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A COMPARATIVE STUDY OF THE SELECTIVITY AND EFFICIENCY OF TARGET TISSUE UPTAKE OF FIVE TRITIUM-LABELED ANDROGENS IN THE RAT

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Summary—A comparative study of the tissue distribution of five tritium-labeled androgens was done in rats to determine the efficiency and selectivity of their uptake by target tissue. Testosterone (T), 5α-dihydrotestosterone (DHT), 19-nortestosterone (nor-T), mibolerone (Mib) and methyltrienolone (R1881) all showed selective uptake by the ventral prostate in one-day castrated rats (250 g) that was 61–90% displaceable by co-injection of an excess of unlabeled steroid. The greatest uptake was with R1881 (0.69% injected dose per gram prostate tissue (%ID/g) at 1 h), and Mib (0.56% ID/g); the other three showed lower uptake (approx. 0.4% ID/g). The target tissue activity remained high for all compounds up to 4 h after injection, and at 2–4 h the prostate to blood ratio for Mib and R1881 exceeded 10 and 20, respectively. The uptake efficiency and selectivity of these five androgens appear to be related to their affinity for the androgen receptor and their resistance to metabolism. Mib and R1881 have substantial affinity for other steroid receptors, which might account for some of their prostate uptake. However, co-administration of triamcinolone acetonide, which has high affinity for progesterone and corticosteroid receptors but not for the androgen receptor, failed to block their uptake significantly, whereas co-administration of DHT, the most selective ligand for the androgen receptor, blocked their uptake as completely as the unlabeled tracer itself. The prostate uptake of Mib and R1881 in intact animals was significantly lower than in castrated animals, but treatment of the intact animals with diethylstilbestrol restored their uptake nearly to the level seen in castrated animals. These uptake patterns are consistent with earlier studies of *in vivo* androgen uptake and with known changes in androgen receptor content and occupancy as a result of castration or diethylstilbestrol treatment. They further suggest that high affinity androgens labeled with suitable radionuclides—particularly derivatives of mibolerone (Mib) or methyltrienolone (R1881)—may be effective receptor-based imaging agents for androgen target tissues and tumors, even when patients are already receiving hormonal therapy.

INTRODUCTION

Androgen receptors, which are present in high concentrations in the prostate [1–5], are also found in some tumors of the prostate [3, 6, 7], and the assay of the androgen receptor content of prostatic tumors is useful in predicting the response to endocrine therapy [3, 8–10]. Since the original demonstration [11] of the androgen dependence of most prostatic carcinomas, treatments have been designed to suppress or remove endogenous androgens: surgical castration to remove the tissue source of androgen production or chemical castration through the administration of estrogens [6, 11, 12], progestins [13], or antiandrogens [13, 14] to suppress androgen biosynthesis, or LHRH agonists to block testicular testosterone secretion [15, 16]. While in the normal male, androgen receptors are nearly fully occupied by the endogenous androgens, they are largely unoccupied in patients receiving hormonal therapy. Thus, androgens with suitable binding properties and labeled with an appropriate radionuclide might be useful as *in vivo* imaging agents for the prostate and for primary and metastatic prostatic tumors, especially in patients on hormonal therapy. Such a method might enable one to assess the extent to which the cancer has escaped the tissue capsule and metastasized, thus placing the selection of alternative therapeutic approaches on a firmer basis.

There have been a number of reports of the use of androgens labeled with gamma-emitting isotopes as prostate imaging agents [17–19]. In most cases, however, selective uptake by target tissues such as the

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Abbreviations: Testosterone (T), 17β-hydroxy-4-androsten-3-one; 5α-dihydrotestosterone (DHT), 17β-hydroxy-5α-androstan-3-one; 19-nortestosterone (nor-T), 17β-hydroxy-4-estren-3-one; mibolerone (Mib), 7α,17α-dimethyl-19-nortestosterone; methyltrienolone (R1881), 17α-methyl-17β-hydroxyestra-4,9,11-triene-3-one; diethylstilbestrol (DES), (E)-3,4-bis(4-hydroxyphenyl)-3-hexene.

prostate has not been demonstrated, and it has not been possible to obtain useful images with these agents (for a review, see Katzenellenbogen [20]). More recently, we [21] and others [22] have analyzed how known structural changes in the testosterone, 19-nortestosterone and 5α-dihydrotestosterone systems affect binding affinity for the androgen receptor, and, as a prelude to the preparation of androgens labeled with the positron-emitting radionuclide fluorine-18 ($t_{1/2}$ = 110 min), we [23] have reported the synthesis and androgen receptor binding affinity of a number of the fluorine-substituted androgens. It was apparent from these studies, however, that compounds with affinities comparable to or greater than that of testosterone would be required to provide adequate target tissue uptake and target to non-target contrast for successful *in vivo* imaging of androgen target tissues.

In this report, we describe the tissue distribution in the orchidectomized rat of five tritium-labeled androgens: testosterone (T), 5α-dihydrotestosterone (DHT), 19-nortestosterone (nor-T), mibolerone (Mib) and methyltrienolone (R1881). All these compounds have high affinity for the androgen receptor, and they all show selective uptake by the prostate, which, in each case, is blocked by co-administration of an excess of unlabeled compound. Their uptake efficiency and selectivity differ considerably. Mib and R1881, which show the greatest uptake by the prostate, were also studied in the presence of other competitors (triamcinolone acetonide and DHT) and in intact and intact-diethylstilbestrol (DES)-treated rats, to verify that their uptake is due to binding to the androgen receptor and that uptake can be observed in intact animals and in intact animals receiving hormone therapy. These findings are helpful in the design of androgen receptor-based imaging agents that are better behaved than those that have been prepared up to now, and in developing suitable model systems in experimental animals to evaluate the uptake behavior of these agents.

EXPERIMENTAL

Materials and methods

Unlabeled steroids were obtained from the following sources: testosterone and 19-nortestosterone (Searle, Skokie, Ill.), 5α-dihydrotestosterone, triamcinolone acetonide, diethylstilbestrol (DES), and estradiol (Sigma, St Louis, Mo.), 17α-methyl-17β-hydroxyestra-4,9,11-trien-3-one (Methyltrienolone) (R1881) and 7α,17α-dimethyl-19-nortestosterone (Mibolerone); RU28362, promegestone (R5020) (DuPont NEN, Boston, Mass), hydrocortisone (Steraloids, Pawling, N.Y.).

The tritium-labeled steroids were [1α,2α(*n*)-^{3}H]testosterone (51.5 Ci/mmol), 5α, dihydro [1α,2α-(*n*)-^{3}H]testosterone (60 Ci/mmol), 19-[6,7-^{3}H]nortestosterone (15.8 Ci/mmol) [6,7-^{3}H]estradiol (43 Ci/mmol) [1,2,6,7-^{3}H]aldosterone (82 Ci/mmol) (Amersham Corp., Arlington Heights, Ill.), methyltrienolone [17α methyl-^{3}H] (81.8 Ci/mmol), mibolerone [17α-methyl-^{3}H] (85 Ci/mmol), [17α-methyl-^{3}H]promegestone (R5020) (86.7 Ci/mmol), [6-methyl-^{3}H]RU28362 (77.5 Ci/mmol), (DuPont NEN). Other chemicals included hydroxylapatite (BioRad Laboratories, Richmond, Calif.) and Nuclear Chicago Solubilizer (Amersham Corp.).

The radiochemical purity of the tritiated androgens was checked by thin layer chromatography using Macherey–Nagel plastic-backed silica gel plates with fluorescent indicator. The solvent systems, R_f values, and radiochemical purity are listed in Table 1.

