INVESTIGATIVE NUCLEAR MEDICINE

Myocardial Imaging with a Radioiodinated Norepinephrine Storage Analog

Donald M. Wieland, Lawrence E. Brown, W. Les Rogers, Kathy C. Worthington, Jiann-long Wu, Neal H. Clinthorne, Charlotte A. Otto, Dennis P. Swanson, and William H. Beierwaltes

University of Michigan Medical Center, Ann Arbor, Michigan

Meta-iodobenzylguanidine (M-IBG), an iodinated aromatic analog of the hypotensive drug guanethidine, localizes in the heart of the rat, dog, and rhesus monkey. A comparative study of tissue distribution in the dog has been performed with five myocardiophilic agents: thallium-201, I-125 16-iodohexadecanoic acid, H-3 norepinephrine, C-14 guanethidine and I-125 M-IBG. The last two compounds give heart concentrations and heart-to-blood concentration ratios similar to those of thallium-201. Planar and tomographic images of the hearts of the dog and rhesus monkey were obtained using I-131 or I-123 labeled M-IBG. Blocking studies with reserpine suggest that a major component of myocardial retention of M-IBG is sequestration within the norepinephrine storage vesicles of the adrenergic nerves. The localization of M-IBG in other organs with rich sympathetic innervation and the relative insensitivity of myocardial uptake to a wide range of loading doses lend additional support for a neuronal mode of retention.

J Nucl Med 22: 22–31, 1981

Despite the widespread use of thallium-201 in myocardial imaging, there remains a compelling need for an alternative radiopharmaceutical (*1,2*). Recent attempts to develop this alternative have focused on receptor-binding agents, such as radiolabeled β-blockers (*3,4*) and muscarinic antagonists (*5*); or substrate analogs, such as ω-iodofatty acids (*6,7*). The latter compounds have been used clinically, but high blood activity levels, due to rapid metabolic release of radioiodide, have posed special imaging problems (*8*).

An approach to myocardial imaging—one that has been for the most part neglected—can be based on the neurotransmitter norepinephrine. The heart is richly supplied by sympathetic nerves and its ability to rapidly concentrate H-3 norepinephrine has been well documented (*9,10*). In 1976, Fowler and coworkers obtained scintiscans of the canine heart with C-11 norepinephrine using a gamma camera (*11*). Thus norepinephrine or a norepinephrine cogener labeled with a radionuclide that is generally available might be useful for routine myocardial imaging. Unfortunately, structure-activity-relationship studies (*12*) suggest that little flexibility exists for introducing a foreign atom other than fluorine (*13*) into the norepinephrine molecule. It appears from studies with dopamine (*14*) that incorporation of radioiodine into the 6 position of the aromatic ring of norepinephrine might provide a chemically stable derivative with a biodistribution similar to that of norepinephrine, but a multistep radiosynthetic scheme would be required.

A plausible alternative to the use of a radiolabeled norepinephrine is the false neurotransmitter guanethidine (Fig. 1). This compound shares the same uptake, storage, and release mechanisms as norepinephrine (*15*), but it is not metabolized by monoamine oxidase (MAO) or catechol-O-methyl transferase (COMT) (*16*). Carbon-14 guanethidine (Fig. 1), when administered in pharmacological doses, selectively concentrates in the rat heart (*17*). Though guanethidine itself is not easily labeled with a gamma emitter, pharmacologically similar aromatic analogs of guanethidine, such as benzyl-

Received June 2, 1980; revision accepted Aug. 26, 1980.

For reprints contact: Donald M. Wieland, PhD, 3685 Kresge I, Univ. of Michigan Med. Ctr., Ann Arbor, MI 48109.

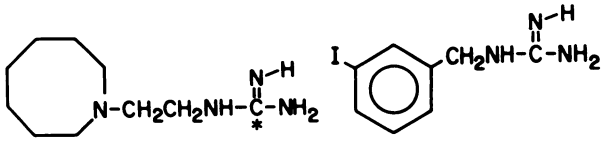

FIG. 1. Chemical structures of guanethidine (*denotes position of C-14 label) and meta-iodobenzylguanidine (M-IBG).

guanidine, can be labeled readily by incorporation of radioiodine into the phenyl ring (*18*).

This paper presents an initial evaluation of radioiodinated meta-iodobenzylguanidine (M-IBG) as a potential radiopharmaceutical for imaging the myocardium. Specifically, we report: (a) tissue distributions of I-125 M-IBG in the rat, dog, and rhesus monkey; (b) comparative tissue distributions of C-14 guanethidine, I-125 M-IBG, H-3 norepinephrine, thallium-201, and I-125 16-iodohexadecanoic acid (I-HDA); and (c) imaging of the dog and rhesus monkey using I-131- and I-123-labeled M-IBG.

