Steroid hormone receptors in prostatic hyperplasia and prostatic carcinoma

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Summary
One hundred and six prostatic tissue samples obtained from transurethral resection were analysed for androgen and estrogen receptors. In 62 of these, progesterone and glucocorticoid receptors were also assayed. Steroid receptors were assayed using single saturation dose 3H-labelled ligand assays. Ninety percent of the 97 prostatic hyperplasia tissues and six of the nine prostatic carcinoma tissues were positive for androgen receptors. Estrogen receptors were only present in 19% and 33% respectively. Progesterone receptors were present in 70% of the tissues, but glucocorticoid receptors were present in only 16% of prostatic hyperplasia and none in prostatic carcinoma.

Key words: Prostate, androgen, estrogen, progesterone and glucocorticoid receptors.

Introduction
Androgens have a direct effect on the growth of prostatic tissue and on the development of prostatic hyperplasia and cancer.1 Gonadotrophins (Follicle stimulating hormone and luteinizing hormone) act indirectly on prostatic tissue by stimulating testicular production of testosterone. Removal of androgenic influence by endocrine therapy would inhibit prostatic growth or the progression of prostatic carcinoma.1 Other types of endocrine therapy consist of the use of antiandrogen, progestin, estrogen and corticosteroid. The antiandrogen cyperterone acetate acts at the receptor level as an antagonist and at the pituitary level by inhibiting pituitary luteinizing hormone secretion.1 Progestins such as medroxyprogesterone which have tumour regressing effect, acts mainly by lowering pituitary gonadotrophins rather than at the progesterone receptors in the prostatic tissues.1,3 The estrogens act probably by suppression of gonadotrophins or directly on the prostatic estrogen receptors.1,4,5 Corticosteroids have also been used in advanced prostatic cancers but their mode of action is unknown and this is not the usual form of endocrine therapy.6 Endocrine therapy and manipulation however is not effective in all cases, and the response rate for carcinoma is between 75–80%.1,6 As steroid hormones act predominantly on specific cytosolic protein receptors, detection of the presence of specific steroid hormone receptors in the tissues could help to determine the potential responsiveness of a tumour.
Assays for steroid receptors in prostatic tissue have always been hampered by the difficulty in obtaining sufficient amount of tissues (eg. by fine needle biopsy per rectum) for saturation assays of androgen and other receptors. This is further complicated by the presence of large amounts of endogenous steroids such as dihydrotestosterone (DHT), and sex-hormone binding globulin SHBG which binds DHT with similar affinity to that of androgen receptors. To overcome these problems and to determine all four steroid receptors within a single specimen, we used prostatic “chips” obtained from transurethral resection of the prostate, and the method of single saturation steroid receptor assays. We also used synthetic steroids as ligands and receptor blockers in the assays. They are the synthetic androgen (R1881) 3H-methyltrienolone (for androgen receptors), and synthetic progestin 3H-Org.2058 (for progesterone receptors) and synthetic glucocorticoid 3H-dexamethasone (for glucocorticoid receptors). The aim of this study was to determine the presence of androgen, estrogen, glucocorticoid and progesterone receptors in prostatic tissues from patients with prostatic hyperplasia and carcinoma.

**Materials and Methods**

**Chemicals:** Radioactive steroid 3H-dexamethasone (specific activity 60 Ci/mmol). 3H-estradiol (specific activity 85 Ci/mmol), 3H-R1881 Methyltrienolone (specific activity 80 Ci/mmol) and 3H-Org 2058 (specific activity 60 Ci/mmol) were obtained from the Radiochemical Centre Amersham UK and New England Nuclear, USA. Non-radioactive steroids dexamethasone, estradiol, DHT, triamcinolone acetomide and progesterone, were obtained from Sigma Chemical Co, St. Louis, Missouri, USA. Other chemicals such as Triton X100, POPOP, PPO, Norit A charcoal, were also from Sigma. Dextran T70 was from Pharmacia Fine Chemical, Sweden.

**Methods:** Fresh prostatic tissue chips were obtained from transurethral resection of prostate (TURP) at the General Hospital, Kuala Lumpur. The tissues were kept in ice and sent to the Steroid Receptor Laboratory, Universiti Kebangsaan Malaysia where they were analysed immediately or kept frozen at −20°C for no longer than 2 weeks.

