I (X = 3-$CH_2OC_6H_4$-3′-$NHCOCH_3$), 79508-86-4; I (X = 3-$CH_2OC_6H_4$-3′-$NHCSNH_2$), 79508-87-5; I (X = 3-$CH_2OC_6H_4$-3′-$NHCONH_2$), 70579-43-0; I [X = 3-$CH_2OC_6H_4$-4′-$(CH_2)_4CH_3$], 87739-81-9; I [X = 3-(methoxycarbonyl)-2-naphthyl], 87871-40-7; I [X = 3-(methoxycarbonyl)-1-naphthyl], 87871-41-8; I (X = 3-$CH_2SC_6H_5$), 80239-83-4; I (X = 3-$CH_2SC_6H_4$-3′-CH_3), 87739-85-3; I (X = 3-$CH_2SeC_6H_5$), 87739-79-5; I (X = 3-$SCH_2C_6H_5$), 87739-83-1; I (X = 3-$SCH_2C_6H_4$-4′-Cl), 87739-84-2; DHFR, 9002-03-3.

Adrenal Medulla Imaging Agents: A Structure–Distribution Relationship Study of Radiolabeled Aralkylguanidines[1]

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Fourteen ^{125}I-labeled aralkylguanidines were synthesized and evaluated as potential imaging agents for the adrenal medullae and tumors of adrenomedullary origin. These guanidines are radiotracer analogues of guanethidine, an antihypertensive agent thought to mediate neuron blockade by uptake into adrenergic nerves. Dog adrenal medullae were used as a model to test radiotracer affinity for catecholamine storage tissue. Tissue distribution studies revealed that a number of radioiodinated guanidines showed pronounced localization in the adrenal medullae following intravenous injection, in certain cases exceeding that of either (−)-[^3H]norepinephrine or [^{14}C]guanethidine. (*m*-[^{125}I]Iodobenzyl)guanidine (*m*-IBG, **2b**) gave the best combination of high concentration and selectivity. The low adrenomedullary affinity observed with [^{14}C]guanidine and *m*-[^{125}I]iodobenzylamine demonstrates the uniqueness of the aralkylguanidine structure. Preliminary evidence suggests that **2b** is a storage analogue of norepinephrine. [^{125}I]**2a** is now being used clinically in imaging and radiotherapy of catecholamine tumors, such as pheochromocytoma.

An imaging agent for the adrenal medulla and its diseases has been actively pursued for more than a decade.[2-13] Recent efforts in our laboratory to develop a clinically useful agent have focused on radioiodinated analogues of the antihypertensive drug guanethidine. This drug inhibits the release of norepinephrine from adrenergic nerve endings, as well as depletes neuronal stores of norepinephrine.[14,15] Both of these effects involve the direct action of guanethidine on the adrenergic nerves.[16] If the adrenal medulla is considered a specialized sympathetic ganglion,[17] then compounds known to have an affinity for adrenergic nerves might be expected to localize in the adrenal medulla.

Studies in dogs in the early 1960's, however, showed that pharmacological doses of guanethidine, although rapidly depleting the heart and spleen of norepinephrine, had little effect on the catecholamine content of the adrenal medulla.[18,19] Nonetheless, our initial studies revealed that [^{14}C]guanethidine had a high affinity for the dog adrenal medulla. Although guanethidine is not readily labeled with a γ-emitting radionuclide suitable for use in scintigraphy, pharmacologically active analogues such as benzylguanidines can be readily labeled by substitution of radioiodine on the aromatic ring. In the benzylguanidine series, Short and Darby[20] have shown that lipophilic aromatic substituents (e.g., CF_3, Br, I) can, in certain cases, enhance neuron blocking potency. However, since pharmacological activity may not be the best correlate of adrenal medulla uptake, we report here a structure–distribution relationship (SDR) study of 14 ^{125}I-labeled (iodoaralkyl)guanidines and 3 [^{14}C]guanidines in dogs. This study focuses on the structural elements of aralkylguanidines necessary for maximum adrenomedullary uptake and retention. One of the most promising compounds, (*m*-iodobenzyl)guanidine (*m*-IBG, **2a**), when radiolabeled with γ-emitting isotopes ^{131}I or ^{123}I, has shown recent clinical success in imaging diseases of the adrenal medulla.[21,22]

Chemistry. At the outset of this investigation, attempts were made to synthesize the (iodoaralkyl)guanidines by reaction of the appropriate amine with 2-methyl-2-thiopseudourea sulfate.[23,24] It was subsequently found that reaction of the appropriate amine hydrochloride with molten cyanamide gave consistently higher yields of

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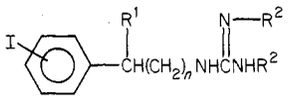
(1) A brief report of part of the present study has appeared: Wieland, D. M.; Brown, L. E.; Mangner, T. J.; et al. Proceedings of the 3rd International Symposium on Radiopharmaceutical Chemistry, *J. Labelled Cmpd. Radiopharm.* **1981**, *18*, 122.
(2) Morales, J. O.; Beierwaltes, W. H.; Counsell, R. E.; et al. *J. Nucl. Med.* **1967**, *8*, 800.
(3) Lieberman, L. M.; Beierwaltes, W. H.; Varma, V. M.; et al. *J. Nucl. Med.* **1969**, *10*, 93.
(4) Anderson, B. G.; Beierwaltes, W. H.; Harrison, T. S.; et al. *J. Nucl. Med.* **1973**, *14*, 781.
(5) Fowler, J. S.; Ansari, A. N.; Atkins, H. L.; et al. *J. Nucl. Med.* **1973**, *14*, 867.
(6) Fowler, J. S.; Wolf, A. P.; Christman, R. D.; et al. In "Radiopharmaceuticals", Subramanian, G.; Rhodes, B. A.; Cooper, J. T., et al., Eds.; Society of Nuclear Medicine: New York, 1975; p 196.
(7) Ice, R. D.; Wieland, D. M.; Beierwaltes, W. H.; et al. *J. Nucl. Med.* **1975**, *16*, 1147.
(8) Fowler, J. S.; MacGregor, R. R.; Wolf, A. P. *J. Med. Chem.* **1976**, *19*, 356.
(9) Counsell, R. E.; Yu, T.; Ranade, V. V.; et al. *J. Med. Chem.* **1973**, *16*, 1038.
(10) Korn, N.; Buswink, A.; Yu, T.; et al. *J. Nucl. Med.* **1977**, *18*, 87.
(11) Wieland, D. M.; Swanson, D. P.; Brown, L. E.; et al. *J. Nucl. Med.* **1979**, *20*, 155.
(12) Wieland, D. M.; Wu, J. L.; Brown, L. E.; et al. *J. Nucl. Med.* **1980**, *21*, 349.
(13) Wieland, D. M.; Brown, L. E.; Tobes, M. C.; et al. *J. Nucl. Med.* **1981**, *22*, 358.
(14) Chang, C. C.; Chang, J. C.; Su, C. Y. *Br. J. Pharmacol.* **1967**, *30*, 213.
(15) Giachetti, A.; Hollenbeck, R. A. *Br. J. Pharmacol.* **1976**, *58*, 497.
(16) For an excellent review, see: Maxwell, R. A.; Wastila, W. B. *Handb. Exp. Pharmacol.* **1977**, *39*, 161.
(17) Stjarne, L. *Handb. Exp. Pharmacol.* **1972**, *33*, 231.
(18) Athos, W. J.; McHugh, B. P., Fineberg, S. E.; et al. *J. Pharmacol.* **1962**, *137*, 229.
(19) Cass, R.; Kuntzman, R.; Brodie, B. B. *Proc. Soc. Exp. Biol. Med.* **1960**, *103*, 871.
(20) Short, J. H.; Darby, T. D. *J. Med. Chem.* **1967**, *10*, 833.
(21) Sisson, J. C.; Frager, M. S.; Valk, T. W.; et al. *N. Engl. J. Med.* **1981**, *305*, 12.
(22) Valk, T. W.; Frager, M. S.; Gross, M. D.; et al. *Ann. Int. Med.* **1981**, *94*, 762.

