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Active Uptake and Extravesicular Storage of *m*-Iodobenzylguanidine in Human Neuroblastoma SK-N-SH Cells[1]

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ABSTRACT

Radio-iodinated *m*-iodobenzylguanidine (MIBG), an analogue of the neurotransmitter norepinephrine (NE), is increasingly used in the diagnosis and treatment of neural crest tumors. Active uptake and subsequent retention of MIBG and NE was studied in human neuroblastoma SK-N-SH cells. Neuron-specific uptake of [^{125}I]MIBG and [^{3}H]NE saturated at extracellular concentration of 10^{-6} M and exceeded by 20–30-fold that by passive diffusion alone. A minimum of 50% of accumulated MIBG remained permanently stored but the SK-N-SH cells were incapable of retaining recaptured [^{3}H]NE. [^{125}I]MIBG was displaced from intracellular binding sites by unlabeled MIBG with 10-fold higher potency than by unlabeled NE. MIBG stored in SK-N-SH cells was insensitive to depletion by the inhibitor of granular uptake reserpine (RSP) and was not precipitated in a granular fraction by differential centrifugation. Only few electron-dense granules were found in these cells by electron microscopy. In contrast, MIBG storage in PC-12 pheochromocytoma cells which contained many storage granules, was sensitive to RSP and part of accumulated drug was recovered in a granular fraction. Accordingly, storage of MIBG in the SK-N-SH neuroblastoma cells is predominantly extravesicular and thus essentially different from that of biogenic amines in normal adrenomedullary tissue or in pheochromocytoma tumors, while sharing with these tissues a common mechanism of active uptake.

INTRODUCTION

Human tumors of neuroectodermal origin such as pheochromocytoma, neuroblastoma, and medullary thyroidoma, have often preserved the capacity of neuronal cells to recapture extracellular NE.[3] MIBG is a functional analog of NE, derived from the neuron-blocking agents bretyllium and guanethidine (1), which competes with biogenic amines for uptake and storage into chromaffin tissues. Radioiodinated [^{131}I]MIBG has been successfully applied for the scintigraphic visualization of adrenergic tissues (2) and neuroendocrine tumors such as pheochromocytoma (3, 4), neuroblastoma (5, 6), and many other tumors of the APUD series (7). Intratumoral radiotherapy with elevated doses has been pioneered in pheochromocytoma (8) and is currently being evaluated in neuroblastoma and carcinoid tumors (9).

Selective uptake of the drug has been studied in bovine adrenomedullary cells (10), human neuroblastoma cells (11), and explants of pheochromocytoma tumors (12). These *in vitro* studies all agree that MIBG enters competent cells predominantly by Uptake-I, a saturable, sodium- and energy-dependent mechanism of catecholamine uptake characteristic of neuronal cells. This uptake is sensitive to specific inhibition by tricyclic antidepressants such as (des)imipramine (13).

Contrary to active uptake, much less is known of the subsequent storage of MIBG. Storage of biogenic amines into chromaffin granules involves an ATP-driven proton pump which is specifically inhibited by the drug reserpine (for reviews, see Refs. 14 and 15). Although it is generally thought that storage of MIBG is similar to that of catecholamines and thus requires intracellular storage vesicles (7, 12, 16), only a fraction of MIBG incorporated by Uptake-I is permanently sequestered in neuroblastoma cells (11). Moreover, the relative contribution of active Uptake-I and of passive diffusion to total uptake and retention in pheochromocytoma (12) and adrenal neurons of the heart (17) is a matter of dispute. In addition, it has been suggested that MIBG may be stored to some extent outside of secretory granules (17). Accordingly, in neuroblastoma the interrelationship between drug uptake and storage, the factors controlling the levels and the biological half-life of retained MIBG as well as the intracellular distribution of the concentrated radiopharmaceutical are poorly understood.

In the present study, we have investigated specific uptake, long-term retention, and the intracellular distribution of radiolabeled MIBG in human SK-N-SH cells as a model for human neuroblastoma.