MATERIALS AND METHODS

Microanalyses were performed commercially. Thin layer chromatography (TLC) was done on precoated silica gel* or cellulose† plastic strips. Tritiated norepinephrine‡ (25 mCi/mg), thallium-201,‡ and sodium iodide (I-125,‖ I-123,§ and I-131‡) were obtained from commercial sources. Proton magnetic resonance (PMR) spectra were obtained on a spectrometer.**

Synthesis of unlabeled compounds. The synthesis of M-IBG·$\frac{1}{2}H_2SO_4$ has been described (*18*). The 16-bromohexadecanoic acid, precursor to I-125 iodohexadecanoic acid, was synthesized by the following method: 1.08 g (4.0 mmole) of 16-hydroxyhexadecanoic.acid,†† dissolved in 10 cc of 30% hydrobromic acid in glacial acetic acid,‡‡ was heated in a Parr pressure reaction vessel for 6 hr at 100°C. After cooling to room temperature, the mixture was rotary-evaporated to dryness and the residue vacuum-distilled. The white solid, which distilled at 170–180°C at 0.3 mm pressure, was recrystallized from 40–60°C petroleum ether to give 1.21 g of white needles, m.p. 69.5–70.0°C; lit. m.p. 70–70.5°C (*19*). Yield was 91%. Anal.: calcd. for $C_{16}H_{31}BrO_2$: C 57.31, H 9.32, Br 23.83; found: C 57.60, H 9.32, Br 23.65; PMR ($CDCl_3$) 1.10–2.05 [m,26,$(C\underline{H}_2)_{13}$], 2.35 (m,2,$C\underline{H}_2CO$), 3.40 (t,2,$C\underline{H}_2Br$), 11.30 (s,1,$COO\underline{H}$).

Synthesis of radiolabeled compounds. *Iodine-125 16-iodohexadecanoic acid.* This radiosynthesis was based on the method of Robinson (*6*). A formulation of 9.4% ethanol, 2.3% polysorbate-80, and 88.3% physiological saline by volume was used. The specific concentration was 0.2 mCi/ml. Radio-TLC on silica gel using n-hexane:ethyl ether:acetic acid (70:30:1; R_f = 0.25) and on cellulose using n-hexane:ethyl ether:acetic acid (320:80:1) (R_f = 0.90) showed >98% radiochemical purity. The radiochemical yield was 85–95%; specific activity was 1.1 mCi/mg.

Carbon-14 guanethidine sulfate. Radiochemical purity was >95% as determined on silica gel, with n-propanol:10% NH_4OH (3:1; R_f = 0.47) and n-butanol:acetic acid:water (50:11:25; R_f = 0.17). The TLC plate was visualized with Dragendorff reagent. Specific activity was 0.01 mCi/mg. Specific concentration in physiological saline was 50 μCi/cc.

Iodine-125 M-IBG. The radiosynthesis, by an iodide exchange technique, is similar to that previously reported for I-125 para-iodobenzylguanidine (*18*). Synthetic details will be published elsewhere. Recent improvements have lowered the radiosynthesis time to 2 hr. Specific activities of 1–2 mCi/mg can be obtained routinely in 90–98% radiochemical yield. Specific activities up to 150 mCi/mg can be achieved in 40–50% yield. Similar results were obtained in the syntheses of I-123- and I-131-labeled M-IBG. Radiochemical purity was >98% as determined by radio-TLC and radio-high pressure liquid chromatography (*18*).

The final product was formulated in 0.9% bacteriostatic saline and the pH adjusted to 5–6 by addition of pH 4 phosphate buffer. The specific concentration was 0.2 mCi/cc. When stored in the dark at 4°C, I-125- and I-131-tagged M-IBG showed <5% radiodecomposition in 3 wk.

Tissue distribution studies. *Dogs.* Tissue distribution studies were performed on female mongrel dogs (14–22 kg). Each animal received a single intravenous injection of one of the following compounds: 100 μCi of Tl-201, 100 μCi I-125 M-IBG, 100 μCi H-3 norepinephrine, 100 μCi I-125 16-I-HDA, or 50 μCi C-14 guanethidine. The dogs were killed at selected time intervals by intravenous injection of sodium pentobarbital. For M-IBG, Tl-201, and I-125 16-I-HDA, duplicate samples of 18 different tissues in each dog were weighed and counted in an autogamma counter with corrections made for radioactive decay, background, and counter efficiency. For H-3 norepinephrine and C-14 guanethidine, duplicate samples of the same tissues were weighed, oxidized in a sample oxidizer,‖‖ then counted in a liquid scintillation counter with corrections made for background and counter efficiency. Blood samples were obtained by cardiac puncture. To normalize for differences in animal weights, tissue concentrations are expressed as percent kilogram dose per gram (% kg-dose/g) (*20*).

Rats. Female Sprague-Dawley rats weighing 200–300 g were anesthetized with ether and injected with 25 μCi of I-125 M-IBG by femoral vein. The rats were anesthetized and killed by decapitation at selected time intervals. Samples of heart, blood, liver, lung, and muscle were analyzed by the above procedures.

Monkeys. Following a restraining i.m. dose of ketamine hydrochloride, monkeys were anesthetized with sodium pentobarbital and injected intravenously with

100 μCi of I-125 M-IBG. Three hours later, duplicate samples of 11 tissues were removed and analyzed by the above procedures.

Blood curves. Blood curves were obtained in dogs anesthetized with sodium pentobarbital. Venous samples were obtained at seven selected time intervals and analyzed, with results being expressed as % kg-dose/g.