The tissues were placed in 2.5 ml of ice cold TEMDG buffer (Tris 10mM, EDTA 1.5mM, dithiothreitol 2mM (added fresh daily), sodium molybdate 10mM, and glycerol 10% v/v, pH 7.4 at 4°C). The tissues were then homogenised in a mechanical tissue homogeniser (Tissumizer, Tekmar Instruments) at 0–4°C for 3 seconds, with 5 second intervals, five times. The homogenised tissues were then centrifuged for one hour at 20,000 rpm at 0–4°C in a Beckman Model J-21 high speed centrifuge. The supernatant cytosol were removed and put into clean containers and protein concentration estimated by Coomassie Blue Method. Protein concentration was then adjusted to about 2–5 mg/ml of cytosol protein. Aliquots (0.2 ml) of cytosol were then added into glass assay tubes appropriately labelled for estradiol, glucocorticoid, androgen and progesterone receptors. Each receptor assay was carried out in duplicates, with two tubes for total binding of 3H-ligands, and two tubes for non-specific binding. Into each receptor assay tube was added 0.2 ml of 3H-steroid prepared in TEMDG buffer to give a final concentration in each tube of 2nM for 3H-estradiol, 3H-Org 2058 and 3H-R1881, and 5nM for 3H-dexamethasone. For androgen receptors. 100nM triamcinolone acetomide was added to block progesterone receptors from binding R1881. For non-specific binding, 5 μl of non-radioactive steroid (as mentioned above) was added to give 100 fold concentration to block 3H-steroid binding to respective receptors. The assay tubes were then vortexed, covered and incubated at 0–4°C overnight.

The following day, 0.4 ml of dextran coated charcoal (DCC), (Dextran T70, and Norit A suspended in TEMDG buffer, pH 7.6) prepared fresh, were added to all the assay tubes. These
were then vortexed, left standing for five minutes, vortexed again and after another five minutes standing, centrifuged at 4000 rpm for 15 minutes in a Damon/IEG CRU-500 refrigerated centrifuge. The supernatant was tipped carefully into scintillation vials containing 8 ml of scintillant fluid (PPO, POPOP, Triton X100 dissolved in toluene). The vials were vortexed, allowed to equilibrate, and the radioactivity was counted in a liquid scintillation counter (Philips) for four minutes per vial at 37% efficiency. The radioactivity of total amount of radioactive steroid added into each vial was counted after adding 0.2 ml of the 3H-steroid preparation in 0.6 ml of buffer to the scintillant fluid. The amount of 3H-steroid bound to receptors was calculated by subtracting counts per minute (cpm) of non-specific binding from cpm of the bound fraction. The specific binding obtained was then expressed per mg of protein present in each tube.

Knowing the total cpm added into each assay and the specific activity of each 3H-steroid, the number of femto moles (fmol) of 3H-steroid bound per milligram (mg.) of cytosol protein could be calculated, assuming all receptors saturated with 3H-steroids and one binding site per receptor. Positive controls were done with each assay. The tissues used for positive controls were rat liver for glucocorticoid receptors, uterus for estrogen and progesterone receptors, and kidneys for androgen receptors. The intra assay coefficient of variation was less than 10% for all four types of receptors in the three types of tissues tested for high values and 15% for low values.

A total of 106 prostatic tissues were analysed. Sixty two samples were assayed for all four steroid receptors, whilst 44 samples were analysed for androgen and estrogen receptors only because of insufficient tissue.

Tissues were considered to be positive for steroid receptors when receptor concentration was 5 fmol/mg. protein or higher for androgen, estrogen and progesterone receptors, and 20 fmol mol/mg protein for glucocorticoid receptor. These values are widely used criteria for steroid receptors in breast tumours and other tissues.7 -1 5

Results
A total of 106 samples of prostatic tissues obtained from TURP were analysed. Out of this 106, nine were prostatic carcinoma and 97 were benign prostatic hyperplasia (BPH). Forty-three samples were from Malays, 49 from Chinese and 14 from Indians. Their mean age was 68 years, ranging from 44 to 83 years.

In the 106 samples assayed for androgen receptors, 93 (88%) were positive. The mean and standard error of mean for the androgen receptor concentration was 21 ± 19 fmol/mg protein. The range was 7-133 fmol/mg. Of the 97 BPH tissues, 87 was positive (89.7%). Of the nine prostatic carcinoma tissues, six (66.7%) were positive for androgen receptors, with receptor concentration of 25 ± 11 fmol/mg protein, ranging from 12 to 41 fmol/mg.

Estrogen receptors were present in 21 of the 106 samples analysed (20%), with a concentration of 22 ± 17 fmol/mg protein, ranging from 5 to 72 fmol/mg. In only 15% of the cases were there both androgen and estrogen receptors positive. (Table 1)

Progesterone receptors were present in 43 of the 62 samples analysed (69%), with mean concentration of 21 ± 14 fmol/mg protein, ranging from 6 to 56 fmol/mg protein. (Table 2) Glucocorticoid receptors however were present in only nine samples of the 62 samples analysed (14.5%) with a mean concentration of 46 ± 28 fmol/mg, ranging from 22–93 fmol/mg. (Table 2)
Table 1

Androgen and Estrogen Receptors in Prostatic Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Androgen Receptors</th>
<th>Estrogen Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Prostatic Hyperplasia</td>
<td>97</td>
<td>87 (90)</td>
</tr>
<tr>
<td>Prostatic Carcinoma</td>
<td>9</td>
<td>6 (67)</td>
</tr>
<tr>
<td>All samples</td>
<td>106</td>
<td>93 (88)</td>
</tr>
</tbody>
</table>

