Development of a Kit-Form Analog of Metaiodobenzylguanidine

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The synthesis and evaluation of two radiopharmaceutical analogs of metaiodobenzylguanidine (MIBG) are described. Unlike MIBG, these analogs are rapidly and conveniently radioiodinated at room temperature in clinically applicable kit form. Radioiodinated 4-amino-3-iodobenzylguanidine (AIBG) for injection is synthesized in 20 min using IODO-GEN as the radioiodide oxidant and an anion exchange filter for purification. AIBG shows an affinity for the heart and adrenal medullae of dog and monkey similar to that of MIBG. In addition, AIBG shows improved selectivity for the adrenergic nerves of the heart as demonstrated by chemical sympathectomy studies. Tomographic images of the dog heart and planar images of the dog adrenal medullae were obtained using [^{123}I]AIBG and [^{131}I]AIBG, respectively. Planar images of the monkey heart using [^{131}I]AIBG were similar in quality to those reported previously with [^{131}I]MIBG. In view of the facile radiosynthesis of AIBG, a clinical evaluation of this new agent is warranted.

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Radioiodinated metaiodobenzylguanidine (MIBG), an aromatic analog of the neuron blocker guanethidine, has been used clinically for detection and radiotherapy of catecholamine tumors (*3–12*) and imaging of adrenergically innervated organs such as the heart (*13–15*). Radioiodinated MIBG is synthesized by an exchange technique that requires heating in the solid state at 140°C for 1–2 hr followed by passage through anion exchange resin (*16*). The goal of the work described here was to develop an analog of MIBG that possessed the biodistribution properties and heart neuronal selectivity of MIBG but could be rapidly synthesized under very mild conditions, hopefully in a self-contained system or "kit form" suitable for a hospital or nuclear pharmacy setting.

The rationale for the development of MIBG as a radiopharmaceutical was in large part based on the structure-activity-relationship (SAR) studies of benzylguanidines which demonstrated their neuron blocking potency and antihypertensive activity (*17*). Accumulation of MIBG in the adrenal medulla and peripheral nerves is dependent on the uptake$_1$ process—the transporter that carries norepinephrine into adrenergic nerve terminals (*18,19*). Although compounds divergent in structure as guanethidine, cocaine, amphetamine, and MIBG bind to the "amine pump," there are nonetheless considerable structural constraints on compounds transported into and retained in adrenergic nerves (*20,21*), the low neuronal accumulation of isoproterenol (*N*-isopropylnorepinephrine) being a prominent example (*21*). We have also observed that subtle structural changes in radiolabeled aralkylguanidines markedly affect their accumulation in adrenergic-rich tissue (*22*). Benzylguanidine derivatives that possess electron-donating groups such as OH or NH_2 on the aromatic ring could be rapidly radioiodinated by an electrophilic process using radioiodide and a mild oxidant. However, the original (SAR) study of benzylguanidines by Short and Darby (*17*) and the subsequent work of Fielden and Green (*23*) did not report the effect of electron-donating substituents such as OH or NH_2 on pharmacologic potency; the implication being that such polar substituents on the aromatic ring lower activity. Thus, at the outset of this study, we predicted that a polar analog of MIBG could be synthesized which was amenable to radiolabeling in kit form but it was highly unlikely that such an analog would bind strongly to the uptake$_1$ transporter and accumu-

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* Brief reports of parts of the present study have appeared (*1,2*).

late selectively in adrenergic-rich tissue. Our first attempt at a kit approach was in the phenethylguanidines series of compounds and the results confirmed our prediction: ^{125}I-3-iodo-4-hydroxyphenethylguanidine showed less accumulation in the heart and adrenal medulla than its more lipophilic counterpart ^{125}I-3-iodophenethylguanidine (unpublished data). Despite this poor prognosis, we proceeded to evaluate the radioiodination and biodistribution of benzylguanidines bearing polar substituents on the aromatic ring; a direction that has unexpectedly proven successful. We report here (a) the radioiodination of 4-amino- and 4-hydroxybenzylguanidine; (b) their pronounced accumulation in adrenergic-rich tissues of the dog; (c) the high neuronal selectivity of ^{125}I-3-iodo-4-aminobenzylguanidine (AIBG) in the rat myocardium; and (d) optimization of the radioiodination of 4-aminobenzylguanidine to produce AIBG for use in a kit process. The results in animals described in this report suggest that a clinical trial comparing AIBG and MIBG should be conducted.

MATERIALS AND METHODS

Microanalyses were performed commercially.† Radio-thin layer chromatography was done using 250-μ silica gel coated glass plates† using a radiochromatogram scanner.‡ The Cellex-D anion-exchange cellulose (hydroxide form),§ IODO-BEADS,¶ IODOGEN,¶ chloramine-T,‡ 3,5-dimethylpyrazole-1-carboxamidine nitrate, 4-aminobenzonitrile,‡ 4-methoxybenzylamine,‡ 4-nitrobenzylamine·HCl,‡, 6-hydroxydopamine hydrobromide** and [^{125}I]sodium iodide†† were obtained from commercial sources. Proton magnetic resonance (PMR)‡‡ and infrared (IR)§§ spectra were obtained; high pressure liquid chromatography (HPLC)¶¶ data were also determined.

Synthesis of Unlabeled Compounds

4-Nitrobenzylguanidine sulfate. A mixture of 4-nitrobenzylamine (obtained by CH_2Cl_2 extraction of a mixture of 10 mmol of the hydrochloride salt and 20 ml of 10% NaOH solution) and 2-methyl-2-thiopseudourea sulfate (1.40 g, 5.0 mmol) in water-ethanol (6 ml, 1:1 v/v) was heated and stirred in an oil bath at 110–120°C under argon for 40 hr. The solvents were evaporated and the residue was treated with boiling water (70 ml) and charcoal, filtered and concentrated to ~40 ml. On cooling, yellow crystals formed. They were filtered and washed with water. Recrystallization from water afforded an analytically pure sample (0.99 g, 41%), mp 244–245°C (dec.); IR (cm^{-1}) Nujol 3470 (NH), 1690 and 1625 (C=N), 1510 and 1350 (NO_2); PMR (CH_3OH-d_4 + two drops CF_3COOH) δ 7.85 (d, 2H, J = 8.5 Hz), 7.16 (d, 2H, J = 8.5 Hz), 3.20 (t, J = 6 Hz, 2H), 2.68 (t, 2H, J = 6 Hz). Anal. Calcd. for $C_8H_{10}N_4O_2 \cdot \frac{1}{2}H_2SO_4$: C, 39.51; H, 4.53; N, 23.05. Found: C, 39.61; H, 4.52; N, 23.18.

