Effect of Uptake-One Inhibitors on the Uptake of Norepinephrine and Metaiodobenzylguanidine*

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The mechanisms underlying the uptake of the radiopharmaceutical metaiodobenzylguanidine (MIBG) and the catecholamine norepinephrine (NE) were studied using cultured bovine adrenomedullary cells as an in vitro model system. Sodium-dependent and sodium-independent uptake systems have been identified and characterized for both MIBG and NE. The sodium-dependent uptake of NE and MIBG was inhibited by the selective Uptake-one inhibitors, desmethylimipramine (DMI) and cocaine, whereas the sodium-independent uptake for NE and MIBG was much less sensitive to inhibition by these agents. The sodium-dependent uptake system fulfills the criteria for the neuronal Uptake-one system, and the sodium-independent uptake system fulfills the criteria for a passive diffusion mechanism. Both NE and MIBG were transported into cultured bovine adrenomedullary cells by both uptake systems; the relative role of each uptake system was dependent upon the concentration of NE and MIBG in the media. Arterial concentrations proximal to the dog adrenal were very small suggesting that the sodium-dependent (Uptake-one) system is predominant in vivo. Consistent with the in vitro observations, the in vivo uptake of MIBG and NE into dog adrenal medullae was effectively blocked by pretreatment with DMI or cocaine. Therefore, iodine-131 MIBG scintigraphy of the adrenal appears to reflect uptake by way of the Uptake-one system.

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Metaiodobenzylguanidine (MIBG) is used clinically in the diagnosis (*3–8*) and treatment (*9,10*) of human pheochromocytomas, adrenomedullary hyperplasia, and neuroblastomas. Iodine-123 (^{123}I) MIBG has also been used to image the human heart (*11*), and this agent is currently being used in our clinic for the evaluation of neuronal-integrity in the heart. Although the use of MIBG is increasing around the world (*8,12–14*), there are limited data on the mechanism(s) of its uptake and retention. MIBG is structurally related to the neuron blocking agent guanethidine (*15*) and thus may act in a similar biochemical manner. MIBG appears to be sequestered within norepinephrine (NE) storage granules based on the evidence of in vivo reserpine depletion of dog adrenomedullary [^{131}I]MIBG content (*16*), in vivo reserpine blocking of [^{125}I]MIBG accumulation into the dog heart (*17*), and subcellular distribution of [^{125}I]MIBG in dog adrenomedullary tissue (*16*). However, little is known about the mechanism(s) of uptake of MIBG into the adrenal medulla or peripheral adrenergically rich tissues.

A preliminary study with DMI and cocaine suggested that MIBG uptake may be by way of the neuronal Uptake-one system (*1*). In this paper, we have used cultured bovine adrenomedullary cells as an in vitro model system to probe the mechanism(s) of MIBG uptake. We now report the effect of the classical Uptake-one inhibitors, desmethylimipramine (DMI) and cocaine, on the in vitro uptake of MIBG and NE. We also report extended studies on the effects of DMI and cocaine on the in vivo uptake of MIBG and NE into the dog adrenal medulla and other adrenergically rich tissues. A scheme is presented which demonstrates the similarities and dissimilarities of MIBG and NE uptake across the cell membrane.

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* A preliminary presentation of some of this work has appeared in abstract form (*1,2*).

MATERIALS AND METHODS

NE and MIBG

Nonradiolabeled *levo*-NE† and *levo*-[ring-2,5,6-^{3}H]NE‡ with a specific activity of 40 to 60 Ci/mmol were obtained from commercial sources. Nonradiolabeled MIBG was synthesized by the method of Wieland et al. (*15*), and [^{125}I]MIBG was prepared at a specific activity of 1.5 to 2.3 Ci/mmol using an iodide exchange technique (*18*). Iodine-131 MIBG was similarly prepared (*18*) with a specific activity of 0.83 Ci/mmol.