Table 2

Progesterone and Glucocorticoid Receptors in Prostatic Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Progesterone Receptors</th>
<th>Glucocorticoid Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Prostatic Hyperplasia</td>
<td>56</td>
<td>40 (71)</td>
</tr>
<tr>
<td>Prostatic Carcinoma</td>
<td>6</td>
<td>3 (50)</td>
</tr>
<tr>
<td>All samples</td>
<td>62</td>
<td>43 (69)</td>
</tr>
</tbody>
</table>

In the nine prostatic carcinoma tissues analysed, six had positive androgen receptors ranging from 12 to 41 fmol/mg, but estrogen receptors were present in only three (33%). The mean estrogen receptor concentration was 26 ± 14 fmol/mg protein, ranging from 12–41 fmol/mg. Progesterone receptor was present in three (50%) of the six samples analysed, with mean concentration of 16 ± 5 fmol/mg protein. None of the prostatic carcinoma analysed had positive glucocorticoid receptor. (Table 1)

As a comparison, in 25 normal rat uterus, estrogen and progesterone receptors were present in all samples (100%). The mean estrogen receptor concentration (± S.E) was 200 ± 35 fmol/mg protein ranging from 12 to 775 fmol/mg. The mean progesterone receptor concentration was 398 ± 62 fmol/mg protein, ranging from 46–1332 fmol/mg. In normal rat kidneys, androgen receptors were present in 100% of samples with a mean concentration of 27 ± 8 fmol/mg protein, ranging from 5 to 33 fmol/mg.

Discussion

Using single saturation analysis method for determining steroid hormone receptors, we were able to determine the concentration of steroid receptors in prostatic tissue chips obtained from transurethral resection of prostates. In 62 of the 106 samples, there was sufficient tissue sample to measure all four steroid receptors, namely androgen, estrogen, progesterone and glucocorticoid receptors. In 44 samples, there was just sufficient tissue to measure androgen and estrogen receptors. Thus androgen and estrogen receptors were measured in all 106 samples. If the method
of Scatchard analysis were used there would be insufficient tissue to measure even the androgen receptors. This is because, to get a proper Scatchard plot, at least six data points of Bound/Free fraction versus bound 3H-steroids are required. The Scatchard analysis however allows the simultaneous determination of the affinity of the steroid receptors and the receptor concentration. In practice, it is not essential to measure affinities of the steroid receptors since these affinities are not altered by various metabolic conditions. Routinely it is only necessary to determine steroid receptor concentration for the assessment of the potential tissue responsiveness to the particular steroid or its antagonist.

The problem of contamination of the tissue cytosol with plasma proteins sex hormone binding globulin (SHBG) and cortisol binding globulin (CBG) which bind dihydrotestosterone, progesterone and cortisol respectively was overcome by using synthetic steroids as outlined in the methods section. Androgens have a direct effect on the growth of prostate tissue, and on the development of prostatic hyperplasia and prostatic carcinoma. These effects are mediated by specific androgen receptors. In prostatic hyperplasia, androgen receptors was present in 89.7% of tissues tested. One would expect all 100% of prostatic hyperplasia tissue to be positive because the prostate is an androgen target organ and prostatic hyperplasia is androgen dependent. The negative tissues could have been due to full occupancy of the androgen receptors in vivo by the excess dihydrotestosterone known to be present in the issue, and/or due to down regulation of the receptors by excessive androgen action. The synthetic steroid methyltrienolone (R1881) used in the assay however has a higher affinity to the receptor than dihydrotestosterone and should be able to displace endogenous steroid. Other possibilities are that the receptors had been denatured by varying degrees of homogenization, or by length of storage before analysis. These variabilities were kept to minimum during the assays. The high variation in receptor content could reflect varying degrees of denaturation, or of endogenous steroid occupancy of receptors, or of dissociation from nuclear compartment of cells or of non-uniform distribution of receptors in tissues. In prostatic carcinoma, 66.7% of the carcinoma tissues had positive androgen receptors. Receptor negative tissues could, in addition to above postulates, have re differentiated to lack androgen receptors. Androgen receptor positive tumours are responsive to endocrine manipulation such as castration or anti androgens. It is therefore important to determine androgen receptor status in prostatic cancers before contemplating hormonal therapy.

Estrogen receptors were only present in small proportion of prostatic tissues. The use of estrogens as hormonal agents to suppress prostatic tissue growth is therefore less likely to succeed in these cases, unless the estrogen receptor status is known. The side effects of estrogen therapy are also less desirable than the use of anti androgens.

Progesterone was present in 70% of benign prostatic tissue as well as in 50% of prostatic carcinoma. Progesterone therapy thus have a higher chance of succeeding in causing tissue regression as compared with estrogen treatment. Progesterone may act by direct effect on the receptors as well as by inhibiting pituitary luteinizing hormone (LH) secretion with secondary inhibition of testosterone production. The antiandrogen cyperterone acetate binds not only to androgen receptors, but also to progesterone receptors, and has additional effect of inhibiting pituitary LH secretion. There is also no estrogenic effects with cyperterone acetate. Anti androgens such as cyperterone acetate would therefore be a good endocrine therapy for receptor positive prostatic tissue and carcinoma because of its multiple effects. The use of endocrine therapy however should only be contemplated after the steroid receptor status of the tissue has been determined.
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References


