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THE SYNTHESIS AND TESTING OF E-17α-(2-IODOVINYL)-5α-DIHYDROTESTOSTERONE AND Z-17α-(2-IODOVINYL)-5α-DIHYDROTESTOSTERONE AS γ-EMITTING LIGANDS FOR THE ANDROGEN RECEPTOR

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Summary—Two iodinated steroids, E-17α-(2-iodovinyl)-5α-dihydrotestosterone and Z-17α-(2-iodovinyl)-5α-dihydrotestosterone were synthesized in a search for a γ-emitting androgen that binds with high affinity to the androgen receptor. Such compounds would be extremely useful research tools for studies of androgen responsive tissues and as *in vivo* probes of androgen responsive tumors such as prostate cancer. These 17α-iodovinyl steroids were synthesized because many 17α-substituents do not interfere markedly with binding to the androgen receptor and because similar analogs of other steroids, estrogens and progestins, have been shown to have the requisite properties for ligands to those receptors. Both of these potential ligands were tested for their ability to compete with [^{3}H]R1881 for binding to the androgen receptor in cytosols from prostate, hypothalamus and pituitary. The relative binding affinities ranged between 5 and 20%, depending upon the tissue and steroid. In order to test the two ligands directly, they were both synthesized labelled with ^{125}I and tested for binding to the androgen receptor in prostatic cytosol and *in vivo* for specific concentration in androgen responsive tissues. While there was considerable binding in the prostatic cytosol, it was not specific because 5α-dihydrotestosterone did not compete. Likewise in the *in vivo* experiment there was no evidence for androgen receptor mediated concentration of the tracers. While on the basis of relative binding affinity, these 2 steroids appeared to be good candidates for androgen receptor ligands, neither were useful for this purpose. These results contribute new information which will be valuable in the design of other γ-emitting androgens and emphasises that, in this process, other factors such as metabolism and nonspecific binding must be considered.

INTRODUCTION

Biologically active γ-emitting steroids that bind to receptors are generally recognized as important probes of hormone action. Such compounds can be used for external monitoring of hormonally-dependent tumors *in vivo* and because of the extremely high specific activity of most of these isotopes, they are extremely useful for the quantification of steroid receptors *in vitro*. However the design and synthesis of such steroidal ligands has generally proved to be extremely challenging, because the more usual isotopes that are available, such as those of iodine, are bulky and electronegative substituents that interfere with the receptor binding of the relatively small steroid molecule. In addition to steric and electronic problems that require the precise placement of the radioisotope, other equally important factors have to be considered, such as nonspecific binding to nonreceptor macromolecules and metabolic and chemical stability. Because of these constraints, of the six families of steroids (glucocorticoids, mineralocorticoids, vitamin D, androgens, progestins, and estrogens) γ-emitting ligands exist for only the latter two [1, 2].

The first gamma emitting steroid synthesized was the estrogen 16α-[^{125}I]iodoestradiol [1, 3, 4]. This steroid labelled with ^{125}I has proven to be an excellent ligand for the estrogen receptor, and is considered to be the ligand of choice for quantifying the estrogen receptor in breast tumor biopsy specimens [5–8]. A similar analog, 11β-methoxy-16α-iodoestradiol, labelled with ^{125}I has been shown to be an excellent *in vivo* probe of the estrogen receptor [9], especially for its sensitive quantification in the brain [10, 11].

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Labelled with ^{123}I, this steroid has properties appropriate for the clinical detection of estrogen receptor containing tumors [12]. Similar compounds labelled with Br^{77} and F^{18} at C-16α have been synthesized to detect tumors containing the estrogen receptor [13–15].

Other estrogenic ligands have been synthesized in which the γ-emitter, ^{125}I, has been inserted into the 2′ position of a vinyl substituent at C-17α of the steroid nucleus [16–18]. These compounds are stable and have good affinity for the estrogen receptor. We have used this substituent to fashion E- and Z-17α- (2-[^{125}I]iodovinyl)-19-nortestosterone, ligands for the progesterone receptor [2, 19]. The ^{125}I-labeled Z-isomer has been used for the *in vivo* autoradiographic analysis of the progesterone receptor in the brain [20]. In addition we synthesized another ^{125}I-ligand, the photoaffinity label Δ^9-(16α-[^{125}I]iodo-19-nortestosterone, for structural studies of the progesterone receptor [21]. Recently a [^{18}F]progestin, has been synthesized labeled at 2′ of a C-16α ethyl substituent, 21-[^{18}F]fluoro-16α-ethyl-19-norprogesterone, for the clinical imaging of tumors containing the progesterone receptor [22].

