Measuring acute changes in adrenergic nerve activity of the heart in the living animal

Changes in the function of the adrenergic neurons of the heart may be important indicators of the adaptations of an animal to physiologic stress and disease. Rates of loss of norepinephrine (NE) from the heart were considered to be proportional to NE secretion and to adrenergic function. In rat hearts, yohimbine induced almost identical increases in rates of loss of ^{3}H-NE and of ^{125}I-metaiodobenzylguanidine (MIBG), a functional analog of NE. Clonidine induced decreases in rates of loss of ^{3}H-NE that were also mimicked by those of ^{125}I-MIBG. In the dog heart, pharmacologically-induced increases and decreases in rates of loss of ^{123}I-MIBG could be measured externally; these values were similar to those obtained for ^{125}I-MIBG in the rat heart. Thus acute changes in the adrenergic neuron activity can be measured in the living heart. The method is applicable to man in determining the capacity of the adrenergic system to respond to provocative challenges. (Am Heart J 1991;121:1119.)

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Adrenergic nerve activity in the heart plays prominent roles in health and disease and therefore is of importance to physiologists, pharmacologists, and clinicians. However, this function has been difficult to measure in living animals and in man. Adrenergic activity can be defined in terms of rate of secretion of norepinephrine (NE) from the neuron terminals, but sampling of the NE in the synaptic space is beyond technical capabilities in the defined circumstances. However, the secreted NE has several fates in addition to interacting with the receptors on effector cells, and one fraction of this NE enters the circulation and is lost to the heart. The quantity of NE leaving the heart is thought to be proportional to the amount secreted; this fraction has been termed "spillover."[1] Measurement of the spillover of NE from the heart requires catheterization of the coronary sinus and establishment of an equilibrium between endogenous NE and infused ^{3}H-NE in the circulation.[1-3] Such assays are not readily applied in most clinical experiments.

If the neuronal pool of NE could be labeled with a radiopharmaceutical that mimicked the movements of NE and that could be measured by instruments outside the animal, this would provide a noninvasive method for monitoring changes in NE rates of loss and of secretion. Radiolabeled metaiodobenzylguanidine (MIBG) appears to be such a radiopharmaceutical: in patterns similar to those of NE, MIBG enters adrenergic cells via the uptake-1 pathway,[4] is stored in adrenergic vesicles,[5,6] and is released by acetylcholine.[7] Without knowledge of the size of the cardiac pool of endogenous NE, measurements of rates of loss cannot be correlated with quantities of secretion, but acute changes in rates of loss of ^{123}I-MIBG from the heart should indicate the direction and the relative magnitude of the acute changes in adrenergic nerve function. These acute changes in adrenergic nerve activity—such as those following exercise or the administration of pharmacologic agents—may also reflect the functional capacities of the nervous system. Reliable measurements of changes in rates of loss of ^{123}I-MIBG will then be valuable in defining how the adrenergic nerves adapt to physiologic stress, to disease, and to treatments of myocardial dysfunction. In this report, we validate the relationship between acute changes in rates of loss of radiolabeled MIBG and NE from the hearts of rats, and demonstrate the feasibility of measuring changes in rates of loss of ^{123}I-MIBG from the hearts of living dogs.

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METHODS

Materials. ^{3}H-NE, with a specific activity of 20 to 21 Ci/mmol, was purchased from E. I. Du Pont de Nemours &

Table I. Residual concentrations and rates of change of adrenergic radiopharmaceuticals in rat hearts

Groups			Concentrations (nCi/gm)*	Concentrations Ratio (Conc ÷ vehicle conc)	Rates of loss over 2 to 6 hours Fractional loss (per hr)	Rates of loss over 2 to 6 hours Ratio (Conc ÷ vehicle conc)
A. ^{3}H-NE (10 μCi/gm at 0 hr)						
Baseline	2 hr	(6)	508 ± 80			
Vehicle	6 hr	(6)	361 ± 90		0.082	
Yohimbine (10 mg/kg)	6 hr	(6)	233 ± 54†‡	0.65	0.177	2.2
Clonidine (0.2 mg/kg)	6 hr	(6)	428 ± 54§	1.19	0.042	0.51
B. ^{125}I-MIBG (10 μCi at 0 hr)						
Baseline	2 hr	(5)	358 ± 10			
Vehicle	6 hr	(6)	205 ± 28		0.130	
Yohimbine (10 mg/kg)	6 hr	(6)	101 ± 26†‖	0.49	0.271	2.1
Clonidine (0.2 mg/kg)	6 hr	(6)	303 ± 64†¶	1.48	0.041	0.32

