Metaiodobenzylguanidine as an Index of the Adrenergic Nervous System Integrity and Function

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The radiopharmaceutical, metaiodobenzylguanidine (MIBG) acts as an analog of norepinephrine (NE). Experiments in rats were carried out to determine how closely the movements of [^{125}I]MIBG in the heart mimicked those of [^{3}H]NE, and if the changes [^{125}I] MIBG concentrations would reflect injury to, and function of, adrenergic neurons in the heart. Injury to adrenergic neurons by 6-hydroxydopamine substantially reduced the uptake of [^{125}I] MIBG into the left ventricle, but the effect was less than that on uptake of [^{3}H]NE uptake and concentration of endogenous NE. Similarly, when desmethylimipramine was given to inhibit the uptake-1 pathway of neurons, the reduction in uptake of [^{125}I]MIBG was statistically significant but less than that of [^{3}H]NE; part of this difference may be attributable to partial uptake of [^{125}I]MIBG into neurons by a diffusion pathway. Substantial fractions of [^{125}I]MIBG and [^{3}H]NE were displaced from the heart by the sympathomimetic drug, phenylpropanolamine. When adrenergic neurons of the heart were stimulated by feeding of rats, the disappearance rates of [^{3}H]NE and [^{125}I]MIBG from the heart were significantly increased. Although not a perfect analog of [^{3}H]NE, [^{125}I]MIBG appears to enter and leave the heart in patterns similar to those of [^{3}H]NE. Thus, movements of [^{125}I]MIBG give indices of adrenergic neuron injury and function in the heart.

J Nucl Med 28:1620–1624, 1987

Acting as a neurotransmitter, norepinephrine (NE) mediates the function of adrenergic neurons. A rise in adrenergic neuron activity is then associated with increased NE secretion by the synaptic terminals of the nerves and with an accelerated loss of NE from not only the neuron but also from the innervated tissue. This concept was validated in rats by measuring the rates of loss of [^{3}H]NE from various organs after stimulation of the adrenergic nervous system (*1,2*).

A synthesized compound, metaiodobenzylguanidine (MIBG), shares many cellular transport properties with NE. Metaiodobenzylguanidine and NE enter adrenergic cells through the same uptake system (*3*), are stored in granules (*4,5*) and are secreted in response to acetylcholine (*6*). Sequestration of radiolabeled MIBG by adrenergic tissues has permitted, through scintigraphic imaging, the detection of pheochromocytomas (*7*), and the portrayal of the heart (*8*) in man. If MIBG acts as an analog of NE, radiolabeled MIBG will also enable the study of adrenergic neuron integrity and function through detection of radioactivity.

We give evidence that iodine-125- (^{125}I) labeled MIBG resides in adrenergic neurons of the heart in rats: uptake of [^{125}I]MIBG is diminished by injury to adrenergic neurons, and by inhibition of a selective uptake system in the neurons; [^{125}I]MIBG is displaced by an indirectly-acting sympathomimetic drug; and increased sympathetic activity is associated with a more rapid rate of loss of [^{125}I]MIBG. Movements of [^{125}I]MIBG in the heart largely mimic those of [^{3}H]NE.

METHODS

Iodine-125 sodium iodide* was obtained at 17.4 Ci/mg (81 GBq/mmol), and radiolabeled MIBG was synthesized as described before (*9*). An exchange reaction produced [^{125}I]MIBG

Received Oct. 29, 1987; revision accepted Apr. 17, 1987.

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with a specific activity of 73 mCi/mg (737 GBq/mmol) and a free ^{125}I content of <2%.

Female Sprague-Dawley rats† weighing ~200 g were given water *ad lib*, but standard chow was removed from the cages 1 hr before beginning experiments. Twenty-five microcuries of [^{125}I]MIBG or [^{3}H]NE (777 GBq/mmol)* were injected through femoral vein under light ether anesthesia. Ether anesthesia may effect the uptakes of the radiopharmaceuticals, but the alternative, injection of animals under restraint, may also affect uptakes of [^{125}I]MIBG and [^{3}H]NE. Uniformity in protocol enabled comparison of results obtained with the two agents.

The rats were killed by rapid thoracotomy and excision of the heart. For uniformity the left ventricle was chosen to represent heart tissues; the right ventricle, generally gave similar results and atria often had slightly higher concentrations of the radiopharmaceuticals.

