Metaiodobenzylguanidine to Map Scintigraphically the Adrenergic Nervous System in Man

J.C. Sisson, B. Shapiro, L. Meyers, S. Mallette, T.J. Mangner, D.M. Wieland, J.V. Glowniak,* P. Sherman, B.S., and W.H. Beierwaltes

Division of Nuclear Medicine, Department of Internal Medicine, The University of Michigan Medical Center, Ann Arbor, Michigan

Metaiodobenzylguanidine (MIBG) localizes in adrenergic neurons; MIBG labeled with ^{123}I then serves as an analog of norepinephrine, and concentrations of [^{123}I]MIBG reflect sites of adrenergic neurons in organs. Movements of [^{123}I]MIBG into and out of organs were measured by quantitative scintigraphy in man. We perturbed adrenergic neuron function in several ways, and [^{123}I]MIBG concentrations in the heart were subsequently altered in patterns consistent with the concept that [^{123}I]MIBG resides mostly in adrenergic neurons. Uptake of [^{123}I]MIBG into the heart was inhibited by the tricyclic drug, imipramine, and this agent also accelerated the rate of loss of [^{123}I]MIBG. Phenylpropanolamine, a sympathomimetic drug that acts by displacing norepinephrine from neurons, increased the rates of loss of [^{123}I]MIBG from the heart. Exercise was followed by a movement of [^{123}I]MIBG into blood and urine. Generalized autonomic neuropathies were associated with marked diminutions of [^{123}I]MIBG uptake into the heart. We conclude that quantitative scintigraphy in patients will enable determinations of regional disturbances in integrity (by measuring uptake of [^{123}I]MIBG) and function (by measuring rates of loss of [^{123}I]MIBG) of the adrenergic nervous system in the heart.

J Nucl Med 28:1625–1636, 1987

The integrity and function of the autonomic nervous system is disturbed in a generalized pattern by a number of diseases (*1–3*). Abnormalities in this nervous system may be regional, and the adrenergic nerves of the heart appear to be particularly vulnerable to effects of disease. Heart failure is associated with diminished adrenergic activity in the myocardium (*4,5*), infarction can disrupt regions of neurons within the heart (*6,7*), and an imbalance of innervation may predispose to arrhythmias (*8*).

However, integrity and function of the sympathetic nervous system are difficult to determine in vivo. Increased function of adrenergic neurons can be related to an increased rate of secretion of norepinephrine (NE) by the neurons that, in turn, is reflected in elevated plasma concentrations of NE (*2,3,9*). Nevertheless, the circulating levels of NE are several steps removed from the adrenergic neurons and, therefore, probably lack sensitivity as measurements of adrenergic function. Moreover, plasma NE is derived from adrenergic activity throughout the body, and a circulating concentration of this neurotransmitter cannot be an accurate index of localized or regional changes in neuron secretions.

Radiolabeled metaiodobenzylguanidine (MIBG), an analog of NE, concentrates in adrenergic neurons (*10*), and, in this position, should enable scintigraphic display of the adrenergic nervous system, and especially the neurons of the heart. In fact, Kline et al. were able to portray the heart in normal subjects after administering MIBG labeled with iodine-123 (^{123}I) (*11*).

We now give evidence that concentrations of [^{123}I]MIBG can be quantified in the heart of man. From pharmacologic perturbations of the sympathetic nervous system, and from observations of patients with autonomic neuropathies, it appears that [^{123}I]MIBG resides largely in the adrenergic neurons of the heart, and changes in [^{123}I]MIBG concentrations reflect neuron integrity and/or function.

Received Oct. 29, 1986; revision accepted May 11, 1987.

For reprints contact: James C. Sisson, MD, University of Michigan Medical Center, Division of Nuclear Medicine, 1500 Medical Center Dr., Ann Arbor, MI 48109-0028.

*Present address: Veterans Administration Medical Center, P.O. Box 1034, Portland, OR 97207.

METHODS

Both normal subjects and patients with autonomic neuropathy were studied using quantified scintigraphy after injections of [^{123}I]MIBG.

Subjects

Each subject signed a consent form approved by The Committee to Review Grants for Clinical Research and Investigation involving Human Beings of the University of Michigan Medical Center.

Normal subjects. Twenty men, 20 to 62 yr of age, were paid volunteers; they were deemed healthy after an interview and a physical examination. Seven subjects were selected to make up a group that spanned a broad age range, 22–62 yr; each gave a normal response in an exercise ECG test and had a normal echocardiogram. Thirteen of the normal subjects were selected for experiments designed to demonstrate by pharmacologic means that [^{123}I]MIBG resided in adrenergic neurons of tissues.

Patients with neuropathy. Five patients exhibited signs of generalized autonomic neuropathy. Table 1 denotes the characteristics of these patients. Each had postural hypotension without compensatory rise in heart rate, lack of heart rate variation during deep breathing, failure of diastolic blood pressure to rise with isometric exercise and abnormal heart rate response to a Valsalva maneuver (*12–14*). Pupil responses to dark adaptation were (*15*) abnormal in each of these patients except for Patient 1 whose pupils responded normally. Scintigraphic studies using radiolabeled meals were previously performed on Patients 1 and 2 and showed delayed emptying of their stomachs. In Patient 4, cystoscopy and cystomanometry had demonstrated a neurogenic bladder. Two of these patients had diabetes mellitus, one manifested a central nervous system disorder typical of the Shy-Drager Syndrome, and two had no known cause for their neuropathy.

Patient 2 gave a history of a myocardial infarction. No other patient had known heart disease or gave clinical evidence for heart failure, and none, except Patient 2, had evidence of disease on ECG tracings. Patients 1 and 2 were taking insulin but neither experienced episodes of hypoglycemia during or in the several days prior to the study. Patient 1 was taking pentazocine intermittently for painful neuropathy. Patient 2 was taking digoxin 0.25 mg daily. Patient 4 was taking metoclopramide, 10 mg bid and l-thyroxine 0.1 mg daily. Patient 5 was taking fludrocortisone acetate (Florinef Acetate) 0.1 mg bid.

Methods

In vitro techniques. [^{123}I]sodium iodide* was prepared by the ^{127}I (p, 5n) ^{123}Xe method; ^{123}I prepared in this manner contained 0.12% ± 0.25% ^{125}I and possibly a trace of ^{121}Te (0.004%), but no ^{124}I. Iodine-123 MIBG was synthesized as described before (*10,16*) giving a specific activity of 5.0 mCi/mg (59 GBq/mmol). A radioenzymatic assay† was used to measure plasma concentrations of NE (*17*).

