a). At the end of the incubation, specifically bound [^{3}H]-DHT (○) was estimated as described in 'Experimental'. (b) Representative Scatchard analysis of specific binding of [^{3}H]-DHT determined similarly to that described in (a).

Table 1. Steroid specificity of nuclear DHT-receptor complex. Rat ventral prostate nuclei from intact rats were incubated for 16 h at 15°C with [^{3}H]-DHT (20 nmol/l) alone or in the presence of a 100-fold excess of indicated unlabelled steroids and the total amount of bound radioactivity determined at the end of incubation

Competitor	d.p.m./100 μg DNA
None	18141 ± 664
DHT	5194 ± 327
Oestradiol	17699 ± 412
Diethylstilboestrol	18597 ± 771
Progesterone	17401 ± 964
Cortisol	19532 ± 1358

Not unexpectedly, level of cytoplasmic and nuclear binding sites decreased markedly after castration (Table 3). Total numbers of cytoplasmic receptors had fallen by 50% 24 h after orchidectomy, by 48 h had decreased to 30% of initial levels, and 72 h after castration the level was 15% of that in prostate cells of intact animals. During this period, accessible cytoplasmic sites increased in number, and then declined. When expressed as a percentage of total sites, those unoccupied by endogenous steroid were increased with longer post-castration times. The number of nuclear receptor sites had decreased by 70% 24 h after castration and by over 90% 72 h after castration.

Administration of testosterone propionate to orchidectomised rats maintained levels of nuclear receptor if given immediately or 24 h after castration in daily doses (Table 4). A single dose of androgen given 48 h after castration raised the level of nuclear receptor to 200% of that observed in the castrated control. Androgen did not affect cytoplasmic sites so dramatically. Over 72 h of treatment, cytoplasmic receptor increased to 300% of control (castrated) levels and the percentage of sites accessible decreased to below 10%, but levels did not attain those observed in non-castrated animals. This lower reaction of cytoplasmic receptor to androgen was obviously reflected in total cellular receptor sites which in 3 day-treated castrated rats were lower than those levels seen in prostates of non-castrated animals.

Table 2. Tissue specificity of nuclear DHT-receptor complex. Nuclei were prepared from the indicated tissues of normal intact rats [6] and were incubated under optimal conditions of exchange with [^{3}H]-DHT (20 nmol/l) and total amount of bound radioactivity was determined

Tissue	d.p.m./100 μg DNA
Prostate	20390 ± 894
Seminal vesicle	16085 ± 1592
Liver	2295 ± 270
Spleen	3206 ± 331
Muscle	1404 ± 406

Table 3. Concentrations of receptors after castration. Subcellular fractions were prepared from the ventral prostate glands of intact rats or from glands of rats castrated 24 h, 48 h and 72 h prior to experimentation. Total and accessible cytoplasmic DHT-binding sites and nuclear DHT-binding sites were determined as described in 'Experimental'

Days after castration	Total receptor sites	Cytoplasmic Receptor sites Total	Accessible	Nuclear receptor sites
0	31771	18445	3282 (18)*	13326
1	13538	9803	6600 (67)	3735
2	7544	6215	5014 (81)	1329
3	3960	3140	2769 (88)	821

* Numbers in parentheses indicate accessible receptor as a percentage of total receptor.

Effects of castration and androgen administration on RNA chain initiation and elongation on rat ventral prostate chromatin

Conventional analysis of chromatin template activity was carried out using saturating concentrations of ventral prostate RNA polymerase B and nucleotides, and showed that incorporation of precursors into RNA was proportional to the amount of template using a fixed amount of polymerase until a titration point or plateau is reached. Homologous RNA polymerase B was used since previously it was observed that bacterial (*E. coli*) RNA polymerase transcribed prostate chromatin with different characteristics to prostate enzyme B [4] and may not respond to hormonal stimuli [8]. Preliminary results (Thomas and Davies, unpublished) showed that ventral prostate chromatin from young mature intact rats contained one binding site for homologous RNA polymerase B per 6000 nucleotide pairs and one initiation site per 120,000 nucleotide pairs. This suggests that 20 RNA polymerase molecules may be bound to chromatin for each RNA chain initiated.

Initiation of RNA chains on rat ventral prostate chromatin by homologous RNA polymerase B was measured by monitoring incorporation of [γ-^{32}P]-purine nucleotides under conditions precluding reinitiation during chain propagation. Elongation of RNA chains was measured by incorporation of [^{3}H]-UMP derived from [^{3}H]-UTP. Under the conditions of assay employed (see 'Experimental'), the preincubation times was sufficient to ensure initiation at all specific sites on chromatin, and incubation in the presence of rifamycin or high-salt was sufficiently long to allow completion of chain propagation.