Radioactivity was measured in a Nuclear Chicago Isocap 300 liquid-scintillation counter, using xylene-based cocktail containing 0.55% 2,5-diphenyloxazole, 0.01% *p*-bis[2-(5-phenyloxazoyl)]benzene, and 25% Triton X-114. Tritium counting efficiency was 25–55%.

Animal treatment and tissue processing

Sprague-Dawley, 250 g male rats (7–9 weeks old) were obtained from the Holtzman Co., Madison, Wis. Some were maintained as intact control animals. Some were orchidectomized 24 h before the experiment, and others were left intact and treated with DES following a modification of the procedure of Symes [24]: 2 mg/ml of DES was dissolved directly in sunflower seed oil (Wesson-Sunlite) and 0.5 ml was injected s.c. (1 mg/rat), 27, 20, and 3 h before the experiment. The tritiated androgens were prepared in 20% ethanol in physiological saline and were injected i.v. (carotid artery) with the animals under ether anesthesia. The dose of radioactivity that was injected (injected dose) was determined as previously described [25], by weighing the injection syringe before and after filling with a solution of known concentration, and counting the dose that remained in the

Table 1. TLC solvent systems, R_f values and radiochemical purity of [^{3}H]androgens

Compound	Chromatography (R_f)	Radiochemical purity
Testosterone (T)	Ethyl acetate:hexane 3:1 (0.33)	89–92%
Dihydrotestosterone (DHT)	Ethyl acetate:hexane 3:1 (0.42)	90–91%
19-Nortestosterone (nor-T)	Ethyl acetate:hexane 3:1 (0.39)	98%
Mibolerone (Mib)	chloroform:methanol 19:1 (0.55)	93%
Methyltrienolone (R1881)	chloroform:ethanol 98:2 (0.33)	93–94%

syringe after injection. Animals receiving radioactive compound alone were given 3–6 μCi in 0.1 ml vehicle. To block receptor-mediated uptake, animals received simultaneously with the tritium-labeled compound 36 μg of unlabeled compound or 36 μg of DHT. To block uptake due to progesterone or corticosteroid receptors, the animals received 1 mg triamcinolone acetonide (0.5 ml of a 2 mg/ml solution in 1:1 ethanol:saline) i.p. 15 min before receiving the tritiated androgen. The animals were provided with food and water *ad libitum*.

At the indicated times, the animals were killed by CO_2 asphyxiation, blood was collected by cardiac puncture, and tissues were excised and weighed immediately. The tissues were dissolved and decolorized, the radioactivity measured as previously reported [25].

Competitive binding assays for steroid receptors and serum binding proteins

Relative binding affinities were determined in several receptor systems. Cytosol was incubated with buffer or several concentrations of unlabeled competitor together with 10 nM tritium-labeled tracer at 0°C. In all cases, the assays were incubated 18–24 h and free steroid removed by the charcoal–dextran method [26]. Assays for androgen receptor utilized prostate cytosol from 1 day orchidectomized rats which was preincubated with 1 μM triamcinolone acetonide to block the glucocorticoid and progesterone receptors; [^{3}H]R1881 was the tracer. Assays for progesterone receptor utilized cytosol from estrogen-stimulated immature rat uteri preincubated with 1 μM hydrocortisone; [^{3}H]R5020 was the tracer. Mineralocorticoid assays utilized kidney cytosol from 3-day adrenalectomized rats with [^{3}H]aldosterone as the tracer; preincubation with 1 μM RU28362 blocked glucocorticoid sites. Glucocorticoid assays used liver cytosol from 3 day adrenalectomized rats with [^{3}H]RU28362 as a glucocorticoid specific tracer. We have published a complete description of the androgen and progesterone receptor assays [23] and the glucocorticoid and mineralocorticoid receptor assays [27].

Assays with sex steroid binding globulin were performed as we reported previously [28], utilizing third-trimester human pregnancy serum with [^{3}H]estradiol as tracer; assays were incubated for 30 min, and hydroxylapatite was used to remove free steroid.

Androgen receptor assay

Androgen receptor was assayed using a modification of the procedure of Boone [29], incorporating some of the suggestions of Hechter [4]. The prostates were homogenized in a glass:glass homogenizer in AR buffer (0.01 M Tris, 0.0015 M EDTA, 0.02% NaN_3, 0.01 M thioglycerol, 20 mM Na molybdate, and 10% glycerol, pH 7.4 at room temperature). Protease inhibitors were added to the homogenate; 5 mg/ml soybean trypsin inhibitor, 0.1 mg/ml leupeptin, and 1 mM PMSF. Nuclei were pelleted at 800 *g* for 20 min. The supernatant was removed and centrifuged at 140,000 *g* for 1 h to yield a high speed cytosol.

The nuclei were washed twice by resuspension and recentrifugation with AR buffer. After the last wash, they were resuspended in AR buffer plus protease inhibitors, as above. Both cytosol and nuclei were incubated with 30 nM [^{3}H]R1881 $\pm$ 100-fold excess unlabeled R1881 in the presence of 1 μM triamcinolone acetonide. After a 2 h incubation at 0–4°C a portion was assayed to determine the receptor that had been "empty". The remainder was incubated at 15°C and assayed after 19 and 43 h of exchange to determine what had been "bound" by endogenous androgens.

The bound [^{3}H]R1881 in the cytosol was determined by the charcoal dextran assay. The bound [^{3}H]R1881 in the nuclear pellet was determined by the hydroxylapatite assay [30]. The nuclear pellet was also assayed for DNA [31] and data reported as fmol androgen receptor/μg DNA. The receptor degradation, over the exchange period, was estimated from the loss of binding seen after 19 and 43 h of exchange in the prostate fractions of a castrate rat, where all receptor was free and the 2 h 0°C, point measured 100% of the receptor.

RESULTS

The five androgens used in this study are commercially available in tritium-labeled form. Their structures and the sites of tritium labeling are shown in Fig. 1.

Binding affinity to steroid receptors and sex steroid binding protein

The binding affinities of the five androgens to four steroid receptors, the androgen receptor (AR), the progesterone receptor (PgR), the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR), and blood sex-steroid binding proteins (SBP) are shown in Table 2. Our binding data is consistent with that which has appeared in the literature [22, 32–34]. Mib and R1881 are the highest affinity ligands for the androgen receptor (AR); DHT binds about 10-fold better than T, and the affinity of nor-T is intermediate. All five androgens show heterologous binding to the other steroid receptors. R1881 and Mib have highest affinities towards PgR, and this has interfered with their use as radiotracers for *in vitro* assay of androgen receptors [33–35]; R1881 binds weakly to GR, but strongly to MR; T, nor-T and Mib have moderate affinity for MR but little affinity for GR. In a practical sense, in humans *in vivo*, only R1881 and Mib binding to PgR might pose a problem, since substantial levels of PgR (but not MR) are present in the prostate [36].

