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FORMATION, NUCLEAR INCORPORATION AND ENZYMATIC DECOMPOSITION OF ANDROGEN–RECEPTOR COMPLEX OF RAT PROSTATE*

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SUMMARY

Specific affinity of the prostate cytosol receptor for 5α-dihydrotestosterone was demonstrated by competitive experiment between testosterone and 5α-dihydrotestosterone. The binding of [1,2-^{3}H]-5α-dihydrotestosterone to the cytosol receptor of rat ventral prostate was not affected by simultaneous administration of nonradioactive testosterone, whereas the binding of [1,2-^{3}H]-testosterone to the receptor was severely suppressed by non-radioactive 5α-dihydrotestosterone. Binding capacity of the cytosol receptor for the tritiated 5α-dihydrotestosterone and testosterone was somewhat greater at 37°C than 4°C. The involvement of the cytosol receptor in retention of 5α-dihydrotestosterone by the nuclei of rat prostate was confirmed at 4° and 37°C. A 3α-hydroxysteroid dehydrogenase fraction which contained no 9S receptor was prepared from the prostate cytosol by precipitation with ammonium sulphate at 60–100% saturation. When [^{3}H]-labeled 5α-dihydrotestosterone bound to the cytosol receptor (9S) was incubated with the 3α-hydroxysteroid dehydrogenase fraction in the presence of NADPH, a remarkable dissociation of the radioactivity from the receptor complex was observed. According to the analysis of the liberated steroids, the major radioactive steroid was identified as 5α-androstane-3α,17β-diol, which bound to the receptor to a very limited extent. On the contrary, the remaining radioactivity bound to the receptor after incubation was principally due to 5α-dihydrotestosterone. The degradation of the androgen–receptor complex by the 3α-hydroxysteroid dehydrogenase in the cytosol suggests a possible process of intracellular decomposition of the complex, in relation to excretion of the steroid metabolite from the prostatic cells.

INTRODUCTION

Testosterone is rapidly converted to 5α-dihydrotestosterone in androgen-dependent tissues such as ventral prostate and seminal vesicle of rat [1–3]. The selective uptake of androgen by rat ventral prostate is probably due to the specific interaction of 5α-dihydrotestosterone with androgen-binding proteins or receptors. Androgen receptors are intracellularly located in the cytosol fraction [4–6] and in the nuclei [7, 8] of the prostate. We previously reported [9] that the cytosol receptor labeled with [^{131}I] was incorporated into the prostatic nuclei in the form of the complex with 5α-dihydrotestosterone and subsequently associated with non-histone protein and DNA of the chromatin. It is postulated [10, 11] that the stimulation of the synthesis of RNA and protein by androgen is a consequence of the above intranuclear binding of the 5α-dihydrotestosterone–receptor complex. The cytosol receptor is therefore of primary importance in the mediation of androgenic actions in the cell. According to our previous experiment [12], there are two types of cytosol receptors in rat ventral prostate, one is a macromolecule with sedimentation coefficient of 9S, and the other with sedimentation coefficient of 5S. Of these two cytosol receptors, only 9S receptor is involved in the transport of 5α-dihydrotestosterone into the nucleus [12]. In this paper, we attempted to reveal a decomposition of 5α-dihydrotestosterone–cytosol receptor (9S) complex by an endogenous enzyme, after formation of the androgen-receptor complex and its nuclear incorporation were established.

MATERIALS AND METHODS

Tissue preparation

Male rats of the Wistar strain, aged 10 weeks, were used in this experiment. Animals were killed by decapitation. The ventral prostates were isolated and dissected free of their capsules. The ventral prostates were homogenized in an ice-cold 0·25 M sucrose solution (pH 7·4) by a Polytron homogenizer (Kinematica, Luzern, Switzerland) and then by a tight-fitting glass homogenizer with a Teflon pestle. The homogenates were filtered through four layers of Nylon net (200 mesh) and centrifuged at 800 *g* for 20 min. After centrifugation of the supernatant fluid at 105,000 *g* for 60 min, the supernatant fluid was used as the cytosol fraction. For preparation of the purified nuclei, the 800 *g* precipitate was suspended in a 0·88 M sucrose solution containing 1·5 mM $CaCl_2$ and layered over

* The following trivial names are used in this paper: testosterone, 17β-hydroxy-4-androsten-3-one and 5α-dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one.

