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Steroid Receptors in Human Prostatic Cancer A Preliminary Evaluation

H. J. de Voogt and P. Dingjan

Departments of Urology and Pathological Chemistry, University Hospital, Leiden, The Netherlands

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Summary. The determination of steroid-receptors in human prostatic tissue from 68 patients with benign prostatic hypertrophy(BPH) and 36 patients with prostatic cancer by the method of cold agar-gel-electrophoresis is described. The results are discussed in the light of several problems inherent to this assay. There are certain indications that there is a correlation between steroid-receptor values and response to endocrine therapy.

Key words: Steroid-receptors - Benign prostatic hypertrophy - Prostate carcinoma - Endocrine therapy.

The determination of steroid receptors in human mammary cancer tissue has been of major importance for the prognosis and the endocrine therapy of that tumour (5, 24, 38, 43). It seemed likely that the same could be true for prostatic cancer, an androgen-dependent tumour in a target organ for androgenic hormone. The significance of androgen receptors for prostate growth has been proven in numerous animal experiments (6, 9, 10, 11, 13, 26, 27, 28, 29, 30, 38, 39, 40, 41), as well as in human tissue (1, 16, 17, 22, 23, 25, 31, 32, 33).

However the assessment of steroid receptors in human prostatic tissue is still a challenge, mainly because of difficulties in getting proper tissue material and the biochemical methods. In our opinion Wagner solved the methodological problem initially with his method of cold agar-gel-electrophoresis (42). Recently, new methods have been developed which hold promise for the future (7, 8, 14).

Since 1975 we have used Wagner's method to determine not only androgen-receptors but also oestrogen binding proteins in human prostatic tissue. The latter were measured because oestrogens are still used on a large scale for the treatment of patients with prostatic cancer and because several publications indicate a specific intracellular action of oestrogens in prostatic tissue itself, thus differing from the antiandrogenic properties, which act via the hypophyseal-gonadal axis (2, 3, 4, 18, 19, 20, 21, 34, 36, 45).

MATERIALS AND METHODS

Collection and Storage of Prostatic Tissue

Directly after the removal of the tissue by the surgeon, it was immersed in ice-cold buffered physiological saline solution in order to achieve rapid cooling and removal of most of the blood. In the room next to the operating-room the tissue was cut into pieces of 3 x 3 x 3 mm, and immediately frozen in liquid nitrogen. The cutting procedure was carried out on ice cooled glass throughout and completed between 1 to 30 min after operation. From any tissue block a representative part was sent for histological diagnosis and morphometric analysis.

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Extraction of cytoplasmic Receptors

The small frozen tissue specimens, 100-2000 mgs, were transferred into pre-cooled 7 ml cylindrical teflon containers, a pre-cooled 9 mm tungsten carbide ball was added, the cylinders capped, immersed again in liquid nitrogen (5 min.) and then vibrated for 45 sec at 50 Hz and maximal excursion in a Mikro-Dismembrator (Braun, Melsungen). The cooling and vibrator sequence was repeated twice. The resulting tissue powder was transferred in 3 to 4 volumes of ice-cold buffer (0.01 M Tris-HCl, pH 7.5, 0.001 M dithiothreitol, 0.005 M NaN_3), by repeated aspiration and discharge with a precooled polyethylene syringe (Primo 2 ml) for 5-10 min. The homogenate was centrifuged for 45 min. at 0-2° C and 37,500 rpm (105.000 $g_{av.}$) in an SW-50.1 rotor.

Incubation

0.125 ml aliquots of freshly prepared extracts were incubated in 3.0 ml glass tubes with a final concentration of 1-2 10^{-8} M labelled steroid at 0° C overnight. 6, 7-^{3}H-oestradiol, (^{3}H-E2, 47.9 Ci/mmol) and 1, 2-^{3}H-Dihydrotestosterone, (^{3}H-DHT, 40-Ci/mmol, purity 98 %), were purchased from New England Nuclear, England. All non-radioactive compounds were commercial preparations of analytical grade.

In control incubations for determination of unspecific binding, mixtures of the radioactive steroid and a 200-fold excess of cold steroid were added. The final incubation volume was 0.175 ml. The time course between suspending the tissue powder and adding the steroids was less than two hours. All pipetting procedures were carried out with disposable plastic tips.

