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SHORT COMMUNICATION

USE OF A NEW RADIOACTIVE LIGAND, 7α, 17α-DIMETHYL[17α-METHYL^{3}H]19-NORTESTOSTERONE FOR THE ESTIMATION OF ANDROGEN RECEPTORS IN RAT LIVER CYTOSOL

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Summary—The use of the testosterone derivative, 7α,17α-dimethyl[17α-methyl ^{3}H] 19-nortestosterone for the estimation of cytosolic androgen receptors in male rat liver is described. Use of this compound demonstrates binding which has a similar dissociation constant, maximum binding and steroid specificity to that seen with other synthetic testosterone derivatives. In contrast to previous data significant binding to the progesterone receptor also occurs and future studies with this ligand should employ triamcinolone acetonide to block such binding.

INTRODUCTION

Mammalian liver was, until recently, thought to be devoid of androgen receptors [1], despite the presence of androgen dependent processes, e.g. production of α2U globulin in the rat liver [2] and variation in the activity of ethylmorphine-*N*-demethylase in the mouse liver [3]. In recent years, however, there have been a number of reports documenting androgen receptors in rat liver cytosol, nucleosol [4] and rat liver microsomes [5].

We report the use of a new synthetic testosterone analogue, Mibolerone (7α, 17α-dimethyl[17α-methyl^{3}H]19-nortestosterone), in the estimation of rat liver cytosolic androgen receptors. This compound is not photosensitive, relatively heat resistant and is claimed not to bind to progesterone receptors or sex hormone binding globulin [6, 7].

EXPERIMENTAL

Tritium labelled Mibolerone, sp. act. 87Ci/mmol, was obtained from Amersham International PLC, Bucks, England. Non-radioactive steroids were obtained from Sigma Chemical Co., U.K. Hydroxylapatite, DNA grade was obtained from Bio-Rad Laboratories (Richmond, California). All other chemicals were of analytical grade and were obtained from BDH Laboratories, England.

Buffers

TEDGM. 10 mM Tris; 1mM EDTA; 1 mM dithiothreitol, 15% glycerol (v/v). 5 mM Na_2MoO_4.

Preparation of cytosol

Liver from male adult Wistar rats was removed, following sacrifice and perfused with ice-cold 0.9% NaCl solution. Four gram portions were homogenised in TEDGM buffer and centrifuged at 800 *g* for 20 min. The supernatant was further centrifuged at 100,000 *g* for 1 h to yield the cytosol. Two-hundred and fifty μl of cytosol was incubated with 10 μl of ethanol containing [^{3}H] mibolerone in the required concentration. A paired sample was incubated with 10 μl ethanol containing [^{3}H]mibolerone plus a 200-fold excess of dihydrotestosterone or unlabelled mibolerone (in saturation analyses) or varying concentrations of other steroid (specificity analyses). Incubation was for 18 h at 0–2°C, an incubation time shown to achieve maximum binding.

Receptor assay

The method of Pavlik and Coulson [8] was used, in which hydroxylapatite is used to bind the receptor–ligand complex. The only modification was to filter the hydroxylapatite and count directly rather than extract the bound steroid in ethanol.

RESULTS

Saturation analysis

Incubation with [^{3}H]mibolerone (0.1–10 nM) showed saturable binding of the cytosol, with a plateau of specific binding at approx 1 nM. Scatchard analysis (Fig. 1) showed a K_d of 0.86 nM (range = 0.27–1.36, SD 0.4) with a maximum binding capacity of 8.36 fmol/mg protein (range 3.8–12.9, SD 2.77, $n = 12$). In rats 24 h post castration the kinetics of binding, the dissociation constants (mean K_d 0.6 nM, $n = 4$) and the maximum binding (9.2 fmol/mg protein) did not differ significantly from intact rats, suggesting that in our routine assay near complete exchange for endogenous steroids occurs.

Steroid specificity

Cytosol was incubated with a 50, 100, 200 or 1000-fold excess of unlabelled competitor steroid (Fig. 2). This showed dihydrotestosterone, testosterone and mibolerone were effective competitors. In addition progesterone, the synthetic progestin R5020,(17,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione) showed significant inhibition of binding. The main metabolite of progesterone, 17-OH-progesterone did not show effective competition. The addition of a × 1000 excess of triamcinolone acetonide (9-fluoro 11-β, 21-dihydroxy 16-α, 17-[1 methyl ethylidine *bis* oxy] pregna-1,4-diene 3,20 dione) reduced the inhibition of binding by progesterone from 60 to 10% but did not completely remove

