7α-Iodine-125-Iodo-5α-Dihydrotestosterone: A Radiolabeled Ligand for the Androgen Receptor

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We describe the preparation of 7α-[^{125}I]iodo-5α-dihydrotestosterone (7α-[^{125}I]IDHT) and its characterization as a ligand for the androgen receptor. **Methods:** We designed a route to prepare the radioiodine-labeled androgen on microscale through treatment of the 7β-tosylate of 7β-hydroxy-5α-dihydrotestosterone-17β-*p*-nitrobenzoate with Na^{125}I, followed by alkaline hydrolysis. The radiolabeled steroid was tested as a ligand for the androgen receptor in cytosol from MCF-7 cells, and for its in vivo tissue distribution in the rat. In addition, we tested 7α-[^{125}I]IDHT as a ligand in a novel assay for the detection and quantification of the ligand activated androgen receptor by in vitro autoradiography. **Results:** The above synthetic route produced the 17β-*p*-nitrobenzoate of 7α-[^{125}I]IDHT in carrier-free form and in good yield. The 17β-ester was removed with alkali and the resulting 7α-[^{125}I]IDHT was purified by HPLC. 7α-[^{125}I]IDHT bound with high affinity, K_d=0.26 n*M*, to the androgen receptor and showed low nonspecific binding. Since the ligand was carrier free and thus of very high specific activity, ~2,200 Ci/mmole, the sensitivity of the assay was much greater than with [^{3}H]R1881, the classical androgen receptor ligand with which it was compared. When tested as a ligand for in vitro autoradiography, 7α-[^{125}I]IDHT produced excellent autoradiograms of the activated receptor with very low nonspecific binding and with only overnight exposure of the film. **Conclusion:** These studies demonstrate that 7α-[^{125}I]IDHT is an excellent ligand for the androgen receptor.

Key Words: radioiodinated androgen; androgen receptor; 7α-iodo-5α-dihydrotestosterone; receptor imaging

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Steroid hormones that bind with high affinity to their receptors and are labeled with high-energy isotopes are important hormonal probes. Radioiodine-labeled steroids are particularly versatile. Steroids labeled with ^{123}I can be used as SPECT imaging agents for the detection of hormonally-dependent breast cancers and metastases. They are excellent analytical agents when labeled with ^{125}I (~2,200 Ci/mmole) because of their high specific activity. The requirements for steroid receptor ligands are stringent and currently only radioiodine-labeled estrogens (*1–4*) and progestins (*5–7*) have been synthesized. It has been apparent that an iodinated androgen would be an extremely useful ligand for studies of the androgen receptor, including imaging of prostatic cancer and the synthesis of androgens labeled with high-energy isotopes, gamma or positron emitters, that are capable of receptor-mediated concentration in androgen target tissues has been a goal of many investigators. While several ^{18}F-labeled androgens have shown potential as imaging agents (*8*), the design of a biologically-active androgen labeled with iodine, however, has proven to be elusive. Although many potential iodinated androgen receptor ligands have been synthesized they have not been useful (*7*).

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We too have synthesized several iodinated steroids as ligands for the androgen receptor: 16α-Iodo-5α-dihydrotestosterone (*9*), E-17α-(2′-iodovinyl)-5α-dihydrotestosterone and Z-17α-(2′-iodovinyl)-5α-dihydrotestosterone (*10*) were selected on the basis of their structural similarity to biologically active D-ring substituted androgens and estrogens. Although estrogens iodinated in the same positions bind with high affinity to the estrogen receptor (*1,11,12*), these substitutions in 5α-dihydrotestosterone produce compounds that do not bind appreciably to the androgen receptor. Conversely, we synthesized 7α-methyl-17α-(E-2′-[^{125}I]iodovinyl)-19-nortestosterone and 7α-methyl-17α-Z-(2′-[^{125}I]iodovinyl)-19-nortestosterone) that have high affinity for the androgen receptor (*13*). However, nonspecific binding was very high, and they did not concentrate in tissues in an androgen receptor mediated manner. Other 17α-(2′-iodovinyl) analogs of testosterone and 19-nortestosterone have been synthesized. Some were found to be good ligands in competition studies of the androgen receptor, but again none showed appreciable receptor-mediated concentration when administered to rats (*7*).

Recently, we synthesized 7α-iodo-5α-dihydrotestosterone (7α-IDHT) and have shown that it competes with high affinity for the binding of the ligand, [^{3}H]R1881, to the androgen receptor (*14*). The binding of the 7-iodinated steroid is specific for the androgen receptor. It does not compete with the binding of other steroid hormones to their receptors, including estrogen, progestin and glucocorticoid receptors. The procedure that we used to incorporate iodine into 7α-IDHT was through reaction of 7β-hydroxy-5α-dihydrotestosterone with NaI and trichloromethylsilane (the reaction was accomplished with the 7β-epimer, as well as a mixture of the 7α-, 7β-epimers), a method developed by Olah et al. (*15*) for the direct conversion of alcohols to iodo compounds. Subsequently, we found that this procedure is not appropriate for the microscale reaction that is required for radiochemical synthesis. We now describe another route for the synthesis of 7α-iodo-5α-dihydrotestosterone through S_N2 attack of the 7β-tosylate intermediate with iodide (Figs. 1 and 2). This strategy permits the radiosynthesis of 7α-[^{125}I]IDHT with carrier-free Na^{125}I. We report the binding characteristics of 7α-[^{125}I]IDHT to the androgen receptor in vitro and in vivo.

Experimental

All of the chemical syntheses for the compounds shown in Figure 1, compounds 2–6, as well as nonradiolabeled 7 are described in detail in the Appendix.

17β-Hydroxy-7α-[^{125}I]iodo-5α-androstan-3-one (7α-[^{125}I]IDHT). The substitution reaction with Na^{125}I was performed as per Method A (see Appendix) with some modifications and carried out in an enclosed hood as described earlier (*3*). In a 100 μl Reacti-vial (Pierce) was placed a solution of Na^{125}I in H_2O (27 μl, 10 mCi, 4.95 nmole, low pH, Dupont New England Nuclear

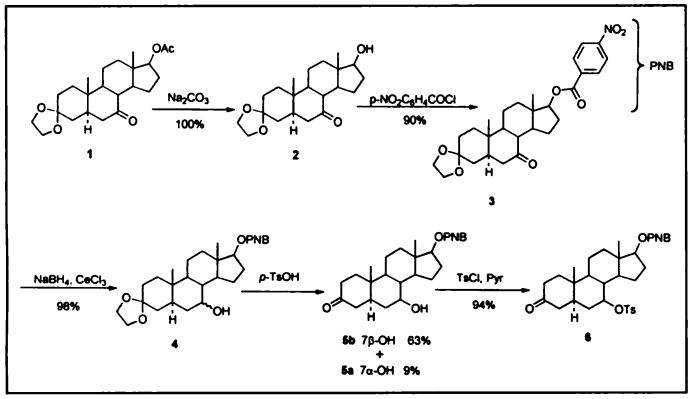

FIGURE 1. The procedures for the synthesis of compounds 2-6 are described in the Appendix.