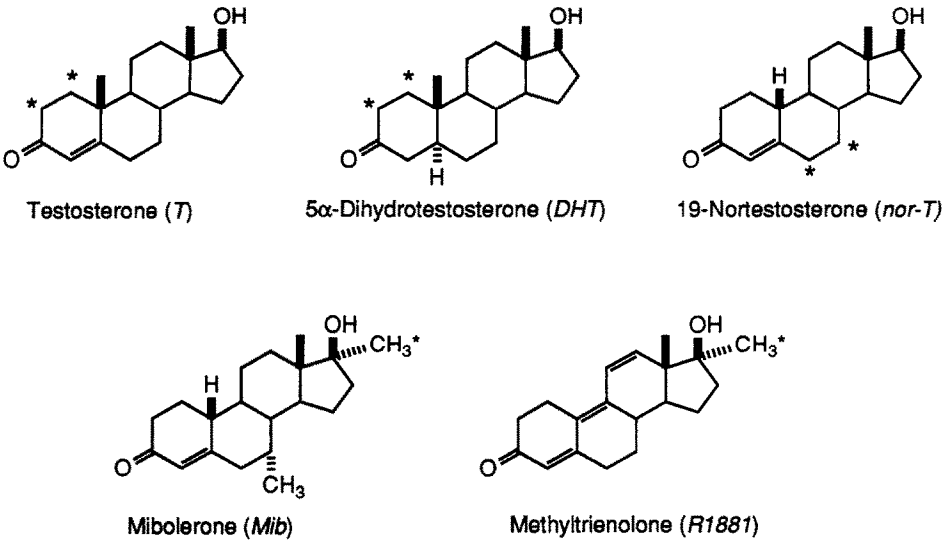

Fig. 1. Structures and sites of tritium-labeling of the five androgens. *Indicates site of ^3H.

The two naturally occurring androgens DHT and T show high affinity for sex steroid binding protein (SBP). The synthetic androgens R1881 and Mib were developed so as to minimize this high serum binding, as well as to reduce the rapid metabolism observed with T, DHT, and nor-T [29, 33, 34, 37]. The consequences of this heterologous and serum binding and experiments to establish their effect on *in vivo* tissue distribution will be discussed later.

Tissue distribution in vivo

The tissue distribution of all five androgens was determined first in 250 g male rats 24 h after castration. The radiotracer was injected i.v. in ethanol-saline, and blood and tissue samples were obtained at 0.5, 1, 2, and 4 h after injection. Radioactivity was determined by liquid scintillation counting after complete solubilization and decolorization of the tissue samples. In order to establish that the uptake was mediated by a limited capacity, high affinity uptake system, one set of animals in each experiment was treated concurrently with a large dose (36 μg) of the corresponding unlabeled tracer. The uptake data for the five androgens are listed in Tables 3–7. The prostate uptake of these five compounds is displayed in terms of % ID/g in Fig. 2, as the ratio with blood in Fig. 3, and as the ratio with non-target tissues (the average of uptake by muscle, esophagus and lung) in Fig. 4. Although androgen receptors have been reported in rat skeletal muscle [38], the concentration is about 60 times less than in prostate, leaving muscle as a reasonable control tissue.

Uptake efficiency and retention of androgens by the prostate. As is evident in Tables 3–7, but shown more graphically in Fig. 2, both Mib and R1881 show significantly greater uptake efficiency in the prostate than do T, DHT, and nor-T: the uptakes of R1881 and Mib average around 0.50–0.69% ID/g over 0.5–4 h, whereas the other three androgens average around 0.2–0.3% over this time period.

With all compounds, uptake by the prostate is rapid; the levels at 0.5 h are nearly as great as those at 1 h. Also, the activity in the prostate is retained, with the levels at 4 h for R1881 and Mib being only slightly below that of the 1 h peak levels; the other three androgens show somewhat greater decline by 4 h. The fact that the uptake in the prostate is mediated by a high affinity, limited capacity uptake system is evident from the fact that the uptake at 1 h is greatly depressed by co-administration of a large excess of the unlabeled androgen (in Tables 3–7, compare column 3 with column 2). This target tissue

Table 2. Relative binding affinity (RBA) of the five androgens to steroid receptors and sex steroid binding protein

	Receptor system RBA% ± SD				
Compound	Androgen receptor (AR) (R1881 = 100)[a]	Progesterone receptor (PgR) (R5020 = 100)[a]	Glucocorticoid receptor (GR) (RU28362 = 100)[a]	Mineralocorticoid receptor (MR) (Aldosterone = 100)[a]	Sex-steroid binding protein (SBP) (Estradiol = 100)[a]
Testosterone	5.99 ± 1.18	0.09 ± 0.02	0.37 ± 0.17	4.19 ± 3.44	417.00 ± 87.68
DHT	60.87 ± 17.23	0.23 ± 0.10	0.22 ± 0.11	0.17 ± 0.14	2125.83 ± 998.55
nor-T	30.55 ± 1.48	3.10 ± 0.99	0.22 ± 0.02	2.00 ± 1.20	29.90 ± 6.79
Mib	117.50 ± 3.54	20.40 ± 4.24	0.19 ± 0.004	5.70 ± 0.47	19.00 ± 8.27
R1881	100	43.65 ± 9.12	1.38 ± 0.40	25.4 ± 1.27	4.04 ± 0.91

[a]The equilibrium dissociation constants (K_d) for the tracer ligand measured under the same conditions are: AR (R1881) 0.6 nM; PgR (R5020) 0.41 nM; GR (RU28362) 11 nM; MR (Aldosterone) 3.9 nM; SBP (Estradiol) 1.6 nM.

Table 3. Tissue radioactivity distribution following i.v. injection of [^{3}H]testosterone into 300 g, castrated male rats %ID/g ± SEM (n = 5)[a]

Tissue	0.5 h	1 h	1 h (blocked)[b]	2 h	4 h
Blood	0.191 ± 0.025	0.152 ± 0.054	0.153 ± 0.019	0.108 ± 0.036	0.070 ± 0.009
Lung	0.104 ± 0.018	0.093 ± 0.015	0.121 ± 0.018	0.127 ± 0.021	0.050 ± 0.007
Spleen	0.098 ± 0.015	0.213 ± 0.148	0.059 ± 0.013	0.055 ± 0.015	0.042 ± 0.004
Muscle	0.128 ± 0.018	0.074 ± 0.008	0.110 ± 0.021	0.080 ± 0.016	0.051 ± 0.008
Prostate	0.319 ± 0.039	0.436 ± 0.059	0.169 ± 0.022	0.266 ± 0.031	0.255 ± 0.021
Liver	1.039 ± 0.196	0.533 ± 0.110	0.633 ± 0.103	0.721 ± 0.114	0.213 ± 0.043
Fat	0.214 ± 0.029	0.128 ± 0.020	0.187 ± 0.016	0.177 ± 0.068	0.039 ± 0.006
Esophagus	0.172 ± 0.030	0.133 ± 0.028	0.185 ± 0.030	0.094 ± 0.018	0.058 ± 0.005
Prostate/blood	1.792 ± 0.301	3.513 ± 0.453	1.149 ± 0.193	3.421 ± 0.903	3.904 ± 0.660
Prostate/n.t.[c]	2.559 ± 0.521	4.480 ± 0.369	1.264 ± 0.114	3.029 ± 0.767	4.933 ± 0.525

[a]%ID/g: percent injected dose per gram tissue.
[b]Blocked: in order to block receptor-mediated uptake, 36 μg of testosterone was added to each injected dose.
[c]n.t. = non-target = average of muscle, esophagus and lung.

