Biochemical Pharmacology, Vol. 36, No. 21, pp. 3571–3577, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

EFFECT OF FLUTAMIDE ON HEPATIC CYTOSOLIC METHYLTRIENOLONE (R1881) BINDING KINETICS AND TESTOSTERONE RESPONSIVE HEPATIC DRUG AND STEROID METABOLISM IN THE ADULT MALE RAT*

GEOFFREY I. SUNAHARA,† RAPHAEL C. K. PAK‡ and GAIL D. BELLWARD§
Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

(*Received* 1 *July* 1986; *accepted* 13 *February* 1987)

Abstract—Flutamide was used to investigate the mechanism involved in androgen responsive hepatic microsomal drug and steroid metabolism. We compared the antiandrogenic action of flutamide on the prostate to its effect on testosterone responsive hepatic microsomal benzo[*a*]pyrene hydroxylase (BPH) and testosterone reductase (TR) activities. Male Wistar rats, castrated as adults, were treated with 5 μmoles $\cdot$ kg^{-1} $\cdot$ day^{-1} of testosterone enanthate subcutaneously for 10 days. Co-administration of increasing doses of flutamide caused a dose-dependent reduction in prostate to body weight ratios and, in the same animals, caused significant alterations in adult male hepatic microsomal BPH and TR activities. These doses of flutamide did not affect the serum testosterone levels. To test the possibility that the action of flutamide on androgen responsive hepatic microsomal drug and steroid metabolism may be similar to that occurring in the prostate, a tissue which contains an androgen receptor, we also studied the effect of flutamide on the binding kinetics of the high affinity hepatic cytosolic [^{3}H]R1881 binding protein *in vivo*. Scatchard analysis of [^{3}H]R1881 binding data revealed a reduction in the binding capacity of the hepatic cytosolic androgen binding protein in castrated animals treated with a combination of flutamide and testosterone enanthate at doses capable of maximally altering hepatic microsomal drug and steroid metabolism. No alteration in binding affinity occurred in this treatment group. However, a decreased binding affinity was found when flutamide alone was given. The binding kinetics of the hepatic cytosolic androgen binding protein were not altered in the castrated adult male with or without testosterone treatment. When flutamide was injected daily into the intact adult female rat, no effect was observed on either hepatic microsomal BPH or TR activities. Taken together, these data indicate that flutamide reduces hepatic cytosolic R1881 binding in the adult male rat, and this may explain some of the effects of this antiandrogen on testosterone-sensitive hepatic microsomal drug and steroid metabolism.

Hepatic microsomal cytochrome P-450-dependent benzo[*a*]pyrene hydroxylase (BPH‖) and testosterone reductase (TR) represent two important microsomal enzyme systems responsible for the metabolism of polycyclic aromatic hydrocarbons and endogenous delta-5 containing steroids. As well as being androgen sensitive in the rat, these enzymes are age and sex dependent [1, 2], and are under hypothalamic-hypophyseal-gonadal control [3]. The exact cellular mechanism of hepatic androgen responsive drug and steroid metabolism remains obscure. The notion that hepatic testosterone responsitivity is mediated by an androgen receptor has been proposed by many workers [4–7]. This hypothesis is in accordance with the steroid hormone receptor occupancy concept which was developed using classical sex end-organ target tissues and has been reviewed [8]. The measurement of the hepatic cytosolic androgen binding protein in past studies has involved the use of high protein concentrations and radiolabeled natural androgens such as dihydrotestosterone (DHT), testosterone, and androstenedione. Unfortunately, these *in vitro* conditions favor metabolism of the natural androgen radioligands [9] and could, therefore, alter the interpretation of the radioreceptor binding data. The recent use of metabolically stable synthetic androgens such as methyltrienolone (R1881) and mibolerone has enabled workers to detect a specific high affinity androgen binding protein in hepatic cytosolic and nuclear fractions in various rodent species [10–15], and in hepatic cytosolic fractions of humans [16]. Although the exact functional role of the hepatic cytosolic androgen binding protein is not clear, the advantages of these synthetic radioligands clearly invite their use as a means of further characterizing this hepatic cytosolic androgen binding protein.

Flutamide (α,α,α-trifluoro-2-methyl-4′-nitro-*m*-propionotoluidide; SCH 15321) is a potent nonsteroidal androgen antagonist in androgen sensitive sex end-organ tissues such as the prostate. A review

* Preliminary reports of the data presented here have appeared in the *Canadian Federation of Biological Societies Proceedings*, Vol. 17, p. 153 (1984) and in the Sixth International Symposium on Microsomes and Drug Oxidations, pp. 1/42 (1984).

