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SPECIFIC BINDING OF [^{3}H]-METHYLTRIENOLONE TO BOTH PROGESTIN AND ANDROGEN BINDING COMPONENTS IN HUMAN BENIGN PROSTATIC HYPERTROPHY (BPH)

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SUMMARY

As previously reported, [^{3}H]-R1881, a stable tracer having high affinity for both androgenic and progestin binding components, binds to BPH cytosol with a predominant progestin-like specificity. However, when incubation was performed in the presence of a large excess of triamcinolone acetonide, a typical androgen binding specificity was demonstrated. This is explained by masking of the progestin binding component by triamcinolone acetonide, a steroid which has high affinity for the progestin receptor and no affinity for the androgen binding component. The present data clearly show that [^{3}H]-R1881, in the presence of triamcinolone acetonide, can be efficiently used for determination of androgen binding in BPH, a tissue which contains both androgen and progestin binding components. A similar approach could be adopted for other tissues containing these two receptors.

INTRODUCTION

Demonstration of an androgen receptor in human benign prostatic hypertrophy (BPH) has met many difficulties. Although specific androgen binding in this tissue has been observed using [^{3}H]-DHT as tracer [1–7], detailed investigation of the characteristics of the androgen binding component was prevented by metabolism of [^{3}H]-DHT and contamination of cytosol by sex binding protein (SBP). Recently, Bonne and Raynaud[8, 9] have suggested the use of [^{3}H]-R1881 as a potential new androgen receptor marker and binding of [^{3}H]-R1881 has been demonstrated in BPH cytosol [9–13]. However, low concentrations of progestins compete with [^{3}H]-R1881 binding [10–12] and when the binding of [^{3}H]-R5020, (a typical marker for the progesterone receptor [14]) was studied in BPH cytosol, its specificity was found to be similar to that of [^{3}H]-R1881 [10]. Moreover, it was also reported that [^{3}H]-R1881 binds with high affinity to the progesterone receptor in uterus [10, 12, 14, 15]. These results suggest that a progestin binding component in BPH could be largely responsible for the reported [^{3}H]-R1881 binding in this tissue.

Since [^{3}H]-R1881 offers many advantages over [^{3}H]-DHT as an androgen receptor tracer (resistance to degradation, higher affinity and absence of binding to SBP), we have examined the possibility of masking the BPH progestin binding component in order to be able to use [^{3}H]-R1881 for detailed characterization of androgen binding. The present data show that this can be achieved by adding an excess of unlabeled triamcinolone acetonide to the incubation mixture. This steroid does in fact saturate the progestin binding component without affecting the androgen receptor.

MATERIALS AND METHODS

Human BPH tissue was obtained as previously described [10] and used fresh or kept frozen at −70°C (less than 2 weeks). Ventral prostates were obtained from Sprague–Dawley rats (Canadian Breeding Farms Laboratories, St. Constant, Quebec) weighing between 175–200 g and castrated 24 h before sacrifice. Uteri were obtained from female rats (175–200 g) castrated and treated for 3 days with estradiol-17β (2 μg/day, s.c.). Uteri were removed 24 h after the last steroid injection.

Tissues were rinsed in ice-cold buffer (25 mM Tris–HCl, 1.5 mM EDTA, 10 mM α-monothioglycerol, 10% glycerol (pH 7.4), minced and homogenized in 2 (BPH), 4 (rat ventral prostate) or 12 (uterus) volumes (v/w) of buffer with a Polytron PT-10 at 0–4°C. Incubations were performed using 200 μl of cytosol and [^{3}H]-R1881 for at least 17 h. At the end of the incubation, the DCC (Dextran-Coated Charcoal) or protamine sulfate method was used as previously described [16] to separate bound and unbound steroids.