Table 4. Tissue radioactivity distribution following i.v. injection of [^{3}H]dihydrotestosterone into 250 g, castrated male rats. %ID/g ± SEM (n = 5)[a]

Tissue	0.5 h	1 h	1 h (blocked)[b]	2 h	4 h
Blood	0.237 ± 0.028	0.152 ± 0.026	0.197 ± 0.023	0.124 ± 0.029	0.118 ± 0.011
Lung	0.271 ± 0.023	0.144 ± 0.009	0.095 ± 0.015	0.082 ± 0.011	0.062 ± 0.005
Spleen	0.093 ± 0.006	0.115 ± 0.016	0.099 ± 0.011	0.073 ± 0.012	0.048 ± 0.005
Muscle	0.106 ± 0.011	0.117 ± 0.011	0.126 ± 0.019	0.081 ± 0.012	0.071 ± 0.009
Prostate	0.367 ± 0.049	0.387 ± 0.063	0.134 ± 0.013	0.416 ± 0.093	0.265 ± 0.023
Liver	1.494 ± 0.102	0.824 ± 0.150	0.606 ± 0.105	0.462 ± 0.062	0.244 ± 0.017
Fat	0.160 ± 0.006	0.184 ± 0.018	0.112 ± 0.012	0.060 ± 0.003	0.039 ± 0.005
Esophagus	0.161 ± 0.010	0.151 ± 0.015	0.116 ± 0.011	0.062 ± 0.009	0.079 ± 0.003
Prostate/blood	1.545 ± 0.145	2.706 ± 0.549	0.700 ± 0.066	3.672 ± 0.593	2.377 ± 0.343
Prostate/n.t.[c]	2.016 ± 0.198	2.767 ± 0.309	1.245 ± 0.193	5.339 ± 0.579	3.802 ± 0.375

[a]%ID/g: percent injected dose per gram tissue.
[b]Blocked: in order to block receptor-mediated uptake, 36 μg of dihydrotestosterone was added to each injected dose.
[c]n.t. = non-target = average of muscle, esophagus and lung.

Table 5. Tissue radioactivity distribution following i.v. injection of [^{3}H]19-nortestosterone into 250 g, castrated male rats. %ID/g ± SEM (n = 5)[a]

Tissue	0.5 h	1 h	1 h (blocked)[b]	2 h	4 h
Blood	0.097 ± 0.012	0.114 ± 0.019	0.058 ± 0.006	0.044 ± 0.007	0.029 ± 0.002
Lung	0.170 ± 0.017	0.131 ± 0.026	0.108 ± 0.010	0.042 ± 0.004	0.020 ± 0.004
Spleen	0.105 ± 0.011	0.086 ± 0.021	0.065 ± 0.006	0.055 ± 0.010	0.025 ± 0.002
Muscle	0.135 ± 0.010	0.142 ± 0.022	0.067 ± 0.005	0.071 ± 0.008	0.056 ± 0.013
Prostate	0.352 ± 0.034	0.378 ± 0.069	0.100 ± 0.004	0.358 ± 0.026	0.148 ± 0.027
Liver	1.451 ± 0.136	1.721 ± 0.276	0.704 ± 0.076	0.561 ± 0.059	0.489 ± 0.098
Fat	0.215 ± 0.021	0.175 ± 0.022	0.135 ± 0.013	0.064 ± 0.009	0.025 ± 0.001
Esophagus	0.161 ± 0.015	0.132 ± 0.017	0.088 ± 0.007	0.065 ± 0.010	0.081 ± 0.008
Prostate/blood	3.857 ± 0.563	3.721 ± 0.339	1.826 ± 0.272	9.160 ± 1.848	5.160 ± 0.876
Prostate/n.t.[c]	2.259 ± 0.112	2.980 ± 0.308	1.152 ± 0.053	6.315 ± 0.810	2.878 ± 0.603

[a]%ID/g: percent injected dose per gram tissue.
[b]Blocked: in order to block receptor-mediated uptake, 36 μg of 19-nortestosterone was added to each injected dose.
[c]n.t. = non-target = average of muscle, esophagus and lung.

retention and target selective uptake can be appreciated more clearly from the presentation of prostate uptake data in Table 8.

In this table, androgen uptake by the prostate (% ID/g) is shown for total uptake at 1 and 4 h (TOT) and blocked or non-specific uptake at 1 h (NS). The "specific" or receptor-mediated uptake (SP) at 1 h is the difference between the TOT and NS uptake. It is clear that the specific uptake (SP) as a percent of the total (TOT) is high; this percentage, shown in column 4 of Table 8, is 61–74% for the androgens T, DHT, nor-T and Mib, and is nearly 90% for R1881.

Since the level of non-specific activity in both the target and non-target tissues decreases more rapidly than the specific (note [Fig. 4] how the ratio of prostate to non-target tissue activity increases with time), it is perhaps most instructive to compare the total uptake at 4 h (which should have little non-specific activity left) with the specific uptake at 1 h. For all the androgens except nor-T, the 4 h activity is equivalent to the 1 h specific uptake; with nor-T the 4 h uptake is about one-half the 1 h specific level. Thus, the component of androgen uptake by the prostate at 1 h that is receptor-mediated is very high, and this component appears to be well retained by the prostate over a 4-h period by all compounds, except perhaps by nor-T.

Target tissue uptake selectivity. Figure 3 and 4 display the prostate uptake of the five androgens relative to blood and non-target tissues (the average uptake of muscle, esophagus, and lung), respectively.

Table 6. Tissue radioactivity distribution following i.v. injection of [^{3}H]Mibolerone into 250 g, castrated male rats. %ID/g ± SEM[a]

Tissue	0.5 h	1 h	1 h (blocked)[b]	2 h	4 h	1 h + TA	1 h + DHT[b]	1 h intact	1 h intact + DES
Blood	0.122 ± 0.014	0.053 ± 0.009	0.056 ± 0.009	0.048 ± 0.005	0.043 ± 0.002	0.052 ± 0.003	0.050 ± 0.007	0.057 ± 0.016	0.046 ± 0.009
Lung	0.208 ± 0.030	0.127 ± 0.017	0.085 ± 0.016	0.090 ± 0.019	0.055 ± 0.005	0.086 ± 0.020	0.076 ± 0.013	0.116 ± 0.016	0.079 ± 0.021
Spleen	0.122 ± 0.010	0.037 ± 0.005	0.068 ± 0.012	0.049 + 0.006	0.030 ± 0.004	0.065 ± 0.017	0.039 ± 0.002	0.058 ± 0.010	0.045 ± 0.004
Muscle	0.191 ± 0.018	0.112 ± 0.014	0.096 ± 0.015	0.080 ± 0.005	0.093 ± 0.009	0.137 ± 0.012	0.061 ± 0.006	0.096 ± 0.013	0.110 ± 0.010
Prostate	0.573 ± 0.048	0.559 ± 0.053	0.186 ± 0.030	0.481 ± 0.048	0.503 ± 0.060	0.690 ± 0.044	0.195 ± 0.080	0.282 ± 0.037	0.726 ± 0.111
Liver	1.220 ± 0.076	0.590 ± 0.175	0.738 ± 0.232	0.478 ± 0.040	0.243 ± 0.022	0.614 ± 0.190	0.428 ± 0.077	0.439 ± 0.067	0.528 ± 0.149
Fat	0.287 ± 0.029	0.187 ± 0.019	0.218 ± 0.019	0.096 ± 0.005	0.046 ± 0.003	0.333 ± 0.012	0.196 ± 0.032	0.206 ± 0.058	0.259 ± 0.008
Esophagus	0.239 ± 0.015	0.130 ± 0.018	0.120 ± 0.016	0.156 ± 0.011	0.095 ± 0.003	0.212 ± 0.009	0.083 ± 0.005	0.135 ± 0.024	0.128 ± 0.011
Prostate/blood	4.842 ± 0.389	12.255 ± 3.017	4.098 ± 1.030	10.693 ± 1.859	11.497 ± 0.955	13.398 ± 1.005	4.331 ± 2.166	5.970 ± 1.570	16.380 ± 2.441
Prostate/n.t.[c]	2.779 ± 0.373	4.442 ± 0.600	2.095 ± 0.358	4.454 ± 0.338	6.246 ± 0.850	4.753 ± 0.173	2.594 ± 0.945	2.438 ± 0.094	6.854 ± 0.965

[a]%ID/g: percent injected dose per gram tissue. Columns 1, 4 and 5: $n = 5$; column 2: $n = 9$; column 3: $n = 8$; columns 6, 8 and 9: $n = 4$; column 7: $n = 3$.
[b]Blocked: in order to block receptor-mediated uptake, 36 μg of mibolerone (column 3) or DHT, (column 7) was added to each injected dose.
[c]n.t. = non-target = average of muscle, esophagus and lung.