Co., North Billerica, MA) followed by 20 μl of a solution containing 100 μg/ml sodium thiosulfate in acetonitrile/H_2O (9:1) and evaporated to dryness under vacuum. To ensure removal of H_2O, 20 μl of acetonitrile was added to the vial and evaporated to dryness under vacuum. Twenty microliters of a solution containing 30 μg of tosylate 6 in acetonitrile was added, the vial was sealed and heated at 105°C for 2 hr. The reaction was allowed to cool to room temperature, vented through a charcoal filter, diluted with 10 μl of the above sodium thiosulfate solution and evaporated under vacuum. The residue was diluted with CH_2Cl_2 (70 μl) and purified by HPLC, system 1: (Hibar-Si, 0.1% iPrOH-CH_2Cl_2, 1 ml/min, 254 nm). Material eluting as a peak at 19 min was collected and concentrated in vacuo to give [^{125}I]-7 (90% radiochemical yield). In this system 6 migrates in 31 min. The mass of the radioactivity was determined by UV absorption in the HPLC by comparison with a standard curve of 7. The specific activity of [^{125}I]-7 calculated from the mass and the amount of radioactivity in the peak was >2,200 Ci/mmole.

The above *p*-nitrobenzoate, [^{125}I]-7, was transferred with acetonitrile into a 1 ml conical Reacti-vial (Pierce), evaporated in vacuo and dissolved in 75 μl of 1% KOH/MeOH. The solution was sealed and stirred at room temperature for ½ hr. The reaction was neutralized with 75 μl of 0.178 *M* aqueous HOAc and injected directly onto the HPLC column in system 2: Altex Ultraspere 5 μm ODS, MeOH/H_2O (65:35), 1 ml/min. Material corresponding to a peak containing the bulk of the radioactivity migrated at 17–18 min. It was collected and evaporated in vacuo to give 7α-[^{125}I]IDHT, 7 mCi (78% yield). The overall radiochemical yield was 70%. The dried residue was dissolved in benzene:ethanol (4/1) and stored at 4°C under argon where it was stable for months. This material co-migrated with authentic 7α-IDHT by TLC (hexane:EtOAc, 2/1), R_f 0.22, and by two HPLC systems: RP-18, acetonitrile-H_2O (1:1), 1 ml/min at 26 min and Ultrasphere, 1% iPrOH-CH_2Cl_2, 1 ml/min at 17.5 min.

Androgen Receptor

Saturation Binding Analysis. Androgen receptor binding experiments were performed with cytosols prepared from human breast cancer MCF-7 cells as we have previously described (*14*). The MCF-7 cells were cultured in phenol red-free RPMI 1640 medium supplemented with 5% charcoal-stripped fetal calf serum. Cells were harvested at approximately 80% confluency and washed in ice-cold PBS. The pelleted cells were resuspended and homogenized in ice-cold TEGDMo buffer (10 m*M* Tris, 1.5 m*M* Na_2-EDTA, 10% (v/v) glycerol, 1.0 m*M* dithiothreitol, 25 m*M* sodium molybdate, pH 7.4 at 4°C) and centrifuged at 105,000 *g* for 45 min at 4°C. The supernatant (cytosol) was frozen on dry ice and stored at -80°C until assay. For assay, the cytosol was defrosted on ice and diluted with TEGTMo buffer to a concentration of 1.0–1.5 mg protein/ml. Aliquots (75 μl) of the cytosol were incubated for 20 hr at 4°C with 50 μl TEGDMo buffer containing a range of concentrations of 7α-[^{125}I]IDHT (0.07–6.18 n*M* final) or [^{3}H]R1881 (0.15–7.76 n*M* final). Nonspecific binding was assessed in parallel incubations containing 1 μ*M* radioinert DHT. All incubates also contained 1 μ*M* triamcinolone acetonide to prevent potential binding to the progesterone receptor (*16*). Bound 7α-[^{125}I]IDHT was separated from free by gel filtration on 7 × 35 mm LH-20 Sephadex columns (*17*). Aliquots of incubate (100 μl) were loaded onto the columns and washed into the column bed with 100 μl TEGDMo. Thirty min after sample application, the macromolecular bound fraction was eluted into 12 × 75 mm borosilicate culture tubes by adding 400 μl TEGDMo to the column. 7α-[^{125}I]IDHT was counted at 70% efficiency using an ICN Micromedic 4/600 Plus γ counter (Huntsville, AL). Column eluates from [^{3}H]R1881 incubates were collected in scintillation vials and 5 ml Betacount scintillation cocktail was added. After overnight extraction, [^{3}H]R1881 was quantified at 55% efficiency using an ICN Micromedic Taurus liquid scintillation counter (ICN). Specific binding was calculated as the difference between total binding and nonspecific binding (measured in the presence of excess radioinert DHT). The resulting data were analyzed by the method of Scatchard using a computer assisted nonlinear curve fitting method (LIGAND) (*18*).

Competition Binding Analysis. Aliquots (75 μl) of MCF-7 cell cytosol were incubated at 4°C for 20 hr with 50 μl TEGDMo buffer containing 7α-[^{125}I]IDHT in the presence or absence of radioinert R1881, 5α-DHT, 7α-IDHT, testosterone, estradiol, progesterone or dexamethasone. The final concentration of 7α-[^{125}I]IDHT was 4.0 n*M* and the competitor concentrations ranged from 0.005 n*M* to 3 μ*M*. Bound radioligand was separated from free by LH-20 Sephadex gel filtration and quantified by counting as described above. Displacement curves were analyzed by a least-squares curve-fitting method with the computer program ALLFIT (*19*).

In Vitro Autoradiography of Occupied Androgen Receptor. The prostate gland was removed from Sprague-Dawley rats (80 days of age) that were gonad-intact or that had been castrated 3 days earlier, mounted onto a cryostat specimen holder and rapidly frozen on crushed dry ice. Thin (20 μm-thick) cryostat sections were adhered to poly-*l*-lysine-coated microscope slides by briefly thawing the sections and then refreezing immediately. Occupied androgen receptors were detected in the tissue sections by an in vitro exchange assay as previously described for [^{3}H]R1881 with the modification that 7α-[^{125}I]IDHT was the ligand (*20*). Under the conditions of this assay the unoccupied androgen receptor is either destroyed or leached from the sections. Briefly, sections were incubated for 72 hr at 4°C in buffer containing 5.0 n*M* 7α-[^{125}I]IDHT in the presence or absence of 1 μ*M* radioinert 5α-DHT. After incubation, sections were washed to remove unbound 7α-[^{125}I]IDHT, as follows: the slides were quickly drained of incubation buffer and loaded into slide racks, then rinsed in PM buffer (3.0 m*M* $MgCl_2$, 1.0 m*M* KH_2PO_4, pH 6.8) for 5 min, rinsed twice in PM buffer containing 0.1% triton X-100 for 5 min and then rinsed twice in PM buffer for 5 min. Finally, the slides were briefly dipped into distilled water to remove buffer salts and fan-dried at 4°C. When completely dry, the sections were allowed to equilibrate to room temperature and placed against high-resolution autoradiographic film (Hyperfilm-3H, Amersham, Oakville, ON) for

TABLE 1
Distribution of Tissue Radioactivity Following Intravenous Injection of 7α-[^{125}I]IDHT into Castrated Male Rats