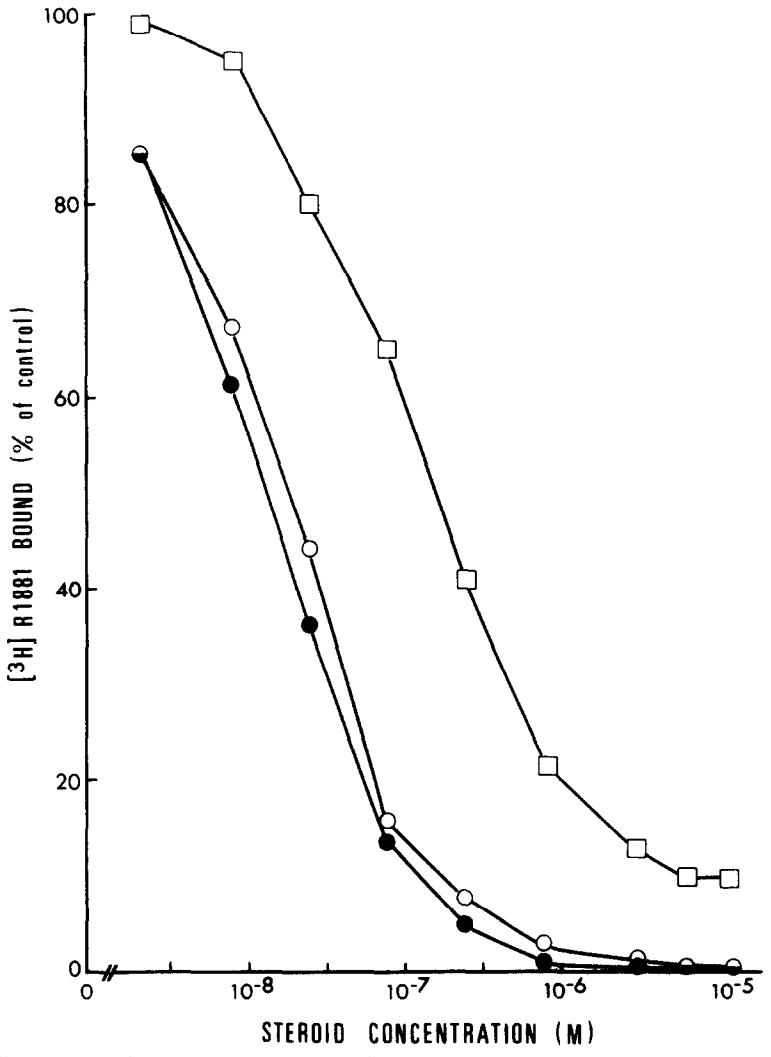

Fig. 1. Effect of increasing concentrations of unlabeled R1881 (●——●), R5020 (○ ··· ○) and triamcinolone acetonide (□——□) on the binding of 10 nM [^{3}H]-R1881 to rat uterine cytosol incubated at 0–4 C for 16 h. The bound fraction was separated by the dextran-coated charcoal (DCC) method. In the absence of unlabeled steroid, 14,000 c.p.m. of [^{3}H]-R1881 were bound per tube.

RESULTS

Masking of the progestin-binding component with triamcinolone acetonide

After screening a large series of compounds, we have found, as illustrated in Fig. 1, that triamcinolone acetonide is a potent competitor of [^{3}H]-R1881 binding in the rat uterine cytosol. Since unlabeled R5020 and R1881 had approximately the same displacing ability on [^{3}H]-R1881 binding, these data already suggest that [^{3}H]-R1881 binds to the progesterone receptor, as previously reported [10, 13–15, 17, 18], and that a large excess of unlabeled triamcinolone acetonide could mask binding of this tracer to the progestin component.

Absence of significant activity of triamcinolone acetonide for the androgen binding component

It can be seen in Fig. 2 that high concentrations of triamcinolone acetonide had no significant effect on [^{3}H]-R1881 binding in a typical androgenic tissue, rat ventral prostate. As previously reported [10], progestins, at high concentrations, compete with binding to the androgen receptor. Consequently, progestins cannot be used to mask the progestin binding component without affecting androgen binding. The absence of effect of triamcinolone acetonide on the androgen receptor in the rat ventral prostate is in agreement with a previous observation in MCF-7 cells [19]. These data clearly indicate that triamcinolone acetonide possesses all the properties required to achieve specific masking of the progesterone receptor, thus permitting the use of [^{3}H]-R1881 for studies of the characteristics of the androgen receptor.

Effect of masking of the progestin binding component on the specificity of [^{3}H]-R1881 binding in human BPH

We then investigated the specificity of [^{3}H]-R1881 binding in cytosol prepared from BPH and incubated