Analysis of radioactivity in the urine. Two dogs were each injected with 1.0 mCi of I-131 M-IBG and placed in a metabolic cage. A 2-ml aliquot of the 24-hr urine collection was spiked with 5 mg of stable M-IBG. The chemical form of the radioactivity in the urine was determined by TLC on silica gel-G using ethyl acetate:95% ethanol (1:1; R_f of M-IBG = 0.25; R_f of iodide = 0.76); also in ethanol:28% NH_4OH (3:1; R_f of M-IBG = 0.14; R_f of iodide = 0.85). The R_f of the radioactive peaks(s) was determined on a radiochromatogram scanner and compared with the R_f of unlabeled compound as determined by fluorescent quenching under ultraviolet light. Control radiochromatograms were performed on 2-ml nonradioactive urine samples spiked with 15 μCi of I-131 M-IBG, 5 μCi of sodium iodide (I-131), and 5 mg of stable M-IBG.

Reserpine blocking studies. Two female mongrel dogs were injected i.m. with 1 mg/kg reserpine followed by a 100 μCi i.v. dose of I-125 M-IBG 4 hr later. Two hours after the M-IBG injection, the animals were killed and tissue analyzed. Blood samples were taken by cardiac puncture. The two female mongrel dogs that served as controls were injected with an equal volume of reserpine vehicle and then treated similarly.

Imaging. Tomographic imaging of the heart was performed using a seven-pinhole collimator on a camera[§§] and a time-modulated coded aperture on a portable camera[¶¶] (*21,22*). In each case, the heart was viewed in the LAO projection.

Specific-activity study. High-specific activity I-125 M-IBG was synthesized by radioiodide exchange of 6.5 mCi of $Na^{125}I$ with 25 μg of M-IBG · $\frac{1}{2}H_2SO_4$ in 0.20 ml of boiling water. Reaction time was 3.5 hr. Purification (*18*) gave 2.8 mCi of I-125 M-IBG with a specific activity of ~130 mCi/mg. Specific activities of 100, 50, 1.0, and 0.01 mCi/mg were obtained by addition of the requisite amounts of stable M-IBG · $\frac{1}{2}H_2SO_4$ to 500-μCi aliquots of the stock solution. Concentrations were determined spectrophotometrically (λ_{max} = 228) using a standard curve.

RESULTS

Tissue distribution studies. Table 1 summarizes the distribution of I-125 M-IBG in selected tissues of the rat, dog, and monkey. The monkey data are limited in scope by the high cost of the animals. In all three species, M-IBG showed selective localization in the heart. The overall distribution patterns in the three species were similar, with the major differences being: (a) more rapid washout of radioactivity from the rat heart than from the dog heart; (b) higher muscle concentrations ([M]) in the rat; and (c) increased liver uptake in the monkey. The

TABLE 1. DISTRIBUTION OF M[^{125}I]IODOBENZYLGUANIDINE (M-IBG) IN RATS, DOGS, AND MONKEYS*

Animal	No. of Animals	Time (hr)	Heart	Blood	Liver	Lung	Muscle	H/B†
			Tissue concentration (% kg dose/g)					
Rats	6	0.5	1.09 (0.87–1.47)	0.03 (0.03–0.04)	0.36 (0.27–0.44)	0.69 (0.44–1.03)	0.25 (0.17–0.40)	36
	5	1.0	0.68 (0.62–0.75)	0.04 (0.03–0.04)	0.27 (0.23–0.32)	0.38 (0.31–0.42)	0.14 (0.12–0.17)	17
	5	4.0	0.19 (0.14–0.23)	0.02 (0.02–0.02)	0.06 (0.03–0.08)	0.11 (0.10–0.12)	0.10 (0.08–0.15)	10
Dogs	2	0.08	0.46 (0.43–0.49)	0.04 (0.04–0.05)	0.57 (0.52–0.62)	0.98 (0.87–1.10)	0.06 (0.05–0.07)	12
	2	0.50	0.47 (0.44–0.50)	0.02 (0.02–0.03)	0.34 (0.29–0.39)	0.22 (0.13–0.32)	0.02 (0.02–0.03)	23
	2	2.0	0.63 (0.60–0.67)	0.03 (0.03–0.03)	0.29 (0.26–0.32)	0.95 (0.64–1.26)	0.05 (0.04–0.06)	21
	2	6.0	0.31 (0.28–0.34)	0.03 (0.02–0.03)	0.09 (0.09–0.09)	0.21 (0.13–0.28)	0.04 (0.04–0.05)	12
Monkeys	3	3.0	0.64 (0.56–0.72)	0.02 (0.02–0.02)	0.76 (0.66–0.82)	0.17 (0.16–0.18)	0.02 (0.02–0.03)	32

* Mean and range.

† Heart/blood. Blood samples were taken by cardiac puncture.

‡ Tissue data for other organs are available from authors on request.