Table 7. Tissue radioactivity distribution following i.v. injection of [^{3}H]methyltrienolone (R1881) into 250 g, castrated male rats. %ID/g ± SEM[a]

Tissue	0.5 h	1 h	1 h (blocked)[b]	2 h	4 h	1 h + TA	1 h + DHT[b]	1 h intact	1 h intact + DES
Blood	0.088 ± 0.010	0.077 ± 0.015	0.050 ± 0.007	0.025 ± 0.003	0.026 ± 0.005	0.047 ± 0.009	0.069 ± 0.006	0.059 ± 0.005	0.053 ± 0.010
Lung	0.251 ± 0.014	0.156 ± 0.022	0.077 ± 0.006	0.100 ± 0.011	0.061 ± 0.009	0.111 ± 0.038	0.072 ± 0.010	0.093 ± 0.007	0.094 ± 0.017
Spleen	0.104 ± 0.007	0.049 ± 0.009	0.042 ± 0.003	0.026 ± 0.004	0.021 ± 0.003	0.057 ± 0.005	0.039 ± 0.008	0.054 ± 0.015	0.055 ± 0.002
Muscle	0.146 ± 0.008	0.125 ± 0.010	0.085 ± 0.009	0.058 ± 0.006	0.032 ± 0.003	0.106 ± 0.025	0.087 ± 0.004	0.093 ± 0.007	0.094 ± 0.003
Prostate	0.633 ± 0.041	0.689 ± 0.078	0.101 ± 0.012	0.467 ± 0.076	0.592 ± 0.146	0.649 ± 0.134	0.263 ± 0.067	0.357 ± 0.084	0.545 ± 0.082
Liver	0.771 ± 0.086	0.499 ± 0.068	0.328 ± 0.028	0.233 ± 0.049	0.190 ± 0.034	0.258 ± 0.068	0.610 ± 0.116	0.357 ± 0.020	0.332 ± 0.030
Fat	0.211 ± 0.013	0.166 ± 0.043	0.117 ± 0.018	0.046 ± 0.005	0.027 ± 0.005	0.157 ± 0.025	0.128 ± 0.016	0.161 ± 0.032	0.207 ± 0.023
Esophagus	0.176 ± 0.020	0.142 ± 0.007	0.055 ± 0.004	0.082 ± 0.012	0.046 ± 0.006	0.102 ± 0.011	0.108 ± 0.009	0.164 ± 0.016	0.133 ± 0.008
Prostate/blood	7.528 ± 0.714	10.111 ± 1.832	2.168 ± 0.488	19.914 ± 4.198	23.404 ± 5.914	15.061 ± 4.361	3.970 ± 1.139	5.824 ± 1.069	11.765 ± 3.062
Prostate/n.t.[c]	3.324 ± 0.194	4.377 ± 0.446	1.263 ± 0.156	5.709 ± 0.698	13.019 ± 3.117	6.887 ± 2.177	3.025 ± 0.850	3.065 ± 0.723	5.145 ± 0.712

[a]%ID/g: percent injected dose per gram tissue. Columns 1, 4 and 5: $n = 5$; column 2, 3: $n = 9$; columns 6, 8 and 9: $n = 4$; column 7: $n = 3$.
[b]Blocked: in order to block receptor-mediated uptake, 36 μg of R1881 (column 3) or DHT (column 7) was added to each injected dose.
[c]n.t. = non-target = average of muscle, esophagus and lung.

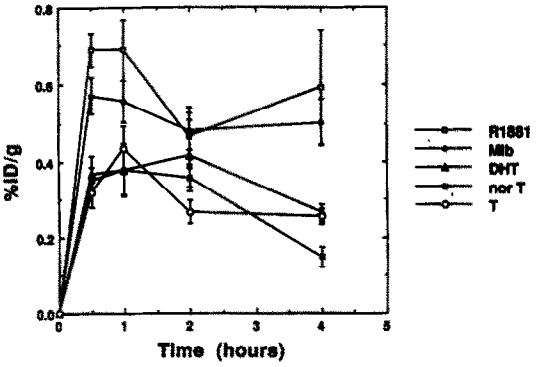

Fig. 2. Uptake into prostate: uptake of the five androgens as percent injected dose/g tissue (%ID/g ± SEM) into the prostate of orchidectomized rats. Each point represents the average uptake in 5 or more animals (see Table 3–7).

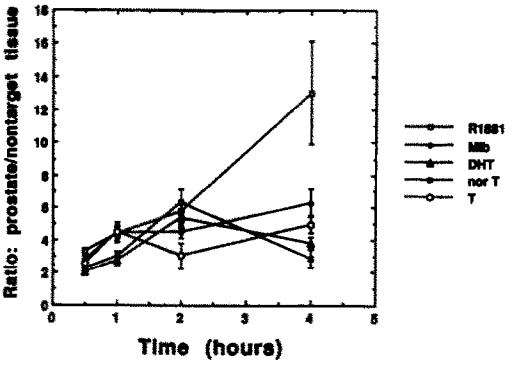

Fig. 4. Uptake-ratio prostate/non-target tissue: uptake of the five androgens in prostate of orchidectomized rats relative to non-target tissues (average of muscle, esophagus, and lung) ± SEM. Each point represents the average of 5 or more animals (see Tables 3–7).

Relative to blood (Fig. 3), prostate uptake of Mib and R1881 is very selective, with ratios being approx. 10 or 10–20, respectively. The other three androgens give significantly lower prostate to blood ratios, with the exception of the 2 h uptake of nor-T. These uptake ratios reflect the known differences in metabolism of these androgens. Those which are rapidly metabolized show higher blood levels (presumed to be mostly metabolites) than those resistant to metabolism. There is a less pronounced difference among the five androgens in terms of prostate uptake relative to non-target tissues (Fig. 4). Only at 4 h is the ratio for R1881 significantly above that of the others.

Uptake by other tissues is not unusual. All compounds show high, but transient uptake by the liver. However, while liver levels equal or exceed those of the prostate at early times, activity falls off more rapidly in liver relative to prostate (except with nor-T), so that by 4 h, liver levels are equal to (for T and DHT) or below that of prostate (Mib, R1881). There is apparent blockage of the 1-h liver uptake of nor-T and R1881 by an excess of unlabeled compound, but this might represent dose-dependent metabolism rather than receptor-mediated uptake, since androgen receptor levels in the liver are low [39].

