Evaluation of Metaiodobenzylguanidine Uptake by the Norepinephrine, Dopamine and Serotonin Transporters

Jerry V. Glowniak, John E. Kilty, Susan G. Amara, Beth J. Hoffman and Fredrick E. Turner

Nuclear Medicine Service, Veterans' Affairs Medical Center and The Vollum Institute, Oregon Health Sciences University, Portland, Oregon; Program in Neuroscience, Yale University School of Medicine, New Haven, Connecticut; and the Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, Maryland

Metaiodobenzylguanidine (MIBG) is taken up by sympathetic neurons, but the precise mechanism of uptake has not been elucidated. Uptake of monoamines by presynaptic neurons is mediated by plasma membrane proteins, the monoamine transporters. The human norepinephrine transporter (hNET), the bovine dopamine transporter (bDAT) and the rat serotonin transporter (r5HTT) have been cloned, sequenced and expressed in various cell lines. This study involves the measurement of MIBG uptake by cell lines that have been transfected with complementary DNAs encoding these monoamine transporters. At 20 n*M* MIBG, hNET transfected cells demonstrate a ninefold greater uptake of MIBG than nontransfected cells. MIBG uptake in hNET transfected cells is inhibited by 3×10^{-6} *M* norepinephrine (87% inhibition) and by hNET transport inhibitors: 10^{-7} *M* desipramine (94% inhibition) and 10^{-7} *M* mazindol (97% inhibition). hNET transfected cells exhibit a K_m for MIBG transport of 264 n*M*. Percent nonspecific uptake rises with increasing concentrations of MIBG while specific uptake is saturable. There is no significant uptake by bDAT or r5HTT. The NET appears to be responsible for the specific uptake of MIBG.

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Metaiodobenzylguanidine (MIBG) has been found to be a useful agent for the evaluation of the sympathetic innervation of the heart. Cardiac uptake and washout of MIBG parallel sympathetic nerve integrity and function. Surgical denervation (*1–3*), chemical sympathectomy with 6-hydroxydopamine (*3–5*) or severe autonomic neuropathy (*6*) produce marked decreases in cardiac MIBG uptake. Fasting, which causes a fall in cardiac norepinephrine (NE) turnover (a measure of cardiac sympathetic nerve function) (*7*), decreases cardiac MIBG washout (*4*).

The mechanism of MIBG uptake by sympathetic neurons has been studied in vitro through the use of bovine adrenomedullary chromaffin cells. Adrenomedullary chromaffin cells have an uptake and storage mechanism similar to sympathetic neurons and therefore serve as an in vitro model for the study of NE transport by sympathetic neurons. The uptake of MIBG in adrenomedullary chromaffin cells is qualitatively similar to the uptake of NE. Two components of MIBG uptake have been identified: (1) specific uptake that is saturable, has a high affinity for MIBG and is sodium-, temperature- and energy-dependent and (2) nonspecific uptake that is nonsaturable, has a low affinity for MIBG and is not sodium-, temperature- or energy-dependent (*8*). Specific uptake of MIBG is blocked by cocaine and desipramine (DMI, a specific inhibitor of NE uptake) in a manner similar to that for NE (*9*).

Previous studies have suggested that uptake of extracellular monoamines (NE, dopamine and serotonin) by monoaminergic neurons is due to specific proteins, the monoamine transporters, which reside in the cell membrane. Within the last 2 yr, the human norepinephrine transporter (hNET) (*10*), the rat (*11–13*) and bovine (*14*) dopamine transporters (rDAT and bDAT) and the rat serotonin (5-hydroxytryptamine, 5HT) transporter (r5HTT) (*15,16*) have been cloned, sequenced and expressed in various cell lines by transfection of complementary DNA (cDNA). In this study, we evaluated MIBG uptake in cell lines expressing the monoamine transporters.

MATERIALS AND METHODS

Cloning and Expression of the Monoamine Transporters

The cDNA encoding hNET was previously cloned from SK-N-SH cells, a human neuroblastoma cell line (*10*). A cDNA for bDAT was generated from mRNA from bovine midbrain, a region rich in mRNA for the DAT (*14*). The r5HTT cDNA clone was prepared from mRNA isolated from a rat basophilic leukemia cell line, RBL 2H3 (*15*). In the present study, hNET was transiently expressed in HeLa cells, a human uterine cancer cell line. bDAT and r5HTT (*17*) have been stably expressed in CV-1 cells (green monkey kidney cells).

