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SPECIFIC PROGESTERONE RECEPTOR IN HUMAN RENAL CANCER

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SUMMARY

It was possible, using a synthetic progestin R5020, to identify a specific progesterone cytosol receptor in six human renal adenocarcinoma. The receptor has a low capacity but a high affinity, with a dissociation constant ranging between 0.13 and 3.72 × 10^{-9} M. Comparison between the results of quantitative experiments obtained with D-Norgestrel and R5020 is reported.

INTRODUCTION

The presence of specific oestradiol and progesterone receptors have been demonstrated in normal human kidney [1] as well as in human renal carcinoma [2–5] by means of the agar gel electrophoresis technique according to Wagner[6].

In progesterone receptor studies we used either labelled progesterone (preincubating the cytosol with cortisol 1 × 10^{-6} M) [2] or tritiated D-Norgestrel (preincubating the cytosol with cortisol 1 × 10^{-7} M) [1, 4]. It is, however, well known that progesterone itself binds to glucocorticoid and androgen receptors, to corticosteroid binding globulin (CBG) and to non specific components as well as to its own receptor. In agar gel electrophoresis CBG migrates in the same anodic area as the progesterone receptor. D-Norgestrel which does not bind to CBG, binds to glucocorticoid and androgen receptors as well as to its own receptor. Therefore a synthetic progestin, R5020, whose binding specificity is restricted to progesterone receptor was used in order to confirm our previous results.

EXPERIMENTAL

Biological material

Six human kidney adenocarcinoma obtained by Dr. Di Silverio (Urology Department of University of Rome) were immediately frozen on dry ice or stored at −22°C until processed.

Radioactive material

[6,7-^{3}H]-R5020 (17,21-dimethyl-19Nor-pregna-4,9-diene-3,20-dione) 51.4 Ci/mmol, kindly supplied by Dr. J. P. Raynaud of Roussel-Uclaf, and [15,16-^{3}H]-D-Norgestrel (13β-ethyl-17α-ethynyl-17β-hydroxy-19Nor-androst-4-en-3-one) 56 Ci/mmol by Schering AG. The labelled compounds were purified before use by means of t.l.c.

Methods

Kidney specimens were cut into small pieces, rinsed four times with 0.9% NaCl in order to remove most of the serum proteins, minced with scissors, dried on filter paper, weighed and homogenized at the maximum speed for three 10 s periods with 30 s rests between periods in two vols of buffer (0.01 M Tris–HCl pH 7.5, 0.001 M NaN_3) using an ice-cooled glass/glass Potter Elvehjem homogeniser. The homogenate was centrifuged at 10,000 *g* for 30 min at 2°C and the supernatant centrifuged again at 200,000 *g* for 90 min at 2°C using a Beckman 65 Ti rotor. The resulting supernatant (cytosol) was then divided into aliquots of 1 ml each and the protein concentration determined by the Folin phenol method of Lowry *et al.*[7].

Four cytosols, tested for progesterone receptor with D-Norgestrel, were preincubated with cortisol (1 × 10^{-7} M) to saturate glucocorticoid binding sites.

Agar gel electrophoresis of cytosol labelled with R5020 or D-Norgestrel with and without the addition of 100-fold excess of equivalent cold steroid was performed according to the technique originally described by Wagner[6].

Saturation analyses were performed by incubating duplicate samples of cytosol (100 μl) with increasing concentrations (0.05–4.00 × 10^{-9} M) of labelled D-Norgestrel or R5020 in the presence or absence of an excess of equivalent radioinert steroid: 1000-fold excess of D-Norgestrel was added to cytosol labelled with D-Norgestrel and preincubated with cortisol; 500-fold excess of R5020 was added to cytosol labelled with R5020. After incubation for 18 h at 4°C the unbound steroids were removed by adding a suspension of dextran-coated charcoal (0.5% Norit A, 0.05% dextran in buffer). The correction for non specific binding suggested by Chamness and McGuire[8] was applied to the Scatchard plot analysis [9].

RESULTS

Progesterone receptor

The migration of CBG in the anodic area of agar gel electrophoresis has been demonstrated in a previous study on plasma from women with breast fibroadenoma [10].