Preparation, maintenance, and characterization of the cultured adrenomedullary cells

Bovine adrenals, obtained at a local slaughter-house immediately after exsanguination, were freed of connective tissue and transported on ice in a Ca^{2+}-free Locke's buffer to the laboratory. The adrenomedullary cells were dispersed and plated as a monolayer as previously described (*19,20*). Briefly, the adrenals were perfused through the adrenal vein with 0.05% collagenase†; the adrenomedullary tissue was subsequently separated, minced and treated in vitro to 0.05% collagenase† and 15% of a 0.25% trypsin† solution (*19*). The cells were centrifuged and plated at densities of 0.6 to 0.8 × 10^6 cells per well§ in Minimum Essential Medium with Earle's salt solution supplemented with 15% fetal calf serum,† penicillin† (100 μg/ml) and streptomycin† (100 μg/ml). The cells were allowed to adapt to culture for 4 days prior to starting an experiment. After this adaptation period, the media was changed every second or third day. All experiments reported herein were performed 4–8 days postdispersion.

Prior to plating the cells and/or commencing an experiment, cell viability was shown to be 95% or greater by trypan blue exclusion. Cell catecholamine content was routinely assayed using the differential assay technique of Anton and Sayre (*21*) and total catecholamines were in the range of 30 to 40 nmol/10^6 cells. Periodically, the cell dispersions were evaluated using histofluorescence to demonstrate the endogenous catecholamines (*22*).

Incubation conditions and procedures

During the uptake studies, the tissue culture plates were placed in a 37°C water bath or an ice water bath (0.5 to 1.5°C) under air atmosphere. Fifteen min prior to commencing the uptake study, the culture media was removed, and the cells were pre-incubated, at either temperature, with 0.5 ml HEPES†¶ buffered Krebs Ringer Glucose (*23,24*), hereafter named H-KRG solution [125 m*M* NaCl, 4.8 m*M* KCl, 2.6 m*M* $CaCl_2$, 1.2 m*M* $MgSO_4$, 5.6 m*M* glucose, and 25 m*M* HEPES], containing 1 m*M* ascorbic acid†, adjusted to a final pH of 7.35. After the pre-incubation, the H-KRG solution was removed, and the cells in each well were incubated with 0.30 ml H-KRG solution containing various concentrations of either H-3-NE or [^{125}I]MIBG alone or in combination with other compounds. At the end of the incubation period, the plates were placed on ice, the H-KRG solution containing H-3-NE or [^{125}I]MIBG was removed, and the cells were washed two times with approximately 1.0 ml of ice-cold Dulbecco's phosphate buffered saline. The Dulbecco's phosphate buffered saline was used because it contains no Ca^{2+} whereas the H-KRG solution does. The presence of Ca^{2+} in the wash solution could facilitate the release of NE and MIBG from the cells during the wash procedure. The H-3-NE and [^{125}I]MIBG taken up by the cells was extracted by the addition of 0.30 ml of 10% trichloroacetic acid. Following the trichloroacetic acid extraction, the cells were washed with 0.30 ml of double distilled water which was also added to scintillation vials or gamma-counting tubes, as appropriate. After the addition of 10 ml of scintillant**, the vials containing the radioactivity due to H-3-NE were mixed well and counted††. The samples were counted for 10 min or to a two sigma error of 0.50%. The tubes containing radioactivity due to [^{125}I]MIBG were mixed well and counted‡‡ at an efficiency of 77.5%. The 37°C incubations were done in quadruplicate, while the 0°C incubations were done in duplicate. The total uptake at a given time point or concentration was determined by correcting the uptake at 37°C for nonspecific uptake at 0°C. All uptake studies were conducted for 14 min and the total uptake was standardized as pmol/10^6 cells/10 min; uptake of each agent was linear to at least 20 min for all concentrations of NE and MIBG studied (*19*).

Sodium dependency of uptake

Uptake studies were done by incubating the cells with either H-3-NE or [^{125}I]MIBG in 0.30 ml of H-KRG solution under air atmosphere while a second, complimentary, uptake study was done by incubating the cells with the same agent at identical concentrations in 0.30 ml zero-sodium H-KRG solution for 14 min. This zero-sodium H-KRG solution was identical to H-KRG except that the NaCl had been replaced by an equimolar concentration of LiCl (i.e., 125 m*M*). Sodium-independent uptake is uptake which occurred in the zero-sodium H-KRG buffer and sodium-dependent uptake is uptake in the presence of NaCl less the sodium-independent uptake. Total uptake is, therefore, the sum of the sodium-independent uptake and the sodium-dependent uptake and measured as the uptake in H-KRG.