MATERIALS AND METHODS

Cells and Culture Conditions. Human neuroblastoma SK-N-SH cells and its congeners SH-SY-5Y and SH-EP were obtained by courtesy of Dr. J. Biedler, Memorial Sloan-Kettering Cancer Center, New York, NY. Cell line NB-100 was obtained from Dr. G. Melino, Rome, Italy, and NB-1 from Dr. D. Kerr, Glasgow, UK. Dr. C. Figdor, Department of Immunology of this Institute, provided us with human LA-N_1 and CHP-212. Rat PC-12 pheochromocytoma and mouse N_1E115 neuroblastoma were given to us by Dr. W. Moolenaar, Utrecht, The Netherlands. The murine neuroblastoma lines NB4A3 and Neuro-2A were obtained commercially. All neuroblastoma cell lines were grown in Dulbecco's modification of Eagle medium supplemented with 10% fetal calf serum and antibiotics in a humidified incubator in 6% CO_2. PC-12 was propagated in Ham's F12:Dulbecco's (1:1) with 10% horse and 10% fetal calf serum.

Incorporation Studies. Uptake studies were performed in cells grown in 6-well cluster dishes of 35-mm diameter to a density of 0.5–1.0 × 10^6 cells/well in 3 ml of medium. After incubation in medium with [^{125}I]MIBG or [^{3}H]NE, the monolayers were washed three times with cold PBS and extracted twice with 0.25 ml of 0.5 N perchloric acid for 20 min at 4°C. The acid extracts were combined for liquid scintillation counting and cell layers were dissolved in alkali for protein determination. Concentration dependency of uptake and storage was determined by incubation in 0.1 μCi/ml of radiolabeled MIBG, supplemented with unlabeled drug at the desired final concentration. The inhibitors imipramine (IMP) and reserpine (RSP) were added from 1000-fold concentrates.

For storage studies, the monolayers were incubated with radiolabeled MIBG or NE for the times indicated, washed three times with PBS, and incubated in prewarmed medium with or without additives. Washing with PBS was repeated and acid extraction was performed at indicated postincubation hours.

Differential Centrifugation. Approximately 10^7 SK-N-SH or PC-12 cells, grown in 13.5-cm petri dishes, were incubated for 2 h in 3 × 10^{-8} M [^{125}I]MIBG with or without IMP, washed, and incubated in fresh medium. After 6 h, the cells were harvested by trypsinization and disrupted by homogenization in a loosely fitting Potter-Elvehjem

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[3] The abbreviations used are: NE, norepinephrine; MIBG: *m*-iodobenzylguanidine; IMP, imipramine; RSP, reserpine; PBS, phosphate-buffered saline.

homogenizer for the minimum number of strokes required to release >90% of the cytosolic LDH marker. Cytoplasmic granules were isolated from a 800 × *g* supernatant by centrifugation at 20,000 × *g* for 45 min as described by Oberlechner *et al.* (18). Aliquots of supernatant and pellet were sampled and specific incorporation was calculated using nonspecific uptake in the presence of IMP in corresponding fractions as blanks.

Electron Microscopy. Cells grown in small petri dishes were fixed in glutaraldehyde and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead oxide and photographed for counting of dense core cytoplasmic granules. Granule density was determined in whole cell sections of three independent fixations.

Chemicals. Unlabeled MIBG was synthesized by us as described (19). [^{125}I]MIBG was a gift of Amersham Buchler, Braunschweig, GFR. Formulated reserpine (Serpacil) was obtained by courtesy of Ciba Geigy, Arnhem, The Netherlands. [^{3}H]Norepinephrine was purchased from Amersham, dissolved according to instructions of the supplier and supplemented with unlabeled NE to the desired final concentrations. All other chemicals were purchased from Sigma (St. Louis, MO).