MIBG Uptake by the Monoamine Transporters

Iodine-125 MIBG uptake by hNET was evaluated in HeLa cells transiently transfected with cDNA encoding hNET. Plas-

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For correspondence and reprints contact: J.V. Glowniak, MD (115), VA Medical Center, PO Box 1034, Portland, OR 97207.

mids containing the cDNA of hNET were introduced into HeLa cells using a vaccinia virus/T7 polymerase transient expression system as previously described (*10,11*). To measure nonspecific uptake, MIBG uptake was also measured in HeLa cells transfected with the same plasmid which did not contain the hNET insert (mock transfected cells). Transfection was carried out in 24 well plates (400,000 cells/well) incubated for 12 hr at 37°C. Cells were then incubated for 20 min with 20 n*M* ^{125}I MIBG. All incubations were performed simultaneously in triplicate. After 20 min, cells were washed three times in ice cold buffer and counted in a gamma counter.

MIBG uptake was also measured in hNET transfected cells in the presence of various compounds: 10^{-7} *M* NE, 3×10^{-6} *M* NE, 10^{-7} *M* mazindol [a specific inhibitor of hNET, $K_i = 1.36 \times 10^{-9}$ *M* for NE transport (*10*)], 10^{-7} *M* DMI ($K_i = 3.88 \times 10^{-9}$ *M* for NE transport (*10*)) and 10^{-7} *M* paroxetine [a specific inhibitor of the 5HTT, $K_i = 3.12 \times 10^{-7}$ *M* for NE transport by hNET (*10*)]. In another set of experiments, ^{125}I MIBG uptake was measured in hNET transfected and mock transfected HeLa cells incubated with various concentrations of MIBG (0.1 n*M* to 50 μ*M*) in order to measure the K_m of MIBG transport for hNET. Uptake in mock transfected cells was subtracted from uptake in hNET transfected cells to give specific uptake (i.e., uptake by hNET).

MIBG uptake by bDAT was evaluated in CV-1 cells stably transfected with bDAT (CV-1 m3.13 cell line). Nonspecific uptake in CV-1 cells was determined by measuring MIBG uptake in CV-1 cells not transfected with bDAT. Nontransfected CV-1 cells do not express monoamine transporters. Cells were incubated in 24 well plates, 100,000 cells per well, with MIBG concentrations ranging from 1.22 n*M* to 502 μ*M* for 5 min. Two experiments were performed in triplicate at each concentration of MIBG in transfected and nontransfected cells. At the end of the incubation, cells were washed three times in ice cold buffer and counted in a liquid scintillation counter. Identical incubations were also performed in the presence of 1 μ*M* of GBR12909, a specific blocker of bDAT (K_i for dopamine, 52 n*M*). As a positive check to ensure bDAT expression, another group of cells was incubated with 105 n*M* H-3 dopamine in both CV-1 transfected and nontransfected cells with and without GBR12909. After incubation and washing, cells were counted in a liquid scintillation counter. Specific MIBG uptake was defined as uptake in transfected CV-1 cells minus uptake in nontransfected cells.

MIBG uptake by r5HTT was evaluated in CV-1 cells stably transfected with r5HTT (*15*). MIBG uptake was measured in transfected and nontransfected cells in a manner similar to the experiments with bDAT. One hundred thousand cells were incubated with MIBG concentrations ranging from 11 n*M* to 550 μ*M* for 5 min. Identical incubations were performed in the presence of 10 μ*M* fluoxetine. Fluoxetine is a specific inhibitor of the 5HTT (K_i for serotonin, 33 n*M*). Specific uptake was defined as uptake in transfected CV-1 cells minus uptake in nontransfected cells. It is possible that if only a small number of transporters were expressed, a large amount of nonspecific uptake would obscure specific uptake by r5HTT. To exclude this possibility, MIBG uptake was measured in JAR cells (human choriocarcinoma cells) and in RBL 2H3 cells (rat basophilic leukemia cells), both of which are known to express moderate to high levels of the 5HTT (*18,19*). MIBG uptake was measured in these cell lines at MIBG concentrations ranging from 7.9 n*M* to 101 μ*M*. Since 5HTTs are endogenously expressed in these cells, it is not possible to measure nonspecific uptake directly, i.e., uptake in the absence of 5HTTs. To measure nonspecific uptake indirectly, cells were incubated with and without 10 μ*M* of fluoxetine. Specific uptake by the 5HTTs was defined as fluoxetine blockable uptake, i.e., uptake measured without fluoxetine (total uptake) minus uptake measured at the same MIBG concentration in the presence of fluoxetine (nonspecific uptake). In all experiments, measurements were done in triplicate. Three separate incubations were performed (9 data points for each measurement). At the end of the incubations, the various cell lines were washed three times in ice cold buffer and counted in a liquid scintillation counter.

