Localization of Radiolabeled Enzyme Inhibitors in the Adrenal Gland

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Tissue distribution studies were performed in rats and dogs at five time intervals between 10 min and 24 hr after the intravenous injection of one of the following radiolabeled adrenocortical enzyme inhibitors: ^{3}H-aminoglutethimide, ^{125}I-3-iodoaminoglutethimide, ^{3}H-SKF-12185, ^{125}I-3-SKF-12185, ^{3}H-metyrapone, ^{3}H-metyrapol, ^{3}H-amphenone B, and ^{3}H-SU-10603. In rats, ^{3}H-SKF-12185 showed the highest uptake in the whole adrenal (3.5% kg dose/gm at 1 hr). In dogs, ^{3}H-metyrapol showed the highest uptake in the adrenal cortex (9% kg dose/gm at 1 hr), and the peak cortex-to-liver concentration ratio was 57 at 2 hr. These peak uptakes were comparable to those obtained with the conventional iodocholesterols, but they were reached much earlier, with elimination of most of the adrenal radioactivity by 24 hr. These properties would permit the use of ^{123}I as the label and a higher tracer dose, resulting in a higher photon flux. Thus, the radiolabeled enzyme inhibitors show promise as adrenal-scanning agents, with a markedly shortened scanning procedure, a lower absorbed radiation dose, and better resolution.

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In spite of numerous publications on adrenal cortex enzyme inhibitors, only Goldman has studied the uptake and release of a radiolabeled enzyme inhibitor in the adrenal cortex (*1*). When he gave ^{14}C-isoxazole to rats, Goldman showed that his competitive inhibitor would bind specifically, tightly, and for a long period of time to 3β-hydroxysteroid dehydrogenase and Δ^{5}-4,3-ketosteroid isomerase. We explored the possibility that these steroid dehydrogenase and isomerase activities might be sufficient in rat and human breast carcinoma to achieve a significant binding of ^{14}C-isoxazole (*2*). The ^{14}C-isoxazole concentrated in the Sprague–Dawley rat adrenal at 2% of the tracer dose per gram of tissue in 2 days and at 1% dose/gm in the ovaries. This concentration in adrenal was 30 times that in liver. The radioactivity in adrenal and ovary remained at one-half to one-third of this level for at least 35 days (*2*). When we treated rats with estradiol (*3*), the concentration of radioactivity from this radiolabeled "irreversible" enzyme inhibitor reached 8% dose/gm in the adrenal gland and 4% dose/gm in the ovary. Unfortunately, isoxazole is not easily labeled with a gamma-emitter.

This experience encouraged us to evaluate the localization of a series of radiolabeled reversible adrenocortical enzyme inhibitors previously used to inhibit human adrenocortical function (*4–7*).

METHODS

We evaluated the localization in the adrenal gland of ^{3}H-aminoglutethimide, ^{125}I-aminoglutethimide, ^{3}H-SKF-12185, ^{125}I-SKF-12185, ^{3}H-metyrapone, ^{3}H-metyrapol, ^{3}H-SU-10603, and ^{3}H-amphenone B in the rat and dog. The chemical structures of these compounds are shown in Fig. 1.

Synthesis of compounds. Aminoglutethimide,* metyrapone,* SU-10603,* SKF-12185,† and amphenone B‡ were labeled with tritium by New