4-Aminobenzylguanidine sulfate. 4-Nitrobenzylguanidine sulfate (0.243 g, 1.0 mmol) was dissolved in water (50 ml) by gentle warming and then cooled to ambient temperature. 500 mg of activated Raney-Nickel catalyst (wet form) was added and the mixture was hydrogenated at 50 p.s.i. for 90 min. The catalyst was filtered and washed with water. To the clear, colorless filtrate was added 2N H_2SO_4 to pH 1.5. The solution was concentrated to ~25 ml; 95% ethanol (150 ml) was added and the solution was stored at 4°C overnight. The colorless needles were filtered and recrystallized from 1*N* H_2SO_4 (8 ml) to give stout prisms (0.205 g, 78%), mp 248–250°C (dec); IR (cm^{-1}) Nujol 3395 and 3260 (NH), 1675 and 1655 (C=N), 1060 (S=O) 835 (1,4-disubstituted benzene); PMR (DMSO-d_6) δ 7.54 (t, 1H, *J* = 5 Hz, CH_2*NH*—), 6.90 (m, 7H) 6.40 (d, 2H, *J* = 8.5 Hz) and 3.98 (d, 2H, *J* = 5 Hz, CH_2NH). Anal. Calcd. for $C_8H_{12}N_4 \cdot H_2SO_4$: C, 36.64; H, 5.34; N, 21.37. Found: C, 36.70; H, 5.30; N, 21.39.

4-Hydroxybenzylguanidine sulfate. A mixture of 0.251 g (1.0 mmol) of 4-hydroxybenzylamine·HI (synthesized by demethylation of 4-methoxybenzylamine with hydroiodic acid) and cyanamide (0.126 g, 3.0 mmol) was heated and stirred at 120°C under argon for 3 hr and cooled to ambient temperature. The residue was dissolved in boiling water (1 ml), a solution of $KHCO_3$ (0.11 g, 1.1 mmol) in 1 ml of water was added and the solution was boiled for 2 min and cooled. The bicarbonate salt of the title compound separated as colorless crystals which were filtered and then suspended in 2 ml of warm water. 2N H_2SO_4 was added dropwise to pH 2. The solution was boiled and concentrated to ~1 ml and cooled. The colorless solid was filtered and recrystallized from water to yield the sulfate salt as colorless crystals (0.108 g, 51%), mp 260–262°C; IR (cm^{-1}) Nujol 3160, 3360 (OH), 1690, 1640 (C=N), 1070 (S=O); PMR (CH_3OH-d_4 and two drops CF_3COOH) δ 6.90 (d, 2H, J = 8 Hz), 6.70 (d, 2H, *J* = 8 Hz), and 3.95 (s, 2H). Anal. Calcd. for $C_8H_{11}N_3O \cdot \frac{1}{2}H_2SO_4$: C, 44.86; H, 5.61; N, 19.63. Found: C, 44.90; H, 5.60; N, 19.58.

4-Amino-3-iodobenzylamine hydrochloride. To a solution of (2.44 g, 10 mmol) 4-amino-3-iodobenzonitrile (synthesized by iodination of 4-aminobenzonitrile with I_2/H_2O_2) in 5 ml of dry THF was added 30 ml of borane-tetrahydrofuran complex solution (30 mmol) with stirring. The solution was heated at reflux temperature for 1 hr under argon. Upon cooling, 2 ml of ethanol was added to quench excess borane. After the vigorous reaction subsided, 30 ml of water and 40 ml of diethyl ether were added and the mixture was separated. The aqueous layer was again extracted with ether (2 × 40 ml) and the combined ether extracts were dried. HCl gas was bubbled through the ether solution for 10 min and the granular white precipitate (3.0 g, 94%) which resulted was collected and recrystallized from

methanol: mp 187–189°C (dec); IR (cm^{-1}) Nujol 3500, 1545, 1595 (amine salt); PMR (DMSO-d_6) δ 8.5 (br s, 2H), 7.8 (m, 3H), 7.2 (br s, 2H), 3.7 (s, 2H). Anal. Calcd. for $C_7H_9NI \cdot 2$ HCl: C, 26.17; H, 3.43; N, 8.72. Found: C, 26.21; H, 3.42; N, 8.75.

4-Amino-3-iodobenzylguanidine nitrate. An ethanol (5 ml) solution of 4-amino-3-iodobenzylamine (0.220 g, 0.887 mmol) and 3,5-dimethylpyrazole-1-carboxamidine nitrate (0.179 g, 0.087 mmol) was heated at reflux temperature for 3 hr under argon. The solvent was evaporated in vacuo and the residue washed with ether and dichloromethane to remove 3,5-dimethylpyrazole and unreacted amine. The crude product was recrystallized from methanol/ether to give fine, pale-yellow needles (0.163 g, 72.5%), mp 135–137°C; PMR (DMSO-d_6) δ 6.62–7.66 (m, 9H), 4.1 (s, 2H); IR (cm^{-1}) Nujol 1660, 1620 (C=N); Anal. Calcd. for $C_{18}H_{11}IN_4 \cdot HNO_3 \cdot 0.5H_2O$: C, 27.20; H, 3.42, N, 19.08. Found: C, 26.70; H, 3.33; N, 19.08.

4-Hydroxy-3-iodobenzylguanidine sulfate. 4-Hydroxybenzylguanidine sulfate (450 mg, 2.1 mmol) was dissolved in 30% ethanol (30 ml) and conc. NH_4OH (30 ml); a solution of I_2 (399.8 mg, 1.575 mmol) in ethanol (3 ml) was added with stirring at 0°C and the mixture was stirred at ambient temperature for 18 hr. The solvent was removed in vacuo and the residue was recrystalized three times from water affording 350 mg (49%), mp 208–210°C (dec); PMR (DMSO-d_6) δ 6.32–7.8 (m, 6H, ArH, NH_2); 4.15 (s, 2H, CH_2) IR (cm^{-1}) Nujol 3350, 3140 (NH_2); 1660, 1630 (C=N).

Synthesis of Radiolabeled Compounds

Radioiodination of 4-aminobenzylguanidine sulfate. Method A: Chloramine-T technique. In a typical run, to a 5 ml Kontes "V" vial containing 0.5 mg of the title compound was added 1 ml of 0.02*M* KH_2PO_4 buffer (pH 4.8) and a spin vane. The mixture was gently warmed (~40°C) and stirred to obtain a clear solution which was cooled to room temperature. Approximately 10 μl of 0.1*N* NaOH solution containing 4.1 mCi [^{125}I]sodium iodide was added and the vial was closed with a Teflon-lined screw cap. Chloramine-T solution (7.5 μg in 30 μl of 0.02*M* KH_2PO_4 solution) was added with vigorous stirring and the reaction mixture was stirred at ambient temperature for 10 min, and then manually shaken for a few seconds. After stirring for another 5 min, 40 μl of aqueous $NaHSO_3$ solution (1.4 mg/ml) was added, the solution was stirred for 5 min and then checked by thin layer chromatography (TLC) [silica gel:EtOH/EtOAc/conc. NH_4OH (20/20/1)]. The reaction had proceeded to the extent of 95–98%. The solution was passed through an anion exchange column (Cellex-D, OH^- form) under partial vacuum in a closed system to remove the free [^{125}I]iodide. Further elution with 0.005*M* sodium acetate buffer (3 × 1 ml) provided the radioactive product which was >98% pure as shown by TLC analysis on silica gel using n-butanol/acetic acid/water (5/2/1), Rf = 0.50, and EtOH/conc. NH_4OH (3/1), R_f = 0.15. Identity of the radioactive peak was confirmed by R_f coincidence with authentic unlabeled material. The radiochemical yield was 3.9 mCi (95%) and the specific activity was 4.3 mCi/mg. The average radiochemical yield for four runs using varying amounts of 4-aminobenzylguanidine was also 95% with the specific activity of the product ranging from 1–80 mCi/mg. Essentially identical results were obtained when [^{131}I]sodium iodide was used.