MIBG Labeling

Unlabeled MIBG was purchased from the University of Michigan. High specific activity ^{125}I sodium iodide was obtained from a commercial source (Amersham, Inc., Cardiff, U.K.). Labeling was performed by the ammonium sulfate, heat-mediated exchange procedure according to published methods (*20*). The reaction product was purified by anion exchange chromatography. The final specific activity ranged from 9 to 34 mCi/mg (107–405 GBq/mmol).

Data Analysis

The means and standard deviations for each incubation were calculated for the hNET experiments. Uptake by hNET in the presence of inhibitors was compared to uptake without inhibitors (control uptake) by the Student t-test, adjusted for multiple comparisons (Bonferroni's method). For the five comparisons (control uptake by hNET compared to five inhibitor experiments), Bonferroni's method yields significance for $p < 0.01$. For the bDAT and r5HTT experiments in transfected cells, specific uptake values significantly greater than zero were considered indicative of MIBG transport. For multiple comparisons, p values were adjusted. For the experiments with the JAR and RBL cells which consisted of five measurements each, significance was accepted at the $p < 0.01$ level.

The K_m for hNET was calculated from the specific uptake values by Eadie-Hofstee kinetic analysis. Only those specific uptake values that were significantly different from zero were used in the calculation. Comparisons were made by the Student t-test, and p values < 0.006 (corrected p value for eight measurements) were accepted as significant.

RESULTS

MIBG uptake (means ± s.d.) in mock transfected and hNET transfected HeLa cells was 0.262 ± 0.0159 and 2.32 ± 0.0986 pmole/400,000 cells/20 min, respectively. Using the mock transfected cells to represent nonspecific uptake, specific uptake (control uptake) was 89% of the total uptake. Results of the inhibitor experiments are shown in Figure 1. For hNET, 10^{-7} *M* NE caused a significant fall in specific MIBG uptake to 76% ± 6.3% of control values ($p < 0.006$ compared to controls). With 3×10^{-6} *M* NE, MIBG uptake was reduced to 13% ± 2.5% of the control values ($p < 0.001$). Mazindol and DMI reduced hNET mediated uptake to 3.3% ± 0.91% and 6.3% ± 0.66% of the control values, respectively ($p < 0.001$ for both measurements). Paroxetine, which has little effect on hNET mediated transport of NE [K_i for NE 3.12×10^{-7} *M* (*10*)], reduced MIBG uptake to 91% ± 5.6% of the control value ($p = 0.16$, not significant).

Figure 2 shows the total uptake in hNET transfected

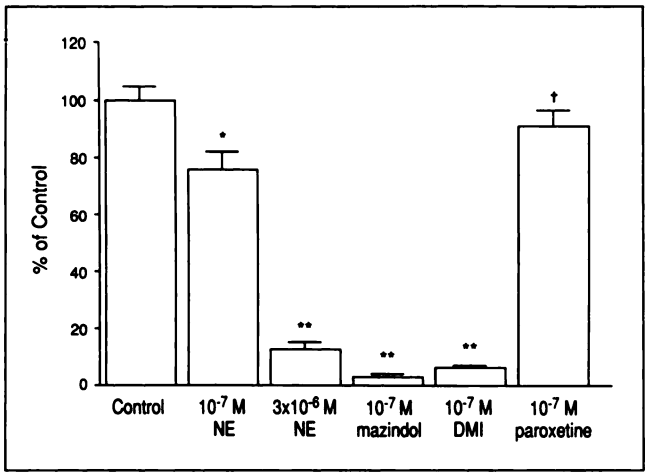

FIGURE 1. Specific uptake of MIBG by hNET in Hela cells. All studies were performed at 20 n*M* MIBG. Specific uptake (uptake by hNET) is defined as uptake in cells transfected with hNET minus uptake in nontransfected cells. Control = specific uptake in hNET transfected cells without inhibitors. DMI = desipramine. An asterisk indicates specific uptake significantly different from control, p = 0.006. Double asterisks indicate specific uptake significantly different from control, p < 0.001. A dagger indicates specific uptake not significantly different from control, p = 0.16.

