The Synthesis of 11β-Methoxy-[16α-^{123}I] Iodoestradiol and Its Interaction with the Estrogen Receptor In Vivo and In Vitro

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In order to produce an estrogen receptor mediated imaging agent, we have synthesized 11β-methoxy-16α-iodoestradiol labeled with ^{123}I, and have studied its interaction with the estrogen receptor and its distribution in rats and rabbits. This ^{123}I-labeled steroid, 11β-methoxy-16α-[^{123}I]iodoestradiol, binds with high affinity, $K_a = 6 \times 10^9\ M^{-1}$, and specificity to the estrogen receptor in uterine cytosol. When tested in vivo, this radiolabeled steroid concentrates by a receptor mediated mechanism, in the estrogen target tissue, the uterus, producing very high target to nontarget tissue ratios. The results of these experiments indicate that 11β-methoxy-16α-[^{123}I]iodoestradiol may be a useful imaging agent for clinically monitoring and detecting estrogen receptor containing tumors.

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A gamma-emitting estrogen has long been recognized as a potentially important clinical probe for the detection and monitoring of estrogen dependent cancers (*1–4*) through noninvasive in vivo imaging. In 1949 the first attempt was made to synthesize a gamma-emitting steroid that could be used for measuring the uptake of an estrogen in target tissues (*5*). It failed, as did most of the others that followed. Subsequently, specific criteria have been recognized as critical for the success of ligands for hormone receptors, these include: high affinity for the receptor; low nonspecific binding; chemical and metabolic stability (*6*).

In 1979, our laboratory produced a biologically active gamma-emitting radiolabeled steroid hormone, 16α-[iodine-125]iodoestradiol (16α-^{125}IE$_2$) (*7*), an estrogen that met all of the above criteria for a receptor ligand (*8,9*). This has been widely confirmed and subsequently, other 16α-halogenated analogs of estradiol were shown to act similarly (*10,11*). 16α-^{125}IE$_2$ has proven to be an excellent ligand for the estrogen receptor and it is now widely used for the in vitro quantification of this important protein (*12–14*).

However, 16α-IE$_2$ does not appear to be suitable as a clinical agent for imaging estrogen responsive tumors. High levels of radioactivity in the liver, as well as circulating metabolites in the blood, mask receptor localized isotopic emissions (*15,16*). Other interesting isotopically labeled estrogens have been synthesized (*17–20*), but none of these compounds appear to have properties that eliminate the high background. Consequently, in order to decrease metabolism and thereby diminish the relatively high levels of radioactive metabolites in the liver and blood, we synthesized another 16α-iodo analog, 11β-methoxy-16α-iodoestradiol (11β-CH$_3$O-16α-IE$_2$) (*21*). We showed that this estrogen labeled with ^{125}I, shares most of the desirable characteristics of the interaction of 16α-^{125}IE$_2$ with the estrogen receptor in vitro, but most importantly, that the 11β-methoxy analog has far superior in vivo characteristics. In the present study, we synthesized 11β-CH$_3$O-16α-IE$_2$ radiolabeled with ^{123}I, an isotope that has excellent characteristics for imaging. We tested this [^{123}I] steroid both in vivo and in vitro, measuring its interaction with the estrogen receptor and the kinetics of its distribution in various tissues in both rats and rabbits.

MATERIALS AND METHODS

11β-Methoxy-16β-bromo-17β-estradiol was synthesized as previously described (*21*). Na^{123}I, produced by the tellurium-124 (^{124}Te)(p,2n) nuclear reaction, (Medi-Physics, Richmond, CA) was used for radiolabeling. It contained ~3% of ^{124}I at noon of the day of delivery (and synthesis). High performance

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liquid chromatography (HPLC) was performed on a Waters instrument (Waters Assoc., Milford, MA): M-45 pump, U6K injector, and 440 uv detector. The reversed phase HPLC system was an Altex Ultrasphere ODS column (Beckman Instruments, Berkeley, CA) (4.6 mm × 25 cm) using CH_3CN:H_2O (40/60) at a flow rate of 1 ml/min. Both of the isotopes, ^{123}I and ^{125}I, were counted in a gamma-4000 gamma-counter Beckman at efficiencies of 89% and 71%, respectively. The counter was calibrated for ^{123}I using a standard supplied by the Medi-Physics Corp.

