PROSTATIC INVOLUTION: EFFECT ON ANDROGEN RECEPTORS AND INTRACELLULAR ANDROGEN TRANSPORT

by

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SUMMARY

In the regressing rat prostate gland the concentration of cytoplasmic receptor declines from a maximal level of 174 ± 24 fmoles/mg protein 1 day after orchiectomy and is virtually undetectable after 7 days. The results of tissue mixing experiments in which equal amounts of prostate from rats orchiectomized 1 day and 7 days previously are incubated and homogenized together indicate that at the latter time the prostate contains factors, presumed to be proteolytic enzymes, which can eradicate the binding of radioactive dihydrotestosterone to cytoplasmic receptor. In the apparent absence of cytoplasmic receptor, the capacity of the cell to transfer androgens into the nucleus is kept intact at a level 30-60% of the experimentally determined maximum, and the competence to form intranuclear receptor is preserved. However, the nuclear receptor observed 7 days after orchiectomy is smaller than the one observed 1 day after orchiectomy.

INTRODUCTION

Recent reports by Jung and Baulieu (1), Mainwaring and Mangan (2), and Sullivan and Strott (3) have drawn attention to the apparent loss of cytoplasmic receptor from rat prostate with increasing time after orchiectomy. We have taken advantage of this effect to determine whether the depletion of cytoplasmic receptor is accompanied by a reduction in the incorporation of androgens into nuclei of prostatic cells. Although the binding of 5α-androstan-17β-ol-3-one (dihydrotestosterone) to cytoplasmic receptor and the subsequent transfer of dihydrotestosterone-receptor complex into the cell nucleus is thought to be an obligatory step in the mechanism of androgen action within prostate (4), this assumption remains unverified principally because of the difficulty of proving that the cytoplasmic and nuclear receptors, which are characterized by different physical properties (4,5-7), are indeed related molecules. Thus, in an attempt to link the nuclear receptor to a cytoplasmic source we also investigated the competence of prostatic cells to form nuclear receptor in the absence of detectable cytoplasmic receptor.

METHODS AND MATERIALS

Male rats of the Wistar strain weighing 250-300 g were routinely anaesthetized with ether and castrated through a scrotal incision.

For in vivo experiments, rats castrated 1 day or 7 days previously were eviscerated, functionally hepatectomized, and then injected intravenously with

300 μCi (6.9 nmoles) of [1,2-^{3}H]testosterone (sp. act. 40 Ci/mmole); 60 min later the animals were killed by decapitation and the prostatic tissue recovered was separated into cytosol and nuclear fractions by centrifugation techniques.

For in vitro experiments, prostatic tissue from orchiectomized rats was minced and incubated in 2 ml of tissue culture medium (CMRL 1415-ATM, Connaught Laboratories, Toronto, Ontario) containing [1,2-^{3}H]dihydrotestosterone (sp. act. 40 Ci/mmole; 750 nM) for appropriate intervals at 37° in an atmosphere of CO_2:O_2 (5:95 v/v).

In each case the cytosol fraction was treated with ammonium sulfate at 80% saturation, and the protein precipate was analysed by gel-exclusion chromatography. This analysis was performed with a dual-column system consisting of a pre-column (0.9 cm x 30 cm) of Sephadex G-25 connected in series with a separating column (0.9 cm x 100 cm) of Sephadex G-200. The columns were equilibrated with 0.01 M Tris-HCl buffer, pH 7.0 containing 0.6 M NaCl and the sample was eluted with the same buffer.

Purified nuclei were disrupted by sonication and extracted with 0.01 M Tris-HCl buffer, pH 7.0 containing 0.6 M NaCl. The extract, usually containing about 95% of the intranuclear radioactivity, was analysed by gel-exclusion chromatography with a single-column (0.9 cm x 100 cm) of Sephadex G-200 which was equilibrated and eluted as above. Details of these procedures are described elsewhere (5-7).

