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HUMAN KIDNEY STEROID RECEPTORS*

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SUMMARY

Saturation analyses, binding kinetics and agar gel electrophoresis performed on normal human kidney tissue revealed the presence of a specific progesterone receptor in all the specimens studied and in one, a specific estradiol receptor was observed,. Progesterone binding had a rapid association and a slow dissociation reaction.

The binding capacity for estradiol and progesterone was not significantly different, whereas the binding affinity for progesterone was lower than that for estradiol.

These results on progesterone receptors in the cytosol of normal human kidney tissue offer an interesting field of investigation considering the estrogen-induced renal adenocarcinoma in animals and the possibility of progestational therapy for tumour regression in humans.

INTRODUCTION

During the last few years many authors have studied steroid receptor molecules in order to obtain further information on the mechanism of action of steroid hormones. These studies were performed mainly on target tissues and hormone-dependent tumours.

De Vries *et al.*[1] recently demonstrated the presence of an estradiol receptor in rat kidney, Bullock and Bardin[2] an androgen receptor in mouse kidney, Pasqualini *et al.*[3, 4] an aldosterone and estradiol receptor in guinea-pig kidney, and Fanestil *et al.*[5] steroid receptors in human kidney adenocarcinoma.

The present report deals with the properties of progesterone and estradiol-17β cytosol receptors of normal human kidney.

EXPERIMENTAL

Biological material

Kidney specimens were obtained from Cases 1, 2 and 3 subjected to surgery for kidney stones: normal kidney tissue was removed after polar resection to avoid urine stasis. Specimens obtained during surgery by Di Silverio (Urology Department of the University of Rome), were immediately frozen on dry ice or stored at −22°C until processed.

Radioactive material and reagents

The following radioactive compounds, purchased from The New England Nuclear Corporation (Frankfurt-Main, Germany), were used: [2,4,6,7-^{3}H]-estradiol 100 Ci/mmol, [1,2,6,7-^{3}H]-progesterone 100 Ci/mmol, [1,β,2β,-^{3}H]-testosterone 43.5 Ci/mmol, [1,2-^{3}H]-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) 42.4 Ci/mmol, [1,2-^{3}H]-aldosterone 45.0 Ci/mmol. The radiochemical purity of labelled compounds was determined either by paper or t.l.c. Unlabelled steroids were purchased from Vister (Como, Italy).

Reagents used were: Tris-hydroxymethyl-aminomethane (THAM) and sodium azide (NaN_3) from Sigma (St. Luis, U.S.A.); dithiothreitol (DTT) from Pfaltzer and Bauer, Inc. (New York, U.S.A.); charcoal and idranal (EDTA) from Riedel–De Haen AG (Seelze-Hannover, Germany); dextran from Schuchardt (München, Germany); nafthaline, PPO, dimethyl-POPOP, sodium diethyl-barbiturate, dioxane, toluene, methanol and HCl from Merck (Darmstadt, Germany); sodium acetate from Carlo Erba (Milano, Italy); Agar purum from Beheringwerke AG (Marburg–Lahn, Germany).

Methods

Kidney specimens were cut into small pieces, rinsed in 0.9% NaCl, minced with scissors and homogenized for 10 s in two vol. of either TN buffer (0.01 M Tris–HCl pH 7.5, 0.001 M NaN_3) or TED buffer (0.01 M Tris–HCl pH 7.5, 0.0015 M EDTA, 0.5 mM DTT). DTT was added freshly each time it was used. 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. The resulting supernatant (cytosol) was then divided into small portions of 1 ml each, and the protein concentration determined by the Folin phenol method of Lowry *et al.*[6].

Cytosols, tested for progesterone receptor, were preincubated in all instances with cortisol ($1–10 \times 10^{-6}$ M) to saturate the binding sites of corticosteroid binding globulin (CBG).

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Binding activity. A constant amount of radioactive steroid was added to increasing portions of cytosol in TN buffer to establish the presence of binding activity. After incubation overnight the charcoal adsorption method of Korenman and Dukes[7] was used.

Displacement curves were prepared with 200 μl of cytosol TN, a constant amount of radioactive steroid and increasing quantities of the equivalent cold steroid up to a 100-fold excess.