11β-CH_3O-16α-$^{125}IE_2$, was synthesized by exchange with $Na^{125}I$ (DuPont Company, No. Billerica, MA) exactly as described (*21*). The purification was modified, using the reversed phase HPLC system described below. The specific activity of each of the [^{125}I] preparations synthesized in this way was determined both by uv absorption in the HPLC detector, and by quantification of the estrogen receptor binding sites in rabbit uterine cytosol in comparison with [^3H]E_2 of known specific activity (*8,21*). In this manner every preparation of 11β-CH_3O-16α-$^{125}IE_2$ was found to be carrier free, ~2,200 Ci/mmol.

Synthesis of 11β-CH_3O-16α-$^{123}IE_2$. The synthetic scheme, exchange of 16β-bromo-11β-methoxy-17β-estradiol with $Na^{123}I$, is a modification of that previously reported for the [^{125}I] ligand (*21*). Ten millicuries of $Na^{123}I$, supplied in 100 μl of an aqueous solution containing 5 μg of $Na_2S_2O_3$, was added to a 20 mm × 55 mm screw cap V-Vial (Wheaton Scientific, Milloilk, NJ), and evaporated close to dryness at 60°C under vacuum with a DuPont rotary evaporator (Micro Spin-Vap). Approximately 1 ml of CH_3CN was added and the evaporation process was repeated. When the vial was dry, 50 μg of 11β-methoxy-16β-bromo-17β-estradiol in 50 μl CH_3CN was added to the residue. The vial was tightly sealed with a Teflon-lined septum and heated at 105°C for 90 min. At the end of the exchange reaction, 70 μl of H_2O was added through the septum, after which the diluted reaction mixture was purified by direct injection onto the reversed-phase HPLC system. A typical chromatogram is shown in Figure 1. Those fractions containing the radioactive product were pooled and evaporated under vacuum. The residue was dissolved in ethanol and an aliquot was counted. The radiochemical yield of the total process in several syntheses ranged from 2.5 to 6 mCi (25% to 60%). The entire synthesis, including purification of the radioactive product, 11β-CH_3O-16α-$^{123}IE_2$, can be completed within 3 hr.

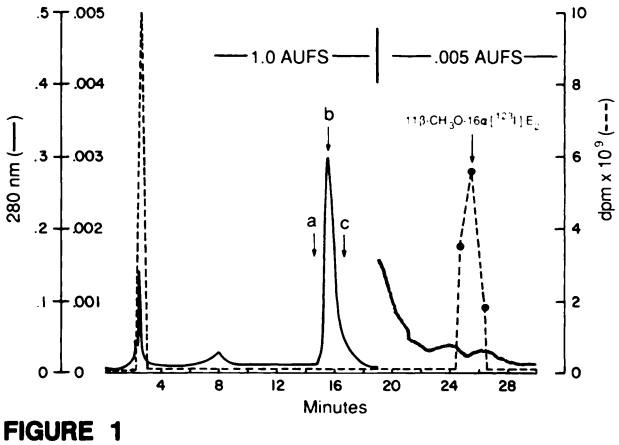


FIGURE 1
HPLC purification of 11β-CH_3O-16α-$^{123}IE_2$. The reaction mixture of the exchange of 11β-methoxy-16β-bromoestradiol with $Na^{123}I$ was diluted with 70 μl of H_2O and chromatographed on an Altex Ultrasphere 5 μm ODS column (4.5 mm × 25 cm) with acetonitrile/H_2O (40:60) at 1 ml/min. AUFS, absorption units full scale. a,b,c Mark the position in the chromatogram where the following steroids migrate: a: 11β-methoxyestrone; b: 11β-methoxy-16β-bromoestradiol; c: 11β-methoxy-16α-bromoestradiol.