TABLE 2. DISTRIBUTION OF VARIOUS RADIOLABELED HEART AGENTS IN DOGS*,†

Agent	Time (hr)	Tissue concentration (% kg dose/g) Heart	Blood	Liver	Lung	Muscle	H/B‡
16[^{125}I]iodo-hexadecanoic acid	0.08	0.58 (0.53–0.62)	0.07 (0.05–0.09)	0.26 (0.25–0.27)	0.28 (0.28–0.28)	0.12 (0.11–0.13)	8
	0.17	0.36 (0.32–0.40)	0.12 (0.11–0.13)	0.25 (0.25–0.25)	0.26 (0.24–0.29)	0.05 (0.03–0.07)	3
	0.33	0.23 (0.17–0.29)	0.15 (0.12–0.18)	0.26 (0.22–0.31)	0.19 (0.17–0.21)	0.08 (0.07–0.09)	2
Thallium-201	0.5	0.38 (0.35–0.41)	0.01 (0.01–0.01)	0.20 (0.12–0.27)	0.29 (0.21–0.36)	0.05 (0.04–0.06)	38
	2.0	0.37 (0.34–0.40)	0.01 (0.01–0.01)	0.14 (0.13–0.15)	0.15 (0.13–0.17)	0.08 (0.07–0.08)	37
	4.0	0.21 (0.16–0.26)	0.01 (0.01–0.01)	0.22 (0.09–0.35)	0.10 (0.08–0.11)	0.10 (0.08–0.12)	21
C-14 guanethidine	0.5	0.44 (0.43–0.44)	0.03 (0.02–0.04)	0.46 (0.33–0.58)	0.46 (0.37–0.58)	0.04 (0.03–0.04)	15
	2.0	0.46 (0.41–0.50)	0.02 (0.01–0.02)	0.30 (0.29–0.31)	0.31 (0.26–0.36)	0.05 (0.04–0.05)	23
	6.0	0.39 (0.31–0.46)	0.01 (0.00–0.02)	0.27 (0.24–0.31)	0.34 (0.26–0.42)	0.03 (0.03–0.03)	39
M[^{125}I]iodobenzyl-guanidine	0.5	0.47 (0.44–0.50)	0.02 (0.02–0.03)	0.34 (0.29–0.39)	0.22 (0.13–0.32)	0.02 (0.02–0.03)	23
	2.0	0.63 (0.60–0.67)	0.03 (0.03–0.03)	0.29 (0.26–0.32)	0.95 (0.64–1.26)	0.05 (0.04–0.06)	21
	6.0	0.31 (0.28–0.34)	0.03 (0.02–0.03)	0.09 (0.09–0.09)	0.21 (0.13–0.28)	0.04 (0.04–0.05)	10
H-3 Norepinephrine	0.5	0.74 (0.69–0.78)	0.04 (0.04–0.04)	0.27 (0.26–0.27)	0.18 (0.12–0.23)	0.07 (0.06–0.08)	18
	2.0	0.39 (0.36–0.44)	0.03 (0.03–0.03)	0.17 (0.12–0.22)	0.07 (0.05–0.08)	0.04 (0.03–0.04)	13
	6.0	0.21 (0.17–0.25)	0.02 (0.02–0.03)	0.27 (0.14–0.40)	0.11 (0.05–0.18)	0.06 (0.02–0.09)	10

* Tissue data for other organs are available from authors on request.
† Two dogs per time interval.
‡ Heart/blood. Blood samples taken by cardiac puncture.

most encouraging features of Table 1 are the high heart concentration ([H]) and the heart-to-blood radioactivity ratio ([H]/[B]) obtained in the monkey at 3 hr after injection.

Table 2 compares the tissue distribution of I-125 M-IBG in the dog with those of C-14 guanethidine and H-3 norepinephrine. Data for Tl-201 and I-125 HDA are included for comparison. The major observations are:

1. Except for differences in lung activity, I-125-M-IBG and C-14-guanethidine show generally similar tissue distribution profiles at 0.5 and 2.0 hours.

2. H-3 norepinephrine showed the highest initial [H] of all the agents.

3. At 2 hr, M-IBG has slightly lower [H]/[B] and [H]/[Lung] values than Tl-201; although the [H] and [H]/[M] values of M-IBG are higher than those of Tl-201 at all time intervals.

4. I-HDA has lower [H]/[B] and shorter myocardial $t_{1/2}$ than the other agents.

A comparison of the time-activity curves of I-125 M-IBG, C-14 guanethidine, and H-3 norepinephrine for the heart and blood of the dog are shown in Figs. 2 and 3, respectively. The three compounds show rapid uptake in the heart and similar efflux patterns up to 24 hr. Although the compounds show their highest [H] at early intervals, the exact time of maximum [H] varies slightly among the three agents. All three compounds are rapidly cleared from the blood; H-3 norepinephrine consistently gives the highest blood values at all time points from 5–120 min.

Urinalysis study. Radio-TLC of the collected 24-hr urines of two dogs administered I-131 M-IBG showed that >95% of the radioactivity was unchanged M-IBG.

Specific-activity study. Figure 4 shows the concen-

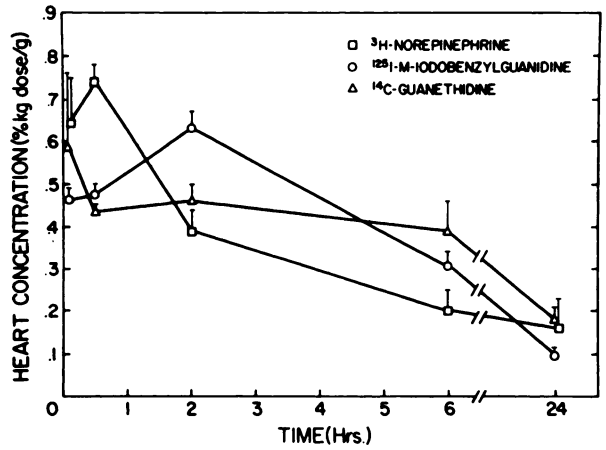

FIG. 2. Time-activity curves comparing three agents in dog's myocardium. Each point represents mean of two dogs.

tration of I-125 M-IBG in the heart and blood as a function of the specific activity of the administered compound. Within the 10,000-fold range of specific activities evaluated, only the lowest specific activity (10 μCi/mg) produced a change in the [H] and [B]; both values increased at this high loading-dose level.