Tissue	% Injected Dose/g 0.5 hr	1 hr	1 hr (blocked)*	4 hr
Prostate	0.29 ± 0.06	0.52 ± 0.03§	0.17 ± 0.03	0.35 ± 0.03
Blood	0.33 ± 0.05	0.23 ± 0.04	0.23 ± 0.05	0.31 ± 0.03
Muscle	0.10 ± 0.02	0.09 ± 0.01¶	0.06 ± 0.004	0.08 ± 0.01
Spleen	0.17 ± 0.03	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01
Fat	0.24 ± 0.02	0.30 ± 0.05	0.19 ± 0.02	0.10 ± 0.01
Lung	0.28 ± 0.04	0.23 ± 0.03	0.22 ± 0.02	0.26 ± 0.01
Kidney	0.47 ± 0.06	0.32 ± 0.05	0.23 ± 0.01	0.25 ± 0.02
Liver	1.02 ± 0.14	0.88 ± 0.10	0.78 ± 0.05	0.44 ± 0.03
Thyroid†	1.43 ± 0.53	6.91 ± 2.59	10.0 ± 3.06	13.2 ± 5.53
Prostate/Blood	0.87 ± 0.09	2.50 ± 0.36	1.02 ± 0.29	1.21 ± 0.12
Prostate/Sp.Mu.Lu.‡	1.57 ± 0.12	3.52 ± 0.47	1.18 ± 0.07	2.15 ± 0.12

*Blocked animals were injected s.c. with 250 μl of a 2.0 mg/ml solution of 5α-DHT in propylene glycol/saline (3:2) 15 min before the injection of the [^{125}I]androgen; n = 5; values are ± s.e.m.

†Thyroid also contained adhering esophagus.

‡Sp.Mu.Lu. = the average of the tissues, spleen, muscle and lung. The tissues from the 1-hr group were compared to the 1-hr blocked group given nonradioactive 5α-DHT by Student Newman-Keuls test.

§Prostate statistically significantly different, $p < 0.01$.

¶Muscle statistically different, $p < 0.05$. There was no significant effect of 5α-DHT blocking in all other tissues.

Castrated male rats were injected in the tail vein with 5 μCi 7α-[^{125}I]IDHT and then killed at the indicated times.

14 hr. The film was photodeveloped by standard procedures using Kodak D-19 photodeveloper.

Distribution In Vivo. Male Sprague-Dawley rats, approximately 250 g, were castrated and on the following day they were injected in the tail vein with 5 μCi (2.3 pmole) of 7α-[^{125}I]IDHT in 100 μl of ethanol/saline (1:4). Control animals were injected subcutaneously with 500 μg of 5α-DHT in 250 μl of propylene glycol/saline (3:2) 15 min before the administration of the [^{125}I]tracer. At 0.5, 1 and 4 hr after the injection of the tracer the animals were anesthetized with ether and killed by decapitation. Immediately afterwards, various tissues were extirpated, weighed and counted. The entire thyroid was taken, which contained some adhering esophagus. The results are presented in Table 1. Data on the uptake of 7α-[^{125}I]IDHT by different tissues were tested for normal distribution and homogeneity of variance by Sigmastat for Windows software (Jandel Corp., Corte Madera, CA). Significant inhomogeneity of variance noted in the raw data was eliminated using a reciprocal transformation of the data.

RESULTS

While several methods for the direct conversion of alcohols to iodo compounds are known, few of these employ NaI as the iodinating agent. The procedure described by Olah et al. (*15*) in which an alcohol is reacted with trichloromethylsilane and NaI in acetonitrile is such a method. Since radioiodination using I^- is preferable to that using I_2,we investigated the possibility of using the Olah method to effect the direct conversion of a 7-hydroxysteroid to the 7-[^{125}I]iodo product as we previously described for radioinert 7α-IDHT. In a number of attempted radiosyntheses with 10 mCi of Na^{125}I and several concentrations (including large molar excess) of 7β-hydroxy-5α-dihydrotesterone-17β-acetate and trichloromethylsilane in a process similar to the method previously described (but on microscale as required by the radiosynthesis) (*14*), we were unable to obtain the desired 7α-^{125}I product (data not shown). In order to determine whether this failure was due to the uncertain nature of the radiochemical reaction, we synthesized the chromogenic reactant, 5b, with which we could follow the course of the microreaction by HPLC using sensitive UV detection. In reactions with concentrations of NaI chosen to duplicate that of the radiochemical synthesis (0.7 μg NaI, 5 equivalents of trichloromethylsilane and 5 equivalents of 5b in 6–20 μl of CH_3CN at 80°C for 2 hr) we did not detect any production of 7α-IDHT-17β-*p*-nitrobenzoate, 7, in HPLC system 1 at 254 nm (not shown). If 7 was formed we could have easily detected a 1% yield. Increasing the concentration of all reactants 10-fold led to the 7α-iodo product in about a 10% yield (based on NaI).

It was apparent that another route was necessary for incorporation of radioiodine at C-7α. The synthetic scheme we chose was nucleophilic displacement of a 7β-tosylate group. We synthesized the 7β-tosylate of 5α-dihydrotestosterone-17β-PNB, 6, (Fig. 1) as the reactant for insertion of iodine by nucleophilic attack of iodide, including radioiodine (Fig. 2). As mentioned above, the *p*-nitrobenzoyl (PNB) ester was selected as a chomophore that is easily detected and quantified in the UV detector of the HPLC system used to purify the radio-labeled product. The starting material 1 was prepared as previously described (*14*). Hydrolysis of the acetate group with Na_2CO_3 produced 2 and esterification with *p*-nitrobenzoyl chloride gave the PNB ester 3 in a 90% overall yield. Reduction with $NaBH_4$ in the presence of $CeCl_3$ (*21*) gave a mixture of epimeric C-7 alcohols 4. This mixture was not separable by TLC in several solvent systems. After removal of the C-3 ketal with *p*-TsOH in acetone, the desired 7β-alcohol 5b (7β-/7α- = 7:1) was isolated in 63% yield. Our assignment of stereochemistry is based on the ^{1}H NMR chemical shift data of 7α- and 7β-alcohols as discussed previously (*14*). The 7β-tosylate 6 was prepared by reacting 5b with 20 equiv. of *p*-TsCl at 4°C for 5 days as described (*22*). It had been reported that 7β-tosylates of 5α-steroids (cholestanes) decompose during chromatography on neutral alumina (*23*), but we found that 6 could be purified by flash chromatography on silica gel. It is stable when stored in a freezer as a solid or dissolved in CH_3CN. Iodination of 6 with NaI produced the 7α-iodosteroid 7 in 82% yield. As described above, compound 7 was also prepared in 40% yield (in mg scale) from the mixture of 5a and 5b by the Olah procedure.