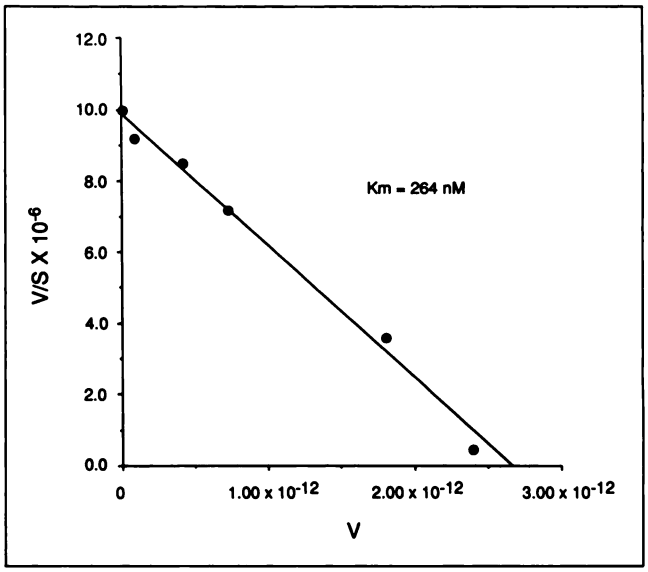

FIGURE 3. Eadie-Hofstee plot of specific uptake data in Figure 2. V (velocity) expressed as pmole/400,000 cells/20 min. S = molar concentration of MIBG. The K_m (concentration of MIBG at half maximal uptake) is given by Km = −1/slope = 264 n*M*. The K_m for NE in this system is 457 n*M* (*10*).

cells, uptake in mock transfected cells (nonspecific uptake) and specific uptake in hNET transfected cells at MIBG concentrations varying from 10^{-10} *M* to 5×10^{-5} *M*. Total uptake was significantly greater than nonspecific uptake at all concentrations of MIBG except at the lowest (10^{-10} *M*) and highest (5×10^{-5} *M*) concentrations which were excluded in the calculation of the K_m. Uptake of MIBG was primarily by hNET at low concentrations of MIBG, accounting for 81% and 82% of uptake at 0.01 and 0.1 μ*M* MIBG, respectively. At 1–2 μ*M*, approximately 50% of uptake was specific uptake. MIBG uptake by hNET saturated at higher concentrations of MIBG. Nonspecific uptake of MIBG as a percent of the total uptake was low at low concentrations of MIBG and increased with increasing MIBG concentration so that MIBG uptake was nearly completely nonspecific (89%) at the highest concentration of MIBG. Figure 3 shows an Eadie-Hofstee plot of the specific uptake in Figure 2. The K_m is obtained from the slope of the fitted curve: $K_m = -1/\text{slope} = 264$ n*M*. hNET demonstrates a K_m for NE transport in this cell system of 457 n*M* (*10*).

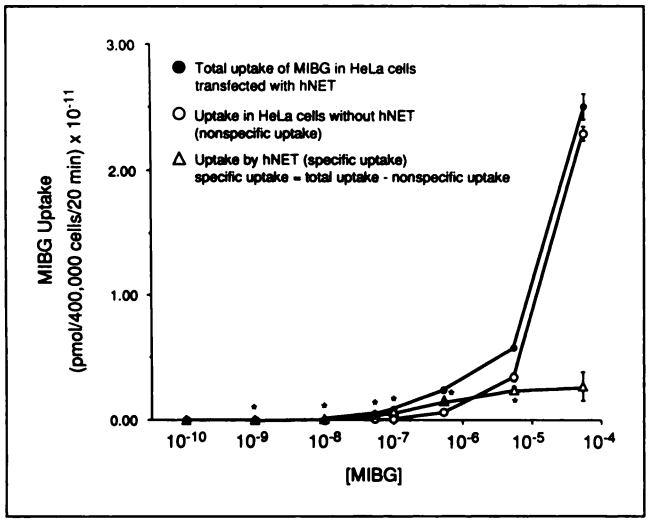

FIGURE 2. Effect of concentration on total, specific and nonspecific MIBG uptake. Specific uptake (uptake by hNET) is defined as uptake in cells transfected with hNET minus uptake in nontransfected cells. Uptake given in units of pmole/400,000 cells/20 min. [MIBG] = Molar concentration of MIBG. An asterisk indicates specific uptake significantly different from zero, p < 0.001.