Scintigraphic studies. To block thyroid uptake of any dissociated [^{123}I]iodide, all subjects and patients were treated with a saturated solution of potassium iodide for 5 days beginning the day before injection of [^{123}I]MIBG. Subjects were injected intravenously with 3 to 10 mCi (111 to 370 MBq) of [^{123}I]MIBG over several minutes. The first seven normal subjects received a relatively larger dose (7–10 mCi or 260–370 MBq) of [^{123}I]MIBG than the other normal subjects to enable comparison with the patients with neuropathy. The timing of their scintigraphic studies also varied slightly from that of the other normal subjects. Thirteen normal subjects received 3–5 mCi of [^{123}I]MIBG. Of these normal subjects, four were randomly chosen to receive imipramine, and three to receive phenylpropanolamine in a second scintigraphic study. The remainder served as additional control subjects.

Sets of scintigraphic images were made at four times after injection of [^{123}I]MIBG. The times for the sets of images were limited by the availability of [^{123}I]MIBG (injected in afternoon after a morning synthesis), the physical half-life of ^{123}I (13.3 hr), and the time required to attain a set of images (over 1 hr). Within these restraints the times were somewhat arbitrarily selected: for the first seven normal subjects and the patients with neuropathy, the times for the sets were: 2 to 4, 16.8 to 23.8, 24.3 to 27.0 and 42.8 to 45.5 hr. For the remaining normal subjects who received a smaller dose and, in some cases, two doses of [^{123}I]MIBG, the times for the sets were: 2 to 4, 17 to 20, 24 to 28, and 42 to 47 hr. The major difference in the two protocols was a longer interval between the second and third set of scintigraphic images in the latter patients, 6.8 ± 0.6 hr versus 3.7 ± 1.7 hr. The longer interval enabled the recording of pharmacologic effects induced by phenylpropanolamine in selected patients during this time. Six of the seven normal subjects in the former group (shorter interval between

TABLE 1
Characteristics of Patients with Generalized Neuropathy

Patient	Etiology of neuropathy	Sex	Age (yr)	Blood pressure (mm of Hg)		Plasma norepinephrine (pg/ml)†	
				Supine	Standing*	Supine	Standing
1	Diabetes	M	35	135/93	82/62	87	112
2	Diabetes	M	55	130/70	80/40	119	104
3	Idiopathic	M	69	143/60	80/58	91	88
4	Idiopathic	F	72	125/70	80/40	47	53
5	Shy-Drager	M	62	130/70	98/70	115	228

* Blood pressure recorded after standing 1 min.
† Blood samples obtained by 10 min of standing or in Patient 4, sitting. To obtain values in pmol/l, multiply by 5.92.

second and third set of images) exercised during this time (see below).

Anterior and posterior (180° opposed) planar images of the chest were obtained using a Searle LFOV gamma camera with a parallel hole, high-energy collimator that produced better defined images than the low-energy collimator. Better images were probably consequent to resolving better the higher energy (0.529 MeV) photons that make up 1.4% of the distintegrations of ^{123}I. Data for each image were collected over pre-set time intervals as follows: for doses of [^{123}I]MIBG of over 7 mCi, 10 min up to 28 hr and 15 min after 28 hr; for doses of <7 mCi, 15 min up to 28 hr and 20 min after 28 hr. Both analog and computer (64 × 64 pixel matrix) images were obtained, the latter for quantitative analysis.

Just before the second set of planar images were made, tomographic images of the heart were obtained by means of single photon emission computed tomography (SPECT) using a rotating gamma camera fitted with a low-energy collimator and connected to a STAR computer system in all subjects and patients who received more than 7 mCi (270 MBq) of [^{123}I] MIBG. Data were obtained in a 180° rotation about the thorax from the 45° right anterior oblique to the 45° left posterior oblique position. The data were stored in a computer and subsequently reconstructed in short axis, horizontal long axis, and vertical long axis views.

Using the computer data, uptakes in the heart and lung were measured in 75 pixel regions of interest (ROIs) drawn to be approximately an equal distance above the left and right diaphragms respectively on the anterior (Fig. 1A) and posterior chest images, and liver uptake was obtained from anterior and posterior 25 pixel ROIs in the upper right lobe, a region that was approximately the same distance below the diaphragm for each subject (Fig. 1A). Uptake refers to radioactivity in counts per minute (cpm) calculated for the geometric mean of the 180° opposed ROIs at the earliest imaging time, 2 to 4 hr. Uptakes were normalized to a 10 mCi (370 MBq) dose and to a body surface area of 1.73 m^2. For rates of loss of radioactivity, only the anterior regions of interest were used after the camera position was fixed; rates of disappearance were corrected for physical decay of ^{123}I. Rates of loss were calculated for the three periods that were separated by the time of scintigraphic images.

The reproducibility of quanitifying data from the same image by different operators and the reproducibility of data acquired from the same subject were evaluated. In processing data from ten separate studies, two operators independently recorded the uptakes of [^{123}I]MIBG in the heart that, in the individual studies, had a mean difference of 2.6% with a standard deviation of 3.1%; the two operators also recorded rates of loss of [^{123}I]MIBG from the heart (during the first period) that, in individual studies, had a mean difference of 2.7% with a standard and deviation of 2.2%. Seven subjects underwent two separate basal studies that were analyzed by a single operator. For the group of seven as a whole, the mean of the uptakes of [^{123}I]MIBG in the heart in the first study (11,412 cpm/75 pixels) differed from the mean of the second study (11,943 cpm/75 pixels) by 1.9%. Individual differences in uptake between the two studies gave a mean of 6.8% with a standard deviation of 3.9%. For the group as a whole, the mean of rates of loss of [^{123}I]MIBG from the heart (during the

