PRELIMINARY NOTE

Imaging the Adrenal Medulla with an I-131-labeled Antiadrenergic Agent

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Tissue distributions of four antiadrenergic agents labeled with iodine-125 have been determined in dogs. [^{125}I] ortho-iodobenzyldimethyl-2-hydroxyethyl ammonium and [^{125}I] ortho-iodobenzyldimethylethyl ammonium show highly selective uptake in the adrenal medulla. Studies of molecular structure-distribution indicate that both the nature of the cationic head and the ring position of the iodine atom greatly influence adrenal specificity. Distinct images of dogs' adrenal medulla have been obtained 4 days after i.v. injection of 1.5 mCi of [^{131}I] ortho-iodobenzyldimethyl-2-hydroxyethyl ammonium.

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Adrenal-medullary imaging agents have been actively pursued by our laboratory and others over the past decade (*1–10*). These efforts have been based primarily on the observation that labeled catecholamines, especially dopamine, concentrate in the adrenal medulla (*1*). Recently, [^{125}I] para-iodobenzyltrimethyl ammonium (compound 5, Table 1)—an analog of the adrenergic neuronal blocking agent, bretylium—was found to concentrate in dogs' medullary tissue (*10*).

Our review of the latter approach revealed discrepancies between the reported adrenal-medulla uptake of bretylium analogs (*10*) and previous pharmacologic studies of their antiadrenergic potency (*11*). We report here a preliminary structure-distribution evaluation of a series of I-125-labeled bretylium analogs and the first imaging of the dogs' adrenal medullae using [^{131}I] ortho-iodobenzyldimethyl-2-hydroxyethyl ammonium (compound 1, Table 1).

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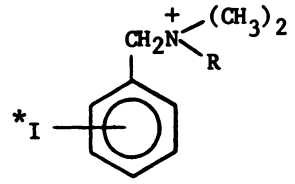
MATERIALS AND METHODS

Radiolabeled compounds: synthesis and purity. The following two-step radiosynthesis of [^{125}I] ortho-iodobenzyldimethylethyl ammonium (compound 2, Table 1) exemplifies the general procedure used in this study.

1. Radioiodide exchange. A solution of carrier-free Na^{125}I (10 mCi) in 4.0 ml of concentrated ammonium hydroxide was added to 12.0 mg of ortho-iodobenzylethylmethyl amine hydrochloride, mp 148–149°C, obtained by the method of Copp and Stephenson (*12*). The reaction was refluxed for 18 hr. Diethyl ether extraction of the free base resulted in 9.2 mCi of product with greater than 98% radiochemical purity as determined by radioactive thin-layer chromatography (TLC) on silica gel G* using either 4/1 benzene:ethyl acetate (R_f = 0.45) or 100% benzene (R_f = 0.13).

2. Quaternization: The ether solution (from above) was evaporated to dryness and the oily residue dissolved in 4.0 ml of absolute ethanol containing 0.2 ml of methyl iodide. The reaction was placed in the dark and stirred for 6 hours at room temperature. Solvent and excess methyl iodide

TABLE 1. TISSUE DISTRIBUTION† OF [^{125}I] IODOBENZYL QUATERNARY AMMONIUM COMPOUNDS IN THE DOG



Compound No.	R	Position of radioiodine	Time (hr)	Tissue concentration (% kg dose/g)‡					
				Adrenal medulla	Adrenal cortex	Liver	Kidney	Bile	Thyroid
1	$-CH_2CH_2OH$	ortho	0.5	2.89	.09	.71	.14	.12	.71
			15.0	2.94	.06	.03	.01	.30	2.56
			24.0	3.55	.07	.05	.02	.43	4.87
			72.0	2.00	.02	.01	.00	.05	13.42
			120.0	1.65	.03	.01	.00	.01	5.42
2	$-CH_2CH_3$	ortho	0.5	2.64	.10	1.24	.56	.54	.20
			2.0	1.11	.05	.50	.22	.83	.15
			6.0	2.43	.06	.15	.10	.53	.39
			15.0	3.74	.08	.13	.04	.36	.48
			24.0	3.01	.05	.04	.02	.65	1.48
			72.0	1.37	.03	.02	.00	.07	2.08
			120.0	.35	.00	.00	.00	.02	3.76
3	$-CH_3$	ortho	2.0	.79	.10	.75	.12	1.07	0.20
			6.0	.63	.12	.24	.07	1.97	0.26
			15.0	.73	.04	.09	.02	1.35	2.57
4	$-CH_2CH_3$	para	0.5	.11	.06	.47	.27	.07	.05
			1.0	.18	.05	.38	.50	.06	.24
			2.0	.11	.07	.16	.25	.41	.07
			6.0	.04	.01	.13	.07	.33	.46
5[I]	$-CH_3$	para	6.0	.42	.08	.30	–	.74	.17
			48.0	.27	.04	.05	–	.03	1.35
			96.0	.29	.01	.02	.01	–	.46

† All other tissues except urine showed concentrations of 0.1% kg dose/g or less at 1–5 days.
‡ Average value for two dogs given for each time interval
[I] Data from Ref. 10

were evaporated with a stream of nitrogen gas and the residue partitioned between 2.0 ml of water and 6.0 ml of chloroform using vigorous stirring. The isolated aqueous solution was passed through a glass column packed with 1.5 g of anion-exchange resin† to eliminate traces of free iodide.

Overall radiochemical yield for compound 2 was 75% (specific activity approximately 0.6–0.8 mCi/mg). The radiochemical purity was greater than 98% as determined by three chromatography systems:

1. 4/1 benzene:ethyl acetate on silica gel G (R_f = 0.0; R_f of unquaternized starting material = 0.45);
2. 1/1 ethyl acetate:ethanol on silica gel G (R_f = 0.1; R_f of free radioiodide = 0.56); and
3. 66/17/17 n-butanol:acetic acid:water on silica gel G (R_f = 0.51).