0022-2623/84/1827-0149$01.50/0

Table I. Characteristics of Guanidinium Salts



compd	position of I	R^1	n	R^2	mp, °C	formula[a]	crystn solvent	yield,[b] %
1a	ortho	H	0	H	230-232[e]	$C_8H_{10}IN_3 \cdot 0.5H_2SO_4$	CH_3OH	63
2a	meta	H	0	H	164-165	$C_8H_{10}IN_3 \cdot 0.5H_2SO_4$	H_2O	84
3a	para	H	0	H	260-262[f]	$C_8H_{10}IN_3 \cdot 0.5H_2SO_4$	H_2O	64
4a	meta	H	0	CH_3	217-220	$C_{10}H_{14}IN_3 \cdot HCl$	$EtOH/Et_2O$	43
5a	para	H	0	CH_3	247-249	$C_{10}H_{14}IN_3 \cdot HCl$	$EtOH/Et_2O$	56
6a	meta	H	1	H	177-179	$C_9H_{12}IN_3 \cdot 0.5H_2SO_4$	$EtOH/H_2O$	42
7a	para	H	1	H	228-229	$C_9H_{12}IN_3 \cdot 0.5H_2SO_4$	H_2O	30
(±)-8a	meta	CH_3	0	H	279-281	$C_9H_{12}IN_3 \cdot 0.5H_2SO_4$	$EtOH/H_2O$	70
(+)-8a[c]	meta	CH_3	0	H	281-283	$C_9H_{12}IN_3 \cdot 0.5H_2SO_4$	$EtOH/H_2O$	
(−)-8a[d]	meta	CH_3	0	H	279-280	$C_9H_{12}IN_3 \cdot 0.5H_2SO_4$	$EtOH/H_2O$	
(±)-9a	para	CH_3	0	H	267-269	$C_9H_{12}IN_3 \cdot 0.5H_2SO_4$	$EtOH/H_2O$	76
10a	3,4-I_2	H	0	H	266-268	$C_8H_9I_2N_3 \cdot 0.5H_2SO_4$	$H_2O/EtOH$	44
11a	3,5-I_2	H	0	H	273-275[g]	$C_8H_9I_2N_3 \cdot 0.5H_2SO_4$	H_2O	39
12a [(*m*-iodophenyl)guanidine]					247-248	$C_7H_8IN_3 \cdot 0.5H_2SO_4$	$EtOH/H_2O$	29

[a] Microanalyses were within ±0.3% of theoretical values. [b] Isolated yields starting from the respective amine. [c] $[M]_D$ −40.1° (H_2O). [d] $[M]_D$ 21.6° (H_2O). [e] Literature[20] mp 229-231. [f] Literature[20] mp 258-260 °C. [g] Decomposes.

pure guanidines. Lower reaction temperatures (120–135 °C) and shorter reaction times (2–4 h) than those suggested in the literature[20,25] were utilized. Workup of the cyanamide reaction was modified from that reported by Short and Darby.[20] The water-soluble guanidine hydrochlorides were converted to their respective bicarbonate salts,[25,26] and the precipitates were isolated and then acidified with 2 N sulfuric acid. The resulting sulfate salts were recrystallized to obtain analytically pure compounds, which were used in subsequent radiolabeling experiments. Attempts were not made to maximize yields, a notable exception being **2a**.

The *N'*,*N''*-dimethyl-*N*-benzylguanidines **4a** and **5a** were synthesized by the method of King and Tonkin.[27] For radiolabeling experiments, the hydriodide salts of the guanidines were converted to their respective hydrochloride salts by passage through Amberlite IRA-400 anion-exchange resin (chloride form). Partial resolution of (+)-(*m*-iodo-α-methylbenzyl)guanidine (**8a**) was achieved by fractional crystallization of its (+)- and (−)-mandelate salts with subsequent conversion to the respective sulfates.

Only five of the precursor amines were unavailable commercially. *m*-Iodophenethylamine was synthesized by diborane reduction of (*m*-iodophenyl)acetonitrile. The 3,4-diiodobenzylamine was obtained by iodination of *p*-iodobenzylamine by the Derbyshire method.[28] The 3,5-diiodobenzylamine was synthesized by diiodination of *p*-aminobenzonitrile with iodine monochloride, followed by reductive deamination with nitrous acid/hypophosphorous acid and subsequent reductive of the nitrile with $BH_3 \cdot Me_2S$. The iodo-α-methylbenzylamines were obtained from *m*- and *p*-iodoacetophenone, respectively, via the Leuckhart reaction.[29]

Radiochemistry. In this work the labeling and biodistribution studies were performed on compounds labeled with ^{125}I. This radionuclide is not only less expensive but its long $T_{1/2}$ (60 days) and low γ energy (36 keV) make it more convenient to handle. The findings reported in this study, however, are equally applicable to other radioiodine isotopes (e.g., ^{123}I and ^{131}I).