Agar Electrophoresis at Low Temperature

The method has been described in detail by Wagner (42) and was used by us in exactly the same manner. The only difference was that we applied a 100 min run at 120 mA per plate and 190-225 V (9 V/cm agar) during which the temperature within the gel did not exceed 6° C.

Measurement of Radioactivity, Data Evaluation and Auxiliary Assays

After the run, gels were sliced lengthwise to separate the individual analyses and then cut into 3 mm wide sections. The 3 x 12.5 x 5 mm agar blocks were transferred to 15 ml glass vials and counted after overnight extraction of the radioactive steroids in 7 ml Rotiszint-33 (Carl Roth, Karlsruhe). The counting efficiency was between 35 and 40 %. Specific binding in the peak fractions was calculated by subtracting the radioactivity left in the excess-cold-steroid controls. Tissue protein was calculated by subtracting the plasma protein contamination from the total protein concentration. Total protein was assayed according to Lowry, serum albumin by immunodiffusion (Partigen-M, Behring Werke, Marburg). Plasma protein contamination was defined as 100/60 of the albumin concentration.

Plasma levels of testosterone (T), oestradiol (E2) and prolactin were determined by radio-immuno-assay, using specific antisera against T and E2 prepared in our own laboratory and WHO-30-222 standard prolactin (Serono, Milan).

Patients

During the years 1975-1977 receptor assays were carried out in tissue from 68 patients with benign prostatic hypertrophy and from 36 patients with different stages of prostatic cancer. The patients with benign prostatic hypertrophy provided the tissue-material necessary to test the method and then formed a tissue-pool, from which further samples could be taken for comparison.

The cancer patients were carefully examined, treated and followed in accordance with a protocol to record as many objective data as possible and using the TNM system for classification. They were divided in two groups:

1. a group of 15 patients who had already been treated with hormones for periods of 2 to 84 months at the time of receptor-determination.

2. a group of 21 patients who had not yet been treated. Trans-urethral resection was performed for those cases showing signs of urinary obstruction which provided the tissue for receptor assay. In the other cases tissue was taken by open or needle biopsy. Diagnosis was proven histologically and cytologically (by needle as well as aspiration biopsy).

The treatment in this group was mainly by oestrogens, but two cases, who had stage T1 tumours underwent radical prostatectomy. So later evaluation was not possible.

The response to endocrine therapy was determined by the following criteria:

Table 1. Results of steroid-receptor determination in prostate "cytosol". (fmol/mg tissue protein)

		n	mean	range	SD	SEM
BPH	DHT-R	63	22	0-276	47	6
	E_2-R	68	798	0-10849	1598	194
Ca. untreated	DHT-R	19	49	0-205	59	13
	E_2-R	19	2791	0-15507	4543	1042
Ca. treated	DHT-R	15	184	0-1621	407	105
	E_2-R	15	135	0-829	226	58

BPH, Benign prostatic hypertrophy; Ca, Carcinoma; n, Number of patients; SD, Standard-deviation; SEM, Standard error of mean

1. regression of prostatic tumour (rectal palpation).
2. improvement of patients welfare and activity.
3. disappearance of difficulty of micturition, bladder residue and pain.
4. return of high serum acid phosphatase levels to normal.
5. reduction of plasma-testosterone below 100 ng/100ml.
6. the appearance of "hormonal effect" in cytological aspiration material.
7. the appearance or disappearance of visible metastases (Skeleton, Lung, Skin or Lymphnodes).

RESULTS

Table 1 gives a survey of the values of androgen- and oestrogen-binding that were found in our patients. All except 3 of the patients in the untreated group were over 65 years of age. From this group of 19 patients, 12 were examined for a second time after six months of oestrogen treatment at which time a second steroid-receptor assay was carried out on biopsy material. In all but 2 of the 19 previously untreated patients and in 13 of 15 already treated patients the plasma-testosterone values (after oestrogen-treatment) were below 100 ng/100 ml, indicating a good suppression of endogenous androgens.

Values of DHT-receptor below 20 fmol/mg tissue protein were considered as negative (because they fall within the margin of error of the method) and values higher than this as positive. For the E_2-receptor the borderline level was arbitrarily taken as 100 fmol/mg tissue protein. Tables 2 and 3 show the values obtained related to the clinical progress of the patients in both groups.

DISCUSSION

There are a great many problems associated with steroid-receptor studies of human prostatic tissue.

