Urol Res (1991) 19:327–332

Urological Research

Original articles

Steroid receptor profile and receptor stability in subfractions of human prostatic tissues

Critical aspects on microassays

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Accepted: February 1, 1991

Summary. Androgen (AR), progesterone (PR), and estrogen (ER) receptor contents in cytosol and salt-extractable nuclear subcompartments from 6 normal, 39 benign hyperplastic (BPH), and 7 malignant prostatic tissue specimens were analyzed by radioligand-binding assay techniques. In addition, the temperature stability of AR and PR was measured in another three BPH specimens. Five punch-needle biopsy samples from prostate cancers were also analyzed for AR and PR content. All receptor data were calculated from saturation analyses. The highest AR content was found in the cytosol and nucleic from malignant prostatic tissues. The highest PR concentrations were found in BPH cytosol, whereas nuclei of all types of tissues were negative with regard to this receptor. Markedly lower concentrations of ER were found in cytosol and nuclei from BPH as compared with malignant and normal tissues. PR was the most temperature-stable receptor; a marked receptor loss at room temperature was not registered until after 12 h. AR was stable for 4–5 h in cytosol and for 8–9 h in nuclei. Needle-biopsy specimens from prostate cancer showed highly variable and confusing results for AR and PR content, indicating that microassay studies using biochemical techniques on small tissue samples are unreliable and should not be recommended.

Key words: Androgen, progesterone and estrogen receptors – Temperature stability – Human prostate – Cancer – Benign prostatic hyperplasia

Steroid receptor assays have not become a routine part of the clinical workup of patients with newly diagnosed prostate cancer, mainly due to difficulties in obtaining sufficient quantities of tissue. Various microassays have been developed, but the results reported have been inconsistent [2, 23]. Steroid receptor activity is generally believed to be highly susceptible to temperature changes and is purportedly rapidly lost due to enzymatic degradation. Therefore, samples used for receptor assays are prepared rapidly and are usually frozen to −80°C until use.

To set standards for steroid receptor content, we measured the androgen (AR), progesterone (PR), and estrogen (ER) receptor profiles in cytosol and nuclei from normal, benign hyperplastic (BPH), and malignant human prostatic tissues. We also attempted to carry out microassays on prostate-cancer biopsy specimens according to the technique we described previously [9]. Furthermore, we wanted to investigate the generally accepted concept of rapid receptor degradation at room temperature.

Materials and methods

Tissue samples

In all, 6 normal, 39 BPH, and 7 malignant prostates were used for analyses of steroid receptor profiles. Hyperplastic tissues were obtained during open adenoma enucleations. Normal and malignant tissues were harvested during radical cystoprostatectomies for bladder carcinoma and radical prostatectomies for prostate cancer. Cancerous biopsy specimens were obtained at the time of castration of five patients exhibiting various grades and stages of prostatic cancer. Representative sections of all tissues were prepared for histological examination. Samples taken from malignant prostatic tissues were accepted for further analyses only when their tumor-cell content represented more than 75% of the total number of cells. These tissue samples were frozen to −80°C within 30 min and were kept frozen until their use.

Samples for studies of receptor stability were obtained from three patients who underwent open surgery for BPH. From each specimen, approximately 25 g tissue was recovered and cut into small pieces weighing around 0.2 g each; these were then randomly collected in vials (2.5–3 g/tube). They were thereafter kept at room temperature (22°C) until being frozen to −80°C. The first tissue sample was frozen after 30 min; the second, after 1 h and the next eight, after 1.5, 2, 3, 4, 6, 8, 12, and 20 h, respectively. The receptor content was considered to be unchanged when the measured amount represented at least 70% of the content of the 30-min sample.

Table 1. Simultaneous measurement of AR, PR, and ER contents in normal, BPH, and malignant human prostate tissues

Tissue	*n*	AR		PR		ER	
		Cytosol	NE	Cytosol	NE	Cytosol	NE
Normal	6	9.5 ± 3.3	23.5 ± 17.8	7 ± 3.1	ND	3.1 ± 1.3	1.2 ± 0.7
BPH	39	12.4 ± 5.4	29.6 ± 19.3	11.4 ± 6.1	ND	0.9 ± 0.5	0.3 ± 0.5
Canser	7	19.1 ± 9	33 ± 21.8	7.4 ± 4.9	ND	2.7 ± 1.2	1.4 ± 0.9

Data represent mean values ± SD (expressed in fmol/mg protein). NE, Nuclear extract; ND, not detectable

Table 2. Simultaneous measurement of AR, PR, and ER contents in normal, BPH, and malignant human prostate tissues

Tissue	*n*	AR		PR		ER	
		Cytosol	NE	Cytosol	NE	Cytosol	NE
Normal	6	382 ± 92	191 ± 105	282 ± 102	ND	147 ± 73	16 ± 11
BPH	39	348 ± 200	324 ± 361	319 ± 231	ND	22 ± 12	1.9 ± 3.6
Cancer	7	475 ± 147	242 ± 134	226 ± 169	ND	77 ± 39	16 ± 11