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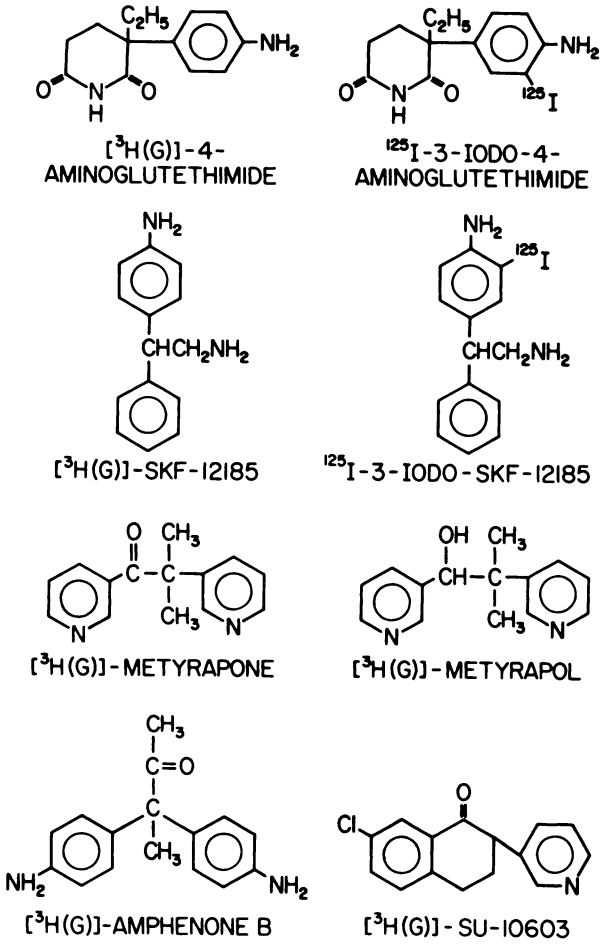

FIG. 1. Chemical structures of eight radiolabeled enzyme inhibitors evaluated in this study.

England Nuclear Corp. (North Billerica, Mass.) using catalytic exchange and were purified in our laboratory by column chromatography on silica gel-G. Specific activities were approximately 200 mCi/m*M*. The ^{3}H-metyrapol was obtained by sodium borohydride reduction of ^{3}H-metyrapone. All tritiated compounds had a radiochemical purity greater than 98% as determined by thin-layer chromatography on silica gel-G.

The ^{125}I-aminoglutethimide was synthesized by reaction of aminoglutethimide with $^{125}I_2$ in 50% ethanol at reflux temperature. The $^{125}I_2$ was initially generated in ether solution by the method of Hallaba and El-Shaboury (*8*). The specific activity of ^{125}I-3-iodoaminoglutethimide ranged from 30–150 mCi/m*M*. Similarly, ^{125}I-3-iodo-SKF-12185 was obtained by direct radioiodination of SKF-12185. The specific activity of the product ranged from 40–100 mCi/m*M*. Following purification by column chromatography on silica gel-G, both radioiodinated compounds had radiochemical purities greater than 96% as determined by thin-layer chromatography. The corresponding stable-iodine compounds were characterized by infrared and nuclear magnetic resonance spectroscopy and the correct carbon, hydrogen, and nitrogen analysis. Further details of the syntheses are being prepared for publication.

The compounds were formulated as the mono- or dihydrochloride salts, the pyridine derivatives in 0.9% saline solution, and the aniline derivatives in 5% dextrose solution.

Injected volumes varied over 0.2–0.5 ml. Sterility was ascertained by means of radiometric bacterial detection|| and freedom from pyrogens by the Limulus lysate test.

Animals. *Rats.* Eight groups of 15 mature female Sprague–Dawley rats, weighing 150–250 gm, were injected with each of the eight radiolabeled enzyme inhibitors. Each rat was given 25 μCi through a tail vein. The rats were killed in groups of three at 10 and 30 min and at 1, 4, and 24 hr after the dose, by excising the heart immediately after administration of ether anesthesia.

Dogs. Eight groups of 12 female mongrel dogs, weighing 8–12 kg, were injected with 400 μCi of each compound through a cephalic vein. Two dogs were killed at each time interval by intravenous administration of 50 mg/kg of sodium pentobarbital.