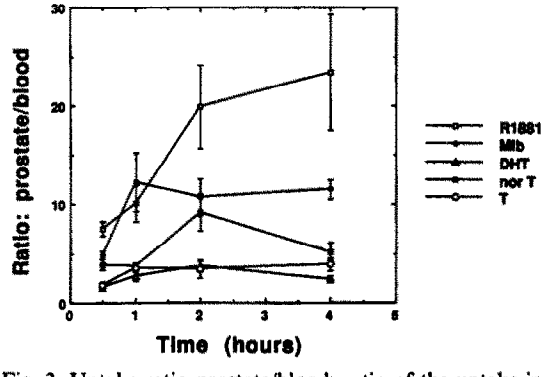

Fig. 3. Uptake-ratio prostate/blood: ratio of the uptake in prostate relative to blood ± SEM in orchidectomized rats for the five androgens. Each point represents the average of 5 or more animals (see Tables 3–7).

R1881 had high (2.9% ID/g) uptake into the bladder at 1 h (data not shown). Since both liver and bladder are organs involved in the metabolism and voiding of drugs from the body, these results were expected.

Examination of the role of other steroid receptors, castration, and diethylstilbestrol treatment on the uptake of mibolerone and R1881 by the prostate

Of the five androgens we have investigated, Mib and R1881 had the highest prostate uptake and seemed the most promising androgens for fluorine labeling. They were also the only ones with substantial binding affinity for other steroid receptor systems (cf. Table 2), in particular, the progesterone receptor and the mineralocorticoid receptor. It is unlikely that either of these receptor systems is responsible for any of the prostate uptake of these compounds, as neither is present at detectable levels in the prostate of the orchidectomized rat [36]. The human prostate, however, does contain receptors for glucocorticoids and for progesterone [40], and ligand binding to the progesterone receptor in particular, can be a serious problem with androgen receptor assays in prostatic human cytosol when Mib or R1881 are used as tracers [33–35]. These tracers are generally used together with a high concentration of triamcinolone acetonide, a glucocorticoid with substantial affinity for the progesterone, glucocorticoid and mineralocorticoid receptors, but only very low affinity for the androgen receptor (<0.0001% relative to R1881)

Table 8. Androgen uptake by the prostate[a]

	1 h (%ID/g)				4 h (%ID/g)
Androgen	TOT[a]	NS[a]	SP	SP/TOT(%)	TOT[a]
T	0.44	0.17	0.27	61%	0.26
DHT	0.39	0.13	0.26	67%	0.27
nor-T	0.38	0.10	0.28	74%	0.15
Mib	0.56	0.19	0.37	66%	0.50
R1881	0.69	0.10	0.59	86%	0.59

[a]Data taken from Tables 3–7. TOT = total (%ID/g prostate at indicated times 1 or 4 h); NS = non-specific (%ID/g prostate 1 h blocked); SP = specific, i.e. TOT − NS; SP/TOT% = specific (1 h) as percent of total (1 h).

(KEC and JAK unpublished), in order to "blank out" binding of these tracers to the other receptors [35].

Although the castrated rat prostate has little if any progesterone receptor, we were concerned that the greater prostate uptake seen with mibolerone and R1881 may have been enhanced by binding to other receptors. Therefore, we also examined the tissue distribution of Mib and R1881 in the castrated rat both in the presence of a large excess of unlabeled triamcinolone acetonide and DHT. If the prostate uptake of these compounds was reduced by triamcinolone acetonide, or if the extent of uptake blockage by DHT, the most androgen selective ligand, was not as great as that effected by the unlabeled ligand itself, this would suggest that other receptor systems were contributing to the uptake. These uptake studies are noted in Tables 6 and 7, columns 6 and 7, and in Figs 5 and 6.

It is clear from these uptake data that prior injection of a large excess of unlabeled triamcinolone acetonide does not result in a significant lowering of prostate uptake of mibolerone or R1881, but does affect the uptake into a glucocorticoid-rich tissue such as liver (in Tables 6 and 7, compare column 6 with column 2; Figs 5 and 6). Also, the extent to which prostate uptake is blocked by DHT is comparable to that achieved by the unlabeled ligand itself (in Tables 6 and 7 compare column 3 with column 7). Thus, it appears that the specific uptake observed for Mib and R1881 in the prostate is due largely, if not exclusively, to binding to the androgen receptor.

The uptake of Mib and R1881 was determined in intact rats and in intact rats treated with diethylstilbestrol, hormonal states with clinical significance. Both these uptake experiments were done in the presence of an excess of unlabeled triamcinolone acetonide, to assure that no uptake was due to the progesterone receptor, since estrogen treatment will induce both progesterone receptor and androgen receptor levels in

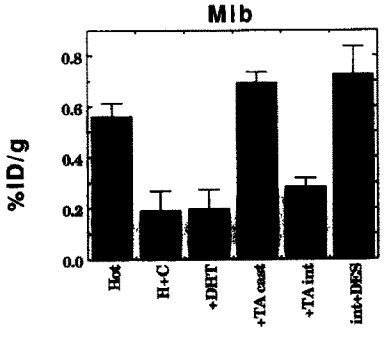

Fig. 5. Uptake of mibolerone into prostate: uptake of [^{3}H]mibolerone at 1 h as percent injected dose/g tissue (%ID/g) into orchidectomized rats (columns 1–4) or intact rats (columns 5 and 6). Column 1, [^{3}H]Mib alone; column 2 and 3, [^{3}H]Mib + 36 μg unlabeled Mib or DHT respectively; Column 4, [^{3}H]Mib alone in animals pre-injected with triamcinolone acetonide. Column 5, uptake into intact animals pre-injected with triamcinolone acetonide; column 6, uptake into intact animals preinjected with triamcinolone acetonide and pretreated with DES. Data from Table 6.

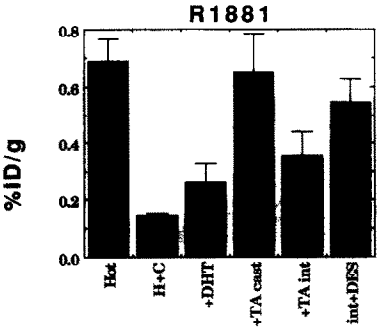

Fig. 6. Prostate uptake of [^{3}H]methyltrienolone (R1881): uptake of [^{3}H] (R1881) into the prostate of orchidectomized animals (columns 1–4) and intact animals (columns 5 and 6). Uptake as percent injected dose/gram of tissue (%ID/g) at 1 h. Column 1, [^{3}H]R1881 alone; column 2 and 3, [^{3}H]R1881 plus 36 μg unlabeled R1881 or DHT; column 4, [^{3}H]R1881 alone into animals preinjected with triamcinolone acetonide. Uptake into intact animals: column 5, uptake into intact rats preinjected with triamcinolone acetonide; column 6, uptake into intact rats preinjected with triamcinolone acetonide and pre-treated with DES. Data from Table 7.

the prostate [12]. It is clear that the high levels of circulating androgens in the intact rats caused a significant depression of the uptake of Mib and R1881 (in Tables 6 and 7, compare column 8 with column 6; Figs 5 and 6). However, treatment of the intact animals with a high dose of diethylstilbestrol, which is used clinically to suppress androgen biosynthesis [8], restores most of the androgen receptor-mediated uptake in the prostate (in Tables 6 and 7, compare column 9 with column 6; Figs 5 and 6).