The number of initiation sites on ventral prostate chromatin decreased after castration (Fig. 4), falling from over 10,000 sites per pg DNA in prostates of intact animals to 6000 sites per pg DNA in prostates of rats castrated 3 days previously. Administration of androgen to castrated rats did not completely maintain the level of initiation sites, although the number was increased over longer treatment time (Fig. 4). Interestingly, androgenic status apparently influenced prostate RNA chain length, since longer chains were synthesised from prostate chromatin from intact animals, or animals receiving an extended supply of androgen, than from prostate chromatin of animals totally deprived of or receiving a more limited dosage of androgen (Fig. 5). In this respect, it is noteworthy that preliminary results showed that the initial rate of elongation of RNA chains off intact-rat ventral prostate chromatin was 10 nucleotides/s, while that off prostate chromatin of 3-day castrates was 7 nucleotides/s. However, under the conditions of assay, RNA chain elongation had ceased in all cases.

Table 4. Concentrations of receptor after androgen administration to castrated rats. Groups of 6 or 7 rats were castrated and left untreated for 72 h, or received daily doses of testosterone propionate (see 'Experimental') beginning immediately or 24 h or 48 h after operation. Animals not receiving the androgen were injected with vehicle alone. Subcellular fractions were prepared from ventral prostate glands of those rats, and the numbers of receptor sites present in these factors were assessed as described in 'Experimental'

Days of androgen administration	Total receptor sites	Cytoplasmic receptor sites Total	Accessible	Nuclear receptor sites
0	4306	2317	2129 (92)*	1989
1	7626	3366	1717 (51)	4260
2	18823	4734	3010 (64)	14089
3	21029	7096	583 (8)	13933

* Numbers in parentheses indicate accessible receptor as a percentage of total receptor.

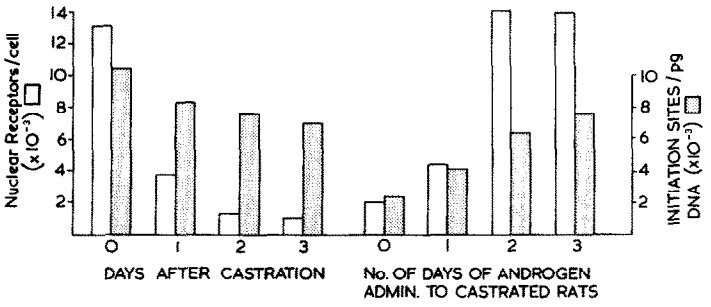

Fig. 4. Concentration of nuclear receptors and RNA initiation sites on chromatin after castration and androgen administration to castrated rats. Experiments were carried out on 8 groups of 6 or 7 rats, which were (a), sham-castrated or castrated 24 h, 48 h or 72 h before use; and (b) castrated and left untreated for 72 h, or receiving androgen (see 'Experimental') in daily doses commencing immediately, or 24 h, or 48 h after operation. Animals not receiving androgen were injected with vehicle alone. The concentration of nuclear receptor sites was determined using the [^{3}H]-DHT exchange assay (see 'Experimental') and initiation sites on chromatin for RNA synthesis were estimated by incorporation of [γ-^{32}P]-purine nucleotides.

DISCUSSION

This report describes preliminary investigations into possible relationships between cellular receptor levels and transcriptional ablility of the rat ventral prostate cell under differing conditions of androgenic status. This involved the development of methods to measure receptor sites, based on the experiences of others.

Certain considerations complicate the quantitation of prostate specific DHT-binding [17]. Dextran-coated charcoal techniques have been developed [18–21], but studies have been directed towards the separation of specific from nonspecific components by preferential precipitation, either by ammonium sulphate [19, 22] or protamine sulphate [17]. The latter method has been used to separate specifically bound DHT for assessment by 'radioimmunoassay' [17], and has now been used to measure occupied receptor by exchange assay. The method is suitable for the estimation of receptor in cytoplasm and nuclei, and apparently measures steroid- and tissue-specific sites, although improvements for the complete elimination of nonspecific binding [17] could be incorporated.

Depletion of nuclear [17] and cytoplasmic [23–25] receptor at various times after castration has been reported and confirmed using the exchange assay. This depletion has been attributed to increased proteolytic activity [24], although decreases have been observed before the onset of proteolysis [17]. Restoration or maintenance of varying levels of receptor by differing dosages of androgen [25, and above] may therefore be accounted for by inhibition of proteolytic activity or by *de novo* synthesis of receptor under the influence of the hormonal steroid. Increase of receptors in the absence of protein synthesis [17] has led to conjecture concerning microsomal [26] or soluble [17] precursors.

Preferential accumulation of nuclear receptor after testosterone treatment (Table 4) may be due to rapid translocation of cytoplasmic receptor, activated by DHT [5, 8], to the nucleus. If this occurs, then although total receptor is diminished, concentration of receptor within the nucleus might be sufficient to stimulate cellular processes during continued replenishment of cytoplasmic receptor. However, certain observations [27, 28] that nuclear receptor can arise without intervention of cytoplasmic receptor suggest

