Uptake of Iodine-123 MIBG by Pheochromocytomas, Paragangliomas, and Neuroblastomas: A Histopathological Comparison

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The percentage uptake of [^{123}I]metaiodobenzylguanidine (MIBG) by tumors of the paraganglion system is compared with the number of neurosecretory granules (assessed by both light and electron microscopy) in the subsequently resected tumors in six patients. Iodine-123 MIBG was injected intravenously; the tumor uptake of [^{123}I]MIBG varied between 0.001% and 0.14% of the injected dose per gram of tumor tissue at 22 hr. The number of neurosecretory granules in tissue sections was scored on a scale of I–III. A direct proportional correlation was found between the percentage uptake of [^{123}I]MIBG by the tumor and the number of neurosecretory granules in the tissue sections but not with plasma or urinary catecholamines. This technique for imaging reflects the storage status of the tumor better than plasma and urinary catecholamine measurements.

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The paraganglion system comprises collections of neuroepithelial cells scattered throughout the body. The definitive common feature of these cells is the presence of numerous neurosecretory granules containing catecholamines in their cytoplasm (*1*). Paragangliomas are the better differentiated tumors arising from this system and their cells contain numerous catecholamine granules. Pheochromocytomas are paragangliomas of the adrenal medulla. Neuroblastomas and ganglioneuroblastomas are poorly differentiated tumors believed to arise from precursors of this system, and these two tumors contain fewer cytoplasmic catecholamine granules.

Neurosecretory granules containing catecholamines have the ability to fix chrome salts and have been described as chromaffin if this property is easily demonstrable and nonchromaffin if it is not. However, chromaffinicity depends very much on type and pH of fixation, does not correspond specifically to adrenaline or noradrenaline, and does not correlate well with secretory activity. This means of classification is therefore of very limited practical value. The best routine light microscopic technique for demonstrating neurosecretory granules in paragangliomas in our laboratory is the Grimelius argyrophil method (*2*). Transmission electron microscopy (TEM) is however, the method of choice for accurate assessment of the numbers and types of such granules in tumor cells.

Metaiodobenzylguanidine (MIBG) is a physiological analog of norepinephrine and guanethidine. It is taken up dominantly in vivo through the neuronal uptake 1 system (*3*), and is stored in the noradrenergic neurosecretory granules; thus the uptake and storage simulate that of norepinephrine. MIBG may be labeled with iodine-123 (^{123}I) or iodine-131 (^{131}I) and used for imaging the adrenal medulla (*4*) and related tumors such as pheochromocytomas (*5*), other paragangliomas (*6*), and neuroblastomas (*7*). Until now there has been no specific comparison between this imaging modality and the detailed histopathological appearances of this group of tumors. In this study, the percentage uptake of [^{123}I] MIBG by the tumor is compared with the number of neurosecretory granules in the tumor cells, and these

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two parameters are compared with plasma and urinary catecholamine levels.

MATERIALS AND METHODS

Of the six patients in this study, two had paragangliomas, three had pheochromocytomas, and one had a ganglioneuroblastoma. In two patients, the clinical diagnosis of malignant pheochromocytoma and malignant paraganglioma was known prior to the imaging protocol. In each case, a drug history was taken to rule out the possibility of reserpine, tricyclic antidepressant, and phenylpropanolamine usage as these drugs interfere with the uptake of MIBG. Before each study the plasma catecholamine levels were measured following an overnight fast, with the patient in a supine and resting state for at least 30 min after venous cannulation. The plasma samples were measured by high performance liquid chromatography with electrochemical detection for catecholamine levels (*8*). The urinary metanephrine and VMA levels were measured on 24-hr urine samples (*9,10*) and were expressed in μmol/24 hr.