Compound 6 is facilely converted into [^{125}I]-7 when treated

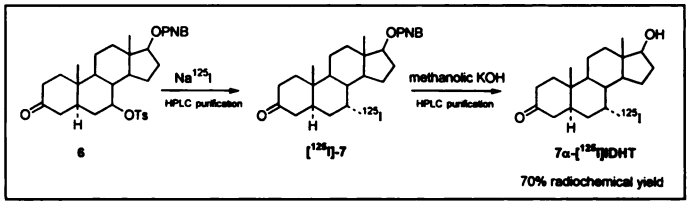

FIGURE 2. The procedures for the synthesis of 7α-[^{125}I]IDHT.

with Na^{125}I in CH_3CN with a yield of 90% (Fig. 2). The product, [^{125}I]-7, was purified by HPLC in system 1 where it is cleanly separated from unreacted 6, which is much more polar. The mass of the radioactive product was quantified by UV absorption and comparison to a standard curve produced with 7. The specific activity of the product as calculated from the mass and the radioactivity showed that [^{125}I]-7 was carrier-free (~2,200 Ci/mmole). The target compound 7α-[^{125}I]IDHT was obtained in 77% yield from [^{125}I]-7 by saponification with methanolic KOH, followed by purification in HPLC system 2 (overall radiochemical yield of 7α-[^{125}I]IDHT from 6 in Figure 2 was 70%). The radioactive products, [^{125}I]-7 and 7α-[^{125}I]IDHT, migrated in HPLC systems 1 and 2 respectively, exactly as predicted from the authentic steroids. The identity of the radioactive peak as 7α-[^{125}I]IDHT was confirmed by its co-migration with standard 7α-IDHT by TLC and in two additional HPLC systems.

7α-[^{125}I]IDHT was tested for binding to the androgen receptor in cytosol from the MCF-7 human breast cancer cell line. As can be seen in Figure 3a, 7α-[^{125}I]IDHT binds in a saturable manner to the cytosol. Nonspecific binding in the presence of excess 5α-DHT is very low. Scatchard analysis of the binding data (Fig. 3b) shows high affinity binding, K_d = 0.260 n*M*, to a single class site. For comparison, the binding of the classical androgen receptor ligand [^{3}H]R1881 was analyzed in parallel. Figure 3d, [^{3}H]R1881, has a higher affinity for the androgen receptor K_d = 0.067 n*M*. However, the analysis of the androgen receptor is much more sensitive with 7α-[^{125}I]IDHT than it is with the [^{3}H]ligand; there is considerably more radioactivity in the receptor bound fraction at saturation with 7α-[^{125}I]IDHT than with [^{3}H]R1881 (compare Fig. 3a with Fig. 3c). This is due to the large difference in the specific activity of the two isotopes with which the individual ligands are labeled. The apparent specific activity of 7α-[^{125}I]IDHT determined in the binding experiment was the same as [^{125}I]-7 determined by UV absorption. The concentration of the [^{125}I]ligand was calculated by assuming that it too was carrier-free, specific activity of 2,200 Ci/mmole. Using this value, the concentration of androgen receptor binding sites in the cytosol was calculated by Scatchard analysis (Fig. 3b) to be 32.7 n*M*. This compares extremely well with 33.0 n*M* that was calculated for the concentration of binding sites in a parallel experiment when the ligand was [^{3}H]R1881 of known specific activity (Fig. 3d). This confirms that 7α-[^{125}I]IDHT is carrier-free.

Competition studies with several types of steroids were also performed with MCF-7 cytosol and 7α-[^{125}I]IDHT as the ligand. These experiments show the expected specificity for androgens as well as the relative binding of the various androgen receptor ligands. A typical experiment is shown in Figure 4 with 5α-DHT ≥ R1881 > testosterone = 7α-IDHT. Other classes of steroids, competed poorly for binding; the relative binding affinity (RBA) of estradiol was 2%, progesterone 1% and dexamethasone <0.1% of 5α-DHT. This is in good

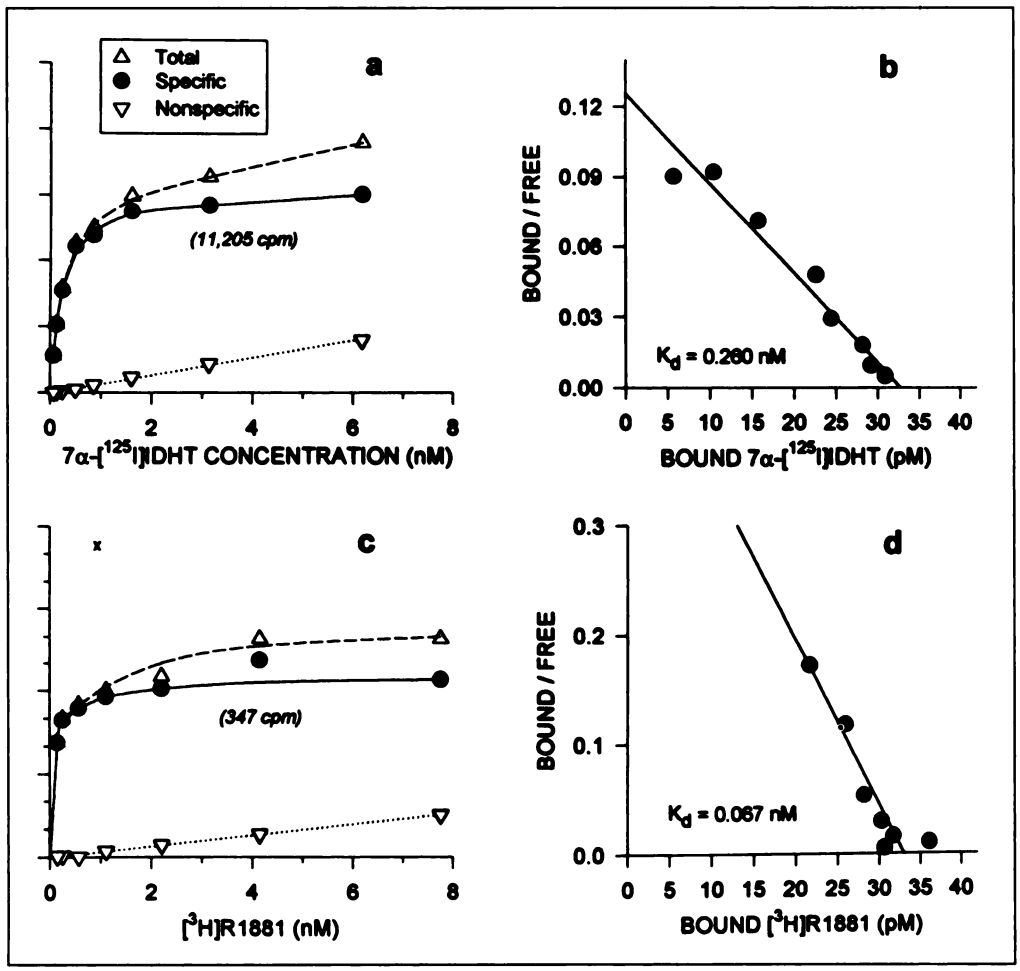

FIGURE 3. Saturation analysis of 7α-[^{125}I]IDHT and [^{3}H]R1881 binding to MCF-7 cell cytosol. Saturation binding curves are shown in panels a and c and Scatchard representation of the data for specific binding are shown in panels b and d. The cpm shown in brackets under the curves in panels a and c are the total cpm of specifically bound radioactivity. They are shown to emphasize the large difference between the two ligands.