Estrogen Receptor

Rabbit uterine cytosol was prepared as previously described (*21*). Cytosol was stored frozen at −80°C. Before use the cytosol was thawed, diluted with TE buffer (0.01 *M* Tris-HCl, pH 7.4, and 0.0015*M* EDTA), usually ~ fivefold (1 mg protein/ml), to produce an initial bound/free ratio of ~0.3–0.7 in the receptor assay. Competitors and radioactive ligands were dissolved in dimethylformamide and diluted with an equal volume of TE buffer. Various concentrations of the steroidal competitors and radioactive steroids were added to test tubes in 20 μl of dimethylformamide-TE solution. Nonspecific binding was determined in parallel incubations with diethylstilbestrol (DES), final concentration 5×10^{-7} *M*. The diluted cytosol was added and the mixture incubated at room temperature for 3 hr, except where specifically noted. Afterwards, the mixture was cooled on ice and 0.5 ml of a suspension of 1% charcoal and 0.01% dextran in TE buffer was added to the incubation tubes, mixed, and immediately centrifuged at 2,600 *g* for 5 min. The radioactivity in the supernatant was counted to determine the bound ligand.

Iodine-123-labeled 11β-CH_3O-16α-IE_2, in vivo. Twenty-one-day-old (~45 g) Sprague-Dawley rats (Charles River, Wilmington, MA) (six per group) were injected in the tail vein with 2 μCi of 11β-CH_3O-16α-$^{123}IE_2$ dissolved in 100 μl of a mixture of ethanol/1% bovine serum albumin (BSA) in saline (1:4). The BSA was added to prevent surface adsorption of the hydrophobic tracer from the aqueous medium. Blocked animals received either 2 μg of 11β-methoxy-17α-ethynyl-1,3,5(10)estratriene-3,17β-diol (Roussel-Uclaf, Romainville, France) (moxestrol) (*22*) or 200 μg of tamoxifen (Sigma Chemical Corp., St. Louis, MO) 0.5 hr before the tracer. In chase experiments, either 2 μg of moxestrol or 200 μg of tamoxifen were injected i.p. 1 hr after the tracer. At the indicated times after the injection of 11β-CH_3O-16α-$^{123}IE_2$, the animals were killed. Tissues were removed, cleaned of extraneous matter, blotted, weighed, and counted. Trunk blood was collected in heparinized tubes and aliquots were counted.

Female New Zealand white rabbits (Millbrook Farms, Amherst, MA) weighing ~3 kg were ovariectomized 2 days before the start of the experiments. Prior to administration of the tracer the animals were anesthetized with Ketamine-HCl (Bristol Laboratories, Syracuse, NY) (35 mg/kg i.m.) and Xylazine (Mobay Corp., Shawnee, KS) (5 mg/kg i.m.). Unless otherwise indicated, the animals were injected in the ear vein with 500 μCi of 11β-CH_3O-16α-$^{123}IE_2$ dissolved in 500 μl of the ethanolic albumin solution (above) and imaged at various times on a large field gamma camera using a high-energy parallel hole collimator (Pho Gamma LFOV, Siemens, Islen, NJ). Afterwards, the rabbits were bled by cardiac puncture, killed by injection of 5 ml Beuthanasia D (Burns Biotechnology, Omaha, NE) and then tissues were removed, dissected,

and counted. In some animals estrogen receptors were saturated by the s.c. administration of 100 μg of moxestrol in 100 μl of ethanol/saline (1:2), 1 hr prior to the radioactive steroid.

RESULTS

The major modification from our previous radiosynthesis using [^{125}I] (*21*) is the purification of the product by reversed-phase HPLC, rather than the DIOL HPLC separation which we originally reported (*8,21*). This system is necessary because although a no carrier added reaction was performed, both uv absorption and estrogen receptor binding (described below) showed that the [^{123}I] product purified on the DIOL column had an apparent specific activity of ~4,000 Ci/mmol, which was much lower than expected. This was probably caused by impurities which bind to the estrogen receptor and do not separate from 11β-CH_3O-16α-$^{123}IE_2$ on the DIOL HPLC system. The reversed-phase HPLC system cleanly separates the 16α-iodo product from the 16β-bromo reactant, as well as possible products of side reactions, such as the 17-ketone and the 16α-bromo steroid. The former formed by dehydrohalogenation and the latter by epimerization. The chromatogram of a typical reaction mixture (Fig. 1) shows that even at the most sensitive setting of the uv monitor very little uv absorbing material could be detected co-migrating in the region of the radioactive peak, indicating a high specific activity tracer.