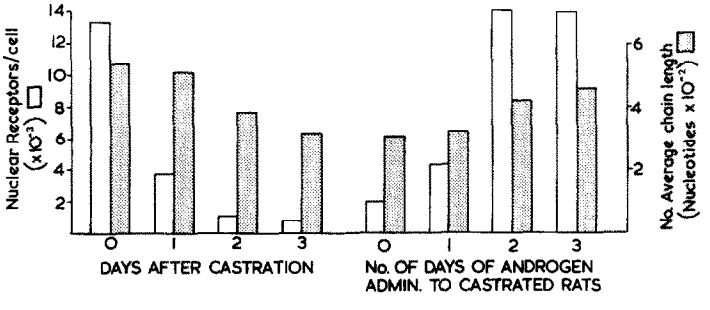

Fig. 5. Concentration of nuclear receptors and elongation of RNA chains after castration or androgen administration to castrates. Groups of rats were used as described in the legend to Fig. 4. During the experiment, the number-average length of polyribonucleotide chains synthesised by previously initiated RNA polymerase under conditions precluding reinitiation was calculated from incorporation of [^{3}H]-UMP from [^{3}H]-UTP assuming that UMP comprises 28% of RNA chains.

that androgen dependent maintenance of cytoplasmic and nuclear receptor may be independent of each other. Nevertheless, there are indications that cytoplasmic receptor has physical properties suggestive of a precursor relationship to nuclear receptor [5], and comparatively low values of cytoplasmic receptor in androgen-treated animals may be the result of destruction of [^{3}H]-DHT binding by residual proteolytic activity [27].

The major purpose of this investigation was to determine any existing link between receptor concentration and RNA initiation and elongation. DHT-receptor complexes influence the relative concentration of protein constituents and template availability of prostate chromatin [5]. Any modification of prostate chromatin by changing hormonal environment does not alter the numbers of binding sites for RNA polymerase B (Thomas and Davies, unpublished observations), so alterations in transcriptive ability [5] should be due to an increased ability of the enzyme to initiate or propagate RNA synthesis.

Studies on the effect of oestradiol on gene expression in the chick oviduct [29, 30] report that the major influence of steroid is exerted towards the number of initiated RNA chains with no significant effects on the rate or extent of chain elongation, with a temporal correlation between nuclear-bound oestradiol receptors and initiation sites for RNA synthesis. However, studies on the effects *in vitro* of DHT-receptor complexes on the transcription of prostate chromatin by homologous RNA polymerase have been shown to be directed toward chain elongation [7, 8]. The present investigation, however, after castration or androgen administration *in vivo*, show effects on chain initiation and elongation, indicating possible diverse actions of the steroid–receptor complexes. Obviously, from the maximum number-average chain lengths shown in Figs. 4 and 5, elongation *in vitro* is severely impaired. This is not due to nuclease action, but possibly to the loss of elongating factors during chromatin isolation [31]. It is difficult to envisage an increasing loss of elongation factors reducing chain length or rate of chain propagation with increased postcastration time unless these factors themselves are androgen-dependent, as described for prostate proteins with high affinity for DNA [32] including a DNA-unwinding protein [32, 33]. The existence of possible factors causing premature termination of RNA chains must also be taken into consideration.

To summarise, castration caused a decrease in ventral prostate receptor sites, chromatin sites for RNA chain initiation, and also the length of RNA chains. Androgen administration maintained all these parameters to some extent. While no stoichometric relationship could be ascertained to exist between nuclear-bound receptor and RNA chain initiation or elongation, it is by no means unlikely that the association of receptor with chromatin produces fundamental effects at the transcriptional level. Whether the DHT-receptor complex acts by displacing chromatin-associated proteins so as to increase the number of available initiation sites, by increasing template activity by increasing unwinding of DNA, by inactivating spurious termination factors, or by a combination of all these processes, awaits a more detailed appraisal of the many interactions essentially involved in these events.

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DISCUSSION

Jensen. When you speak of the number of nuclear sites decreasing after castration and being restored, I take it you do not mean what we call acceptor sites or the capacity to bind transformed complex but rather the actual number of androgen–receptor complexes that are translocated to the nucleus.

Davies. Yes, this is what I mean, that's what I was referring to.

Spelsberg. I just want to throw out a word of caution. Even though one has a ribonuclease inhibitor, you can't be sure that you are not cleaving any RNA. One or two cleavages can reduce the size of molecular weight tremendously. Secondly, did you analyze your chromatin after these incubations. For instance, histone integrity, etc. With long incubations at high temperatures, nuclease/protease action can be significant.

Davies. We only made preliminary observations on this, but under the conditions of assay we don't lose histones to any great extent.

Hamilton. I have two questions to ask you of a methodological nature, but I think you can forgive me for asking this because we are trying to do similar things with chromatin of uterus from the ovariectomized rat. I'd like to know how much DNA in the form of chromatin you add to each reaction vial in your measurement of initiation sites and then what is the percent of variation in replicate experiments?

Davies. We add about 10 micrograms. We try to get 10 μg exactly but it varies with chromatin DNA. We have an interassay variation about 10–12%.

McGuire. Your comments about the receptors disappearing rapidly after castration possibly partly being due to polymerase activity, have you taken any 0 and 3 day extract from cytoplasm and nuclei and mixed them and assayed? Have you done a classical mixing experiment?

Davies. No, we have not done that.