RESULTS

In order to precisely characterize the decline in the concentration of cytoplasmic receptor after orchiectomy, minces of prostate from animals castrated 1-7 days previously were incubated with radioactive dihydrotestosterone as described. The protein recovered from cytosol was then analysed by gel-exclusion chromatography, and a typical positive result is shown in Fig. 1A. The principal peak of radioactivity, eluted in fractions 30-45 represents saturable, high-affinity binding of dihydrotestosterone to a cytoplasmic receptor possessing a Stokes radius of 48 Å and a sedimentation coefficient of 4.4S measured in 0.6 M NaCl (7). From the results shown in Fig. 1B, it is clear that this receptor is not detected in prostate of animals orchiectomized 7 days previously. Estimates of the concentration of cytoplasmic receptor in normal and involuting prostate are presented in Fig. 2. From a level of 65 femtomoles/mg protein in normal tissue, the concentration of cytoplasmic receptor rises to a maximal level of 174 ± 24 femtomoles/mg protein (mean ± S.E. of 4 determinations) after 1 day of orchiectomy and then declines to a level close to zero after 7 days. The amount of receptor then remains very low for as long as 13 days after castration (data not shown).

The possibility that the regressed prostate contains receptor inactivating

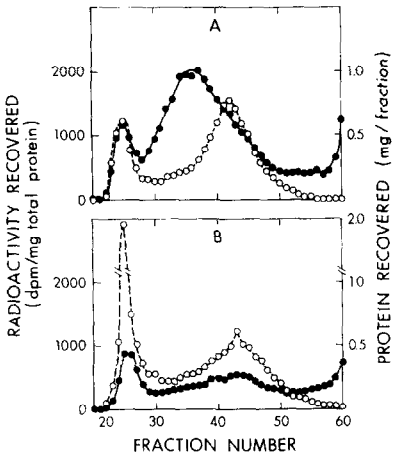

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Fig. 1 Androgen Receptor in Cytoplasm

Prostatic tissue was minced and incubated with [1,2-^{3}H]dihydrotestosterone (750 nM) for 2 min at 37°. Protein recovered from cytosol was analysed by gel-exclusion chromatography (dual-column method). Fraction size was 1.4 ml. Radioactivity, ●——●. Protein (18), O---O. A, 1 day after orchiectomy; B, 7 days after orchiectomy.

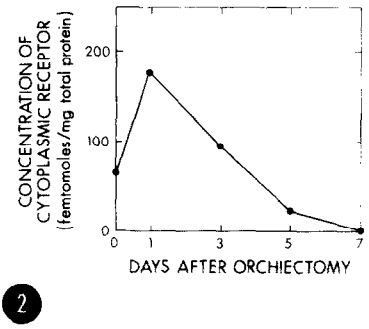

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Fig. 2 Concentration of Cytoplasmic Receptor after Orchiectomy

Radioactivity recovered in the receptor peak (and displaced by a one-hundred fold excess of non-radioactive dihydrotestosterone) was determined at 0, 1, 3, 5 and 7 days after orchiectomy. It is assumed that there is only 1 androgen binding site per receptor, and the results are expressed as fmoles of receptor per mg of total cytosol protein.

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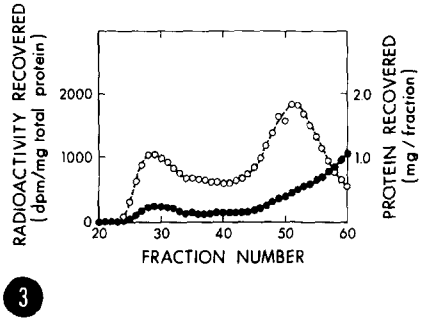
factors was investigated by incubating and analysing (as in Fig. 1) pooled tissue from rats orchiectomized 1 day and 7 days previously. Since no cytoplasmic receptor is recovered (Fig. 3), it is clear that such factors are present in prostate 7 days after orchiectomy and are capable of eradicating the binding of dihydrotestosterone to cytoplasmic receptor at least during homogenization procedures. Intense protease activity following orchiectomy has been noted (10-12) and may account for this effect.

