Iodine-131 Metaiodobenzylguanidine Intra- and Extravesicular Accumulation in the Rat Heart

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In order to establish the appropriate time for [^{123}I]MIBG human myocardial imaging to assess the adrenergic nerve activity, the time courses of metaiodobenzylguanidine (MIBG) intra- and extravesicular accumulation in the rat heart were estimated by using [^{131}I]MIBG and reserpine. In the heart, the intravesicular accumulation was relatively constant, while the extravesicular accumulation decreased rapidly from 5 min to 6 hr. The intravesicular percentage of the total cardiac tissue concentration reached a plateau value of 50% at 4 hr after i.v. injection of [^{131}I]MIBG. In the spleen, similar time courses were observed as those in the heart, both of these organs being richly innervated by adrenergic nerves. Along with the time activity difference previously observed in the human hearts, these results suggest that at 4 hr post i.v. injection, [^{123}I]MIBG myocardial imaging will best express the neuronal accumulation of the tracer and may be useful for the assessment of adrenergic function in various pathological conditions of the human heart.

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Investigators at the University of Michigan Medical Center developed radioiodinated metaiodobenzylguanidine (MIBG) with affinity for adrenal medullae, an analog of adrenergic neuron-blocking agents (*1,2*). Iodine-131 MIBG ([^{131}I]MIBG) imaging has made it possible to visualize human pheochromocytomas of all types (*3–5*) and neuroblastomas (*6,7*). In addition, initial promising results were also reported on the treatment of malignant pheochromocytomas with [^{131}I]MIBG in selected cases (*8*).

MIBG is thought to share the same uptake, storage, and release mechanisms as norepinephrine (NE) in the adrenergic nerve terminals; however, it is not metabolized by catechol-*o*-methyl transferase and monoamine oxidase and thus can be viewed as a "nonmetabolizable" NE (*9*). Therefore, aside from such clinical utility of this agent in nuclear oncology, radioiodinated MIBG also has a potential value as an imaging agent in the assessment of such organs as the heart (*10,11*) and salivary gland (*12*), both of which are richly innervated by the adrenergic nerves (*13*). Especially in the heart, [^{123}I]MIBG imaging has the potential to be a new diagnostic tool in the assessment of various heart diseases from the standpoint of adrenergic nerve activity (*9*). Although it has been shown that [^{123}I]MIBG can be used for myocardial imaging in man (*14*), the dependence of image activity on such factors as myocardial perfusion, neuronal and extraneuronal uptake remains to be elucidated. It is crucial for future clinical use of this agent to determine the time courses of MIBG accumulation in various cardiac tissue compartments, because what the image means may differ according to the time at which the image is obtained after i.v. injection of the tracer. If one wants to assess the adrenergic nerve activity of the heart by using [^{123}I]MIBG, it seems important to determine the time when the maximal or stable state of neuronal accumulation of the tracer is reflected in the image.

The aim of this investigation is to discriminate the time course of MIBG in the vesicles, which are the specific NE storage sites in adrenergic neurones (intravesicular), from that of all the other tissue compartments (extravesicular) in the rat heart by a pharmacological method and thereby determine the appropriate time after i.v. injection of [^{123}I]MIBG for myocardial

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imaging to assess the adrenergic nerve activity in a clinical setting.

MATERIALS AND METHODS

Radiopharmaceutical

We used [^{131}I]MIBG as a tracer of MIBG. It was obtained from a commercial laboratory.* The specific activity was 1.5 μCi/μg and radiochemical purity was >95% as determined by thin layer chromatography (silica gel support, acetate/ethanol (1:1, Rf [^{131}I]MIBG = 0.07, Rf [^{131}I]iodide = 0.75).

Method for Blocking the Uptake of MIBG into NE Storage Vesicles

We employed reserpine as a pharmacological tool to estimate the intra- and extravesicular accumulation of MIBG in the organs, because reserpine is well known to selectively block the uptake of NE and guanethidine into storage vesicles of adrenergic nerves (*15–18*). Bhaget et al. studied the time courses of depletion of endogenous catecholamines and uptake of exogenous NE in the rat heart following intraperitoneal administration of reserpine at a dose of 1.25 mg/kg (*19*). Depletion of endogenous catecholamines was noted at 1 hr and the maximal depletion (90%) occurred at 4 hr with gradual increase of endogenous catecholamines thereafter. Maximal inhibition of the uptake of exogenous NE is observed between 1 and 24 hr in the rat heart. We selected the 4-hr interval between reserpine and MIBG administration, because this interval appears to produce maximal blocking effect on adrenergic nerve terminals.