RESULTS

Specific and Nonspecific Uptake of MIBG. Uptake of MIBG by SK-N-SH cells was measured in the presence of various concentrations of imipramine (an inhibitor of Uptake-I, see Ref. 13). The results (Fig. 1) revealed dose-dependent inhibition. MIBG uptake (<5% of controls) at IMP concentrations above 0.6×10^{-6} M was of the same level as determined in the presence of an excess (10^{-4} M) of unlabeled NE and thus fulfilled the criteria of nonspecific uptake by passive diffusion (10). Several other human and murine neuroblastoma lines were screened for specific uptake of MIBG, similarly sensitive to IMP or an excess of unlabeled NE. Only SK-N-SH and its derivatives and NB-100 were positive. NB-1, LA-N_1, CHP-212, N_1E115, NB4A3, and Neuro-2A all showed low levels of incorporation, not affected by the inhibitors. Based on these results, the SK-N-SH cells were selected for further studies.

Effect of Extracellular Concentration. Specific and nonspecific uptake of MIBG were determined as a function of extracellular drug concentration. The results (Fig. 2) revealed that specific uptake saturated at about 10^{-6} M with a half-maximal value of 0.8×10^{-7} M whereas nonspecific incorporation increased linearly with extracellular drug concentration. Moreover, both active and passive uptake saturated in 2–3 h (not shown). Specific and nonspecific uptake of [^{3}H]NE was fully comparable with that of [^{125}I]MIBG. At steady state (incubations for 2 h at 2×10^{-6} M) maximal incorporation amounted to 0.80 nmol/mg P for [^{3}H]NE *versus* 1.10 nmol/mg P for [^{125}I]MIBG. As for MIBG, the uptake of [^{3}H]NE was sensitive to inhibition by IMP.

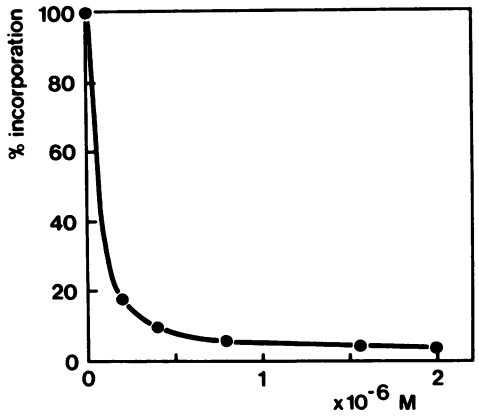

Fig. 1. Inhibition of [^{125}I]MIBG uptake in SK-N-SH cells by imipramine at the indicated concentrations. Incubation time was 2 h in 10^{-8} M radiolabeled MIBG. Mean values from two experiments.

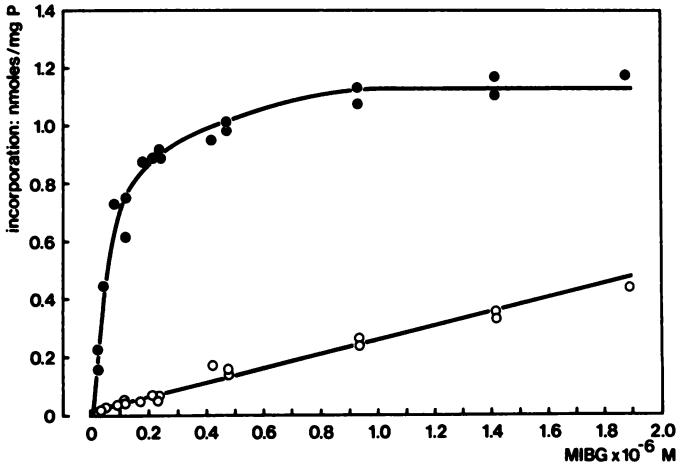

Fig. 2. Specific (●) and nonspecific (○) uptake of [^{125}I]MIBG by SK-N-SH cells as a function of extracellular concentration. Nonspecific uptake was determined in the presence of 1.25×10^{-6} M imipramine and specific uptake was calculated as total-nonspecific. Data from triplicates in five experiments.