The endogenous content of NE in the heart was determined initially by radioenzymatic assay of NE‡ (*10*) and later by a modification of a method (*11*) of high performance liquid chromatography (HPLC). The tissues were homogenized in iced 0.4*M* perchloric acid, 1 g to 9 ml, to extract the NE. For the radioenzymatic assay, glutathione was added to the perchloric acid to a concentration of $10^{-3}M$. Homogenates were centrifuged at 2°C and at 20,000 *g* for 20 min. For the radioenzymatic assay, supernatants were diluted 1:100 with the kit buffer and then processed. For the HPLC measurements, 1.0 ml of Tris/EDTA buffer (1.5*M*, pH 8.6) was added to 400 μl of supernatant, then the NE adsorbed to alumina and subsequently extracted with 200 μl of 0.1*M* perchloric acid.

In the experiments where only [^{3}H]NE or [^{125}I]MIBG were measured, the hearts were quickly removed, weighed and the radioactivity determined: ^{3}H in a liquid scintillation counter after tissue oxidation, and ^{125}I in an automated gamma counter. In some experiments NE was measured in animals different from those receiving the radiolabeled compounds. For the experiments in which ^{3}H-NE/NE ratios were determined, aliquots of the alumina eluates were assayed for ^{3}H (liquid scintillation) and NE (HPLC). When [^{125}I]MIBG/NE ratios were determined, ^{125}I was assayed in an aliquot of tissue homogenate (gamma counting), and NE measured by HPLC as described above. The possible metabolism of [^{125}I]MIBG was estimated from the fraction of ^{125}I in the homogenate that was in the form of [^{125}I]iodide. After centrifugation of the homogenate, an aliquot of supernatant was added to a SEP-PAK C-18 cartridge and the [^{125}I]iodide eluted with potassium iodide and counted (*12*).

The functional integrity of adrenergic neurons was impaired in groups of rats by the intraperitoneal injection of 6-hydroxydopamine§ 100 mg/kg (*13*). Five days later [^{125}I]MIBG or [^{3}H]NE were injected, and rats were killed 2 hr later. (The time of maximum effects of 6-hydroxydopamine on adrenergic neurons is uncertain, but injury to neurons is manifest for days after the agent is given.)

The selective uptake of NE by adrenergic neurons was inhibited in another group of rats by the intraperitoneal injection of desmethylimipramine,¶ 10 mg/kg (*14,15*), 2 hr before administration of the radiolabeled agents. These animals were also killed 2 hr after receiving [^{125}I]MIBG or [^{3}H]NE. (The time of peak action of desmethylimipramine after intraperitoneal injection is uncertain, but the action of the agent is well manifest and uniform 2 hr later).

The sympathomimetic drug, phenylpropanolamine,§ was used to displace [^{125}I]MIBG and [^{3}H]NE from neurons. Phenylpropanolamine has been shown to displace [^{3}H]NE from the hearts of mice (*16*), and its selection gave an analogy to future experiments in human subjects. Beginning 2 hr following the injection of [^{125}I]MIBG and [^{3}H]NE, phenylpropanolamine, 50 mg/kg, was injected intraperitoneally every 15 min for four doses [a protocol that was found to give greater depletion of tissue catecholamines than a single injection (*17*)]; the animals were killed 15 min after the last injection.

Groups of rats were fasted for 40 hr to decrease adrenergic neuron activity in the heart (*1*). During the fast the animals had access to a dilute electrolyte solution containing 78 mEq/l of sodium and 15 mEq/l of potassium; fed rats were given regular chow. Fasting or feeding continued after injection of [^{3}H]NE or [^{125}I]MIBG into these rats. The rats were killed in groups at 1.5, 4, 8, and 20 hr after injection of a radiopharmaceutical.

RESULTS

The experiments were designed to determine how well radiolabeled MIBG will serve as an index of adrenergic neuron integrity and function. Two different but related questions were asked.

1. Following pharmacologic perturbations of the adrenergic nervous system, how closely do changes in [^{125}I]MIBG concentrations within the heart mimic those of [^{3}H]NE?

2. Will changes in [^{125}I]MIBG concentration reflect injury and stimulation to regions of the adrenergic nervous system?

Pharmacologic Perturbations of the Adrenergic Nervous System

Effects of 6-hydroxydopamine. Treatment of rats with a single injection of 6-hydroxydopamine was designed to impair function of the adrenergic nerve terminals in all tissues (*13*). In consequence, the endogenous NE concentrations in the heart were markedly reduced (Table 1A); the NE concentration in the left ventricle fell to 0.09 of the control value. The uptake of [^{3}H]NE was comparably diminished to 0.12 of control values by 6-hydroxydopamine (Table 1A). The uptake of [^{125}I]MIBG was also inhibited by treatment with 6-hydroxydopamine (Table 1A), but to a lesser degree (0.31 of control value) than those of endogenous NE and [^{3}H]NE.