MIBG was labeled with ^{123}I using a modified solid phase method (*11*). The radiochemical yield was between 80% and 95% resulting in a specific activity of 1-5 mCi/mg. Free iodine was always <2%. Half an hour before each study 400 mg of potassium perchlorate was administered orally to block the thyroid uptake of ^{123}I. The activity of the injection was measured in an ionization chamber before and after the injection, using the appropriate calibration factor. Between 3.5 and 7 mCi of [^{123}I]MIBG was given intravenously over 20–30 sec with the patient in a supine position. Using a gamma camera* with a high resolution collimator, 400,000 count images were acquired for anterior abdomen, anterior chest, posterior abdomen, posterior chest; and skull at 10 min, 4 hr, and 22 hr after injection.

TUMOR UPTAKE MEASUREMENT

Tumor uptake measurement was calculated by identifying a region of interest around the tumor in the image obtained at 22 hr and recording the total counts in the region. A background correction was made using an interpolative method (*12,13*) to subtract any counts within the region that came from surrounding normal tissue rather than the tumor. The depth and mass of the tumor was calculated using x-ray computed tomography (CT). In cases where the tumor was resected, the tumor was measured and weighed following removal of attached connective tissue. This confirmed that the CT estimates of tumor size were reasonably accurate. A correction was done for the loss of counts caused by attenuation of the radiation from the tumor by tissue overlying it. The factors used in this correction were obtained from experimental measurements of the attenuation of radiation from ^{123}I in tissue equivalent material. The background- and depth-corrected tumor counts were then corrected for isotope decay between the time of injection and the time of imaging, and expressed as a percentage of the total counts injected per gram of tumor tissue. This percentage was used as the measure of tumor uptake.

HISTOLOGICAL TECHNIQUES

Four of the tumor specimens were received fresh and unfixed straight from the operating theatre (Cases 1, 3, and 6 in Table 1). Blocks of ~1 mm^3 were taken and immersed immediately in 3% glutaraldehyde in 0.1*M* cacodylate buffer pH 7.4 containing 5% sucrose. The remainder of the tumor was then immediately fixed in 10% formalin. The other three specimens (Cases 2, 4, and 5) were received already fixed in 10% formalin, but again 1 mm^3 blocks were taken and placed in a glutaraldehyde solution as above.

The formalin fixed tissue was routinely embedded in paraffin wax, and sectioned at 4 μm. Sections were all stained with hematoxylin and eosin and, for the purposes of this study, by the argyrophil Grimelius method (*2*). These were examined by light microscopy and scored on a scale of I–III: I = virtually all cells Grimelius positive, II = many Grimelius positive cells, III = occasional Grimelius positive cells.

The glutaraldehyde fixed tissue was then embedded in premix resin (by TAAB), sectioned at 80 nm and stained with aqueous uranyl acetate and Reynolds lead citrate. The sections were then viewed in a Jeol 100 CX electron microscope. The whole section was inspected and then representative micrographs were taken at magnifications ranging from 1,800× to 35,000×. The photographs of the five tumors were then compared and scored for numbers of neurosecretory granules on a scale of I–III: I = virtually all tumor cells containing granules and some cells packed with granules, II = tumor cells containing moderate numbers of granules easy to find, III = small minority of tumor cells containing granules. Both the light and electron microscopic assessments of numbers of granules were made without prior knowledge of the MIBG uptake results.

RESULTS

Table 1 summarizes the findings in the six patients investigated.

Patient 1, a male age 26 yr, had a malignant paraganglioma, known from previous resection; the CT showed a soft-tissue mass in the posterior mediastinum measuring 3 × 2.2 × 2.5 cm; the biopsy at the time of study was taken from this metastatic lesion in the mediastinum. It showed the appearances of pleomorphic granular cells arranged in cords, sheets, and clumps with a fibrovascular stroma. A Grimelius stained section was strongly positive on virtually all cells (grade I) (Fig. 1A). TEM showed most cells to be stuffed with neurosecretory granules (grade I) mostly with a dense core and narrow halo and of very variable size (Fig. 2A).