Since uv absorption is too insensitive to measure the specific activity of the tracer, it was necessary to use an estrogen receptor based radio-receptor assay. When the [^{123}I] product was compared to carrier-free 11β-CH_3O-16α-$^{123}IE_2$ for binding to the estrogen receptor in rabbit uterine cytosol, it was apparent that as expected the specific activity of the [^{123}I] steroid was much higher than that synthesized with [^{125}I]. Since the specific activity of the [^{125}I] steroid was accurately determined (see Materials and Methods), a comparison of the binding of both tracers, [^{123}I] and [^{125}I], (Fig. 2) allows the determination of the specific activity of the [^{123}I] labeled estrogen. In the synthesis shown, a specific activity of 44,200 Ci/mmol ± 3,600 Ci/mmol (mean ± s.d.) was measured using the graphical method (*8,23*) displayed in Figure 2. In addition, the specific activity was also calculated with the analytical program LIGAND (*24*). In this procedure the number of estrogen receptor binding sites was also calculated from data obtained simultaneously with 11β-CH_3O-16α-$^{123}IE_2$. The specific activity determined with this program, 45,100 Ci/mmol, agreed well with the graphical method. The specific activity of all preparations of 11β-CH_3O-16α-$^{123}IE_2$ which were synthesized using the procedure described above were about the same, ranging from 40,000 to 50,000 Ci/mmol.

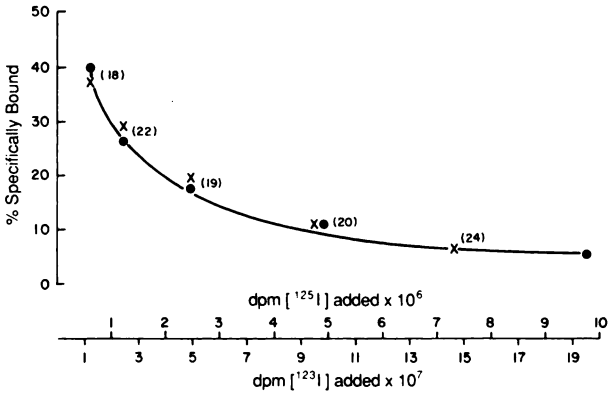
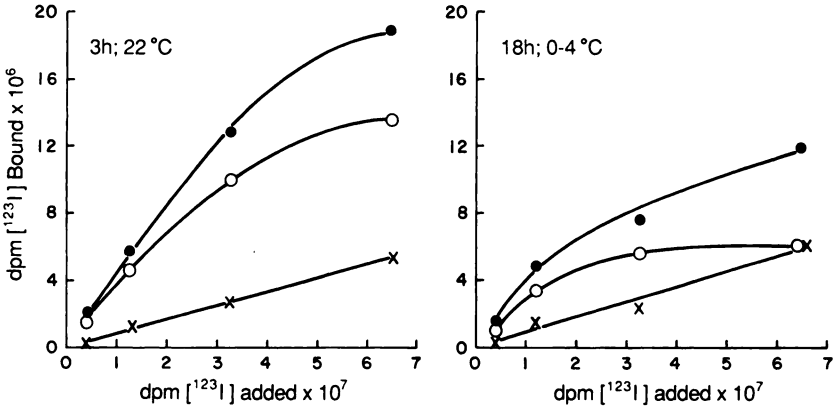


FIGURE 2
Binding of ^{125}I- and ^{123}I-labeled 11β-CH_3O-16α-IE_2 in rabbit uterine cytosol. Both isotopes were incubated in the presence of uterine cytosol for 3 hr at 22°C ± 5 × 10^7 *M* DES. X, 11β-CH_3O-16α-$^{123}IE_2$; ●, 11β-CH_3O-16α-$^{125}IE_2$. The values in brackets at each point are the ratios in dpm of the specifically bound steroids ([^{123}I]/[^{125}I]). Dpm of [^{123}I] are corrected for decay of the tracer. The line represents the best fit relationship for the data obtained with 11β-CH_3O-16α-$^{125}IE_2$. Each point is the mean of determinations performed in duplicate.