Progesterone receptor and CBG migrate in the same area of the agar gel electrophoresis and therefore CBG contamination could interfere with the identification of the progesterone receptor. By using D-Norgestrel this inconvenience is partially overcome inasmuch as it does not bind to CBG but to glucocorticoid and androgen receptors (Raynaud, personal communication) as well as to its own receptor [3, 4].

In all the six human renal cancers studied with the use of labelled R5020 a specific progesterone cytosol receptor was demonstrated by means of agar gel electrophoresis and by the addition of 100-fold excess of unlabelled R5020 (Fig. 1), which reduces the bound radioactivity recovered in the anodic area. The specificity of R5020 for progesterone receptor was further demonstrated by the competitive experiments in which the amount of bound radioactivity was unaffected by the addition of 1000-fold excess of oestradiol-17β, cortisol and dexamethasone.

Progesterone concentration

The Scatchard plot of the progesterone cytosol receptor from human renal cancers obtained by incubating increasing concentrations of labelled D-Norgestrel with and without adding an excess of radioinert D-Norgestrel is reported in Fig. 2: a low capacity and high affinity component ($K_D = 1.8 \times 10^{-10}$ M) was found.

Since receptors other than the progestational receptor can be evaluated with D-Norgestrel, R5020 was used in preference to other progestins for quantitative

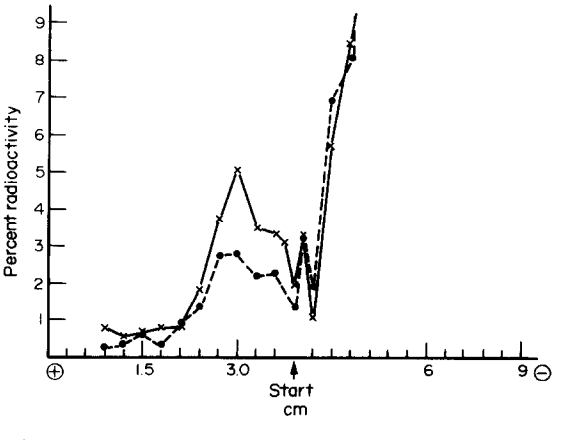

Fig. 1. Agar gel electrophoresis of cytosol from human renal adenocarcinoma: presence of progesterone receptor complex. Incubation for 18 h at 4°C with 2 nM tritiated R5020 in the presence (dotted line) or absence (solid line) of 200 nM unlabelled R5020. 1% agar gel in 0.05 μ sodium diethyl-barbiturate/acetate pH 8.2, run 180 V for 3 h, 50 μl samples applied for analysis, 30 sections of 3 mm.

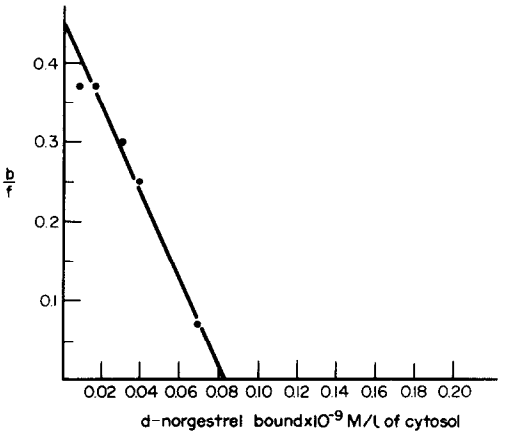

Fig. 2. Scatchard plot of progesterone cytosol receptor from human kidney tumour. Cytosol preincubated with 1×10^{-7} M unlabelled cortisol. Specific binding determined by the addition of a 1000-fold excess of unlabelled D-Norgestrel to increasing concentrations (0.05–4.00 nM) of tritiated D-Norgestrel. Incubation for 18 h at 4°C.

studies on account of the well known advantages. The Scatchard plot of progesterone cytosol receptor from human renal cancer obtained by incubating increasing concentrations of labelled R5020 with and without the addition of an excess of cold R5020 is reported in Fig. 3: a low capacity and high affinity component ($K_D = 1.3 \times 10^{-10}$ M) was found. The number of binding sites and the dissociation constant (K_D) measured in six human renal tumours are given in Table 1.