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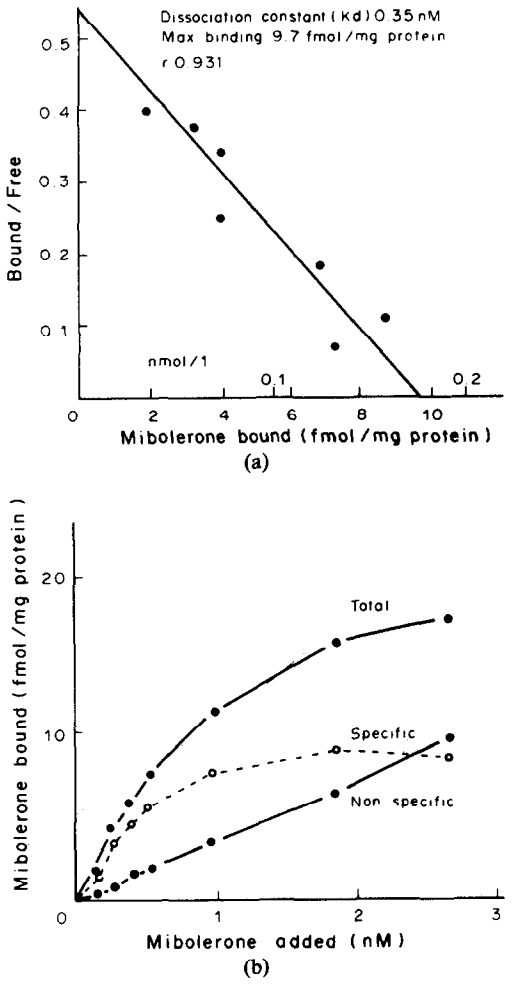

Fig. 1. Scatchard plot analysis (a) of [^{3}H]mibolerone binding in rat liver cytosol and (b) the data from which it was derived.

the suppression of binding. Further saturation analyses in the presence of × 1000 excess of triamcinolone acetonide did not show significant changes in dissociation constants (K_d = 1.02 nM, n = 6) or maximum binding (9.2 fmol/mg protein), though both total and non specific binding were reduced by approx 10%.

COMMENT

[^{3}H]Mibolerone has been shown to be an effective ligand for demonstrating saturable binding in male rat liver cytosol. Our reported mean K_d of 0.86 nM and maximum binding of 8.3 fmol/mg protein are in agreement with data published using R1881 (17βhydroxy 17α-methyl-4,9,11-estratriene-3-one) for rat liver [9, 11], and the dissociation constant is close to that seen for the classical prostatic androgen receptor employing mibolerone as the active ligand [6].

Mibolerone has significant advantages over other ligands for androgen receptor research in that it does not bind to SHBG (we have not investigated this for rats have no SHBG [6]), and is light and relatively heat stable.

Our finding of competition for binding by progestins, including the specific progestin R5020 [10], is at variance with previous reports and suggests that mibolerone like R1881 may bind to progesterone receptors. The addition of a × 1000 excess of triamcinolone acetonide to block the progesterone but not androgen receptor [12], reduces the

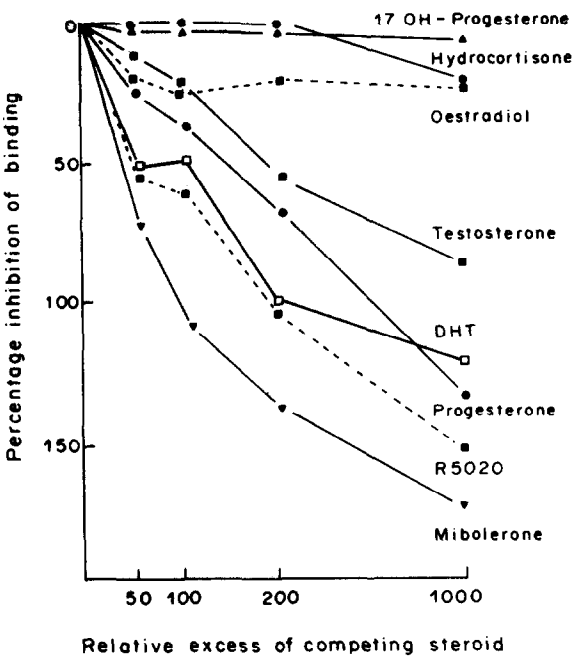

Fig. 2. Steroid inhibition studies of mibolerone binding to rat liver cytosol. Specific binding of [^{3}H]mibolerone in the presence and absence of a 200-fold excess of unlabelled dihydrotestosterone (DHT) was set equal to 100%.

competition by progesterone but does not remove it completely. This residual competition may reflect true binding of progesterone to the androgen receptor, as has been reported in other tissues [13, 14]. We would recommend the use of triamcinolone acetonide to reduce this problem in future studies with this radioligand, particularly if rat liver is to be used as the experimental model.

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