Table 1 gives the values for MIBG uptake by CV-1 cells transfected with bDAT and by nontransfected CV-1 cells. Specific uptake by bDAT was obtained by subtracting uptake in nontransfected cells from uptake in transfected cells. There was no significant uptake at low concentrations of MIBG (1 and 10 n*M*). Although uptake at the next highest concentration was significant, no significant uptake was seen at the micromolar level (0.917 μ*M*). At higher values (10 μ*M* and above) uptake was again significant. These results suggest that there may be a low affinity uptake mechanism for MIBG by bDAT. The pattern of uptake, however, is unusual. The percent specific uptake at the various MIBG concentrations, unlike MIBG uptake by hNET, was low at low concentrations of MIBG and rose with increasing MIBG concentration. Because uptake did not saturate with increasing MIBG concentrations, a K_m could not be calculated. To confirm that specific uptake seen in these experiments was due to transport by bDAT, identical incubations were performed with GBR12909, a specific blocker of bDAT (Table 2). Somewhat unexpectedly, GBR12909 produced substantial blockade of nonspecific uptake. If specific uptake by bDAT was occurring,

TABLE 1
MIBG uptake in CV-1 Cells With and Without The Bovine Dopamine Transporter

[MIBG]	Total uptake		Specific uptake	% Specific
	bDAT CV-1	CV-1	(bDAT CV-1–CV-1)	uptake
1.22 n*M*	$2.31 \pm 0.39 \times 10^{-2}$	$2.22 \pm 0.39 \times 10^{-2}$	$8.7 \pm 5.5 \times 10^{-4}$	3.8
10.5 n*M*	$6.35 \pm 0.25 \times 10^{-2}$	$6.14 \pm 0.29 \times 10^{-2}$	$2.03 \pm 3.87 \times 10^{-3}$	3.2
98.7 n*M*	0.405 ± 0.012	0.339 ± 0.012	0.066 ± 0.017*	14.8
0.917 μM	3.16 ± 0.0072	3.21 ± 0.174	—	0
10.4 μM	37.5 ± 1.25	32 ± 0.29	5.5 ± 1.3*	14.7
101 μM	328 ± 12.8	240 ± 11.8	88.1 ± 17.4*	26.8
502 μM	1258 ± 41.8	939 ± 47.8	319 ± 63.5*	25.4
105 n*M* H-3 DA	0.386 ± 0.0217	0.0541 ± 0.00671	0.332 ± 0.0227*	86.0

*Specific uptake significantly different from zero, $p < 0.003$.

[MIBG] = concentration of MIBG, M = molar, DA = dopamine. Uptake is given in units of pmole/100,000 cells/5 min and is expressed as the mean ± standard error (n = 6 for all measurements). Nonspecific uptake is represented by total uptake in nontransfected CV-1 cells. Values for specific MIBG uptake are not significantly greater than zero at MIBG concentration of 1.22 n*M*, 10.5 n*M* and 0.917 μM. Percent specific uptake is specific uptake divided by total uptake in bDAT CV-1 cells times 100%.

GBR12909 should have produced a greater fall in percent MIBG uptake in bDAT transfected cells than in nontransfected cells, since both specific and nonspecific uptake would be blocked. At MIBG concentrations where specific uptake was seen (98.7 n*M*, 10.4 μM, 101 μM and 502 μM), GBR12909 produced equal or greater blockade of MIBG uptake in nontransfected than in transfected cells suggesting that there was no uptake of MIBG by bDAT.

To ensure that functional bDAT was present, a control incubation was performed using H-3 dopamine, 105 n*M* (K_m for dopamine by bDAT in CV-1 m3.13 cells is 800 n*M*, Hoffman B, unpublished data). Specific uptake as a percent of total uptake was 5.8-fold higher for dopamine than for MIBG (86% versus 14.8%) at a similar MIBG concentration (98.7 n*M*). After incubation with GBR12909, there was a 99.8% blockade of bDAT uptake (specific uptake) in bDAT CV-1 cells. There was no effect on uptake in CV-1 cells without bDAT (dopamine uptake with and without GBR12909, 0.0645 pmole and 0.0540 pmole, respectively).

TABLE 2
Percent Blockable Uptake in CV-1 Cells by GBR12909

[MIBG]	bDAT CV-1	CV-1
1.22 n*M*	13%	8.3%
10.5 n*M*	31%	30%
98.7 n*M*	38%	39%
0.917 μM	38%	45%
10.4 μM	38%	46%
101 μM	38%	42%
502 μM	34%	34%
105 n*M* H-3 DA	85.8%	0%

The values represent the percent of total MIBG uptake that is blocked by 1 μM GBR12909, a specific inhibitor of the dopamine transporter. GBR12909 blocked MIBG uptake to the same extent in cells with and without the dopamine transporter. Although blockade of MIBG uptake at [MIBG] = 1.22 n*M* appears higher in the bDAT CV-1 cells, the difference was not statistically significant. DA = dopamine.

In contrast, there was a 0% blockade of specific uptake of MIBG and a 39% blockade of nonspecific uptake (Table 2).