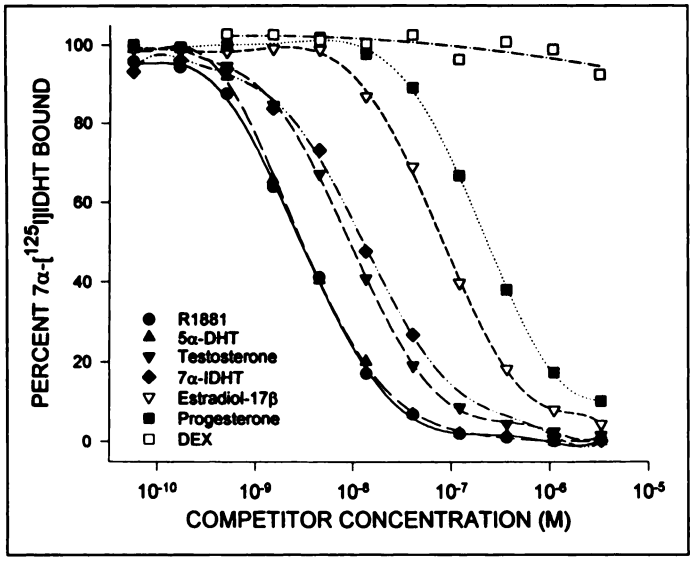

FIGURE 4. Competition of various ligands for 7α-[^{125}I]IDHT binding to MCF-7 cytosol. Data are presented as the percentage of 7α-[^{125}I]IDHT specifically bound in the absence of competitor. Nonspecific binding was taken as the level of 7α-[^{125}I]IDHT binding in the presence of 1.0 m*M* 5α-DHT. RBA values relative to R1881, determined at the 50% binding level as calculated by a least-squares curve-fitting method were: R1881 = 100%, 5α-DHT = 103%, testosterone = 36.3%, 7α-IDHT = 21.4%, estradiol = 3.72%, progesterone = 1.39%, dexamethasone < 0.001%.

agreement with the competition of these classes of steroid to the androgen receptor in MCF-7 cell cytosol with [^{3}H]DHT or rat prostatic cytosol with [^{3}H]R1881 as ligands (*24,25*).

The experiment performed on the in vitro autoradiography of the occupied androgen receptor shows that 7α-[^{125}I]IDHT is an excellent ligand for this analysis. High levels of radioactivity are seen in prostate sections from noncastrate rats that were incubated with 7α-[^{125}I]IDHT (Fig. 5A), and was very low in both tissue from castrated rats (Fig. 5C) or in sections from intact animals incubated with both 7α-[^{125}I]IDHT and saturating concentrations of 5α-DHT (nonspecific binding) (Fig. 5B). The prostate sections were exposed to film for only 14 hr. This compares to 10 wk in similar experiments with [^{3}H]R1881 as ligand (*20*). The conditions used for binding of the prostatic sections allows exchange of androgens that have bound to the receptor in vivo, e.g., the ligand activated androgen receptor. The unoccupied receptor that has not bound androgen in vivo is very labile and is thought to be lost under the conditions of the exchange incubation due to its loose association with chromatin (*20*). Consequently, the section obtained from a castrate animal shows no specifically bound ligand (Fig. 5C). The unbound ligand is removed from the sections by extensive washing, including washing with buffers containing triton X-100 (see Materials and Methods section).

We determined whether 7α-[^{125}I]IDHT is concentrated in vivo by a receptor mediated mechanism. Five μCi of 7α-[^{125}I]IDHT was injected into castrated male rats and the concentration of radioactivity in various tissues was determined at several times after the injection. The results in Table 1 show that at 1 hr after injection there was significantly more radioactivity in the prostate gland than the other tissues studied with the exception of liver and thyroid. Analysis of variance revealed significant differences between the tissues ($F = 32.83$ d.f. 9,80 $p < 0.0001$) as well as significant effects of the unlabeled 5α-DHT treatment. ($F = 13.80$ d.f. 1,80 $p < 0.0004$). The effect of 5α-DHT appears to be tissue specific as indicated by the significant interaction effect between the tissue and 5α-DHT treatment factors ($F = 2.52$ d.f. 9,80 $p = 0.0136$). Newman-

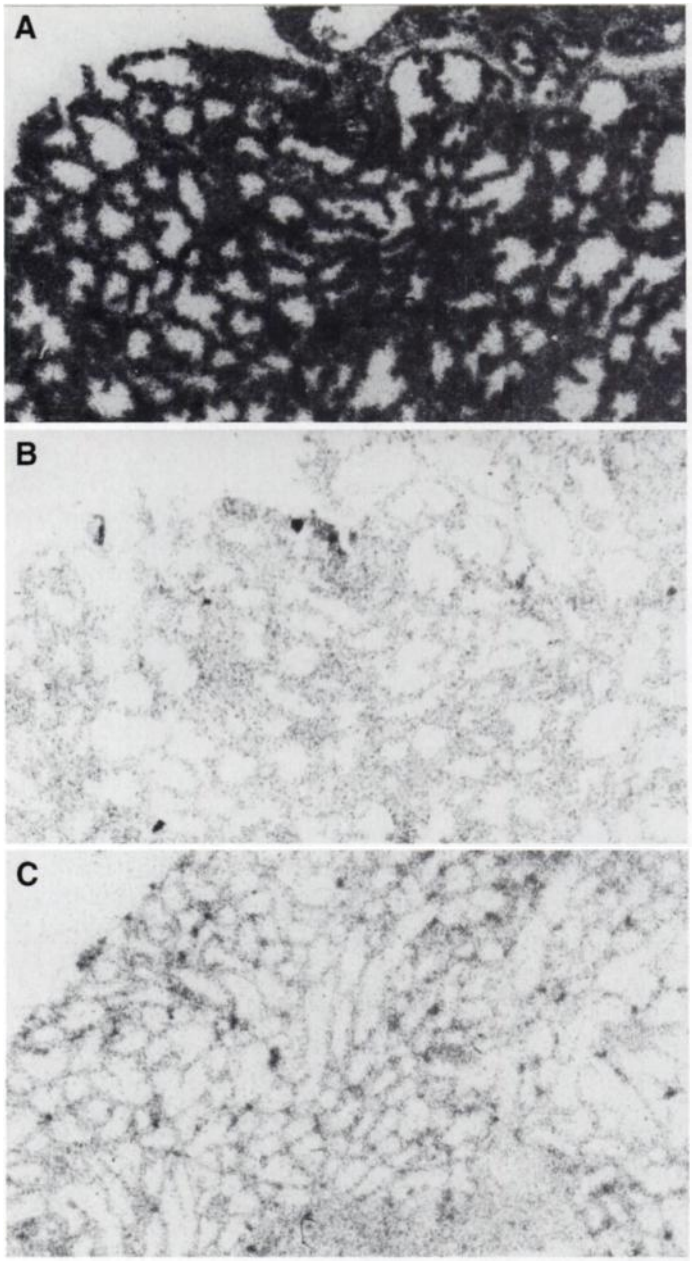

FIGURE 5. In vitro autoradiographic images of binding of 7α-[^{125}I]IDHT to rat prostate. (A) Prostate from a gonad-intact rat. (B) Adjacent sections (nonspecific binding) from the intact rat were incubated in parallel with the radiolabeled androgen and 1 μ*M* unlabeled DHT. (C) Prostate from a rat 3 days after castration.