† Present address: National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

‡ Present address: Department of Pharmacology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, N.T. (Hong Kong).

§ To whom correspondence should be addressed.

‖ Abbreviations: BPH, benzo[*a*]pyrene hydroxylase; TR, testosterone reductase; K_d, equilibrium dissociation constant; and B_{max}, maximum binding capacity.

of the properties and pharmacology of flutamide can be found elsewhere [17]. Raynaud [18] has reported that rapidly-dissociating antiandrogens such as hydroxyflutamide (SCH 16423), a metabolite of flutamide, competitively displace androgens from the prostrate cytosolic androgen receptor. Recently, we provided similar evidence that hydroxyflutamide significantly displaces [^{3}H]R1881 binding from the rat high affinity hepatic cytosolic androgen binding protein *in vitro* [10]. These data suggest that flutamide or its active metabolite, hydroxyflutamide, may also inhibit the nuclear uptake of the androgen–receptor complex in androgen sensitive target tissues (such as the prostrate and perhaps the liver) and ultimately lead to decreased androgen responsiveness. To further characterize the role of the hepatic androgen binding protein, we studied the antiandrogenic effects of flutamide on testosterone responsive hepatic microsomal BPH and TR activities and the high affinity hepatic cytosolic [^{3}H]R1881 binding protein in the adult rat. We report here that flutamide inhibited androgen responsive hepatic microsomal BPH and TR metabolism and prostate to body weight ratios, and lowered high-affinity hepatic cytosolic R1881 binding *in vivo*. These data provide further evidence that androgen responsive hepatic drug and steroid metabolism may be modulated by a hepatic androgen receptor.

MATERIALS AND METHODS

Chemicals. Flutamide was a gift from the Schering Co. (Bloomfield, NJ). Glycerol and dextran T70 were obtained from British Drug House (Vancouver, BC) and Pharmacia Fine Chemicals (Uppsala, Sweden) respectively. Unlabeled methyltrienolone (R1881) and 17α-[^{3}H]R1881 (87 Ci/mmol, >99% pure) were obtained from New England Nuclear (NEN) Canada Ltd. (Montreal, PQ) under licensed agreement with Roussel-Uclaf (Roumainville, France). All other solvents and reagents were purchased from the Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Vancouver, BC). Benzo[*a*]pyrene was further purified by solubilization in benzene, filtration, and finally recrystallization in cold methanol.

Animals and treatments. Adult (60–85 days old) male and female Wistar rats were purchased from the Canadian Breeding Laboratories (La Praire, PQ). Castration was done under light ether anaesthesia using the scrotal route. Treated animals were then injected daily with subcutaneous doses of testosterone enanthate alone or in combination with flutamide for 10 days prior to the day of the experiment. Dose regimens are described in the text. All drugs were dissolved in 0.1 to 0.2 ml corn oil vehicle; controls received the vehicle alone. Animals were housed under standard laboratory conditions and were maintained on Purina Lab Chow and water *ad lib*. The last injection of testosterone enanthate and/or flutamide was given 24 hr prior to the experiment.

Preparation of hepatic cytosol and microsomes. Each animal was stunned by a blow to the head and decapitated; blood samples were taken by cardiac puncture. The liver and ventral prostate gland were quickly dissected out, and wet weights were determined. Hepatic cytosolic and microsomal fractions were prepared separately from each animal, using the methods described elsewhere [10, 19]. Briefly, cytosol was prepared following homogenization with ice-cold TED buffer (pH 7.4) containing 50 mM Tris-base, 1.5 mM EDTA-sodium, 0.5 mM dithiothreitol, 10% glycerol and 20 mM sodium molybdate, using a precooled Potter–Elvehjem homogenizer and Teflon pestle. All subsequent procedures were carried out at 4°. The crude cytosol was prepared individually following differential centrifugation at 10,000 *g* for 10 min, and then the supernatant fraction was centrifuged at 105,000 *g* for 65 min. The resulting supernatant or cytosolic fraction was diluted with ice-cold TED buffer to the desired protein concentration (1–3 mg/ml) for the radioligand binding assays.

Microsomes were prepared separately following a similar methodology as described above, except that an ice-cold 1.15% aqueous KCl solution was used for the homogenization step. Each homogenate was centrifuged at 10,000 *g* for 20 min, and the supernatant fraction was then centrifuged at 105,000 *g* for 65 min. After the final centrifugation step, each pellet (microsomal precipitate) was carefully washed and resuspended in ice-cold aqueous phosphate buffer (pH 7.2) to the desired protein concentration (5–10 mg/ml) for the enzyme assays. All enzyme and binding assays were done on fresh preparations. Cytosolic and microsomal protein concentrations were determined using the protein-dye method described by Bradford [20] using bovine serum albumin as the standard.