Imaging studies. Figures 5–8 show representative heart images obtained in the dog and monkey using either I-123 M-IBG or I-131 M-IBG. Standard as well as tomographic images of the dog heart are included. Heart images of consistent quality were obtained from 15 min to 3 hr in both species. The wall of the right ventricle can be seen clearly in some of the tomographic slices. Using I-131 M-IBG, the monkey myocardium could still be imaged at 24 and 48 hr after injection with either a pinhole or parallel-hole collimator.

Of the four monkeys evaluated, only one failed to give a clear heart image. Use of Tl-201 in this same monkey also failed to image the heart. Subsequent injection of Tc-99m macroaggregated albumin showed decreased

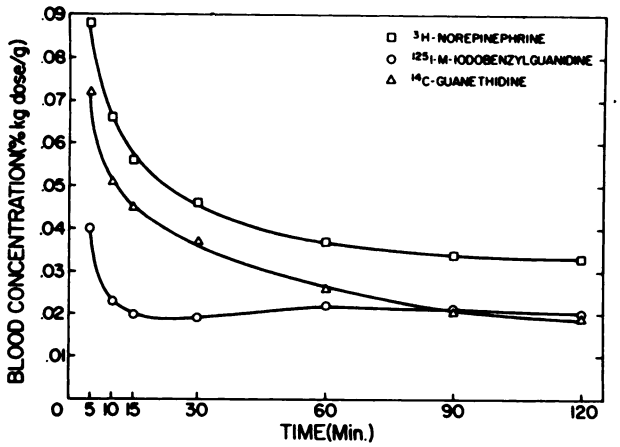

Fig. 3. Time-activity curves comparing three agents in dog's blood. Five dogs were killed at each time point. S.e.m. was too small to be shown. Blood was obtained by venous sampling.

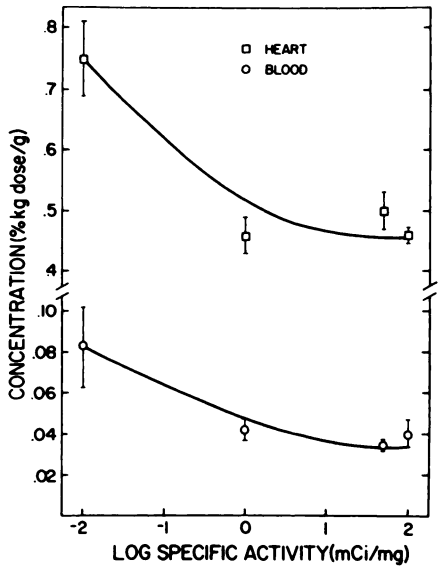

FIG. 4. Concentration of I-125 M-IBG in dog's heart and blood as function of specific activity. Each point represents mean of two dogs. Animals were killed 5 min after injection. Blood samples were obtained by cardiac puncture.

uptake of radioactivity in the left lung, suggesting the presence of lung disease, a common occurrence in captive primates (*23*).

Reserpine blocking study. As shown in Table 3, pretreatment of dogs with reserpine caused a 30% decrease in the myocardial concentration of I-125 M-IBG. Blood activity levels in the reserpine-treated dogs were higher than the controls.

DISCUSSION

All four chambers of the heart have a rich sympathetic

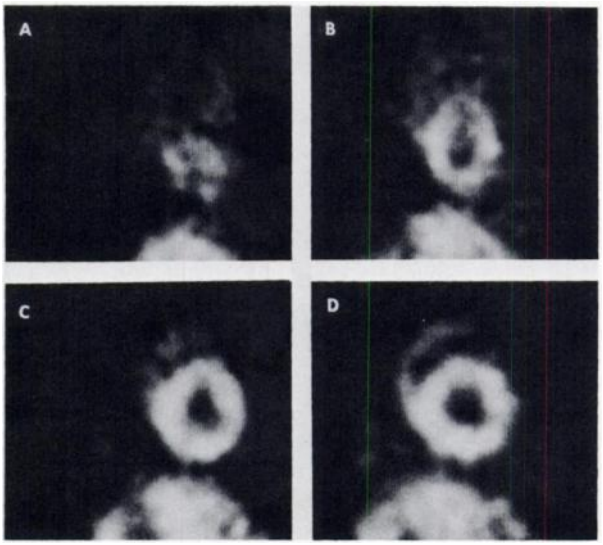

FIG. 5. Coded-aperture tomograms of normal dog's heart (Dog 1) obtained with 5 mCi *m*-[^{123}I]iodobenzylguanidine. Data were obtained 1 hr after injection, with imaging time 7 min. Images were reconstructed at 1-cm intervals, beginning at apex of heart.