Keuls multiple range tests reveals significant differences only in prostate and muscle between the 1 hr. group and the 1 hr group treated with 5α-DHT to occupy all of the androgen receptors. It is clear from this experiment that the androgen target organ, prostate, has significantly more radioactivity than most other tissues and that this concentration of radioactivity is receptor mediated since it is obliterated when the androgen receptors are saturated with a potent androgen. Although blood had relatively low levels of radioactivity, it should be borne in mind that rats, unlike humans, have no sex hormone binding globulin in their blood (*26*). Muscle contained only low levels of radioactivity and showed a statistically significant decrease when blocked with 5α-DHT. This most probably reflects its status as an androgen target organ that contains comparatively low levels of androgen receptor (*27*). The specific concentration of ^{125}I in the prostate is also seen at 0.5 and 4 hr but to a lesser degree.

DISCUSSION

The synthesis of 7α-[^{125}I]IDHT through attack of the 7β-tosylate by $^{125}I^-$ has several advantages: the intermediate tosylate is stable, the reaction proceeds quickly and in high yield and the product is carrier free. Also important, the reagent is readily available radioactive NaI. Although we used a two step procedure to purify the [^{125}I]product, the first HPLC of [^{125}I] 7 in system 1 can be eliminated. We isolated [^{125}I]-7 in order to determine its specific activity by UV adsorption. The mixture from the exchange reaction can be saponified directly, followed by purification in HPLC system 2. This makes the synthesis and purification sufficiently rapid for use with ^{123}I. 7α-IDHT has characteristics that make it an excellent ligand for the androgen receptor. It binds with high affinity to the androgen receptor with a K_d of 0.26 n*M*, about the same as that of testosterone (Fig. 4). In addition, 7α-[^{125}I]IDHT shows low nonspecific binding (Fig. 3a), a characteristic that encumbers the E and Z isomers 7α-methyl-17α-(2′-[^{125}I]iodovinyl)-19-nortestosterone, ligands that have a high affinity for the androgen receptor (*13*). The advantage of 7α-[^{125}I]IDHT as a ligand are readily apparent from Figures 3 and 5; its high specific activity allows the very sensitive and specific detection of the androgen receptor; it does not bind to other steroid receptors (*14*). Morever, 7α-[^{125}I]IDHT is particularly useful for the detection of activated androgen receptor by in vitro autoradiographic analysis (Fig. 5). Through this unique analysis, the receptor that is physiologically (in vivo) activated by binding circulating androgens can be quantified in tissue sections (*20*). It thus provides a unique determinant of steroid action. A limiting factor in this technique has been the long exposure time (months) against film that is required to produce an image when classical [^{3}H]ligands are used. The use of 7α-[^{125}I]IDHT (2,200 Ci/mmole) decreases this exposure period to only overnight (~14 hr). While this obviously facilitates studies of the androgen receptor, its importance for a clinical evaluation of prostatic tissue samples would be critical.

CONCLUSION

While the uptake/retention in the prostate relative to other tissues is not large, it is apparent that substitution of radioiodine into the steroid nucleus at the 7α-position produces a ligand with many favorable characteristics. As can be seen in Table 1, 7α-[^{125}I]IDHT is concentrated in the prostate by an androgen receptor regulated mechanism. Iodination at 7α- of other steroid analogs that are better protected from metabolism and have inherently higher affinity for the androgen receptor would be expected to lead to radioiodo-androgens that are excellent imaging agents.

APPENDIX

Synthesis of Nonradioactive Steroidal Intermediates and Products

General. Thin-layer chromatography (TLC) was performed using Analtech silica gel plates (GHLF, 0.25 mm) and visualized using phosphomolybdic acid or UV illumination. All nonradioactive reactions were performed under an atmosphere of dry N_2. Melting points were obtained in a Mel-temp apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer Model 1600 FT-IR or Beckman AccuLab 4 instruments. High-performance liquid chromatography was performed isocratically on a Waters 600E system equipped with an Omniscribe recorder, a Waters 484 variable wavelength detector set at 254 nm and an analytical silica gel column (Altex Ultrasphere 5μm Si, 4.6 mm i.d. × 25 cm). Radiolabeled compounds were purified by HPLC using a Waters modular system consisting of a U6K injector, an *M*-45 pump, a Model 440 detector set at 254 nm and either a reverse-phase column (Altex Ultrasphere 5 μm ODS, 4.6 mm i.d. × 25 cm) or an analytical silica gel column (Merk Hibar 5μm Si-60, 4.6 mm i.d. × 25 cm). Purification by flash-column chromatography was performed according to the procedure of Still (*28*) using 230–400 mesh silica gel (EM Science). Solvents for chromatography and extraction were reagent grade. Low- and high-resolution fast atom bombardment (FAB) mass spectra were obtained on a VG instrument (ZAB SE) using a matrix of *m*-nitrobenzyl alcohol by Dr. Walter J. McMurray at the Yale University Comprehensive Cancer Center. Elemental analyses were performed by Schwarzkopf Micro Analytical Laboratory, Woodside NY.

Tetrahydrofuran (THF) was distilled from benzophenone ketyl under N_2 immediately before use. Acetonitrile and pyridine were distilled from CaH. Steroids were obtained from Steraloids (Wilton, NH), Na^{125}I and [^{3}H]R1881, methyltrienolone, (86 Ci/mmole) from Dupont New England Nuclear Co. (North Billerica, MA) and other reagents from Aldrich (Milwaukee, WI) were used without further purification.

3,3-(Ethylenedioxy)-17β-hydroxy-5α-androstan-7-one (2). A solution of ketal 1 (14)(1.05 g, 2.68 mmole) in 110 ml of 1/5 (v/v) saturated aqueous Na_2CO_3. MeOH was stirred and heated at 90°C for 3 hr. The reaction was allowed to cool to room temperature, diluted with H_2O (100 ml), and extracted with CH_2Cl_2 (3 × 100 ml). Combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to give 1.01 g (100%) of 2 as a white solid. The compound was pure by TLC and used in the next step without further purification. Data for 2: mp 198–199°C (MeOH/petroleum ether); TLC R_f 0.1 (hexane/EtOAc, 3:1); ^{1}H NMR (490 MHz, $CDCl_3$) δ 3.91 (8-line signal, 4H, 3-ketal), 3.66 (dt, 1H, J_t = 8.2 Hz, J_d = 5.5 Hz, H-17α), 1.09 (s, 3H, H-19), 0.74 (s, 3H, H-18); IR ($CHCl_3$) 3500, 1700, 1170, 1083 cm^{-1}; MS (FAB) 610 [MH]+.