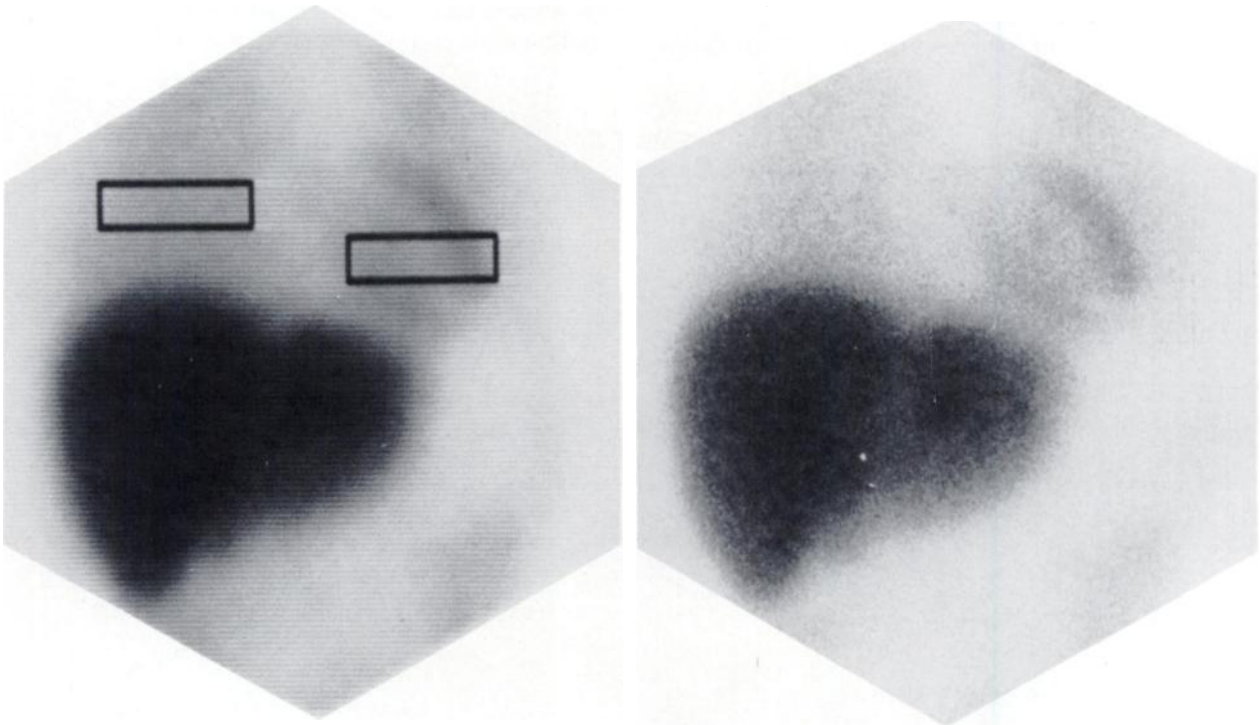

FIGURE 1
A: Computer enhanced image of an anterior view of the chest and abdomen of a control subject obtained 2.5 hr after injection of the tracer dose; regions of interest are inscribed for the heart, lung, and liver for quantification of radioactivity. B: Analog image of subject from which data shown in A were derived.

first period) in the first study (0.0252 as a fraction per hour) differed from the mean of the second study (0.0239) by 5.4%. Individual differences in rates of loss between the two studies gave a mean of 17.6% with an s.d. of 13.2%.

Radioactivity in the spleen and adrenal glands was assessed in a semiquantitative manner from the final posterior analog images because these organs were not visualized 2 to 4 hr after injection of [^{123}I]MIBG. Urine excretion data are included only for the subjects in the phenylpropanolamine experiment because in the other groups an occasional subject gave an incomplete collection.

Except for Patient 4, the patients with generalized autonomic neuropathy were also injected with 3 mCi (111 MBq) of thallium-201 (^{201}Tl) thallous chloride to portray the myocardium in a separate study after the collection of [^{123}I]MIBG data. Planar and tomographic images of the hearts were made within 3 hr of injection of ^{201}Tl. The patients were not exercised for any study.

Imipramine. A tricyclic antidepressant, imipramine, was given to four normal subjects in an oral dose of 25 mg tid for 7 days before a second scintigraphic study to inhibit selectively the uptake of NE by adrenergic neurons (*18,19*).

Phenylpropanolamine. An indirectly acting, sympathomimetic, phenylpropanolamine, was ingested by three normal subjects in a single dose of 75 mg 4 hr before the third set of scintigraphic images (during the third period) of a second scintigraphic study to displace NE and [^{123}I]MIBG from adrenergic neurons (*20*). (The time of peak action of oral phenylpropanolamine is uncertain, but the effects begin after 30 min and persist for longer than 1 hr.)

Exercise. Six of the seven normal subjects who received 7–10 mCi of [^{123}I]MIBG and who had shorter intervals (3.7 hr mean) between the second and third set of scintigraphic images exercised to give a brief physiologic stimulus to adrenergic neuron activity (*21*). In a supine position, they peddled a bicycle ergometer for 15 min to a peak work load of 200 W.

Statistical analysis. Comparisons were by the Student's t-test.

RESULTS

Normal Subjects

Basal data. Scintigraphic images made 2 to 4 hr after injection of [^{123}I]MIBG portrayed myocardial walls; ventricle cavities were relatively deficient in radioactivity (Fig. 1B), indicating a low blood concentration of [^{123}I]MIBG when the first images were made. Since <16% of radiolabeled MIBG was metabolized over several days (*22*), it was assumed that the detected radioactivity in these subjects represented [^{123}I]MIBG.

Uptakes of [^{123}I]MIBG at 2 to 4 hr are tabulated for heart, lung, and liver of 20 control subjects in Table 2, A and B. The times of earliest measurements could not be made more uniform, but there was no appreciable variation in uptake related to differences in time of measurement (between 2 and 4 hr) after injection of [^{123}I]MIBG. Similarly, when the uptake values were adjusted to a 10-mCi dose and to a surface area of 1.73 m^2, they did not appear to be affected by the different doses actually given to the subject. The uptake values did not correlate with the ages of the subjects (Table 2A).

The appropriate background radioactivity for each ROI was uncertain, given the variation in background about different regions. Some portion of lung radioac-

TABLE 2
Uptake of [^{123}I]MIBG at 2 to 4 hr in Normal Subjects

Subject	Age (y)	Surface area (m^2)	Dose [^{123}I]MIBG (mCi)	Time after dose (hr)	Radioactivity in organs (cpm/25 pixels*) Heart	Lung	Liver
A. Subjects spanning years in age and receiving 7-10 mCi of [^{123}I]MIBG							
1	22	2.05	10.0	2.5	3,542	2,927	9,098
2	22	1.87	10.0	2.5	4,162	3,110	9,936
3	31	1.80	7.6	2.5	3,852	3,197	8,670
4	33	1.99	8.7	3.0	3,334	3,072	9,418
5	38	1.91	10.0	4.0	2,517	2,355	9,312
6	47	1.98	7.25	3.5	4,811	3,586	8,557
7	62	1.91	10.0	2.5	3,894	3,485	9,296
(7) Mean		1.93	9.08	2.93	3730	3105	9184
s.d.		0.08	1.23	0.61	714	404	468
B. Subjects receiving 3–5 mCi of [^{123}I]MIBG							
(13) Mean	24.8	2.04	4.83	2.5	3,784	3,158	9,148
s.d.	4.4	0.31	0.63	0.59	587	621	1,137

* Adjusted to [^{123}I]MIBG dose of 10 mCi and body surface area of 1.73 m^2; expressed as geometric mean of anterior and posterior ROIs.