Method B: IODO-GEN technique. To a 5-ml Kontes "V" vial was added a solution of 0.5 mg of IODO-GEN in 0.5 ml of methylene chloride. The solvent was carefully evaporated under a stream of argon while stirring the solution vigorously with a magnetically driven spin vane; the procedure left a uniform coating of IODO-GEN on the walls of the vial. A solution of 4-aminobenzylguanidine sulfate, 0.18 mg in 0.36 ml of 0.04*M* sodium acetate buffer (pH 4.5) was added to the vial. The reaction vial was closed with a teflon-lined cap. Approximately 10 μl of 0.1N NaOH solution containing 5.0 mCi [^{125}I]sodium iodide was dissolved in 0.14 ml of 0.04*M* sodium acetate buffer (pH 4.5) and added to the reaction vial by a Hamilton syringe with vigorous stirring. The reaction solution was then stirred gently at room temperature for 30 min. A purity check by radio-TLC [silica gel: EtOH/EtOAc (1/1)] showed that the radioiodination was greater than 90% complete. The reaction solution was drawn into a 2.5 ml sterile syringe. The needle of the syringe was removed and a Swinnex-13 Filter Unit*** fitted with an Acropor Ion Exchange Filter SB-6407††† was attached. The solution was slowly forced through the filter and into a sterile 10-ml, multidose vial containing 2.0 ml of 0.005*M* sodium acetate buffer. Radio-TLC on silica gel using *n*-butanol/acetic acid/water (5/2/1), R_f = 0.50, and EtOH/EtOAc (1/1), R_f = 0.15, revealed a radiochemical purity >98%. The radiochemical yield was 4.2 mCi (85%) and the specific activity was ~20 mCi/mmol.

Radioiodination of 4-hydroxybenzylguanidine sulfate. This reaction proceeded smoothly by using the chloramine-T technique described above for the 4-amino analog. For a typical run, the radiochemical yield was 92% and the specific activity was 5.1 mCi/mg. Radio-TLC analyses on silica gel using *n*-butanol/acetic acid/water (5/2/1), R_f = 0.40, and EtOH/EtOAc/conc. NH_4OH (20/20/1), R_f = 0.05, showed >97% radiochemical purity.

HPLC purity of [^{125}I]AIBG. A Waters Model 272 liquid chromatograph equipped with a Radiomatic Flo-one radioactive flow detector (200 μl solid scintillation cell) was used which employed simultaneous ultraviolet (254 nm) and radioactivity detection. Two C18 col-

umns‡‡‡ (3.9 × 300 mm each) were used in tandem for the analysis with 0.2*M* $NH_4H_2PO_4$:THF (85/15) as the eluting solvent at pH 4.6 and a flow rate of 1.50 ml min^{-1}.

In Vitro radiodeiodination of [^{125}I]AIBG and [^{125}I]HIBG. Two separate batches of [^{125}I]AIBG with specific activities of 3.6 and 3.8 mCi/mg, respectively, were dissolved in 0.005*M* sodium acetate buffer (pH 4.5) and stored in the dark at 4°C. The radioactive concentration of the two batches was ~530 μCi/ml. TLC of small aliquots was performed at 5, 9, and 16 days using silica gel coated glass plates with EtOH/EtOAc/conc. NH_4OH: 20/20/1 as eluant. Radiochromatograms were obtained on a radiochromatogram scanner. In this solvent system, radioiodide has an $R_f \simeq 0.80$ and [^{125}I]AIBG has an $R_f \simeq 0.05$. In a similar manner, two additional batches of [^{125}I]AIBG with specific activities of 40 mCi/mg and 110 mCi/mg were evaluated by radio-TLC at various time intervals. A single batch of I-131-AIBG, 77 mCi/mg, was evaluated at 30 hr and 6 days at a specific concentration of ~1 mCi/ml.

A single preparation of [^{125}I]HIBG, 310 mCi/mg, was tested as described above at 42 hr and 8 days. The R_f of [^{125}I]HIBG is ~0.05 in the above-mentioned TLC system.

Animal Studies

Tissue distribution studies. Dogs. Tissue distribution studies were performed on female mongrel dogs (14-22 kg). Each animal received a single i.v. bolus injection of 100 μCi of ^{125}I-labeled tracer in 2.0 ml of 0.005*M* sodium acetate buffer, pH 4.5. The dogs were killed at selected time intervals by large i.v. injections of sodium pentobarbital. Duplicate samples of 22 different tissues in each dog were weighed and counted in an autogamma counter with corrections made for radioactive decay, background, and counter efficiency. Blood samples were obtained by cardiac puncture. Isolation of adrenal medullary tissue was performed as previously described (*22*). To normalize for differences in animal weight, tissue concentrations are expressed as percent kilogram dose per gram (% kg-dose/g) (*24*).

Monkeys. Following a restraining i.m. dose of ketamine hydrochloride (5.0–10.0 mg/kg), monkeys were anesthetized with sodium pentobarbital (20–33 mg/kg) and injected intravenously with 100 μCi of I-125-AIBG in 2.0 ml of sodium acetate buffer, pH 4.5. Three hours later, the animals were killed by a large i.v. injection of sodium pentobarbital and duplicate samples of 11 tissues were removed and analyzed by the procedures described above for dogs.

Imaging–Adrenal glands. Dogs. A male dog (18.4 kg) was injected intravenously with a bolus of 825 μCi of [^{131}I]AIBG (10 mCi/mg). With the dog anesthetized with sodium pentobarbital, planar posterior adrenal images were obtained at 1, 3 and 6 days after injection using a Pho Gamma camera (high-energy, parallel hole collimator) interfaced to a minicomputer.

Monkeys. Two rhesus monkeys, a male (10.2 kg) and a female (12.0 kg), were each given a restraining dose of ketamine hydrochloride (5.0–10.0 mg/kg, i.m.) and then anesthetized with sodium pentobarbital (12–25 mg/kg, i.v.). Each monkey received a bolus i.v. injection of 850 μCi of [^{131}I]AIBG (46 mCi/mg) in 0.5 ml sodium acetate buffer, pH 4.5. Planar posterior images were obtained at 1, 5 and 8 days using a Pho Gamma camera fitted with a high-energy, parallel hole collimator. Confirmation of adrenal images was made by subsequent injection of 1 mCi of technetium-99m diethylenetriaminepentaacetic acid into the immobolized dog without altering position. The superimposed renal images were subtracted from the adrenal scans using the computer.

Imaging—Heart. Dogs. A conscious female dog (27.7 kg) was given a bolus i.v. injection of 6.5 mCi of [^{123}I]AIBG (7.0 mCi/mg) in 4.0 cc of pH 4.2 acetate buffer. The animal was then anesthetized with 30 mg/kg i.v. sodium pentobarbital; anesthesia was maintained by additional pentobarbital as needed. Using a GE 400-AT tomographic imager, 32 anterior chest images were obtained over 180° at 3 and 24 hr postinjection.

Monkeys. A female rhesus monkey (10.0 kg) was anesthetized as described above and then given a bolus i.v. injection of 5.4 mCi of [^{123}I]AIBG (9 mCi/mg) in 3.0 ml of pH 4.5 sodium acetate buffer. A 5° LAO pinhole image was obtained at 6 hr postinjection using a Pho Gamma camera. Tomographic images were also obtained at 4 and 24 hr using SPRINT, an experimental single photon imager (*25*).