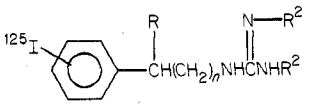
Initially, the radiosyntheses of the ^{125}I-labeled guanidines listed in Table II represented a formidable task. The "cold" or nonradioactive iodoguanidines posed no synthetic difficulty—the precursor amines are either commercially available or readily synthesized. Optimally, radioiodine should be introduced in the final synthetic step, preferably by an isotopic exchange technique. However, at the outset of this investigation, no method existed to exchange label iodo aromatics that were not activated either by strong electron-withdrawing or -donating groups. Indeed, our first attempts to exchange label **2a** with $Na^{125}I$ failed under all classical exchange conditions.[30] A new technique for the synthesis of aryl radioiodides of high specific activity was then developed, which involves a mild, solid-phase exchange under mildly acidic, oxidizing conditions. The in situ thermal decomposition of $(NH_4)_2SO_4$ facilitates the exchange.[30] The technique has permitted the rapid, high-yield radiosyntheses of all the ([^{125}I]iodoaralkyl)guanidines in Table II. Exchange reactions were conducted in the solid state at 120–140 °C for 2–4 h with ammonium sulfate as a promoter. Purification was achieved by dissolving the reaction mixture in 13.5 mM sodium acetate buffer (pH 4.5) and passing the solution through a column of Cellex-D anion-exchange resin to remove unreacted radioiodide. Radiochemical yields ranged from 60 to 98%. Specific activities were routinely 0.25–0.80 Ci/mmol, although values as high as 100 Ci/mmol could be achieved if desired, albeit in slightly lower yield. Radiochemical purity for all ^{125}I-labeled compounds was greater than 98%, as determined by radio-TLC (three solvent systems) and, in a few select cases, by radio-HPLC.

The hydriodide salts of iodoguanidines **4a** and **5a** gave poor exchange yields; carrier iodide likely competes with [^{125}I]iodide for exchange. The respective hydrochloride salts, however, gave good exchange yields.

The exchange reaction of $Na^{125}I$ with *m*-IBG (**2a**) was chosen for more detailed purity studies, both radiochemical and nonradiochemical. Potential radioactive impurities, such as [^{125}I]iodide, *m*-[^{125}I]iodobenzylamine, (*m*-[^{125}I]-iodobenzyl)urea, (3,4-[^{125}I]diiodobenzyl)guanidine and

(23) Rathke, B. *Ber. Dtsch. Chem. Ges.* **1881**, *14*, 1774.
(24) Braun, C. E. *J. Am. Chem. Soc.* **1933**, *55*, 1280.
(25) Bobeck, R. *Justus Liebigs, Ann. Chem.* **1931**, *487*, 294.
(26) Fielden, R.; Green, A. L.; Willey, G. L. *Br. J. Pharmacol.* **1965**, *24*, 395.
(27) King, H.; Tonkin, I. M. *J. Chem. Soc.* **1946**, 1063.
(28) Barker, I. R. L.; Waters, W. A. *J. Chem. Soc.* **1952**, *150*.
(29) Ingersoll, A. W. In "Organic Syntheses"; Wiley: New York, 1943; Collect. Vol. II, p 503.
(30) Mangner, T. J.; Wu, J. L.; Wieland, D. M.; et al. *J. Org. Chem.* **1982**, *47*, 1484.

Table II. SDR Data on Radiolabeled Guanidines in Dogs[a]



compd	position of radioiodine	R^1	n	R^2	adrenal medulla concn[b] 30 min	adrenal medulla concn[b] 72 hr	[adrenal medulla]/[liver] at 72 h
1b	ortho	H	0	H	1.7 ± 0.1	1.3 ± 0.1[c]	130[c]
2b	meta	H	0	H	5.4 ± 0.7	13.0 ± 1.5	720
3b	para	H	0	H	3.2 ± 0.6	13.9 ± 1.6	545
4b	meta	H	0	CH_3	0.4 ± 0.0		
5b	para	H	0	CH_3	0.1 ± 0.0		
6b	meta	H	1	H	4.4 ± 0.3	4.8 ± 0.7	190
7b	para	H	1	H	1.4 ± 0.2	2.4 ± 0.6	200
(±)-8b	meta	CH_3	0	H	5.4 ± 0.4	7.3 ± 1.0	430
(+)-8b	meta	CH_3	0	H	4.2 ± 0.5	9.3 ± 0.9	390
(−)-8b	meta	CH_3	0	H	6.4 ± 1.2	7.3 ± 1.6	300
(±)-9b	para	CH_3	0	H	2.9 ± 0.1	7.2 ± 0.5	190
10b	3,4-I_2	H	0	H	2.1 ± 0.3	4.9 ± 0.1	115
11b	3,5-I_2	H	0	H	0.4 ± 0.0	0.5 ± 0.0	5.2
12b [(*m*-iodophenyl)guanidine]					3.0 ± 0.1	1.3 ± 0.3	45
13 ([^{14}C]guanethidine)[d]					2.6 ± 0.8	5.1 ± 0.6	70
14 ([^{14}C]guanidine)					0.2 ± 0.0	0.2 ± 0.0[d]	1.4[e]
15 ([^{14}C]benzylguanidine)[f]					9.3 ± 0.8	8.1 ± 2.2	2700
16 [(*m*-iodobenzyl)amine]					0.2 ± 0.0		
17 [(−)-[^{3}H]norepinephrine][g]					13.8 ± 0.5	8.0 ± 0.6	

[a] Tissue distribution data for all compounds in 18 selected tissues can be obtained upon request from the author. [b] Concentration is given in percent kilogram dose per gram, plus or minus the standard error of the mean; N ranged from 4 to 12. [c] 48 h. [d] [2-(Octahydro-1-azocinyl)ethyl][^{14}C]guanidine sulfate. [e] 2 h. [f] [*methyl*-^{14}C]Benzylguanidine. [g] L-[7-^{3}H(N)]-Norepinephrine.

N,N'-bis(*m*-[^{125}I]iodobenzyl)urea, were shown to be absent by separate synthesis and analysis by radio-HPLC.[31] The absence of the radiochemical impurities is also evidence for their absence as the respective nonradiolabeled impurities, since they have been shown in separate experiments to undergo radioiodide exchange under the reaction conditions employed. To further demonstrate the nonradiochemical purity of *m*-[^{125}I]IBG (**2b**), a mock exchange reaction using Na^{127}I was conducted. The chemical purity (>98%) of *m*-IBG (**2a**) was then determined by HPLC using ultraviolet detection (254 nm). The four iodo aromatics listed above, as well as benzylguanidine, were shown to be absent under the HPLC conditions employed. Scrambling of the radioiodine label during the exchange process did not occur (<2%), as evidenced by the HPLC chromatogram shown in Figure 1, which compares the radioactive trace of *m*-[^{131}I]IBG with the ultraviolet trace of a mixture of (*o*-, *m*-, and *p*-iodobenzyl)guanidines.