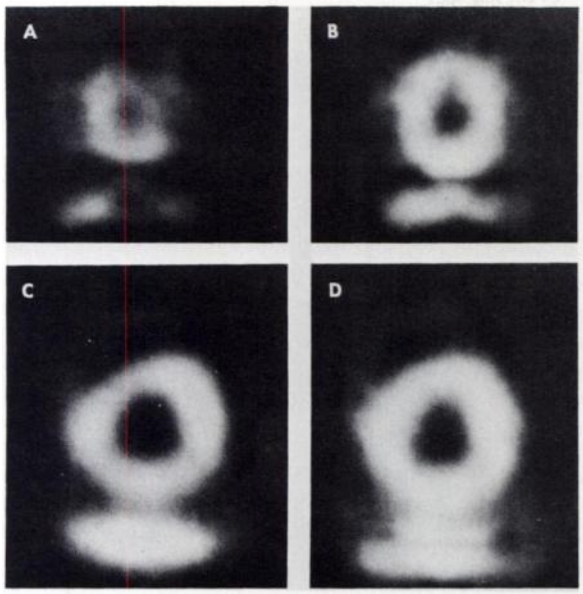

FIG. 6. Seven-pinhole tomograms of a normal dog's heart (Dog 1) with 5 mCi of *m*-[^{123}I]iodobenzylguanidine. Data were obtained 0.5 hr after injection, with reconstructions beginning at apex of heart.

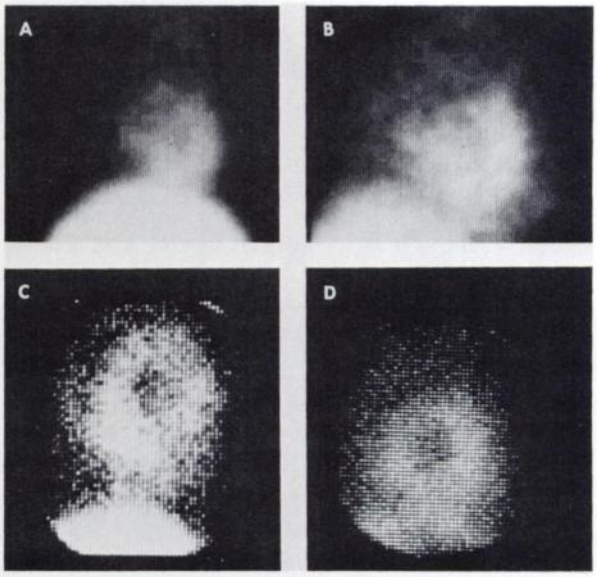

FIG. 8. Representative parallel hole and pinhole images obtained with 1 mCi of *m*-[^{131}I]IBG: (A) Parallel-hole image of a normal monkey's heart. (B) Parallel-hole image of a normal dog's heart. (C) Pinhole-image of a normal monkey's heart. (D) Pinhole image of a normal dog's heart.

nerve supply, which serves as a link between the brain and contracting heart muscles. Although 80% of the norepinephrine present in the heart is synthesized there (*10,24*), the myocardium can avidly accumulate exogenous norepinephrine. The high initial concentrations of H-3 norepinephrine observed in the dog heart (Table 2) confirm the latter point. Studies have indicated that 50–70% of small intravenous doses of norepinephrine are rapidly metabolized by COMT and MAO (*9*). M-IBG can thus be viewed as a "nonmetabolizable" norepinephrine; it shares the same uptake, storage, and release mechanisms as norepinephrine in adrenergic nerve terminals, but does not suffer the same metabolic fate (*15*). Use of radioiodinated M-IBG as a tracer could thus serve to simplify the complex disposition of norepinephrine by focusing on only a few, but nonetheless important, determinants of its in vivo distribution.

Should subsequent studies confirm that M-IBG resembles guanethidine at both pharmacological and tracer levels, the radiopharmaceutical evaluation of M-IBG becomes a straightforward task, since the disposition and actions of guanethidine in the peripheral sympathetic nervous system have been extensively studied (*25–27*). Guanethidine is both a neuron-blocking agent and a depletor of norepinephrine. Both actions, though mechanistically different, require uptake of guanethidine into sympathetic nerves. Blockade seemingly entails binding to some cytoplasmic component of the neuron, whereas depletion involves uptake into the intraneuronal vesicles with subsequent release of nor-

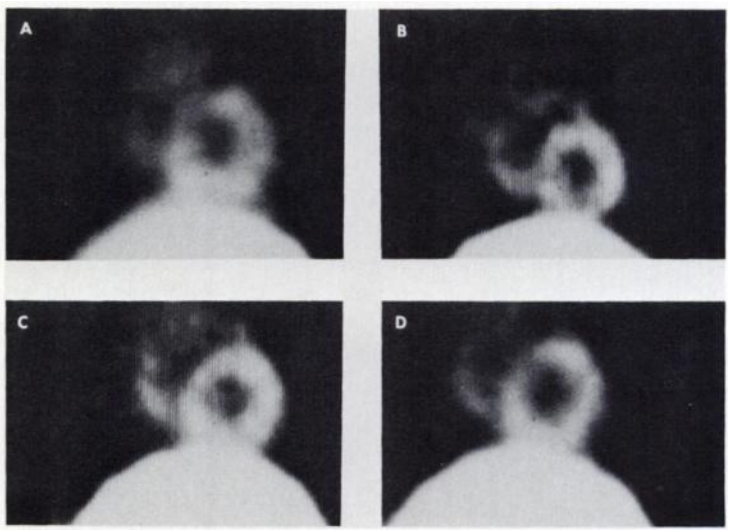

FIG. 7. Coded-aperture tomograms of normal monkey's heart, obtained with 2 mCi *m*-[^{123}I]iodobenzylguanidine. Data were obtained 1 hr after injection, with imaging time 20 min. Images were reconstructed at 1-cm intervals, beginning at apex of heart.