Effects of desmethylimipramine. Desmethylimipramine was given to inhibit the selective uptake-1 pathway in adrenergic neurons through which NE that has left the neurons is recaptured (*14,,15*). Uptake of ^{3}H-NE by the heart was impaired (0.06 of control value) by desmethylimipramine to a greater extent than that produced by 6-hydroxydopamine (Table 1B). Desmethylamipramine also reduced the uptake [^{125}I]MIBG

but again to a lesser extent (0.5 of control value) than the uptake of [^{3}H]NE (Table 1B). In contrast to the pattern observed for uptakes of [^{3}H]NE, the effect of desmethylimipramine on the uptake of [^{125}I]MIBG was less than that obtained with 6-hydroxydopamine. Less than 2% of ^{125}I within the heart was in the form of iodide.

Effects of phenylpropanolamine. The indirectly-acting sympathomimetic drug, phenylpropanolamine, was given to displace norepinephrine from adrenergic neurons (*16*). Injection of this agent caused statistically significant depletions of [^{3}H]NE from the left ventricle; residual [^{3}H]NE was 0.63 of control value (Table 1C). Phenylpropanolamine also depleted a significant fraction of [^{125}I]MIBG from the left ventricle leaving a residual of 0.47 of the control value (Table 1C). In contrast to the actions of 6-hydroxydopamine and desmethylimipramine, phenylpropanolamine had a slightly greater effect on the concentration of [^{125}I] MIBG than on that of [^{3}H]NE.

Stimulation of the Adrenergic Nervous System

Feeding of rats has been shown to stimulate the activity of adrenergic neurons of the heart, and the rate of loss of NE has been an index of this activity (*1*). The rates of disappearance of NE from the heart were expressed as the specific activity of [^{3}H]NE (nCi of [^{3}H] NE/μg endogenous NE or [^{3}H]NE/NE) in both ventricles over time (Fig. 1). The disappearance rate of ^{3}H-NE/NE from the heart of fed rats (T1/2 = 12.8 hr) was significantly different ($p < 0.003$) than that from fasted rats (T1/2 = 23.6 hr) (Fig 1A).

The rates of disappearance of [^{125}I]MIBG were recorded in an analogous manner, as ratios of nCi of [^{125}I] MIBG/μg of endogenous NE ([^{125}I]MIBG/NE) and were more rapid than those of [^{3}H]NE/NE. However, the pattern of disappearance of [^{125}I]MIBG/NE was similar to that of [^{3}H]NE/NE in that the rate of disappearance of [^{125}I]MIBG/NE in fed rats (T1/2 = 6.0 hr) was significantly greater ($p < 0.001$) than the rate in fasted rats (T 1/2 = 10.3 hr) (Fig 1B). The percent of ^{125}I as iodide in the hearts was always < 2.

DISCUSSION

The data of the experiments in rats show that pharmacologic treatments known to alter the function of adrenergic neurons reduce the concentration of both [^{3}H]NE and [^{125}I]MIBG in the heart. Although the effects on [^{3}H]NE and [^{125}I]MIBG were qualitatively similar, the quantitative changes in tissue concentrations of the two agents differed. Uptake of [^{3}H]NE was consistently more reduced than that of [^{125}I]MIBG by 6-hydroxydopamine and desmethylimipramine. Some of this difference in uptake may be accounted for by a greater proportion of [^{125}I]MIBG than of [^{3}H]NE entering non-neuronal sites of the tissues, but probably another mechanism, neuronal uptake by a diffusion pathway described below, plays an important role.

The uptake of [^{3}H]NE was reduced in the heart more

TABLE 1
Concentrations of [^{125}I]MIBG, [^{3}H]NE and Endogenous NE in Left Ventricle Administration of Drugs Directed at Adrenergic Neurons in Rats

	Concentration of agents in tissues								
	Uptake of radiolabeled agents (% kg dose/g*) mean ± s.d.						Endogenous NE (ng/g wet weight) mean ± s.d.		
Tissue	Control	Experimental	E/C‡	Control	Experimental	E/C‡	Control	Experimental	E/C‡
A. Injury to neurons by 6-hydroxydopamine§									
	(4)0.734 ±0.086	(6)0.225¶ ±0.035	0.31	(5)1.241 ±0.088	(5)0.146¶ ±0.032	0.12	(4)688 ±175	(5) 63¶ ±24	0.09
B. Inhibition of uptake by desmethylimipramine§									
	(5)0.649 ±0.069	(5)0.322§ ±0.036	0.50	(5)0.562 ±0.036	(5)0.031** ±0.011	0.06	(5)550 ±60	(5)691 ±137	1.26
C. Displacement by phenylpropanolamine§									
	(5)0.484 ±0.065	(5)0.288§ ±0.013	0.47	(5)0.670 ±0.081	(5)0.419** ±0.068	0.63			

* Reference 18.