The saturation analysis of the receptor is shown in Figure 3. It can be seen in the 3 hr room temperature incubation (left hand panel) that as would be expected for the estrogen receptor, the nonsteroidal estrogen, DES, markedly inhibits binding of 11β-CH_3O-16α-$^{123}IE_2$. The steroid bound in the presence of DES, which is termed nonspecifically bound, is relatively low. In the incubation performed at 0°C overnight (corrected for decay) the specifically bound estrogen is much lower, while the nonspecifically bound steroid remained the same. Thus it appears that in long-term exposure, radioautolysis had a deleterious effect on the receptor in the in vitro incubations. This did not occur in the incubations with the ^{125}I-labeled steroid. In fact there was a slight increase in the specifically bound hormone in the overnight incubation compared to that performed at 3 hr (data not shown). The association constant, K_a, calculated with the LIGAND program (3 hr incubation) was 5.97 ± 1.54 × 10^9 M^{-1}, which agreed well with the K_a obtained for the [^{125}I] ligand in simultaneous experiments, and with those studies which we previously reported, K_a = 4.95 × 10^9 M^{-1} (*21*). The specificity of the binding of 11β-CH_3O-16α-$^{123}IE_2$ was determined in a competition assay shown in Table 1. In this experiment several types of steroid hormones were tested for their ability to compete with the binding of 11β-CH_3O-16α-$^{123}IE_2$. None of the nonestrogenic steroids: mineralocorticoids; glucocorticoids; androgens or progestins had any effect on the binding of 11β-CH_3O-16α-$^{123}IE_2$. All of the estrogens inhibited the binding of this [^{123}I] ligand. The inhibition agreed well with the affinity of these estrogens for the estrogen receptor, with DES > E_2 > 11β-CH_3O-16α-IE_2 > E_1 > E_3 (*25*).

FIGURE 3
Binding of 11β-CH_3O-16α-$^{123}IE_2$ in rabbit uterine cytosol. In the panel at the left the incubation was carried out for 3 hr at 22°C, and in the right panel for 18 hr at 0 to 4°C. Free steroid was absorbed with charcoal as described in the text. ●, total bound steroid. X, nonspecifically bound, determined in the presence of 5×10^{-7} *M* DES. O, specifically bound steroid = total − nonspecifically bound. The tracer, [^{123}I] is corrected for decay. Each point is the mean of determinations performed in duplicate. The coefficients of variance for the determination of dpm bound are as follows: 22°C, total bound = 22%, nonspecific = 5.8%; 0–4°C, total bound = 5.2%, nonspecific = 16.3%.



It can be seen in Table 2 that when the [^{123}I] estrogen was administered to immature female rats, extremely high levels of radioactivity were present in the uterus, a major target tissue for estrogens. Of the time points studied, maximal levels were reached in 1 hr, although the levels in the uterus at 6 hr after the injection were only minimally decreased (after correcting for decay). The amount of radioactivity in all other tissues was far lower, and when compared to the uterus gave very high target tissue to background ratios, ranging at 1 hr, from 34 to 1 for the blood, to ~10 to 1 for the liver and kidney. The uterus of animals injected with a large dose of the potent estrogen, moxestrol, prior to the tracer, contained much less radioactivity, down to nontarget tissue levels, which strongly suggests a receptor mediated concentration. There were minimal changes "if any" in other tissues. In the chase experiment in which the estrogen was administered 1 hr after the isotope and the distribution measured 2 hr later (3 hr after the isotope was administered) there was a 50% fall in the radioactivity in the uterus compared to animals that did not receive the nonradioactive steroid. There was no change in any other tissue, with the possible exception of the kidney. If instead of moxestrol, the antiestrogen, tamoxifen, was administered either prior to (block), or after (chase), similar results were obtained (Table 3).