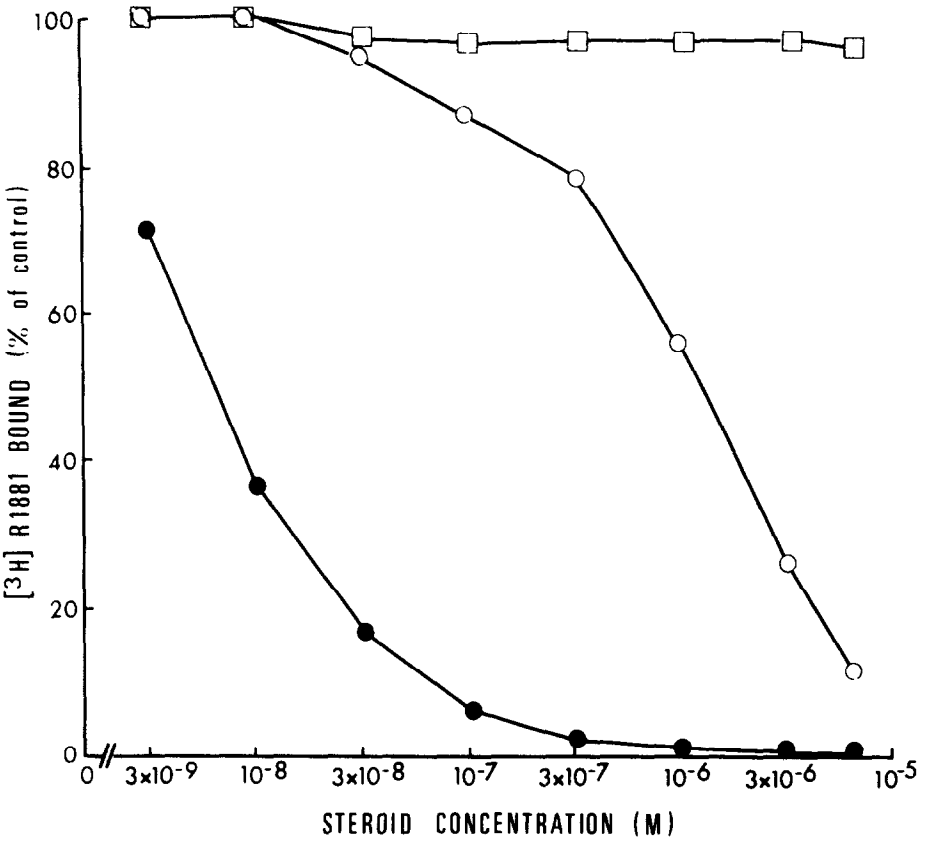

Fig. 2. Effect of increasing concentrations of unlabeled R1881 (●——●), R5020 (○——○) and triamcinolone acetonide (□——□) on the binding of 5 nM [^{3}H]-R1881 in cytosol prepared from rat ventral prostate. After incubation at 0–4°C for 17 h, the bound fraction was separated by the DCC method. In the absence of unlabeled steroid, 9000 c.p.m. of [^{3}H]-R1881 were bound per tube.

Table 1. Specificity of [^{3}H]-R1881 binding in BPH cytosol

Unlabeled steroid	Displacing ability (%)	
	Control	(+triamcinolone acetonide) 700-fold excess
R1881	100	100
Provera	90	10
D-Norgestrel	90	9
Progesterone	25	2
R5020	23	<1
Cyproterone acetate	10	2
Triamcinolone acetonide	2	<1
DHT	1	42
Testosterone	1	9
Estradiol-17β	<1	1
R2956	<1	<1
Dexamethasone	<1	<1
5α-androstane 3α-17β-diol	<1	<1
Cortisol	<1	<1

[^{3}H]-R1881 (7 nM) was incubated with BPH cytosol in the presence or absence of six increasing concentrations of the indicated unlabeled steroids for 24 h at 0–4°C. DCC or protamine sulfate were used for separation of bound and unbound hormones. Displacing ability was calculated as the molar concentration of radioinert R1881 required for 50% displacement of [^{3}H]-R1881 divided by the molar concentration of test compound required for a similar displacement multiplied by 100. 5000 c.p.m. were bound in the absence of unlabeled steroids and R1881 led to a 50% displacement at 7 nM.

in the presence or absence of an excess of unlabeled triamcinolone acetonide. Six increasing concentrations of each unlabeled steroid were used in order to obtain precise ED_{50} values. As demonstrated in Table 1, in the absence of triamcinolone acetonide, progestins were potent competitors, thus suggesting the presence of a progestin binding component in human BPH [9–12]. However, addition of a large excess of triamcinolone acetonide to BPH cytosol decreased by 50–75% [^{3}H]-R1881 binding levels but, more importantly, it led to a marked increase of the displacing ability of androgens and almost prevented competition by pure progestins such as progesterone and R5020 [20]. These data clearly indicate that the addition of an excess of triamcinolone acetonide has masked the progestin binding component in BPH tissue and that the resulting [^{3}H]-R1881 binding is androgen specific.

DISCUSSION

Although [^{3}H]-R1881 binding has been previously described in BPH, somewhat variable results have been obtained [9–13]. Properties characteristic of a progestin binding component have been described [9–12] although one report dealt with a typical androgen binding specificity [13]. Using [^{3}H]-R1881 in the presence or absence of unlabeled triamcinolone acetonide, the present observations clearly demonstrate both specific androgen and progestin binding components in BPH cytosol. Moreover, it is now possible to use [^{3}H]-R1881 (in the presence of excess unlabeled triamcinolone acetonide) to investigate in detail the characteristics of the androgen binding component in a tissue such as human BPH which contains both androgen and progestin binding components. The pure progestin [^{3}H]-R5020 can then be used to study the progestin binding component. The availability of two synthetic and stable tracers, [^{3}H]-R5020 and [^{3}H]-R1881, should be of great help for studies on the mechanisms of progestin and androgen action in BPH and other tissues containing these two receptors.

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