Similar γ-emitting androgens would likewise be valuable ligands for quantifying the androgen receptor *in vitro* and for detecting and monitoring androgen sensitive tumors, such as prostate cancer. However the androgen receptor has resisted several efforts to synthesize such radioactive ligands. The 2α-brominated derivative of 5α-dihydrotestosterone (5α-DHT) has been shown to be rapidly dehalogenated *in vivo* [23, 24]. Similarly the 2α-[^{125}I]iodo derivative of 5α-DHT[25] has been synthesized as an *in vivo* probe of the androgen receptor, but this α-haloketone is also too reactive to be chemically and metabolically stable. Several A- and B-ring substitutions with iodine, bromine, fluorine, as well as selenium, produced compounds that compete with 5α-DHT for binding to the androgen receptor, but they are either unstable or it has not been shown that they have the requisite properties for receptor ligands when radiolabelled [23, 26, 27]. We suspected that 16α-iodo-5α-dihydrotestosterone would be a likely candidate for an androgen receptor ligand but when we synthesized this iodinated C_{19}-steroid we found that it was neither androgenic nor did the ^{125}I-labeled tracer bind to the androgen receptor [28].

Since iodinated vinyl groups which have been used for the synthesis of radioiodine labelled estrogens [16–18] and progestins [2, 19] are chemically and metabolically stable, and because steroids bearing short alkyl 17α-substituents are good ligands for the androgen receptor [29, 30], we have synthesized the E- and Z-17α-(2-iodovinyl)analogs of 5α-dihydrotestosterone (IVDHT) and tested these iodinated steroids as competitors of the binding of [^{3}H]R1881 to the androgen receptor in the cytosol of prostate, pituitary and hypothalamus. In addition we have synthesized both steroids labelled with the radioisotope ^{125}I and tested each for binding to the androgen receptor *in vitro* and for their concentration in androgen target tissues *in vivo*.

EXPERIMENTAL

Melting points were obtained in a Koffler hot stage and are uncorrected. Infrared spectra were recorded in potassium bromide disks on a Beckman Acculab 4 spectrophotometer. NMR spectra were obtained at 100 MHz with an IBM/Bruker WP 100SY FT instrument. Mass spectra were recorded on a Hewlett-Packard model 5890A spectrometer at 70 eV with a direct-insertion probe. High-performance liquid chromatography (HPLC) was performed on a Beckman Model 334 gradient system equipped with model 421 controller, Altex CR-1A integrator-recorder and Hitachi model 100-10 variable wavelength detector, and for radioactive compounds, a Waters modular system consisting of U6K injector, M45 pump and model 440 detector.

Z- and E-17α(-2-tri-n-butylstannylvinyl)-5α-androstane-17β-ol-3-one

A mixture of 100 mg (0.318 mmol) of 17α-ethynyl-5α-androstane-17β-ol-3-one (Steraloids, Wilton, N.H.), 150 mg (0.515 mmol) of Tri-n-butyltin hydride, 25 mg of 2,2′-azobisisobutyronitrile (AIBN) and 10 ml of dry benzene was placed in a screw capped test tube, purged with dry nitrogen and then heated at 80°C for 24 h. After cooling, HPLC analysis of 100 μl of this solution (25 × 1 cm Silica column, Rainin, 0.25% isopropyl alcohol in methylene chloride at 5 ml/min) showed a 2:1 ratio of the E- and Z-addition products migrating at 10.3 and 12.3 min respectively. The remainder of the solution was iodinated directly as described below.