Tissue samples and ^{3}H radioactivity measurements. Representative tissue samples, weighing 30–50 mg, were placed in counting vials containing 0.3 ml of 10% NaOH, left to digest overnight, and dissolved by warming to 70°C. After cooling, the samples were neutralized with two drops of glacial acetic acid, decolorized with 3–6 drops of 30% hydrogen peroxide, and vortexed. Ten milliliters of liquid-scintillation fluid (PCS, Amersham/Searle, Arlington Heights, Ill.) and 2.5 ml of distilled water were added to each vial, which was again vortexed. After cold and dark adaptation, the samples were counted for 10 min in a liquid-scintillation system. Quench corrections were made using the channels-ratio counting technique. Tissue concentrations were expressed as percent kilogram dose per gram (% kg dose/gm), calculated as follows:

$$\frac{\mu\text{Ci/gm of tissue} \times \text{kg body wt} \times 100\%}{\mu\text{Ci administered dose}}.$$

Tissue samples and ^{125}I radioactivity measurements. Representative tissue samples, weighing 100–200 mg, were placed in 5-ml gamma tubes to which 2.5 ml of distilled water was added. Each tube was counted for 10 min in a scintillation spectrometer. Corrections were made for radioactive decay, background counts, and counter efficiency, and the results were again expressed as percent kilogram dose per gram.

TABLE 1. RELATIVE TISSUE DISTRIBUTION OF EIGHT RADIOLABELED ADRENOCORTICAL ENZYME INHIBITORS AT THE TIME OF PEAK ADRENAL UPTAKE IN RATS*
(% kg dose/gm tissue; mean ± s.e.m.)

Compound	Interval after dose	Adrenal			Ovary	Liver	Blood	Thyroid	Kidney	Urine
		Whole	Cortex	Medulla						
^{3}H-aminoglutethimide	30 min	2.02			0.19	0.23	0.23	0.09	0.17	0.75
(230 mCi/mM)					±0.02	±0.02	±0.03	±0.01	±0.02	±0.08
^{125}I-aminoglutethimide	30 min	0.82			0.09	0.03	0.04	0.15	0.11	
(30 mCi/mM)		±0.03			±0.01	±0.00	±0.00	±0.02	±0.01	
^{3}H-SKF-12185	1 hr	3.49	3.87	1.33	0.10	0.32	0.05	0.10	0.15	
(255 mCi/mM)		±0.61	±0.78	±0.30	±0.01	±0.01	±0.11	±0.02	±0.02	
^{125}I-SKF-12185	4 hr	2.59	1.98	0.57	0.09	0.27	0.03	8.56	0.12	
(85 mCi/mM)		±0.20	±0.39	(1 sample)	±0.01	±0.02	±0.00	±0.26	±0.01	
^{3}H-metyrapone	10 min	0.94	0.84	0.29	0.08	0.27	0.08	0.05	0.16	
(225 mCi/mM)		±0.08	±0.23	±0.14	±0.01	±0.01	±0.00	±0.02	±0.00	
^{3}H-metyrapol	10 min	1.39	1.43	1.37	0.17	0.38	0.08	0.08	0.28	
(225 mCi/mM)		±0.14	±0.21	±0.30	±0.02	±0.00	±0.00	±0.01	±0.02	
^{3}H-SU-10603	10 min	0.31	0.24	0.13	0.03	0.13		0.01	0.09	
(260 mCi/mM)		±0.11	±0.08	±0.08	±0.01	±0.03		±0.00	±0.03	
^{3}H-amphenone B	10 min	0.55	0.67	0.62	0.11	0.34	0.13	0.11	0.12	
(250 mCi/mM)		±0.10	±0.07	±0.05	±0.01	±0.03	±0.03	±0.03	±0.01	

* Peak adrenal uptake occurred at different time intervals with the different compounds.

TABLE 2. RELATIVE TISSUE DISTRIBUTION OF EIGHT RADIOLABELED ADRENOCORTICAL ENZYME INHIBITORS AT THE TIME OF PEAK ADRENAL UPTAKE IN DOGS*
(% kg dose/gm tissue; mean ± s.e.m.)