DMI and cocaine inhibition of sodium-dependent and sodium-independent uptake: determination of IC_{50} (μ*M*) values

Cells were incubated in zero-sodium H-KRG or H-

KRG containing either 0.5 or 20 μM H-3-NE or [^{125}I]MIBG. Within a given experiment (i.e., one substrate at one concentration), a single inhibitor, either DMI or cocaine, was present in both buffers at concentrations from 0.001 to 10,000 μM. Controls were done in the absence of either inhibitor. The effects of DMI or cocaine in the zero-sodium buffer directly reflected its inhibitory capacity upon the sodium-independent uptake system. The inhibitory capacity of DMI or cocaine upon the sodium-dependent uptake system was calculated after subtracting the sodium-independent uptake from the total uptake. The IC_{50} (μM) values were obtained from the linear portion of semilogarithmic plots of percent inhibition versus concentration.

Determination of the inhibition kinetics of DMI upon the sodium-dependent uptake of NE and MIBG

Cells were incubated in zero-sodium H-KRG or H-KRG containing 0.25, 0.50, 1.00 or 2.00 μM H-3-NE in the presence or absence of 0.03 μM DMI. In parallel experiments, cells were incubated in zero-sodium H-KRG or H-KRG containing 0.25, 0.50, 1.00 or 2.00 μM [^{125}I]MIBG in the presence or absence of 0.10 μM DMI. Therefore, at any of the four concentrations of H-3-NE, cells were incubated under four conditions: zero-sodium H-KRG, zero-sodium H-KRG containing 0.03 μM DMI, H-KRG and H-KRG containing 0.03 μM DMI. Cells were incubated under similar conditions at any of the four concentrations of [^{125}I]MIBG. As described above, the inhibitory capacity of DMI upon the sodium-dependent uptake system was calculated after subtracting the sodium-independent uptake from the total uptake. The kinetics of these studies were analyzed using the double-reciprocal equation of Lineweaver-Burk, and a standard deviation of each parameter was calculated (*25*).

Determination of the inhibition kinetics of NE and MIBG upon the sodium-dependent uptake of each other

Cells were incubated in zero-sodium H-KRG and H-KRG containing 0.25, 0.50, 1.00, or 2.00 μM H-3-NE in the presence or absence of 1.6 μM MIBG. In a parallel experiment, cells were incubated in zero-sodium H-KRG and H-KRG containing 0.25, 0.50, 1.00, or 2.00 μM [^{125}I]MIBG in the presence or absence of 2.5 μM NE. The sodium-dependent uptake was calculated as previously explained. The kinetics of these studies were analyzed using the double reciprocal equation of Lineweaver-Burk, and a standard deviation of each parameter was calculated (*25*).

Arterial concentrations of MIBG to the adrenal

Following anesthetization with sodium pentobarbital, three female mongrel dogs, 17.5, 24.0, and 24.1 kg, were catheterized in the abdominal aorta just above the branch of the left phrenicoabdominal artery to the adrenal. Arterial blood samples were obtained at various time intervals before, during, and following the 2-ml injection into the right cephalic vein with 101 to 109 μCi of [^{131}I]MIBG. The [^{131}I]MIBG was administered over a 2 min time interval. Blood samples were weighed and counted in an autogamma counter[‡‡] with corrections made for radioactive decay, background and counter efficiency. Blood concentrations, μM, were calculated on the basis of the specific activity of the injected [^{131}I]MIBG. The specific activity of the injected dose was determined by assaying the radioactivity and ultraviolet absorption of the [^{131}I]MIBG.

DMI and cocaine inhibition of the in vivo uptake of NE and MIBG

Female mongrel dogs (12 to 23 kg) were injected intravenously with DMI (10 mg/kg) followed by a 100 μCi dose of [^{125}I]MIBG or H-3-NE 60 min later. Similarly, dogs were injected intravenously with cocaine (5 mg/kg) followed by a 100 μCi dose of [^{125}I]MIBG or H-3-NE 5 min later. The DMI and cocaine were dissolved in approximately 1 ml of 0.9% saline solution and injected over 1 min. Dogs that served as controls were injected with saline followed by a 100 μCi dose of either agent; no differences were observed in controls administered saline at 5 or 60 min prior to the radiotracer dose. Five min after receiving the 100 μCi dose of either radiotracer, the dogs were killed by rapid intravenous injection of sodium pentobarbital. Both adrenals were removed from each dog, freed of fat and connective tissue, and immediately placed on dry ice. The adrenals were sliced in half to expose the medullae, and the medullary tissue was removed with a 2-mm Meyhoffer curette. Duplicate samples of 11 different tissues in each dog were excised, washed free of blood with saline solution, blotted dry, and quickly weighed (15 to 100 mg). For [^{125}I]MIBG, the tissue samples were counted in an autogramma counter[‡‡] with corrections made for radioactive decay, background, and counter efficiency. For H-3-NE, the tissue samples were oxidized in a sample oxidizer[§§] and then counted in a liquid scintillation counter[¶¶] with corrections made for background and counter efficiency. Blood samples were obtained by cardiac puncture. To normalize for differences in animal weights, tissue concentrations are expressed as percent kilogram dose per gram (% kg dose/g) (*26*).