Z- and E-17α-(2-Iodovinyl)-5α-androstane-17β-ol-3-one, (Z- and E-IVDHT)

Iodine, 152 mg (0.6 mmol), dissolved in 2 ml of benzene was added to the solution of 17α-(2-tri-n-butylstannylvinyl)-5α-androstane-17β-ol-3-one described above. After stirring for 30 min, the mixture was diluted with 10 ml of a solution containing 10% sodium bisulfite and 1% potassium fluoride and shaken. After removal of the aqueous layer, the organic solution was washed with two 10 ml portions of water, transferred to another tube and dried over anhydrous sodium sulfate. Preparative HPLC in six separate applications was conducted on the mixture using a 25 × 1 cm, Silica Column, Rainin (0.25% isopropanol in methylene chloride, 5 ml/min). This gave 25 mg (17.8% yield) of the Z-isomer (eluting in 13–17 min) and 56 mg (39.8% yield) of the E-isomer (eluting in 18–24 min). Samples of each isomer were further purified on a 25 × 4.6 mm Lichrosorb-Diol column (EM Science) using 75% isoctane, 25% methylene chloride at 1 ml/min. Under these conditions the Z-isomer eluted at 12 min and the E-isomer eluted at 22 min.

Z-IVDHT: m.p. 140–144 (acetone-petroleum ether); IR(KBr): 3450 cm^{-1} (broad, 17β-OH), 1700 (C=O) MS: 442 (0.7, parent), 424 (3.21, M-H_2O), 315 (31.8, M-I), 297 (40.1, M-I-H_2O), 55 (89.1, A-ring cleavage); NMR ($CDCl_3$): δ6.75 and 6.30 (AB pattern, 2, *cis*-CH=CH-, J = 8.6 Hz), 0.99 (s, 3, H-19), 0.91 (s, 3, H-18); High resolution M_r for $C_{21}H_{31}O_2I$, calcd. 442.1360, found 442.1378.

E-IVDHT: m.p. 100.0–101.5 (acetone-petroleum ether); IR (KBr): 3450 cm^{-1} (broad, 17β-OH), 1700 (C=O), 965 (*trans*-CH=CH-out of plane), MS: 442 (0.99, parent), 424 (32, M-H_2O), 315 (35.2, M-I), 297 (45.1, M-I-H_2O), 55 (base, A-ring cleavage); NMR ($CDCl_3$): δ6.70 and 6.22 (AB pattern, 2, *trans*-CH=CH-, J = 14.5 Hz), 0.99 (s, 3, H-19), 0.88 (s, 3, H-18); high resolution M_r for $C_{21}H_{31}O_2I$, calcd. 442.1360, found 442.1365.

E- and Z-17α-(2-[^{125}I]iodovinyl)-5α-androstane-17β-ol-3-one

While a thoroughly dried 13 × 100 mm test tube equipped with a screw cap was being constantly swept with a stream of N_2, the following were added: 10 mg (31.8 μmol) of E-17α-ethynyl-5α-dihydro-testosterone, 2.5 mg of 2, 2'-AIBN. 1 ml of dry, freshly distilled benzene and 15 mg (13.8 μmol) of tri-n-butyltin hydride. The tube was sealed and heated for 21 h at 80°C in a thermostat controlled heating block. The reaction mixture was allowed to cool to room temperature and a 100 μl aliquot was purified by HPLC on a 25 cm × 4.6 mm Lichrosorb-Diol column (EM Science) with 75% isooctane, 25% methylene chloride at 1 ml/min. In this sytem Z-17α-(2-tri-n-butylstannylvinyl)-5α-dihydrotestosterone elutes in 5.5 min, the E-isomer in 8.4 min, and the starting material, 17α-ethynyl-5α-dihydrotestosterone in 23.7 min. The fractions containing the Z- and E-isomers of the tin adducts were collected (the ratio of E- to Z- was always about 2 to 1) and iodinated with ^{125}I as described below.