Hepatic cytosolic androgen binding protein exchange assay. The cytosolic androgen binding assays were carried out according to the method outlined by Sunahara *et al.* [10]. In brief, 0.5-ml aliquots of cytosol were separately incubated with various concentrations of [^{3}H]R1881 (final concentration, 0.05 to 5 nM, unless otherwise stated) in the presence (non-specific binding) or absence (total binding) of 100-fold molar ratio excess competitor. Unlabeled triamcinolone acetonide (100-fold excess) was also added to each incubation mixture, in order to block non-specific binding [10]. Incubations were carried out for 3 hr at 4°. These assay conditions were linear with respect to protein and were carried out under equilibrium conditions [10]. Incubation was terminated by addition of 2.5% dextran coated charcoal in TED buffer (2.5% charcoal, 0.25% dextran), according to the method of Beato and Feigelson [21]. This mixture was centrifuged at 1500 *g* for 10 min. A 0.5-ml aliquot of the supernatant fraction was added to separate vials containing 10 ml Biofluor (NEN Canada) scintillation fluid. The radioactivity was counted and analyzed using a Searle Mark III Liquid Scintillation System model 6880 (40–47% efficiency) interfaced to an Apple II-plus microcomputer system (Apple Computer Inc., Cupertino, CA). Specific bound [^{3}H]R1881 was calculated as the difference between total and non-specific bound fractions. Data were analyzed according to the method of Scatchard [22]. The apparent equilibrium dissociation constant (K_d) and the apparent maximal binding capacity (B_{max})

were expressed in nM and fmoles R1881 per mg cytosolic protein respectively.

Microsomal enzyme assays. The BPH fluorimetric assay was carried out according to the method of Nebert and Gelboin [23]. The BPH activities are expressed as nmol 3-hydroxybenzo[*a*]pyrene formed per min per mg microsomal protein. TR activities were measured following the method described by McGuire and Tomkins [24] and expressed as nmol testosterone reduced per min per mg microsomal protein.

Other assays. Serum testosterone concentrations were determined using the protocol outlined in the RSL nosolvex [^{125I}]testosterone RIA kit (Radioassay System Laboratories Inc., Carson, CA). The assay was specific (cross-reactivity to DHT was 10.5% and to other steroids <0.01%) and was sensitive to testosterone from 0.05 to 25 ng/ml. Radioactivity was measured using a Nuclear Chicago Series 1185 Autogamma Counting System (70–80% efficiency). All steroids were routinely checked for purity using TLC as described in the product specifications.

Statistical analysis. Data are expressed as the mean ± SE; the numbers in parentheses denote the number of experiments. Differences between control and test groups were analyzed using the two-tailed Student's *t*-test for unpaired data and the Duncan's Multiple Range test. Differences were considered significant at $P < 0.05$. All samples were analyzed in duplicate or triplicate; individual data presented are representative samples from two or more experiments.

RESULTS

Serum testosterone levels and tissue:body weight ratios. The effects of ten days post-castration and the daily administration of testosterone enanthate (5 μmol/kg, s.c.) alone or in combination with flutamide (5–50 μmol/kg, s.c.) for 10 days, on adult male rat serum testosterone levels are summarized in Table 1. Serum testosterone concentrations in the sham-operated adult male rat varied from 2.4 to 7.6 ng/ml (average 4.6 ± 1.7 ng/ml) and were within physiological range for the rat [25]. Castration reduced testosterone levels to 0.3 ± 0.2 ng/ml compared to the sham-operated controls. Administration of testosterone enanthate (5 μmol/kg) to castrated adult males increased serum testosterone levels to 1.2 ± 0.2 ng/ml. In preliminary experiments this dose of testosterone enanthate maintained normal prostate:body weight (P:BW) ratios and consistently induced hepatic microsomal BPH and TR activities in the castrated adult male rat. The serum testosterone levels in castrated adult male rats that were treated daily with flutamide (5–50 μmol/kg, s.c.) and testosterone enanthate for 10 days were not altered.