RESULTS AND DISCUSSION

This section is divided into three parts. First, we describe the in vitro studies with cultured bovine adrenomedullary cells and discuss the in vivo implications of these studies. Second, we describe the in vivo studies in dogs and discuss how they correlate with the in vitro studies. We then conclude with an overall discussion of

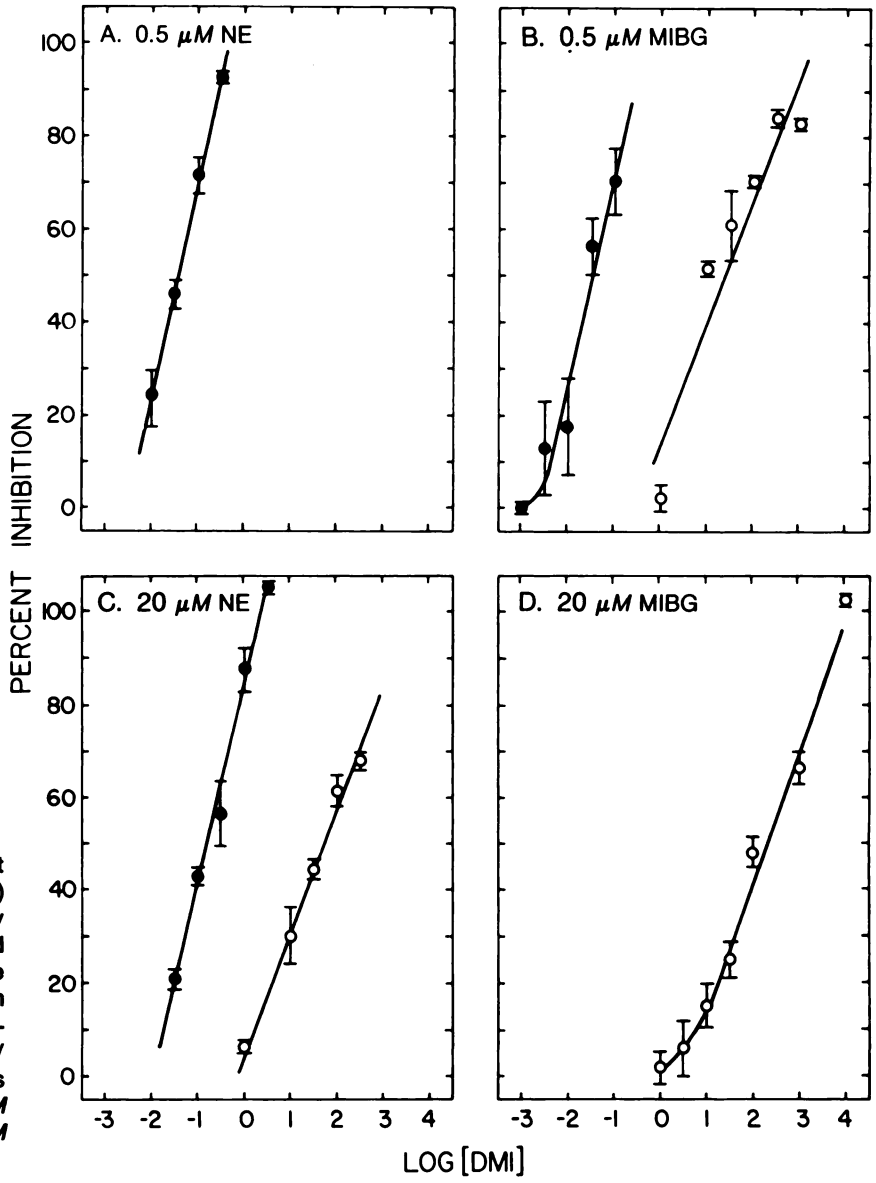

FIGURE 1
Inhibition of the sodium-dependent (-●-) and sodium-independent (-O-) uptake systems for NE and MIBG by DMI, a tricyclic antidepressant and selective Uptake-one blocker. The concentrations of DMI ranged from 0.001 to 10,000 μM. The percent inhibition of the sodium-dependent and/or sodium-independent uptake is shown for: A. 0.5 μM NE, B. 0.5 μM MIBG, C. 20 μM NE, and D. 20 μM MIBG