Biological Evaluation. The choice of the animal model(s) is a difficult one. The ultimate goal of this work was to produce a radiodiagnostic agent to image tumors of the human adrenal medullae. These tumors (pheochromocytoma) synthesize and store catecholamines and generally possess the properties of the adrenomedullary tissue from which they originate. A rat pheochromocytoma model is available,[32,33] but we have had little success in showing consistent uptake of adrenal-avid compounds in these tumors. The normal adrenal medulla would serve as a more convenient model for screening compounds. However, rat adrenal medullae are small and difficult to clearly dissect from cortical tissue. We have chosen the dog as our model because their medullae are large and easier to isolate, and follow-up imaging experiments with

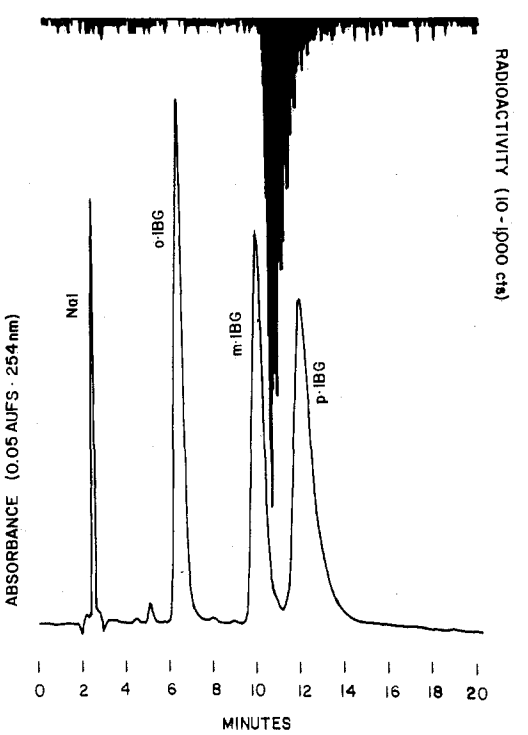


Figure 1. HPLC, employing radioactivity and ultraviolet (254 nm) detection of an aqueous solution of [^{131}I]**2a** spiked with **1a** (*o*-IBG), **2a** (*m*-IBG), **3a** (*p*-IBG), and NaI. Analysis performed on a Waters μ-Bondapak C-18 column eluted with 0.2 M $NH_4H_2PO_4$/THF, 80:20, at 1.5 mL/min. The t_R difference between the UV peak for carrier *m*-IBG and the radioactive peak for *m*-[^{131}I]IBG represents the time lag between the UV and radioactive detectors, which are in series.

^{131}I-labeled analogues can be conveniently performed with dogs.[11]

The norepinephrine (NE)–epinephrine (E) concentration of adrenomedullary tissue is an indicator of the catecholamine storage capacity and/or chromaffin granule

(31) Mangner, T. J.; Inbasekaran, M.; Wieland, D. M., unpublished findings.
(32) Warren, S.; Chute, R. *Cancer* **1972**, *29*, 327.
(33) Oberlechner, E.; Westhead, E.; Neuman, B.; et al. *J. Neurochem.* **1982**, *38*, 615.

density in this tissue. Numerous estimates of the NE and E content of the adrenal of various mammals have shown that the total concentration of catecholamines and the ratio of E to NE varies from species to species.[34] Although the NE plus E concentration in the normal dog adrenal is higher than in the human adrenal, pathological conditions in humans, such as pheochromocytoma, can exhibit exceedingly high catecholamine levels.[35]

Results and Discussion

Table II summarizes the biodistribution data obtained for the 17 radiolabeled guanidines evaluated in this study. The tissue concentrations in Table II are given as the mean of a minimum of four tissue samples (duplicate samples from two dogs). The concentrations are expressed as percent kilogram dose per gram.[36] Data for *m*-[^{125}I]-iodobenzylamine (**16**) and (−)-[^{3}H]norepinephrine (**17**) are also included in Table II. More detailed tissue distribution data for compounds **1b–3b** have been published elsewhere.[12] The concentration of radioactivity in the adrenal medulla 30 min after intravenous injection provides an estimate of the initial uptake of the compound. The 72-h data give an assessment of the retention of the compound in the adrenal medulla. In the absence of extensive metabolic studies, it must be emphasized that the tissue concentrations compiled in Table II represent the concentration of radioactivity, not necessarily the concentration of the radioactive compound injected. Metabolic studies of certain guanidine derivatives have shown significant, species-dependent metabolism.[16] Guanethidine,[37,38] N,N′,N″-trisubstituted guanidines, such as bethanidine,[39] and cyclic analogues, such as the isoquinoline carboxamidine debrisoquin,[40,41] are all metabolized to some extent. The results in Table II, especially for compounds **4b**, **5b**, and **13**, should be considered with possible metabolism in mind. Detailed metabolic studies of *N*-benzylguanidines are lacking. However, radio-TLC analysis of adrenomedullary tissue of dogs injected with **3b** has revealed that >95% of the extracted radioactivity is unchanged **3b**.[12] A similar study of **2b** using radio-HPLC has shown that >98% of the adrenomedullary radioactivity at 24 h postinjection is due to unchanged **2b**.[31] The in vivo stability of the carbon–iodine bond in the ^{125}I-labeled compounds in Table II was good, as indicated by low concentrations of radioactivity in the thyroid, an organ that efficiently sequesters iodide. By comparison of the thyroid concentrations of radioactivity obtained with **1b–3b** and **6b–12b** to a standard graph of thyroid radioactivity concentration vs. time in dogs injected intravenously with Na^{125}I, we estimate that at 24 h postinjection none of these compounds show >4% deiodination. However, at 72 h, the values ranged from 3 to 25%; compounds **3b**, **6b**, and **10b** in particular showed a high degree of deiodination at this time interval.

Since a homogeneous distribution of a radiochemical evenly throughout the body would result in a concentration of 0.1% kg dose/g in all body tissues,[36] it can be seen that many of the guanidines show high affinity for the adrenal medulla. In screening for potential adrenomedullary radiopharmaceuticals, high concentrations of radioactivity in the adrenal medulla are desirable, as well as low concentrations in surrounding organs, such as adrenal cortex, kidneys, liver, intestines, stomach, and gallbladder. The liver is particularly troublesome because of its large size (2600 times heavier than the adrenal medulla) and high metabolic activity. Thus the 72-h adrenal medulla to liver concentration ratio is given in Table II as an index of the degree of selectivity of the radiolabeled compounds for the adrenal medulla. Markedly high concentration ratios were obtained for a majority of the radiolabeled guanidines evaluated.

The following observations can be made based on the data in Table II: (1) Of the radioiodinated compounds, **2b** shows the best combination of high retention and pronounced selectivity for the adrenal medulla. (2) Two structural modifications of **2b** result in a striking diminution in adrenomedullary affinity, methyl substitution on the N′,N″ atoms (e.g., **4b** and **5b**) and incorporation of a second iodine atom in the 5-position (**11b**). (3) The low values obtained with [^{14}C]guanidine (**14**) and *m*-[^{125}I]-iodobenzylamine (**16**) stress the combined importance of both the aralkyl and guanidino portions of *m*-IBG to adrenomedullary localization. (4) Comparison of **2b** with [^{14}C]benzylguanidine (**15**) suggests that incorporation of radioiodine into benzylguanidine increases the 72-h concentration of radioactivity in the adrenal medulla but lowers the adrenal medulla to liver concentration ratio. However, the definitive experiment will be to compare the biodistributions of [*methyl*-^{14}C]benzylguanidine with [*methyl*-^{14}C]-*m*-IBG of the same specific activity.

