Evaluation of Metaiodobenzylguanidine Heart and Lung Extraction Fraction by First-Pass Analysis in Pigs

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Metaiodobenzylguanidine (MIBG) is a norepinephrine analog that can be used to study cardiac sympathetic innervation. Most of the kinetic data on MIBG, however, have been obtained in vitro from adrenal chromaffin cells. To elucidate MIBG cardiac kinetics in vivo, we measured the first-pass extraction fraction (EF) of MIBG in pig heart and lungs and determined the relationship between the cardiac EF and myocardial blood flow (MBF) before and after dipyridamole, cocaine and imipramine. The first-pass lung EF was 24% ± 0.80% (mean ± s.e.). The baseline cardiac EF of MIBG was 79% ± 1.6%. With dipyridamole, MBF increased significantly and the EF fell (82% ± 2.5% to 71% ± 3.5% baseline compared to 0.03 mg/kg/min dipyridamole, $p < 0.001$), indicating that the cardiac EF of MIBG is dependent on MBF. Cocaine infusion had no effect on MBF or EF. Imipramine caused a significant increase in the EF (72% ± 3.5% versus 77% ± 2.5%, baseline versus imipramine $p = 0.032$) without a change in MBF. In adrenal chromaffin cells, cocaine and imipramine decrease MIBG uptake, suggesting that adrenal chromaffin cells may be an inappropriate model for studying MIBG kinetics in cardiac sympathetic neurons.

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The sympathetic nervous system (SNS) plays a major role in the regulation of cardiac function under physiologic and pathophysiologic conditions (*1*). While the SNS seems to play an important role in cardiac function, it has been difficult to quantify the contribution of the cardiac SNS to these processes because of the difficulty in studying cardiac sympathetic innervation noninvasively in isolation of the rest of the SNS. Over the last 10–15 yr, several radiotracers have been developed which are taken up by the sympathetic neurons in a relatively specific manner and thus allow assessment of cardiac sympathetic nerve function (*2–5*).

Of the several agents that have been evaluated, radioiodinated metaiodobenzylguanidine (MIBG) has been the most widely used [for recent reviews, see references (*6,7*)]. In vitro studies have shown that MIBG is taken up and stored in adrenergic synaptosomal vesicles (*8*). Studies in man and animals indicate MIBG is a marker of cardiac sympathetic nerve function. In the rat heart, MIBG follows qualitatively the kinetics of norepinephrine (NE) in the fed and fasting state (*9*). In dogs, destruction of cardiac sympathetic nerves by phenol application (*10,11*), stellate ganglion sympathectomy (*10,11*) and myocardial infarction (*12*) cause decreased MIBG uptake in regions of sympathetic nerve destruction. Cardiac transplantation in man (in which all sympathetic nerves are destroyed) causes nearly complete loss of cardiac MIBG uptake (*13*). Patients with severe diabetic autonomic neuropathy (*14*) showed decreased MIBG cardiac uptake that paralleled sympathetic cardiac dysfunction. MIBG has been used in humans to study congestive heart failure (*13,15,16*), myocardial infarction (*17*) and hypertrophic cardiomyopathy (*18*).

In order to properly interpret the results of cardiac MIBG studies, the basic kinetics of MIBG must be understood. Detailed kinetic studies of MIBG have been performed only in bovine adrenal chromaffin cells in vitro (*7,19*). While these cells manufacture and store catecholamines, they are morphologically and functionally distinct from cardiac sympathetic neurons, and results in vitro may not reflect behavior in an intact animal. In this study, we measured the first-pass extraction fraction (EF) of MIBG in pig heart and lung and determined the effects of cocaine, dipyridamole and imipramine on the cardiac first-pass EF of MIBG.

METHODS

First-Pass Extraction Fraction of MIBG

The first-pass EF of MIBG was calculated according to the methods of Weich, Strauss and Pitt (*20*). In this method, two tracers are injected simultaneously upstream from the organ of interest: a nonextractable reference or indicator tracer (in this study, ^{99m}Tc-human serum albumin, HSA) and the test substance, [^{131}I]MIBG. Arterial (a) and venous (v) blood samples are simultaneously drawn as the bolus transits the organ. The EF is

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calculated by the formula:

$$EF = \frac{[MIBGa/HSAa - MIBGv/HSAv]}{[MIBGa/HSAa]} \times 100\%$$

$$= (1 - [MIBGv * HSAa]/[MIBGa * HSAv]) \times 100\%.$$

Tracers

Technetium-99m-HSA was used as the reference tracer and was prepared from a commercial kit (Amersham, London, England) immediately prior to injection. After labeling, thin-layer chromatography was used to ensure that greater than 95% of the activity was protein bound (average for the entire study, 98%).

Unlabeled MIBG was purchased from the University of Michigan and labeled with high-specific activity ^{131}I-sodium iodide. MIBG labeling was performed according to published methods using the ammonium sulfate decomposition, solid-phase, heat-mediated exchange method (*21*). Free iodide was removed from the reaction product using anion exchange chromatography. At the time of injection, radiochemical purity averaged 97.6% ± 3.2% (mean ± s.e.). The remainder of the activity was free iodide. Specific activity was in the range of 4.2–31.8 GBq/mmol (0.35–2.65 mCi/mg) at the time of injection.