The synthesis with radioiodide was carried out in an enclosed hood outfitted with charcoal filters. The following synthetic procedure is representative of several similar reactions which we have carried out. A solution of 5.0 mCi (2.2 nmol) of [^{125}I]NaI (low pH, Dupont New England Nuclear Co.) was added to a 300 μl Microflex reaction vial (Kontes Glass Co.). In order to avoid a release of radioactivity, radioactive I_2 which might be produced by air oxidation was converted into iodide by adding 5 μl of an ethanolic solution containing 2.5 μg of $NaBH_4$ to the vial and allowed to react for 10 min. The aqueous mixture was evaporated under vacuum using a semi-micro scale rotary evaporator inside of the enclosed hood. The resulting residue was dissolved in 100 μl of acetone (destroying any residual $NaBH_4$) and the vial was closed with a cap containing a Teflon septum. Chloramine T, 20 μg, (71 nmol) was injected into the vial in 4 μl of tetrahydrofuran (THF). The appropriate fraction from the HPLC (described above) containing either the Z-isomer of the E-isomer of the tin adduct was evaporated, the residue dissolved in 100 μl of methylene chloride and injected into the sealed vial. The reaction mixture was left to stand for 30 min at room temp., and then quenched with 100 μl of an aqueous containing 10 mg of sodium bisulfite and 1 mg of KF.

After the termination of the reaction, the vial was opened and the contents transferred to a 16 × 100 mm screw capped test tube with the aid of several washings with methylene chloride; a total volume of 4 ml. The aqueous layer was removed with a pipet and the organic phase was washed 3 times with 1 ml portions of H_2O. The organic solution was dried over anhydrous sodium sulfate, transferred to a conical flask and evaporated to dryness on the rotary evaporator. The residue was dissolved in 200 μl on benzene and purified by HPLC on a 25 cm × 4.6 mm Diol column (Waters Co.) with 80% isooctane, 20% methylene chloride at a flow rate of 1 ml/min. The column effluent was monitored with the flow cell of the u.v. detector at 280 nm (C-I bond) with a setting of 0.005. The sensitivity of the u.v. detector was approximately 150 ng for both the E- and Z-iodosteroid. Fractions of 1 ml were collected and the content of ^{125}I determined in a γ-counter. Most of the radioactivity eluted as a symmetrical peak: either Z-[^{125}I]IVDHT eluting at 11 min, or E-[^{125}I]IVDHT eluting at 19 min. Those fractions containing radioactive Z-IVDHT or E-IVDHT were combined, evaporated and dissolved in 20 ml of 10% ethanol in benzene. The total radiochemical yield usually ranged between 20 and 30%. The radioactive products were stored at 4°C in the alcoholic benzene solution. Analysis by thin-layer chromatography (system T-1: silica gel: benzene: ethyl acetate, 6/1), R_f: E-isomer 0.31, Z-isomer 0.36) showed the materials to be stable under these conditions for a period of at least 2–3 months.

The [^{125}I]isomers were considered to be carrier free, having a specific activity of approximately 2200 Ci/mmol. This specific activity was consistent with the mass of the [^{125}I]steroid determined with the HPLC u.v. detector. TLC analysis with system T-1 of several different lots of the purified [^{125}I]steroids (ranging from 20 to 80% of the entire radioactive product) showed no signs of any other steroidal contaminant.

In vivo androgen uptake

The concentration of the two [^{125}I]steroids into androgen target tissues and control tissues, as well as localization in nuclei, was determined in castrated male rats as previously described [31]. Male Sprague–Dawley rats, 30 days of age, were castrated 24 h prior to injection of the tracers. Animals were injected with 5.1 μCi of either Z- or E-[^{125}I]IVDHT (approx. 2.3 pmol) in 100 μl of a vehicle consisting of propylene glycol/saline (1:1) via the lateral tail vein. For controls, two groups received 50 μg of 5α-DHT

dissolved in 100 μl of the same vehicle, 1 min prior to the isotope. The animals injected with isotope alone received vehicle without the displacing androgen. 1 h after injection of the isotope, the animals were killed and prostate, seminal vesicles, spleen, pituitary and blood were collected. Tissues were weighed and counted. The blood was allowed to clot, serum was separated by centrifugation and 100 μl aliquots taken for counting. One half of the spleen and prostate were taken for the isolation of cell nuclei, as follows: tissues were homogenized in 2 ml of N1 buffer (1 mM potassium phosphate, pH 6.5, 3 mM $MgCl_2$, 0.3 M sucrose, 2.5% Triton X-100, v/v), and centrifuged at 800 *g* for 10 min. The pellets were washed once with 2 ml of N2 buffer (as for N1, but without the triton X-100). The resulting crude nuclear pellet was counted for ^{125}I in a γ-counter. Essentially all of the DNA was recovered in this pellet [32].