Patient 2, a female age 28 yr, had a malignant pheochromocytoma known from previous resection of left adrenal gland. The CT scan showed an abnormal area in the liver which measured 1 × 0.5 × 1 cm. The biopsy taken from this metastatic lesion showed similar histologic features to the above. A Grimelius stained section was strongly positive on all cells (grade 1). TEM showed most cells full of neurosecretory granules (grade 1) with a dense core and narrow halo and of variable size.

TABLE 1
Comparison of Biochemical, Histopathologic, and Scintigraphic Data in Pheochromocytomas and Paragangliomas

Patient no.	Clinical condition	Plasma NE* pmol/ml	Plasma E† pmol/ml	Urinary VMA‡ μmol/24 hr	Urinary MET§ μmol/24 hr	Neurosecretory granules grade (TEM)** and (LM)¶	% Uptake of [^{123}I] MIBG/g tumor
1	Malignant paraganglioma	26	0.21	70	25	I	0.14
2	Malignant pheochromocytoma	3.87	0.72	120	22	I	0.13
3	Pheochromocytoma	67	1.2	86	28	II	0.01
4	Paraganglioma	3.37	0.23	29	6	II	0.02
5	Pheochromocytoma	17.16	1.46	66	44	II	0.01
6	Ganglioneuroblastoma	2.14	0.38	68	4	III	0.001
	Normal range	0.6–2	0.05–0.6	10–35	<5	—	—

*NE = Norepinephrine.
†E = Epinephrine.
‡VMA = Vanillyl mandelic acid.
§MET = Metanephrines.
¶LM = Light microscopy.
**TEM = Transmission electron microscopy.

Patient 3, a female age 14 yr, had a solitary left sided pheochromocytoma. CT showed a mass measuring 4.2 × 3.3 × 1.7 lying medial to the upper pole of the kidney; after injection of i.v. contrast this mass enhanced peripherally but remained lucent centrally. Histologically it appeared similar to the above. A Grimelius stain showed many positive cells (grade II) (Fig. 1B). TEM showed moderate numbers of cells containing neurosecretory granules (grade II) with dense cores and large lucent haloes typical of norepinephrine (Fig. 2B).

Patient 4, a female age 17 yr, had a solitary extra-adrenal paraganglioma removed from the retroperitoneum. CT showed a 3.2 × 3.8 × 3 cm mass in the region of the right sympathetic chain below and slightly behind the pancreas; it was shown to be highly vascular after contrast. A Grimelius stain showed many positive cells (grade II), and TEM showed moderate numbers of cells, containing neurosecretory granules (grade II) measuring 133 nm average diameter with dense cores and narrow haloes.

Patient 5, a female age 49 yr, had a solitary left-sided pheochromocytoma. CT showed a soft-tissue mass measuring 5.2 × 1.5 × 6 cm; after i.v. contrast rim enhancement was seen; the center of the mass appeared to contain fluid. Histologically it was very similar to Patients 1 and 3. Grimelius staining was again grade II