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a discontinuous sucrose gradient, as described by Maggio *et al.*[13]. After centrifugation of the above at 36,000 *g* for 90 min, the nuclear pellet was resuspended in a 0·25 M sucrose solution (pH 7·4) containing 0·5 mM $CaCl_2$.

Radioactive steroids and other chemicals

[1,2-^{3}H]-5α-Dihydrotestosterone (S.A. 44 Ci/mmol) and [1,2-^{3}H]-testosterone (S.A. 45 Ci/mmol) were obtained from New England Nuclear, Boston, Mass. Non-radioactive steroids used in this experiment were purchased from Steraloids, Pawling, New York. Their radiochemical purities were carefully checked by t.l.c., just before use. NADPH was purchased from Boehringer, Mannheim, Germany.

Gel filtration

Separation of the steroid–macromolecule complex from the incubation mixture was performed with a column (2·5 cm. × 39·0 cm.) of Sephadex G-100 (Pharmacia Fine Chemicals, Uppsala, Sweden). The gel was equilibrated and eluted with 0·01 M Tris–HCl buffer (pH 7·4) containing 0·05 mM EDTA, 0·5 mM 2-mercaptoethanol and 0·1 M NaCl. Samples were eluted with the same buffer as described above at a flow rate of 0·5 ml per min. Every 5 ml of the eluate was collected, unless otherwise indicated. Void volume of the column was calibrated with Blue Dextran 2000 (Pharmacia Fine Chemicals).

Preparation of 3α-hydroxysteroid dehydrogenase fraction

3α-Hydroxysteroid dehydrogenase (E.C. 1.1.1.50) fraction was prepared from the prostate cytosol fraction by precipitation with 60–100% saturation of ammonium sulphate, as the dehydrogenase activity was exclusively localized in the cytosol [14]. This subfraction was found to be devoid of appreciable androgen receptor (9S) [12].

Analysis of steroids

Radioactive steroids in each fraction obtained by the gel filtration were extracted with methylene chloride. The extract was dried with anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. Extracted steroids were subjected to t.l.c., after addition of unlabeled 5α-dihydrotestosterone and 5α-androstane-3α,17β-diol. Spots of the carrier steroids detected by iodine vapour were scraped off and exhaustively extracted with methylene chloride. Identification of the metabolites was carried out by the methods as described previously by Nozu and Tamaoki[15]. The solvent of the extract in the counting vial was evaporated off and radioactivity in the residue was measured.

Measurements of radioactivity and protein content

Extracted radioactive steroids were dissolved in 11 ml of a regular toluene scintillator containing 0·4% (w/v) 2,5-diphenyloxazole and 0·01% (w/v) 1,4-bis-2-(5-phenyloxazolyl)-benzene. Radioactivity of the sample was measured in a liquid scintillation spectrometer (Beckman LS-250, Fullerton, California). For counting of aqueous samples, a mixture of Triton X-100 and the normal liquid scintillator (1:3, v/v) was used as a scintillator. Counting efficiency of tritium was about 35%. Corrections for quenching samples were made with external standardization, where necessary. Protein content in tissue preparation was determined by the biuret method [16], using bovine serum albumin as the standard.

RESULTS

Specific binding of cytosol receptor to 5α-dihydrotestosterone

The cytosol fraction of the rat ventral prostates was incubated with [^{3}H]-5α-dihydrotestosterone at 37°C for 20 min. Then the incubation mixture was put on a column of Sephadex G-100 and eluted with the 0·01 M Tris–HCl buffer (pH 7·4). In the elution profile, two radioactive peaks which bound to macromolecules were observed (Fig. 1a). One corresponding to the 9S receptor was eluted immediately after the void volume, whereas the other or 5S receptor was retained by the gel for some time, and then eluted at the position close to BSA. When non-radioactive testosterone was added together with the [^{3}H]-5α-dihydrotestosterone into the medium (100 times more than the 5α-dihydrotestosterone), the sizes of these two