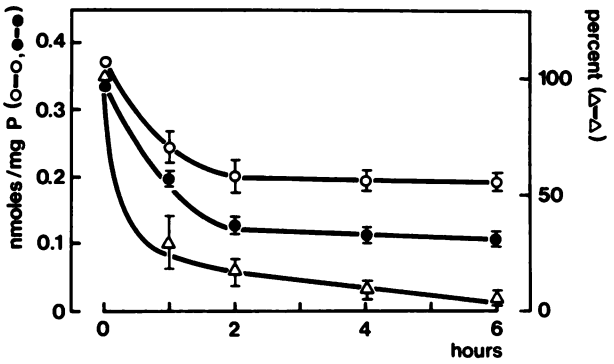

Fig. 3. Retention of [^{125}I]MIBG and [^{3}H]norepinephrine by SK-N-SH cells, preloaded for 2 h in 10^{-8} M [^{125}I]MIBG (○, specific uptake), in 4×10^{-7} M [^{125}I]MIBG plus 1.25×10^{-6} M imipramine (●, nonspecific uptake) or in 2×10^{-6} M [^{3}H]NE (△, represented in % of T_0 value). Mean ± SD from three experiments.

Storage of MIBG and NE. Retention of [^{125}I]MIBG and [^{3}H]-NE by SK-N-SH cells was determined as the residual, cell-bound radioactivity in preloaded cells. Preloading with MIBG for 2 h was performed under two conditions of drug uptake, namely by specific Uptake-I at 10^{-8} M and by passive diffusion at 4×10^{-7} M in the presence of IMP, yielding comparable levels of uptake by either route (compare with Fig. 2). Preloading with ^{3}H-NE was performed at a single concentration of 2×10^{-6} M, *i.e.*, under saturating conditions. About 60% of specifically incorporated MIBG but also a considerable fraction (35%) of drug entering by passive diffusion was retained after removal of drug-containing medium (Fig. 3). However, [^{3}H]NE was not retained to a significant degree by the SK-N-SH cells.

Displacement of stored [^{125}I]MIBG was assayed by postlabeling incubations in graded concentrations of unlabeled MIBG or NE. Unlabeled MIBG removed cell-bound [^{125}I]MIBG about 10-fold more efficiently than did NE (Fig. 4). The difference tended to be larger at low concentrations of the competing drugs and below 10^{-7} M, displacement of stored MIBG by NE was undetectable.

Granular and Extragranular Storage. The inability of SK-N-SH cells to retain [^{3}H]NE (compare with Fig. 3) suggested that these cells lack functional granules for the storage of accumulated neurotransmitter, protecting it from degradation in the cytosolic compartment. Therefore, SK-N-SH cells were com-

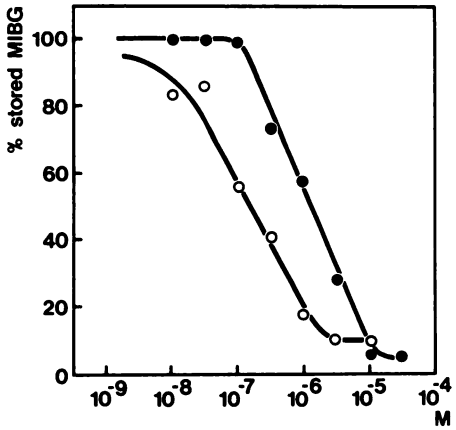

Fig. 4. Displacement of stored [^{125}I]MIBG from SK-N-SH cells, preloaded for 2 h, washed, and reincubated for 4 h with unlabeled MIBG (○) or NE (●). From triplicates in two experiments.

Table 1 *Subcellular distribution of stored [^{125}I]MIBG in SK-N-SH neuroblastoma and PC-12 pheochromocytoma cells*

Cells were loaded by incubation for 2 h in [^{125}I]MIBG-containing medium, washed, and incubated for 6 h in regular tissue culture medium. Cytosols, prepared as described in "Materials and Methods," were centrifuged for 45 min at 20,000 × *g*, and then the radioactivity of supernatant and pellet was determined.

