jnm/ PRELIMINARY NOTE

A Radioiodinated Bretylium Analog as a Potential Agent for Scanning the Adrenal Medulla

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Studies with radioiodinated bretylium analogs (RIBA) suggested that the p-isomer was capable of concentrating in adrenal medulla. The present studies with ^{125}I-p-RIBA in rats and dogs confirm this property and show its marked and persistent affinity for the adrenal medulla. Analogous studies with ^{14}C-p-IBA indicate that it is the quaternary form of the drug that is retained by the adrenal and that high thyroidal radioactivity following ^{125}I-p-RIBA administration is due to in vivo deiodination of the drug.

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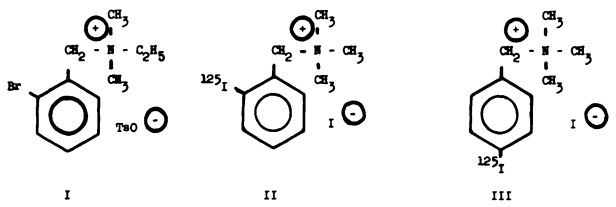
While it is now possible to image the adrenal gland with agents that concentrate in the adrenal cortex (e.g., ^{131}I-19-iodocholesterol) and thus to diagnose adrenocortical diseases (*1,2*), there has been no success to date in the search for agents that concentrate in the adrenal medulla and that would be potentially useful in the diagnosis of neural-crest tumors, such as pheochromocytoma and neuroblastoma.

One approach has been the use of catecholamines as carriers for gamma-emitting nuclides since these compounds are known to be synthesized and stored in the medulla (*3*). While ^{14}C-p-tyramine showed a high level of uptake in the dog's adrenal medulla (*4*), this was not the case with several radioiodinated analogs (*5*). ^{11}C-dopamine has been synthesized but the short half-life of carbon-11 severely limits clinical utility of this radiopharmaceutical (*6*).

The approach in this laboratory has focused on drugs known to bind at adrenergic sites, such as the adrenergic neuron-blocking drug bretylium tosylate (I in Fig. 1). Replacement of the ortho-bromine in this drug with radioiodine represents a minimal alteration in chemical structure. Previously published studies from this laboratory (*7*), using the radioiodinated bretylium analogs (^{125}I-RIBA), indicated their ability to concentrate in rat and dog adrenergic neurons and in such organs as heart ventricle, adrenal medulla, and spleen. Ortho-RIBA (II) showed a marked affinity for the myocardium at early time periods, while p-RIBA (III) displayed an affinity for adrenal medulla and spleen. The present study has been undertaken to confirm the affinity of p-RIBA for the adrenal medulla and to ascertain its potential as an imaging agent.



I II III

FIG. 1. Alterations in chemical structure of bretylium tosylate (I) result in ^{125}I-o-RIBA (II) and ^{125}I-p-RIBA (III).

MATERIALS AND METHODS

Both compounds used in these experiments (^{125}I-p-RIBA and ^{14}C-p-IBA) were synthesized by methods previously reported (*7*). Chemical purity was ascertained initially and confirmed before use by thin-layer chromatography. Single spots were obtained with 2:1 chloroform–methanol (R_f = 0.4) and with 1:1 benzene–ether (R_f = 0.32) solutions.

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Each spot was coincident with a single radioactive peak on the radiochromatogram.

Tissue distribution studies. The radioiodinated compounds were dissolved in isotonic saline and administered by intravenous or subcutaneous route to male Sprague–Dawley rats weighing 190–340 gm and to mongrel male dogs weighing 8–21 kg. Rats received a dose of either 23–27 μCi/rat or 60 μCi/kg and the dogs received 10 μCi/kg.

Twenty rats (five per group) received subcutaneous injections and were killed by exsanguination under ether anesthesia at 6 hr, 48 hr, 4 days, and 8 days after injection. Immediately after death samples of blood were taken and major organs removed, rinsed to remove blood, blotted dry, and weighed. Large organs were minced with scissors. Samples of tissue were placed in polyethylene microfuge tubes and assayed according to the method of Ashcroft (*8*) in a liquid-scintillation spectrometer.* In the intravenous series, 28 rats were injected and killed: six rats each at 6, 18, and 48 hr, and five rats each at 4 and 8 days after injection. Samples of tissue were assayed for radioactivity in a well scintillation spectrometer. An additional five rats were intravenously injected with 9.8 μCi/rat (39 μCi/kg) of ^{14}C-p-IBA (the para-iodinated bretylium analog with stable iodine and a carbon-14 label on the N-methyl group). These rats were killed at 18 hr after injection. Tissue samples were placed in preweighed liquid-scintillation vials and 0.3 ml of 2 *N* NaOH was added. After standing at least 16 hr, the vials were heated at 40°C for 5–10 min and then allowed to cool. Hydrogen peroxide (0.05 ml of 30% solution) was added and the vials heated at 40°C for 1–3 min. Sufficient 0.5 *M* acetic acid to neutralize the base and 13.5 ml of PCS† were added and the vials shaken on a vortex mixer. Radioactivity was assayed on a liquid-scintillation spectrometer.‡