Electrophoresis. For the identification of the specific steroid receptors agar gel electrophoresis was used using the method of Wagner[8]. Tissue extract in TN or TED were treated overnight at 4°C with a suspension of charcoal-Dextran (1 mg/mg protein) to remove the endogenous free steroids [9]. Incubations were then carried out with radioactive steroids (1×10^{-9} M) in the absence and in the presence of unlabelled steroids. The agar gel was made up in sodium diethylbarbiturate/acetate buffer pH 8.2 μ0.05. Electrophoresis in a refrigerate chamber used sodium diethyl-barbiturate/acetate buffer pH 8.2 μ 0.1. Connections to the edges of the agar plate were made with MN 604 filter paper and filter paste board kindly supplied by Dr. R. K. Wagner. After 2–3 h at 260–280 V the temperature within the gel was 4°C. Samples (5 to 7) of 40 μl were run and the agar cut into 1.5 or 3.0 mm sections. Radioactivity was assayed with a Tricarb β-scintillation counter (Packard, mod. 3380) by adding 10 ml of Bray's[10] solution to the agar gel fragments.

Binding kinetics. For the progesterone receptor binding, cytosol (50 μl) in TN buffer was added to a duplicate series of tubes containing identical amounts of radioactive progesterone in 50 μl of TN. The reaction was stopped after 0, 5, 10, 15, 30, and 60 min by adding a suspension of Dextran-coated charcoal (0.5% Norit A, 0.05% Dextran in TN buffer) to the tubes. After centrifugation at 1600 *g* for 10 min the bound radioactivity was calculated by counting a fraction of the supernatant.

For the dissociation rate of progesterone receptor binding, the cytosol was labelled with radioactive progesterone (1×10^{-9} M) overnight and, after addition of a suspension of Dextran-coated charcoal in TN, was centrifuged at 1600 *g* for 10 min. The dissociation experiment was then started by adding unlabelled progesterone (1×10^{-6} M) in TN to the supernatant. At varied intervals, portions (0.5 ml) were removed and assayed for bound radioactivity after adding 0.5 ml of a suspension of Dextran-coated charcoal in TN.

Saturation analyses were carried out by incubating cytosol (100 μl) with increasing quantities of [^{3}H]-progesterone and [^{3}H]-estradiol-17β ($1–8 \times 10^{-9}$ M) according to the method described by De Vries *et al.*[1] and McGuire and Delagarza[11]. After 18 h incubation at 4°C the unbound steroids were removed by adding a suspension of Dextran-coated charcoal in TN. Blank values, obtained by incubating buffer instead of cytosol, were subtracted from all the values. Results were analyzed by the method of Scatchard[12]. Saturation analysis in one specimen was performed using two different techniques i.e. charcoal adsorption and agar gel electrophoresis.

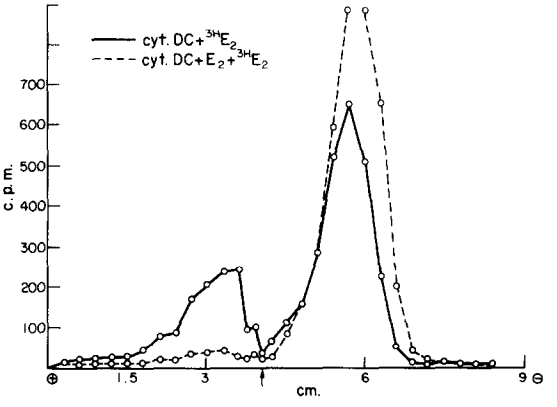

Fig. 1. Agar gel electrophoresis of cytosol from normal human kidney (Case 2): presence of specific estradiol-17β receptor complex. Incubation with 1×10^{-9} M [^{3}H]-estradiol-17β for 18 h at 4°C (solid line); incubation with 1×10^{-9} M [^{3}H]-estradiol-17β plus 1×10^{-7} M cold estradiol-17β for 18 h at 4°C (dotted line). 1% agar gel in 0.05 μ sodium diethyl-barbiturate/acetate buffer pH 8.2; run 280 V for 3 h; 50 μl samples applied for analysis, 60 sections of 1.5 mm.