Tissue Distribution Studies

Male Sprague-Dawley rats weighing 200–300 g were used in this study. In the reserpinized group, rats were injected i.p. with 4 mg/kg reserpine 4 hr before the i.v. injection of the tracer. This dose of reserpine is enough to deliver its maximal pharmacological effect on adrenergic nerve terminals (*20*), as employed in the guanethidine study by Chang et al. (*21*). Control rats received no pretreatments. They were injected with 25 μCi of [^{131}I]MIBG by the tail veins, the dose of which was determined by reference to the report of Wieland et al. (*9*). The rats were killed at 5 and 30 min, and at 1, 2, 3, 4, 5, 6, 8, 12, and 24 hr after the injection of the tracer. Five rats were studied for tissue concentration at each selected time. Several organs including the heart and spleen were removed. Blood samples were obtained from drips of the head. The organs were rinsed in physiologic saline and blotted to remove blood. Blood clots in the cardiac cavities were also removed to eliminate the activity of the blood pool. After weighing the organs, radioactivity was counted in an autogamma counter with corrections made for radioactive decay, background and counter efficiency. Tissue concentrations are expressed as percent kilogram dose per gram (% kg dose/g) to normalize the differences in rat weights (*22*).

Estimation of Intra- and Extravesicular MIBG Accumulation

Total tissue concentrations in the control group were viewed as the sum of the intra- and extravesicular accumulation. Because of selective blockade of vesicular uptake by reserpine, tissue concentrations in the reserpinized group were regarded as extravesicular accumulation. Therefore, the intravesicular accumulation in the rat heart and spleen equaled mean tissue concentration in the control group minus mean tissue concentration in the reserpinized group.

The percentage of the intravesicular accumulation to the total tissue concentration was estimated as a function of time in the rat heart and spleen, from which the appropriate time for human myocardial imaging with [^{123}I]MIBG to assess the adrenergic nerve activity was extrapolated. The percentage at each selected time was calculated as follows: (mean tissue concentration in the control group − mean tissue concentration in the reserpinized group)/mean tissue concentration in the control group × 100%.

Statistical analyses were performed using unpaired t-test.

RESULTS

Table 1 summarizes the tissue concentrations of [^{131}I]MIBG in the control and reserpinized rat groups as functions of time. In the heart, the tissue concentrations were significantly higher in the control group than in the reserpinized group at every selected time from 5 min through 24 hr. The spleen showed the same difference between the two groups as observed in the heart at 1 hr and thereafter. However, such difference in tissue concentration between both groups was not observed in the liver and lung.

Figure 1 shows the intra- and extravesicular accumulation as functions of time in the heart and spleen. The intravesicular accumulation was relatively constant for both organs. The value ranged between 0.09 and 0.13% kg dose/g in the heart from 5 min to 6 hr and between 0.06 and 0.08% kg dose/g in the spleen from 1 to 6 hr, respectively. On the other hand, the extravesicular accumulation decreased rapidly from 5 min to 6 hr; from 0.53 to 0.08% kg dose/g in the heart. In the spleen, it decreased from 0.09% kg dose/g at 1 hr to 0.03% kg dose/g at 4 hr.

Figure 2 shows the percentage of the intravesicular accumulation to the total tissue concentration as a function of time in the heart and spleen. In both organs,

TABLE 1
Tissue Concentrations of [^{131}I] MIBG in Control (C) and Reserpinized (R) Rats with Time