Assuming that nuclear receptor is derived from cytoplasmic receptor, the loss of cytoplasmic receptor should be paralleled by a reduction in the capacity of the cell to transfer both androgens and androgen receptors into the nucleus. This prediction was tested by comparing the incorporation of radioactive androgens into nuclei of prostatic cells at 1 day and 7 days after orchiectomy. Experiments were performed both in vivo and in vitro under conditions described in the "Methods" section which promote maximal incorporation of androgens and



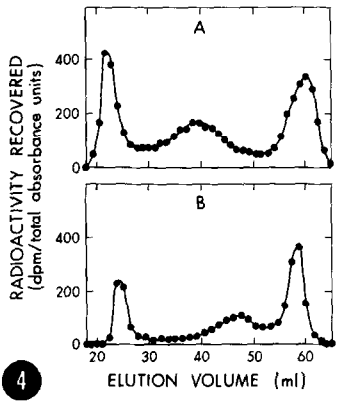


Fig. 3 Inactivation of Cytoplasmic Receptor

Prostates (1 g) from rats orchiectomized 1 and 7 days previously were pooled, and processed as in Fig. 1. Analysis of cytosol protein was accomplished by gel-exclusion chromatography (dual-column method). Fraction size was 1.4 ml. Radioactivity, ●—●. Protein, O---O .

Fig. 4 Nuclear Receptors

Prostatic tissue (1 g) was processed as in Fig. 1 and nuclear extracts were analysed by gel-exclusion chromatography (single-column method). The radioactivity in each fraction was divided by the total absorbance units at 260 nm. Radioactivity recovered: A, 1 day after orchiectomy; b, 7 days after orchiectomy.

androgen receptors into nuclei of prostatic cells 1 day after orchiectomy (7). From the results presented in Table I, it is evident that the incorporation of androgens into cytoplasm is virtually the same at 1 day and 7 days following orchiectomy whether *in vivo* or *in vitro* labelling techniques are used. By contrast, the *in vivo* incorporation of androgens into nuclei is reduced approximately 70% from $115.6 \pm 15.9 \times 10^{-4}$ dpm/60 min/nucleus to $35.3 \pm 10.0 \times 10^{-4}$ dpm/60 min/nucleus, while the *in vitro* incorporation is reduced approximately 40% from $26.0 \pm 3.9 \times 10^{-4}$ dpm/10 min/nucleus to $16.5 \pm 4.2 \times 10^{-4}$ dpm/10 min/nucleus. Therefore, despite the apparent lack of cytoplasmic receptor, the capacity of the cell to transfer androgens into the nucleus is kept intact at a level 30-60% of the experimentally determined maximum.

Since the potential for intracellular androgen transport is partially sustained by regressing cells, we next examined the question whether the competence of these cells to form intranuclear receptor is also preserved. Extracts of nuclei recovered in experiments described in Table I were analysed by gel-exclusion chromatography with Sephadex G-200 (single-column method). The results of analyses of nuclear extracts of cells labelled *in vitro* are shown in Fig. 4 and are the same as the results obtained with nuclear extracts of cells labelled

TABLE I

Incorporation of Androgens

Experiment	Days After Orchiectomy	Radioactivity Recovered	
		Cytoplasm (dpm/g x 10^{-5})	Nucleus (dpm/nucleus x 10^{4})
In Vivo	1	20.3 ± 1.3 (3)	115.6 ± 15.9 (3)
	7	17.6 ± 0.8 (3)	35.3 ± 10.0 (3)
In Vitro	1	336.4 ± 18.2 (6)	26.0 ± 3.9 (5)
	7	334.0 (2)	16.5 ± 4.2 (3)

In vivo Groups of 3-14 rats (250-300 g) orchiectomized 1 and 7 days previously received intravenous injections of 300 µCi (6.9 nmoles) of [1,2-^{3}H] testosterone. 60 min later the animals were killed and the radioactivity in cytoplasmic and nuclear fractions of prostate was measured. Both at 1 day and 7 days after orchiectomy dihydrotestosterone is the principal intracellular metabolite of testosterone.