RESULTS

Binding activity. Cytosols from normal human kidney specimens showed binding activity for testosterone, dihydrotestosterone, aldosterone, estradiol and progesterone. Agar gel electrophoresis revealed that this binding activity was related to a specific receptor only for estradiol and progesterone.

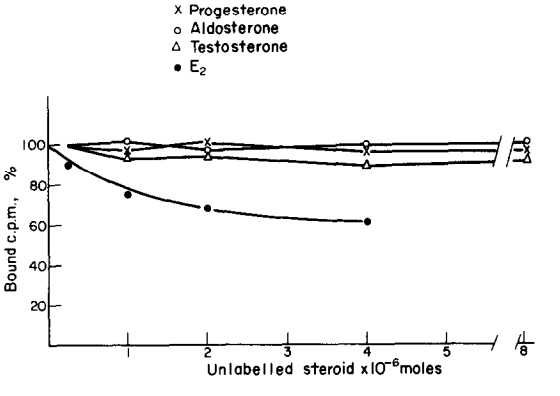

Fig. 2. Displacement of bound [^{3}H]-estradiol-17β in normal human kidney (Case 2) by unlabelled estradiol-17β. Unlabelled aldosterone (empty circles), testosterone (triangles) and progesterone (asterisks) show no affinity for estradiol receptor complex. Incubation for 18 h at 4°C with 1×10^{-8} M of [^{3}H]-estradiol-17β and $0.5–4 \times 10^{-6}$ M of unlabelled estradiol-17β, or equivalent amount of aldosterone, testosterone, progesterone in presence of 200 μl of human kidney cytosol (Case 1).

Table 1. Total radioactivity bound to steroid cytosol receptors of normal human kidney

Steroids incubated (a)	Case 1	Case 2	Case 3
	(d.p.m. recovered in the anodic area)		
[^{3}H]-Progesterone	2730	4388	1709
[^{3}H]-Progesterone + Cortisol	402	1039	235
[^{3}H]-Progesterone + Cortisol + Progesterone	139	753	119
[^{3}H]-Estradiol	—	2225	—
[^{3}H]-Estradiol + Estradiol	—	1039	—

(a) Incubation with [^{3}H]-progesterone 1×10^{-9} M alone, or with cortisol 1×10^{-6} M, or with cortisol 1×10^{-6} M plus cold progesterone 1×10^{-6} M. Incubation with [^{3}H]-estradiol 1×10^{-9} M alone, or with cold estradiol 1×10^{-7} M. Identification of receptors by agar gel electrophoresis.

Estradiol receptor. In one kidney only (Case 2) was it possible to demonstrate the presence of an estradiol cytosol receptor. Fig. 1 shows the behaviour of the [^{3}H]-estradiol macromolecular complex which migrated into the anodic area. Addition of 100-fold excess of cold estradiol-17β confirmed the specificity of the binding. The amount of [^{3}H]-estradiol bound in the anodic area in the absence and in the presence of cold estradiol-17β is shown in Table 1. From Fig. 2 it can be seen that estradiol competed in the formation of the [^{3}H]-estradiol-complexes in the cytosol, but that other unlabelled stroids (progesterone, aldosterone, testosterone) had no effect.

Concentration of estradiol receptor. The Scatchard plot of the specific binding of various concentrations of [^{3}H]-estradiol in the cytosol fraction of normal human kidney (Case 2) is shown in Fig. 3. One binding component was found with a $K_d = 7.40 \times 10^{-9}$ M and a number of sites of 32.04 fmol./mg protein (Table 2).

Progesterone receptor. A specific progesterone cytosol receptor was demonstrated by means of agar gel electrophoresis and by the addition of 1000-fold excess of unlabelled progesterone in all three specimens of normal human kidney examined. The effect of presaturation of CBG and the specificity of the progesterone receptor complex are shown in Table 1.

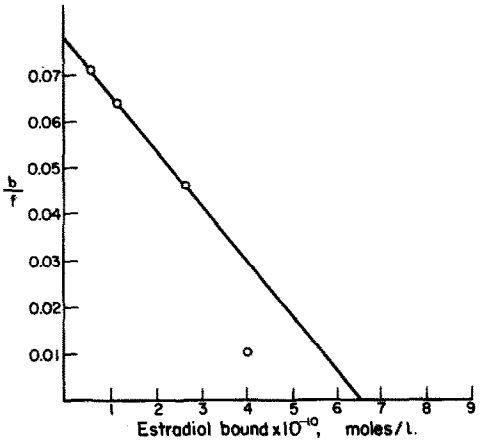

Fig. 3. Scatchard plot of estradiol-17β cytosol receptor from normal human kidney (Case 2): specific binding determined by the addition of 100-fold excess of non-radioactive estradiol-17β.