6-Hydroxydopamine (6-OHDA) sympathectomy study. Female Sprague-Dawley rats weighing ~200 g were each injected i.p. with 100 mg/kg 6-OHDA hydrobromide (26) freshly prepared in 0.9% saline solution (100 mg/ml). Five days later the rats were each injected i.v. with 25 μCi of tracer and killed 90 min later. Control animals were injected with an equal volume of physiological saline instead of 6-OHDA hydrobromide solution.

RESULTS

Synthesis of "Cold" Compounds

The synthesis of 4-nitrobenzylguanidine has been reported previously (*27*) but the method presented here has been more reliable in our hands. The synthesis of 4-aminobenzylguanidine (ABG) was straightforward but the synthesis of AIBG was more elusive. Direct iodination of ABG with a variety of agents under numerous reaction conditions resulted in inseparable mixtures of

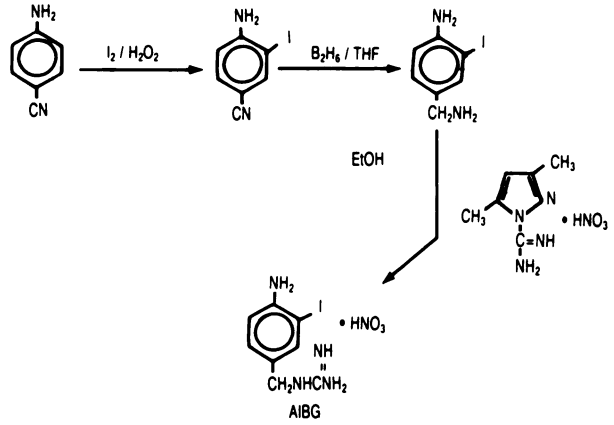

FIGURE 1
Synthetic scheme for 3-iodo-4-aminobenzylguanidine (AIBG)

the 3-iodo and 3,5-diiodo products. The iodine atom was thus introduced early in the synthetic sequence as shown in Fig. 1. The synthesis of "cold" HIBG, however, was accomplished by direct iodination of 4-hydroxybenzylguanidine. Further details on this chemistry, including the synthesis of related 3,4-disubstituted benzylguanidines will be published elsewhere (*28*).

Synthesis of Radiolabeled Compounds

The radiosyntheses of [^{125}I]AIBG and [^{125}I]MIBG are presented for comparison in Fig. 2. Radioiodination of ABG with no-carrier-added [^{125}I]iodide at a molar ratio of ABG to [^{125}I]iodide of >6,000 to 1 gives no-carrier-added [^{125}I]AIBG containing a >6,000-fold molar equivalent of ABG. Thus the term "specific activity" as used in this report refers to "effective specific activity." In other words, ABG is considered to be AIBG for purposes of calculating specific activity. In the radioiodination of ABG, the amount of ABG was kept as low as possible without compromising the radiochemical yield. This was done to minimize possible pharmacological effects of ABG. The amount of chloramine-T used was also of concern since it is converted to *p*-toluenesulfonamide upon quench reduction with sodium bisulfite. The use of water-insoluble oxidants such as IODO-BEADS and IODO-GEN would obviate the problem of the small amount of *p*-toluenesulfonamide contaminant. We achieved satisfactory radiochemical yields of [^{125}I]AIBG using IODO-GEN (80–95%); however, radiochemical yields using IODO-BEADS were considerably lower (data not shown). The radiosynthesis of [^{125}I]AIBG is a mild and rapid method which is readily adaptable to a kit system. The amount of unreacted radioiodide in the final product is generally small (<5%), and if necessary, can be readily removed by passage through an anion exchange membrane filter fitted to a syringe, such as the Acropor Ion Exchange Filter SB-6407 reported here. The kit synthesis of [^{123}I]AIBG has not yet been optimized for clinical use. However, the following stepwise summary of the kit labeling procedure should serve as a general guide.

1. To a sterile septum sealed V-vial precoated with 0.5 mg of IODO-GEN is added a solution of ABG sulfate (0.18 mg) in pH 4.5 acetate buffer (0.36 ml).
2. Commercial NaI-123 (10–15 mCi) in 0.1*N* NaOH is dissolved in 0.14 ml of pH 4.5 acetate buffer and added to the V-vial by shielded syringe.
3. The V-vial is mechanically shaken for 20–30 min at ambient temperature.
4. The contents of the V-vial are drawn into a 2.5 ml syringe. A filter unit containing both anion exchange and 0.22 μ micropore filters is attached and the solution is forced through the filters into a 5-ml sterile multidose vial containing 1.0 ml of 0.005*M* sodium acetate buffer.
5. Radiochemical purity can be rapidly assessed by radio-TLC on SiO_2-G (i.e., Gelman ITLC SG). Using EtOH/EtOAc (1/1) as elutant, the R_f of AIBG is $\simeq$0.15; R_f of radioiodide is $\simeq$0.7.

The mechanism of retention of [^{125}I]AIBG in adrenergic neurons does not likely involve binding to low capacity receptors (*29*); thus extremely high specific activity [^{125}I]AIBG should not be necessary for optimal concentration in adrenergic-rich tissue. Although [^{125}I]AIBG at specific activities ranging from 4–50 mCi/mg was used in our studies, specific activities of 180 mCi/mg (86% radiochemical yield) and 400 mCi/mg (40% radiochemical yield) have been obtained.

HPLC Analysis of Radiochemical and Chemical Purity of [^{125}I]AIBG

Iodine-125 AIBG preparations were analyzed for radiochemical as well as nonradiochemical impurities

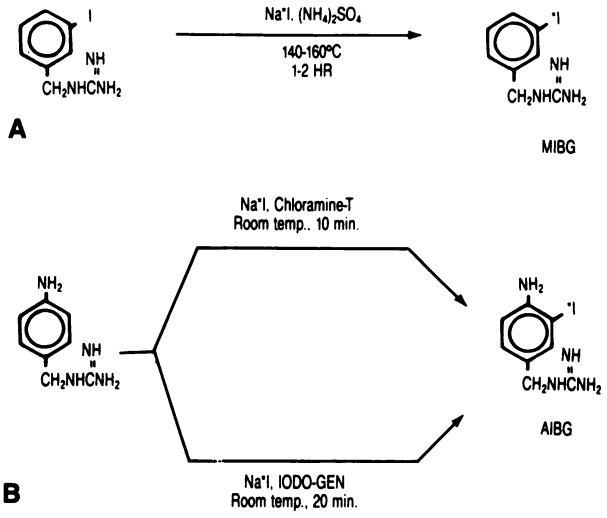

FIGURE 2
Radioiodination methods for (A) MIBG and (B) AIBG

by HPLC. The radiochemical purity using a C18 column with 0.2*M* $NH_4H_2PO_4$:THF (85/15) was routinely >98%. However, the low concentration of AIBG produced in a typical radioiodination experiment were not sufficient for accurate detection of unlabeled impurities by a uv detector unless a large amount of radioactivity was injected on the column. To avoid this problem, the iodination was conducted with $Na^{127}I$ in place of $Na^{125}I$ and a large aliquot of the reaction solution was injected on the column. The chemical purity of the [^{125}I]AIBG preparations was determined to be >98%. A potential nonradioactive impurity that could arise through competitive chlorination of ABG by chloramine-T is 3-chloro-ABG (*30*). The absence of 3-chloro-ABG was confirmed by separate synthesis and HPLC analysis. In addition to 3-chloro-ABG, 3,5-diiodo-ABG and 3-iodo-4-aminobenzylamine have been synthesized and shown to be absent by HPLC and radio-HPLC analysis (Fig. 3). Details of this extensive purity determination will be published in the full chemistry paper (*28*).