In order to determine the effects of myocardial blood flow on the EF under basal and test conditions, measurement of myocardial blood flow using radiolabeled microspheres was performed immediately prior to each EF determination. Since up to four EF measurements were performed in each pig, four sets of microspheres were used. Each set was labeled with a radionuclide that had distinct energies that could be differentiated on a multichannel analyzer: ^{103}Ru, ^{141}Ce, ^{51}Cr, ^{95}Nb (Dupont Pharma, N. Billerica, MA).

Animal Preparation

All experiments were reviewed and approved by the animal care facilities at the Portland Veterans Affairs Medical Center and the Oregon Health Sciences University. Domestic pigs weighing 35–45 kg were used. Animals were preanesthesized with xylazine (2 mg/kg) and ketamine (10 mg/kg) intramuscularly. An ear vein was then cannulated, and an anesthetic loading dose of ketamine (5 mg/kg) and morphine (2 mg/kg) was given as a bolus. Pigs were then intubated and ventilated on a Harvard volume-controlled ventilator (tidal volume 12 ml/kg, 14 cycles/min). Adequacy of ventilation was checked at periodic intervals by arterial blood gas measurements and ventilator settings were adjusted to maintain the pH 7.35–7.45, pCO_2 35–45 mmHg and pO_2 > 120 mmHg. After documentation of the adequacy of anesthesia by the absence of a corneal reflex, 3 mg of d-tubocurarine were given intravenously to prevent involuntary muscle contraction during thoracotomy. Maintenance anesthesia consisted of ketamine (10 mg/kg/min) and morphine (1 mg/kg/min). A median sternotomy was then performed and the heart suspended in a pericardial cradle. Catheters were placed into the left atrium through the left atrial appendage for injection of tracers for heart studies (cardiac EF and myocardial blood flow) or for the collection of blood for the lung EF measurements. A catheter was placed in the great cardiac vein (the coronary sinus was not used because the azygos vein enters the coronary sinus in the pig) to collect cardiac venous outflow. Another catheter (internal diameter 0.5 mm, external diameter 1.0 mm) was inserted into the proximal left anterior descending coronary artery. Separate catheters were introduced by cutdown into the left femoral artery and vein: the former for monitoring blood pressure and withdrawing blood samples for blood flow measurements, the latter for infusing pharmacologic agents, tracers for measuring pulmonary EF and volume as needed to maintain blood pressure. Another catheter was placed in the pulmonary artery for collection of blood to measure pulmonary EF. Heart rate, blood pressure and ventilation were continuously recorded on a strip chart.

Measurements of MIBG Extraction Fraction

To minimize the contribution of the downscatter of ^{131}I photons into the ^{99m}Tc window during blood sample counting, ^{99m}Tc-HSA and [^{131}I]MIBG were injected in a 10:1 ratio. In the nine pigs in which pulmonary EF was measured, 50 μCi of [^{131}I]MIBG and 500 μCi of ^{99m}Tc-HSA were first injected through the lungs before cardiac studies were performed. For the cardiac studies, blood flow measurements were performed immediately before the HSA/MIBG injection.

To measure EF, HSA/MIBG was injected into the left atrium (for the cardiac EF) or right atrium (lung EF) 10 sec after the start of blood withdrawal. Blood from the arterial and venous catheters was collected into separate pre-weighed tubes at 5-sec intervals for 50 sec. Blood was withdrawn at a rate of 30 ml/min. At the end of the experiment, tubes were reweighed and the net sample weight calculated.

Tubes were counted in a gamma counter on the same day as the experiment. Energy windows (100 keV) were centered about the ^{99m}Tc and ^{131}I photopeaks (140 keV and 364 keV, respectively) and counted for 10 min. Downscatter from ^{131}I was subtracted from the ^{99m}Tc counts. In experiments where multiple injections of HSA/MIBG were performed, background counts measured in the first two tubes (before tracer injection) were subtracted from counts in the eight following tubes. Counts were divided by sample weight and results expressed as cpm/g.

The arterial sample and the venous sample with the highest counts were used to calculate the peak instantaneous EF by the above formula. The instantaneous EF was also calculated for any sample whose arterial counts were within one-third the value of the peak arterial counts. For any given injection, one to three samples were used. The instantaneous EFs were averaged to give a mean EF.

Measurements of Myocardial Blood Flow

Myocardial blood flow was measured by standard technique (*22*). Twenty-five microcuries of a different set of microspheres were used before each cardiac EF measurement in a given pig. The tracer was injected in a bolus fashion into the left atrium and blood was collected continuously for 2 min from an arterial catheter in the distal aorta during the entire passage of the bolus.