	SK-N-SH		PC-12	
Experiment	Supernatant	Pellet (%)	Supernatant	Pellet (%)
I	993,800	24,700 (2.4)	693,500	633,900 (47.7)
II	936,250	37,390 (3.8)	337,390	209,530 (38.3)

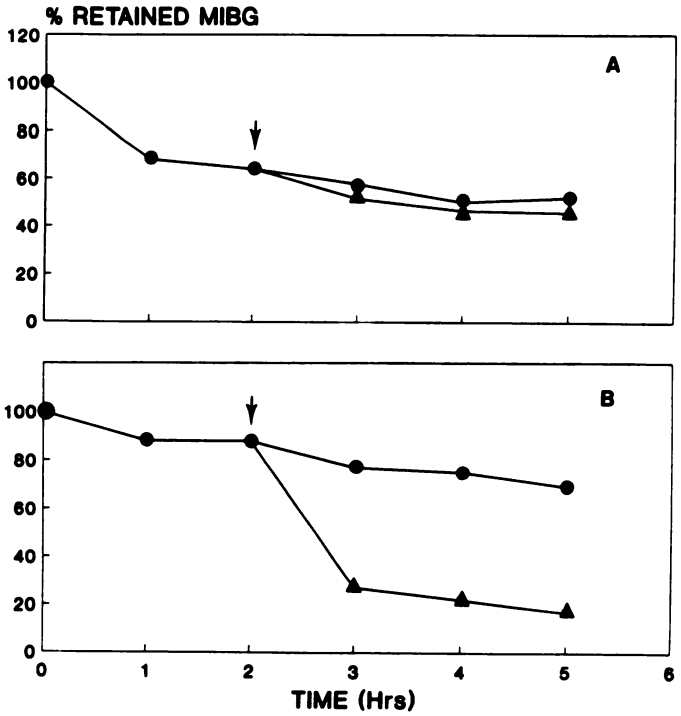

Fig. 5. The effect of reserpine (2×10^{-6} M) on the retention of [^{125}I]MIBG by SK-N-SH (*A*) and PC-12 (*B*) cells. Reserpine was added as indicated by *arrows* 2 h after washing the cultures, preloaded for 2 h in 2×10^{-8} M [^{125}I]MIBG. Mean values from triplicate determinations of control (●) and RSP-treated (▲) cultures.

pared with PC-12 pheochromocytoma for various properties associated with granular storage of catecholamines. Electron microscopic investigations revealed that electron dense storage granules were sparse in SK-N-SH cells, amounting to 8–12 granules per whole cell section as compared to 80–110 in PC-12. Table 1 summarizes the results of differential centrifugation experiments. About 40% of [^{125}I]MIBG stored by PC-12 cells could be recovered in a 20,000 × *g* fraction as compared 2–4% in similar experiments with SK-N-SH. Finally, the effect of the granular uptake inhibitor reserpine, which inhibits the uptake of catecholamines (14, 15) and MIBG (16) by isolated chromaffin granules, was tested. RSP inhibited the uptake of MIBG in PC-12 cells by about 50% (not shown) and largely depleted these cells from stored MIBG when given at any postlabeling time. On the other hand, the drug had no effect on MIBG uptake in SK-N-SH cells and caused only marginal depletion of stored MIBG. Fig. 5 represents a typical set of experiments in which RSP was added 2 h after removal of [^{125}I]MIBG containing medium. Rapid depletion of ~75% of stored drug was observed in PC-12 whereas RSP exerted only a marginal effect on SK-N-SH cells.

DISCUSSION

The specific uptake and retention of the novel radiopharmaceutical MIBG was studied in SK-N-SH neuroblastoma cells. The results (Figs. 1 and 2) confirmed previous studies with these and related cells, namely that MIBG accumulates *via* the neuron-specific Uptake-I mechanism up to 30-fold over the incorporation by passive diffusion alone (10–12). Moreover, it was established that this accumulation saturated in about 2 h and at extracellular concentration of 10^{-6} M.