To verify that the treatment of intact animals with DES was effective in reducing androgen levels and leaving unbound AR, we assayed the amount and distribution of AR in each treatment group. The data is presented in Fig. 7. The orchidectomized animals had no androgen receptor that was bound with endogenous androgens. We measured only "empty" receptor, 7.3 fmol/μg DNA, of which 94% was in the cytosol. The intact rats had the receptor divided between the cytosol (53%) and the nucleus (47%). Most of the receptor was filled with endogenous androgens (82% in the cytosol and 93% in the nucleus), so that only a small portion was free and therefore available to the injected tritium-labeled ligand. The total receptor in the intact animals both free and that bound with endogenous androgens (measured by exchange) was 11.7 fmol/μg DNA.

The intact but DES-treated rats presented an intermediate picture of filled and empty, nuclear and cytosolic receptor. The receptor was mostly cytosolic (78%), of this 56% was filled. The receptor that was in the nucleus was mostly filled (85%). The total receptor, 8.5 fmol/μg DNA, was also an intermediate value.

Since the prostate is androgen dependent, removal of the androgens, either surgically or chemically, results in atrophy of the organ as well as loss of androgen receptor. We see about a 10% loss in

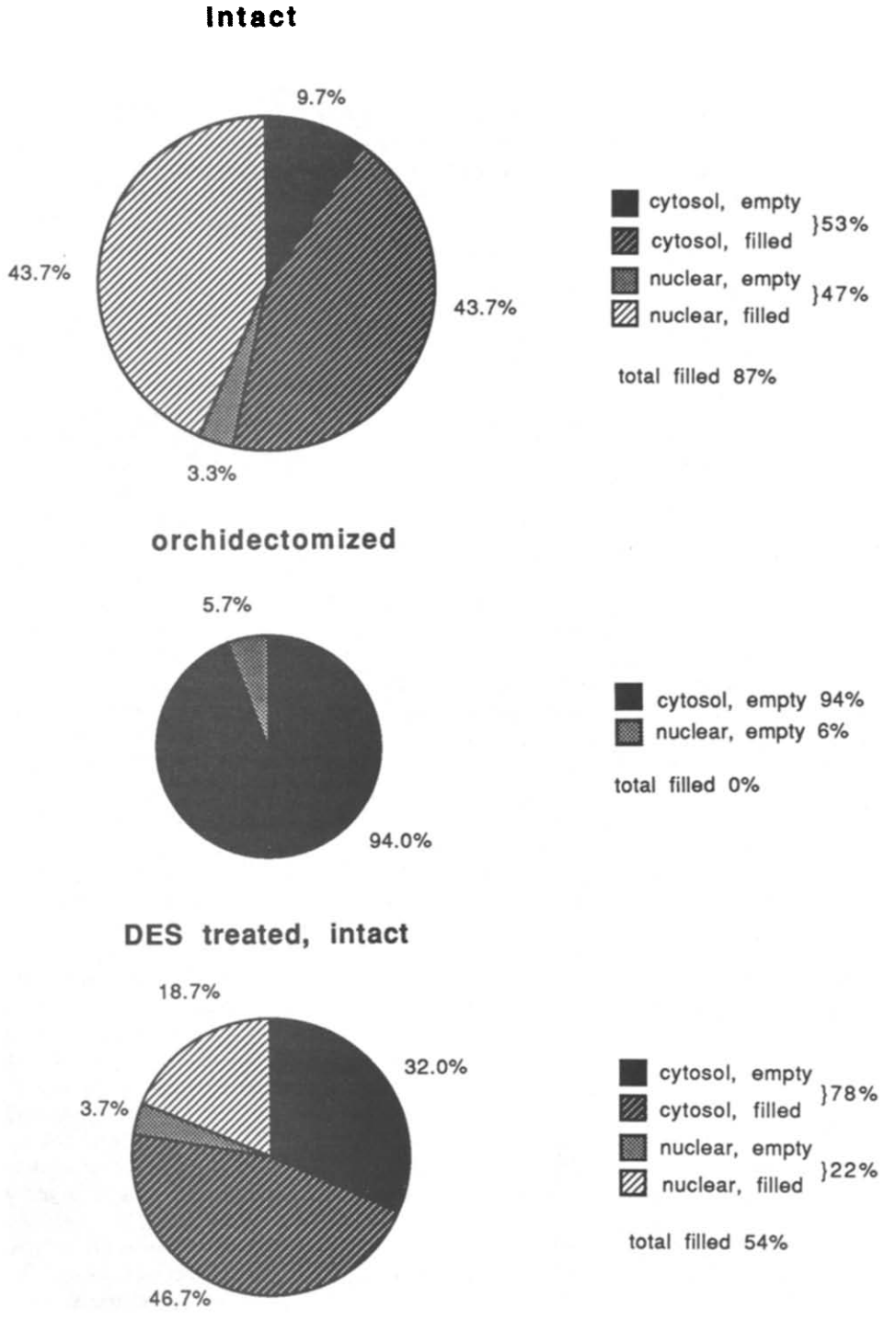

Fig. 7. Concentration, occupancy and distribution of androgen receptor in the prostate of orchidectomized, intact, and intact-DES-treated rats. The areas of the circles are proportional to the average AR concentration in each treatment group: intact, 11.68 fmol/μg DNA; orchidectomized, 7.27 fmol/μg DNA; DES treated, intact, 8.47 fmol/μg DNA. The areas of the segments are proportional to the average concentrations of empty and occupied cytosol AR, and empty and occupied nuclear AR.

prostate weight and a 35 and 19% loss of androgen receptor, respectively, in the orchidectomized and DES-treated animals compared to the untreated intact animals, consistent with values in the literature [6, 14] for 24 h castration.

DISCUSSION

The development of imaging agents for androgen receptor-positive prostatic cancer depends on the availability of radioligands for the androgen receptor

that have suitable receptor binding affinity and *in vivo* distribution properties and that are labeled with an appropriate radionuclide. To assist in the development of such agents, we have investigated the tissue distribution in the rat of five commercially available androgens labeled with tritium at high specific activity. We have found that all of these compounds demonstrate selective, receptor-mediated uptake and prolonged retention by the prostate. In a number of respects (% ID/g, prostate uptake ratio to blood and non-target tissues, and uptake selectivity), the best two compounds are R1881 or methyltrienolone, a high affinity, synthetic androgen prepared by the Roussel Co. [33], and mibolerone (Upjohn Co.), another synthetic androgen.

If one corrects for the differences in animal weights (% ID-kg/g), then the 1 h uptake of the androgens by the prostate (0.09–0.20% ID-kg/g) is within the same range as that of estrogen (0.16–0.4% ID-kg/g) [41] and progestins (0.03–0.26% ID-kg/g) [42] by the uterus. The equivalent uptake of these target sites cannot be explained by receptor content, since the androgen receptor content of the 1 day castrated rat prostate (120 fmol/mg protein or 5.4 pmol/g tissue) is only 10–15% of the estrogen receptor content of the uterus (1000 fmol/mg protein or 30 pmol/g tissue), or the progestin receptor content of the estrogen-primed uterus (800 fmol/mg protein or 40 pmol/g tissue). It is likely in such receptor-rich target sites, that receptor-mediated uptake is flow limited [43]; thus, the equivalency of uptake in these three systems may be more a reflection of the roughly equivalent blood flow rates in the target sites and the similar tissue permeabilities of the radiopharmaceuticals.