In view of the close ontogenetic relationship between chromaffin cells and sympathetic nerves, it is not surprising that the adrenal medulla removes and retains circulating catecholamines. The ability of the adrenal medullae to efficiently accumulate tracer amounts of radioactive catecholamines was first demonstrated by the early work of Axelrod and co-workers.[42,43] Although the adrenomedullary uptake of radioactivity at 30 min from a bolus intravenous injection of (−)-[^{3}H]norepinephrine is higher than that of any radiolabeled guanidine studied, the retention (or more appropriately the net accumulation) of radioactivity in the medullae at 72 h is less than observed with **2b** or **3b**. Although speculative at this point, this reversal may be due to the continuous release of **2b** or **3b** into the blood pool from the peripheral adrenergic nerves and partial reuptake in the adrenal medullae. This systemic, steady perfusion is likely a less efficient process in the case of (−)-[^{3}H]norepinephrine due to its rapid metabolism by monoamine oxidase or catechol *O*-methyltransferase following release from the peripheral adrenergic nerves. Alternatively, the adrenomedullary concentration pattern of the two radiotracers may be due, at least in part, to a slower release of *m*-IBG (**2b**) from the adrenal medullae.

A potential problem exists in that the three ^{14}C-labeled compounds evaluated in this study have up to 100-fold lower specific activities than the radioiodinated compounds. The specific activity or loading dose of a radio-

(34) Holzbauer, M.; Sharman, D. F. *Handb. Exp. Pharmacol.* **1972**, *33*, 110.
(35) Winkler, H.; Smith, A. D. *Handb. Exp. Pharmacol.* **1972**, *33*, 900.
(36) Kirschner, A. S.; Ice, R. D.; Beierwaltes, W. H. *J. Nucl. Med.* **1975**, *16*, 248.
(37) Bisson, G. M.; Muscholl, E. *Nauyn-Schmedebergs Arch. Exp. Pathol. Pharmacol.* **1962**, *244*, 185.
(38) McMartin, C.; Rondel, R. K.; Vinter, J.; et al. *Clin. Pharmacol. Ther.* **1970**, *11*, 423.
(39) Turnbull, L. B.; Teng, L.; Newman, J.; et al. *Drug Metab. Disp.* **1976**, *4*, 269.
(40) Mahgoub, A.; Idle, J. R.; Dring, L. G.; et al. *Lancet* **1977**, *2*, 584.
(41) Medina, M. A.; Giachetti, A.; Shore, P. A. *Biochem. Pharmacol.* **1969**, *18*, 891.
(42) Axelrod, J.; Weil-Malherbe, H.; Tomchick, R. *J. Pharmacol. Exp. Ther.* **1959**, *127*, 251.
(43) Whitby, L. G.; Axelrod, J.; Weil-Malherbe, H. *J. Pharmacol. Exp. Ther.* **1961**, *132*, 193.

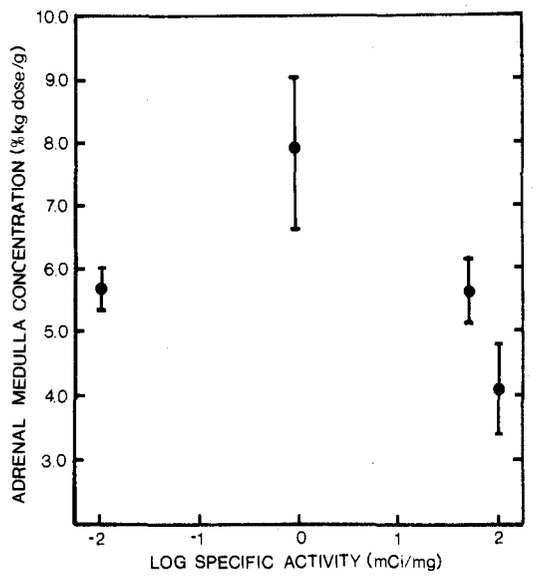


Figure 2. Semilog plot of the specific activity of *m*-[^{125}I]IBG (**2b**) vs. concentration of radioactivity in dog adrenal medulla 5 min after a 100-μCi intravenous injection of **2b**: mean ± SEM (N = 4) for each point.

labeled compound can greatly affect its biodistribution pattern, especially if the tracer binds to tissues by a low capacity, receptor-mediated process.[44] Fortunately, however, as shown in Figure 2, the capacity of the adrenal medulla for radiolabeled guanidines, exemplified by **2b**, is very high: through a 1000-fold variation in specific activity the percent of the injected dose localizing in adrenomedullary tissue shows little change. The adrenal medulla concentration of **2b** reaches the millimolar range at the lowest specific activity injection used (i.e., 3.25 mCi/mmol).

In order to gain a perspective on the striking selectivity that many of the radiolabeled guanidines in Table II have for the adrenal medulla, the whole-body tissue distribution of **2b** in the dog at 24 and 72 h postinjection is given in Table III. Besides the adrenal medulla, only the thyroid gland and urine show a comparatively high concentration of radioactivity. The thyroid activity is due mainly to radioiodide, as evidenced by the large decrease in this value when dogs were maintained on oral KI before and after injection of **2b**. It is also notable at 24 h that organs with relatively dense adrenergic innervation such as spleen and heart have higher concentrations of radioactivity than organs with lesser innervation, such as kidney and liver.[34]

It is tempting to seek out a possible correlation between the adrenomedullary concentration of the compounds in Table II and their ability to act as neuron blockers and/or norepinephrine depletors. Though the pharmacological action of these compounds on the sympathetic nervous system was not determined in this study, Short and Darby[20] have evaluated the neuron blocking activity of (*o*-iodobenzyl)guanidine (**1a**) and (*p*-iodobenzyl)guanidine (**3a**) and found only the latter compound to be active. As noted in Table II, radiolabeled (*p*-iodobenzyl)guanidine (**3b**) displays a 10-fold higher adrenomedullary concentration than (*o*-iodobenzyl)guanidine (**1b**) at 72 h. Phenylguanidines seemingly lack appreciable neuron blocking activity,[45] and the one phenylguanidine evaluated in this