Suitable normal prostatic tissue is virtually impossible to obtain, since it has to be frozen immediately, with a minimal warm ischaemia time since receptor molecules are thermolabile. For practical purposes BPH-tissue, which is available in large amounts, can be used to test the methods and to find out whether steroid-receptors are present or not in prostatic tissue. However one has to keep in mind that BPH is itself a tumour, probably originated by hormonal imbalance (1, 23, 25, 27, 37). This is especially

Table 2. Steroid-receptor values (fmol/mg tissue protein) and clinical response in previously untreated patients (Group 2)

	Number of patients	DHT-R>20 (pos.)	DHT-R>20 (neg.)	E_2-R>100	E_2-R<100
Good response	11	6	5	9	2
Stationary	5	4	1	4	1
No response	3	2	1	1	2
Totals	19	12	7	14	5

DHT-R, Dihydrotestosterone-receptor; E_2-R, Oestradiol-receptor

Table 3. Steroid-receptor values (fmol/mg tissue protein) and clinical progress in patients who had received oestrogen prior to study (Group 1)

	Number of patients	DHT-R>20 (pos.)	DHT-R<20 (neg.)	E_2-R>100	E_2-R<100
Progression	11[a]	7	4	2	9
Regression	4	2	2	3	1
Totals	15	9	6	5	10

[a] 9 of the 11 patients who showed progression died within a short period after the receptor assay

Table 4. Diagnoses of BPH and carcinoma in material obtained by trans-urethral resection compared with original diagnoses on needle biopsies

Needle biopsy diagnosis	N	Malignancy in TUR-material		
		Positive	Negative	Suspect
BPH	16	0	16	0
Carcinoma (untreated)	14	12	2	0
Carcinoma (treated)	9	7	1	1

relevant when BPH-tissue is used as comparison for carcinomatous prostatic tissue.

Except when an open biopsy, trans-urethral resection or a radical prostatectomy is done it is usually not possible to get more than a needle biopsy from a given prostatic cancer. The amount of tissue obtained by a needle biopsy ranges from 50-100 mg. As the original biochenical procedures require 100-300 mg tissue, we had to prove in several experiments that the small amount of tissue in a needle biopsy could yield detectable steroid-binding, or that the material obtained by T. U. R. could be used. Figure I shows an example of E_2-receptor values in large pieces of a prostatic adenoma compared to the values found in a needle biopsy of the same tissue. Comparison of receptor-assays in tissue removed by T. U. R. and removed surgically from the same prostatic adenoma also showed the same results. Table 4 compares the histological diagnoses in T. U. R. material and in needle biopsies of BPH and prostatic carcinoma, and indicates that histologically carcinomatous tissue is usually present in resected tissue. Enzyme histochemistry showed that the layer of coagulation necrosis is seldom more than 0.2μ thick, while the remainder of the tissue was not affected.

We finally concluded that tissue provided by needle biopsy or by T. U. R. can be used for steroid-receptor studies (12).

The amount of endogenous androgen steroid in men is relatively high and it seems likely that most of the binding sites of cytoplasmic receptors are occupied by these endogenous androgens (35, 44). The steroid-receptor complexes then translocate rapidly to the nucleus. This may account for the fact that only small amounts of free binding sites are found and sometimes none at all. The fact that the values for DHT-receptors were higher in patients treated with oestrogens can be partly explained by the presence of more free binding sites due to suppression of endogenous androgens (2).

Another important problem is the presence of sex-hormone binding globulin (SHBG) in all samples of human target tissue.

This binding protein which serves as transport medium for steroids in plasma, contaminates the tissue samples and is probably even partly synthesised or concentrated by the tissue (25). Therefore, for the determination of cytoplasmic receptors it is necessary to use a method which clearly distinguishes SHBG-binding from real receptor-binding. Wagner's method deals with this problem quite effectively and satisfactorily. Most other methods fail in various points in this respect (43).