Conc, Concentration.
*Mean ± SD.
†In a one-way ANOVA, these values differed from those in respective vehicle groups, $p < 0.05$
In a two-tailed, unpaired t test, comparisons were with the respective vehicle group: ‡$p = 0.014$, §$p = 0.15$, ‖$p = 0.0001$, and ¶$p = 0.008$.

Table II. Concentrations of endogenous NE in rat hearts before and after treatments

Groups		NE (ng/gm)* ^{3}H-NE experiment		^{125}I-MIBG experiment	
Baseline	2 hr	(6)	720 ± 130	(5)	899 ± 133
Vehicle	6 hr	(6)	788 ± 146	(6)	973 ± 158
Yohimbine (10 mg/kg)	6 hr	(6)	624 ± 158†	(6)	675 ± 219†
Clonidine (0.2 mg/kg)	6 hr	(6)	832 ± 140†	(6)	1253 ± 141†

*Mean ± SD.
†In one-way ANOVA, these values differed from those in respective vehicle groups, $p < 0.05$.

Company, North Billerica, Mass. ^{125}I-MIBG and ^{123}I-MIBG were synthesized by methods previously described[8-10] with specific activities of 21 Ci/mmol and 1.6 Ci/mmol, respectively. Yohimbine hydrochloride and clonidine hydrochloride were obtained from the Sigma Chemical Company, St. Louis Mo. The agents were in physiologic saline except for yohimbine, which was dissolved in sterile water.

Female Sprague-Dawley rats, weighing 180 to 225 gm, were purchased from Charles River Breeding Laboratories, Portage, Mich., and were given rat chow and water ad lib. Four mongrel dogs, 16.5 to 22.6 kg in weight, were obtained from a commercial vendor. The dogs were "conditioned" for multiple studies by observation over 30 days; they were clincally normal, immunized against diseases, free of parasites, and were fed regular chow and water ad lib.

Rat experiments. To validate that the movements of NE are mimicked by those of MIBG, we adopted the following protocol. Rats were injected with 10 μCi of either ^{3}H-NE or ^{125}I-MIBG via an exposed femoral vein while under light anesthesia induced by intraperitoneal chloral hydrate. Two hours were allowed for mixing of the selected agent with the endogenous pool of NE; by this time the levels of MIBG in the blood are relatively low compared with those in the heart.[11] One group of animals, designated the baseline group, was put to death at this time. Yohimbine, an α_2-adrenergic receptor antagonist, increases the function of the adrenergic system,[12] and clondine, an α_2-agonist, causes the opposite effect.[13] To produce these effects, additional groups of rats were injected intraperitoneally at 2 hours with: yohimbine, 10 mg/kg; clonidine, 0.2 mg/kg; or the vehicle, physiologic saline. These three groups of animals were put to death 4 hours after the intraperitoneal injection.

The rats were killed by rapidly opening their chest cavities during chloral hydrate anesthesia. The hearts were quickly excised, washed free of blood with physiologic saline, and placed on ice. Within 1 hour the hearts were homogenized in ice-cold 0.4 mol/L perchloric acid, 9 ml/gm. The homogenate was centrifuged at 20,000 g at 4° C for 20 minutes, and the supernatant was assayed for endogenous NE by high-pressure liquid chromatography after extraction by alumina.[9] When ^{3}H-NE was used as the radiopharmaceutical, an aliquot of the eluate from the alumina was assayed for ^{3}H in a liquid scintillation counter; the activity was considered to represent unmetabolized ^{3}H-NE. When ^{125}I-MIBG was used, aliquots of the homogenate were taken before centrifugation and were counted in a well

counter for ^{125}I; previously we had demonstrated that less than 2% of the radiopharmaceutical was metabolized to iodide in the heart.[9]