The concentrations of binding sites of progesterone cytosol receptor measured with D-Norgestrel and R5020 are reported in Table 2.

Protein concentration was in the range of 8–10 mg/ml.

DISCUSSION

Using R5020 a synthetic progestin Philibert and Raynaud[10] confirmed the presence of a progester-

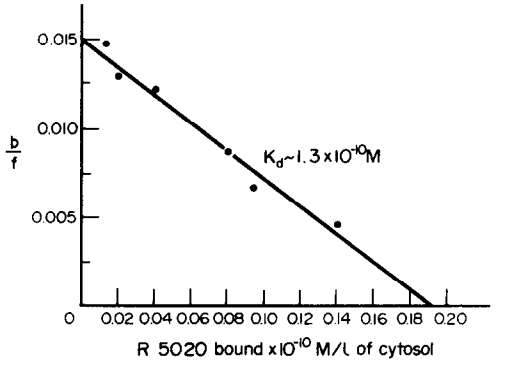

Fig. 3. Scatchard plot of progesterone cytosol receptor from human kidney tumour. Specific binding determined by the addition of a 500-fold excess of cold R5020 to increasing concentrations (0.05–4.00 nM) of labelled R5020. Incubation for 18 h at 4°C.

Table 1. Binding capacity and binding affinity of progesterone receptor from cytosol of human renal tumours measured with R5020

Tumour	n* (fmol/mg protein)	K_D ($M \times 10^{-9}$ M)
T_7	5.50	0.95
T_8	6.50	3.72
T_{10}	1.49	0.39
T_{11}	4.27	—
T_{12}	1.95	0.13
T_{14}	0.73	0.46

* n = number of binding sites.

Table 2. Binding capacity of progesterone cytosol receptor from human renal cancer: comparison of results obtained with D-Norgestrel and R5020

	D-Norgestrel	R5020
Tumour	n* (fmol/mg protein)	
T_7	0.28	5.50
T_8	3.57	6.50
T_{12}	1.50	1.95
T_{14}	0.86	0.73

* n = number of binding sites.

one receptor in immature rat uteri. Horowitz and McGuire[11] used the same compound to demonstrate the presence of a progesterone receptor in human breast cancer.

A systemic study of progesterone receptor in human breast cancer using R5020 was performed also by Raynaud *et al.*[12] who demonstrated the usefulness of the synthetic progestin not only for qualitative studies but also for measuring the number of binding sites in these cancers.

Horwitz and McGuire[11] demonstrated that the progestin receptor of the human mammary tumour cytosol estimated with R5020 had two components sedimenting in the 8*S* and 4*S* regions of the gradient and that the concentrations of these two components differed in the tumours.

In agar gel electrophoresis 8*S* and 4*S* molecules migrate in the same anodic area. R5020, which binds to a specific progesterone receptor distinguishable from CBG and glucocorticoid receptors, was used in the present studies on the progesterone cytosol receptor from human renal cancers. In previous experiments progesterone binding in these tumours was measured using tritiated progesterone (S.A. 100 Ci/mmol) with an excess of unlabelled cortisol to eliminate CBG binding of progesterone. A high capacity low affinity binding was measured with this compound in many cancers; tritiated progesterone binding approached saturation with a small number of binding sites and with high affinity only in some of the renal tumours, the dissociation constant (K_D) being estimated to be in the order of 1×10^{-9} M.

It was possible using the synthetic progestin R5020 to identify a specific progesterone cytosol receptor with a high affinity ($K_D = 0.13$–3.72×10^{-9} M) but with a low capacity in all six tumours studied. The amount of receptor found is quite small and this must be evaluated bearing in mind the studies of Jensen and De Sombre[13]: i.e. "target" tissues have a greater concentration of steroid receptors than "non target" tissues, the difference being quantitative rather than qualitative.

From the quantitative experiments performed with D-Norgestrel and R5020 in four out of the six tumours (Table 2) it can be seen that the concentration of binding sites measured with D-Norgestrel is comparable with that obtained with R5020.

The results obtained in these investigations confirmed our previous studies on the presence of a progesterone cytosol receptor from human kidney adenocarcinoma obtained using progesterone and D-Norgestrel. Receptor studies may provide a useful tool in the hormonal treatment of kidney cancer.

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