At the end of the experiment, the LAD catheter was injected with methylene blue to outline the myocardium supplied by the LAD. After the animal was killed, an approximate 10-g sample of myocardium was removed from the center of the LAD territory for tissue counting. Since the half-lives for ^{103}Ru, ^{141}Ce, ^{51}Cr and ^{95}Nb are all greater than 27 days (compared to half-lives of 8 days and 6 hr for ^{131}I and ^{99m}Tc, respectively), tissue and blood samples were counted 40 days after injection at which time ^{131}I activity was negligible (3% of initial activity in the sample) and ^{99m}Tc had decayed to background. For the tissue sample, the entire spectrum of the sample was obtained using a multichannel analyzer and stored on a computer. The spectrum from a pure sample of each of the nuclides used in this experiment had been previously

recorded. The activity of each nuclide from the tissue sample was calculated from the composite spectrum and the individual spectra of each nuclide. This methods corrects for downscatter from the other radionuclides. Blood flow was expressed as cc/min/g.

Measurements of MIBG Extraction Fraction After Pharmacologic Intervention

After baseline measurement of the myocardial blood flow, 50 μCi of [^{131}I]MIBG and 500 μCi of ^{99m}Tc-HSA were used to measure the baseline cardiac EF in 27 pigs. To determine if the cardiac EF is dependent on myocardial blood flow, dipyridamole, a potent coronary vasodilator, was given in increasing doses and the cardiac EF repeated in 12 pigs. After baseline measurements, dipyridamole was infused at 0.03 mg/kg/min for 10 min and measurements repeated. Measurements were repeated in a similar fashion after infusion rates of 0.06 mg/kg/min and 0.14 mg/kg/min for 10 min each. Total activity of ^{131}I plus ^{99m}Tc for each EF measurement was given in a ratio of 1:1:2:4 for the four sets of cardiac injections.

Similar studies were performed with cocaine and imipramine, both of which block the catecholamine uptake pathway of sympathetic neurons (*23,24*), to see if the initial myocardial uptake of MIBG follows NE kinetics. In six pigs, imipramine was infused at a constant rate in doses ranging from 0.01 to 0.07 mg/kg/min for individual pigs for 30 min. Doses were increased in successive experiments to determine if there was a dose response relationship between the EF and imipramine dose. In five pigs, imipramine blood levels were measured at 15 and 30 min after the start of the infusion. In nine pigs, cocaine was given at a fixed dose of 0.25 mg/kg/min over 30 min. Cocaine blood levels were measured at 15 and 30 min after the start of the infusion. In both experiments, EF and blood flow were determined before and at 15 and 30 min after the start of the intravenous infusion. Total activity of ^{99m}Tc plus ^{131}I was given in a ratio of 1:1:2 for the three sets of injections.

Data Analysis

Data analysis was performed by a commercial statistical software program (SPSS/PC+, version 1.0, SPSS Inc., Chicago, IL). The mean lung EF and standard error were calculated for the nine pigs that had this measurement performed. The baseline cardiac EFs and baseline myocardial blood flows for all 27 pigs in the study were subjected to linear regression analysis. The correlation coefficient was calculated and significance accepted at the $p < 0.05$ level.

Due to the different nature of the drug intervention studies, data were analyzed differently for the various groups. In the imipramine experiments, complete data sets were obtained for all pigs. The EFs, blood flow, blood pressure and heart rate measurements were analyzed by repeated analysis of variance (ANOVA) measurements. Significance was accepted at the $p < 0.05$ level. When the overall repeated ANOVA measurements for a set of measurements was significant, Student-Newman-Keuls post hoc comparison tests were performed to test for significance between the different groups (*25*). Significance was accepted at the $p < 0.05$ level. In order to ascertain relationships between dose or blood levels of imipramine and EF or blood flow, linear regression analysis was performed at the 15-min time point. To validate this finding, this analysis was repeated at the 30-min time point. Significance was accepted at the $p < 0.05$ level.

For the dipyridamole and cocaine experiments, data sets were incomplete because some of the pigs died before the end of the experiment, or in some cases data were not obtained. In the cocaine experiments, three of nine pigs died of ventricular fibrillation before the 30-min measurements could be obtained. In the dipyridamole experiments, 6 of 12 pigs died from refractory hypotension or cardiac arrhythmias before measurements could be obtained at the highest level of dipyridamole infusion (0.14 mg/kg/min). In other pigs, measurements at the 0.03 (three pigs), 0.06 (one pig) and 0.14 (two pigs) mg/kg/min infusion rates were not obtained due to problems with blood sampling. In these experiments, repeated ANOVA measurements were inappropriate due to the quantity of missing data. For both experiments, the paired t-test adjusted for multiple comparisons (Bonferroni's method) was used (*26*). In the dipyridamole experiment, the EF, blood flow, blood pressure and heart rate were compared between the baseline and each level of drug infusion (six comparisons). Using Bonferroni's correction, significance was accepted at the $p < 0.05/6 = 0.0083$ level. In the cocaine experiments, similar comparisons were made between baseline, 15-min, and 30-min measurements (three comparisons). Significance was accepted at the $p < 0.05/3 = 0.017$ level. Linear correlation was performed between the EFs and blood flow versus cocaine blood levels at 15 and 30 min. Significance was accepted at the $p < 0.05$ level.