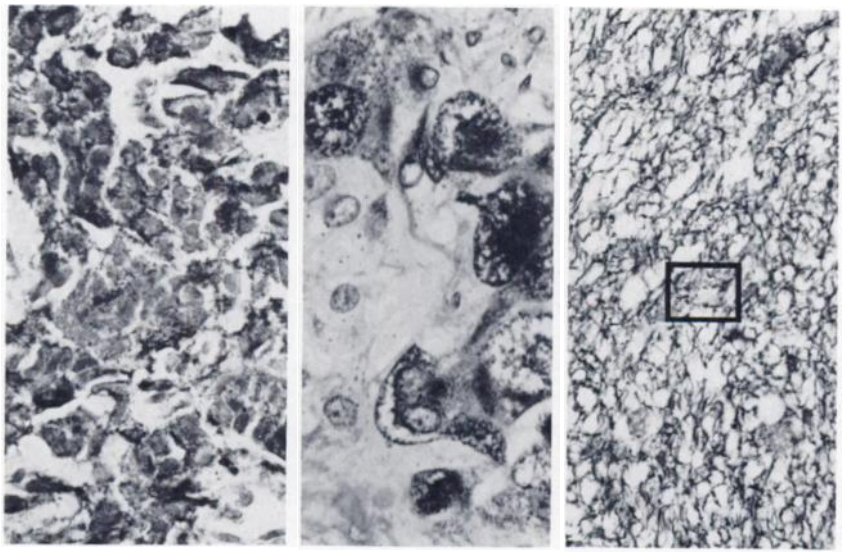

FIGURE 1
A: Patient 1. Grade I Grimelius positivity. B: Patient 3. Grade II Grimelius positivity. Though individual cells stain even more strongly than in (A) fewer cells per unit area are positive. C: Patient 6. Grade III Grimelius positivity. Only occasional cells contain positive cytoplasmic granules (boxed area). Nerve fibers are responsible for the background staining. All photos × 500.

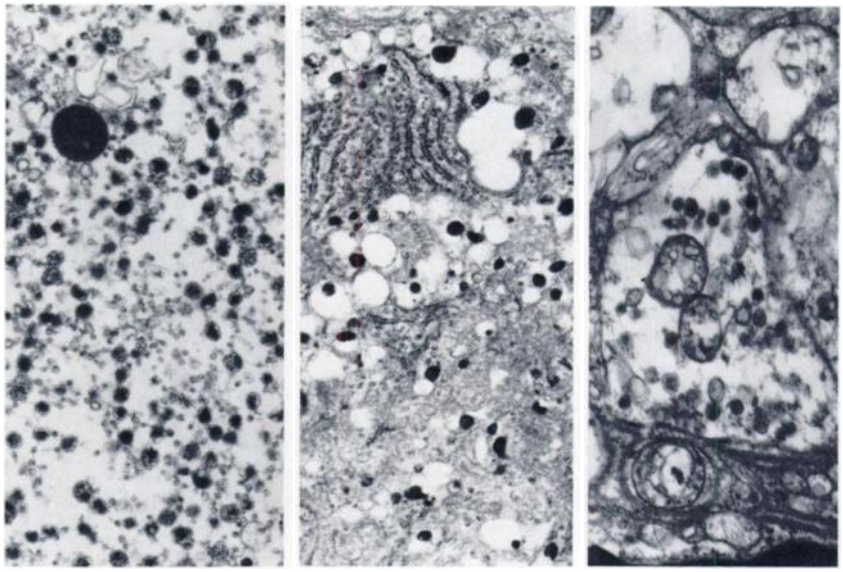

FIGURE 2
A: Patient 1. Grade I transmission electron microscopic (TEM) positivity. These neurosecretory granules mostly have a dense core, a narrow halo and are of very variable size. B: Patient 3. Grade II TEM positivity. Fewer neurosecretory granules than in (A) with dense cores and broad lucent halos typical of norepinephrine. C: Patient 6. Grade III TEM positivity. One of very few such fields showing a nerve fiber with neurosecretory granules 50–100 nm in diameter as expected in nerve endings. All micrographs × 20,000.

and TEM also grade II for the numbers of granules which averaged 160 nm in diameter with thin dense cores and narrow halos.

Patient 6, a male age 19 yr, had a mass in the region of the right adrenal. CT showed a mass measuring 6 × 6 × 4.2 cm, after i.v. contrast the periphery of this mass showed enhancement with two lucent areas placed centrally. Pathologic examination of the operative specimen showed a partly cystic tumor weighing 75 g. Most sections showed numerous ganglion cells set in a neurofibrillar background, but occasional sections also contained foci of immature neuroblasts. These are the appearances of a ganglioneuroblastoma. A Grimelius stain showed occasional cells containing positive granules (grade III) (Fig. 1C) in both mature and immature areas of the tumor. TEM showed mostly nerve fibers and only scattered nuclei. The only neurosecretory granules seen were in scattered nerve fibers (grade III). These granules measured from 50 to 100 nm in diameter as expected in nerve endings (Fig. 2C).