Estradiol-17β showed no affinity for the kidney progesterone cytosol receptor. The radioactivity recovered in the anodic area after electrophoresis of cytosols incubated with [^{3}H]-progesterone alone, or with [^{3}H]-progesterone plus 100-fold excess of cold estradiol-17β, remained unchanged (11.17 vs 12.24%).

Kinetics studies of progesterone binding are shown in Figs. 4 and 5: the association reaction is rapid while dissociation is slow.

Concentration of progesterone receptor. The number of binding sites and the dissociation constant in two of the tissues examined are given in Table 2 (there was insufficient tissue to perform the analysis in Case 1). The number of sites for progesterone was lower in Case 2 (11.22 fmol./mg protein) than in Case 3

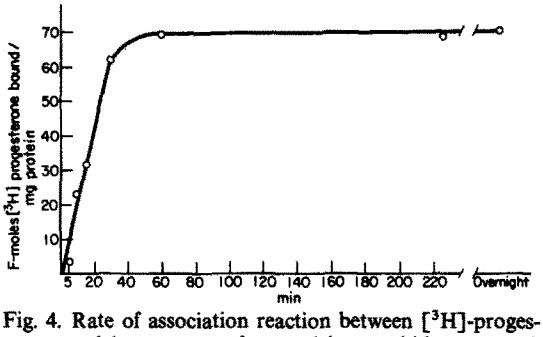

Fig. 4. Rate of association reaction between [^{3}H]-progesterone and its receptor of normal human kidney cytosol.

Table 2. Binding sites and dissociation constants of progesterone and estradiol-17β cytosol receptors in normal human kidney derived from Scatchard plot analysis

	Progesterone		Estradiol-17β	
	n* (fmol/mg protein)	K_d ($M \times 10^{-9}$)	n* (fmol/mg protein)	K_d ($M \times 10^{-9}$)
Case 2	11.22	3.60	32.04	7.40
Case 3	39.83 (a)	64.30	—	—
	45.26 (b)	15.40	—	—

* Number of binding sites.
(a) Saturation analysis performed with charcoal adsorption method.
(b) Saturation analysis performed with agar gel electrophoresis.

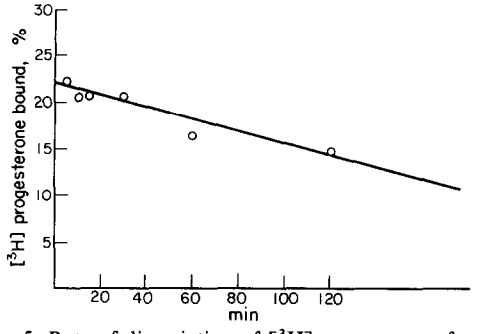

Fig. 5. Rate of dissociation of [^{3}H]-progesterone from its receptor in normal human kidney.

(39.83 fmol./mg protein). The dissociation constant for progesterone in Case 2 ($K_d = 3.60 \times 10^{-9}$ M) is lower than that in Case 3 ($K_d = 6.43 \times 10^{-8}$ M) i.e. the affinity of the progesterone receptor in Case 2 is higher than that in Case 3.

Saturation analysis of Case 3 was repeated using agar gel electrophoresis, and the Scatchard plot of the specific binding of various concentrations of [^{3}H]-progesterone in the cytosol fraction is given in Fig. 6. One binding component was found with a $K_d = 1.54 \times 10^{-8}$ M and a number of sites of 54.26 fmol./mg protein (Table 2).

DISCUSSION

The results of this investigation clearly indicate the presence of specific estradiol and progesterone receptors in the normal human kidney as already demonstrated in several animals.