Castration of adult male rats led to a reduction in P:BW ratios (Fig. 1A), from 75 ± 10 mg/100 g in sham-operated adult males to nondetectable levels in the adult castrates. This effect was fully reversed following androgen replacement using testosterone enanthate (5 μmol/kg). Flutamide administration (5–50 μmol/kg) caused a dose-dependent reduction in prostate:body weight ratios in the testosterone enanthate treated castrated adult male rats. These effects were maximum at flutamide doses of 25 μmol/kg or greater. In contrast, this flutamide dose did not alter the P:BW ratios in the sham-operated or the castrated males compared to their respective corn oil controls. Liver to body weight ratios were not altered in the sham or the castrated animals treated with either testosterone enanthate alone or in combination with flutamide (Table 1).

Hepatic microsomal enzyme activities. Microsomal

Table 1. Effects of flutamide and testosterone enanthate treatment on serum testosterone levels and liver:body weight ratios

	Serum testosterone (ng/ml)	Liver:body weight (g/100 g)
Sham-operated adult male rats		
Corn oil	4.6 ± 1.7 (6)	4.16 ± 0.14 (4)
Testosterone enanthate (5 μmol/kg)	ND*	4.17 ± 0.13 (4)
Flutamide (25 μmol/kg)	ND	4.20 ± 0.03 (4)
Castrated adult male rats		
Corn oil	0.3 ± 0.2* (8)	4.40 ± 0.30 (4)
Testosterone enanthate		
1.3 μmol/kg	ND	4.50 ± 0.12 (4)
2.5 μmol/kg	ND	4.72 ± 0.07 (4)
5.0 μmol/kg	1.2 ± 0.2‡ (4)	4.42 ± 0.09 (4)
Flutamide (25 μmol/kg)	ND	3.67 ± 0.23 (3)
Testosterone enanthate (5 μmol/kg) plus flutamide:		
5 μmol/kg	1.4 ± 0.2‡ (4)	3.91 ± 0.15 (8)
15 μmol/kg	ND	4.01 ± 0.05 (8)
25 μmol/kg	1.0 ± 0.1‡ (3)	4.12 ± 0.13 (4)
50 μmol/kg	0.9 ± 0.1‡ (4)	4.37 ± 0.09 (4)

Injections were administered daily in a 0.1 to 0.2 ml corn oil vehicle. Values are mean ± SEM; number of experiments carried out is given in parentheses.

* Not determined.

† Significant difference compared with corn oil sham-operated control ($P < 0.05$), using Student's *t*-test.

‡ Significant difference compared to the respective castrated control ($P < 0.05$) using Student's *t*-test.