the mechanisms underlying MIBG scintigraphy of human pheochromocytomas, adrenomedullary hyperplasia, neuroblastomas, and adrenergic-rich organs such as the salivary gland and heart.

Sodium-dependency of uptake

Using cultured bovine adrenomedullary cells, we have observed the uptake of NE and MIBG to be affected by two uptake systems (*19*). These two uptake systems were identified by their respective sodium-dependency, ouabain sensitivity, and energy dependency (*19*). The sodium-dependent uptake system was characterized to be temperature dependent, of high affinity and low capacity, saturable, ouabain sensitive, and energy dependent. The sodium-independent uptake system was characterized to be temperature dependent, unsaturable out to 5 mM, ouabain insensitive, and energy independent. Nonspecific binding does not explain the sodium-independent uptake system since uptake increased linearly as the substrate concentration increased out to 5 mM (*19*). The uptake of both NE and MIBG occurred concurrently by way of both systems, but the preference for one system relative to the other was both concentration and agent dependent. In these studies, we selected 0.5 and 20 μM NE and MIBG to evaluate the effects of selective Uptake-one inhibitors because these concentrations enabled us to examine the sodium-dependent and sodium-independent uptake systems in isolation or in combination. The uptake of 0.5 μM NE was 92.5% sodium-dependent and 7.5% sodium-independent (N = 12); whereas, the uptake of 20 μM NE was 48.1% sodium-dependent and 51.9% sodium-independent (N = 20). The uptake of 0.5 μM MIBG was 52.3% sodium-dependent and 47.7% sodium-independent (N = 16); whereas, the uptake of 20 μM MIBG was 14.8% sodium-dependent and 85.2%