Dog experiments. The experiments in dogs were designed to demonstrate that in vivo scintigraphy will detect acute changes in adrenergic nerve activity; the protocols for producing the acute changes were based on the data derived from the rat experiments. Throughout the 5 hours of each study, the dogs were kept under light anesthesia by intravenous thiamylal, and 500 ml of physiologic saline was infused to maintain hydration. For each study, 9 to 10.6 mCi of ^{123}I-MIBG was injected at 0 hours. Intravenous treatments were begun at 2 hours: yohimbine, 125 μg/kg as a bolus, followed by a pump-governed infusion of 1 μg/kg/min thereafter—a dose found to produce effects on the adrenergic nervous system of human subjects[14]; clonidine, 20 μg/kg, followed by 10 μg/kg at 3.5 hours[15]; or vehicle, which was only the physiologic saline infusion since the preparations of neither yohimbine nor clonidine added appreciably to the total volume of fluid administered.

A GE 400AT gamma camera (GE Medical Systems, Milwaukee, Wisc.) was used to locate the ^{123}I-MIBG in the heart. Beginning between 2¼ and 2¾ hours after the injection of the ^{123}I-MIBG, data in regions-of-interest were collected[10] over 10-minute periods; generally counts were obtained until 5 hours, but there were brief interruptions to obtain tomographic images[10, 16] to ascertain if one region of the heart lost more ^{123}I-MIBG than another. Regions-of-interest were the heart and an upper region of lung that approximated the thickness of lung behind the heart; the counts/pixel/10 minutes of lung activity were subtracted as background from the respective heart activity.[10] Counts were corrected for physical decay of the radionuclide.

Data analysis. Statistical comparisons were by analysis of variance and, between selected groups, by the unpaired, two-tailed *t* test. Fractions were transformed into logarithms before analysis.

RESULTS

Rat experiments. The residual concentrations of ^{3}H-NE in the hearts of rats 4 hours after treatment with yohimbine were significantly lower than those in the hearts of vehicle-treated animals (Table I). The residual concentrations of ^{3}H-NE in the clonidine-treated rats were greater but not significantly different from those in the vehicle-treated animals ($p = 0.15$ in the *t* test). The changes in the levels of ^{3}H-NE in rat hearts over the 4-hour period after the treatments were also calculated as monoexponential rates of loss (Table I), along with the rates of loss of ^{125}I-MIBG from rat hearts (Table I) for comparison with the rates of loss of ^{123}I-MIBG from dog hearts (see below, Table III). By this calculation, the rate of loss of ^{3}H-NE from the heart was increased about 2× after yohimbine treatment and decreased to 0.5× after clonidine treatment.

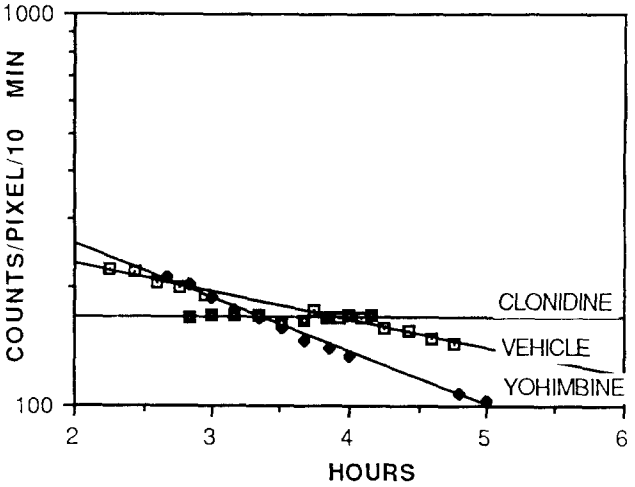

Fig. 1. Rates of loss of ^{123}I-MIBG from heart of dog No. 2. Analysis of data of individual curves shows: *VEHICLE*—$r^2 = 0.990$, fractional rate of loss = 0.153; *YOHIMBINE*—$r^2 = 0.992$, fractional rate of loss = 0.272; *CLONIDINE*—$r^2 = 0.0$, fractional rate of loss = 0.0.