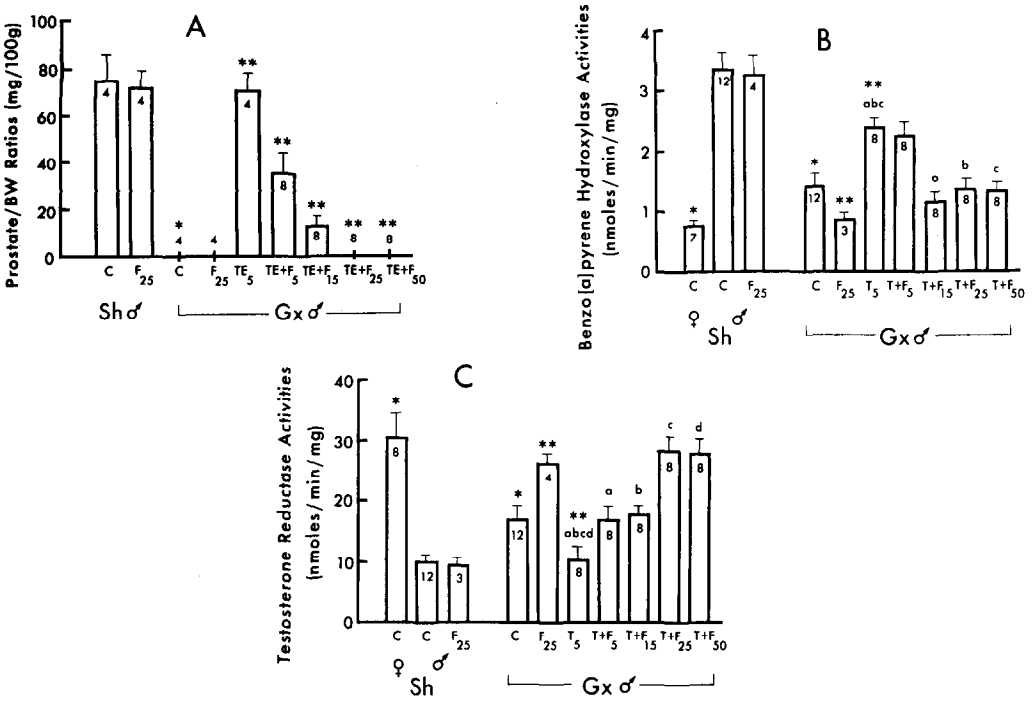

Fig. 1. Effects of testosterone enanthate and flutamide on prostate to body weight ratios (panel A, expressed as mg per 100 g body weight), hepatic microsomal benzo[*a*]pyrene hydroxylase (panel B, in nmol per min per mg) and testosterone reductase (panel C, in nmol per min per mg) activities in the sham-operated (Sh♂) and 10 days castrated (Gx) adult male and intact female (♀) Wistar rat. Animals were treated daily with testosterone enanthate (T; 5 μmol/kg, s.c., 10 days) alone or in combination with various doses of flutamide (F; 5–50 μmol/kg, s.c., 10 days); control animals received the corn oil vehicle (0.1 to 0.2 ml) or a single daily dose of flutamide (25 μmol/kg, s.c., 10 days). Individual doses are indicated by subscripts. The last injection was 24 hr prior to sacrifice. Enzyme activities were determined as described in Materials and Methods. Data are expressed as the average ± SE, with *n* given in the column. Key: (★) significant difference ($P < 0.05$) compared with sham-operated adult males of the same treatment group; and (★★) significant difference ($P < 0.05$) compared to the appropriate castrated adult male control. For comparisons between the testosterone plus flutamide treated and the flutamide untreated control groups, the Duncan multiple range test was used. Significant differences ($P < 0.05$) between groups are indicated by common letters (a,b,c,d).

BPH (Fig. 1B) and TR activities (Fig. 1C) in the sham-operated adult male and female rats were within the range reported in previous studies [19, 26]. Consistent with these earlier reports, castration of the adult male rat also led to a decrease in BPH activities, but not as low as that observed in adult females (Fig. 1B). A daily dose of flutamide alone (25 μmol/kg, s.c., for 10 days) to the castrated adult males reduced BPH activities to those of the adult female. This dose, however, did not alter the BPH activities in the sham-operated animal. Daily androgen replacement using testosterone enanthate (5 μmol/kg) reversed the castration effect on BPH activities towards the levels of the sham-operated adult male control. Flutamide administration from 5 to 50 μmol/kg, s.c., to testosterone enanthate treated castrated adult male rats inhibited androgen responsive BPH activities compared to the testosterone treated castrated control. Maximal inhibition of these BPH activities occurred at flutamide doses equal to or greater than 15 μmol/kg, s.c., for 10 days.

Like BPH, hepatic microsomal TR is a sex-dependent enzyme in the adult rat [3]. However, unlike BPH, TR activities were higher in the adult female than in the adult male (Fig. 1C). In the adult male, 10 days after castration TR activities were increased towards those of the female, an effect which was fully reversed following testosterone enanthate treatment (5 μmol/kg, s.c.). Daily administration of flutamide (5–50 μmol/kg) stimulated TR activities in the testosterone enanthate treated castrated adult males towards the levels of the adult female. Maximal effects of flutamide on these TR activities occurred at doses equal to or greater than 25 μmol/kg. This dose of flutamide stimulated TR activities in the castrated male, but it did not have a significant effect on the TR activities in the sham-operated animals.

As a further control, BPH and TR activities were also measured in the adult female treated with these antiandrogens. Similar to its effects in the intact male, flutamide administration (25 μmol/kg, s.c.) had no effect on hepatic microsomal BPH or TR activities in the intact adult female (data not shown); that is, there was neither induction nor inhibition of enzyme activity.

Hepatic cytosolic androgen binding protein. To determine if the flutamide-induced alterations of the