TABLE 1
Specificity of the Binding of 11β-CH_3O-16α-$^{123}IE_2$ in Rabbit Uterine Cytsol

Competitor	B/B_o × 100	
	0.5 n*M*	5 n*M*
11β-CH_3O-16α-IE_2	84	47
E_3	96	74
E_2	75	39
E_1	91	73
DES	74	18
Testosterone	96	98
5α-DHT	98	96
DHIA	96	97
Progesterone	96	96
17α-OH progesterone	93	96
Cortisol	—	97
Corticosterone	94	97
Aldosterone	95	97
Dexamethasone	95	97

Rabbit uterine cytosol was incubated for 3 hr at room temperature with 1.76×10^7 dpm of 11β-CH_3O-16α-$^{123}IE_2$ + the two indicated concentrations of competitors. Unbound ligand was removed by absorption with charcoal as described in the text. B_o = dpm specifically bound when only radioactive steroid was present, 7.33×10^6 dpm. B = dpm specifically bound in the presence of competitor. Nonspecifically bound in the presence of 0.5 μ*M* DES 4.3×10^5 cpm. E_3 = estriol; E_2 = estradiol; E_1 = estrone; DES = diethylstilbestrol; 5-DHT = 5α-dihydrotestosterone; 17αOH-Progesterone = 17α-hydroxyprogesterone. Data are the mean of duplicate determinations.

In experiments to determine whether an estrogen target organ could be imaged, the tracer, 11β-CH_3O-16α-$^{123}IE_2$ was administered to adult ovariectomized rabbits. At various times after the administration of the tracer (ranging from 15 min to 20 hr) imaging was performed in attempts to visualize the uterus. However, radioactivity emanating from the kidneys, ureters and bladder, obscured visualization of the uterine activity. Nevertheless, there was extremely high concentration of radioactivity in the uterus when counted directly and compared to non-target tissues (Table 4). This was also evident when the uterus, liver, and muscle were removed from the animal and equal weights of the three tissues were imaged. Under these conditions the uterus could be imaged easily and clearly differentiated from the other two tissues (not shown).

When the various rabbit tissues were counted (Table 4), the distribution of activity was similar to what was

TABLE 2
Tissue Uptake of 11β-CH_3O-16α-$^{123}IE_2$ in Immature Rats

Tissue	0.5 hr	1 hr	3 hr	6 hr	1 hr Blocked*	3 hr Chase†
Uterus	21.20 ± 3.83	40.35 ± 4.15	30.78 ± 3.97	29.83 ± 2.93	1.57 ± 0.13	14.04 ± 2.02
Blood‡	1.46 ± 0.20	1.19 ± 0.11	0.83 ± 0.04	1.02 ± 0.06	1.30 ± 0.15	0.81 ± 0.08
Liver	5.97 ± 0.51	3.83 ± 0.46	1.58 ± 0.18	1.58 ± 0.10	4.86 ± 0.39	1.62 ± 0.17
Kidney	4.84 ± 0.64	4.53 ± 0.31	2.37 ± 0.24	1.66 ± 0.08	2.94 ± 0.27	1.30 ± 0.21
Spleen	1.67 ± 0.15	1.24 ± 0.11	0.56 ± 0.04	0.61 ± 0.03	1.20 ± 0.10	0.62 ± 0.05
Muscle	1.92 ± 0.33	1.70 ± 0.13	0.91 ± 0.05	0.79 ± 0.05	1.02 ± 0.11	0.65 ± 0.04
Fat	3.19 ± 0.28	2.10 ± 0.20	0.76 ± 0.06	0.77 ± 0.03	2.20 ± 0.16	0.78 ± 0.05
Uterus/blood	15	34	37	29	1	17

Twenty-one-day-old female rats were injected with 2 μCi of 11β-CH_3O-16α-$^{123}IE_2$ at the indicated times prior to kill time. Data are the mean ± s.e. and are corrected for decay (n = 6).

* Blocked: animals were injected i.p. with 2μg moxestrol in 100 μl saline/ethanol (4:1) 0.5 hr before the administration of the tracer.

† Chase: animals were injected i.p. with 2μg moxestrol 1 hr after receiving the tracer.