In Vitro Radiodeiodination Studies

The two batches of [^{125}I]AIBG with specific activities of 3.6 and 3.8 mCi/mg showed less than 1% free radioiodide at 5 days, 8–9% at 9 days, and 10–11% at 16 days. The sample of I-125-AIBG with a specific activity of 40 mCi/mg was 5% deiodinated at 14 days; a second sample, 110 mCi/mg, showed ~2% deiodination at 2 and 3 days. The evaluation of a single batch of [^{131}I]AIBG, 77 mCi/mg, showed 5% free radioiodide at 30 hr and 16% at 6 days. The preparation of [^{125}I]HIBG that was evaluated at a specific activity of 310 mCi/mg showed 1–2% free radioiodide at 42 hr and approximately 5% at 8 days.

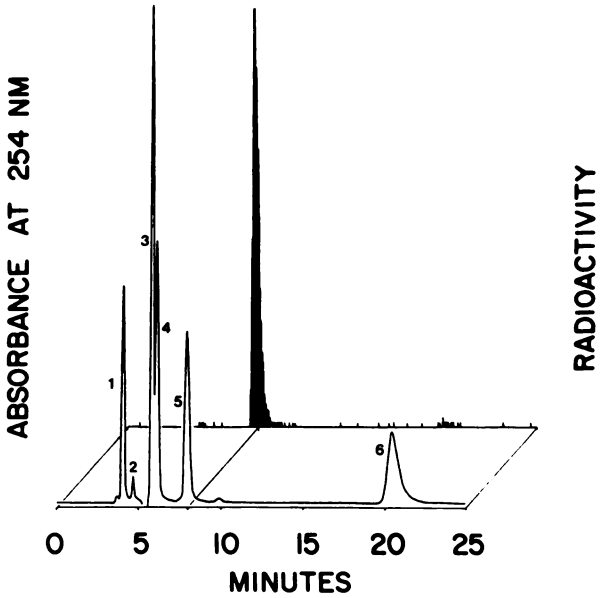

FIGURE 3
HPLC chromatogram of 750 nCi of [^{125}I]AIBG solution spiked with 8 nmol each of: 4-aminobenzylguanidine (1), NaI (2), 3-iodo-4-aminobenzylamine (3), 3-chloro-4-aminobenzylguanidine (4), 3-iodo-4-aminobenzylguanidine ([^{127}I]AIBG, 5) and 3,5-diiodo-4-aminobenzylguanidine (6). Radioactivity trace is shown in black, the ultraviolet trace in white. Two C-18 μ Bondapak columns were used in series with 0.2*M* $NH_4H_2PO_4$/THF (80/20) at flow rate of 1.5 ml min^{-1}

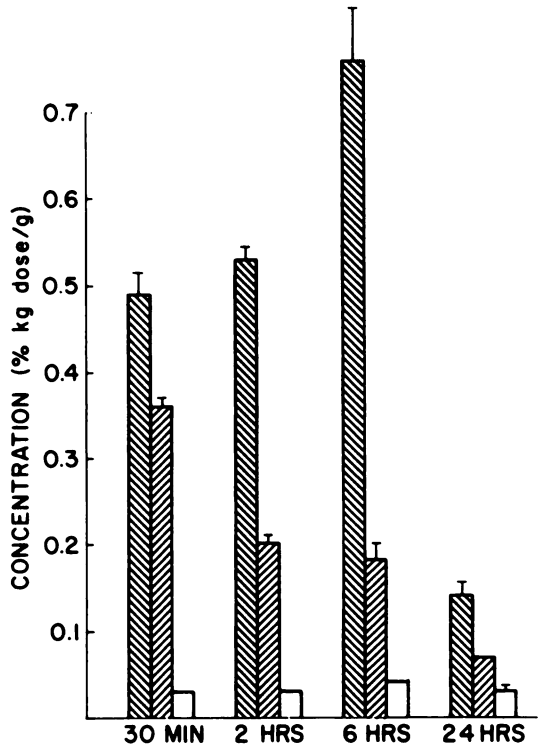

FIGURE 4
Time-activity [^{125}I] concentrations in heart (LV), liver and blood following i.v. injection of [^{125}I]AIBG in dog (N = 3 per time point)

Tissue Distribution Studies

The tissue concentrations of [^{125}I]AIBG and [^{125}I]HIBG in dogs at three time intervals postinjection are given in Table 1. Data for [^{125}I]MIBG are included for comparison. Concentrations of radioactivity in the dog heart, liver, and blood following administration of [^{125}I]AIBG are compared at four time points in Fig. 4. Comparison of radioactivity concentration ratios of heart-to-nontarget tissues is given in Table 2 for AIBG and MIBG. The nontarget tissues presented in Table 2—blood, lung, and liver—are the tissues most likely to produce interfering radioactivity in myocardial imaging studies. Table 3 compares the tissue distribution of [^{125}I]AIBG and [^{125}I]MIBG in eight selected tissues of the monkey at 3 hr postinjection.

Based on these tissue distribution studies, the following observations can be made: (a) like MIBG, AIBG

TABLE 1
Comparisons of Tissue Distribution of [^{125}I]AIBG, [^{125}I]HIBG and [^{125}I]MIBG in 3 Dogs*†

Compound	N	Time (hr)	Adrenal medulla	Adrenal cortex	Kidney	Liver	Blood	Heart (LV)	Lung	Spleen	Thyroid
AIBG	3	2	10.4 (12.0–9.5)	0.51 (0.78–0.27)	0.18 (0.33–0.09)	0.20 (0.21–0.17)	0.03 (0.04–0.02)	0.52 (0.56–0.47)	1.27 (1.65–1.00)	0.52 (0.62–0.44)	0.85 (1.03–0.65)
	3	24	11.6 (12.7–10.4)	0.94 (2.22–0.22)	0.04 (0.05–0.03)	0.06 (0.07–0.04)	0.04 (0.05–0.02)	0.20 (0.26–0.13)	0.19 (0.25–0.14)	0.65 (0.91–0.50)	3.05 (5.54–1.40)
	6	72	14.7 (21.8–9.3)	0.39 (1.88–0.06)	0.04 (0.07–0.02)	0.06 (0.10–0.04)	0.02 (0.03–0.01)	0.06 (0.11–0.04)	0.09 (0.14–0.03)	0.42 (0.64–0.27)	9.63 (17.8–1.54)
HIBG	3	2	5.89 (8.17–4.22)	0.56 (0.90–0.21)	0.13 (0.16–0.09)	0.22 (0.27–0.16)	0.03 (0.04–0.02)	0.45 (0.50–0.43)	0.25 (0.29–0.17)	0.66 (0.85–0.54)	0.53 (0.78–0.34)
	3	24	6.36 (8.46–3.89)	0.13 (0.17–0.08)	0.03 (0.05–0.01)	0.07 (0.07–0.06)	0.02 (0.02–0.01)	0.22 (0.26–0.17)	0.03 (0.04–0.02)	0.52 (0.62–0.45)	1.67 (2.77–0.39)
	3	72	8.42 (10.9–6.30)	0.23 (1.10–0.14)	0.01 (0.02–0.01)	0.03 (0.04–0.02)	0.02 (0.03–0.02)	0.18 (0.38–0.04)	0.02 (0.04–0.01)	0.39 (0.54–0.29)	1.15 (2.02–0.33)
MIBG	2	2	6.29 (6.70–5.88)	0.39 (0.48–0.30)	0.26 (0.30–0.21)	0.17 (0.26–0.08)	0.02 (0.03–0.02)	0.50 (0.67–0.33)	0.05 (0.08–0.03)	0.29 (0.37–0.21)	0.56 (0.65–0.51)
	2	24	7.89 (8.50–7.28)	0.17 (0.27–0.07)	0.04 (0.05–0.04)	0.03 (0.03–0.02)	0.02 (0.03–0.02)	0.10 (0.11–0.08)	0.05 (0.07–0.03)	0.20 (0.23–0.18)	3.37 (4.66–2.07)
	5	72	13.0 (20.3–8.6)	0.17 (0.32–0.05)	0.03 (0.05–0.02)	0.02 (0.03–0.01)	0.01 (0.03–0.01)	0.04 (0.07–0.01)	0.04 (0.08–0.02)	0.08 (0.18–0.03)	10.0 (35.9–1.39)