testosterone responsive hepatic microsomal enzymes were related to changes in the hepatic androgen binding protein *in vivo*, the effects of flutamide on the binding kinetics of the hepatic cytosolic high affinity–low capacity [^{3}H]R1881 binding protein in the adult male rat were investigated. Panels A and B of Fig. 2 illustrate representative saturation and Scatchard data, respectively, for the high affinity hepatic cytosolic R1881 binding protein of individual castrated adult rats that were treated with the corn oil vehicle, testosterone enanthate (5 μmol/kg, s.c., 10 days) or flutamide (25 μmol/kg, s.c., 10 days) alone or in combination. Incubation of hepatic cytosol taken from castrated adult males treated with testosterone enanthate or corn oil vehicle with increasing concentrations of [^{3}H]R1881 alone or in the presence of excess competitor led to increasing displaceable, saturable binding (Fig. 2A). In contrast, displaceable [^{3}H]R1881 binding in hepatic cytosol taken from a castrated adult male treated with flutamide alone reached saturation at much higher concentrations. Scatchard analysis (Fig. 2B) of the high affinity hepatic R1881 binding site for both the corn oil treated castrated adult male controls and testosterone treated castrates revealed similar binding kinetics: apparent K_d value of 0.19 nM and an apparent B_{max} value of 5.95 fmol [^{3}H]R1881/mg cytosolic protein and $K_d = 0.15$ nM and $B_{max} = 4.79$ fmol/mg respectively. Administration of flutamide only to a castrated adult male rat caused an increase in the apparent K_d value (0.42 nM), reflecting a decrease in binding affinity, with little change in the apparent B_{max} value (4.50 fmol/mg) compared to either the corn oil or testosterone treated control. Interestingly, concurrent administration of testosterone enanthate plus flutamide to a castrated adult male rat led to a dramatic decrease in high affinity [^{3}H]R1881 binding to near nondetectable levels (Fig. 2A). Accordingly, Scatchard analysis did not produce reliable binding data for the combined treatment.

The combined results of several of the above experiments are presented in Table 2. Scatchard analyses of [^{3}H]R1881 binding to the high affinity hepatic cytosolic androgen binding site resulted in the following binding data (K_d, B_{max}): intact male, 0.20 ± 0.04 nM, 4.50 ± 0.07 fmol/mg; castrated adult male, 0.24 ± 0.03 nM, 3.90 ± 1.26 fmol/mg; treated with 5 μmol/kg testosterone enanthate, 0.29 ± 0.06 nM, 3.24 ± 0.40 fmol/mg. These values are consistent with our earlier results [10], that is, 10 days post-castration with or without testosterone replacement did not alter significantly the high affinity hepatic cytosolic androgen binding protein in the adult male rat. Flutamide administration (25 μmol/kg, s.c., 10 days) to castrated adult males increased the average K_d value, i.e. decreased the binding affinity, of the hepatic cytosolic R1881 binding protein without altering the average binding capacity ($K_d = 0.81 \pm 0.2$ nM; $B_{max} = 4.27 \pm 0.92$ fmol/mg) compared to the corn oil treated castrated controls. Moreover, the treatment of flutamide in combination with testosterone enanthate to adult male castrates greatly reduced the high affinity [^{3}H]R1881 binding in five of the eight animals tested. In all eight of these animals, there was a reduction in the R1881 binding capacity (0.89 ± 0.52 fmol/mg) compared to the controls treated either with flutamide or testosterone enanthate alone. Of the three flutamide–testosterone treated animals that exhibited detectable R1881 binding, there was a slight but not significant increase in the average K_d value ($K_d = 0.53 \pm 0.11$ nM), compared to controls. The alteration was actually much larger than that presented since those instances where there was nondetectable [^{3}H]R1881 binding could not be incorporated in the analysis of the K_d.