RESULTS

The lung EF for nine pigs was 24% ± 0.80% (mean ± s.e.). Figure 1 shows the relationship between the baseline cardiac EFs and baseline myocardial blood flow for the 27 pigs used in this study. The mean ± s.e. baseline myocardial blood flow was 0.658 ± 0.043 cc/min/g with a range from 0.347 to 1.190 cc/min/g. The EF was 79% ± 1.6% with a range from 61% to 94%. The r value for the regression line was −0.368, which had a borderline value for significance, $p = 0.059$.

Table 1 lists the hemodynamic data for all the experiments. For the dipyridamole experiment, when a paired t-test analysis using the Bonferroni correction was performed between the different doses of dipyridamole, a significant ($p < 0.001$) fall in systolic blood pressure was

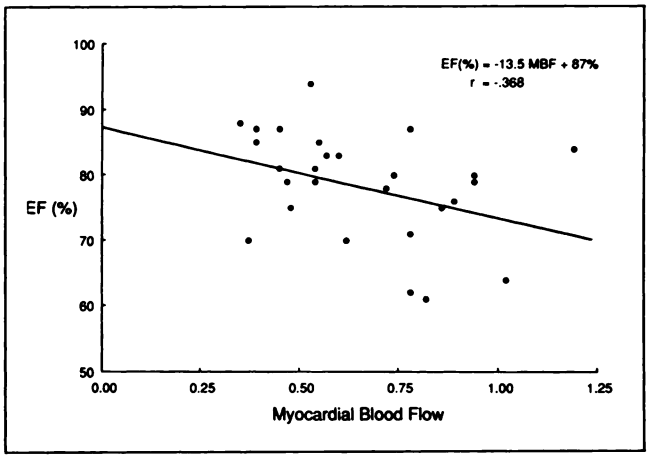

FIGURE 1. Relationship between the baseline cardiac extraction fraction (EF) and myocardial blood flow (MBF) in 27 pigs. r = the correlation coefficient, p = 0.059.

TABLE 1
Hemodynamic Variables (mean ± s.e.)

Dipyridamole mg/kg/min	Blood Pressure (mmHg)		Heart rate bpm
	Systolic	Diastolic	
0 (12)	92 ± 5.7	50 ± 3.1	85 ± 4.9
0.03 (9)	77 ± 8.0*	43 ± 4.5	84 ± 5.0
0.06 (11)	59 ± 7.1*†	33 ± 3.3*†	91 ± 5.0
0.14 (4)	42 ± 5.3*	28 ± 3.0	95 ± 17
Cocaine, Minutes			
0 (9)	82 ± 4.2	43 ± 1.9	95 ± 7.0
15 (9)	69 ± 5.0	40 ± 3.0	108 ± 6.1
30 (6)	68 ± 7.0	38 ± 2.6	108 ± 9.0
Imipramine, Minutes			
0 (6)	70 ± 5.3§	36 ± 2.6	93 ± 3.9¶
15 (6)	66 ± 4.7	34 ± 8.3	95 ± 5.4††
30 (6)	62 ± 5.3**	36 ± 2.3	101 ± 4.8**

Number in parentheses = number of animals.
* Significantly different from baseline.
† Significantly different from 0.03 mg/kg/min.
§ Repeated measures ANOVA significant at p = 0.038.
¶ Repeated measures ANOVA significant at p = 0.036.
** Significantly different from baseline, p < 0.05.
†† Significantly different from 30-min heart rate, p < 0.05.

found between the baseline pressure, 97 ± 6.8 mmHg, and the pressure at 0.03 mg/kg/min, 77 ± 80 mmHg (values are different from those in Table 1 since analysis was performed on only those values for which there were paired extraction fraction and blood flow measurements. Table 2 lists the number of animals for which paired values were available). Values between baseline and the 0.06 and 0.14 doses were also significantly different (p < 0.001 and p = 0.002, respectively). Similarly, a significant (p = 0.002) fall in systolic pressure was seen between the 0.03 mg/kg/min dose (76 ± 9.0 mmHg) and 0.06 mg/kg/min dose (63 ± 9.1 mmHg). No significant change in the systolic blood pressure was found between the 0.14 dose level and the 0.03 and 0.06 dose levels. For diastolic pressure, significant differences were found between baseline and the 0.06 dose (p < 0.001) and between the 0.03 dose and the 0.06 dose (p = 0.002). No other significant differences were seen in the diastolic pressures. For heart rate, no significant change was seen at any drug level.

Table 2 shows the EFs and myocardial blood flow for the dipyridamole experiments. There was a significant (p < 0.001) fall in the baseline EF from 82% ± 2.5% to 71% ± 3.5% at the 0.03 dose. Blood flow increased significantly (p = 0.0080) from 0.654 ± 0.098 at baseline to 0.957 ± 0.158 cc/min/g at the 0.03 mg/kg/min dose. The only other significant differences were between the baseline EF and the 0.06 dose EF (82% ± 2.0% versus 68% ± 2.7%, p < 0.001) and the myocardial blood flow between baseline and the 0.06 dose (0.587 ± 0.086 versus 1.091 ± 0.125, p < 0.001).