* Concentrations represent the mean of two to five separate determinations performed in duplicate and are expressed in % kg dose/g; ranges are given in parentheses below mean.
† Tissue data for all major organs are available from authors on request.

TABLE 2
Concentration Ratios of [^{125}I]AIBC and [^{125}I]MIBG for Heart-to-Nontarget Tissues in Dog*

Time (hr)	Heart/Blood		Heart/Lung		Heart/Liver	
	AIBG	MIBG	AIBG	MIBG	AIBG	MIBG
0.5	17	20	0.6	2.1	1.3	1.4
2	17	22	0.4	1.1	2.6	3.0
6	20	12	1.4	1.5	4.1	3.3
24	5.0	3.9	1.1	1.9	3.5	3.9

* Heart tissue is from left ventricle. Concentration ratios were calculated from tissue distribution data from three dogs per time point for AIBG and two dogs per time point for MIBG; all tissue samples were taken in duplicate.

(and to a lesser extent HIBG) exhibits pronounced accumulation in the dog adrenal medullae (Fig. 5); (b) higher concentrations and/or longer retention of radioactivity in adrenergic-rich organs (i.e. spleen and heart) were observed in the dog and monkey with AIBG and HIBG; (c) slightly higher liver concentrations were obtained with AIBG and HIBG than with MIBG; (d) based on dog thyroid radioactivity levels, HIBG shows the greatest in vivo resistance to deiodination.

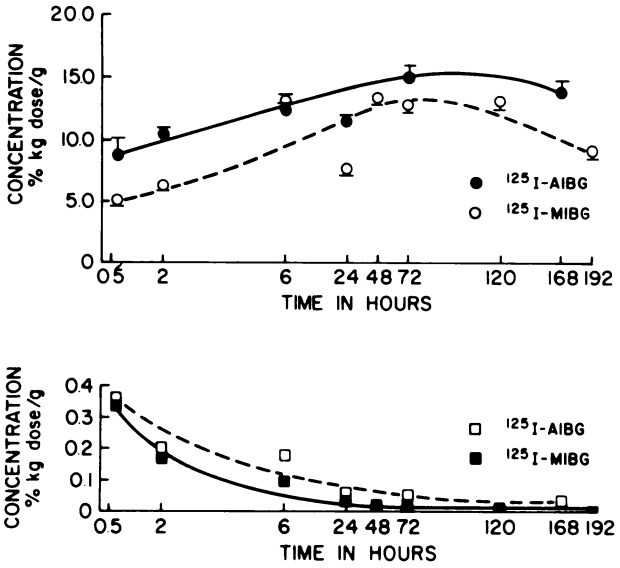

FIGURE 5
Time-activity curves for [^{125}I]AIBG and [^{125}I]MIBG in dog adrenal medulla (top) and liver (bottom)

Imaging Studies

The slightly higher liver radioactivity concentrations obtained with AIBG compared to MIBG suggested that follow-up planar imaging studies with [^{131}I]AIBG would give scintophotos of the adrenal medullae and heart inferior in quality to those reported earlier with [^{131}I]MIBG (*29,31*). Such was the case, although both dog adrenals were nonetheless visualized as shown in Fig. 6 and tomographic heart images of the dog using [^{123}I]AIBG were of satisfactory quality (Fig. 7). In subsequent imaging studies in monkeys with [^{131}I]AIBG, good heart images were obtained (Fig. 8); the left adrenal medulla was plainly visible in monkey scintiphotos but the right adrenal medulla was obscured by radioactivity from the lower right lobe of the liver (Fig. 9).

6-Hydroxydopamine (6-OHDA) Sympathectomy Study

The effects of chemical sympathectomy on radiotracer accumulation in the rat heart are summarized in Table 4. The extent of the sympathectomy in the ventricles was determined using H-3-NE, the accumulation of which was decreased ~90%. All three ^{125}I-labeled tracers showed diminished heart accumulation in sympathectomized rats. The decrease in accumulation of [^{125}I]AIBG in the ventricles, although not as large as that determined for H-3-NE, was 20% higher than both [^{125}I]HIBG and [^{125}I]MIBG. Thallium-201, a blood flow marker, showed little change in heart accumulation following sympathectomy.

TABLE 3
Tissue Concentrations of [^{125}I]AIBG and [^{125}I]MIBG in Monkeys* at 3 hr

Radiotracer	N	Tissue concentration† (% kg dose/g)							
		Adrenal medulla	Adrenal cortex	Kidney	Liver	Blood	Heart‡	Lung	Spleen
[^{125}I]AIBG	2	1.98 (2.12–1.85)	0.60 (0.64–0.56)	0.13 (0.18–0.08)	0.17 (0.22–0.13)	0.03 (0.04–0.03)	0.85 (0.96–0.74)	0.99 (1.52–0.47)	0.55 (0.76–0.34)
[^{125}I]MIBG§	3	2.69 (3.45–1.28)	0.44 (0.58–0.24)	0.15 (0.19–0.11)	0.76 (0.82–0.66)	0.02 (0.02–0.02)	0.64 (0.72–0.56)	0.17¶	0.24 (0.30–0.20)

* Five animals used in this study were killed as part of larger nonradioisotopic study conducted by other researchers. Three animals in MIBG study were rhesus monkeys (9.0 kg female, 6.7 kg female, 5.8 kg male); rhesus monkey (female, 5.2 kg) and pigtail monkey (male, 11.7 kg) were used in AIBG study.
† Mean values with ranges in parentheses. Values for each animal were determined from duplicate samples.
‡ Left ventricle.
§ Date for [^{125}I]MIBG taken in part from Ref. (*31*).
¶ Data from one animal.