Table 3 shows MIBG uptake in nontransfected CV-1 cells and in cells transfected with r5HTT. In transfected and nontransfected cells, the percent blockable uptake by fluoxetine is also given. Uptake in transfected and nontransfected cells was not significantly different at any concentration of MIBG (all p values > 0.49) indicating no significant uptake by r5HTT. Fluoxetine blocked MIBG uptake in transfected and nontransfected cells to nearly the same degree, a result which would be expected if there was no MIBG uptake by r5HTT. Fluoxetine had an even larger blocking effect on nonspecific MIBG uptake than did GBR12909.

A further test of MIBG transport by the 5HTT was performed in JAR and RBL cells in which 5HTTs are expressed in moderate to high amounts. The specific (fluoxetine blockable) uptake of MIBG by the JAR and RBL cell lines at various concentrations of MIBG is given in Table 4. Unlike the data for hNET, percent nonspecific uptake was high even at low concentrations of MIBG and specific uptake averaged about 16% but never exceeded 30% of the total uptake at any concentration of MIBG. For the JAR cell line, specific uptake was significantly greater than zero only at the lowest MIBG concentration. For the RBL cells, specific uptake was not significantly greater than zero at any MIBG concentration.

DISCUSSION

Monoamines (norepinephrine, dopamine and serotonin) are major neurotransmitters in the peripheral and central nervous systems. The primary means by which the action of released neurotransmitters is terminated at synapses is by reuptake into the presynaptic terminal. All the monoamines exist as charged (protonated) species in vivo. The pK (pH at which the charged to uncharged species exist in a one-to-one ratio) for NE is 8.58; for dopamine, 8.93; and for serotonin, 9.8. At physiologic pH, greater than 93% of

TABLE 3
MIBG Uptake in CV-1 Cells With and Without the Rat Serotonin Transporter

MIBG (μM)	r5HTT Transfected cells		Nontransfected cells	
	Total uptake	% Uptake blockable by fluoxetine	Total uptake	% Uptake blockable by fluoxetine
0.011	0.018 ± 0.002	51	0.019 ± 0.001	47
0.10	0.13 ± 0.01	61	0.13 ± 0.01	43
1.0	1.2 ± 0.10	61	1.3 ± 0.10	57
16	15 ± 2.0	66	14 ± 1.0	58
140	180 ± 20	67	170 ± 20	65
550	483 ± 50	66	440 ± 40	64

Uptake is given in units of pmole/100,000 cells/5 min and is expressed as the mean ± s.e.m. (n = 9 for all measurements). There are no significant differences in total uptake between transfected and nontransfected cells at any concentration of MIBG, indicating no uptake by r5HTT. Fluoxetine concentration: 10 μM.

NE is in the charged form. Since charged molecules penetrate the lipid bilayer of the cell membrane very poorly, carrier molecules, the monamine transporters, are responsible for the vast majority of monoamine uptake at presynaptic terminals at physiologic concentrations of the monoamines. Until recently, the individual monoamine transporters have been characterized by physical and chemical means. With the cloning, sequencing and expression of these transporters, their properties can be studied in greater detail.

The specific, high affinity uptake of MIBG by adrenomedullary chromaffin cells is qualitatively similar to that of NE suggesting that they share the same uptake system. In the present study, we have provided evidence that specific MIBG uptake is mediated by the NET and not by the DAT or the 5HTT. In cells transfected with hNET, specific uptake of MIBG has the following characteristics:

1. Uptake is inhibited by NE.
2. Uptake is inhibited by DMI at a concentration (10^{-7} M) that blocks transport of NE (*10*).
3. Uptake is inhibited by mazindol, a potent blocker of hNET (*10*).
4. Uptake is not inhibited by paroxetine, a specific inhibitor of the 5HTT.
5. Specific uptake of MIBG by hNET is greater than nonspecific uptake at MIBG concentrations less than 1–2 μM.
6. hNET has a high affinity for MIBG.

No significant MIBG uptake was seen by bDAT or r5HTT. Furthermore, MIBG uptake was strongly inhibited by 3 μM NE. Since NE in the synaptic cleft is probably in the range of 1–10 μM, this concentration of NE is physiologically relevant. NE (100 nM) caused only a 24% inhibition of MIBG uptake and thus, it is unlikely that plasma norepinephrine levels in the normal physiologic range (1–3 nM) would inhibit MIBG uptake by the NET. Plasma levels of NE, even in severe congestive heart failure, rarely exceed 10 nM and would not be expected to block MIBG uptake. However, plasma levels of NE (except in pheochromocytoma) are a reflection of sympathetic neuronal activity. Increased sympathetic neuronal activity increases levels of NE in the synaptic cleft secondarily increasing plasma NE levels. Increased levels of NE in the synaptic cleft could reach levels that would significantly inhibit MIBG uptake.