Following yohimbine treatment, the residual concentrations of ^{125}I-MIBG in the heart were also significantly less than those observed after vehicle treatment (Table I). With ^{125}I-MIBG as the radiopharmaceutical, clonidine treatment produced residual levels that were higher than those produced by vehicle treatment ($p = 0.008$), a response greater than when ^{3}H-NE was used. For the residual ^{125}I-MIBG, the ratio of the yohimbine-treated group to the vehicle-treated group (0.49) was less than the respective ratio for the residual ^{3}H-NE (0.65), and the ratio of the clonidine-treated group to the vehicle-treated group for ^{125}I-MIBG (1.48) was greater than the respective ratio for residual ^{3}H-NE (1.19). Thus the patterns of change were the same for ^{3}H-NE and ^{125}I-MIBG, but changes from the effects of yohimbine and clonidine were relatively greater for ^{125}I-MIBG.

However, the changes in the fractional rates of loss of ^{3}H-NE and ^{125}I-MIBG were more comparable. In terms of fractional rates of loss, the ratio of yohimbine/vehicle was 2.2 for ^{3}H-NE and 2.1 for ^{125}I-MIBG (Table I), indicating almost identical indices for the acute increases in adrenergic nervous system function. The ratio of clonidine/vehicle was 0.51 for ^{3}H-NE and 0.32 for ^{125}I-MIBG, pointing to some discrepancy in these indices of acute decreases in adrenergic nerve activity.

The concentrations of endogenous NE in the rat hearts represented the sum of the actions of the treatments and the compensatory changes in the synthesis of NE. The net effects of treatments with yohimbine and clonidine were altered endogenous

Table III. Rates of change of ^{123}I-MIBG in dog heart

Agent	Fractional loss (per hr)				
	Dog 1	*Dog 2*	*Dog 3*	*Dog 4*	*Mean ± SD*
Vehicle	0.150	0.160*	0.083	0.156	0.137 ± 0.036
Yohimbine (125 μg/kg; 1 μg/kg/min)	0.463	0.272	0.187	0.186	0.277 ± 0.0130†
Clonidine (20 μg/kg; 10 μg/kg)	0.0	0.0	0.048	0.0	0.012 ± 0.024†
	$T_{1/2}$ (hr)				
Agent	*Dog 1*	*Dog 2*	*Dog 3*	*Dog 4*	
Vehicle	4.3	4.0	8.0	4.1	
Yohimbine	1.1	2.2	3.4	3.4	
Clonidine	∞	∞	14.0	∞	

*Mean value of two vehicle studies: fractional losses were 0.153 and 0.167 per hour.
†By ANOVA there were overall differences among groups ($p = 0.005$), and the difference was significant at the $p < 0.05$ level between the vehicle and clonidine groups. By unpaired, two-tailed t test, the difference between the vehicle and yohimbine groups gave $p = 0.047$, and the difference between vehicle and clonidine groups gave $p = 0.007$.

NE levels (Table II), indicating that changes in synthesis of NE were not fully compensatory for the changes in rates of loss over the times of observation. The changes were relatively modest, but nevertheless, compared with the values in the vehicle-treated group, the levels of endogenous NE in the yohimbine-treated group were significantly diminished, and those in the clonidine-treated group were significantly increased.

Dog experiments. The changes in the ^{123}I-MIBG over time were fitted to monoexponential rates of loss (Fig. 1). There was moderate variability in the fractional rates of loss for the four dogs. Yet the mean fractional rates of loss after vehicle and yohimbine treatments, 0.14/hr and 0.28/hr, respectively (Table III), were similar to those obtained for rates of loss of ^{125}I-MIBG from the rat hearts, 0.13/hr and 0.27/hr, respectively (Table I). The effect of clonidine in the dogs was much more striking than in the rats in that, over the periods of observation, clonidine appeared to inhibit all release of ^{123}I-MIBG from the dog heart, giving rates of loss that approached zero and $T_{1/2}$ values that approached infinity. In the tomographic images, the losses of ^{123}I-MIBG appeared to be uniform throughout the heart after treatments with vehicle and yohimbine.