The selectivity of uptake (activity ratios of prostate to blood or prostate to non-target tissues) for the best two androgens, R1881 and Mib, is reasonably high, but is still somewhat less than we have seen for the best estrogens and progestins. These tissue activity ratios, however, are not entirely dependent on the binding properties of the radiopharmaceutical, but also on radiochemical purity and metabolic stability. The activity in the blood and non-target tissues is generally largely due to metabolites, which with these tritium-labeled compounds could be, in addition to polar and conjugated steroid species, tritiated water released by hydroxylation at the sites of tritium substitution. The sites of tritium substitution on the five androgens are indicated in Fig. 1. It is likely that a portion of the activity situated at carbon-2 in T and DHT and carbon-6 in nor-T is released as a result of hydroxylation at these sites. The tritium label on the 17α-methyl group of Mib or R1881 could well be more metabolically stable.

While there have not been other systematic, comparative studies of the uptake of androgens *in vivo*, there are reports of the *in vivo* uptake of androgens in rats [2, 24, 44, 45]. In 1968 Tveter and Attramadal [45] reported that i.m. injection of [^{3}H]T in castrated rats gave a peak uptake in the prostate of 0.29 %ID/g at 1–2 h, with a ratio to blood and muscle of 3 and 7, respectively. Uptake in intact animals was reduced, and injection of 500 μg of unlabeled testosterone 0.5 h prior to [^{3}H]T reduced prostate uptake in castrated animals by approx. 90%. These results are commensurate with ours. Symes[24] found that [^{3}H]T administered i.p. gave a maximum uptake of 0.73% ID/g at 1 h in somewhat larger animals than we used (350–450 g). The route of injection (i.p.) used in Symes' study may provide a more efficient presentation of the radioligand to the prostate than did our route (i.v.). Prostate to blood and muscle ratios were approx. 10:1 after 2 and 4 h. [^{3}H]DHT, however, showed significantly lower peak uptake 0.18 %ID/g at 2 h, with ratios to blood and muscle of 3–5. Symes also reported a decreased uptake of T by the prostate of intact animals, and an increased uptake selectivity (prostate to blood ratio) in intact animals treated with diethylstilbestrol. We have also noted that the uptake of Mib and R1881 is reduced in intact vs castrated animals, and also, like Symes, that diethylstilbestrol treatment of intact animals results in increased uptake, which in our case, was nearly equivalent to that seen in castrated animals. This latter point is important, since many human prostatic cancer patients are treated with diethylstilbestrol to suppress biosynthetic production of androgens. So, the "intact but diethylstilbestrol treated" state is the one likely to be encountered with human patients.

Mobbs[6] has done a careful study of the effect of castration and diethylstilbestrol treatment on the content and distribution of androgen receptor in human prostate carcinoma (between occupied vs unoccupied states, nuclear vs cytosolic fractions). She finds that upon castration, androgen receptor levels actually decrease modestly, but shift markedly from the nuclear and occupied states to the cytosolic-unoccupied state. Long-term treatment of patients with diethylstilbestrol causes a pronounced increase in androgen receptor content, especially in orchidectomized patients; in these cases, 80–90% of receptor is unoccupied. We also found that the receptor is mostly occupied in the intact rat, whereas in the castrated or DES-treated animal, a large percentage is free.

The endogenous androgens in the occupied receptors, especially in the nucleus, cannot be readily exchanged with titium-labeled ligand *in vitro*. We found, in experiments to validate the assay, that the common procedure for exchange (15°, 24 h) resulted in only ~50% exchange of bound ligand (data not shown). At the same time, 16–20% of the receptor was being degraded (Ref. [29] and our data, not shown). Since the "empty" receptors are readily filled with tritium-labeled ligand at 0°C, and the occupied receptors are slow to exchange and continously degraded, many estimates of receptor occupancy underestimate the number of "filled" receptors. After 43 h of exchange at 15°C, we measure 7.3–11.7 fmol of

total androgen receptor/μg DNA, similar to estimates by radioimmunoassay of 13 fmol/μg DNA [5] and by steroid binding [4]. A recent publication, using an affinity ligand [46] reports 172 fmol/μg DNA in the ventral prostate of intact rats.

While heterologous binding, that is the binding of one class of steroid hormones by the receptors from another class, is not a major issue with estrogens, the two synthetic androgens R1881 and Mib, demonstrate very substantial heterologous binding, especially towards the progesterone receptor (cf. Table 2); R1881 is the least discriminating, having substantial affinity for the estrogen, progestin, glucocorticoid and mineralocorticoid receptors. Nevertheless, despite their capacity for heterologous binding, the uptake of Mib and R1881 by the prostate is due to their interaction with androgen receptors in the target tissue. We have demonstrated this in two ways, by showing that there is not significant competition for prostate uptake by triamcinolone acetonide, a ligand with high affinity for progesterone, glucocorticoid and mineralocorticoid receptors, but very low affinity for the androgen receptor, and by showing that when DHT, the most selective ligand for the androgen receptor, is used as a competitor, the uptake of Mib and R1881 is depressed to a similar extent as when the unlabeled tracer itself is the competitor.

In humans, circulating androgens and estrogens are bound extensively by the serum globulin sex steroid binding protein (SBP). While the level of this protein is relatively low in males, its high affinity for the androgens T, DHT, and nor-T can interfere with *in vitro* assays of androgen receptor and could affect the tissue distribution of these androgens *in vivo*. There is no counterpart to SBP in rats, so in this sense, rats are an imperfect model for humans. We have shown by independent competitive binding assays, however, that the two androgens that show the most efficient and selective uptake by the prostate also have low affinity for SBP. Thus, one would not anticipate that SBP would affect the *in vivo* distribution properties of these compounds in humans. Alphafetoprotein, a fetal albumin of rats, has high affinity for estrogens [47], and has a significant effect on the binding distribution of radiolabeled estrogen in immature rats [28]. However, it does not bind androgens, nor is it present in adult animals [47, 48].

Although T is the major circulating androgen, it is rapidly metabolized intracellularly by reduction to the higher affinity DHT, such that DHT is the predominant form that is bound by androgen receptors in target tissues [2, 49]. DHT and nor-T are also metabolized rapidly [50,51]. This rapid metabolism of the androgens, which limits their potency as therapeutic agents and even interferes with their use in *in vitro* binding assays, prompted the development of derivatives and analogs more resistant to chemical alteration *in vivo*. Both Mib and R1881 have greatly reduced metabolism *in vitro* and *in vivo* [29, 33, 34, 37]. A reduced rate of metabolism could result in a reduced clearance rate which would extend the blood activity curve. This could account for the higher uptake efficiency of these two compounds compared to T, DHT, and nor-T.

The comparative study of androgen uptake that we have presented here represents an initial step in the development of androgens labeled with appropriate gamma- or positron-emitting radionuclides whose binding and distribution properties are suitable for imaging androgen receptor-positive target sites and tumors. The androgens present a more complex case than did the estrogens, because they show substantial heterologous binding, are rapidly metabolized and bind with high affinity to certain proteins. Nevertheless, their uptake by target tissues in the rat—particularly that of Mib and R1881—demonstrates adequate efficiency and promising selectivity.

Earlier, we described the preparation of fluorine-substituted derivatives of T, DHT, and nor-T as agents that might be labeled with fluorine-18 and studied as potential androgen receptor-based imaging agents [23]. The binding affinity of most of these fluorine-substituted derivatives was somewhat, and in some cases considerably, less than that of the five tritium-labeled androgens studied here; so, their utility as imaging agents may be limited. On the basis of the studies presented here, we are undertaking the preparation of certain fluorine-substituted analogs of the two tritium-labeled tracers that demonstrated the best uptake properties, mibolerone (Mib) and methyltrienolone (R1881). The results of these studies, involving the preparation of these compounds in fluorine-18 labeled form and their selective uptake by target tissues in the rat, will be presented elsewhere [52].

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