Binding to the cytosolic androgen receptor

The binding affinity of E- and Z-IVDHT for the androgen receptor was determined by competition for the binding of [^{3}H]R1881 in cytosol from prostate, pituitary and the combined preoptic area–hypothalamus regions of the brain. Three days prior to the experiment, adult male rats were castrated under ether anesthesia. The animals were sacrificed by decapitation and the tissues removed and immediately placed on an ice-cold Petri dish. The hypothalamus and preoptic area were dissected from the brain as previously described [32] and pooled. The prostate tissue was finely minced with scissors. Tissues were homogenized in TEGDMo buffer (10 mM Tris–HCL, pH 7.4, 1.5 mM EDTA, 1 mM dithiothreitol, 10 mM sodium molybdate, containing 10% glycerol v/v) in a motor-driven Teflon-glass homogenizer, using approximately 10 volumes of buffer for the brain tissues and 25 volumes for the pituitary and ventral prostate. Homogenates were centrifuged for 1 h at 105,000 *g*, the supernatants decanted and frozen at −80°C until used.

For binding studies, the cytosols were thawed on ice and 100 μl aliquots were added to the radiolabeled steroid, 2 nM final concentration, dissolved in 50 μl of TEGDMo buffer. Unlabeled steroids, 5α-DHT, E-IVDHT, Z-IVDHT, were added to final concentrations of 0–250 nM in 50 μl of TEGDMo buffer. Triamcinolone acetonide, 1 mM, was included in all incubations to suppress binding to the progesterone/glucocorticoid receptors [33]. In the first experiment, [^{3}H]R1881 was used as the labeled ligand for the androgen receptor in order to measure the relative binding affinity of the two iodinated steroids. In the second experiment Z-[^{125}I]IVDHT and E-[^{125}I]IVDHT were used as the labeled ligands. Since there is uncertainty about the specific activity of the [^{125}I]steroids (see Discussion), several incubations were performed at lower concentrations of ligand, approximately 0.1 M (calculated as carrier free). Incubations were performed for 4 h at 0–4°C and then bound steroid in 100 μl of incubate was separated by Sephadex LH-20 gel filtration [34] and quantified in a γ-counter.

RESULTS AND DISCUSSION

The E- and Z-isomers of 17α-(2-iodovinyl)-5α-dihydrotestosterone were synthesized by iodine cleavage of the respective E- and Z-adducts of tri-n-butyltin hydride and 17α-ethynyl-5α-dihydrotestosterone. The synthetic procedure is similar to that previously described by us to synthesize the progestins E- and Z-(2-iodovinyl)-19-nortestosterone [2, 19]. The reaction sequence is shown in Fig. 1. The radioiodine labeled steroids were made in a similar manner, from iodine produced *in situ* by oxidation of [^{125}I]iodide with chloramine T. The tin adducts are relatively stable in solution and once they are prepared, the complete synthesis and purification (by HPLC) of the radiolabeled steroids is accomplished in less than 2 h. A rapid and simple synthetic scheme is important for minimizing the exposure to radiation and essential when working with isotopes of short half-life.

There is some uncertainty about the specific activity of both the E- and Z-[^{125}I]isomers, although it is likely that they are carrier free, with a specific activity of 2200 Ci/mmol. This specific activity is consistent with the u.v. absorption obtained in the HPLC detector, but the low mass obtained in these syntheses was close to the limit of detectability and so there is some imprecision in the determinations. There is also the possibity that non u.v. adsorbing contaminants that bind to the androgen receptor were present in the HPLC fractions containing the [^{125}I]ligand. However, we consider this to be unlikely for two reasons. First TLC analysis (described above) or large portions of the isotopically labeled steroids failed to reveal any such contaminant. Secondly, our previous experience with an identical iodination of similar progesterone receptor ligands produced carrier free material. As we have previously described [2], the best way to confirm the specific activity is with a binding analysis, by comparison to a ligand of known specific activity. However, as described below, in this case it was not possible. While there is uncertainty in the specific activity, we calculate from the limits of detectability of the HPLC and TLC (assuming that the unknown contaminant would have an RBA equal to 5α-DHT) that the apparent specific activity of each preparation must be greater than 500 Ci/mmol. Additional binding experiments were performed to adjust for the possibility of a lower specific activity.