Table III. Biodistribution of 2b in Female Dogs

tissue	concn[a] 24 h	concn[a] 72 h
adrenal medulla	7.89 ± 0.52	12.99 ± 1.48
adrenal cortex	0.17 ± 0.06	0.17 ± 0.04
ovary	0.07 ± 0.02	0.03 ± 0.01
liver	0.03 ± 0.00	0.02 ± 0.00
kidney	0.04 ± 0.00	0.03 ± 0.00
spleen	0.20 ± 0.01	0.08 ± 0.02
pancreas	0.05 ± 0.01	0.03 ± 0.00
heart	0.10 ± 0.01	0.04 ± 0.01
lung	0.05 ± 0.02	0.04 ± 0.01
thyroid	3.36 ± 0.75[b]	3.57 ± 0.70[b]
small intestine	0.04 ± 0.01	0.04 ± 0.02
large intestine	0.03 ± 0.00	0.02 ± 0.00
stomach	0.05 ± 0.00	0.03 ± 0.00
muscle	0.04 ± 0.00	0.02 ± 0.00
adipose	0.00 ± 0.00	0.00 ± 0.00
bile	0.14 ± 0.02	0.03 ± 0.01
blood	0.02 ± 0.00	0.01 ± 0.00
urine	2.37 ± 0.98	0.54 ± 0.10

[a] Percent kilogram dose per gram, plus or minus the standard error of the mean; N = 4 at 24 h; N = 10 at 72 h. [b] This value averaged <0.20 in two dogs maintained on a regimen of KI before and after injection.

study (i.e., **12b**) showed relatively low affinity for the adrenal medulla. Though there are many structural and functional similarities between chromaffin cells and sympathetic neurons, it has been shown that the high affinity uptake system for catecholamines is structurally less specific in chromaffin cells.[46,47] Thus, caution should be taken in drawing a correlation between a pharmacological action (i.e., neuron blockade), which is dependent on uptake in sympathetic neurons, and radiotracer localization, which is dependent on uptake by chromaffin cells. This point is illustrated by benzylguanidine (**15**), a compound which at tracer levels shows excellent adrenomedullary localization but at pharmacological levels is known to have only weak neuron-blocking activity.[48] On the other hand, derivatives **4b** and **5b**, which are radioiodinated analogues of bethanidine, a potent neuron-blocking drug, show very little affinity for the adrenal medullae.

A subcellular distribution study has shown that *m*-IBG (**2b**) is stored mainly in the chromaffin granules of the dog adrenal medulla.[13] Recent studies, to be published elsewhere, show that the uptake-1 antagonists desmethylimipramine and cocaine also effectively block (>85%) the sequestration of *m*-IBG in the dog adrenal medullae in vivo.[49]

The adrenal medulla concentrations observed with racemic **8b** were similar to those obtained with (+)-**8b** and partially resolved (−)-**8b**. This finding is consistent with the lack of marked stereochemical specificity for uptake of (−)-norepinephrine exhibited in vitro by chromaffin cells.[47]

Based on the foregoing studies, at least three processes are seemingly involved in the localization of radiolabeled guanidines in the adrenal medullae: (1) active transport through the plasma membrane of the adrenomedullary cell via a process similar to uptake-1, (2) transport into the chromaffin storage granules, and (3) retention in the granules. The relative importance of these three processes in determining the concentration of radiolabeled guani-

(44) Eckelman, W. C. In "Computed Emission Tomography"; Ell, P. J.; Holman, B. L., Eds.; Oxford University Press: Oxford, England, 1982.
(45) Shapiro, S. L.; Isaacs, E. S.; Bandurco, V.; et al. *J. Med. Pharm. Chem.* **1962**, *5*, 793.
(46) Slotkin, T. A.; Bareis, D. L. *Pharmacology* **1980**, *21*, 109.
(47) Kenigsberg, R. L.; Trifaro, J. M. *Neuroscience* **1980**, *5*, 1547.
(48) Fielden, R.; Green, A. L.; Willey, G. L. *Br. J. Pharmacol.* **1965**, *24*, 395.
(49) Wieland, D. M.; Brown, L. E.; Sherman, P., unpublished findings.

dines in the medulla of the adrenal gland is presently under study. In view of the low pH (5.5) within chromaffin granules,[50] a priori it would be plausible that the guanidines, in free-base form, passively transit the membranes and are encumbered within the granules as the protonated cations. Such a pH-trapping mechanism has been postulated to explain the high brain retention of certain organic amines.[51,52] However, aralkylguanidines are extremely strong bases ($pK_a > 13$)[53] and at physiological pH would exist almost exclusively (99.9999%) in the protonated form. We have observed high concentrations of radiolabeled aralkylguanidines in the dog adrenal medulla as early as 5 min following intravenous injection.[12] Thus, their transport into adrenomedullary cells, whether it is by uptake-1 or some other process, likely involves the guanidinium cation. This hypothesis is also consistent with the known adrenomedullary accumulation of obligatory cations, such as the structurally similar radiolabeled bretylium analogues.[11] Also, the results of the drug intervention studies mentioned above are not consistent with a simple pH-trapping mechanism. The net accumulation of biogenic amines in isolated chromaffin granules is dependent on both an electrochemical proton gradient and a reserpine-sensitive carrier.[54] We are presently studying the extent to which these two systems are involved in the adrenomedullary accumulation of radiolabeled guanidines.

The work described here has led to a clinically useful radiopharmaceutical. *m*-IBG (**2a**), when labeled with the γ-emitting radionuclides ^{131}I or ^{123}I, successfully images catecholamine-containing tumors,[21,55,56] hyperfunctioning adrenal medullae,[22] and adrenergically innervated organs, such as the heart.[57] In addition, the high, selective affinity of *m*-[^{131}I]IBG for catecholamine tumors has led to its use in radiotherapy.[58]

This work represents an example of how previously published SAR studies can guide a subsequent SDR study with radiotracers, leading to a clinically useful radiopharmaceutical. The utility of [^{123}I]- and [^{131}I]**2a** as general adrenergic probes awaits determination of the extent to which they share the uptake, storage, and release characteristics of norepinephrine in normal and disease states.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. NMR data were recorded on a Varian EM-360A spectrometer with Me_4Si as the internal standard. Infrared spectra were recorded on either a Perkin-Elmer 283B or a Beckman Acculab-8 spectrophotometer. Elemental analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI.

Materials. Cyanamide, 3-iodobenzylamine hydrochloride, 3-iodoaniline, and 4-aminobenzonitrile were purchased from Aldrich Chemical Co. The 2-iodobenzylamine, (3-iodophenyl)-acetonitrile, and 4-iodoacetophenone were purchased from Pfaltz and Bauer, Inc. The 4-iodobenzylamine, 2-(4-iodophenyl)-ethylamine, and 3-iodoacetophenone were obtained from K & K, Sapon, and Eastman Organic Chemistry, respectively.