Time (hr)	Tissue concentration (% kg dose/g)*									
	Heart		Spleen		Liver		Lung		Blood	
	C	R	C	R	C	R	C	R	C	R
0.08	62(5)	53(3)†	13(1)	16(2)§	11(1)	15(1)‡	45(3)	51(6)	10(1)	10(2)
0.5	61(4)	51(4)†	14(2)	12(1)	12(2)	13(2)	38(6)	42(9)	4(0)	4(0)
1	48(3)	37(3)‡	16(2)	9(2)‡	9(2)	8(1)	25(3)	32(5)¶	3(1)	4(0)
2	39(4)	28(4)†	13(1)	6(1)‡	7(1)	6(1)	17(5)	16(1)	3(0)	3(1)
3	31(2)	18(3)‡	12(2)	4(1)‡	5(1)	5(1)	13(3)	13(3)	3(0)	3(0)
4	24(2)	13(1)‡	11(1)	3(0)‡	5(1)	4(1)	8(1)	11(2)§	3(0)	3(0)
5	19(1)	10(2)‡	9(1)	3(0)‡	4(0)	3(0)	8(2)	9(2)	2(0)	3(0)
6	17(1)	8(1)‡	8(1)	2(0)‡	3(1)	3(0)	6(1)	7(1)	2(0)	3(0)
8	11(1)	6(1)‡	7(1)	2(0)‡	3(0)	2(0)	5(0)	4(0)	2(0)	2(0)
12	9(1)	4(1)‡	6(1)	1(0)‡	2(0)	2(0)	4(0)	3(0)	2(0)	2(0)
24	4(1)	2(0)§	4(1)	1(0)‡	2(0)	1(0)	2(0)	1(0)	1(0)	1(0)

* N = 5, all values are expressed as 10^{-2} × % kg dose/g. Numbers in parentheses give s.d.
† $p < 0.01$.
‡ $p < 0.001$.
§ $p < 0.02$.
¶ $p < 0.05$ compared with control rats.

it increased and reached a constant value after 4 hr. The plateau value was 50% in the heart and 75% in the spleen.

DISCUSSION

MIBG is an analog of guanethidine. It has been suggested that guanethidine shares the same uptake, storage and release mechanisms as NE in the adrenergic nerve terminals (*18,21,23–26*). Previous animal and human studies have provided considerable evidence for adrenergic neuronal uptake of MIBG. Reserpine pretreatment caused a 30% decrease in the canine myocardial concentration of [^{125}I]MIBG at 2 hr after i.v. injection of the tracer (*9*). Iodine-131 MIBG heart intensity in the 24- and 48-hr images was inversely related to plasma concentrations of catecholamines suggesting competitive uptake of the tracer by the heart with circulating catecholamines (*10*). Salivary gland accumulation of [^{131}I]MIBG in the 24 hr or later images was blocked by administration of tricyclic antidepressants, known to inhibit the neuronal uptake of NE and guanethidine in the ipsilateral salivary glands in a patient with Horner's syndrome and by the bilateral

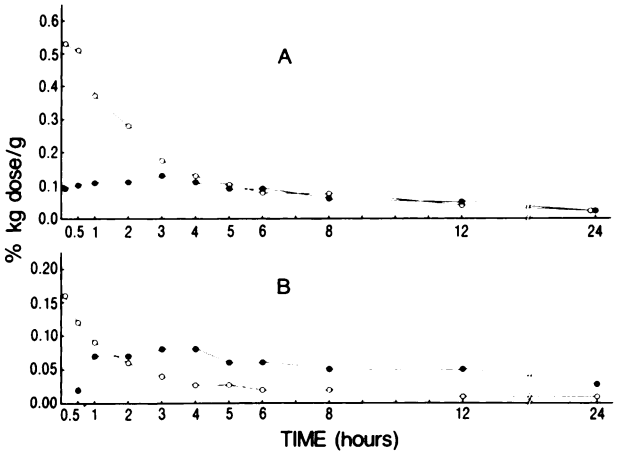

FIGURE 1
Iodine-131 MIBG intra- and extravesicular accumulation as functions of time in rat heart (A) and spleen (B). Intravesicular accumulation (●) was estimated as difference of mean tissue concentration between control and reserpinized rat groups. Mean tissue concentration of reserpinized rat group was regarded as extravesicular accumulation (O). Intravesicular accumulation is relatively constant from 5 min in heart and from 1 hr in spleen, while extravesicular accumulation decreased rapidly during first 6 hr in heart and 4 hr in spleen, respectively