The non-radioactive steroids, E-IVDHT and Z-IVDHT were tested as ligands for the androgen receptor by comparison to 5α-DHT as competitors for the binding of [^{3}H]R-1881. This competition experiment was performed using three different cytosol preparations which contain the androgen receptor: prostate, pituitary and the combined pre-

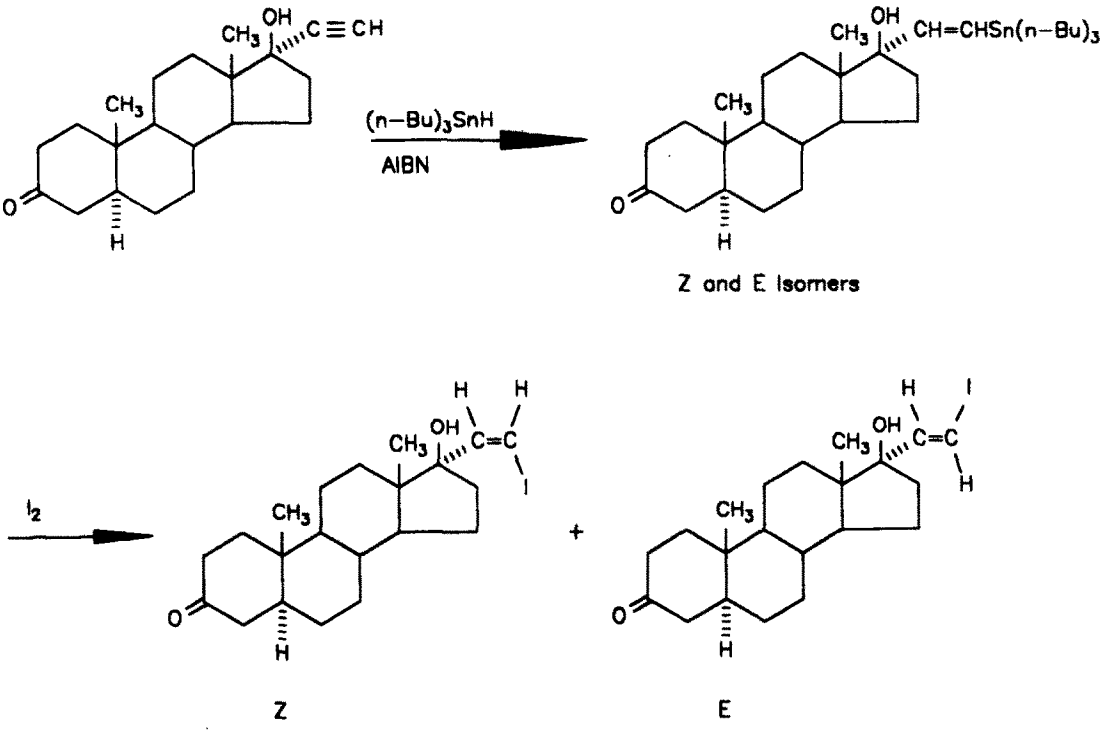

Fig. 1. Synthetic scheme for the synthesis of E-17α-(2-iodovinyl)-5α-dihydrotestosterone and Z-17α-(2-iodovinyl)-5α-dihydrotestosterone.

optic–hypothalamic regions (HPo) of the brain. The displacement curves are shown in Fig. 2, and the relative binding affinities (RBA) calculated by a computerized curve fitting program [35] in Table 1. In the prostate the RBA compared to 5α-DHT is 5% for the E-isomer and 3% for the Z-isomer. However, both iodinated ligands were better competitors in the pituitary and the HPo. In the pituitary the RBA for the E-isomer is 10% and for the Z-isomer 17%. Similar results were found in the HPo cytosol where the RBA for the E-isomer is 12% and for the Z-isomer 20%. The large difference in RBA between the prostate and the brain and pituitary while unexpected has previously been reported for other ligands, such as testosterone [36–38]. The reason for this relatively large tissue specific difference in RBA is not clear but it is probably not due to a differential receptor affinity. It could be caused by any of a number of factors, including a substantial tissue variation in the nonspecific binding of the ligands. If true, then it is probable that the higher RBAs more closely reflect the affinity of these iodinated ligands for the androgen receptor.