TABLE 3
Rates of Loss of [^{123}I]MIBG in Normal Subjects
(Fraction of Residual Radioactivity Lost per hr*)

	Heart†			Lung			Liver		
A. Subjects spanning years in age									
Subject	2 to 24 hr	17 to 27 hr	24 to 46 hr	2 to 24 hr	17 to 27 hr	24 to 46 hr	2 to 24 hr	17 to 27 hr	24 to 46 hr
1	0.027	0.021	0.017	0.031	0.047	0.015	0.031	0.050	0.021
2	0.017	0.039	0.011	0.025	0.046	0.013	0.039	0.069	0.020
3	0.025	0.055	0.023	0.030	0.044	0.023	0.034	0.031	0.025
4	0.030	0.023	0.019	0.033	0.031	0.016	0.037	0.010	0.023
5	0.026	0.018	0.021	0.032	0.019	0.018	0.035	0.044	0.019
6	0.014	0.021	0.018	0.035	0.010	0.019	0.034	0.020	0.019
7	0.025	0.024	0.016	0.032	0.004	0.017	0.036	0.005	0.020
(7) Mean	0.023	0.029	0.018	0.031‡	0.029	0.017	0.035§	0.033	0.021
s.d.	0.006	0.014	0.004	0.003	0.018	0.003	0.003	0.023	0.002
B. Subjects receiving 3–5 mCi of [^{123}I]MIBG: Data from control studies									
	2 to 20 hr	17 to 27 hr	24 to 47 hr	2 to 20 hr	17 to 27 hr	24 to 47 hr	2 to 20 hr	17 to 24 hr	24 to 47 hr
(13) Mean	0.027§	0.027	0.019	0.0035§	0.024	0.017	0.042§	0.030	0.021
s.d.	0.005	0.006	0.003	0.003	0.009	0.005	0.006	0.014	0.006

* Anterior view of ROI; values corrected for physical decay of ^{123}I.

† Periods differed somewhat for Subjects in Group A, particularly the middle period which was 3.74 ± 1.71 hr for Group 1 and 6.83 ± 0.61 hr for Group B.

Statistical comparisons for larger Groups A and B: First period (2 to 20 or 2 to 24 h) compared with last period (24 to 46 or 24 to 47 hr):

‡ $p < 0.01$;

§ $p < 0.001$.

tivity could serve as background for the heart (Fig. 1A), but, since background could not be calculated accurately, it was decided not to subtract background but to make note of differences in change of radioactivity over time between heart and lung (see below).

Tomographic images of the heart demonstrated both right and left ventricle walls (Fig. 2). Lesser concentrations of [^{123}I]MIBG were found at the apex than in the middle and basal region of the heart.

Fractional rates of loss of radioactivity from the regions of the heart, lung, and liver are tabulated in Table 3, A and B. The heart exhibited a greater rate of loss of [^{123}I]MIBG, 2.3% per hour in subjects in Group A and 2.7% per hour in subjects in Group B, during the first period than during the third period, 1.8 to 1.9% per hour, respectively. The patterns of loss from lung and liver resembled those of the heart, but the loss from the lung was more rapid than from the heart during the first period while the rates of loss were similar for the two organs during the third period.

Imipramine. Administration of imipramine reduced the uptake of [^{123}I]MIBG, measured 2 to 4 hr after injection, in the region of the heart to 50%, a statistically significant inhibition of uptake (Table 4A). Imipramine

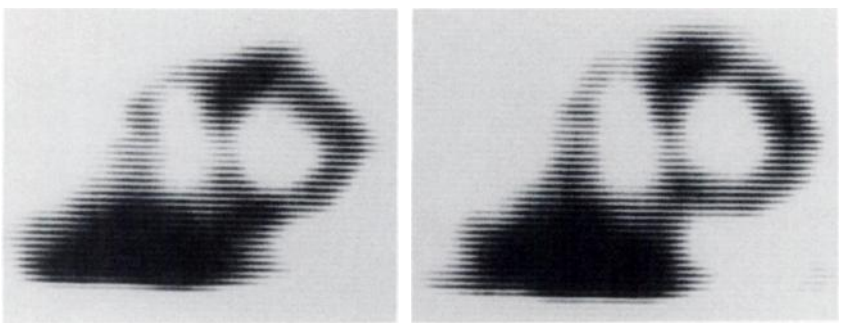

FIGURE 2
Single photon emission tomography of the heart of a control subject. Data for the tomographs were acquired 18 hr after injection of 10 mCi of [^{123}I] MIBG slices are ~1.2 cm thick. Shown are contiguous tomographic slices parallel to the short axis and at about the middle of the heart. Right ventricle (on the left in the image) and left ventricle walls are seen. Radioactivity in the liver appears below the heart.

TABLE 4
Effects of Imipramine* on Uptake and Rate of Loss of [^{123}I]MIBG†

A. Uptake of [^{123}I]MIBG at 2 to 4 hr (cpm/25 pixels)

	Heart			Lung			Liver		
	Control	Imipramine	I/C‡	Control	Imipramine	I/C‡	Control	Imipramine	I/C‡
(4) Mean	4,032	2,001**	0.50	3,077	1,828¶	0.59	8,886	7,452§	0.84
s.d.	321	195		668	414		482	1,135	

B. Rate of loss of [^{123}I]MIBG (fraction/hr)

	Heart			Lung			Liver		
	Control	Imipramine	Increment in imipramine study	Control	Imipramine	Increment in imipramine study	Control	Imipramine	Increment in imipramine study
Period 2 to 2 hr									
(4) Mean	0.022	0.039**	0.017	0.033	0.038§	0.005	0.043	0.047	0.004
s.d.	0.003	0.003		0.003	0.004		0.004	0.007	
Period 24 to 47 hr									
(4) Mean	0.017	0.020	0.003	0.013	0.023	0.010	0.022	0.027§	0.005
s.d.	0.002	0.010		0.004	0.011		0.003	0.003	

* Imipramine given 25 mg orally tid × 7 d.
† [^{123}I]MIBG given ~5 mCi i.v.
‡ I/C: Imipramine/control.
Statistics: Control versus imipramine values.
§ $p < 0.05$.
¶ $p < 0.01$.
** $p < 0.001$.

inhibited uptake of [^{123}I]MIBG into lung and liver to lesser extents, 56 and 84%, respectively.

Imipramine also increased the rate of loss of [^{123}I]MIBG from the heart. In the first period of measurement (2 to 20 hr), the rate of loss was significantly greater after imipramine when compared with values before treatment (Table 4B). This response to imipramine was consistent with inhibition of on-going uptake and re-uptake of the [^{123}I]MIBG by neurons in this period. Rates of loss of [^{123}I]MIBG from the heart during the third period (24 to 47 hr) was also increased, but not to the point of statistical significance.

Phenylpropanolamine. A single dose of phenylpropanolamine produced dramatic changes in the distribution of [^{123}I]MIBG in three normal subjects (Table 5, A, B, and C). Four hours after the ingestion of phenylpropanolamine, given during the second period, the rate of loss of [^{123}I]MIBG from the heart was significantly increased (Table 5A). The concentration of [^{123}I]MIBG virtually disappeared from the heart so that the radioactivity in this region was then less than that in the surrounding lung, and the rate of loss during the third period (24 to 47 hr) was the same as that of the lung.