Compound	Interval after dose	Adrenal			Ovary	Liver	Blood	Thyroid	Kidney	Urine
		Whole	Cortex	Medulla						
^{3}H-aminoglutethimide	1 hr		0.68	0.88	0.16	0.41	0.45	0.16	0.20	2.81
(230 mCi/mM)			±0.08	±0.16	±0.02	±0.08	±0.08	±0.02	±0.05	±0.51
^{125}I-aminoglutethimide	10 min	0.17	0.18	0.13	0.10	0.71	0.05	0.17	0.17	0.20
(120 mCi/mM)		±0.01	±0.01	±0.01	±0.01	±0.02	Δ	±0.02	±0.01	±0.06
^{3}H-SKF-12185	30 min	1.79	1.86	1.47	0.16	0.64	0.06	0.29	0.23	1.62
(255 mCi/mM)		±0.01	±0.03	±0.27	±0.01	Δ	±0.04	±0.04	±0.01	±0.13
^{125}I-SKF-12185	1 hr	2.81	4.47	2.23	0.20	1.29	0.04	0.78	0.29	0.84
(30–70 mCi/mM)		±0.22	±0.14	±0.26	±0.04	±0.03	Δ	Δ	±0.01	±0.01
^{3}H-metyrapone	45 min	2.75	2.75	1.20	0.10	0.44	0.17	0.07	0.28	31.30
(225 mCi/mM)		±0.29	±0.17	±0.11	±0.01	±0.04	±0.01	±0.04	±0.04	±7.10
^{3}H-metyrapol	1 hr	6.87	8.91	6.81	0.11	0.52	0.10	0.07	0.19	20.85
(225 mCi/mM)		±1.13	±0.89	±0.62	±0.01	±0.06	Δ	±0.02	±0.03	±9.10
^{3}H-SU-10603	1 hr	0.56	0.65	0.59	0.22	0.86	0.27	0.18	0.47	9.71
(260 mCi/mM)		±0.01	±0.06	±0.02	±0.02	±0.04	±0.10	±0.01	±0.04	±0.06
^{3}H-amphenone B	30 min	0.38	0.41	0.41	0.13	0.36	0.73	0.13	0.23	11.35
(250 mCi/mM)		±0.02	±0.02	Δ	±0.02	±0.01	±0.34	Δ	±0.05	Δ

* Peak adrenal uptake occurred at different time intervals with the different compounds. (Δ) Less than 0.01.

RESULTS

Table 1 summarizes the relative tissue localization of radioactivity, as % kg dose/gm, for each of eight radiolabeled enzyme inhibitors in the female rat's whole adrenal, adrenal cortex, adrenal medulla, ovary, liver, blood, thyroid, kidney, and urine at the time of peak uptake in the adrenal. The uptake in all other tissues sampled (parathyroid, brain, intestine, spleen, heart, lung, pancreas, adipose tissue, and muscle) was less than in any of the former group and is therefore not presented here. Ranking by uptake in whole adrenal of the rat, ^{3}H-SKF-12185 came first (3.5% kg dose/gm at 1 hr), followed by ^{125}I-SKF-12185 (2.6% kg dose/gm at 4 hr), and ^{3}H-metyrapone (1% kg dose/gm at 10 min). In every instance where radioactive content in the adrenal cortex was analyzed separately from that in the adrenal medulla, the mean concentration in

adrenal medulla was higher than that in the liver but less than that in the cortex.

The radiolabeled SKF-12185 compounds were different from all the other radiolabeled enzyme inhibitors in two respects: the uptake in whole adrenal peaked later (1–4 hr) and remained in the gland in a concentration about six times greater than all the other enzyme inhibitors at 24 hr.