Active Uptake-I, however, was not a necessary nor a sufficient condition for subsequent storage. Passively incorporated MIBG could be retained to a considerable extent whereas actively incorporated NE was not retained at all (Fig. 3).

It is generally believed that MIBG, like natural catecholamines, is exclusively stored in the specialized secretory granules of chromaffin tissues and tumors derived of them (7, 12, 16). However, in the SK-N-SH cells no evidence of such a storage mechanism was obtained. Reserpine at concentrations which effectively block granular translocation of MIBG (*i.e.*, 2×10^{-6} M; Ref. 16) had minor effects on [^{125}I]MIBG uptake and storage by SK-N-SH cells while markedly depleting PC-12 pheochromocytoma (Fig. 5). In the latter reference cells the involvement of secretory granules in uptake and storage of catecholamines and the depleting effect of RSP on catecholamine stores has been well documented (20). Stored MIBG could not be precipitated in a particulate fraction by ultracentrifugation of the cytosol of the neuroblastoma cells using methods which did precipitate about 40% of MIBG stored by PC-12 cells (Table 1), similar to natural catecholamines from broken PC-12 pheochromocytoma tumors cells (18). This evidence, together with the observation of few cytoplasmic storage granules, strongly supports the notion that the storage of [^{125}I]-MIBG in SK-N-SH cells is predominantly extravesicular.

Catecholamines are stored in complexes with ATP and chromogranin A (21), an element of the granular matrix (22, 23). NMR studies have revealed that MIBG mimicks catecholamines in the interaction with ATP *in vitro*.[4] Whether in SK-N-SH cells the radiopharmaceutical is sequestered in the cytoplasm either in complexes with diffusely distributed chromogranin or with as yet unidentified macromolecules remains to be established. The failure of SK-N-SH cells to retain [^{3}H]NE by a mechanism similar to that involved in MIBG storage may be due to the apparently low affinity of NE for these hypothetical cytoplasmic binding sites (Fig. 4) and to rapid degradation of extravesicular NE by cytoplasmic enzymes. The metabolic

[4] J. P. Grivet, communicated at the International Workshop in MIBG and Catecholamines, Tours, France, March, 1987.

stability of MIBG relative to that of NE has been well documented (25, 26).

In human neuroblastoma immunohistochemical staining for chromogranin A is mostly weak and focal, associated with few secretory granules (24, 27). Yet, these tumors are successfully portrayed by [^{131}I]MIBG scintigraphy in the majority of patients. Apparently, the abundant presence of storage granules is not a necessary prerequisite for long term retention of the radiopharmaceutical in this tumor category. The present study demonstrates for the first time extensive extravesicular storage of MIBG in a single *in vitro* model of human neuroblastoma. Obviously, nongranular storage will have an important, although as yet unknown, impact on the biological half-life of MIBG and the susceptibility to pharmacological modulation of its retention in neuroblastoma tumors. However, it remains to be established in forthcoming studies whether this mechanism contributes significantly to MIBG storage by clinical tumors.

The kinetics of MIBG uptake, being quite similar to those reported in other studies (10–12), suggest that optimal *in vivo* loading of neuroblastoma tumors will be obtained with short-time administrations and at plasma concentrations as high as 10^{-6} M. Neither condition seems to be met with in current clinical practice. In most studies, [^{131}I]MIBG is administered by infusions extending from 4 to 30 h. Moreover, from available pharmacokinetic data, plasma concentrations well below 10^{-7} M can be calculated for patients receiving therapeutic doses of [^{131}I]MIBG (26).[5] Such values are more than 10-fold below the saturating concentration and lie on the steep part of the curves relating extracellular concentration with uptake and storage (Fig. 2). From the present and other studies, it appears that the use of radioiodinated MIBG in the therapy of neuroblastoma is amenable to significant improvements at several levels. In this laboratory a realistic tumor model of SK-N-SH cells in nude mice has been recently developed (28) in which some of the conclusions emerging from the *in vitro* studies will be tested.