In vitro Prostatic tissue (1 g) was minced and incubated with [1,2-^{3}H] dihydrotestosterone (750 nM) for 10 min at 37°. The tissue was extensively washed and then separated into cytoplasmic and nuclear fractions which were analysed for content of radioactivity. The results are expressed as the mean ± S.E. of the number of experiments shown in parentheses.

in vivo (data not shown). In prostatic nuclei obtained 1 day after orchiectomy, radioactivity is recovered in 3 peaks (Fig. 4A): the first peak is eluted in a volume of 20-30 ml, corresponding to the excluded fractions, and represents binding to a large molecular weight component of the nucleus presumed to be chromatin (8); the second peak is eluted in a volume of 30-50 ml and represents binding to nuclear receptor possessing a sedimentation coefficient of 3.3S (5-7); the third peak is eluted in a volume of 55-65 ml and represents free androgen. In prostatic nuclei obtained 7 days after castration (Fig. 4B) the distribution of radioactivity is similar but the elution of receptor is delayed. Since the distribution coefficient (K_D) of this molecule is 0.635 ± 0.015 (mean ± S.E. of 4 determinations) as compared to the K_D of the receptor in Fig. 4A which is 0.527 ± 0.019 (mean ± S.E. of 5 determinations), it is clear that a significant change ($p < 0.005$) in the size of receptor to smaller dimensions takes place after orchiectomy. From the K_D values obtained above, it can be estimated by chromatographic methods (9) that the Stokes radius of nuclear receptor observed 1 day after orchiectomy is 24-25 Å, while that of the receptor observed 7 days after orchiectomy is only 19-20 Å.

DISCUSSION

The results presented in this report indicate that the capacity to trans-

port androgens into the nucleus, and the competence to form nuclear receptor are reasonably well preserved in regressing prostatic cells despite an apparent reduction in the concentration of cytoplasmic receptor to a very low level. If the assumption that cytoplasmic receptor is required for the transfer of androgens and androgen receptors into the nucleus is valid, then, on the one hand, our results showing that such transfer reactions are preserved in regressing cells suggest that cytoplasmic receptor is present even though it is not detectable by currently employed methods. On the other hand, although the actual concentration of cytoplasmic receptor may be underestimated because of the inactivation of receptor during homogenization procedures, it is very likely that the intracellular environment during prostatic involution is inimical to the survival of this molecule (10-12). Thus, the detection of nuclear receptor in cells which may be devoid of cytoplasmic receptor raises the possibility that nuclear receptor may arise from a source other than cytoplasmic receptor. This impression is supported by the additional observation that the nuclear receptor observed 7 days after orchiectomy is smaller than the one observed 1 day after orchiectomy. Such a result is unexpected if it is assumed that nuclear receptor is derived directly from the same cytoplasmic source at both times after orchiectomy.

Certain other deductions underline the difficulty of proving that the cytoplasmic receptor is an exclusive determinant of the presence of androgens and androgen receptors in the prostatic nucleus. For example, from the data presented in Table I, it is calculated that 70,000 androgen molecules are transferred into the nucleus in vivo of which about one-third or 23,000 are bound to receptor 1 day after orchiectomy (Fig. 4). In the same tissue the maximal concentration of cytoplasmic receptor at 174 femtomoles/mg protein is equivalent to only 8,000 receptor molecules per cell (Fig. 2) corresponding to the level determined for other hormone responsive organs (13-17). A similar discrepancy appears 7 days after orchiectomy when the nucleus contains 22,000 androgen molecules and 7,000 nuclear receptors under conditions which mitigate against the existence of any cytoplasmic receptor. Thus, in both cases, it seems that the quantity of cytoplasmic receptor is insufficient to account for the total influx of androgens and androgen receptors into the nucleus if a mole to mole relationship is assumed.

These results when viewed in the light of the different physical properties of cytoplasmic and nuclear receptors (4-7), are compatible with the idea that cytoplasmic receptor reduces the threshold of the barrier imposed by the nuclear membrane to the passage of androgens, but they fail to provide unequivocal evidence for the hypothesis that nuclear and cytoplasmic receptors are directly related.

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