DISCUSSION

In examining the validity of using movements of radiolabeled MIBG to mimic the changes in NE, the physiologic neurotransmitter, it seemed appropriate to compare the rates of loss of ^{125}I-MIBG with those of ^{3}H-NE from the heart. The basal rates of loss (after vehicle treatment) of ^{125}I-MIBG from rat hearts were found to be greater than those of ^{3}H-NE. This observation is in keeping with our previous results.[9] This difference probably arose from the relatively greater concentration of ^{125}I-MIBG than of ^{3}H-NE residing in the cytoplasm as opposed to the storage vesicles in the adrenergic nerve terminals, a discrepancy found between MIBG and NE in adrenergic cells.[17] The consequence of such differing intracellular distributions would be that more ^{125}I-MIBG than ^{3}H-NE would leave the neurons by a route other than exocytosis. Still, the rates of loss of ^{123}I-MIBG from the dog heart appeared to be monoexponential in the above studies and in prior experiments with rat hears,[9] thereby giving a pattern of ^{125}I-MIBG loss that was similar to that of ^{3}H-NE. The pattern of loss of each agent indicates an exit from the heart through a predominant pathway or through more than one pathway, with the same rates of loss.

Although there were differences in the basal rates of loss of ^{125}I-MIBG and ^{3}H-NE, the increases and decreases in adrenergic nerve function induced by yohimbine and clonidine were accompanied by changes in rates of loss of the two radiopharmaceuticals that were in the same and appropriate directions. Moreover, following yohimbine treatment, the magnitudes of change in rates of ^{125}I-MIBG and ^{3}H-NE loss were virtually identical. Following clonidine treatment, the magnitude of change in rate of loss of ^{125}I-MIBG was more than that of ^{3}H-NE, but this difference appeared to occur because clonidine treatment caused the rates of loss of both radiopharmaceuticals to approach zero, and the change for ^{125}I-MIBG, which started from a larger basal rate of loss, was then greater.

As agents that are active on the adrenergic nervous system, yohimbine and clonidine also produced

changes in the concentrations of endogenous NE. These changes were in patterns similar to those of ^{3}H-NE but of lesser magnitude, probably because they reflected the sum of the loss of endogenous NE and the compensations in the synthesis of NE that were incomplete.

The data clearly demonstrate that rates of loss and acute changes in rates of loss of ^{123}I-MIBG can be measured in living dog hearts by scintigraphy. In addition, the rates of loss of ^{123}I-MIBG from the canine hearts were nearly the same as those of ^{125}I-MIBG from the rat hearts, both during the basal state and during the acute increase in the activity of the adrenergic nervous system produced by yohimbine treatment. It can be inferred that the relative change in rate of loss of ^{123}I-MIBG from the dog heart after yohimbine, which is similar to that of ^{125}I-MIBG and that of ^{3}H-NE in the rat, should be similar to the relative change in rate of loss of ^{3}H-NE from the dog heart. The decrease in rate of loss of ^{123}I-MIBG from the dog heart after clonidine treatment was more profound than that observed for ^{125}I-MIBG from the rat heart, but this may reflect a greater response of the adrenergic nerves in the dog to the dose of clonidine given.

In conclusion, the use of scintigraphy to quantify ^{123}I-MIBG over time in the heart offers a method to detect acute changes in the activity of the adrenergic nervous system in living animals. The method should be applicable to the hearts of human subjects. The doses of yohimbine and clonidine given to dogs may be administered to man to increase and decrease the function of adrenergic nerves, and the observable changes in the rates of loss of ^{123}I-MIBG from these agents may be indices of the capacity of the neurons to respond. In turn, the results may predict events in patients with diseases leading to heart failure where the adrenergic nervous system becomes progressively disordered.[3, 18] In addition, the acute effects on the adrenergic neurons of other provocative maneuvers, such as exercise, therapeutic agents for heart disease, and cardiotoxic agents, may be tested.

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