TABLE 3. I-125 M-IBG: RESERPINE BLOCKING STUDY IN DOGS (% kg dose/g)

	Time (hr)	Heart	Blood*
Control	2	0.46 (0.46–0.47)	0.03 (0.03–0.03)
Reserpine†	2	0.31 (0.25–0.37)	0.05 (0.04–0.06)

* Blood samples were taken by cardiac puncture.
† 1 mg/kg i.m.; 4 hr later I-125 M-IBG given i.v.

epinephrine stores. The dissociation of the two mechanisms is best demonstrated by certain aralkylguanidines that show predominantly one action or the other (*28,29*). From a radiopharmaceutical standpoint, recognition of these two pharmacological effects as distinct actions that involve binding to separate loci within the adrenergic neuron is important: aralkylguanidines that exhibit both actions should potentially show higher concentrations in the neurons and consequently higher uptake in sympathetically innervated organs, such as the heart. Structure-distribution relationship (SDR) studies now in progress are focusing on radioiodinated aralkylguanidines that possess this dual action.

Guanethidine is thought to share the same neuronal uptake mechanism as norepinephrine. The uptake, often referred to in the older literature as uptake$_1$, is mediated by an active transport process (*30*). This amine pump processes a variety of endogenous amines and basic drugs. Both guanethidine and M-IBG are extremely strong organic bases, with pKa's around 13.5 (*31*), so their neuronal uptake most likely occurs as the protonated forms. The relative affinity of guanethidine for the amine pump is one fourth that of norepinephrine (*30*). Note, however, that relative pump affinity will not be the major determinant of overall neuronal accumulation of a compound, but rather its affinity for intraneuronal components, especially the storage vesicles. Reserpine is generally thought to block selectively the vesicular uptake of norepinephrine in adrenergic neurons (*32*). In addition, reserpine blockade of guanethidine uptake in the rat myocardium has been observed by Brodie and coworkers (*33*). The results of the reserpine blocking study (Table 3) indicate that a considerable portion of the myocardial accumulation of M-IBG is by entrapment within the neuronal storage vesicles. Other, more indirect clues for the neuronal uptake of M-IBG in the heart are:

1. The efflux curve (Fig. 2) of M-IBG from the heart appears to be monophasic with $t_{1/2} \simeq 5$ hr. If considerable extraneuronal (uptake$_2$) or nonspecific binding had occurred in the heart, a biphasic efflux curve would have been observed, the first component having a $t_{1/2}$ of 10–15 min. Such a pattern has been obtained when large loading doses of labeled norepinephrine are injected (*34*). Nonspecific binding at non-neuronal sites has also been observed with guanethidine (*35*), but apparently is a major factor only at pharmacological dose levels.

2. Long retention of M-IBG has been observed in the spleen and adrenal medulla (*18*). The spleen has dense sympathetic innervation, and the adrenal medulla is essentially a large sympathetic ganglion specialized for synthesis and storage of adrenergic hormones.

3. The tissue distribution of M-IBG (Table 2) resembles that of C-14 guanethidine.

There is abundant evidence that the neuronal uptake of norepinephrine is a saturable process that obeys Michaelis–Menten kinetics (*36*). In vivo work with guinea pigs has shown that saturation of heart uptake occurs with intravenous doses $\gtrsim 170$ μg/kg (*10*). Although M-IBG and norepinephrine do not necessarily have the same saturation levels for the carrier molecule, it is noteworthy that the routine loading dose of M-IBG was 10 μg/kg. In addition, the curve of specific activity plotted against heart concentration shown in Fig. 4 is strong evidence that the loading doses used in this study were well below the saturation level. The enhanced heart concentration observed at the highest loading dose (specific activity = 10 μCi/mg) is consistent with the involvement of an extraneuronal uptake process. At this high dose level, saturation of neuronal uptake has most likely occurred, resulting in an incremental accumulation of M-IBG in non-neuronal sites, such as the myocardial muscle cells (*37*). Thus an attractive feature of radioiodinated M-IBG is that only at very low specific activities is non-neuronal uptake in the heart observed. This obviates the synthetic difficulties and stability problems sometimes associated with agents requiring high specific activity. Radioiodinated M-IBG with specific activities of 1–100 mCi/mg can be attained in consistently high radiochemical yields by a rapid exchange technique. The use of radioiodinated M-IBG in this range of specific activities also provides a loading dose well below the pharmacological levels of substituted benzylguanidines (*38*).

The rapid, high-yield radiosynthesis and in vitro stability of radioiodinated M-IBG make it a particularly convenient agent. Metabolic studies are still in progress, but in vivo deiodination of M-IBG, as indicated by the thyroid activity levels, is very low at all time intervals studied. The pronounced stability of M-IBG parallels the high thermodynamic stability of meta-substituted aromatics (*39*); the respective ortho and para isomers show considerably higher in vivo deiodination (*18*). Radio-TLC of the urine of dogs administered I-131 M-IBG has shown no major metabolites, but proof of the absence of metabolites—especially those arising from liver microsomal hydroxylation of the benzylic carbon (*40*)—must await detailed analysis by high-pressure liquid chromatography.