Since the competition of the two iodinated ligands for the androgen receptor in the brain and pituitary indicated that either might be a useful ligand we synthesized both labeled with ^{125}I and tested them for binding *in vitro* and *in vivo*. In the *in vitro* experiment, both E-[^{125}I]IVDHT and Z-[^{125}I]IVDHT at 2 nM, were incubated separately with prostatic cytosol which was shown to contain active androgen receptor by incubation with [^{3}H]R-1881. The [^{125}I]steroids were incubated in the presence and absence of varying concentrations of 5α-DHT, E-IVDHT and Z-IVDHT. As can be seen in Table 2, both iodinated steroids exhibited high levels of

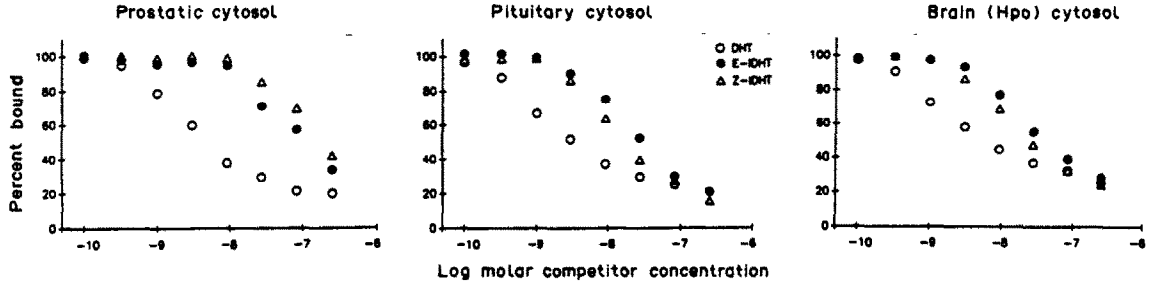

Fig. 2. Competition for binding of [^{3}H]R1881 to the androgen receptor.

Table 1. Competition of E- and Z-IVDHT for binding to the androgen receptor

Competitor	RBA Prostate	Pituitary	Brain (Hpo)
E-IVDHT	5	10	12
Z-IVDHT	3	17	20

RBA (relative binding affinity), 5α-DHT = 100, were calculated from the data in Fig. 2 by a least squares curve-fitting method. Both steroids were compared to 5α-DHT for the binding of 2 nM [^{3}H]R1881 to the androgen receptor in the indicated cytosols. Brain cytosol is a preparation prepared from the combined hypothalamic–preoptic areas.

binding in prostatic cytosol. However, the binding of both γ-emitting steroids was not inhibited by any of the three added steroids. In order to adjust for the possibility of a lower apparent specific activity (see above), several incubations were performed with one fifth to one tenth the concentrations of ^{125}I-labeled steroid used in the experiments above. The results (not shown) were similar to those in Table 2. Thus there was no evidence for specific binding of either E-[^{125}I]IVDHT or Z-[^{125}I]IVDHT.

In the *in vivo* experiment, the [^{125}I]steroids were administered to castrated male rats and after 1 h the concentration of radioactivity was determined in several androgen target tissues, prostate, seminal vesicles and pituitary. The concentration of radioactivity in those tissues was compared to a non-target tissue, spleen, as well as blood. In order to separate the contributions from non-specific and androgen receptor mediated uptake, in separate animals the receptors were saturated by prior injection of a large dose of 5α-DHT. Nuclear radioactivity was also measured in order to detect androgen receptor bound radioactivity, which would be localized in this subcellular fraction. As can be seen in Table 3, there appears to be no *in vivo* androgen receptor-mediated concentration of [^{125}I]steroid. With the Z-isomer it is obvious that there is no concentration of tracer in any of the androgen target tissues in comparison to the control tissue, spleen, nor any concentration of isotope in the prostate nucleus. With the E-isomer there is more radioactivity in the prostate than spleen, and in addition the animals blocked with 5α-DHT had less radioactivity in the prostate. However, this was probably due to animal-to-animal variability because the other androgen target tissues, seminal vesicle and pituitary did not have higher levels of