As the [^{123}I]MIBG was depleted from the heart by phenylpropanolamine, the ^{123}I (assumed to be [^{123}I]MIBG) increased significantly in the blood to a maximum of 1.15 times the baseline value 2 hr after the phenylpropanolamine (Table 5B). In addition, the quantity of [^{123}I]MIBG in the urines of the three subjects increased significantly during the 12-hr period following phenylpropanolamine (Table 5C). Although there was a rise in the mean plasma level of NE 2 hr after phenylpropanolamine, the change was not significant, probably because the natural variability in plasma concentrations approached the expected modest rise in NE.

Exercise. To demonstrate simultaneous movements of [^{123}I]MIBG and endogenous NE, six normal subjects were exercised. Immediately following a moderate degree of exercise, blood concentrations of both [^{123}I]MIBG and of NE were significantly elevated, and 30 min after exercise levels of both had returned to baseline values (Table 6). This level of physical activity did not appreciably diminish tissue concentrations of [^{123}I]MIBG which were quantified within a half hour following exercise.

Patients with Neuropathy: Neuron Injury

Heart. Uptakes of [^{123}I]MIBG measured 2 to 4 hr after injection are shown for ROIs in the patients with neuropathy in Table 7A. The uptake of the radiopharmaceutical by the heart of each of the five patients with generalized neuropathy was too low to be accurately measured. Nevertheless, there were detectable concentrations of radioactivity in the region of the anterior wall of the left ventricle in Patients 1, (Fig. 3) 2, and 3.

TABLE 5

Effects of Phenylpropanolamine* on [^{123}I]MIBG† in Heart

Periods	2 to 20 hr Control‡	2 to 20 hr Phenylpropanolamine	2 to 20 hr Increment in phenylpropanolamine Study	17 to 27 hr Control‡	17 to 27 hr Phenylpropanolamine	17 to 27 hr Increment in phenylpropanolamine Study	24 to 47 hr Control‡	24 to 47 hr Phenylpropanolamine
Rate of loss per hr								
(3) Mean	0.030	0.030	0.000	0.026	0.071¶	0.045	0.020	—
s.d.	0.003	0.006		0.008	0.007		0.002	
Rate of loss per entire period								
(3) Mean	0.470	0.480	0.010	0.191	0.553¶	0.362	0.375	—
s.d.	0.069	0.112		0.065	0.098		0.045	

B. Concentrations in circulation: [^{123}I]MIBG in blood and endogenous NE in plasma (sitting position)

	Fractional changes (2 hr after/before phenylpropanolamine‡) [^{123}I]MIBG	Norepinephrine
Control study		
(3) Mean	0.98	1.25
s.d.	0.04	0.32
Phenylpropanolamine study		
(3) Mean	1.15¶	1.31
s.d.	0.04	0.33

C. Urine concentrations: Rates of excretion of [^{123}I]MIBG (fraction of dose)

	12 hr prior to drug	12 hr after drug
Control study		
(3) Mean	0.110	0.069
s.d.	0.051	0.055
Phenylpropanolamine		
(3) Mean	0.114	0.183§
s.d.	0.019	0.049

* Phenylpropanolamine (Dexatrim) 75 mg orally 3 hr before third scintigraphic study (during 17 to 24 hr period).

† ^{123}I-MIBG given i.v., ~5 mCi. No heart radioactivity was discernible; on scintigrams, heart region activity was less than in lungs.

‡ No drug was given in Control Study; ^{123}I-MIBG was measured as radioactivity in whole blood and NE as pg in plasma; two samples were measured in each assay before the time of phenylpropanolamine administration except in one subject for whom there was only one sample.

Comparisons with respective Control Study Value.

§ $p < .05$.

¶ $p < .01$.

That the impairment of [^{123}I]MIBG uptake in the heart did not relate to perfusion of the myocardium was shown in images made with ^{201}Tl, an agent which concentrates in myocardial fibers. Thallium-201 images of Patients 1, 3, and 5 were normal. The old myocardial infarct in Patient 3 produced a relatively minor defect in the image made with ^{201}Tl, and, even allowing for disruption of neuronal axons on the apical side of this defect, the infarct was not a likely cause of the marked diminution in [^{123}I]MIBG uptake by this man's heart. Tomographic images of the [^{123}I]MIBG in the heart of Patient 1 showed the residual radioactivity in the region of the anterior wall of the left ventricle and will be shown elsewhere.

Lung and liver. Uptakes of [^{123}I]MIBG in the lungs of the patients with neuropathy were the same or higher than those of the control subjects. However, early concentrations in the liver were abnormal for each patient, and abnormal in an unexpected pattern: Patients 1, 2, and 3 exhibited high uptakes, and Patients 4, 5, and 6 had low uptakes. A search for correlations between uptakes and rates of loss of [^{123}I]MIBG from the regions of interest was then sought in these patients (Table 7B). Of note were the abnormally slow rates of loss from the lung, and liver of Patient 4, who had severe clinical manifestations of neuropathy and low uptakes of [^{123}I] MIBG. Patient 3, who had a high uptake in his liver, exhibited a rapid rate of loss of [^{123}I]MIBG from his

TABLE 6
Effect of Exercise on Plasma Levels of Norepinephrine and Blood Levels of [^{123}I]MIBG in Control Subjects

	Norepinephrine (pg/ml)*					[^{123}I]MIBG (dpm/ml)				
		1 min		30 min			1 min		30 min	
Subject	Before† pg/ml	pg/ml	% of before	pg/ml	% of before	Before† dpm/ml	dpm/ml	% of before	dpm/ml	% of before
1	175	404	231	175	100	8,944	10,858	121	9,106	102
2	261	614	235	207	79	14,184	18,047	127	15,133	107
3	341	1,665	488	497	146	12,026	16,303	136	11,824	98
4	122	329	270	148	121	10,775	12,005	111	10,626	99
5	117	1,344	1,149	149	115	13,609	16,596	122	14,301	99
6	129	571	443	137	106	17,711	22,604	128	18,375	104
Mean			469‡		111‡			128§		104§
s.d.			351		22			8		4

*Multiply pg/ml by 5.92 to obtain pmol/l.
† Before exercise value is mean of specimens obtained 30 to 60 min after recumbency; these two values varied for norepinephrine < 44%, and for [^{123}I]MIBG < 5.6%. Exercise graded to 200 W in recumbent position.
‡ Percent at 1 min differs from % at 30 min $p < 0.05$.
§ Percent at 1 min differs from % at 30 min $p < 0.01$.

liver and lung during the third period. Thus, for these two patients, low uptakes were associated with slow rates of loss, and high uptakes with fast rates of loss of [^{123}I]MIBG. Moreover, Patients 1 and 2 also had high uptakes in their livers, and the rates of loss from their livers were either high or borderline high.