In the cocaine experiments, there were no significant differences between the baseline, 15-min, or 30-min systolic or diastolic blood pressures. There was, however, a significant increase in heart rate between baseline and 15 min (95 ± 7.0 bpm versus 108 ± 6.1 bpm, p = 0.007) but not between baseline and 30 min (97 ± 11 versus 108 ± 9.0 bpm, p = 0.217).

The EFs and myocardial blood flow for the cocaine experiments are given in Table 3. There was no significant change in the EFs or blood flow between any of the time points. The EF did not fall with cocaine infusion and actually appeared to increase at 15 (p = 0.032) and 30 (p = 0.037) min over baseline, but changes were not significant when adjusted for multiple comparisons. Cocaine serum levels ranged from 1.20 to 4.45 μg/ml (4.0–14.7 μmol; greater than 0.1 μg/ml is considered toxic in humans). No correlation was found between serum levels and blood flow or between serum levels and the EF.

For the imipramine experiments (Table 4), there was a significant (p = 0.038) fall in the systolic blood pressure with drug infusion. To determine if there was a significant fall in systolic blood pressure between different time points, Student-Newman-Keuls post hoc comparisons were performed between all pairs of time points. A significant fall in systolic blood pressure was seen between the baseline and 30-min value (p < 0.05). No change in diastolic pressure was seen. The pulse rose significantly (p = 0.036) after drug infusion. Comparison of the different

TABLE 2
Extraction Fractions and Myocardial Blood Flow: Baseline and Dipyridamole Infusion

Comparison* (no. of studies)	Extraction Fraction (mean ± s.e.)	p Value†	Blood flow (cc/min/g) (mean ± s.e.)	p Value†
Baseline vs. 0.03 (9)	82% ± 2.5% vs. 71% ± 3.5%	<0.001§	0.654 ± 0.098 vs. 0.957 ± 0.158	0.0080§
Baseline vs. 0.06 (11)	82% ± 2.0% vs. 68% ± 2.7%	<0.001§	0.587 ± 0.086 vs. 1.091 ± 0.125	<0.001§
Baseline vs. 0.14 (4)	86% ± 2.9% vs. 68% ± 6.2%	0.012	0.507 ± 0.040 vs. 1.029 ± 0.185	0.051
0.03 vs. 0.06 (8)	72% ± 3.8% vs. 70% ± 2.7%	0.676	0.901 ± 0.180 vs. 1.097 ± 0.176	0.065
0.03 vs. 0.14 (2)	78% ± 7.5% vs. 77% ± 7.4%	0.031	0.475 ± 0.058 vs. 0.742 ± 0.080	0.036
0.06 vs. 0.14 (4)	69% ± 7.0% vs. 68% ± 6.2%	0.784	0.924 ± 0.170 vs. 1.029 ± 0.185	0.131

* Dipyridamole doses are in mg/kg/min.
† Significance accepted at p < 0.0083 after correction for multiple comparisons.
§ Significant difference between groups.

TABLE 3
Extraction Fractions and Myocardial Blood Flow: Baseline and Cocaine Infusion

Comparison* (no. of studies)	Extraction Fraction (mean ± s.e.)	p Value*	Blood flow (cc/min/g) (mean ± s.e.)	p Value*
Baseline vs. 15 min (9)	78% ± 8% vs. 82% ± 1.8%	0.032	0.675 ± 0.070 vs. 0.691 ± 0.087	0.753
Baseline vs. 30 min (6)	77% ± 3.4% vs. 82% ± 2.1%	0.037	0.770 ± .073 vs. 0.753 ± 0.166	0.909
15 vs. 30 min (6)	80% ± 2.2% vs. 82% ± 2.1%	0.185	0.768 ± .117 vs. 0.753 ± 0.166	0.943

* Significance accepted at $p < 0.017$.

time points showed that there was a significant ($p < 0.05$) rise from the baseline (93 ± 3.9) and 15-min (95 ± 5.4) heart rates compared to the 30-min heart rate (101 ± 4.8). The EF rose significantly ($p = 0.017$) after drug infusion. Comparison of individual time points showed that both the 15- and 30-min values were significantly greater than baseline ($p < 0.05$). No change in myocardial blood flow was seen. To determine if the dose level of imipramine had any affect on blood flow or the extraction fraction, linear correlation was performed between the imipramine dose and EF and between the dose and myocardial blood flow at 15 and 30 min. No correlation was found between the dose of imipramine infused and EF at 15 and 30 min ($p = 0.515$ and 0.293, respectively) or between the dose of imipramine and blood flow at 15 and 30 min ($p = 0.199$ and 0.421, respectively). Imipramine serum levels ranged from 87 to 1512 ng/ml (0.27 to 5.0 μmol; therapeutic levels in humans: 120–500 ng/ml; 0.38–1.58 μmol). No correlation was found between imipramine serum levels and EF or blood flow.