Dogs received subcutaneous injections and were killed at 6 hr (2 dogs), 1 day (2), 2 days (3), 3 days (4), 4 days (2), and 6 days (2) after injection. Four dogs (one dog per interval) received intravenous injections and were killed at 6 hr, 48 hr, 4 days, and 8 days after injection. Samples of blood and major organs were obtained as described above and gamma activity was assayed in a well scintillation spectrometer.

RESULTS

When ^{125}I-p-RIBA is administered to either dog or rat, comparison between subcutaneous and intravenous routes showed that the former is better: the drug or some metabolite of it attained a higher concentration in the adrenal medulla, reached the peak uptake level later, and remained in the target tissue for a longer period of time. This may be due to the slower absorption and clearance of the drug when given subcutaneously. Generally, the profiles of distribution of radioactivity were similar and therefore only the data for the intravenous route will be discussed.

The data from rats (Table 1) confirm that the adrenal medulla concentrates and retains the radioactivity for long periods. The spleen and thyroid were the only other tissues to retain significantly high levels of radioactivity. The prolonged high levels in the thyroid raised the question whether this was due to in vivo deiodination or to retention of the compound per se. This question was answered by comparison with the same drug labeled with carbon-14. Qualitatively both ^{125}I-p-RIBA and its carbon-14 counterpart gave very similar distribution patterns at 18 hr (Table 1). Quantitatively, however, the ^{14}C-p-IBA gave about 45% more radioactivity in the adrenal medulla and spleen. Undoubtedly this reflects the greater in vivo stability of the carbon-14

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN RATS FOLLOWING INTRAVENOUS ADMINISTRATION OF ^{125}I-p-RIBA OR ^{14}C-p-IBA (% administered dose/gm tissue ± s.e.m.)*

	^{125}I-p-RIBA					^{14}C-p-IBA
Tissue	6 hr	18 hr	48 hr	4 day	8 day	18 hr
Adrenal:	0.364 ± 0.048		0.275 ± 0.020			
cortex		0.201 ± 0.057	0.144 ± 0.036	0.166 ± 0.045	0.059 ± 0.010	0.162 ± 0.042
medulla		1.056 ± 0.217	0.561 ± 0.035	0.957 ± 0.119	0.654 ± 0.061	1.931 ± 0.547
Blood	0.051 ± 0.003	0.036 ± 0.001	0.016 ± 0.002	0.009 ± 0.002	<0.001	0.046 ± 0.003
Heart ventricle	0.026 ± 0.002	0.012 ± 0.001	0.003 ± 0.001	0.003 ± <0.001	<0.001	0.021 ± 0.002
Kidney	0.070 ± 0.003	0.038 ± 0.002	0.017 ± 0.001	0.002 ± <0.001	<0.001	0.008 ± <0.001
Liver	0.030 ± 0.002	0.015 ± 0.001	0.007 ± 0.001	0.002 ± <0.001	<0.001	0.012 ± 0.001
Lung	0.059 ± 0.002	0.040 ± 0.003	0.018 ± 0.003	0.007 ± 0.001	0.001 ± <0.001	0.034 ± 0.003
Spleen	0.134 ± 0.006	0.097 ± 0.013	0.041 ± 0.004	0.028 ± 0.005	0.001 ± <0.001	0.139 ± 0.010
Thyroid	0.108 ± 0.014	0.186 ± 0.012	0.124 ± 0.014	0.074 ± 0.014	0.047 ± 0.010	0.020 ± 0.001

* Standardized for animal weight (kg).

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN DOGS FOLLOWING INTRAVENOUS ADMINISTRATION OF ^{125}I-p-RIBA (% administered dose/gm tissue)*

Tissue	6 hr	48 hr	4 day	8 day
Adrenal:				
cortex	0.080	0.039	0.008	0.010
medulla	0.424	0.274	0.290	0.354
Bile	0.740	0.025	—	—
Blood	0.042	0.044	0.020	0.012
Heart ventricle	0.139	0.012	0.005	0.004
Kidney:	—	—	0.011	0.007
cortex	0.101	0.015	—	—
medulla	0.200	0.019	—	—
Liver	0.302	0.052	0.018	0.013
Lung	0.501	0.139	0.048	0.019
Muscle	0.099	0.007	—	—
Pancreas	0.143	0.021	—	—
Spleen	—	3.434	0.148	0.134
Thyroid	0.165	1.345	0.464	0.433

* Standardized for animal weight (kg).

label, which is therefore a better measure for uptake of a quaternary form of the drug. Moreover, the much lower level of radioactivity in the thyroid following administration of ^{14}C-p-IBA supports the contention that radioactivity in the thyroid following administration of ^{125}I-p-RIBA is the result of in vivo deiodination. The similar distribution patterns for the radioiodinated and carbon-14-labeled tracers also support the view that the drug is retained in the tissues in the quaternary form.