It is not too surprising that MIBG is transported only by hNET and not by bDAT or r5HTT. bDAT and r5HTT are relatively specific for their respective monoamines, whereas hNET can transport a variety of monoamine substrates in-

TABLE 4
Specific MIBG Uptake by Human and Rat Cell Lines Expressing Serotonin Transporters

[MIBG]	JAR	% Specific uptake	RBL 2H3	% Specific uptake
7.9 nM	0.017 ± 0.0049*	30	0.0090 ± 0.0050	16
79 nM	0.069 ± 0.035	18	0.11 ± 0.041	22
398 nM	0.38 ± 0.29	9.6	0.49 ± 0.30	16
10 μM	9.4 ± 5.1	17	7.3 ± 3.8	17
101 μM	32 ± 41	7.3	21 ± 27	7

*p = 0.003.

Specific uptake is given in units of pmole/100,000 cells/10 min and is expressed as the mean ± s.e.m. (n = 9 for all measurements). Percent specific uptake is the percent of the total uptake blockable by fluoxetine (10 μM). Specific uptake is significantly different from zero after adjustment for multiple comparisons for p values < 0.01. The only uptake significantly different from zero is at [MIBG] = 7.9 nM for JAR cells. JAR cells are human choriocarcinoma cells. RBL are rat basophilic leukemia cells.

cluding NE, dopamine and epinephrine. In fact, hNET appears to have a high affinity for dopamine. Dopamine strongly inhibits NE transport by hNET (K_i of dopamine for NE transport by hNET = 139 n*M* (*10*)). In contrast, bDAT and r5HTT have low affinities for NE (K_m > 10 μM for bDAT (*14*) and r5HTT (*15*)).

The cloning of hNET has allowed a more detailed analysis of its properties. hNET is a 617 amino acid protein (*10*). Based on hydrophobicity analysis, hNET is predicted to have 12 transmembrane segments. This property of multiple transmembrane segments is shared by the other monoamine transporters, rDAT (*11–13*), bDAT (*14*) and r5HTT (*15,16*). Transport of NE by cloned hNET is also sodium- and temperature-dependent. The energy required to concentrate NE intracellularly is felt to be derived from the potential energy stored in the Na^+ and Cl^- gradients (for a recent review of the possible mechanisms involved in NE transport, see reference (*21*)). These gradients are established by the Na^+-K^+ ATPase, a membrane protein. Hydrolysis of ATP by the Na^+-K^+ ATPase provides the energy necessary for the exchange of intracellular Na^+ for extracellular K^+. Inhibition of the Na^+-K^+ ATPase by ouabain or of glucose metabolism (which blocks ATP synthesis) blocks transport of MIBG and NE (*8*). Thus, most of the characteristics of specific MIBG uptake (sodium, temperature and energy dependence) can be explained by the known properties of the NET.

There is very little data in the literature regarding regulation of the NET. A study of experimentally induced right heart failure in dogs found decreased mazindol binding in the right ventricular myocardium compared to the normal left ventricular myocardium suggesting a down regulation of the NET (*22*). Physiologically, such a down regulation could benefit the failing heart by providing a longer duration of action of NE at the postsynaptic membrane. Because the monoamine transporters have only recently been cloned and sequenced, no details on the endogenous regulation of these transporters are available. Their gene structures have not been determined and as of yet there is no evidence that common regulatory mechanisms, i.e., phosphorylation or interaction with other membrane proteins, are operative in their regulation. It is possible that there are other monoamine transporters that have not been identified.

Confusion exists in the literature regarding the terminology of MIBG uptake by sympathetic neurons. Iversen first proposed the terms $uptake_1$ and $uptake_2$ to refer to neuronal and nonneuronal uptake of catecholamines, respectively (*23,24*). In studies of MIBG uptake in adrenal chromaffin cells, it was found that there was a relatively large component of nonspecific uptake of MIBG. The term $uptake_1$ was used to represent only the specific uptake by adrenal chromaffin cells (*8,9*). The term $uptake_2$ referred to nonneuronal uptake (*25*). Some authors, however, use the term $uptake_1$ to refer to total neuronal uptake (*26,27*), whereas other authors refer to $uptake_2$ as nonspecific uptake of MIBG by sympathetic neurons (*28*). Given the confusion over the terms $uptake_1$ and $uptake_2$ and their nondescriptive nature, the following change in terminology regarding MIBG uptake is suggested. Specific MIBG uptake by sympathetic neurons should be referred to as uptake by the NET. Other types of uptake should be specifically described, i.e., nonspecific neuronal uptake or nonneuronal uptake.