DISCUSSION

MIBG is the most widely used tracer to study the sympathetic innervation of the heart noninvasively. MIBG has been useful in evaluating cardiac sympathetic nerve function in congestive heart failure (*13,15,16*), myocardial infarction (*12,17*), autonomic dysfunction (*14*) and surgical denervation (*10,11,13*). The underlying assumption in all studies employing MIBG is that activity of the tracer within the heart is a direct measure of sympathetic nerve number, function, and integrity.

TABLE 4
Extraction Fractions and Blood Flow: Baseline and Imipramine Infusion (mean ± s.e.)

Time (min)	EF*	p Value	Blood flow§ (cc/min/g)
0	72% ± 3.5%		0.708 ± 0.078
15	77% ± 2.5%	<0.05†	0.693 ± 0.146
30	77% ± 3.1%	<0.05†	0.623 ± 0.055

* Repeated measures ANOVA significant at $p = 0.017$.
† Significant for post hoc comparison to baseline by Student-Newman-Keuls test.
§ Not significant by ANOVA, $p = 0.522$.

The only datum, however, that can be obtained from a scintigraphic image at a given time after injection is the number of counts from the heart. After correction for physical decay, this depends upon the total uptake of tracer minus the total washout since injection. By obtaining serial images, some kinetic parameters can be inferred. Since MIBG clears from the blood rapidly after bolus injection (*2*), most of the uptake by the heart occurs within the first few minutes after injection. Thus, activity at any later time point will depend strongly upon initial uptake.

Total uptake (U) of a tracer by an organ over a given time period (assuming constant blood flow and ignoring physical decay) is given by the equation:

$$U(\mu Ci) = EF \times BF(ml/min) \times A(\mu Ci \times min/ml),$$

where A is the integrated arterial blood concentration of tracer over the given time period and BF is blood flow.

If the EF is constant over a range of blood flows [as is the case with thallium (*20*)], then uptake will be proportional to blood flow. In our study, we calculated the effect of blood flow on EF. When we compared the baseline blood flow to EF, a borderline correlation ($r = -0.368$, $p = 0.059$) was found. Although the results suggest EF is not related to blood flow, given the borderline value of the correlation, it is difficult to draw a strong conclusion regarding the effect of blood flow on EF. One way of analyzing the results is to consider the r^2 value, which relates the degree to which variation in EF is due to variation in blood flow. In this case, $r^2 = 0.135$, suggesting that over the range of resting blood flows (0.347 to 1.190 cc/min/g), only 13.5% of the variability in EF can be attributed to changes in blood flow. Thus, it seems that EF is largely independent of blood flow over this range of flows and in this range cardiac uptake of MIBG would be proportional to blood flow. When blood flow was increased with dipyridamole above the resting range, EF fell significantly. These results suggest that the EF of MIBG is relatively constant in the range of 0.35 to 1.2 cc/min/g but falls as flow increases above this range.

The fall in EF, however, was not proportional to the increase in blood flow. A 46% increase in blood flow (from 0.654 to 0.957 cc/min/g) from baseline to the 0.03 mg/kg/min dose of dipyridamole caused an 11% decrease in EF (from 82% to 71%). At the 0.06 mg/kg/min dose, blood flow increased 85% over baseline, but EF fell only

14% (82% to 68%). Although an even larger fall in EF (18%) occurred at the highest level of dipyridamole infusion (0.14 mg/kg/min), too few measurements were made at this level to reach statistical significance. It is unlikely that the fall in blood pressure caused the fall in the EF, since blood pressure fell with infusion of imipramine, but the EF actually increased.

Since total uptake rather than EF is measured scintigraphically (at least soon after injection before appreciable washout occurs), it would be interesting to measure the effect of dipyridamole on uptake. Although uptake was not measured in this experiment, one can calculate a normalized (for blood tracer concentration) "instantaneous uptake" for each time point in the study (EF × blood flow). As Table 5 shows, for a given dose of dipyridamole, the uptake is higher than at any lower dose. Although only the baseline versus the 0.06 dose is significant, a small increase in uptake operating over several minutes could give a large increase in total uptake. The results suggest that the net effect of a decreased EF and increased tracer delivery from increasing blood flow is a net increase in tracer uptake with increasing blood flow.

The results of the baseline studies suggest that the EF of MIBG is relatively independent of blood flow at low flow rates (less than 1.2 cc/min/g), but decreases as flow increases above this level. Since EF decreases more slowly than the increase in blood flow, the net effect would be to increase MIBG uptake as blood flow increases. The practical effect of these studies is that blood flow must be taken into account when interpreting MIBG uptake by the heart, especially when blood flow is changing (as with exercise) or when there is differential blood flow to different parts of the heart (as in stenotic coronary artery disease).