In Patients 1 and 2, [^{123}I]MIBG revealed metastases in lung, liver, and bones not shown by CT and ultrasonography. In Patient 1, uptake in the mediastinal metastases (Fig. 3A) was 0.14% of the injected dose per gram of tumor tissue and corresponded to grade I on histopathology. In Patients 3, 4, and 5 the uptake in the tumor varied between 0.01% and 0.02% per gram of tumor tissue (Fig. 3B) and the tumors corresponded to grade II on histopathological examination. In Patient 6 the tumor uptake was low, 0.001% per gram of tumor tissue (Fig. 3C) and corresponded to grade III on histopathology.

The table shows the percentage uptake of [^{123}I]MIBG by each of the tumors, the plasma levels of epinephrine and norepinephrine and urinary levels of metanephrine and vanillylmandelic acid. The uninvolved adrenal medulla was visualized in all cases in the posterior view (Fig. 3A). The tumor uptake varied between 0.001% and 0.14% of the injected dose and there is a direct proportional correlation between the percentage uptake of [^{123}I]MIBG and the number of neurosecretory granules in tumor cells. As the illustrations show, this correlation is easier to make on electron microscopy than light microscopy. There is poor correlation between the plasma levels of catecholamines and the percentage uptake of [^{123}I]MIBG and the number of neurosecretory granules.

DISCUSSION

The mechanism underlying the uptake and storage of [^{123}I]MIBG has been identified. Two shared uptake mechanisms are present (*3*): one is a sodium dependent process which is characterized by high affinity, low capacity, saturability, and temperature and ouabain sensitivity. It can be inhibited by cocaine and dismethylimipramine thus fulfilling the criteria for the neuronal uptake 1 system. The second mechanism is sodium independent and is demonstrated to be a passive diffusion process. The relative role of each uptake system is dependent on the concentration of MIBG present. However, uptake 1 is the predominant mechanism if MIBG concentrations are low, as is the case with a bolus injection of [^{123}I]MIBG presently used for imaging. With both mechanisms the storage of MIBG is in the catecholamine containing neurosecretory granules. In concordance with these phenomena we have found that the uptake of [^{123}I]MIBG by paragangliomas and related tumor is proportional to the number of neurosecretory granules in the tumor cells.