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REFERENCES

1. Wieland, D. M., Wu, J., Brown, L. E., Mangner, T. E., Swanson, D. P., and Beierwaltes, W. H. Radiolabeled adrenergic neuron blocking agents: adrenomedullary imaging with [^{131}I]iodobenzylguanidine. J. Nucl. Med., *21:* 349–353, 1980.
2. Wieland, D. M., Brown, L. E., Tobes, M. C., Rogers, W. L., Marsh, D. D., Manger, T. J., Swanson, D. P., and Beierwaltes, W. H. Imaging the primate adrenal medulla with [^{123}I] and [^{131}I] metaiodobenzylguanidine: concise communication. J. Nucl. Med., *22:* 358–364, 1981.
3. Sisson, J. C., Frager, M. S., Valk, T. W., Gross, M. D., Swanson, D. P., Wieland, D. M., Tobes, M. C., Beierwaltes, W. H., and Thompson, N. W. Scintigraphic localization of pheochromocytoma. N. Engl. J. Med., *305:* 12–17, 1981.
4. Shapiro, B., Copp, J. E., Sisson, J. C., Eyre, P. L., Wallis, J., and Beierwaltes, W. H. 131-I-metaiodobenzylguanidine for the locating of suspected pheochromocytoma: experience with 400 cases (441 studies). J. Nucl. Med., *26:* 576–585, 1985.
5. Treuner, J., Feine, U., Niethammer, D., Mueller-Schauenburg, W., Meinke, J., Eibach, E., Dopfer, R., Klingebiel, T. H., and Grumbach, S. T. Scintigraphic imaging of neuroblastoma with m-[131]iodobenzylguanidine. Lancet, *1:* 333–334, 1984.
6. Hoefnagel, C. A., Voûte, P. A., De Kraker, J., and Marcuse, H. R. Total-body scintigraphy with 131-I-metaiodo-benzylguanidine for detection of neuroblastoma. Diagn. Imag. Clin. Med., *54:* 21–27, 1985.
7. Von Moll, L., McEwan, A. J., Shapiro, B., Sisson, J. C., Gross, M. D., Lloyd, R., Beals, E., Beierwaltes, W. H., and Thompson, N. W. Iodine-131 MIBG scintigraphy of neuroendocrine tumors others than pheochromocytoma and neuroblastoma. J. Nucl. Med., *28:* 979–988, 1987.
8. Sisson, J. C., Shapiro, B., Beierwaltes, W. H., *et al.* Treatment of malignant pheochromocytoma with a new radiopharmaceutical. Trans. Assoc. Am. Phys., *96:* 209–217, 1983.
9. Hoefnagel, C. A., Voûte, P. A., De Kraker, J., and Marcuse, H. R. Radionuclide diagnosis and treatment of neural crest tumors using 131-I- metaiodobenzylguanidine. J. Nucl. Med., *28:* 308–314, 1987.
10. Jaques, S., Tobes, M. C., Sisson, J. C., Baker, J. A., and Wieland, D. Comparison of the sodium dependency of uptake of meta-iodobenzylguanidine and norepinephrine into cultured bovine adrenomedullary cells. Mol. Pharmacol., *26:* 539–546, 1984.
11. Buck, J., Bruchelt, G., Girgert, R., Treuner, J., and Niethammer, D. Specific uptake of *m*-[^{125}I]iodobenzylguanidine in the human neuroblastoma cell line SK-N-SH. Cancer Res., *45:* 6366–6370, 1985.
12. Jaques, S., Tobes, M. C., and Sisson, J. C. Sodium dependency of uptake of norepinephrine and *m*-iodobenzylguanidine into cultured human pheochromocytoma cells: evidence for uptake-one. Cancer Res., *47:* 3920–3928, 1987.
13. Arita, M., Wada, A., Takara, H., and Izuma, F. Inhibition of ^{22}Na infux by tricyclic and tetracyclic antidepressants and binding of [^{3}H]imipramine in bovine adrenal medullary cells. J. Pharmacol. Exp. Therap., *243:* 342–348, 1987.