In order to determine if MIBG follows NE kinetics, the MIBG EF was measured after treatment with cocaine and imipramine, agents that block the reuptake of NE by sympathetic neurons (*23,24*). Cocaine and desmethylimipramine have been shown to be specific blockers of MIBG and NE uptake in vitro in bovine adrenal cells and in vivo in dog adrenal medullae as reported by Tobes et al. (*19*). In our study, cocaine was given in a total dose of 7.5 mg/kg over 30 min which approximated the dose (5 mg/kg) that caused an 81% reduction in MIBG uptake and an 86% reduction in ^{3}H NE uptake by the dog adrenal medullae (*19*). Our results show that cocaine had no effect on the cardiac EF of MIBG or possibly increased it. Cocaine infusion had no effect on myocardial blood flow. While these results seem surprising in view of the known effects of cocaine on NE uptake, they are similar to results obtained in the previously quoted study (*19*) in which cocaine caused a 63% increase in cardiac uptake of MIBG while causing a 42% fall in cardiac ^{3}H NE uptake. As Table 5 shows, the instantaneous uptake of MIBG was greater during cocaine infusion. If these instantaneous uptake results are representative of the entire period when there are significant concentrations of MIBG in the blood, our results would support the finding of Tobes et al. that cocaine increases the cardiac uptake of MIBG.

The results of imipramine infusion gave similar results, except that EFs were significantly elevated above baseline after 15 and 30 min of infusion. In the first two pigs given imipramine, infusions of 0.01 mg/kg/min (total dose 0.30 mg/kg) caused an increase in EF after 15 and 30 min of imipramine infusion compared to baseline. Blood levels of imipramine were 87 ng/ml (0.27 μmol) and 135 ng/ml (0.42 μmol) in the two pigs, which is below or at the lower therapeutic limits in humans (120–500 ng/ml, 0.38–1.58 μmol). Imipramine doses were increased stepwise in the next four pigs: 0.033, 0.05, 0.06, and 0.07 mg/kg/min, until clearly toxic blood levels were attained (1512 ng/ml, 5.0 μmol) to see if a fall in EF would occur. In every instance, 15- and 30-min EFs were the same or higher than the baseline value. As with the cocaine experiments, no change in myocardial blood flow was seen. There was no correlation at 15 and 30 min between EF and infusion rate or EF and the blood levels of imipramine. Table 5 shows the instantaneous uptake values. There is a greater cardiac uptake of MIBG at 15 min than at baseline, but not at 30 min because myocardial blood flow fell to a greater extent than the EF increased. Since imipramine probably does not cause a fall in cardiac blood flow in intact, unanesthetized animals, the net effect of an increased EF would be an increased uptake. In the study by Tobes et al. (*19*), desmethylimipramine (10 mg/kg) caused a 97% reduction in ^{3}H NE uptake and a 90% reduction in MIBG uptake by the adrenal medullae in dogs, while heart uptake of ^{3}H NE fell 41% and MIBG uptake rose 55%. Other studies give dissimilar results. In rats given 10 mg/kg desmethylimipramine 4 hr before they were killed, cardiac activity of ^{3}H NE was reduced by 94%, while MIBG activity was reduced 50% compared to con-

TABLE 5
Instantaneous Uptake (mean ± s.e.): Paired t-Tests

Comparison	Normalized Uptake (ml/min)	p value
Dipyridamole mg/kg/min		
0 vs. 0.03	0.535 ± 0.079 vs. 0.663 ± 0.105	0.070
0 vs. 0.06	0.488 ± 0.071 vs. 0.739 ± 0.081	<0.001*
0 vs. 0.14	0.436 ± 0.035 vs. 0.664 ± 0.072	0.044
0.03 vs. 0.06	0.645 ± 0.125 vs. 0.770 ± 0.112	0.075
0.03 vs. 0.14	0.374 ± 0.079 vs. 0.565 ± 0.010	0.278
0.06 vs. 0.14	0.603 ± 0.77 vs. 0.664 ± 0.022	0.092
Cocaine		
0 vs. 15 min	0.516 ± 0.040 vs. 0.555 ± 0.057	0.041
0 vs. 30 min	0.583 ± 0.043 vs. 0.616 ± 0.137	0.780
15 vs. 30 min	0.604 ± 0.080 vs. 0.613 ± 0.135	0.960
Imipramine†		
0 min	0.512 ± 0.600	
15 min	0.531 ± 0.041	
30 min	0.472 ± 0.35	

* Significant difference between groups.
† Repeated ANOVA measurements, p = 0.659.

trols when measured 2 hr after injection of the tracers (*9*). In four human subjects given imipramine 25 mg for 7 days, MIBG activity in the heart 2–4 hr after injection was 50% of the values obtained without imipramine (*14*). Since our study and each of the above quoted studies that measured the effect of cocaine (*19*), desmethylimipramine (*9,19*) or imipramine (*14*) on cardiac uptake of MIBG were all performed in different species, it is possible that the different results reflect species differences in the handling of MIBG by cardiac sympathetic neurons. What effects anesthetics or the open chest preparation used in our experiments had on the results is unknown. While these interventions can clearly alter sympathetic nerve activity, it is impossible to quantify these effects.