† [^{125}I]MIBG or [^{3}H]NE, 25 μCi given i.v. in 0.2–0.3 ml.

‡ E/C: Experimental/control mean.

§ All drugs given i.p. in 0.3 ml. Experimental groups: 6-Hydroxydopamine, 100 mg/kg, 5 days before [^{125}I]MIBG and [^{3}H]NE and killed 2 hr later; Desmethylimipramine, 10 mg/kg, 2 hr before [^{123}I]MIBG and [^{3}H]NE, and 4 hr before killing; Phenylpropanolamine, 50 mg/kg q 15 min × 4, beginning 2 hr after [^{125}I]MIBG and [^{3}H]NE, and killed 3 hr after [^{125}I]MIBG and [^{3}H]NE. Control groups received saline.

Statistical comparison of Experimental and Control groups:

¶ $p < 0.01$.

** $p < 0.001$.

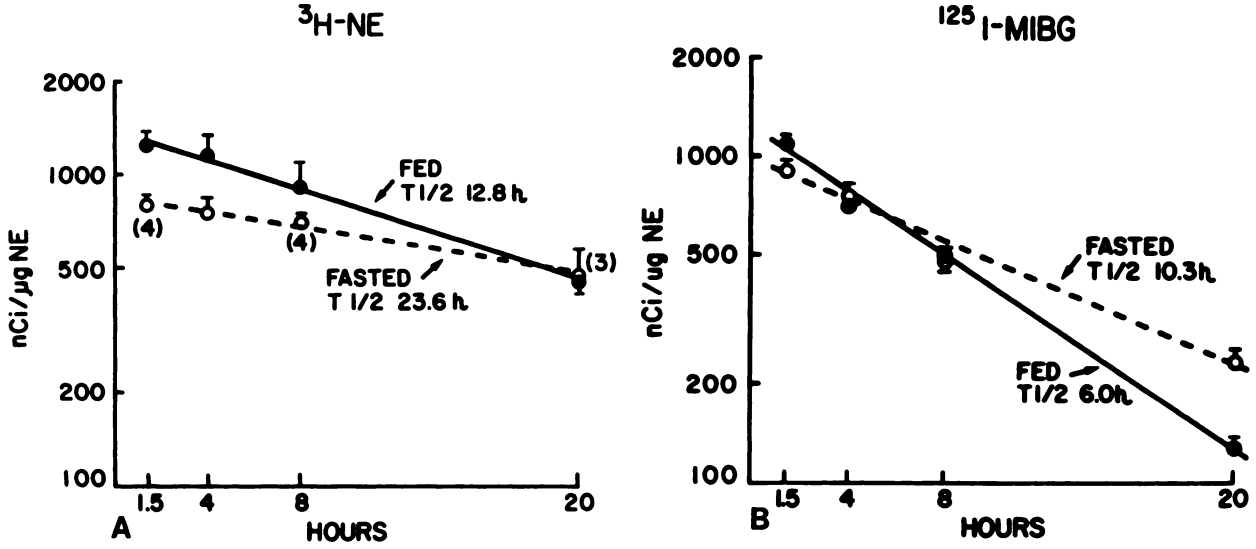

FIGURE 1

Rates of disappearance of [^{3}H]NE and [^{125}I]MIBG from rat heart. Each group consisted of five rats except where indicated by numbers in parentheses. A: Specific activity of [^{3}H]NE as nCi of [^{3}H]NE/μg endogenous NE. B: nCi of [^{125}I] MIBG/μg endogenous norepinephrine. Fasted rats received an electrolyte solution (see text) for 40 hr before intravenous injection of radiopharmaceuticals and thereafter until killing; fed rats received regular chow throughout. Data are shown for means and s.e.m. Slopes of the curves were determined by least squares in a polynomial regression (*19*). The slopes of fed and fasted rats were significantly different in A, $p < 0.003$, and in B, $p < 0.001$.