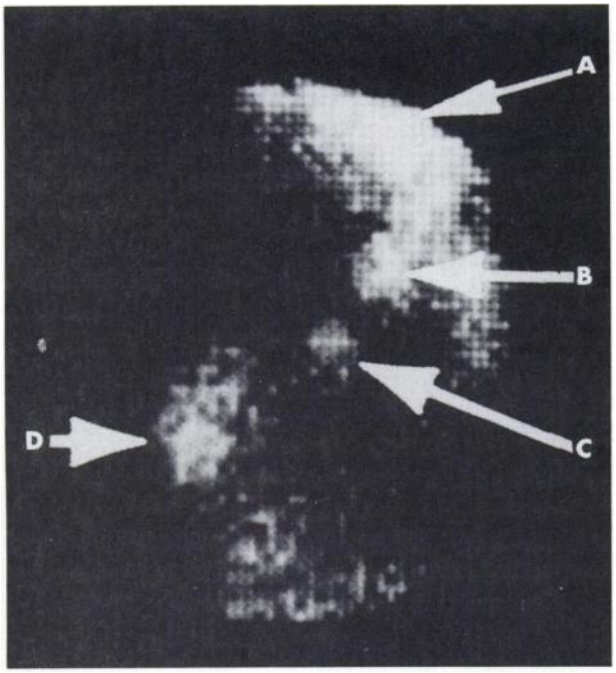

FIGURE 6
Posterior image of 18.4 kg male dog obtained with gamma camera 6 days after injection of 825 μCi of [^{131}I]AIBG. Shown is liver (A), right adrenal medulla (B), left adrenal medulla (C), and spleen (D)

DISCUSSION

From a technical standpoint, the radiosynthesis of AIBG reported here has a number of advantages over the radiosynthesis of MIBG. In our laboratory the preparation of MIBG for diagnostic use in the clinic requires 1.5–2.0 hr. The largest portion of that time is devoted to the radiochemical reaction—a solid-phase exchange requiring relatively high temperature (*16*). In contrast, the radiosynthesis of AIBG involves the rapid, electrophilic radioiodination of a noniodinated precursor (ABG) in buffered solution; the procedure requires less than 15 minutes at ambient temperature. A comparison of the two reaction sequences is presented in Fig. 2. More importantly, the radiosynthesis of AIBG and subsequent preparation for patient injection can be accomplished in kit form. This obviates the many problems associated with transporting a pre-made radiopharmaceutical. The user, with kit in hand, can order the desired radionuclide (^{131}I or ^{123}I) when the need for the radiopharmaceutical arises. The use of water-insoluble IODO-GEN as oxidant in place of chloramine-T would eliminate possible problems associated with the presence of *p*-toluenesulfonamide, the reduction product of chloramine-T. Also, the use of an anion exchange filter attached to a sterile syringe to eliminate free radioiodide is a procedure that could be readily performed in clinical nuclear pharmacies.

In addition, based on our preliminary studies, higher specific activities of AIBG may be more routinely achievable than has been possible with MIBG. Intravenous doses of guanethidine and structurally similar neuron blockers such as the benzylguanidine bethanidine can cause an initial release of norepinephrine from the nerve endings producing transient hypertension and tachycardia (*32*). This short-lived pressor phase is followed by sympathetic blockade of long duration resulting in hypotension and slowing of heart rate (*32*). The amount of carrier MIBG in diagnostic preparations using 0.5–1.0 mCi of [^{131}I]MIBG or 10 mCi of [^{123}I]MIBG is ~20 times lower than oral doses (i.e., 5 mg) of bethanidine generally administered for treatment of hypertension. Although the absorption of orally administered bethanidine is nearly complete (*33*), its initial pressor effect is likely less pronounced by this route than by the i.v. bolus route. In otherwords, MIBG carrier levels in diagnostic preparations are closer to pharmacological levels than might be predicted from prescribed oral doses of structurally similar neuron blockers. The amount of carrier MIBG in therapy doses administered to patients with malignant pheochromocytoma represents pharmacologic levels. Presently a 200 mCi therapy dose of [^{131}I]MIBG contains approximately 5 mg of carrier MIBG thus necessitating an intravenous infusion of the radiopharmaceutical over 90 min to avoid pressor effects of the carrier (*12*). However, the actual need for AIBG of high effective specific activity must await an assessment of the pharmacological potency of ABG, the major component of the AIBG radiopharmaceutical preparation.

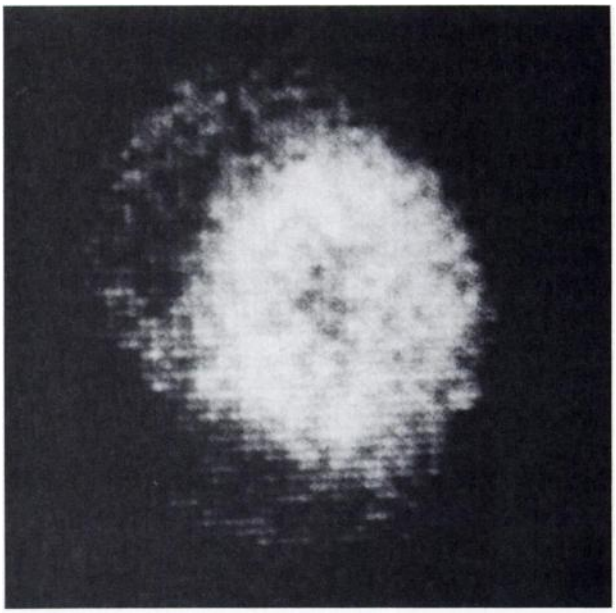

FIGURE 7
Tomographic image of upper chest of 27.7 kg dog obtained 24 hr after injection of 6.5 mCi of [^{123}I]AIBG. Image is mid-heart slice perpendicular to long axis of heart

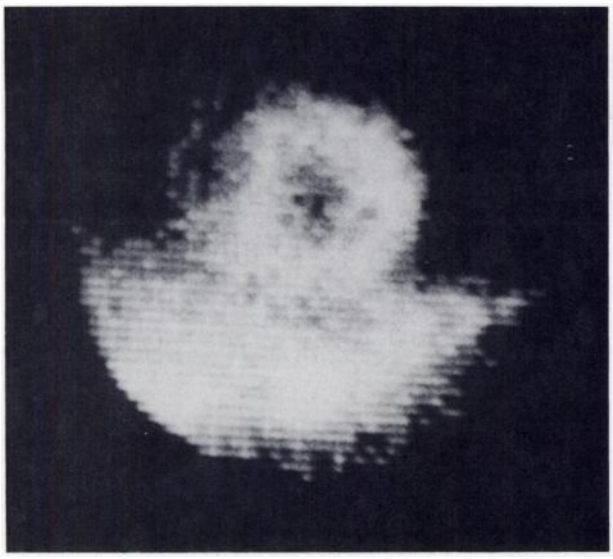

FIGURE 8
Gamma camera images of upper chest of 10.0 kg rhesus monkey 6 hr after injection of 5.4 mCi of [^{123}I]AIBG. 5° LAO pinhole image shows both heart (left ventricle wall and faint trace of right ventricle wall) and liver. Similar image was also obtained at 24 hr postinjection