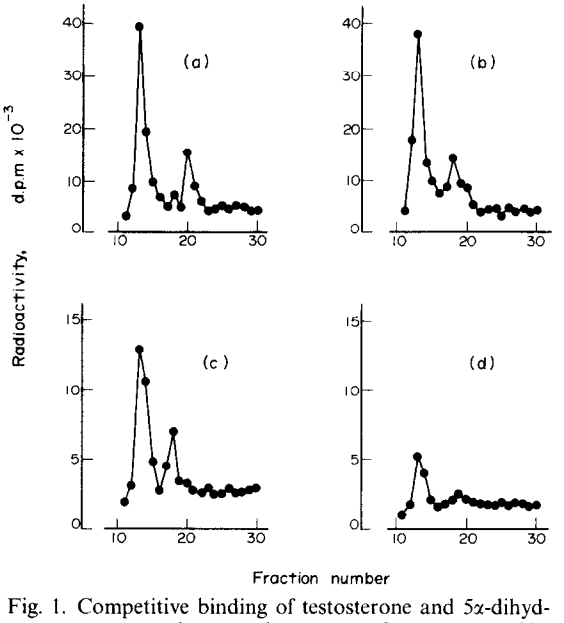

Fig. 1. Competitive binding of testosterone and 5α-dihydrotestosterone to the cytosol receptors of rat prostate. The cytosol fraction (25 mg of protein) of rat ventral prostates was incubated at 37° for 20 min as follows: (a) with [1,2-^{3}H]-5α-dihydrotestosterone (35 pmol) alone, (b) together with non-radioactive testosterone (3·5 nmol), (c) with [1,2-^{3}H]-testosterone (35 pmol) alone, and (d) together with non-radioactive 5α-dihydrotestosterone (3·5 nmol). After the incubation, the mixtures were chromatographed on a column of Sephadex G-100 with 0·01 M Tris–HCl buffer (pH 7·4), containing 0·05 mM EDTA, 0·5 mM 2-mercaptoethanol and 0·1 M NaCl.

peaks due to the radioactive 5α-dihydrotestosterone–receptor complex were hardly affected, as shown in Fig. 1b. After incubation of the [^{3}H]-testosterone alone with the cytosol fraction, the radioactive peaks similar to those above were observed, but the amounts of the radioactivity bound to the receptors were relatively smaller than those in the case of the tritiated 5α-dihydrotestosterone (Fig. 1c). By addition of non-radioactive 5α-dihydrotestosterone, the binding of the tritiated testosterone to the receptors was severely suppressed (Fig. 1d).

Effect of temperature on the binding of testosterone and 5α-dihydrotestosterone to the receptor

[^{3}H]-Testosterone and [^{3}H]-5α-dihydrotestosterone were individually incubated with the cytosol fraction of the rat ventral prostate at 4° and 37°C for 20 min. Thereafter, they were subjected to a gel filtration of Sephadex G-100. Each gel filtration was carried out at the same temperature as that of the incubation. In both cases of tritiated testosterone and 5α-dihydrotestosterone, the cytosol receptor showed a somewhat larger capacity to binding to these steroids at 37°C than at 4°C, but the binding at 4°C was still not negligible. As compared with the binding affinity of the cytosol receptors between [^{3}H]-testosterone and [^{3}H]-5α-dihydrotestosterone at the same temperature, the cytosol receptors exhibited an extremely high specific affinity toward 5α-dihydrotestosterone at the both temperatures (Fig. 2).

Involvement of the cytosol receptor in the incorporation of 5α-dihydrotestosterone into the nuclei of prostate

After incubation of the cytosol fraction with [^{3}H]-5α-dihydrotestosterone, the 5α-dihydrotestosterone–cytosol receptor complex was obtained by the Sephadex G-100 column chromatography. The cytosol receptor complex with [^{3}H]-5α-dihydrotestosterone was incubated with the purified nuclei of rat ventral prostate to study the participitation of the cytosol receptor in the retention of the 5α-dihydrotestosterone by the nuclei. As control, free tritiated 5α-dihydrotestosterone alone was incubated with the nuclei under the same condition. As shown in Table 1, [^{3}H]-5α-dihydrotestosterone which bound to the cytosol receptor was significantly incorporated into the nuclei. The ratio of the radioactivity bound to the nuclei was found to be 18·2 and 17·9% of the total radioactivity at 4° and 37°C, respectively. Meanwhile, binding of free [^{3}H]-5α-dihydrotestosterone to the nuclei was observed only in about 1% of the total radioactivity. No influence of temperature upon the incorporation of [^{3}H]-5α-dihydrotestosterone into the nuclei was observed between 4° and 37°C.