To confirm the use of R1881 as a stable radioligand

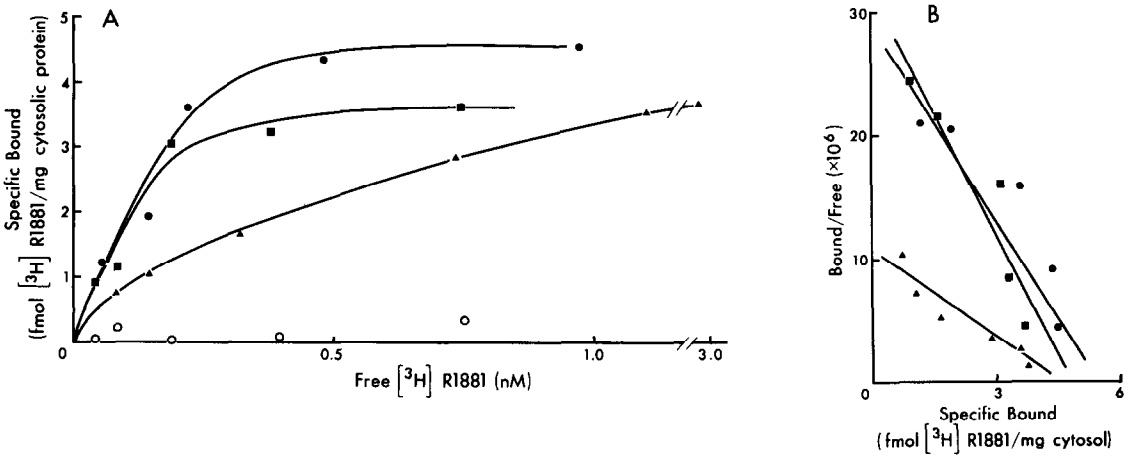

Fig. 2. Representative examples of saturation curves (A) and Scatchard plots (B) of the high affinity hepatic cytosolic [^{3}H]R1881 binding protein taken from adult male rats castrated and treated daily for 10 days with: 5 μmol/kg, s.c., testosterone enanthate (■); 25 μmol/kg, s.c., flutamide alone (▲) or in combination (○); or 0.1 to 0.2 ml of the corn oil vehicle (●). Cytosol was prepared and incubated as described in Materials and Methods. Scatchard analysis of the [^{3}H]R1881 binding data resulted in the following apparent binding kinetics (K_d, B_{max}) for these individual animals: corn oil control (●); 0.18 nM and 5.65 fmol/mg cytosolic protein); testosterone enanthate treated (■); 0.15 nM and 4.79 fmol/mg); flutamide treated (▲; 0.42 nM and 4.50 fmol/mg). Animals treated with testosterone enanthate plus flutamide yielded nondetectable levels of high affinity hepatic cytosolic [^{3}H]R1881 binding protein.

Table 2. Effect of flutamide administration on male rat hepatic cytosolic high affinity [^{3}H]R1881 binding protein

Animal model used	Apparent [^{3}H]R1881 binding kinetics	
	K_d (nM)	B_{max} (fmol/mg)
Intact adult (70 days) male	0.20 ± 0.04 (4)	4.50 ± 0.07 (4)
Castrated adult male (10 days after castration)	0.24 ± 0.03[a] (4)	3.90 ± 1.26 (4)
Testosterone enanthate treated castrated adult male	0.29 ± 0.06 (4)	3.24 ± 0.40[a] (4)
Flutamide treated castrated adult male	0.81 ± 0.20[a] (4)	4.27 ± 0.92[b] (4)
Testosterone enanthate plus flutamide treated castrated adult male	0.53 ± 0.11 (3)	0.89 ± 0.52[a,b] (8)

Samples of crude cytosol were incubated with various concentrations of [^{3}H[R1881 for 3 hr, alone or in the presence of a competitor. Following dextran coated charcoal treatment, aliquots of supernatant fraction were assessed for bound radioactivity. Experimental procedures are described in Materials and Methods. Testosterone enanthate dose used: 5 μmol/kg, s.c., for 10 days. Flutamide dose used: 25 μmol/kg, s.c., for 10 days. Values are means ± SE with N in parentheses. Differences between groups were considered significant at 0.05 using the Duncan's Multiple Range test. Significant differences between groups are indicated by common letters.

and competitor, preliminary studies were undertaken using Mibolerone (70–85 Ci/mmol; Amersham Co., Oakville, Ontario), another metabolically stable synthetic androgen. Using the same incubation conditions as described above, we found that the use of Mibolerone either as a ligand or competitor gave similar binding kinetic values as those for R1881, e.g. $K_d = 0.54$ nM; $B_{max} = 3.5$ fmol/mg protein. Together with the results of Levinson and Decker [11] who reported minimal R1881 degradation following cytosolic incubation for 18 hr at 4°, our results suggest that R1881 degradation following cytosolic incubation is unlikely.

DISCUSSION

The hypothesis that androgen responsive hepatic microsomal drug and steroid metabolism is mediated by a hepatic androgen receptor was investigated using flutamide, a potent nonsteroidal androgen antagonist in classical male sex end-organ tissues. We report that daily flutamide administration (5–50 μmol/kg, s.c., 10 days) reduced testosterone responsive BPH and increased TR activity in testosterone enanthate treated castrated adult males, without significantly altering serum testosterone levels or liver : body weight ratios compared to controls. The doses of flutamide that produced maximal enzyme alterations were 15 μmol/kg for BPH and 25 μmol/kg for TR. These enzyme alterations were produced within the same dosage range as those that reduced ventral prostate to body weight ratios. These data suggest that androgen sensitive hepatic microsomal drug and steroid metabolism may be regulated by an androgen receptor-mediated mechanism similar to that found in the prostate. Stimulation of a hepatic cytosolic androgen receptor leading to increased androgen inducible hepatic microsomal BPH activities is similar to the concept introduced by Poland *et al.* [27], who suggested that occupation of another hepatic cytosolic protein, the Ah receptor, led to the regulation of hepatic microsomal cytochrome P_1-450 dependent drug metabolism. Although these two receptors are unrelated, the general concept of receptor-mediated control of drug metabolizing enzymes is an interesting possibility.