Table 2 presents the results of the same studies on whole adrenals in female dogs. Here the rank orders for highest uptake and for % kg dose/gm uptake differ from those in rats. The ^{3}H-metyrapol shows the highest uptake (7% kg dose/gm at 1 hr, twice the maximum uptake of any radiolabeled enzyme inhibitor in the rat), followed by ^{125}I-SKF-12185 (3% kg dose/gm at 1 hr), ^{3}H-metyrapone (3% kg dose/gm at 10 min), ^{3}H-SKF-12185 (2% kg dose/gm at 30 min), aminoglutethimide, SU-10603, and amphenone B. Here again, the uptake in the adrenal cortex was always greater, where determined, than in the medulla and both were always higher than in liver. At 1 hr, the concentration of ^{3}H-metyrapol radioactivity in adrenal cortex exceeded that in liver by a factor of 17, and at 2 hr by a factor of 57. Furthermore, the retention of these compounds in the adrenal at 24 hr always fell to less than 50% of the peak uptake. The concentration in the liver exceeded that in all tissues other than the adrenal.

It is unique in our experience with radioiodinated analogs, in comparison with their tritiated counterparts, that the iodinated SKF-12185 was taken up to a greater degree in the adrenal cortex in spite of a specific activity one-fourth to one-ninth that of the tritiated compound.

DISCUSSION

With ^{3}H-metyrapol, the maximum uptake in the dog's adrenal cortex of 9% kg dose/gm (equivalent to 0.13% dose/gm in a 70-kg man) and 0.1% dose/gm in the adrenal medulla in 1 hr compare favorably with the uptake of ^{131}I-19-iodocholesterol, which has been used successfully for diagnosing a wide variety of adrenal diseases (*9–13*). We have discretely imaged aldosteronomas 2 cm in diameter as a "white spot" superimposed on a "grey adrenal" as a result of the increased count rate furnished by the tumor (*12*). The highest uptake observed in these patients was 0.1% dose/gm. The ratio of adrenocortical radioactivity concentration to liver concentration with ^{3}H-metyrapol is 17 at 1 hr. This is comparable to the ratio of 16 at 1 day with ^{131}I-6-iodomethyl-19-norcholesterol (NP-59) (*14*), but the metyrapol ratio reaches 57 at 2 hr.

If the uptake in human adrenals of any ^{131}I- or ^{125}I-labeled analog of these reversible adrenocortical enzyme inhibitors proves to be equal to, or better than that in dogs, these compounds will have additional advantages for the diagnosis of adrenocortical and adrenal-medullary disease. The principal limitations of both our original ^{131}I-19-iodocholesterol (*9–13*) and the newer NP-59 (*14*) are that no imaging can be done before 24 hr after the tracer dose and that the variable retention of radioactivity in the human adrenal (for as long as 23 days after the administered dose) delivers a larger than desirable radiation dose to the adrenals, ovaries, and testes. If the radiolabeled enzyme inhibitors show the same % dose/gm uptake and target-to-nontarget ratios in humans as the iodocholesterols, they will allow adrenal imaging to be completed within 1 hr after administration. The rapid elimination of radioactivity from all organs within 24 hr will markedly decrease the absorbed radiation dose. Dexamethasone suppression scans could then be performed with a second tracer dose within 24 hr after the first. In preliminary experiments in dogs we have found that the uptake of radioactivity from ^{3}H-metyrapol is inhibited by the administration of dexamethasone, similar to the decreased uptake of ^{131}I in the radioiodinated cholesterols.

The radioiodination of aniline inhibitors such as SKF-12185 can be accomplished in less than 3 hr by the chloramine-T method. This facile synthesis, combined with the rapid localization of the enzyme inhibitor in the adrenal cortex, would allow radiolabeling with ^{123}I, which has an optimum gamma energy and short physical half-life. The resulting admissibility of higher tracer doses, leading to increased photon flux and better resolution, would aid in the detection of smaller adrenal tumors.

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FOOTNOTES

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|| Bactec (Johnston Laboratories, Baltimore, Md.).

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