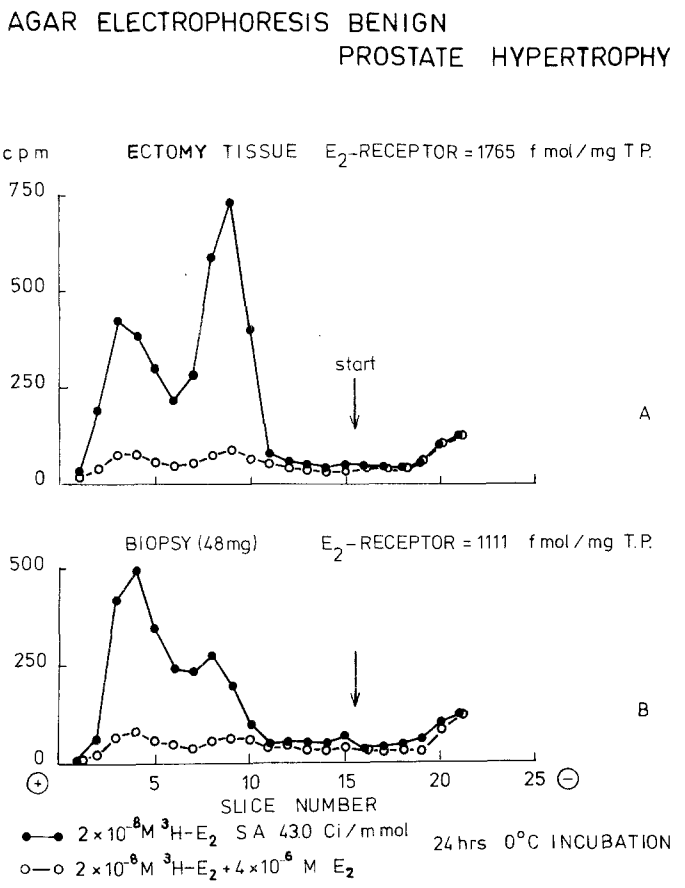

Fig. 1. E_2-binding in prostatic adenoma, determined in large piece of tissue (BPH) and in a biopsy (48 mg). The receptor "peak" (slice numbers 5-10) is similar in both experiments

The oestrogen binding that we found, shows all the characteristics of a receptor and does not differ from the binding protein found by Bashirelahi and Hawkins (3, 4, 20, 21). Bashirelahi moreover proved that this oestradiol-receptor complex moved to the nucleus (4). The quantities of specific oestrogen-binding in our material were in most instances much higher than those of androgen receptor. They also differed from the values found by Wagner (43) and Hawkins (20, 21). The reasons for these controversial findings can for the greater part be explained by technical differences, such as:

1. Wagner used only biopsy material. Although we have demonstrated that this can be used to determine receptors qualitatively, one has to bear in mind that a certain

dilution-factor is introduced, which can change quantitative measurements considerably.
2. Not all investigators have used the same amount of protein concentration in the supernatant. Hawkins worked with concentrations up to 25 mg/ml and furthermore in some of his experiments he used tissue extracts rather than cytosols (21). In our experiments we aimed at the lowest protein concentration possible (3-5 mg/ml) to minimise non-specific binding.
3. We omitted the dextran-coated charcoal treatment after we found that this can remove much of the specific binding depending on the duration of this treatment. It is not necessary when the agar-gel-electrophoresis method is used.

All these purely methodological differences are to be discussed in detail in a further publication (12). However it is also possible that stromal cells as well as carcinoma (epithelial) cells possess oestrogen-receptors.

It is necessary for any steroid-receptor assay in the human prostate to know the histological composition of the piece of tissue in order to assess the ratio of stroma to epithelial elements. All pieces of tissue in our study were divided and a representative portion sent to the pathologist for morphometric analyses.

Our study has shown that there are differences in receptor content in BPH- and carcinomatous tissues and that a relationship exists between steroid-receptor values and response to endocrine therapy in carcinoma patients.

Considering DHT-R values in the 16 previously untreated patients that showed a good response or remained stationary, 10 were DHT-R positive and 6 negative. Of the 11 previously treated patients that progressed, 7 were DHT-R positive and 4 negative. Thus, in itself, the DHT-R value does not seem to indicate response to endocrine therapy. However of the E_2-R values from the same 16 previously untreated patients, 13 were high and 3 low, while from the 11 treated patients with progressive disease only 2 were high and 9 low. From the group of 4 patients that still showed regression, 3 had high E_2-R values and only 1 low. A relationship between response and E_2-R values seems to be present.

It may be that a combination of both receptor assays will give more reliable results as Wagner has indicated for mammary cancer (43, 44).

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Dr. H. J. de Voogt
Department of Urology
University Hospital
Leiden
The Netherlands