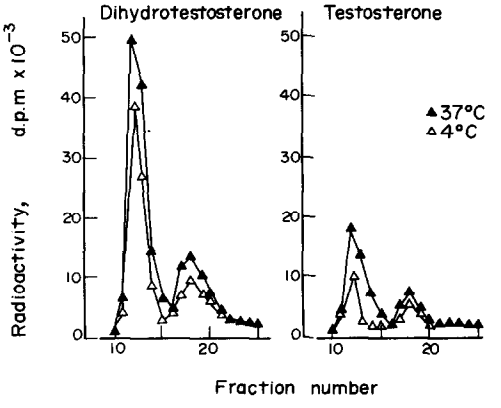

Fig. 2. Effect of temperature on the complex formation of testosterone and 5α-dihydrotestosterone with the cytosol receptor of rat prostate. The cytosol fraction (55 mg of protein) was individually mixed with [1,2-^{3}H]-testosterone and [1,2-^{3}H]-5α-dihydrotestosterone (35 pmol, each). Incubations were carried out at 4° and 37°C for 20 min. The incubated mixtures were subjected to chromatography on a Sephadex G-100 gel filtration of the same temperature as the incubation.

Table 1. Incorporation of 5α-dihydrotestosterone into the prostatic nuclei. The cytosol 9S receptor-[^{3}H]-5α-dihydrotestosterone complex (1·4 mg of protein, 14,000 d.p.m. of the tritium) obtained by the gel filtration and also free [^{3}H]-5α-dihydrotestosterone (14,000 d.p.m.) were incubated separately with the purified nuclei (2·9 mg of protein) at 4° and 37°C for 20 min. Immediately after the incubations, the nuclei were sedimented at 10,000 *g* for 5 min and washed twice with 0·25 M sucrose solution containing 0·5 mM $CaCl_2$. The radioactivity in the nuclei was measured and expressed as d.p.m. and ratio (%) relative to the total radioactivity administered

Fraction	Temperature of incubation (C)	Radioactivity in nuclei (d.p.m.)	Radioactivity in nuclei ratio* (%)
[^{3}H]-5α-Dihydrotestosterone alone	4°	153	1·1
	37°	151	1·1
[^{3}H]-5α-Dihydrotestosterone bound to cytosol receptor	4°	2,540	18·2
	37°	2,500	17·9

* Ratio of the radioactivity incorporated into the nuclei to the administered radioactivity.

Influence of the 3α-hydroxysteroid dehydrogenase upon the 5α-dihydrotestosterone–receptor complex

A series of experiments was carried out to examine the metabolism of the 5α-dihydrotestosterone–receptor complex by the cytosol 3α-hydroxysteroid dehydrogenase fraction, and the binding capacity of the consequent metabolite to the cytosol receptor. The whole cytosol fraction of rat ventral prostate incubated with [^{3}H]-5α-dihydrotestosterone at 0°C was filtered through Sephadex G-100 column and then the fractions corresponding to the radioactive peak of 9S receptor which was eluted immediately after the void volume were collected. This preparation was used as the cytosol receptor-[^{3}H]-5α-dihydrotestosterone complex. The androgen–receptor complex was incubated with the 3α-hydroxysteroid dehydrogenase fraction in the presence of NADPH, and as a control, in the absence of the cofactor. The incubations were performed at 37°C for 5, 10 and 30 min. After the incubations, the mixtures were immediately cooled down to 0°C in an ice-water bath and subjected to the Sephadex G-100 gel filtration chromatography.