The uptake of MIBG by the heart is complex and includes both neuronal and nonneuronal components. It is difficult to measure these components separately in the intact heart, and denervation studies probably provide the most accurate measurements. In humans, cardiac transplantation which causes total denervation reduced cardiac activity of MIBG by 94% compared to controls at 1 hr after injection (*13*), indicating a small nonneuronal component. Values immediately after MIBG injection may be higher. Dae et al. (*11*) found that immediately after MIBG injection in dogs, innervated and denervated portions of the heart had similar uptake suggesting a large nonneuronal uptake immediately after injection. At 3 hr, most of the activity in the denervated portion of the heart had washed out compared to the innervated portion. If a large proportion of first-pass uptake was due to a nonneuronal mechanism, blockage of neuronal uptake might produce little change in the whole heart extraction fraction of MIBG. Our experimental design did not allow us to measure nonneuronal uptake, and we cannot rule out this mechanism for the lack of change in extraction fraction in the cocaine experiments. This, however, would not explain the increase seen in our imipramine experiments. It is possible that imipramine and cocaine increase nonneuronal MIBG uptake so that a blocking effect on sympathetic neurons is masked. There is, however, no data describing drug effects on nonneuronal MIBG uptake.

In adrenal chromaffin cells (an in vitro model for catecholamine synthesizing cells), MIBG uptake has been shown to have two components: (1) a high affinity, saturable, sodium- and energy-dependent component, and (2) a high capacity, nonsaturable, sodium- and energy-independent component (*8*). The first component (referred to as uptake-1) is felt to be due to a specific uptake system, while the second component has been attributed to diffusion (*8,19*). A specific protein in the cell membrane appears to be the high affinity MIBG transporter (*27*). The gene for this transporter has recently been cloned and sequenced and appears to be identical to the NE transporter (*28*). This transporter can be blocked by cocaine and antidepressants. At low concentrations of MIBG, most (approximately 60%) of the uptake by adrenal chromaffin cells is by the high affinity mechanism (*8*). Above a concentration of 1 μmol, most of the uptake is by the nonspecific mechanism. We calculated the MIBG concentration in the peak arterial sample in each experiment. In the baseline and first drug intervention measurements (identical MIBG doses were given for these two measurements), all MIBG concentrations were less than 1.5 μmol, and all but five were less than 1.0 μmol. Thus, approximately 50% of neuronal uptake was presumably by the specific uptake mechanism. According to the data of Tobes et al. (*19*), at the serum levels of cocaine (4–14.7 μmol) and imipramine (0.27 to 14.8 μmol) attained in our studies, the specific uptake would be inhibited by 70%–90% and 40%–100%, respectively, and nonspecific uptake would be inhibited by 20%–32% and 0%–10%, respectively [using a desmethylimipramine to imipramine potency ratio of 16:1 for blockade of the NE transporter (*28*). A similar ratio is assumed for blockade of the nonspecific uptake of imipramine]. Thus, even at the lowest drug concentration, cocaine should have produced a 45% blockade and imipramine a 20% blockade of neuronal uptake of MIBG if cardiac sympathetic neurons have the same kinetics as adrenal chromaffin cells. Whether species differences, organ differences, or drug effects on nonneuronal uptake can account for the discrepancies between our results and those obtained with adrenal chromaffin cells cannot be answered by this study.

A last possible mechanism to consider is intraneuronal handling of MIBG. NE in the cytoplasm of adrenergic cells is transported into storage vesicles by a transporter located in the vesicle membrane. This transporter, the monoamine vesicular transporter, has been characterized pharmacologically (*29,30*) but has not been cloned as of this writing. This transporter has a high affinity for MIBG (*31*), although the binding kinetics for MIBG differ from those of NE. Neither imipramine or cocaine have any known effects on this transporter, and it is unlikely that effects at the vesicle membrane could account for the results of our experiment.

Perhaps the most general conclusion that can be drawn from all the data is that the cardiac kinetics of MIBG differ substantially from the kinetics of adrenal chromaffin cells in vitro or the adrenal medulla in vivo. Adrenal chromaffin cells are the only adrenergic tissue that can be readily studied in vitro. However, the results of MIBG kinetic studies in adrenal chromaffin cells in vitro may not be applicable to cardiac sympathetic neurons. The difference in the manner in which the heart handles MIBG and NE emphasizes the point that while MIBG may be a marker of cardiac sympathetic neuronal function, it does not act as an analog of NE.

Finally, we have found that MIBG has a moderate (24%) first-pass uptake by the lungs. Similar values have been reported in rat (*32*) and sheep (*33*) lungs. The lungs are a major, though highly selective, organ for removal of circulating monoamines. In humans, serotonin (*34*), NE (*35*)

and epinephrine (*35*) have first-pass lung extraction fractions of 62%, 25% and 0%, respectively. Whether MIBG will be useful in evaluating pulmonary pathophysiology requires further study.

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