Overall radiochemical yields for 1, 3, and 4 were 30%, 85%, and 20%, respectively. Specific activities of 1 and 3 ranged from 0.7–0.9 mCi/mg, and 0.1–0.2 mCi/mg for 4. The structures of the respective stable compounds were confirmed by infrared spectroscopy, nuclear magnetic resonance, and correct carbon-hydrogen analysis. Normal saline was used to formulate the final products.

Animal studies. Tissue-distribution studies were performed on female mongrel dogs injected intravenously with 100μCi of the I-125-labeled compounds in an average volume of 2 ml. Two dogs were injected with each compound and were killed at each time interval shown in Table 1 by i.v. injection of sodium pentobarbital. Representative samples of 17 different tissues were counted in an autogamma counter with corrections made for radioactive decay, background, and counter efficiency. To normalize for differences in animal weights (*13*), tissue concentrations were expressed as percent kilogram dose per gram.

For the imaging, dogs were injected intravenously with 1.5 mCi of I-131-labeled compound. A large-field-of-view camera (high-energy collimator) interfaced to a minicomputer was used to obtain

posterior images at daily intervals up to 4 days postinjection. Confirmation of adrenal localization was made by subsequent injection of 2 mCi of Tc-99m DTPA into the immobilized (sodium pentobarbital) dog without altering position. The resultant renal images were superimposed over the adrenal scans using the computer.

RESULTS

Table 1 summarizes the tissue distributions of the four I-125-labeled bretylium analogs synthesized for this study. The distribution of compound 5, previously reported by Korn et al. (*10*), is included for comparison.

High concentrations of radioactivity from compounds 1 and 2 appeared in the adrenal medulla early and persisted throughout the 5 days of study. The peak medullary uptakes of 1 and 2 were greater than eight times the peak uptakes reported at various time intervals for compound 5 (*10*).

In addition to the adrenal medulla, the four tissues representing the greatest potential source of background activity are presented in Table 1. The thyroid is also included to assess the degree of in vivo deiodination. Although the peak medullary activity from compounds 1 and 2 occurred at 24 and 15 hr, respectively, higher target-to-nontarget concentration ratios (T/NT) were observed at 72 hr postinjection. With compound 1, the 72-hr T/NT ratios for adrenal medulla to adrenal cortex, liver, kidney, and bile averaged 100, 200, 500, and 40, respectively. The background activities from compound 1 were initially lower and declined more rapidly than from compound 2. Comparison of the 24-hr thyroid activities in Table 1 with the 24-hr thyroid uptake of $Na^{125}I$ (123.9% Kg dose/g), administered intravenously to dogs, reveals the in vivo deiodination of compounds 1 and 2 to be only 3.9% and 1.2%, respectively. Bretylium itself is known to be metabolically stable in both cats and humans (*14*).

Figure 1 shows a representative scintigram of dog adrenal medullae (50,000 counts over a 50-min interval) obtained 4 days after the i.v. injection of 1.5 mCi of compound 1 labeled with iodine-131. Successful imaging, with only a slightly higher background activity, was also possible at 3 days postinjection. The major deterrent to distinct visualization of the adrenals in less than 3 days was the presence of background activity in the gallbladder and digestive tract. Interference from liver activity was minimal, even at 24 hr postinjection.

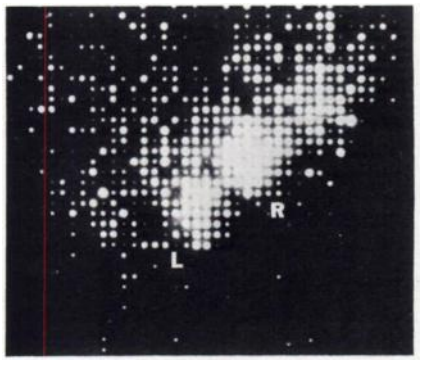


FIG. 1. Computer display, obtained 4 days after injection of compound 1 labeled with I-131, showing posterior image of dogs' adrenal medullae.

DISCUSSION

In their original paper on the pharmacology of benzyl quaternary ammonium compounds, Boura and coworkers (*11*) found that antiadrenergic potency was greatly influenced by the structure of the cationic head. With $R=CH_3$ (i.e., compound 3), no activity was observed, whereas with $R=CH_2CH_3$ (i.e., compound 2) or $R=CH_2CH_2OH$ (i.e., compound 1), high activity resulted. These pharmacologic observations are qualitatively consistent with the adrenal-medulla concentrations observed with compounds 1-3. Further studies are in progress to determine the structure of the cationic head that will result in optimal adrenal-medullary localization.

The position of the iodine atom also appears to be crucial to adrenal-medulla uptake. Moving the iodine atom from the ortho (compound 2) to the para position (compound 4) markedly decreased adrenal-medulla localization. This effect was not as pronounced in the less specific trimethyl series (compound 3 in contrast to 5). These observations differ from the previous report that the para series was more adrenospecific than the ortho series (*10*).

Based on the similarity between tissue distributions of compounds 1 and 2, the latter compound, labeled with I-131, should also provide images of the adrenal medullae. The more rapid background clearance of compound 1 may possibly be due to its more polar nature and its potential for glucuronide formation through the 2-hydroxy group. Both the I-131 compounds are undergoing preliminary clinical evaluation in humans as potential imaging agents for the adrenal medulla, neuroblastoma, and pheochromocytoma.

FOOTNOTES

* Eastman 13181 Silica Gel, Eastman Kodak Co., Rochester, NY

† Cellex D, Bio Rad Laboratories, Richmond, CA

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