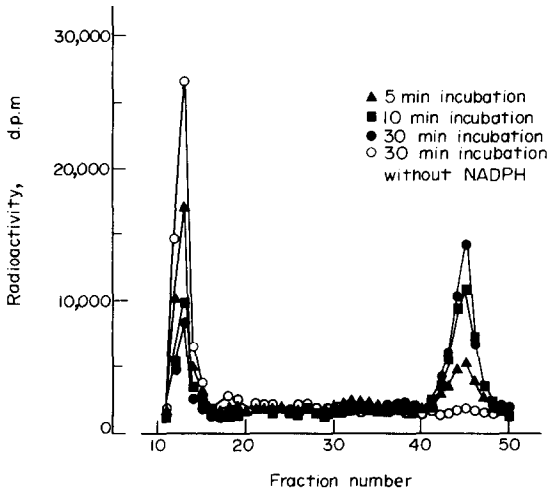

Fig.3. Influence of the 3α-hydroxysteroid dehydrogenase upon the complex of 5α-dihydrotestosterone with the cytosol receptors of rat prostate. After incubation of the cytosol fraction with [1,2-^{3}H]-5α-dihydrotestosterone, the cytosol 9S-receptor-[^{3}H]-5α-dihydrotestosterone complex which was eluted just after the void volume of the Sephadex G-100 gel filtration was separated. The complex (1·4 mg of protein, 60,000 d.p.m. of the tritium) was mixed with the 3α-hydroxysteroid dehydrogenase fraction (0·5 mg of protein, see in Materials of the text). Incubations were carried out at 37°C for several time intervals in the presence or absence of NADPH (1·27 μmol/2 ml). After the incubation, the mixtures were cooled at 0°C and then subjected to the gel filtration chromatography at 4°C.

When NADPH was added into the incubation medium, apparent disappearance of the radioactivity which had bound to the receptor was observed, as shown in Fig. 3. The decreased portion of the radioactivity bound to the receptor was detected in the free steroid region (Fraction Nos. 43–48). With the elapsed time of incubation, the radioactivity in the free steroid region increased gradually, and simultaneously marked disappearance of the radioactivity in the form of the complex with the receptor (Fraction Nos. 12–14) was observed. In the absence of NADPH, however, the radioactive peak bound to the receptor was left almost unchanged, and the radioactivity recovered as free steroid was negligible, even after 30 min of incubation. According to t.l.c. analysis of the radioactive steroids obtained by the incubation, the radioactivity associated with the receptor was essentially identified as 5α-dihydrotestosterone in each case, as shown in Table 2. In the region which corresponded to free steroids, the major steroid was identified as 5α-androstane-3α,17β-diol, but the quantity of free 5α-dihydrotestosterone in the medium was very small. In the absence of NADPH, conversion of [^{3}H]-5α-dihydrotestosterone to 5α-androstane-3α,17β-diol was restricted to a very small extent and dissociation of the radioactivity from the receptor complex was also insignificant.

Table 2. Conversion of the complex of 5α-dihydrotestosterone with the cytosol receptor to 5α-androstane-3α,17β-diol by the 3α-hydroxysteroid dehydrogenase. The steroids associated with the cytosol 9S receptor and in the free steroid regions shown in Fig. 3 were analyzed by t.l.c., after extraction of steroids with methylene chloride

Time of incubation (min)		Ratio of 5α-androstane-3α,17β-diol/5α-dihydrotesterone	
		Bound to the receptor	Free
5	with NADPH	0·16	4·41
10	with NADPH	0·28	4·90
30	with NADPH	0·37	6·85
30	without NADPH	0·07	–*

* Neither 5α-androstane-3α,17β-diol nor 5α-dihydrotestosterone were detectable.