14. Rudnick, G. ATP-driven H^+ pumping into intracellular organelles. Ann. Rev. Physiol., *48:* 403–413, 1986.
15. Johnson, R. G. Accumulation of biological amines into chromaffin granules: a model for hormonal and neurotransmitter transport. Physiol. Rev., *68:* 232–307, 1988.
16. Gasnier, B., Roisin, M-P., Scherman, D., Coornaert, S., Desplanches, G., and Henry, J-P. Uptake of meta-iodobenzylguanidine by bovine chromaffin granule membranes. Mol. Pharmacol., *29:* 275–280, 1986.
17. Sisson, J. C., Wieland, J. M., Sherman, P., Manger, T. J., Tobes, M. C., and Jacques, S. Metaiodobenzylguanidine as an index of the adrenergic nervous system integrity and function. J. Nucl. Med., *28:* 1620–1624, 1987.
18. Oberlechner, E., Westhead, E., Neuman, B., Schmidt, W., Fischer-Colbrie, R., Weber, A., Sperk, G., and Winkler, H. Characterization of catecholamine-storage organelles in transplantable pheochromocytoma and adrenal glands of rats. J. Neurochem., *38:* 615–624, 1982.
19. Smets, L. A., Bout, B., and Wisse, J. Cytotoxic and antitumor effects of the norepinephrine analogue meta-iodo-benzylguanidine (MIBG). Cancer Chemother. Pharmacol., *21:* 9–13, 1988.
20. Green, L. A., and Rein, G. Release, storage and uptake of catecholamines by a clonal cell line of nerve growth factor (NGF) responsive pheochromocytoma cells. Brain Res., *128:* 247–263, 1977.
21. DaPurda, M., Berneis, K. H., and Pletscher, A. Storage of catecholamines in adrenal medullary granules: formation of aggregates with nucleotides. Life Sci., *10:* 639–646, 1971.
22. Winkler, H. The composition of adrenal chromaffin granules: an assessment of controversial results. Neurosci., *1:* 65–80, 1976.
23. Aunis, D., Hesketh, J. E., and Devilliers, G. Immunohistochemical and immunocytochemical localization of myosin, chromogranin A and dopamine-β-hydroxylase in nerve cells in culture and in adrenal glands. J. Neurocytol., *9:* 255–274, 1980.
24. Wilson, B. S., and Lloyd, R. V. Detection of chromogranin in neuroendocrine cells with a monoclonal antibody. Am. J. Pathol., *115:* 458–468, 1984.
25. Manger, T. J., Tobes, M. C., Wieland, D. W., Sisson, J. C., and Shapiro, B. Metabolism of Iodine-metaiodobenzylguanidine in patients with metastatic pheochromocytoma. J. Nucl. Med., *27:* 37–44, 1986.
26. Ehninger, G., Klingebiel, T., Kumbier, I., Schuler, U., Feine, U., Treuner, J., and Walter, H. D. Stability and pharmacokinetics of *m*-[^{131}I]iodobenzylguanidine in patients. Cancer Res., *47:* 6147–6149, 1987.
27. Bomanjii, J., Levinson, W. D., Flatman, T., Horne, T., Bouloux, P. M-G., Ross, G., Britton, K. E., and Besser, G. M. Uptake of Iodine-123 MIBG by pheochromocytomas, paragangliomas, and neuroblastomas: histopathological comparison. J. Nucl. Med., *28:* 937–978, 1987.
28. Huiskamp, R., Hoefnagel, C. A., and McVie, J. G. Xenographted human neuroblastoma in nude mice: an animal model for 131-I-MIBG therapy. (Abstract 1028). Proc. ASCO, *7:* 265, 1988.

[5] R. Huiskamp, personal communication.

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