‡ % injected dose per ml whole blood.

found in the rat; much higher in the uterus than in other nontarget tissues. At 2 hr comparative ratios to most tissues were ~20 to 1 and at 3 hr closer to 30 to 1. Most importantly, the activity in the liver was relatively low from 2 hr on. Much more radioactivity was present in the kidney, producing lower uterine to kidney ratios and confirming what was detected in the imaging experiments. The high level of activity in the kidney of the rabbit is most probably the result of renal clearance of steroidal metabolites. The rabbit, but not the rat, has an entero-hepatic circulation. Thus in rabbits, steroidal metabolites reabsorbed from the intestine after hepatobiliary clearance are subsequently eliminated by the kidney (*26*). Alternatively, more metabolic deiodination may be occurring in the rabbit, which would lead to free iodide in the kidney. In the rat, maximal uterine isotope levels occurred at 1 hr; in the rabbit maximal levels were not noted before 2 hr and the highest uterine to nontarget tissue ratios were not until 3 hr. While in the rabbit, the percent uptake per gram of tissue was much lower than that of the rat, this is entirely consistent with the large difference in body weight.

Tissue radioactivity in animals that were blocked by the prior treatment with moxestrol was measured 3 hr after the injection of the tracer. In these rabbits there was no target tissue concentration of isotope (data not shown). Uterine levels were 20-fold lower than in animals that did not receive the blocking estrogen, and were the same as most of the nontarget tissues. The experiments shown in Table 4 were performed with animals that were injected with 500 μCi of 11β-CH_3O-16α-$^{123}IE_2$. In addition, several animals were injected with lower doses of tracer, 20 and 100 μCi. There was no difference in the distribution of the radioactivity, nor the % of injected dose taken up per gram of tissue when compared at the appropriate times (data not shown).

TABLE 3
Effect of Tamoxifen on the Distribution of 11β-CH_3O-16α-$^{123}IE_2$ in Immature Rats

	% Injected dose/g		
	Control	Tamoxifen block*	Tamoxifen chase†
Uterus	28.99 ± 2.30	0.91 ± 0.29	14.50 ± 1.62
Blood‡	0.61 ± 0.06	0.44 ± 0.06	0.44 ± 0.04
Liver	1.57 ± 0.10	0.92 ± 0.09	0.63 ± 0.07
Kidney	1.56 ± 0.06	0.63 ± 0.07	1.14 ± 0.08
Spleen	0.48 ± 0.03	0.26 ± 0.04	0.41 ± 0.02
Muscle	0.76 ± 0.06	0.23 ± 0.04	0.66 ± 0.13
Fat	0.65 ± 0.04	0.38 ± 0.06	0.57 ± 0.08

Twenty-one-day-old female rats were injected with 2μCi of 11β-CH_3O-16α-$^{123}IE_2$ 3 hr before kill time. Data are the mean ± s.e. (n = 6).

* Animals were injected i.p. with 200 μg tamoxifen in 200 μl saline/ethanol (3:1) 0.5 hr. before administration of the tracer.

† Animals were injected with 200 μg tamoxifen 1 hr after receiving tracer.

‡ % Injected dose per ml whole blood.

DISCUSSION

Like the ^{125}I-labeled analog, 11β-CH_3O-16α-$^{123}IE_2$ is an excellent ligand for the estrogen receptor, binding with high affinity, $K_a = 6 \times 10^9\ M^{-1}$. As would be expected, only estrogens and no other type of steroid inhibited the binding of 11β-CH_3O-16α-$^{123}IE_2$ to the receptor in rabbit uterine cytosol (Table 1). The synthetic procedure, including the purification, produces this [^{123}I] steroid in <3 hr and in high specific activity, >40,000 Ci/mmol. A rapid synthesis is a requirement for working with ^{123}I, which has a $t_{1/2}$ of 13 hr. The theoretical specific activity of ^{123}I, containing 5% ^{124}I, is calculated to be ~230,000 Ci/mmol. The nature of the trace contaminant which results in the fivefold reduction of the apparent specific activity of 11β-CH_3O-16α-$^{123}IE_2$ is not known. However, 40,000 Ci/mmol is