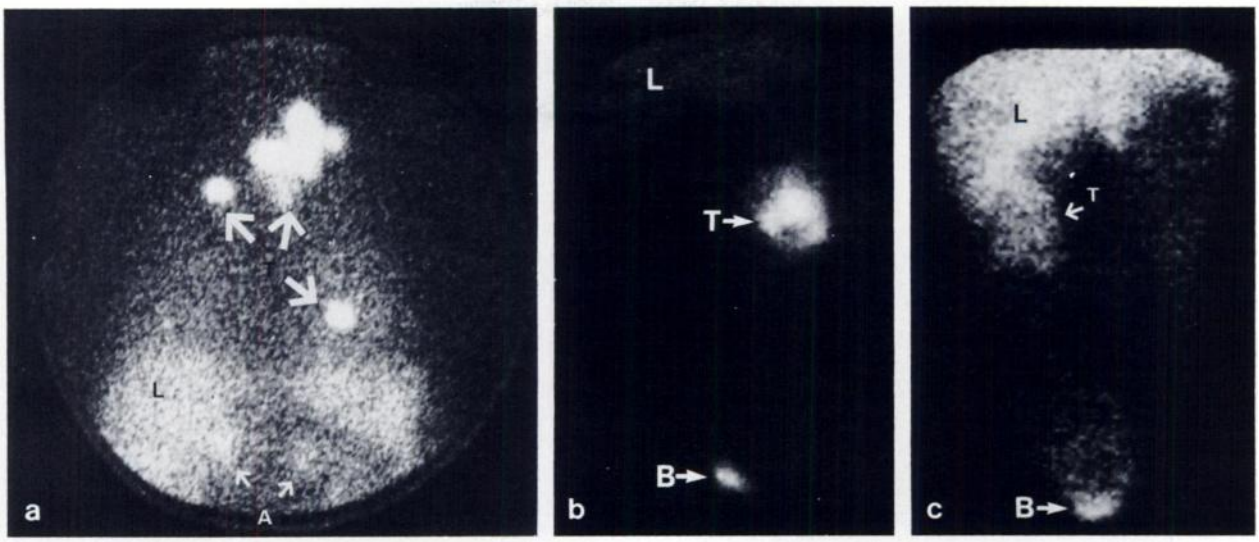

FIGURE 3
[^{123}I]MIBG uptake at 22 hr. (T) = Tumor, (L) = Liver, (B) = Bladder, (A) = Uninvolved adrenals. a: Patient 1. Posterior image of the chest; Paraganglioma metastases in mediastinum and lung; uptake by the whole tumor in the mediastinal metastases is 2% of the injected dose. Grade I on microscopy. b: Patient 3. Anterior image of the pelvis; left phaeochromocytoma; whole tumor uptake is 0.3% of the injected dose. Grade II on microscopy. c: Patient 6. Anterior image of the pelvis; Ganglioneuroblastoma; whole tumor uptake is 0.1% of the injected dose. Grade III on microscopy.

The uninvolved adrenal medulla is not commonly visualized when diagnostic imaging is carried out with [^{131}I]MIBG but it is visualized with [^{123}I]MIBG, and normal adrenal uptake can be quantitated (*14*). This visualization can be explained on the basis of the superior imaging characteristic of ^{123}I when compared with ^{131}I. Iodine-123 has a near optimum photon energy of 159 keV for imaging, combined with a short half-life of 13.2 hr, a lack of particulate radiation, and better radiation dosimetery per μCi administered. This allows the administration of up to 10 mCi of [^{123}I]MIBG, as this amount of activity gives a radiation dose similar to 0.5 mCi of [^{131}I]MIBG (*15*).

The lack of a relationship between the uptake of [^{123}I]MIBG and the plasma and urinary levels of catecholamines has also been observed by Shapiro et al. (*16*). This possibly reflects the well-known phenomenon that the number of neurosecretory granules in endocrine tumors is not a good indicator of the secretory status of that tumor, but rather indicates the amount of hormone stored. High MIBG uptake thus indicates that a particular tumor is likely to contain abundant hormone. It is possible that paragangliomas with abundant stored hormone might release more hormone than those with fewer granules on mechanical stimulation such as pressure induced by posture or operative handling. Also a high uptake of [^{123}I]MIBG by the tumor might well prove of value in predicting a likely response in cases where therapy with [^{131}I]MIBG is considered.

Apart from mechanical factors, little is known about the mechanism of catecholamine release from paragangliomas. There is no pharmacological evidence that these tumors are innervated (*17,18*) hence the release of catecholamines into the plasma is almost certainly not under the influence of neuronal stimulation. Production of hormone, its storage and release are presumably relatively autonomous in these tumors but what governs whether the excess hormone is released or stored is quite unknown. We have shown that the capacity of the normal adrenal medulla to take up [^{123}I]MIBG is not affected by the concomitant presence of an actively secreting paraganglioma (*19*). Thus the uptake and storage of MIBG by the normal adrenal medulla does not appear to be affected by a catecholamine governed feedback mechanism.

In conclusion, this study shows that the percentage uptake of [^{123}I]MIBG by paragangliomas and related tumors correlates well with the number of catecholamine containing granules in tumor cells; this in vivo technique reflects the storage status of these tumors better than measurements of plasma and urinary catecholamines levels.

NOTE

* (Siemens ZLC) Searle-Siemens Medical Systems, Inc., Des Plaines, IL.

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