Molecular biologic techniques offer a new and more precise method of analyzing radiotracer kinetics at the cellular level. Previously, investigation of uptake mechanisms required the use of blocking drugs whose effects on other aspects of cellular uptake or cellular metabolism were unknown. In particular, our studies have shown that drugs which specifically block the DAT (GBR12909) and the 5HTT (fluoxetine) have a significant blocking effect on nonspecific MIBG uptake. Because we were able to measure nonspecific uptake directly, we were able to quantify the effect of these drugs on nonspecific uptake. In the absence of the ability to selectively express transporters in cell lines, specific uptake is usually defined by the ability of selective antagonists to block uptake. If this approach had been used to define specific MIBG uptake in our study, it would have been erroneously inferred that MIBG is transported by bDAT and r5HTT. Since many nuclear medicine studies rely upon cellular uptake of radiopharmaceutical (e.g., MAG-3, IDA agents, TcO_4^-), the cloning and expression of the transporters responsible for the cellular uptake of these radiopharmaceuticals should enhance our knowledge of their biokinetics.

The nature and in vivo significance of the nonspecific uptake of MIBG are unclear. MIBG with a pK probably around 13 (*29*) is more highly protonated in vivo that NE and would not be expected to cross the lipid bilayers of cell membranes. NE, however, is not only charged but also contains polar hydroxyl groups that decrease the lipophilicity of the phenyl ring. MIBG lacks these hydroxyl groups. Thus, the phenyl ring of MIBG may be able to associate with cell membranes without being able to cross these membranes. The in vitro studies performed to date (*8,9*) as well as our studies would be unable to distinguish between membrane binding versus diffusion across the membrane into the cytoplasm. These two mechanisms of nonspecific uptake would have different effects on the way MIBG is retained by the heart in vivo. MIBG which enters the cytoplasm of cardiac sympathetic neurons, whether by specific uptake or by diffusion, would be subsequently taken up by chromaffin granules and stored. Activity in the heart would then fall gradually with time. If MIBG associates with cell membranes in a nonspecific fashion, initial cardiac uptake of MIBG would be high (since the vast majority of cell membranes in the heart are non-neuronal) soon after intravenous injection but would fall rapidly as plasma MIBG levels fell. In this latter case, cardiac sympathetic denervation would produce little effect on the initial cardiac uptake of MIBG. In vivo studies are more suggestive of a nonspecific membrane binding effect as an explanation of nonspecific uptake than diffusion through the plasma cell membrane. Dae et al. (*3*) have shown that sympathectomy in dogs produces no decrease in cardiac

MIBG uptake at 15 min after injection but a marked decrease in cardiac MIBG retention at 3 hr. More recent studies have shown that denervated dog hearts show very little MIBG retention by 1 hr after injection (Dae M, *personal communication*). Similarly, drugs which block the specific uptake of MIBG would be expected to have little effect on the initial cardiac uptake of MIBG if there is a large component of nonspecific membrane binding. Again, this effect is seen in vivo. Neither cocaine nor imipramine decreases the first pass uptake of MIBG in the pig heart (*30*). In the dog, cocaine and DMI appear to increase cardiac MIBG uptake by 5 min after injection, while NE uptake is significantly decreased (*9*). In humans, cardiac denervation by transplantation causes complete loss of cardiac uptake by 15 min after injection, which suggests complete clearance of non-neuronal uptake by this time (*3*). In aggregate, these studies suggest that nonspecific cardiac uptake of MIBG in vivo is a transient phenomenon, and clearance of nonspecific uptake is probably complete by 15 min after injection in humans and by 1 hr after injection in dogs. There may be large differences in nonspecific uptake in different species and conclusions drawn from studies in animals may not be applicable in humans.

CONCLUSIONS

In this study, we have evaluated MIBG kinetics in cell lines transfected with cDNAs encoding the human norepinephrine, bovine dopamine and rat serotonin transporters. Dopamine and serotonin transporters do not appear to be responsible for MIBG uptake. MIBG uptake by the NE transporter shows a K_m similar to that for NE and can explain all the known properties of the specific uptake mechanism for MIBG. On the basis of the present work, we propose that the specific uptake of MIBG by sympathetic neurons is mediated solely by the NET.

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