by desmethylimipramine than by 6-hydroxydopamine, an observation that suggests that damage to the neurons by 6-hydroxydopamine was incomplete. Compared to the control value, the residual uptake of [^{125}I]MIBG after 6-hydroxydopamine in the heart was 0.31; this fraction would then represent the *maximum* non-neuronal uptakes of [^{125}I]MIBG. However, since the injury imposed by 6-hydroxydopamine was incomplete, the true non-neuronal fraction was probably less. Desmethylimipramine imposed a rather complete inhibition of the uptake-1 pathway as indicated by the reduction of [^{3}H]NE uptake into the heart to 0.06 of control level, yet this agent lowered the [^{125}I]MIBG uptakes to only 0.50 giving residual concentration that could not be accounted for solely by non-neuronal uptake. Thus, a substantial fraction of [^{125}I]MIBG must have entered neuronal sites in these organs by a route other than the uptake-1 pathway.

The diffusion pathway that facilitated entry of a greater proportion of [^{125}I]MIBG than of [^{3}H]NE into adrenal medulla cells (*3*) may also operate in adrenergic neurons. If such a diffusion type pathway were more resistant than the uptake-1 pathway to the destructive effect of 6-hydroxydopamine, the pattern of the uptake data in rat heart would be explained. The diffusion type of pathway then may facilitate a substantial proportion of [^{125}I]MIBG into adrenergic neurons.

The depletions of [^{125}I]MIBG and [^{3}H]NE from the left ventricle following administration of phenylpropanolamine were probably caused by displacements of the radiolabeled agents by the sympathomimetic drug. The effect of phenylpropanolamine [^{125}I]MIBG was slightly greater than that on [^{3}H]NE, a difference that could relate to a slightly larger fraction of [^{125}I]MIBG residing in extra-vesicular sites within the neuron, positions that may be more accessible to displacement.

Feeding of rats appears to activate the adrenergic nervous system in the heart, and the rate of disappearance of [^{3}H]NE/NE then serves as an index of NE turnover in that organ (*1,2*). In our experiments, [^{3}H] NE/NE disappeared more rapidly from the heart of fed than from the heart of fasted rats giving values similar to those reported by Landsberg and Young (*1*). The disappearance of [^{125}I]MIBG/NE was also faster in the fed animals, and this pattern suggests that rates of disappearance of [^{125}I]MIBG reflect functional activity of adrenergic neurons in the heart. However, the differences in rates of disappearance between [^{125}I]MIBG and [^{3}H]NE/NE require explanation. Although [^{125}I]MIBG could be preferentially sequestered in the adrenergic vesicles that are in ready position for exocytosis, there is neither theoretical nor experimental support for this concept. Alternatively, a greater proportion of [^{125}I] MIBG than of [^{3}H]NE may be in extra-vesicular sites (neuronal and non-neuronal) so that [^{125}I]MIBG "leaks" out of the heart more rapidly than does [^{3}H] NE. Although this latter explanation means that some of the loss of [^{125}I]MIBG is unrelated to neuron function, measurable changes in the rates of disappearance of [^{125}I]MIBG nevertheless may still faithfully reflect changes in adrenergic activity.

Although not a perfect analog of NE, MIBG, through

measurements of its uptake and rate of release, has the potential to provide indices of adrenergic neuron integrity and function. In its disappearance from tissues, MIBG may be, in some ways, more reliable than NE since MIBG is metabolized to only a small extent (*12*) and does not bind to postsynaptic receptors (*4*). Therefore, radiolabeling MIBG with ^{123}I should give a radiopharmaceutical that can be detected and quantified external to the animal, and thereby enable in vivo estimates of adrenergic function in specific organs such as the heart of man. Such data have heretofore been impossible to obtain.

NOTES

* DuPont Company, No. Billerica, MA.

† Charles River Breeding Laboratories, Inc., Wilmington, MA.

‡ Cat-A-Kit, Upjohn Co., Kalamazoo, MI.

§ Sigma Chemical Co., St. Louis, MO.

¶ Revlon Health Care Group, Tuckahoe, NY.

ACKNOWLEDGMENTS

The authors thank Annise Johnson for expert typing, Carl Dmuchowski for help with statistical analysis, and Susan Fisher, Jon Johnson, and Douglas Heady for outstanding technical assistance.

This work was supported by the NIH Grants AM21477 and HL2755 and the Diabetes Research and Training Center at the University of Michigan, MDRTC-#5P60DK20572-10.

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