Data represent mean values ± SD (expressed in fmol/mg DNA). NE, Nuclear extract; ND, not detectable

Chemicals

[17-methyl-^{3}H]-Methyltrienolone (MT; sp. act., 87 Ci/mmol) and [2,4,6,7-^{3}H]-estradiol (E_2; sp. act., 92 Ci/mmol) were purchased from New England Nuclear (Boston, Mass). Radioinert steroids were obtained from Steraloids, Inc. (Pershing, N.Y.). Activated charcoal, dextran (mol. wt., 73,200 Da), phenylmethylsulfonylfluoride (PMSF), triamcinolone acetonide, and Triton X-100 were purchased from Sigma Chemicals Co. (St. Louis, Mo.).

Radioligand-binding assays

Homogenization and subcellular fractionation were carried out as described previously [9]. Binding assays were carried out at 4°C for 18–20 h, resulting in a 90%–95% exchange of endogenously bound steroid. Saturation analyses [18] were carried out of [^{3}H]-MT using six different concentrations ranging from 0.3 to 10 nM for simultaneous measurement of the AR and the PR content as described elsewhere [9]. For ER assays, [^{3}H]-E_2 was used at eight different concentrations ranging from 0.05 to 6 nM [10].

The microassays were carried out in triplicate as single-concentration analyses using a ligand concentration of 5–10 nM.

Incubations of cytosol and nuclear salt extracts were terminated by the dextran-coated charcoal technique [7, 9]. DNA was extracted and assayed by the diphenylamine method using calf-thymus DNA as a standard [4]. The protein content of soluble extracts was assayed according to the method of Bradford [3] using bovine γ-globulin as a standard.

Statistical analyses

For comparison of differences in the receptor contents of various tissues, Student's *t*-test was used.

Results

Steroid receptor profile in bulky tissue

Malignant prostatic tissue exhibited markedly higher cytosolic AR levels than did normal tissue (mean values, 19.1 vs 9.5 fmol/mg protein; Table 1); the difference was statistically significant ($P < 0.05$). When the AR content in cytosol was expressed in femtomoles per milligram of DNA, malignant tissue contained the highest amounts, whereas the lowest amounts were found in BPH (Table 2); however, these differences were not statistically significant. Nuclear AR content was highest in malignant tissue when the former was expressed in femtomoles per milligram of protein, but when it was expressed in femtomoles per milligram of DNA, BPH contained the highest amounts (Tables 1, 2). The lowest nuclear AR content was found in normal prostatic tissue, regardless of whether the data were expressed in relation to protein or to DNA (Tables 1, 2). No statistically significant difference was registered.

The PR content was higher in BPH (319 fmol/mg DNA) than in normal (282 fmol/mg DNA) or malignant (226 fmol/mg DNA) tissues, but not no statistically significant difference was found (Tables 1, 2). In all tissue samples the nuclei seemed to be devoid of PR. In agreement with previous findings [10], the ER content was markedly lower in BPH (22 fmol/mg DNA) than in normal (147 fmol/mg DNA) or malignant tissues (77 fmol/mg DNA, Table 2). The difference between normal tissue and BPH was statistically significant ($P < 0.01$). This relationship was seen in nuclei as well as in cytosol.

Table 3. AR and PR contents in Subcellular fractions of prostatic cancer biopsies

Patient	Weight of tissue (g)	AR		PR	
		Cytosol	NE	Cytosol	NE
Ca SI	0.03	0	238	0	348
Ca SII	0.08	129	0	0	506
Ca BI	0.06	513	0	57	389
Ca BII	0.08	1,296	110	576	0
Ca KI	0.07	138	75	444	27

Samples hat to be diluted 20–30 times rather then 5–10 times (vol/wt) to ensure that sufficient material was available for triplicate assays; hence, the protein content varied from 0.42 to 1.57 mg/ml. Data indicate the specific binding (expressed in fmol/g tissue). NE, Nuclear extract

Prostatic cancer biopsies

As the tissue weight of these specimens varied between 0.03 and 0.08 g, the samples had to be diluted up to 30 times (vol/wt); therefore, the protein content was markedly reduced (to 0.5 mg/ml). The results are given in Table 3 and demonstrate a confusing variability in the steroid receptor profile. The cystol AR content varied from 0 to 1,300 fmol/g tissue. In contrast to bulky tissue, four of the five biopsies exhibited considerable nuclear PR content.