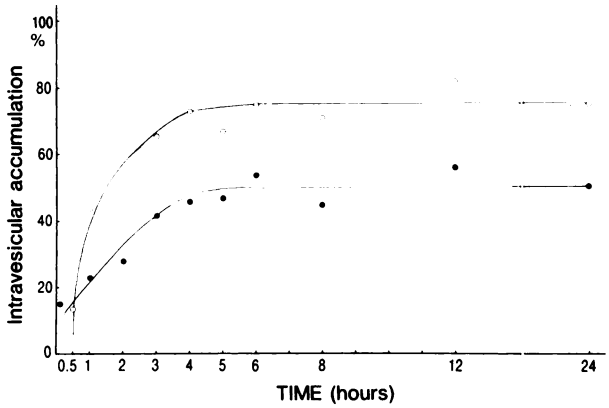

FIGURE 2
Percentage of [^{131}I]MIBG intravesicular accumulation to total tissue concentration (tissue concentration of control group) as function of time in rat heart (●) and spleen (O). Percentages reached plateau values, 50% in heart and 75% in spleen, at 4 hr after i.v. injection of tracer

salivary glands in a patient with severe idiopathic sympathetic neuropathy (*12*). However, the fate of MIBG in the neuronal and extraneuronal tissue compartments with time has not been directly demonstrated. If MIBG has the same in vivo kinetics as NE after i.v. injection, clearance of MIBG from extraneuronal compartments should be more rapid than that from neuronal compartments, because the efflux of NE located extraneuronally is more rapid than that of NE located in the neurons in the heart (*16,27–29*).

The present reserpine blocking studies not only support the neuronal uptake of MIBG but also discriminate the time course of MIBG in the storage vesicles of adrenergic neurones from that of MIBG in the other tissue compartments in the rat heart and spleen. The reserpine blocking effect on MIBG accumulation was observed in the heart and spleen but not in the liver and lung. The former two organs are richly innervated by adrenergic nerves, while in comparison the latter two organs have much less sympathetic innervation (*13*). In the heart and spleen, intravesicular accumulation of [^{131}I]MIBG is relatively constant and appears to be saturable, while the extravesicular accumulation decreases rapidly in a period of 6 hr after i.v. injection of the tracer. These time courses are consistent with those observed for guanethidine in the rat heart (*21*). This means that MIBG as well as guanethidine is bound much more firmly in the NE storage vesicles than in the other cardiac tissue compartments and suggests that MIBG is retained in sympathetically innervated tissues by two types of processes: a nonspecific adsorption as well as a specific retention in the NE storage vesicles.

Five-minute tissue concentrations of the spleen, liver, and lung showed higher values in the reserpinized group than in the control group (Table 1). This may be due to reserpine effects on the cardiovascular system such as hypotension and bradycardia (*30*).

Equilibrium in the [^{131}I]MIBG accumulation between intra- and extravesicular compartments in the rat heart and spleen was observed 4 hr after i.v. injection of the tracer. The intravesicular accumulation occupied 50 and 75% of the total accumulation in the heart and spleen, respectively, thereafter. The adrenergic neuronal accumulation of [^{131}I]MIBG, may be higher than the percentages in these organs for later periods, because an undefined amount of [^{131}I]MIBG may be nonspecifically retained in axoplasm.

The major purpose of this study was to determine the appropriate time for [^{123}I]MIBG myocardial imaging for an assessment of the adrenergic nerve activity of the heart in a clinical setting. Although it has been shown that [^{123}I]MIBG produces a clear myocardial image in man (*14*), the image may express the sum of activity in various cardiac tissue compartments. Therefore, it is critical for an assessment of adrenergic nerve activity to determine the time after i.v. injection of [^{123}I]MIBG when the neuronal accumulation of the tracer is best represented on the image. The present rat study suggests that it will be at 4 hr or later, because the intravesicular accumulation of [^{131}I]MIBG in the heart reached a maximum plateau value of 50% at 4 hr. In other words, it was after 4 hr that the reserpine blocking effect on cardiac adrenergic neurones was best recognizable in tissue concentrations of the reserpinized rats compared to those of the control rats, as shown in Table 1 and Fig. 2. The decrease in the rat myocardial concentration of [^{131}I]MIBG caused by pretreatment with reserpine was measured as 50% after 4 hr, while it was expressed as 30% or less before 2 hr. This 30% decrease in the myocardium at 2 hr is the same percentage as observed in the canine myocardium in the reserpine blocking study with [^{125}I]MIBG (*9*).