3,3-(Ethylenedioxy)-17β-(p-nitrobenzoyloxy)-5α-androstan-7-one (3). A solution of ketone 2 (116 mg, 0.334 mmole) and *p*-nitrobenzoyl chloride (310 mg, 1.67 mmole) in pyridine (20 ml) was stirred at room temperature for 2 hr. The reaction mixture was diluted with H_2O (40 ml) and extracted with CH_2Cl_2 (3 × 30 ml). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (30 ml). The aqueous layers were back extracted with CH_2Cl_2 (3 × 30 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to give 150 mg (90%) of 3 as a white solid. Data for 3: mp 298–299°C (acetone/trace pyridine); TLC R_f 0.28 (1% MeOH/CH_2Cl_2); ^{1}H NMR (490 MHz, $CDCl_3$) δ 8.28 (d, 2H, J = 8.8 Hz, Ar-H), 8.18 (d, 2H, J = 8.8 Hz, Ar-H), 4.92 (t, 1H, J = 8.2 Hz, H-17α), 3.92 (8-line signal, 4H, 3-ketal), 1.11 (s, 3H, H-19), 0.93 (s, 3H, H-18); IR ($CHCl_3$) 1720, 1710, 1530, 1290, 1280, 1130, 1105, 1090 cm^{-1}; MS (FAB) 498 [MH]+; HRMS (FAB) calculated for $C_{28}H_{36}NO_7$ 498.2492, found 498.2507. Anal. Calcd for $C_{28}H_{35}NO_7$ (497.588): C, 67.59; H, 7.09; N, 2.81. Found: C, 67.25; H, 7.36; N, 2.80.

3,3-(Ethylenedioxy)-17β-(p-nitrobenzoyloxy)-5α-androstan-7α-ol (4a) and 3,3-(Ethylenedioxy)-17β-(p-nitrobenzoyloxy)-5α-androstan-7β-ol (4b). This procedure is based on the literature method (*21*). Ketone 3 (300 mg, 0.620 mmole) and cerium(III) chloride heptahydrate (3.25 g, 8.72 mmole) were stirred in a mixture of THF (68.8 ml), MeOH (68.8 ml) and H_2O (6.2 ml) at room temperature as sodium borohydride (330 mg, 8.72 mmole) was added in five portions over 5 min. The reaction mixture was stirred at room temperature for 30 min, diluted with saturated aqueous NH_4Cl solution (150 ml) and H_2O (50 ml), and extracted with CH_2Cl_2 (3 × 150 ml).

Combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to give a white solid weighing 300 mg (98% crude yield), which was composed of a 1:5 mixture of 7α-hydroxy (4a) and 7β-hydroxy (4b) epimers as determined by inspection of the ^1H NMR spectrum. This mixture was inseparable by TLC in various solvent systems and was used in the next step without purification. TLC R_f 0.18 (hexanes/EtOAc, 2:1); IR ($CHCl_3$) 3500, 1719, 1531, 1279, 1120, 1096 cm^{-1}; MS (FAB) 500 [MH]+; HRMS (FAB) calcd. for $C_{28}H_{38}NO_7$ 500.2648, found 500.2629. ^1H NMR data attributable to 4a: (490 MHz, $CDCl_3$) δ 4.91 (dd, 0.17H, H-17α), 0.93 (s, 0.5H, H-19), 0.84 (s, 0.5H, H-18). ^1H NMR data attributable to 4b: (490 MHz, $CDCl_3$) δ 8.28 (d, 2H, J = 9.0 Hz, Ar-H), 8.19 (d, 2H, J = 9.0 Hz, Ar-H), 4.87 (dd, 0.83H, J = 9.0, 7.7 Hz, H-17α), 3.94 (8-line signal, 4H, 3-ketal), 3.43 (m, 1H, half-width 22.2 Hz, H-7α), 0.96 (s, 2.5H, H-19), 0.87 (s, 2.5H, H-18).

7α-Hydroxy-17β-(4-p-nitrobenzoyloxy)-5α-androstan-3-one (5a) and 7β-Hydroxy-17β-(4-p-nitrobenzoyloxy)-5α-androstan-3-one (5b). A solution of the crude mixture of alcohols 3a and 3b (295 mg), *p*-toluenesulfonic acid (9.5 mg, 50 μmole) in acetone (9.5 ml) was heated in a sealed screw-capped tube at 60°C for 3 hr. Another portion of *p*-TsOH (14 mg, 73 μmole) was added and the reaction was heated at 70°C for an additional 3 hr. The reaction mixture was diluted with H_2O (5 ml), concentrated to about 5 ml, diluted with saturated aqueous $NaHCO_3$ solution (30 ml), and extracted with CH_2Cl_2 (4 ×, 50 ml). Combined organic extracts were washed with H_2O (20 ml), dried (Na_2SO_4), and concentrated in vacuo. Purification of the residue by flash chromatography on a 3 × 19 cm column of silica gel using EtOAc-isooctane (2:1) as eluent gave 27 mg (9%) of the 7α-hydroxy compound 5a and 179 mg (63%) of the 7β-hydroxy compound 5b.

Data for 5a: mp 234–235°C (acetone/petroleum ether); TLC R_f 0.18 (hexane/EtOAc, 2:1); ^1H NMR (490 MHz, $CDCl_3$) δ8.29 (d, 2H, J = 8.9 Hz, Ar-H), 8.19 (d, 2H, J = 8.9 Hz, Ar-H), 4.93 (dd, 1H, J = 8.9, 7.5 Hz, H-17α), 3.92 (m, 1H, half-width 8.9 Hz, H-7β), 1.04 (s, 3H, H-19), 0.97 (s, 3H, H-18); IR ($CHCl_3$) 3500, 1720, 1350, 1280, 1120, 1105 cm^{-1}; MS (FAB) 456 [MH]+ HRMS (FAB) calculated for $C_{26}H_{34}NO_6$ 456.2386, found 456.2397. Anal. Calcd for $C_{26}H_{33}NO_6$ (455.548): C, 68.55; H, 7.30; N, 3.07. Found: C, 68.41; H, 7.59; N, 3.10.

Data for 5b: mp 214–216°C (acetone/petroleum ether); TLC R_f 0.13 (hexane/EtOAc, 2:1); ^1H NMR (490 MHz, $CDCl_3$) δ 8.28 (d, 2H, J = 8.9 Hz, Ar-H), 8.19 (d, 2H, J = 8.9 Hz, Ar-H), 4.88 (dd, 1H, J = 8.9, 7.5 Hz, H-17α), 3.44 (m, 1H, half-width 21.9 Hz, H-7α), 1.07 (s, 3H, H-19), 1.00 (s, 3H, H-18); IR ($CHCl_3$) 3500, 1710, 1520, 1345, 1275, 1120, 1100 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 260 (4.1) nm; MS (FAB) 456 $[MH]^+$; HRMS (FAB) calculated for $C_{26}H_{34}NO_6$ 456.2386, found 456.2400. Anal. Calcd for $C_{26}H_{33}NO_6$ (455.548): C, 68.55; H, 7.30; N, 3.07. Found: C, 68.58; H, 7.46; N, 3.07.