Other organs. Scintigraphic images of the spleen and adrenal medullas were also examined. Neither spleen nor adrenal glands were visible on the images made 2 to 4 hr after injection of [^{123}I]MIBG. Probably the total quantities of [^{123}I]MIBG in these adrenergic structures were modest in early images but declined much less rapidly than did background radioactivity. Thus, both spleen and adrenal medullas were best visualized on the last images, 43 to 46 hr after injection of the radiopharmaceutical (Table 8). By semiquantitative assessment, [^{123}I]MIBG in the spleens and adrenal glands of Patients 1, 3, and 5 appeared normal, but Patients 2 and 4 had diminished or absent levels of [^{123}I]MIBG in these organs. In fact, the adrenal glands were never visualized in these two patients with generalized autonomic neuropathy. Uptake of [^{123}I]MIBG in salivary glands may be into nonneuronal sites to a large extent and is the subject of another communication (*24*).

DISCUSSION

Normal Subjects

Basal data. The scintigraphic images and the quantified data recorded above demonstrate localization of [^{123}I]MIBG in the heart, an organ known to be innervated by adrenergic fibers. In addition, [^{123}I]MIBG was concentrated in the myocardial walls but less in the apex than in the base of the human heart giving a pattern that was similar to the concentrations of norepinephrine in mammalian myocardium (*24–26*). Therefore, the anatomic distribution of [^{123}I]MIBG in the heart corresponds to that of the adrenergic nervous system.

In the current studies, an emphasis was placed on the quantification of [^{123}I]MIBG in terms of uptake into, and rates of loss from the heart because such measurements may indicate the status of adrenergic nervous system within that organ. Data from the first period of observation (~2 to 24 hr after injection of [^{123}I]MIBG) will probably reflect the integrity of neuron function and, with further experience, the maximum uptake reached in this period may become an index of neuron density within the heart. The net rate of loss of [^{123}I]MIBG from a tissue was the result of several forces acting on the radiopharmaceutical: neuronal uptake, neuronal release, neuronal re-uptake, and probably a small contribution in the form of a net loss from non-neuronal sites. However, the overall net rate of loss of [^{123}I]MIBG during the third period (~25 to 47 hr after injection and when the contributions of uptake and non-neuronal loss are minimized) should best reflect neuronal release or secretion. The small decrease in net rate of loss during the third period may have been the consequence of a decreasing exit of [^{123}I]MIBG from non-neuronal sites over time. The rate of loss of norepinephrine from tissues, particularly the heart, has been shown to correlate with adrenergic activity (*27*), and, although [^{123}I]MIBG is an imperfect analog of norepinephrine, it would still seem reasonable to conclude that rates of loss during the third period gave an index of neuron activity.

Imipramine. The relatively brief second period must

TABLE 7
Patients with Neuropathy

A. Uptake of [^{123}I]MIBG at 2 to 4 hr

Patient	Radioactivity in organs* (cpm/25 pixels)		
	Heart	Lung	Liver
Patients with Generalized Neuropathy			
1	—	3,173	10,659
2	—	2,308	10,763
3	—	3,941	10,161
4	—	4,424	5,983
5	—	3,627	6,318
Range of values from seven control subjects‡ (Table 2 Group A)	2,517–4,811	2,355–2,586	8,557–9,936

B. Rates of loss of [^{123}I]MIBG for first and third periods (fraction/hr)†

Patient	Heart		Lung		Liver	
	2–24 hr	24–46 hr	2–24 hr	24–46 hr	2–24 hr	21–46 hr
Patients with generalized neuropathy						
1	—	—	0.031	0.025	0.034	0.026
2	—	—	0.031	0.023	0.038	0.023
3	—	—	0.032	0.025	0.034	0.028
4	—	—	0.018	—	0.020	—
5	—	—	0.027	0.014	0.026	0.022
Range of values from seven control subjects‡ (Table 3)	0.014–0.027	0.011–0.023	0.025–0.035	0.013–0.023	0.031–0.037	0.019–0.025

* Given 9.3–10 mCi of [^{123}I]MIBG; body surface areas 1.69–2.18 m^2. Values adjusted to [^{123}I]MIBG dose of 10 mCi and body surface area of 1.73 m^2; expressed as geometric mean of anterior and posterior regions of interest.

† Anterior view region of interest; corrected for physical decay of ^{123}I.

‡ Compared with values of seven subjects spanning year in age (Tables 2 and 3; Group A) patient values outside this range are underlined.

represent a transition in the kinetics of [^{123}I]MIBG. However, since changes in rates of loss were not normally abrupt, this period offered an opportunity to measure alterations in the rates of loss when adrenergic neuron function was perturbed. In the doses given to the normal subjects, imipramine should have produced a substantial inhibition of the uptake of NE by the neurons; uptake of [^{123}I]MIBG was reduced to ~50% in the heart.

Two types of uptake systems for NE and MIBG have been identified in adrenergic tissues: an uptake-1 system that dominates at low concentrations of the substrates and is inhibited by tricyclic antidepressants; and a second diffusion-type system that dominates at higher concentrations of norepinephrine and especially of MIBG. This diffusion pathway is little inhibited by tricyclic agents (*28,29*). The neurons of the heart may sequester [^{123}I]MIBG by the diffusion pathway, but in the studies carried out, at least half of the radiopharmaceutical appeared to enter through the uptake-1 pathway.

Phenylpropanolamine. [^{3}H]NE has been displaced from the hearts of mice (*20*) and rats (*10*) by phenylpropanolamine. The relative safety of this nonprescription sympathomimetic drug and its effects in rats (*10*) led to the study in normal subjects. Within 4 hr, a single dose of phenylpropanolamine abolished all measurable [^{123}I]MIBG in the heart. Such a response to a sympathomimetic drug is consistent with the concept that [^{123}I]MIBG in the heart largely resides in adrenergic neurons. Moreover, the [^{123}I]MIBG appeared to move in the expected pattern after phenylpropanolamine: to blood and to urine.

Exercise. The autonomic nervous system provides for almost immediate responses to stimuli. Yet, the system must also be capable of sustaining its nervous control of organs over many minutes to hours. Thus, even a potent stimulus to adrenergic system, if brief, would not be expected to deplete markedly the pool of norepinephrine in neuron terminals. Such would appear to be the case for physical exercise of 15 min duration. If [^{123}I]MIBG acts as an analog of NE, exercise