In our previous study (*31*), we observed a rapid clearance of [^{131}I]MIBG from the heart and liver of patients with adrenergic dysfunction and pheochromocytoma 4 hr after i.v. injection of the tracer when compared with controls. The largest difference in time activity over the heart and liver was observed at 4 hr between the patients and controls. However, there was no significant difference in the rate of decrease in these organs between controls and patients in the intervals subsequent to 4 hr. We postulate that this difference between controls and patients might be due mainly to the difference in the relative amount of neuronal compartments with slow efflux and extraneuronal compartments with rapid efflux in the heart and liver. In patients with adrenergic dysfunction, the heart and liver may have a reduction in the MIBG uptake and storage capacity of their adrenergic neurones compared to the controls. In patients with pheochromocytoma, high levels of circulating catecholamines may compete with [^{131}I]MIBG for adrenergic neurones. The present study supports this hypothesis by the different time courses of intra- and extravesicular accumulation of [^{131}I]MIBG. However, there is a difference in time activity of the liver between the present rat study and clinical observation mentioned above: The liver as well as the heart of these patients showed more rapid clearance of [^{131}I]MIBG than that of controls, while no significant difference in hepatic time activity is observed between the control and reserpinized rats after 30 min (Table 1). This difference of hepatic time activity between human and rat may be due to the fact that the rat liver has the least NE concentrations of several animals (*32*), suggesting less sympathetic innervation in the rat liver than in the human liver.

The present reserpine blocking study in the rat and the phenomenon observed in man (*31*), strongly suggests that at 4 hr or later, [^{123}I]MIBG myocardial imaging will best represent the neuronal accumulation of the tracer and may best provide useful information on the state of adrenergic neurones in various patholog-

ical conditions of the human heart. More detailed compartmental analysis of this tracer in comparison with NE would further enhance the utility of this agent in the in vivo assessment of such pathological conditions as autonomic denervation, cardiomegaly, congestive heart failure, and hyperthyroidism, all of which may alter myocardial catecholamine kinetics (*33*).