The 2-(*m*-iodophenyl)ethylamine hydrochloride (mp 201–204 °C; lit.[59] mp 204 °C), 3-iodo-α-methylbenzylamine hydrochloride (mp 182.5–184 °C) [Anal. ($C_8H_{11}NClI$) C, H, N], 4-iodo-α-methylbenzylamine hydrochloride (mp 234–236 °C) [Anal. ($C_8H_{11}NClI$) C, H, N], 3,5-diiodobenzylamine hydrochloride (mp 290–292 °C) [Anal. ($C_7H_8NClI_2$) C, H, N], and 3,4-diiodobenzylamine hydrochloride (mp 271–272 °C dec) [Anal. ($C_7H_8NClI_2$) C, H, N] were obtained according to the routes described previously under the Chemistry section. The free amines were converted to their respective hydrochloride salts by bubbling freshly generated, dry HCl gas through a solution of the free amine in methylene chloride. The white precipitate was collected, washed with methylene chloride, and stored in a vacuum dessicator until used.

General Synthesis of Guanidinium Salts. A mixture of the amine hydrochloride (2.0 mmol) and cyanamide (3.0 mmol) is stirred and heated by oil bath to a temperature at which a molten solution is obtained; generally, 150 °C or less is required. The reaction is then maintained at 100–130 °C for 2–4 h. The resulting glassy solid is dissoved in 1–3 mL of hot H_2O, and a solution of $KHCO_3$ (2.0 mmol) in 1–2 mL of H_2O is added dropwise with vigorous stirring. After the solution is briefly cooled to 4 °C, the precipitated guanidinium bicarbonate is collected, washed with cold H_2O, and air-dried. The solid is resuspended in 5–10 mL of H_2O, and an equivalent of 2 N H_2SO_4 is added dropwise with stirring. The resulting suspension is warmed to solution; cooling to room temperature generally results in crystallization of the desired guanidinium sulfate. The crystals are collected, washed with cold H_2O, dried in vacuo, and finally recrystallized from the solvents noted in Table II.

Specific Example of the Synthesis of a Guanidinium Salt: [2-(*m*-Iodophenyl)ethyl]guanidinium Sulfate (6a). A mixture of 2-(*m*-iodophenyl)ethylamine hydrochloride (425 mg, 1.5 mmol) and cyanamide (93 mg, 2.2 mmol) were heated under N_2 to 150 °C to a molten solution. The temperature was then maintained at 120 °C. The progress of the reaction was monitored by TLC using 2.5 × 20 cm silica gel coated glass plates (Whatman K6F) and EtOH/concentrated NH_4OH (5:1) as solvent. No starting amine was observed after 2 h. The temperature was reduced to 90 °C, and the viscous, colorless liquid was dissolved in 1.5 mL of water. To this solution was added slowly, with stirring, a solution of $KHCO_3$ (150 mg, 1.5 mmol) in 1 mL of water to produce a white precipitate, which was collected, washed with cold water, and dried in vacuo. Yield of the bicarbonate of **6a** was 363 mg (69%), mp 89–92 °C dec. A portion of this solid (281 mg, 0.8 mmol) was suspended in 2.5 mL of water, and 0.4 mL of 2 N H_2SO_4 was added slowly. The resulting suspension was heated with stirring, a small amount of EtOH was added, and the colorless solution was filtered hot. The filtrate was cooled to room temperature and then to 5 °C, which resulted in the separation of a colorless oil, which resisted attempts at crystallization. The oil finally crystallized when seeded with solid obtained from subjecting a small portion of the oil to high vacuum. The suspension was then cooled at 5 °C for 2 h. The white solid was collected, washed with cold water, and dried in vacuo to give 164 mg (61% yield), mp 174–177 °C. A 100-mg portion of this solid was recrystallized from 2 mL of water and 0.2 mL EtOH. The solution was filtered hot and allowed to cool to room temperature. Addition of a seed crystal produced colorless needles. After 1 h at room temperature and 4 h at 5 °C, the needles were collected, washed with cold water, and dried in vacuo to give analytically pure **6a** sulfate (69 mg): mp 178–179 °C; IR (Nujol) 1662 (C=N), 1104 (S=O) cm^{-1}; ^1H NMR (CD_3OD) δ 2.87 (t, 2, $PhCH_2$), 3.40 (t, 2, CH_2N). Anal. ($C_9H_{12}N_3I \cdot {}^1/_2H_2SO_4$) C, H, N.

Radiochemistry. The iodine-125 used in this study was a no-carrier-added solution of $Na^{125}I$ (ca. 500 mCi/mL) in reductant-free 0.1 N NaOH obtained from Union Carbide or New England Nuclear. The [^{14}C]guanethidine (14), 2.5 mCi/mmol,

(50) Casey, R. P.; Njus, D.; Radda, G. K.; et al. *Biochemistry* **1977**, *16*, 972.
(51) Kung, H. F.; Tramposch, K. M.; Blau, M. *J. Med. Chem.* **1983**, *24*, 66.
(52) Kung, H. F., Blau, M. *J. Nucl. Med.* **1980**, *21*, 147.
(53) Davis, T. L.; Elderfield, R. C. *J. Am. Chem. Soc.* **1932**, *54*, 1499. Hall, N. F. *J. Am. Chem. Soc.* **1930**, *52*, 5115.
(54) Johnson, R. G.; Carty, S.; Scarpa, A. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1982**, *41*, 2746.
(55) Kalff, V.; Shapiro, B.; Lloyd, R.; et al. *Arch. Intern. Med.* **1982**, *142*, 2092.
(56) Shapiro, B.; Sisson, J. C.; Beierwaltes, W. H. Proceedings of the Third World Congress of Nuclear Medicine and Biology, "Nuclear Medicine and Biology"; Raynaud, C., Ed.; Pergamon Press: Paris, 1982; Vol. II, pp 1265–1268.
(57) Kline, R. C.; Swanson, D. P.; Wieland, D. M.; et al. *J. Nucl. Med.* **1981**, *22*, 129.
(58) Sisson, J. C.; Shapiro, B.; Beierwaltes, W. H.; et al. *Clin. Res.* **1983**, *31*, 547A (abstr).
(59) Hughes, J. L.; Liu, R. C.; Enkoji, T.; et al. *J. Med. Chem.* **1975**, *18*, 1077.

was a generous gift from M. F. Bartlett of CIBA-GEIGY Corp. The [^{14}C]guanidine (**15**), 48 mCi/mmol, was purchased from Moravek Biochemicals, Brea, CA. The [*methyl*-^{14}C]benzylguanidine, 14 mCi/mmol, was synthesized from [*methyl*-^{14}C]benzylamine (ICN), the details of which will be published elsewhere.[60] The D-[7-^{3}H(N)]norepinephrine, 3.2 Ci/mmol, was purchased from New England Nuclear.