Temperature stability of steroid receptors in bulky tissue

Split tissue samples from three patients with BPH were analyzed for their AR and PR content in cytosol and nuclei. A total of 27 samples were examined and the receptor contents for specific binding were expressed n femtomoles per milligram of protein. The AR content in cytosol showed no remarkable decline until after 6 h (Table 4), and the nuclear AR seemed to be stable for up to 8 h (Table 5). Even after 12 h, the AR content in nuclei of

Table 4. Cytosolic AR and PR contents in BPH tissues after various periods of storage at room temperature

Storage period	Case 1		Case 2		Case 3	
	AR	PR	AR	PR	AR	PR
30 min	15.01	28.18	14	15.8	10.65	7.03
1 h	15.15	38.89	13.6	15.5	11.1	11.72
1.5 h	17.99	12.79	13.72	17.38	13.87	11.92
2 h	15.42	13.76	11.03	11.1	13.98	11.4
3 h	13.4	9.37	11.8	9.8	12	7.57
4 h	13.4	14.95	12.2	12.2	8.44	7.23
6 h	–	–	10.79	10.3	3.34	9.77
8 h	5.59	7.29	8.4	12.2	6.12	5.75
12 h	4.62	7.82	5.5	9.5	2.1	9
20 h	4.52	5.92	–	–	–	–

Data indicate the specific binding (expressed in fmol/g protein)

Table 5. Nuclear AR and PR contents in BPH tissues after various periods of storage at room temperature

Storage period	Case 1		Case 2		Case 3	
	AR	PR	AR	PR	AR	PR
30 min	44.78	ND	28.11	ND	31.2	ND
1 h	34.78	ND	29.57	ND	23.73	ND
1.5 h	43.26	ND	22.48	ND	27.73	ND
2 h	36.86	ND	16.86	ND	24.49	ND
3 h	38.32	ND	27.79	ND	9.64	ND
4 h	30	ND	27.7	ND	18.93	ND
6 h	–	–	25.8	ND	20.77	ND
8 h	35.22	ND	27.8	ND	24.56	ND
12 h	40.83	ND	24.4	ND	1.2	ND
20 h	0	ND	–	–	–	–

Data indicate the specific binding (expressed in fmol/g protein). ND, Not detectable

two of the cases remained unchanged (Table 5). The PR in cytosol seemed to be even more stable, and a significant drop (>30%) did not occur until after the tissue samples had been kept at room temperature for 12 h (Table 4).

Discussion

The mean AR content seemed to be higher in malignant than in normal prostatic tissues. However, the higher nuclear AR contents recorded in relation to protein or to DNA and the higher cytosolic AR contents measured in relation to DNA were not statistically significant, which may be attributable to the early stage [24] and low grade (WHO grades I–II) [17] of all cancers tested. Prostate cancer contains relatively more epithelial cells than does the normal gland; therefore this difference could be explained by the higher abundance of AR in the epithelium. It is also possible that the malignant transformation starts in androgen-overstimulated prostatic epithelial cells.

In contrast, the PR content was highest in BPH. The importance of PR in the human prostate has never been adequately explained. Human BPH is dominated by a hyperplastic transformation of the stroma [11, 13]. Therefore, since the PR is mainly localized in the stroma [15], it may play a role in BPH development. It is noteworthy that BPH can be efficiently treated with progestational agents, leading to a reduction in the size of the gland [8].

Confirming previous observations [10], we found that the ER content in BPH was significantly lower than that in normal prostatic tissue. This finding is remarkable, since estrogens purportedly play an important role in the development of BPH, partly via aromatization of androgens, which can take place in human prostatic stroma [20]. The lower ER levels found in BPH in previous investigations [10, 19] as well as in the present study challenge the hypothesis that estrogens are of vital importance for BPH development.

The levels of AR and PR measured in biopsy samples from prostatic cancer tissues using a microassay technique were highly variable in cytosol as well as in the nuclei. It cannot be excluded that the high contents of PR found in the nuclei may reflect an altered cellular biology; however, the confusing data much more likely indicate that single-concentration, biochemical, steroid receptor assays cannot be carried out on small tissue samples. In previous studies, we have demonstrated the hazards of diluting samples to protein contents of 1 mg/ml, since this leads to a marked underestimation of the specific AR and PR binding [9]. Therefore, results in the literature that are based on receptor studies of biopsy material must be interpreted with great caution.

Tissues harvested for receptor studies are immediately frozen to −80°C to avoid enzymatic receptor degradation. The present study shows that a marked loss of steroid receptor content does not occur until after 6–8 h storage of the prostatic tissue at room temperature. This suggests the possibility that autopsy material can be used for steroid receptor assays.

Several recent studies using monoclonal antibodies to the receptors have failed to demonstrate any receptor content in the cytosol, indicating that steroid receptors are present only in the nuclei [5, 19, 21, 22, 25]. This issue is a matter of controversy [1, 6, 12, 14, 16]; using identical biochemical assay techniques, we have demonstrated a marked shift of AR content from the nuclei to the cytosol in prostatic cancer metastase [7]. The inability of some immunohistochemical techniques to detect cytosolic AR content warrants further investigation.

Acknowledgements. The skillful technical assistance of Mrs. I.-L. Larsson is gratefully acknowledged. This study was supported by grant B85-1706860-02 from the Swedish Medical Research Council, by grant 1192/B90/01X from the Swedish Cancer Society, and by generous support from Gunvor and Josef Anér's Foundation, Sven and Dagmar Salén's Foundation, and the Foundation for Aging Research.

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