Table 2. Binding of E- and Z-[^{125}I]IVDHT in rat prostatic cytosol

Ligand	Competitor concentration (nM)	Competitor (B/B$_0$ × 10^2)[a] E-IVDHT	Z-IVDHT	5α-DHT
E-[^{125}I]IVDHT	0	100	100	100
	0.1	104	101	102
	0.3	108	96	105
	1	106	99	97
	3	106	97	97
	9	105	97	98
	28	94	92	105
	83	98	91	105
	250	97	89	102
Z-[^{125}I]IVDHT	0	100	100	100
	0.1	107	107	107
	0.3	103	92	101
	1	105	78	103
	3	93	114	99
	9	104	104	98
	28	108	102	106
	83	103	100	97
	250	105	100	--

Cytosol from rat prostate were incubated with 2n of [^{125}I]steroid as described in the text. [a]B is the [^{125}I] bound in the presence of the indicated amount of competitor and B$_0$ is the [^{125}I] bound in the absence of any competing steroid. B$_0$ for the E-isomer was 14% of the incubated ligand, and 50% for the Z-isomer.

radioactivity. In addition, only a small portion of the prostatic radioactivity was present in the nucleus and this was not suppressed by prior treatment with 5α-DHT, as would be expected for an androgen receptor bound ligand [31]. The concentration of radioactivity in the serum of animals receiving either tracer was far greater, at least 10-fold, than that in any tissue. Obviously, neither tracer would be useful for imaging androgen sensitive tissues or tumors.

The inhibition experiment (Table 1) showed some promise that the iodinated steroids might be suitable ligands for the androgen receptor, but when each [^{125}I]steroid was tested directly in prostate cytosol there was no evidence of specific binding (Table 2). The apparent discrepancy between the two types of experiments can be explained by a combination of high nonspecific binding and low K_a. The former produces high background and the latter, stripping of specifically bound steroid during the procedure to remove free ligand. The combination of both factors leads to undetectable receptor bound steroid. In confirmation, the *in vitro* experiment showed no evidence of androgen receptor mediated tissue uptake and in addition the high blood levels of radioactivity makes it likely that extensive metabolism of the steroid occurred.

Table 3. Uptake of E- and Z-[^{125}I]IVDHT in tissues of the rat

Compound	DPM/g tissue Pituitary	Prostate	Prostate (Nuclei)	S.Ves	Spleen	Spleen (Nuclei)	Serum
E-[^{125}I]IVDT	45,500	90,200	7,400	40,000	41,700	1,200	870,000
E-[^{125}I]IVDT + 5α-DHT	47,000	53,500	5,800	23,900	17,200	1,000	840,000
Z-[^{125}I]IVDT	82,500	99,700	7,600	102,400	76,700	1,800	1,560,000
Z-[^{125}I]IVDT + 5α-DHT	119,900	135,400	10,000	155,000	64,700	1,500	1,750,000

Castrated male rats were injected i.v. with 5.1 μCi of either [^{125}I]steroid and the tissues were obtained 1 h later. Animals receiving a blocking dose of androgen were injected with 50 μg of 5α-DHT 1 min before the tracer. S. Ves. = seminal vesicle. Results are the means of 3 observations for animals receiving tracer alone and 2 for animals injected with 5α-DHT. Standard deviations ranged from 12–41% of the mean. The radioactivity in the nuclei of spleen and prostate are expressed in DPM/g of tissue.

In the design of ligands for the estrogen and progestin receptors, the 17α-2-iodovinyl group has been utilized as a means of incorporating radioiodine into both types of steroids [2, 16–19]. This group is attractive because it allows the facile incorporation of the radiohalogen into a chemically and metabolically stable bond at a position in the steroid which does not interfere markedly with the binding of these types of ligands with their respective receptors. While all of these factors appeared to hold for E-IVDHT and Z-IVDHT, nevertheless when labelled with ^{125}I they could not be used as ligands for the androgen receptor, either *in vivo* or *in vitro*. We had previously found that substitution of iodine at C-16α of 5α-DHT almost completely abolishes binding to the androgen receptor [28]. Since in this case, however, the iodovinyl group does not completely suppress binding affinity (as shown by the competition data in pituitary and brain, Table 1) it is possible that with further modification of the steroid nucleus to decrease nonspecific binding and metabolism, this substituent may be useful in the synthesis of a γ-emitting ligand for the androgen receptor.

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