FOOTNOTE

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REFERENCES

1. Wieland DM, Wu Jl, Brown LE, et al: Radiolabeled adrenergic neuron-blocking agents: Adrenomedullary imaging with [^{131}I] iodobenzylguanidine. *J Nucl Med* 21:349-353, 1980
2. Wieland DM, Brown LE, Tobes MC, et al: Imaging the primate adrenal medulla with [^{123}I] and [^{131}I] meta-iodobenzylguanidine: Concise communication. *J Nucl Med* 22:358-364, 1981
3. Sisson JC, Frager MS, Valk TW, et al: Scintigraphic localization of pheochromocytoma. *N Engl J Med* 305:12-17, 1981
4. Nakajo M, Shapiro B, Copp J, et al: The normal and abnormal distribution of the adrenomedullary imaging agent m-[I-131] iodobenzylguanidine (I-131 MIBG) in man: Evaluation by scintigraphy. *J Nucl Med* 24:672-682, 1983
5. Shapiro B, Sisson JC, Lloyd R, et al: Malignant pheochromocytoma: Clinical, biochemical and scintigraphic characterization. *Clin Endocrinol* 20:189-203, 1984
6. Kimmig B, Brandeis WE, Eisenhut M, et al: Scintigraphy of a neuroblastoma with I-131 meta-iodobenzylguanidine. *J Nucl Med* 25:773-775, 1984
7. Fein U, Treuner J, Müller-Schauenburg W, et al: Scintigraphic imaging of neuroblastoma with I-131-meta-iodobenzylguanidine (I-131-MIBG). *J Nucl Med* 25: P 75, 1984 (abstr)
8. Sisson JC, Shapiro B, Beierwaltes WH, et al: Radiopharmaceutical treatment of malignant pheochromocytoma. *J Nucl Med* 25:197-206, 1984
9. Wieland DM, Brown LE, Rogers WL, et al: Myocardial imaging with a radioiodinated norepinephrine storage analog. *J Nucl Med* 22:22-31, 1981
10. Nakajo M, Shapiro B, Glowniak J, et al: Inverse relationship between cardiac accumulation of meta-[^{131}I]iodobenzylguanidine (I-131 MIBG) and circulating catecholamines in suspected pheochromocytoma. *J Nucl Med* 24:1127-1134, 1983
11. Mangner WM, Hoffman BB: Heart imaging in the diagnosis of pheochromocytoma and assessment of catecholamine uptake. *J Nucl Med* 24:1194-1196, 1983
12. Nakajo M, Shapiro B, Sisson JC, et al: Salivary gland accumulation of meta-[^{131}I]iodobenzylguanidine. *J Nucl Med* 25:2-6, 1984
13. Whitby LG, Axelrod J, Weil-Malherbe H: The fate of H^3-norepinephrine in animals. *J Pharmacol Exp Ther* 132:193-201, 1961
14. Kline RC, Swanson DP, Wieland DM, et al: Myocardial imaging in man with I-123 meta-iodobenzylguanidine. *J Nucl Med* 22:129-132, 1981
15. Campos HA, Shideman FE: Subcellular distribution of catecholamines in the dog heart. Effect of reserpine and norepinephrine administration. *Int J Neuropharmacol* 1:13-22, 1962
16. Malmfors T: Fluorescent histochemical studies on the uptake, storage, and release of catecholamines. *Circulation Res* (Suppl III) XX-XXI: 25-42, 1967
17. Iversen LL, Glowinski J, Axelrod J: The uptake and storage of H^3-norepinephrine in the reserpine-pretreated rat heart. *J Pharmacol Exp Ther* 150:173-183, 1965
18. Maître L, Staehelin M: Guanethidine uptake and noradrenaline depletion in noradrenaline storage particles of the rat heart. *Biochem Pharmacol* 20:1233-1242, 1971
19. Bhagat B, Shideman FE: Repletion of cardiac catecholamines in the rat: Importance of the adrenal medulla and synthesis from precursors. *J Pharmacol Exp Ther* 143:77-81, 1964
20. Bhagat B: Effect of reserpine on cardiac catecholamines. *Life Sci* 3:1361-1370, 1964
21. Chang CC, Costa E, Brodie BB: Interaction of guanethidine with adrenergic neurons. *J Pharmacol Exp Ther* 147:303-312, 1965
22. Kirshner AS, Ice RD, Beierwaltes WH: Radiation dosimetry of ^{131}I-19-iodocholesterol. The pitfalls of using tissue concentration data. (Reply). *J Nucl Med* 16:248-249, 1975
23. Chang CC, Costa E, Brodie BB: Reserpine-induced release of drugs from sympathetic nerve endings. *Life Sci* 3:839-844, 1964
24. Brodie BB, Chang CC, Costa E: On the mechanism of action of guanethidine and bretylium. *Br J Pharmacol* 25:171-178, 1965
25. Mitchell JR, Oates JA: Guanethidine and related agents. I. Mechanism of the selective blockade of adrenergic neurons and its antagonism by drugs. *J Pharmacol Exp Ther* 172:100-107, 1970
26. Nickerson M, Collier B: Drugs inhibiting adrenergic nerves and structures innervated by them. In *The Pharmacological Basis of Therapeutics*, 5th Ed., Goodman LS, Gilman A, eds. New York, MacMillan Publishing Co., 1975, pp 553-564
27. Farnebo L-O, Malmfors T: Histochemical studies on the uptake of noradrenaline and α-methyl-noradrenaline in the perfused rat heart. *Eur J Pharmacol* 5:313-320, 1969
28. Trendelenburg U: The extraneuronal uptake and metabolism of catecholamines in the heart. In *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines*, Paton DM, ed. New York, Raven Press, 1976, pp 259-280
29. Lindmar R, Löffelholz K: Neuronal and extraneuronal uptake and efflux of catecholamines in the isolated rabbit heart. Naunyn-Schmiedeberg's *Arch Pharmacol* 284:63-92, 1974
30. Bhagat BD: *Role of Catecholamines in Cardiovascular Diseases*, Springfield, 1974, pp 163-196
31. Nakajo M, Shimabukuro K, Miyaji M, et al: Rapid clearance of iodine-131 MIBG from the heart and liver

of patients with adrenergic dysfunction and pheochromocytoma. *J Nucl Med* 26:357-365, 1985

32. Anton AH, Sayre DF: The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. *J Pharmacol Exp Ther* 145:326-336, 1964

33. Wurtman RJ: *Catecholamines*, Boston, Little Brown and Co., 1966, pp 61-100