In the dog (Table 2), elevated levels of radioactivity persisted only in the adrenal medulla, spleen, and thyroid. Radioactivity in the thyroid was higher than that seen in the rat, suggesting that if the high thyroid radioactivity is due to in vivo deiodination, the dog is more efficient in performing this metabolic change. The initially high level of uptake in the lung at 6 hr diminished rapidly and was not seen in the rat studies. This high uptake in lung is typical of many amines and other drugs capable of existing in a cationic form at physiologic pH (*9*).

Experience with ^{131}I-19-iodocholesterol suggests that diagnostic imaging in humans can be achieved with as little as 0.02% dose/gm in the adrenal gland when the adrenal-cortex-to-liver ratio is greater than 50 (*10*). Initial studies in this laboratory with ^{125}I-19-iodocholesterol, administered intravenously to rats, gave values of 22.46% dose/gm and an adrenal-to-liver ratio of 57.44 at 48 hr (*11*). For ^{14}C-p-IBA, 18 hr after intravenous administration to rats, the % dose/gm in the adrenal medulla was 10.86 and the adrenal medulla-to-liver ratio was 170. Similar values were also obtained for ^{125}I-p-RIBA for as long as 8 days after injection, suggesting that the adrenal medulla can be visualized with ^{131}I-p-RIBA.

These and previous studies with radioiodinated bretylium analogs confirm and extend knowledge of the marked affinity of p-RIBA for adrenal medulla of rats and dogs. Strong support for further studies with this agent, labeled with a suitable gamma-emitter, is afforded by the high target-to-nontarget ratios observed within 2 days of administration to rats or dogs, the high level of radioactivity persisting for as long as 8 days, and a favorable comparison with the adrenal uptake of ^{131}I-19-iodocholesterol. Studies now in progress with ^{131}I-p-RIBA will, we hope, confirm that this is a potentially useful imaging agent.

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FOOTNOTES

* Beckman LS-150 (Palo Alto, Calif.).
† Amersham/Searle (Arlington Heights, Ill.).
‡ Beckman LS-150.

REFERENCES

1. LIEBERMAN LM, BEIERWALTES WH, CONN JW, et al.: Diagnosis of adrenal disease by visualization of human adrenal glands with ^{131}I-19-iodocholesterol. *N Engl J Med* 289: 1387–1393, 1971

2. CONN JW, MORITA R, COHEN EL, et al.: Primary aldosteronism photoscanning of tumors after administration of ^{131}I-19-iodocholesterol. *Arch Intern Med* 129: 417–425, 1972

3. WURTMAN RJ: *Catecholamines.* Boston, Little, Brown, and Co., 1966, pp 1 and 22

4. MORALES JO, BEIERWALTES WH, COUNSELL RE, et al.: The concentration of radioactivity from labeled epinephrine and its precursors in dog adrenal medulla. *J Nucl Med* 8: 800–809, 1967

5. COUNSELL RE, SMITH TD, RANADE VV, et al.: Potential organ or tumor imaging agents. 11. Radioiodinated tyramines. *J Med Chem* 16: 684–687, 1973

6. CHRISTMAN DR, HOYTE RM, WOLF AP: Organic radiopharmaceuticals labeled with isotopes of short half-life. 1: ^{11}C-1-dopamine hydrochloride. *J Nucl Med* 11: 474–478, 1970

7. COUNSELL RE, YU T, RANADE VV, et al.: Radioiodinated bretylium analogs for myocardial scanning. *J Nucl Med* 15: 991–996, 1974

8. ASHCROFT J: Gamma counting of iodine-125 using a metal-loaded liquid scintillator. *Anal Biochem* 37: 268–275, 1970

9. SAUNDERS L: *The Absorption and Distribution of Drugs.* Baltimore, Williams & Wilkins, 1974, pp 129, 139–142, 168–171, 183

10. MORITA R, LIEBERMAN LM, BEIERWALTES WH, et al.: Percent uptake of ^{131}I radioactivity in the adrenal from radioiodinated cholesterol. *J Clin Endocrinol Metab* 34: 36–43, 1972

11. COUNSELL RE, RANADE VV, KULKARNI PG, et al.: Potential organ or tumor-imaging agents. 12. Esters of 19-radioiodinated cholesterol. *J Nucl Med* 14: 777–780, 1973