TABLE 4
Tissue Uptake of 11β-CH_3O-16α-$^{123}IE_2$ in Ovariectomized Rabbits

Tissue	% Injected dose/g tissue 1 hr	Ut/T	2 hr	Ut/T	3 hr	Ut/T	5 hr	Ut/T
Uterus	0.155 ± 0.035	—	0.210 ± 0.045	—	0.195 ± 0.027	—	0.185 ± 0.051	—
Blood*	0.008 ± 0.003	19	0.009 ± 0.003	23	0.007 ± 0.003	28	0.008 ± 0.001	23
Liver	0.025 ± 0.004	6	0.019 ± 0.002	11	0.011 ± 0.003	18	0.012 ± 0.002	17
Kidney	0.181 ± 0.093	1	0.137 ± 0.012	2	0.091 ± 0.043	2	0.134 ± 0.007	1
Muscle	0.016 ± 0.005	10	0.015 ± 0.003	14	0.011 ± 0.002	18	0.007 ± 0.001	28
Fat	0.028 ± 0.004	6	0.017 ± 0.001	12	0.009 ± 0.002	22	0.006 ± 0.000	31
Lung	0.012 ± 0.007	13	0.010 ± 0.001	21	0.007 ± 0.002	28	0.007 ± 0.001	16
Heart	0.010 ± 0.006	16	0.011 ± 0.001	20	0.007 ± 0.002	28	0.006 ± 0.000	31
Urine*	0.305 ± 0.328	0.5	1.300 ± 0.113	0.2	0.897 ± 0.124	0.2	0.774 ± 0.617	0.2

Ovariectomized rabbits were injected with 0.5 mCi of 11β-CH_3O-16α-$^{123}IE_2$. At the indicated times the animals were killed and the tissues dissected and counted. * % injected dose/ml. Ut/T is the ratio of the radioactivity in the uterus compared to the specified tissue. At 1 hr, n = 4; 2 hr, n = 2; 3 hr, n = 3; 5 hr, n = 2.

Data are the mean ± s.d. and are corrected for decay.

more than acceptable in order to administer a limiting (nonsaturating) mass of hormone in a reasonable dose of isotope. So for example, 2 mCi of 11β-CH_3O-16α-$^{123}IE_2$ would contain ~50 pmol of estrogenic steroid. This is equivalent to only a very small percentage of the circulating estradiol in a cycling human female; about the amount present in 100 ml of blood during mid cycle (*27*). On a weight basis, 2 mCi administered to an adult human would be considerably less than that given to the rats and rabbits in these studies.

The in vivo distribution of the 11β-CH_3O-16α-$^{123}IE_2$ administered to both rabbits and rats is similar to that which we originally found with 11β-CH_3O-16α-$^{125}IE_2$ (*21*). The injection of 11β-CH_3O-16α-$^{123}IE_2$ results in extremely high levels of radioactivity in the estrogen target tissue, uterus. At optimal times the radioactivity in most nontarget tissues was far lower than that in the uterus, resulting in comparative ratios of uterus to nontarget tissues of ~30 to 1. While these relative concentrations of radionuclide tagged steroid in the rabbit should have allowed imaging of the uterus, this was not accomplished because of the quantity of radioactivity in the neighboring kidneys, ureters, and bladder (Table 4).

Nevertheless, there is reason to be optimistic that 11β-CH_3O-16α-$^{123}IE_2$ will be useful clinically for imaging human estrogen receptor containing tumors, such as breast cancer. In the rabbit, the high level of radioactive metabolites in the urine (kidney, bladder, etc.) prevented imaging of the uterus. This problem was magnified because of the small size of this animal as well as the proximity of the target tissue to the urinary tract. In humans, the distance between tumors and the kidneys is likely to be much greater, and urinary radioactivity can be removed immediately with a catheter. Clinical imaging can be made more unambiguous through the use of a saturating chase antiestrogen and the specifically decreased radioactivity in the target tumor (Table 3) can be detected by computerized subtraction of the images. Finally, ^{123}I synthesized by $^{127}I(p,5n)^{123}Xe \rightarrow {}^{123}I$ nuclear reaction is now commercially available, and the use of this isotope preparation (which is contaminated with only small amounts of ^{125}I and not the more energetic ^{124}I) would improve the quality of the ^{123}I images.

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