Radioactivity from Na^{125}I and ^{125}I-labeled compounds was quantified with a Capintec Model CRC-2 radioisotope calibrator and a Packard Model 5260 Autogamma counter. Both instruments were calibrated against a NBS standard solution of Na^{125}I. Radioactive samples of ^{14}C- or ^{3}H-labeled compounds were oxidized on a Model 306 Packard Tri-carb sample oxidizer and then counted on a Packard 3330 liquid scintillation counter. Twelve milliliters of Ox-carb-scint (Romac) liquid scintillation fluid containing Ox-carb-sorb (Romac) were used per ^{14}C-labeled sample. Twelve milliliters of Ox-trit-scint (Romac) was used per tritium sample. The ^{14}C counting efficiency ranged from 36 to 41%; ^{3}H counting efficiency ranged from 37 to 39%.

Radio-TLC analyses were performed on 2.5 × 20 cm silica gel coated glass plates (Whatman K6F). The labeled material was spotted on top of unlabeled compound. The TLC plates were analyzed on a Packard Model 720 radiochromatogram scanner immediately after development and drying. All radiolabeled compounds evaluated in this study were greater than 98% pure as determined on at least two TLC systems; the only exception was [^{14}C]guanethidine, which was 85% pure. The latter compound was not further purified due to the very limited supply. The three TLC systems used were (1) EtOH/EtOAc/H_2O (5:2:1), (2) EtOH/concentrated NH_4OH (3:1), and (3) *n*-BuOH/HOAc/H_2O (5:2:1). In system 1, the R_f's of most of the (iodoaralkyl)guanidine salts were 0.0, and the R_f of the free iodide was 0.75. With system 2, the R_f's of the iodoguanidine salts ranged from 0.10 to 0.20, the R_f's of the respective amine salts were 0.30–0.50, and the R_f of free iodide was 0.90. In system 3, the R_f of the free iodide was 0.65, and the R_f's of the guanidine salts as well as the respective amine salts ranged from 0.4 to 0.7. This latter TLC system was mainly used to check for the possibility of very polar impurities that would remain in the 0.0–0.3 R_f range with systems 1 and 2.

Radio-HPLC was performed on a Waters Model 272 liquid chromatograph equipped with a Radiomatic Flo-one radioactive flow detector (200-μL solid scintillator cell) employing, in series, both ultraviolet (254 nm) and radioactivity detection. Analysis was performed on a Waters μ-Bondapak C-18 column (4.6 × 250 mm) with a Brownlee 3 cm C-18 precolumn eluted with 0.2 M $NH_4H_2PO_4$/THF, 80:20, at 1.5 mL/min. The chromatogram in Figure 1 was obtained from a 35-μL injection of an aqueous solution containing 1 mM each of *o*-IBG, *m*-IBG, and *p*-IBG sulfate and NaI to which was added freshly prepared *m*-[^{131}I]IBG (**2b**) to a final concentration of ~300 nCi/mL.

The general preparative radioiodide exchange technique has been described.[30] A specific example follows: To a 10-mL round-bottom flask was added 3.0 mg of **6b** sulfate, 3.7 mg of $(NH_4)_2SO_4$, and 7.0 mCi of Na^{125}I in 0.30 mL of deionized, distilled water. The solution was heated to dryness, and the dry reaction mixture was maintained at 145 °C for 1.5 h. The oil bath temperature was reduced to 60–70 °C, 1 mL of water was added with stirring, and the solution was passed through a Cellex-D (Bio-Rad) anion-exchange column (1 × 4 cm) using 13.5 mM sodium acetate buffer (pH 4.5) as eluant. This latter treatment removed free radioiodide from the preparation. Approximately 6.6 mCi of ^{125}I radioactivity was isolated in 4–5 mL of acetate buffer (radiochemical yield 94%). Radio-TLC on SiO_2-G using 3:1 EtOH/concentrated NH_4OH (R_f 0.20) and 1:1 EtOAc/EtOH (R_f 0.0; R_f of free iodide 0.75) showed >98% radiochemical purity. Specific activity of the product was ≥0.74 Ci/mmol.

Tissue Distribution Studies. These were performed on female mongrel dogs (14–22 kg). Animals received 100 μCi of the appropriate ^{125}I-labeled compound or 50–60 μCi of ^{14}C-labeled compound. For each time interval evaluated, two to six dogs received bolus intravenous injections of radiolabeled compound in 2.0 mL of sterile acetate buffer (pH 4.5). The dogs were sacrificed 30 min or 72 h later by rapid intravenous injection of sodium pentobarbital. Both adrenals were removed from each dog, freed of fat and connective tissue, and immediately placed on dry ice. The adrenals were sliced in half to expose the medullae, and the medullary tissue was removed with a 2.0-mm Meyhoefer curette. In the ^{125}I studies, duplicate samples of 18 different organs or tissues from each dog were excised, washed free of blood with saline solution, blotted dry, quickly weighed (15–80 mg), and counted on a Packard 5260 Autogamma counter. For the ^{14}C- or ^{3}H-labeled compounds, duplicate samples of the same tissues were weighed, oxidized in a Packard 306 Tri-Carb sample oxidizer, and then counted in a Packard 3330 liquid scintillation counter with corrections made for background and counter efficiency. In order to normalize for differences in animal weights, tissue concentrations are expressed as percent kilogram dose per gram (% kg dose/g).[36]

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Registry No. **1a**, 16125-86-3; **1b**, 74075-14-2; **2a**, 87862-25-7; **2b**, 74075-13-1; **3a**, 14629-13-1; **3b**, 74075-12-0; **4a**, 87862-26-8; **4b**, 87862-27-9; **5a**, 87862-28-0; **5b**, 87862-29-1; **6a**, 87862-30-4; **6b**, 80664-16-0; **7a**, 87862-31-5; **7b**, 80664-17-1; (±)-**8a**, 87862-32-6; (+)-**8a**, 87900-85-4; (−)-**8a**, 87900-86-5; (±)-**8b**, 80664-14-8; (+)-**8b**, 80734-43-6; (−)-**8b**, 80734-42-5; (±)-**9a**, 87862-33-7; (±)-**9b**, 80664-15-9; **10a**, 87862-34-8; **10b**, 80664-13-7; **11a**, 87862-35-9; **11b**, 80664-25-1; **12a**, 87862-36-0; **12b**, 80664-18-2; **13**, 87862-38-2; **14**, 87862-39-3; **15**, 87862-40-6; **16**, 87862-41-7; **17**, 62600-61-7.

Supplementary Material Available: The 24- and 72-h dog thyroid radioactivity concentrations of all ^{125}I-labeled compounds reported in this study and a standard graph of thyroid radioactivity concentration vs. time after injection with Na^{125}I (2 pages). Ordering information is given on any current masthead page. Also, the tissue distribution data for all compounds in Table II in 18 selected tissues can be obtained upon request from the author.

(60) Inbasekaran, M.; Mangner, T. J.; Wieland, D. M. submitted for publication in *J. Labelled Compd. Radiopharm.*