The presence of steroid macromolecular complexes for estradiol-17β and progesterone was demonstrated by agar gel electrophoresis. With this technique, in addition to the detachment of the steroid from albumin during the run and simultaneous migration towards the anode of the two receptor entities which on sucrose gradient centrifugation sediment in separate regions, discrimination is also made between the estradiol-17β receptor and the specific sex hormone binding globulin (SHBG) migrating towards the cathode.

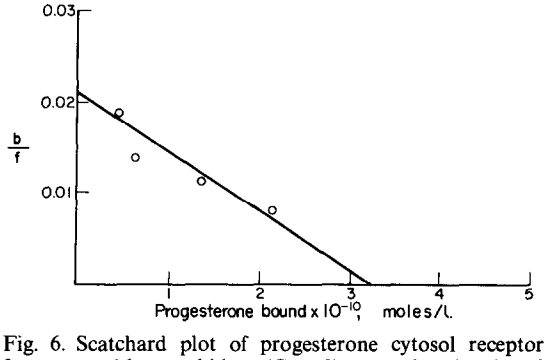

Fig. 6. Scatchard plot of progesterone cytosol receptor from normal human kidney (Case 3): cytosol preincubated with cortisol 1×10^{-6} M; identification of progesterone receptor by agar gel electrophoresis.

Since the progesterone receptor and CBG migrate together cytosol must be preincubated with cortisol to saturate the binding sites of CBG contaminating the cytosol preparations. The presence of this contaminating protein was demonstrated by the displaced radioactivity from the anodic area after presaturation of CBG with cortisol (by 85, 76, 86%, respectively, in Cases 1, 2, and 3). The specificity of estradiol and progesterone receptors was demonstrated by the addition of a 100-fold excess of cold estradiol and of a 1000-fold excess of cold progesterone, which reduces the amount of the tritiated macromolecular complexes by 53% for estradiol in Case 2 and by 65, 28, 49% for progesterone in Cases 1, 2, and 3, respectively. The specificity of these receptor molecules was further demonstrated by competitive experiments in which the amount of estradiol binding was unaffected by the addition of progesterone, aldosterone and testosterone, and progesterone binding was unaffected by the addition of estradiol.

Studies of the kinetics of progesterone binding demonstrated a rapid association and a slow dissociation of this hormone from its receptor.

No substantial differences were observed in the values of number of binding sites and K_d obtained using the charcoal adsorption method and agar gel electrophoresis.

In Case 2, presenting both estradiol and progesterone receptors, the application of the Scatchard method demonstrated that in the cytosol the number of binding sites of estradiol receptor was greater than that of the progesterone receptor (32.04 fmol/mg protein vs 11.22 fmol/mg protein), thus indicating that in this normal human kidney the binding capacity for estradiol is higher than that for progesterone. The dissociation constant was of the same order for both receptors.

The high values of K_d and the specificity observed imply the existence in the kidney of receptors similar to those found in other "target tissues" [13]. The difference between "target" and "non target" tissue, as far as steroid receptor protein is concerned, seems to be quantitative rather than qualitative [14].

Quantitative studies demonstrated that the progesterone receptor in Case 3 had a higher capacity and lower affinity than that in Case 2.

The role of progesterone and estradiol receptors in the human kidney has not yet been fully elucidated. Estrogens reduce urinary sodium excretion in the human [15]. The affinity of renal estradiol binding molecules, able to remove estradiol from serum estradiol binding protein, however has a physiological significance in the human. The affinity of progesterone receptor binding appears to indicate the physiological role of this steroid receptor in the kidney. It is possible that progesterone acts at kidney level in combination with estradiol-17β on electrolyte balance [16]. It is also known that progesterone acts as an antagonist of aldosterone at renal tubule level, thus producing natriuresis [17]. Therefore progesterone recep-

tor in the kidney can be considered as important as other more potent mineralcorticoid receptors.

Studies on the progesterone binding capacity of the normal human kidney cytosol fraction—possibly increased by estrogen exposure, as demonstrated for the rat uterus progesterone receptor [18]—offers an interesting field of investigation considering that experimental renal adenocarcinoma is induced with prolonged administration of estrogens and in view of the possible use of progesterone and progestagen therapy proposed by Bloom[19] and Bracci[20].

The present investigations are being extended to study these two steroid receptors in hormone dependent renal adenocarcinoma in man.

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