The fact that M-IBG has a tissue distribution similar to that of guanethidine is in itself an encouraging finding, demonstrating that large structural variations, including radioiodine incorporation, do not adversely alter the desired biodistribution. To date, studies reveal that the overwhelming determinant of heart specificity is the guanidine group itself.

The fatty-acid analog, 16-iodohexadecanoic acid, was used in this study for comparison with the other myocardial agents. The unsaturated analog, 16-iodo-9-hexadecenoic acid, would be a more logical choice for comparison since it has been more extensively studied, but the synthetic precursor of the latter compound, 16-bromo-9-hexadecenoic acid, though commercially available in crude form, is difficult to purify. The possible presence of both cis and trans isomers further complicates the purification. In contrast, 16-bromohexadecanoic acid, the precursor of the 16-iodohexadecanoic acid, is conveniently synthesized in analytically pure form from the commercially available alcohol, juniperic acid. The 16-iodo-9-hexadecenoic acid and 16-iodohexadecanoic acid have similar affinities for the myocardium (*6*).

Functional aspects aside, an I-123-labeled myocardial agent offers certain advantages over Tl-201 ($t_{1/2}$ = 3 days). The shorter half-life of I-123 (13.3 hr) and its 159 keV gammas, with 83% abundance, provide a higher photo flux per rad of absorbed dose at a desirable energy level. In addition, the tissue attenuation coefficient for the 70–90 keV photons of Tl-201 is 25% greater than for the 159 keV gammas of I-123. It should be noted that I-123 has a 2.25% abundance of photons ranging from 183 to 784 keV, which must be considered when selecting collimators. It is also important to obtain I-123 free of the positron emitting I-124 contaminant (*41*).

Although most of the images reported in this study were obtained by tomographic techniques, note that standard pinhole images (Fig. 8) of the dog and monkey hearts were of good quality. In fact the pinhole provides high resolution and good discrimination against the high-energy gammas mentioned above. The higher liver uptake seen in the monkey, if also observed in humans, might present difficulties for standard gamma imaging, but this should be minimized with tomographic techniques.

The intent of this initial paper is not to suggest the use of M-IBG as a myocardial perfusion agent but to stress its potential application to heart diseases characterized by alterations in sympathetic nerve function. The tissue distribution data on M-IBG and other radiolabeled compounds in Table 2 should not be seen as a comparison of their potential for anatomical myocardial imaging. Thallium-201, 16-I-HDA and M-IBG are measuring different physiological processes—most likely blood flow, metabolism, and adrenergic nerve activity, respectively. No single radiopharmaceutical can serve to measure the array of physiological processes of the heart.

FUTURE WORK

Attempts to document the noradrenaline-mimicking properties of M-IBG are continuing in hope of building a framework for its future use as a dynamic imaging agent. A comparison of the regional distribution of H-3 norepinephrine and M-IBG in the heart should provide further evidence for the specific neuronal localization of M-IBG. The change in M-IBG heart accumulation in response to various physiological and pharmacological interventions known to modify adrenergic nerve function should also be instructive. An ongoing SDR study of aralkylguanidines may produce a radioiodinated derivative with higher heart-to-liver and heart-to-lung concentration ratios without sacrificing neuronal specificity. In addition, such studies could possibly lead to guanidine analogs amenable to rapid labeling with radionuclides, such as fluorine-18. Such an analog, if quantified by positron-emission tomographic techniques, could potentially be used to measure noninvasively the kinetics of storage and turnover in adrenergically innervated tissues in a variety of physiologic and pathophysiologic states.

FOOTNOTES

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

† Eastman Cellulose 13255, Eastman Kodak Co., Rochester, NY.

‡ New England Nuclear, North Billerica, MA.

‖ Union Carbide, Tuxedo, NY.

§ Crocker Nuclear Laboratory, Davis, CA.

¶ CIBA—Geigy Corp., Ardsley, NY.

** Varian EM360A.

†† Aldrich Chemical Co.

‡‡ Eastman Chemical Co.

‖‖ Packard 306.

§§ Searle LFOV.

¶¶ Ohio Nuclear Series 420.

ACKNOWLEDGMENTS

This work was supported by Grant No. CA-09015-02, Cancer Research Training in Nuclear Medicine, from National Cancer Institute, DHEW; by ERDA Contract No. EY-76-S-02-2031; and by the Nuclear Medicine Research Fund.

The C-14-guanethidine was provided through the efforts and courtesy of Dr. M.F. Bartlett of the CIBA-GEIGY Corporation.

The authors thank D. D. Marsh for technical assistance and Vicki Van Wasshnova and Sally Synhaivsky for help in preparing the manuscript.

A special thanks is extended to Dr. J. A. McNamara, Jr., Dr. D. S. Carlson, and J. C. Ungerleider of the University of Michigan for donating the rhesus monkeys used in this study.

Several aspects of this work are the subject of a pending patent application from the University of Michigan.

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Abstracts should not exceed 300 words and should contain a statement of purpose, the method used, results, and conclusions. The name of the author presenting the paper must be underlined.

Original abstracts and four copies should be sent to:

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Dept. of Nuclear Medicine
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Colorado Springs, CO 80907
Tel: (303) 630-5242