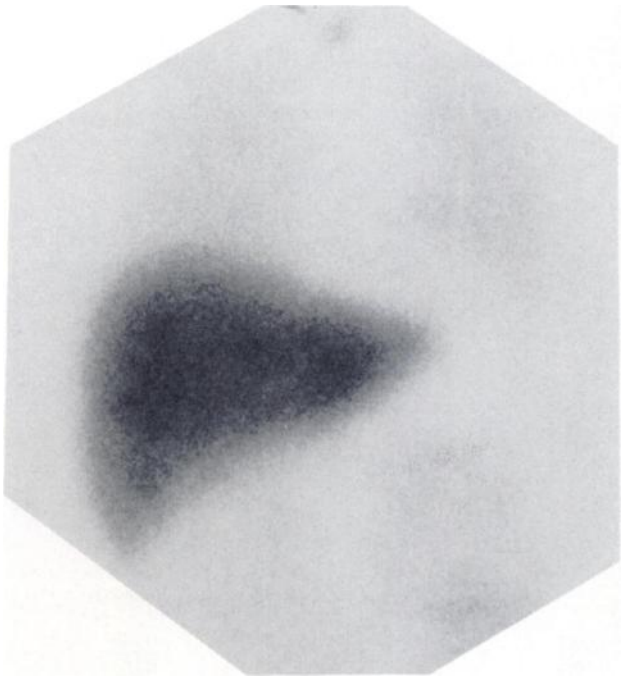

FIGURE 3
Analog image of the anterior chest and abdomen of Patient 1 obtained 2.5 hr after injection of the tracer dose. Only a small concentration of radioactivity is seen in the region of the heart. The appearance is distinctly different from that of the control subject shown in Figure 1B, particularly when heart-to-lung activity is compared.

of magnitude should not, and did not cause a substantial release of the radiopharmaceutical from the heart. Yet, as there were measurable increments in the endogenous NE concentration in plasma, so simultaneously there were significant rises in the levels of [^{123}I]MIBG in the circulation. These results are consistent with a common site of origin of [^{123}I]MIBG and NE. The increase in the blood level of [^{123}I]MIBG was not the same magnitude as that of NE, but differences may be expected if the quantity of [^{123}I]MIBG in tissues had become low (being unreplenished) relative to the quantity in blood.

TABLE 8
Semiquantitative Assessment of [^{123}I]MIBG Activity in Spleen and Adrenal Medulla at 43 to 46 hr: Patients with Generalized Neuropathy*

Patient	Spleen†	Adrenal medulla†
1	3+	2+
2	1+	0
3	2+	1+
4	0	0
5	3+	1+
Range of values from seven control subjects spanning years in age*	+2–+3	+1–+2

* Patients and control subjects who received more than 7 mCi of [^{123}I]MIBG.

† Scoring code: 0—No visible activity; 2+—Easily visible; 1+—Just visible; 3+—Intense.

The difference between the effect of phenylpropanolamine and exercise on [^{123}I]MIBG and NE in tissues may derive from the duration of the respective activities. Phenylpropanolamine taken orally probably acted on neurons over several hours. The consequences of phenylpropanolamine were a marked but gradual displacement of [^{123}I]MIBG (and probably NE) from tissues; the changes in blood concentrations of [^{123}I]MIBG and NE at any one time would be expected to be modest, and, in the case of the NE, the changes were not significant. Such a gradual action of phenylpropanolamine assures its safety and has led to its nonprescription status. In contrast, exercise produced a more potent stimulus to the adrenergic neurons but the action was too brief to deplete measurably the tissue [^{123}I]MIBG (and NE). A more sustained increase in adrenergic activity in the heart should be detected in measurements of rates of loss of [^{123}I]MIBG.

Patients with Neuropathy: Neuron Injury

Heart. If [^{123}I]MIBG is concentrated by adrenergic neurons, then diseases of these neurons should be associated with distinct alterations in [^{123}I]MIBG uptake into innervated tissues. In fact, the difference between the patients with generalized autonomic neuropathy and the control subjects was striking: there was little or no uptake of [^{123}I]MIBG observable in the hearts of the patients. This abnormality is not only derived from injury to postganglionic fibers, but also, as demonstrated by the patient with Shy-Drager Syndrome, from disorders affecting the preganglionic components of the sympathetic nervous system. In some instances, impaired uptake of [^{123}I]MIBG may reflect only a functional abnormality and not necessarily a destruction of postganglionic neurons.

The small, localized concentrations of [^{123}I]MIBG in the hearts of some of the patients with generalized autonomic neuropathies suggest that all neurons of the heart are not equally impaired, and a consequential imbalance of adrenergic stimulation may be the cause of fatal arrhythmias (Kahn JK, Sisson JC, Vinik AI: unpublished data). Sudden death is reported to be unusually frequent in patients with the generalized autonomic neuropathy of diabetes mellitus (*30,31*).

Other organs. The generalized neuropathies, as expected, reduced uptake of [^{123}I]MIBG into organs in addition to the heart in some patients. Less than normal concentrations of [^{123}I]MIBG were found in the spleen and adrenal glands—almost certainly the adrenal medullas (*32*)—of Patient 4 and Patient 2 (diabetic neuropathy).

No conclusion can now be made about the associations of uptake and rates of loss in the regions of interest within the patients with neuropathies. It is impossible

to know the sizes of the catecholamine pools into which [^{123}I]MIBG enters, and, therefore, if an increased rate of loss of [^{123}I]MIBG reflected a normal quantity of catecholamine leaving a small pool or a large release of catecholamine from a pool of normal or increased size. The relatively rapid rates of initial loss of [^{123}I]MIBG from lung and liver may reflect large non-neuronal pools of the radiopharmaceutical. If [^{123}I]MIBG follows the path of norepinephrine, probably substantial quantities will enter the hepatic cells which metabolize norepinephrine to normetanephrine (*33*); however, the hepatic cells would probably subsequently release [^{123}I] MIBG largely unchanged (*22*). The influence of concentration of [^{123}I]MIBG in organs such as the liver and lung on the uptake of MIBG by the heart is undetermined; patterns will evolve as experience is gained with the scintigraphic method. Unless there are marked changes in the uptake of [^{123}I]MIBG by other organs, or in excretion by the kidney, probably the extracardiac influences will be modest.

SUMMARY

Radiolabeled MIBG enters adrenergic neurons, and the quantitative uptake of the radiopharmaceutical is an index of the functional integrity of the neurons in the heart. Iodine-123 MIBG appears to be released from human adrenergic neurons along with the neurotransmitter, NE. Beginning ~24 hr after injection, rates of loss of [^{123}I]MIBG from heart may give an index of adrenergic function in this organ.

Unusual patterns of innervation of the heart may predispose to arrhythmias. Indeed, one of the patients with diabetic neuropathy and an imbalance in innervation described above was found to have died unexpectedly. Because the adrenergic nervous system plays an important role in the adaptation of the normal heart to stress (*34*) and is much altered by heart failure (*4,5*) and myocardial infarction (*6,7*), scintigraphic mapping with [^{123}I]MIBG should be a valuable tool to investigate physiology and disease of the heart.

NOTES

* Crocker Nuclear Laboratories; Davis, CA.

† Cat-A-Kit, Upjohn Company, Kalamazoo, MI.

‡ (General Electric 400AT) General Electric, Milwaukee, WI.

§ (Tofranil) Geigy Pharmaceuticals, Ardsley, NY.

¶ (Dexatrim, caffeine-free) Thompson Medical Company, Inc., New York, NY.