Our results are similar to those reported by Brown *et al.* [28] who showed that flutamide causes an inhibition of androgen-sensitive hepatic microsomal ethylmorphine *N*-demethylase activity in intact prepubertal male rats treated with testosterone. However, our results differ from previous studies [28, 29] in that the flutamide doses used in our study failed to have a significant effect on the enzyme activities or P : BW ratios in the intact adult rat. This may be due to compensatory increases in serum testosterone levels in the adult male as suggested by Clark *et al.* [30]. Clinical findings indicate that similar compensatory increases in serum testosterone levels occur in human subjects being treated with flutamide [31]. Lax and Schriefers [29] utilized prepubertally-castrated adult male rats as their experimental model. The discrepancies between their results and ours with regard to TR activity may be due to the lack of androgen in their animals during pubertal period.

To further delineate the mechanism underlying flutamide's inhibition of androgen responsive hepatic microsomal drug and steroid metabolism, we considered the possibility that flutamide or its active metabolite may be influencing the binding properties of the high affinity hepatic cytosolic androgen binding protein *in vivo*. We report that the dose of flutamide which caused maximal alterations in testosterone responsive hepatic microsomal BPH and TR enzyme activities in androgen treated males also altered the binding kinetics of the hepatic cytosolic high affinity [^{3}H]R1881 binding protein. There was a reduction in the number of binding sites, B_{max}, and an increase in the K_d value, i.e. decreased binding affinity. A reduction or loss of hepatic androgen

binding reflected by decreased binding affinity and/or reduction in binding capacity may explain the alterations in testosterone responsive hepatic microsomal drug and steroid metabolism *in vivo*. This is consistent with the observation that the pseudohermaphroditic adult male rat whose hepatic drug-metabolizing system is insensitive to pharmacological doses of testosterone [32] also lacks a high affinity hepatic cytosolic R1881 binding protein [11].

Flutamide treatment (25 μmol/kg, s.c.) to animals that were not treated with testosterone exogenously reduced BPH and stimulated TR activities in the castrated adult male rat towards those levels found in the adult female. These results may be explained by the antiandrogenic action of flutamide or its active metabolite on residual circulating androgens, such as adrenally synthesized testosterone. The same dose of flutamide also led to an increase in the average K_d values, or decreased binding affinity, of the high affinity R1881 binding protein in the castrated adult males compared to the corn oil treated controls. These data further support the above binding studies involving exogenously administered testosterone in which the alteration in the high affinity hepatic cytosolic [^{3}H]R1881 binding kinetics was more dramatic. Therefore, our enzyme data may be explained by the action of flutamide or its active metabolite to competitively displace endogenous or exogenous androgens from the hepatic androgen receptor. Alternatively, flutamide may also be acting elsewhere upon other processes responsible for the regulation of physiological levels of the hepatic androgen receptor. Either explanation would lead to reduced hepatic androgen receptor occupancy and ultimately decreased hepatic androgen responsivity.

In an earlier report, we demonstrated that the active metabolite of flutamide, hydroxyflutamide, competitively displaces [^{3}H]R1881 from the high affinity hepatic cytosolic androgen binding protein *in vitro* [10]. It is doubtful that the presence of this antiandrogen in the hepatic cytosol preparation from the flutamide injected animals resulted in reduced [^{3}H]R1881 binding in the present studies. Our experiments were carried out 24 hr following the last injection of flutamide. In addition, since the binding affinity of hydroxyflutamide to the high affinity hepatic cytosolic R1881 binding protein is about 2 orders of magnitude less than R1881, DHT or testosterone [11], coupled with a 40- to 50-fold dilution of the hepatic cytosol, this would imply that the presence of hydroxyflutamide, however minimal, would not alter significantly our assay of the hepatic cytosolic R1881 binding site in animals that were tested previously with flutamide.

In summary, we have shown that *in vivo* administration of the antiandrogen, flutamide, to adult castrated male Wistar rats resulted in a decrease in the number of hepatic high affinity [^{3}H]R1881 binding sites or their decreased binding affinity. This occurred at doses which produced maximal alterations in the androgen-responsive hepatic microsomal enzymes, BPH and TR. These findings are consistent with the hypothesis that a hepatic androgen receptor modulates androgen-sensitive microsomal drug and steroid metabolism.

Acknowledgements—The authors wish to express their gratitude to Dr. Paul Rennie (Cancer Control Agency of BC) for his expert advice during the course of this study. This work was supported by the Medical Research Council of Canada (Grant MA-5944).

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