DISCUSSION

In the present competitive experiment between testosterone and 5α-dihydrotestosterone, it was shown that the receptors in the cytosol fraction of the rat ventral prostate had specific affinities for 5α-dihydrotestosterone. It was reported [4] that, when [^{3}H]-testosterone was injected into rats, 5α-dihydrotestosterone which was formed from the tritiated testosterone was the major component associated with the receptors in the cytosol fraction of the ventral prostate. The present result obtained by experiment *in vitro* was essentially in agreement with the finding *in vivo*. In this experiment, relatively large amount of added testosterone did not effect formation of 5α-dihydrotestosterone–cytosol receptor complex, while 5α-dihydrotestosterone severely inhibited formation of testosterone–receptor complex. In the case of rat epididymis [17], testosterone did not suppress binding of 5α-dihydrotestosterone to the cytosol receptor *in vitro*, either. Furthermore, it was previously found [12] that, when the mixture of [^{3}H]-testosterone and [^{3}H]-5α-dihydrotestosterone was incubated with the cytosol fraction of rat ventral prostate, the radioactivity bound to the receptors was mainly due to [^{3}H]-5α-dihydrotestosterone, and the binding of the testosterone was found to a far less extent. Strong inhibition of binding of [^{3}H]-testosterone to the cytosol receptors by non-radioactive 5α-dihydrotestosterone was suggested as being caused by a competitive binding of these two steroids to the same receptor protein at its same site. In this regard, however, it should be noted [18] that the testosterone-bound protein in the cytosol fraction appeared to migrate more slowly than the 5α-dihydrotestosterone-bound protein in the sucrose density gradient centrifugation, indicating that the different receptor protein with the different grade of the affinity to the steroids were present in the cytosol fraction.

In the present experiment, no clear temperature-dependency on formation of the androgen–cytosol receptor complex could be demonstrated, but attaching process of the androgen–nuclear receptor to nuclear acceptor sites was reported as temperature-dependent [19, 20].

In agreement with the results on distribution of 3α-hydroxysteroid dehydrogenase activity [14], the dehydrogenase activity was detected in different fractions from the cytosol receptors according to the gel

filtration of the cytosol [21]. In the present study, the complex of 5α-dihydrotestosterone with the cytosol receptor was markedly decomposed by the presence of the 3α-hydroxysteroid dehydrogenase. This result implies that 5α-dihydrotestosterone bound to the cytosol receptor was converted to 5α-androstane-3α,17β-diol by the dehydrogenase in the presence of NADPH, and the 5α-androstanediol became free from the complex. In fact, the cytosol receptor possessed markedly smaller affinity for 5α-androstane-3α,17β-diol than 5α-dihydrotestosterone [12, 22]. This enzyme reaction was therefore suggested as being involved as a step of androgen excretion from the prostatic cells as follows: 5α-dihydrotestosterone which was formed from testosterone by 5α-reductase in the endoplasmic reticula or nuclear outer membrane of the prostate [23] formed a complex with the cytosol 9S receptor. If the complex was present in the cytoplasm, the 3α-hydroxysteroid dehydrogenase in the cytosol fraction would convert the complex to free 5α-androstane-3α,17β-diol, in the presence of NADPH. The 5α-androstanediol was excreted from the cell membrane, as macromolecules in the cell showed little affinity to this metabolite. In this way, the 3α-hydroxysteroid dehydrogenase might be involved in a regulatory mechanism of the action of the androgens. With regard to 5α-androstane-3α,17β-diol, this metabolite was reported as not being retained by the nuclei [1, 2], and only 5α-dihydrotestosterone bound to the cytosol receptor was effectively transferred into the nuclei (Table 2).

In the present experiments, evidence presented confirmed that [^{3}H]-5α-dihydrotestosterone was incorporated into the nuclei in the form of the complex with the cytosol receptor, but not in its free form. This clear contrast of the complex with the free steroid seems to be a result of the specific permeability of the nuclear membrane to the androgen-receptor complex. The complex incorporated into the nuclei was probably bound to a specific acceptor site in the nuclei. The nuclear acceptor site for the complex of 5α-dihydrotestosterone with the cytosol receptor was localized in the chromatin [24]. Recently, we found using [^{131}I]-labeled cytosol receptor [9] that the cytosol receptor was incorporated into the nuclei as the complex with 5α-dihydrotestosterone and that the nuclear receptor was associated with both non-histone protein and DNA of the chromatin. The non-histone proteins of the chromatin appeared to be important in tissue-specific retention of 5α-dihydrotestosterone, since non-histone proteins are sufficiently tissue-specific and heterogeneous [25]. The binding of 5α-dihydrotestosterone–receptor complex to these acceptor sites of the chromatin is possibly a step of initiation of RNA synthesis *in vivo* [26] and *in vitro* [27].

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