ACKNOWLEDGMENTS

A preliminary report of this work was presented at the Annual Meeting of the Midwest Section of the American Federation of Clinical Research, Chicago, IL, November 1984. An abstract was published in Clinical Research 32: 730A, 1984.

The authors thank Holly Anderson-Davis for the manufacture of [^{123}I]MIBG, the Phoenix Memorial Laboratory for the use of their radiochemistry facilities, and Annise L. Johnson and Michele Bell for the typing of the manuscript.

The work was supported by the following NIH Grants: 5 MO1RR0042, AM 21477, T32 CA09015, and HL27555, DE-AC02-76EV02031 and the Nuclear Medicine Research Fund.

REFERENCES

1. Duchen LW, Anjorin A, Watkins PJ, et al. Pathology of autonomic neuropathy in diabetes mellitus. *Ann Intern Med* 1980; 92 (Part 2):301–303.
2. Cryer PE. Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. *N Engl J Med* 1980; 303:436–444.
3. Kopin LJ, Lake RC, Ziegler, M. Plasma levels of norepinephrine. *Ann Intern Med* 1978; 88:671–680.
4. Swedberg K, Viquerat C, Rouleau J-L, et al. Comparison of myocardial catecholamine balance in chronic congestive heart failure and in angina pectoris without failure. *Am J Cardiol* 1984; 54:783–786.
5. DeQuattro V, Toshiharu N, Mendez A, et al. Determinants of cardiac noradrenaline depletion in humans. *Cardiovas Res* 1973; 7:344–350.
6. Mathes P. Changes in norepinephrine stores in the canine heart following experimental myocardial infarction. *Am Heart J* 1971; 81:211–219.
7. Barber MJ, Mueller TM, Henry DP, et al. Transmural myocardial infarction in the dog produces sympathectomy in noninfarcted myocardium. *Circulation* 1983; 67:787–796.
8. Crampton R. Preeminence of the left stellate ganglion in the long Q-T syndrome. *Circulation* 1979; 59:769–778.
9. Silverberg AB, Shah SD, Haymond MW, et al. Norepinephrine: hormone and neurotransmitter in man. *Am J Physiol* 1978; 234:E252–E256.
10. Sisson JC, Wieland DM, Sherman P, et al. *Meta*-iodobenzylguanidine as an index of the adrenergic nervous system integrity and function. *J Nucl Med* 1987; 28: 1620–1624.
11. Kline RC, Swanson DP, Wieland DM, et al. Myocardial imaging in man with I-123 meta-iodobenzylguanidine. *J Nucl Med* 1981; 22:129–132.
12. Ewing DJ, Campbell IW, Burt AA, et al. Vascular reflexes in diabetic autonomic neuropathy. *Lancet* 1973; 2:1354–1356.
13. Page MM, Watkins PJ. The heart in diabetes: autonomic neuropathy and cardiomyopathy. *Clin Endocrinol Metab* 1977; 6:377–388.
14. Ewing DJ, Campbell IW, Murray A, et al. Immediate heart rate response to standing: simple test for autonomic neuropathy in diabetes. *Br Med J* 1978; 1:145–147.
15. Smith SA, Smith SE. Evidence for a neuropathic aetiology in the small pupil of diabetes mellitus. *Br J Ophthalmol* 1983; 67:89–93.
16. Wieland DM, Wu J-L, Brown LE, et al. Radiolabeled adrenergic neuron-blocking agents: adrenomedullary imaging with [^{131}I] iodobenzylguanidine. *J Nucl Med* 1980; 21:349–353.
17. Peuler JD, Johnson GA. Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epi-

nephrine and dopamine. *Life Sci* 1977; 21:625–636.
18. Axelrod JG, Hertting G, Potter L. Effect of drugs on the uptake and release of ^{3}H-norepinephrine in the rat heart. *Nature* 1963; 194:297.
19. Daly JW, Greveling CR, Witkop B. The chemorelease of norepinephrine in mouse hearts. Structure-activity relationships. II. Drugs affecting the sympathetic and central nervous systems. *J Med Chem* 1966; 9:280–284.
20. Daly JW, Creveling CR, Witkop B. The chemorelease of norepinephrine from mouse hearts. Structure-activity relationships. I. Sympathomimetic and related amines. *J Med Chem* 1966; 9:273–280.
21. Dimsdale JE, Hartley LH, Guiney T, et al. Postexercise peril: plasma catecholamines and exercise. *JAMA* 1978; 251:630–632.
22. Mangner TJ, Tobes MC, Wieland DM, et al. Metabolism of iodine-131 meta-iodobenzylguanidine in patients with metastatic pheochromocytoma. *J Nucl Med* 1986; 27:37–44.
23. Sisson JC, Wieland DM, Jaques S Jr, et al. Radio *meta*-iodobenzylguanidine and the adrenergic neurons of salivary glands. *Am J Phys Imag* 1987; 2:1–9.
24. Angelakos ET. Regional distribution of catecholamines in the dog heart. *Circ Res* 1985; 16:39–44.
25. Pierpont GL, Master EG, Reynolds S, et al. Ventricular myocardial catecholamines in primates. *J Lab Clin Med* 1985; 106:205–210.
26. Chilian WM, Boatwright RB, Shoji T, et al. Regional uptake of [^{3}H] norepinephrine by the canine left ventricle. *Proc Soc Exp Biol Med* 1982; 171:158–163.
27. Landsberg L, Young JB. Fasting, feeding and regulation of the sympathetic nervous system. *N Engl J Med* 1978; 298:1295–1300.
28. Jaques S Jr, Tobes MC, Sisson JC, et al. Comparison of the sodium dependency of uptake of meta-iodobenzylguanidine and norepinephrine into cultured bovine adrenomedullary cells. *Molec Pharmacol* 1984; 26:539–546.
29. Tobes MC, Jaques S Jr, Wieland DM, et al. Effect of uptake-one inhibitors on the uptake of norepinephrine and meta-iodobenzylguanidine. *J Nucl Med* 1985; 26:897–907.
30. Watkins PJ, Mackay JD. Cardiac denervation in diabetic neuropathy. *Ann Intern Med* 1980; 92:304–307.
31. Ewing DJ, Campbell IW, Clarke BF. Assessment of cardiovascular effects in diabetic autonomic neuropathy and prognostic implications. *Ann Intern Med* 1980; 92:308–311.
32. Wieland DM, Brown LE, Tobes MC, et al. Imaging the primate adrenal medullae with [^{123}I] and [^{131}I] meta-iodobenzylguanidine: concise communication. *J Nucl Med* 1981; 22:358–364.
33. Axelrod J, Tomchick R. Enzymatic O-methylation of epinephrine and other catechols. *J Biol Chem* 1958; 233:702–705.
34. Braunwald E, Sonnenblick EH, Ross J Jr. Contraction of the normal heart. In: Braunwald E, ed. Heart disease. A textbook of cardiovascular medicine. Philadelphia: W. B. Saunders Co., 1984: 437.