7β-p-Toluenesulfonyloxy-17β-(4-nitrobenzoyloxy)-5α-androstan -3-one (6). A solution of 7β-hydroxy steroid 5b (169 mg, 0.372 mmole) and p. toluenesulfonyl chloride (1.42 g, 7.45 mmole) in anhydrous pyridine (50 ml) was allowed to stand at 4°C for 5 days in a sealed screw-capped tube (*22*). The reaction mixture was poured into CH_2Cl_2 (80 ml) and washed with H_2O (50 ml). The aqueous layer was back-extracted with CH_2Cl_2 (2 × 50 ml). Combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. Purification of the residue by flash chromatography on a 2 × 15 column of silica gel using hexane-EtOAc (2:1) as eluent gave 213 mg (94%) of 6 as a yellow oil. Crystallization from acetone/petroleum ether gave the analytical sample as white needles. Data for 6: mp 160–162°C dec.; TLC R_f 0.2 (hexane/EtOAc, 2:1); ^1H NMR (490 MHz, $CDCl_3$) δ 8.28 (d, 2H, J = 9.0 Hz, Ar-H), 8.18 (d, 2H, J = 9.0 Hz, Ar-H), 7.78 (d, 2H, J = 8.1 Hz, Ar-H), 7.34 (d, 2H, J = 8.1 Hz, Ar-H), 4.86 (dd, 1H, J = 9.4, 7.3 Hz, H-17α), 4.48 (m, half-width 27.8 Hz, H-7α), 2.45 (s, 3H, Ar-CH_3), 1.03 (s, 3H, H-19), 0.94 (s, 3H, H-18); IR ($CHCl_3$) 1710, 1520, 1280, 1170, 1120, 1110 cm^{-1}: MS (FAB) 610 [MH]+; HRMS (FAB) calcd. for $C_{33}H_{40}NO_8S$ 610.2475, found 610.2450. Anal. Calcd for $C_{33}H_{39}NO_8S$ (609.736): C, 65.00; H, 6.45; N, 2.30; S, 5.26. Found: C, 64.93; H, 6.67; N, 2.24; S, 5.24.

7α-Iodo-17β-(4-nitrobenzoyloxy)-5α-androstan-3-one (7). Method A. Nucleophilic Substitution Reaction of Tosylate (6) with Sodium Iodide. A solution of tosylate 6 (71.0 mg, 0.116 mmol) and sodium iodide (174 mg, 1.16 mmole) in anhydrous acetonitrile was stirred and heated at 105°C for 3 hr. The reaction mixture was allowed to cool to room temperature, diluted with CH_2Cl_2 (75 ml), and washed with 10% aqueous sodium thiosulfate solution (20 ml) and H_2O (20 ml). The organic phase was dried (Na_2SO_4) and concentrated in vacuo. Purification of the residue by flash chromatography on a 2 × 13 cm column of silica gel using hexane-EtOAc (3:1) as eluent gave 54.2 mg (82%) of 7 as a white solid. Data for 7: mp 168–172°C (acetone/petroleum ether); TLC R_f 0.52 (hexane/petroleum ether, 2:1); ^1H NMR (490 MHz, $CDCl_3$) δ 8.29 (d, 2H, J = 8.8 Hz, Ar-H), 8.19 (d, 2H, J = 8.8 Hz, Ar-H), 4.92 (dd, 1H, J = 9.0, 7.7 Hz, H-17α), 4.65 (m, 1H, half-width 5.5 Hz, H-7α), 1.09 (s, 3H, H-19), 1.01 (s, 3H, H-18); IR ($CHCl_3$) 1705, 1520, 1340, 1270, 1110, 1095 cm^{-1}; MS (FAB) 566 [MH]+. Anal. Calcd for $C_{26}H_{32}NO_5I$ (565.447): C, 55.23; H, 5.70; N, 2.48; I, 22.44. Found C, 55.12; H, 5.68; N, 2.27; I, 22.65.

Method B. Reaction of a Mixture of 5a and 5b with Trichloromethylsilane and Sodium Iodide. The following iodination procedure (*15*) was based on the Olah method we previously used for the related 17β-acetates (*14*). A 95:5 mixture of 7α- and 7β-hydroxy steroids 5a and 5b (76.0 mg, 0.167 mmole) was placed in a screw capped test tube followed by chloroform (115 μl), an 0.8 *M* solution of sodium iodide in acetonitrile (328 μl, 0.262 mmole), and trichloromethylsilane (23 μl, 29 mg, 0.196 mmole). The tube was sealed and the reaction was stirred and heated at 85°C for 2 hr. The reaction was allowed to cool to room temperature, diluted with CH_2Cl_2 (100 ml), and washed with a 10% aqueous solution of sodium thiosulfate (20 ml) and H_2O (20 ml), The organic phase was dried (Na_2SO_4) and concentrated in vacuo. Purification of the residue by flash chromatography on a 1 × 14 cm column of silica gel eluting with CH_2Cl_2 gave 38.4 mg (40%) of 7 as a white solid.

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Variables Influencing Tumor Dosimetry in Radioimmunotherapy of CEA-Expressing Cancers with Anti-CEA and Antimucin Monoclonal Antibodies

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In this study, we examined the factors that may influence tumor dosimetry in the radioimmunotherapy of solid, CEA-expressing cancers. **Methods:** Data from 119 tumors in 93 patients with CEA-expressing cancers were analyzed. The patients underwent radioimmunotherapy with the ^{131}I-labeled IgG_1 anti-CEA antibodies NP-4 ($K_a = 10^8\ M^{-1}$) or MN-14 ($K_a = 10^9\ M^{-1}$), its humanized form hMN-14, as well as the anticolon-specific antigen-p (CSAp) antibody, Mu-9. For dosimetry, the biodistribution, targeting kinetics and cumulated activity of tumors and organs were determined from planar and SPECT imaging. **Results:** An inverse logarithmic relationship between tumor size and antibody uptake was found for both anti-CEA antibodies, whereas no such relationship was found for Mu-9. The absolute tumor uptake was identified as the most important factor determining the radiation dose to the tumor ($r = 0.9$), with the biological half-life of the antibody in the tumor being of secondary importance ($r = 0.5$). No significant difference in tumor uptake was found between both anti-CEA antibodies, despite their tenfold difference in affinity. At comparable masses, colorectal and medullary thyroid cancers had significantly higher tumor uptakes ($p = 0.02$), as well as tumor-to-red marrow dose ratios, than other cancer types. The tumor half-lives of the anti-CEA antibodies were significantly lower in colorectal than in all other tumor types ($p = 0.01$). **Conclusion:** In radioimmunotherapy, tumor uptake appears to be the most important dose-determining factor. Differences in antibody affinity are reflected by differences in the biological half-life, not the absolute uptake. Especially favorable conditions for anti-CEA antibodies seem to prevail in colorectal cancer patients having minimal disease, as well as in medullary thyroid cancer, where cytotoxic tumor doses might be expected. Antimucin antibodies may have a particular advantage in the treatment of patients with larger colorectal tumors.

Key Words: radioimmunotherapy; monoclonal antibodies; carcinoembryonic antigen; tumor dosimetry

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Although radioimmunotherapy (RAIT) of lymphoma and other hematological tumors is increasingly being accepted as a potent new mode of treatment (*1,2*), its success in solid tumors is still limited (*2,3*). In preclinical models, RAIT of colorectal cancer has been shown to be more effective than an equitoxic chemotherapy of 5-fluorouracil and leucovorin (*4*). In an adjuvant (minimal metastatic disease) model, RAIT was shown to be highly effective for achieving even long-term cures (*5*). The biological, physiological, biophysical and biochemical conditions in such animal models, however, can be fundamentally different from the clinical situation (*6*). Mathematical

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