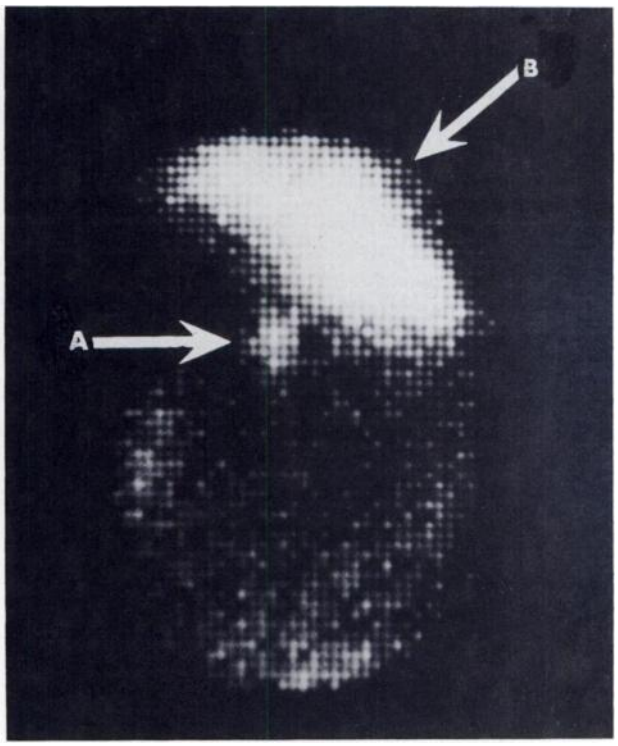

FIGURE 9
Posterior image of 10.2 kg male rhesus monkey obtained with gamma camera 8 days after injection of 900 μCi of [^{131}I]AIBG. Shown is liver (A) and left adrenal medulla (B). Right adrenal medulla is obscured by radioactivity in liver

The advantages discussed above will hold little importance if AIBG fails to provide images of the heart or tumors of neural crest origin of a quality comparable to those obtained with MIBG in humans. Radiotracer accumulation in the dog adrenal medulla and the monkey adrenal medulla have served as a suitable model for predicting successful imaging in man. The fact that AIBG shows an affinity for the dog and monkey adrenal medullae similar to that of MIBG strongly suggests that radioiodinated AIBG could be used to image human neoplasms such as pheochromocytoma and neuroblastoma. However, concentrations of radioactivity in the liver of the dog following AIBG administration are generally 2–3 times higher than those observed with MIBG. This is not a trivial difference since clear delineation of the right adrenal medulla of the dog is slightly compromised by liver activity as shown in Fig. 6. Although AIBG 3 hr postinjection shows lower radioactivity concentrations in the liver of the monkey than observed with MIBG (Table 3), this pattern is reversed at longer time points when adrenal imaging is performed. As shown in Fig. 9, liver activity completely obscures the right adrenal region of the monkey. If high liver concentrations are also observed in humans with AIBG, it may adversely affect the successful detection of tumors located in the peritoneal cavity; only comparative studies of the two agents in the same patients will provide definitive answers as to the value of AIBG. Similar concerns are true regarding the utility of AIBG in imaging the heart. Although superior scintigrams of the heart were obtained with MIBG in the dog, myocardial images obtained in the monkey were of comparable quality using the two agents. The use of tomographic imaging techniques will likely enhance the utility of AIBG in human heart studies.

The high lung activities associated with AIBG in both dogs and monkey (Tables 2 and 3) are also of possible concern regarding followup myocardial imaging studies in man. The lung accumulation of AIBG may be related to the known ability of the lungs to selectively remove vasoactive amines from the blood (*34,35*). The 4-amino group of AIBG is seemingly

TABLE 4
Effect of 6-Hydroxydopamine (6-OHDA) Sympathectomy on Radiotracer Concentration in Rat Heart

Tracer	N	% Decrease in radioactivity in heart*		
		L. Vent. (LV)	R. Vent. (RV)	LV+RV
^{3}H-(−)NE	15	89	88	88
[^{125}I]AIBG	11	73	72	72
[^{125}I]HIBG	9	59	49	54
[^{125}I]MIBG	5	61	48	54
^{201}Tl+	6	7	7	7

* 100 mg/kg (i.p.) of 6-OHDA were given 5 days prior to tracer injection (i.v.). Rats were killed 1.5 hr after tracer injection. Control rats received equal volume i.p. injections of 0.9% saline solution instead of 6-OHDA. Tissue concentrations are expressed in % Kg dose/g.

linked to the lung sequestration process since neither MIBG nor HIBG show similarly high lung levels. Radioactivity in the spleen, an exceptionally large organ in the dog, also degraded the adrenal images.

General use of the amino group to activate the aromatic ring of a drug towards electrophilic radioiodination may have merit. Most often the hydroxy group is utilized for this purpose (*36*). Based on thyroid radioactivity levels observed in this study, the in vivo resistance of AIBG to radiodeiodination is comparable to MIBG. Especially noteworthy is HIBG, which unlike many *ortho*-radioiodinated phenols (*37,38*), shows exceptional in vivo stability. This behavior may be related in part to the sequestration of HIBG in sympathetic neurons remote from tissue dehalogenases. The resistance of HIBG to in vivo radiodeiodination and its amenability to kit production make it a strong candidate for clinical evaluation as a pheochromocytoma and neuroblastoma localizing agent.

From a physiologic standpoint, a key finding in this study is that AIBG shows higher neuronal selectivity in the left ventricle of the heart than MIBG. The value of the 6-OHDA rat model in predicting neuronal selectivity of radiotracers in the human heart is not yet known. Nonetheless, the indication that hydrophilic substituents on the aromatic ring of MIBG may enhance heart neuronal selectivity has encouraged the evaluation of radiolabeled 3,4-dihydroxybenzylguanidine and other polar aralkylguanidines as highly selective neuronal mapping agents for the myocardium.

CONCLUSION

If ^{123}I-labeled radiopharmaceuticals are to become routine diagnostic tools in clinical nuclear medicine, the availability of Na^{123}I must be increased and kit methods for their on-site synthesis must be developed. Unlike most ^{99m}Tc-labeled agents, ^{123}I-labeled tracers can be used to study specific biochemical-based properties such as receptor density, enzyme concentrations and metabolic processes. The structural studies reported here have led to the kit synthesis of radioiodinated AIBG for possible localization of catecholamine tumors and assessment of neuronal defects in the myocardium (*39*). Based on comparative studies (*18,19*) with MIBG and norepinephrine, AIBG most likely enters neuronal tissue by the amine pump and accumulates in the catecholamine storage vesicles. Clinical evaluation of [^{123}I]AIBG, and possibly [^{123}I]HIBG, are warranted in light of their adaptability to kit synthesis and their high affinity for neuronal-rich tissues.

FOOTNOTES

† Spang Microanalytical Laboratory, Eagle Harbor, MI.

‡ Aldrich Chemical Co., Milwaukee, WI.

§ BioRad Laboratories, Richmond, CA.

¶ Pierce Chemical Co., Rockford, IL.

** Sigma Chemical Co., St. Louis, MO.

†† DuPont NEN Medical Products, No. Billerica, MA (NEZ-033H).

‡‡ Varian EM360A Spectrometer.

§§ Perkin-Elmer 283B Spectrophotometer.

¶¶ Waters Model 272 chromatograph equipped with A Radiomatic Flo-one radioactive flow detector (200-μl solid scintillator cell) and an ultraviolet detector (254 nm) in tandem.

*** Millipore Corp., Bedford, MA.

††† Gelman Sciences, Inc., Ann Arbor, MI.

‡‡‡ Waters Bondapak.

ACKNOWLEDGMENTS

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