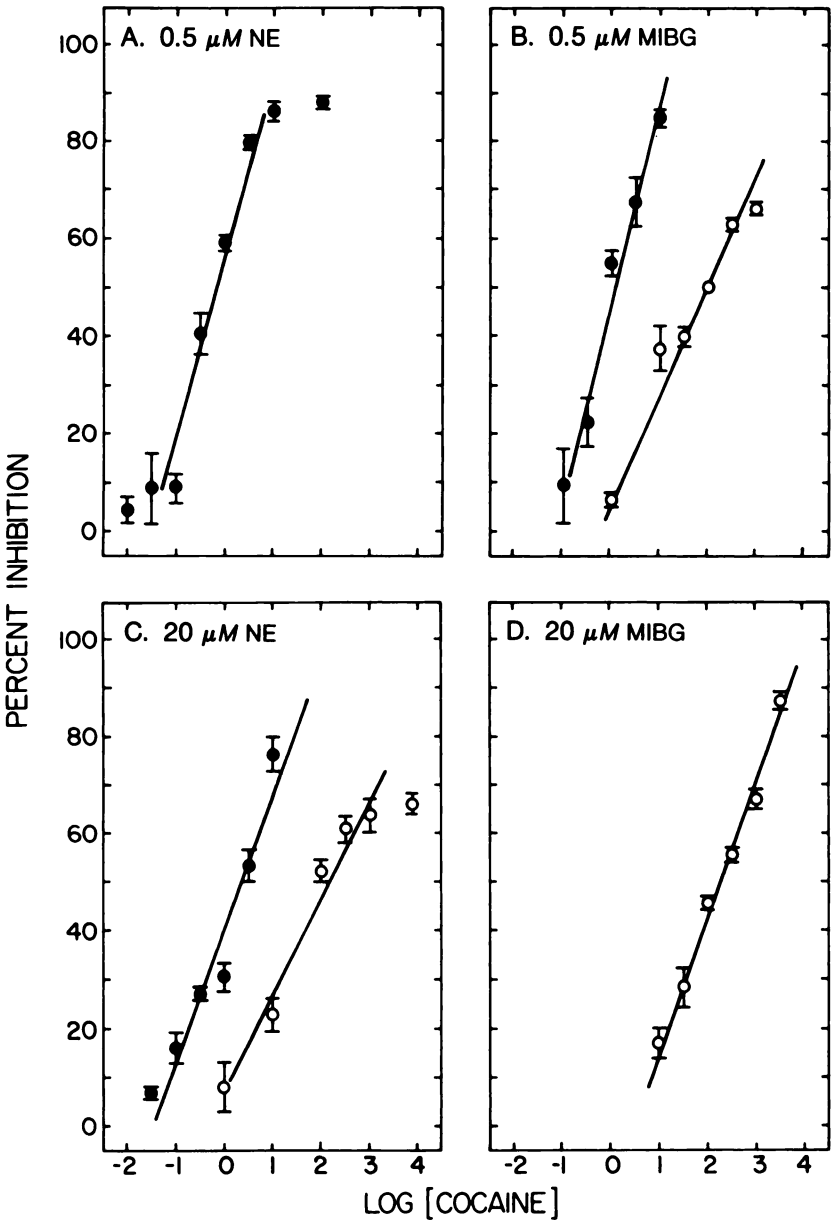

FIGURE 2
Inhibition of the sodium-dependent (-●-) and sodium-independent (-O-) uptake systems for NE and MIBG by cocaine, a selective Uptake-one blocker. Concentrations of cocaine ranged from 0.001 to 10,000 μM. Percent inhibition of the sodium-dependent and/or sodium-independent uptake is shown for: A. 0.5 μM NE, B. 0.5 μM MIBG, C. 20 μM NE, and D. 20 μM MIBG

sodium-independent (N = 6). In all cases, a greater percentage of MIBG, relative to NE, was taken up by the sodium-independent uptake system at any given concentration.

DMI and cocaine inhibition of sodium-dependent and sodium-independent uptake

DMI and cocaine are selective inhibitors of the neuronal Uptake-one system (*27–29*). Since the neuronal Uptake-one system has been characterized to be sodium-dependent and sodium-specific (*27,28,30,31*), these inhibitors were expected to be selective for the sodium-dependent system in the cultured bovine adrenomedullary cells. The effect of DMI on the sodium-dependent and sodium-independent uptake of 0.5 and 20 μM NE and MIBG is shown in Fig. 1. Similarly, the effect of cocaine on the sodium-dependent and sodium-independent uptake of 0.5 and 20 μM NE and MIBG is shown in Fig. 2. The effects of these Uptake-one inhibitors were not studied for the sodium-independent uptake of 0.5 μM NE or the sodium-dependent uptake of 20 μM MIBG; since they are such a small component of the total uptake at these concentrations, reliable statistics could not be obtained. Table 1 is a summary of all the data in Figs. 1 and 2.

As shown in Figs. 1 and 2 and Table 1, the sodium-dependent uptake system for NE and MIBG was 60- to 680-fold more sensitive to these selective Uptake-one inhibitors than the sodium-independent uptake system. DMI was 12- to 35-fold more effective than cocaine as an inhibitor of the sodium-dependent uptake system and 1- to 4-fold more effective than cocaine as an

TABLE 1
DMI and Cocaine Inhibition of Sodium-Dependent and Sodium-Independent Uptake: Determination of IC_{50} (μM) Values*

Agent	Concentration (μM)	IC_{50}(μM):DMI Sodium-dependent	IC_{50}(μM):DMI Sodium-independent	IC_{50}(μM):Cocaine Sodium-dependent	IC_{50}(μM):Cocaine Sodium-independent
NE	0.5	0.037	—	0.65	—
	20.0	0.161	51.4	2.08	131.3
MIBG	0.5	0.032	21.8	1.13	95.7
	20.0	—	180.9	—	177.5

* IC_{50}(μM) values are micromolar concentration of the inhibitor needed to give 50% inhibition. Correlation coefficients were greater than 0.95.

inhibitor of the sodium-independent uptake system. This observation that DMI is a more effective inhibitor than cocaine is consistent with reports of their relative effectiveness in the neuronally characterized Uptake-one system (*27–29*). The relatively poor inhibition of the sodium-independent uptake system for NE and MIBG by DMI and cocaine also supports the concept that the sodium-independent uptake system is distinct from the sodium-dependent uptake system.

Inhibition kinetics of DMI upon the sodium-dependent uptake

Although DMI and cocaine are structurally dissimilar, both of these inhibitors interact with the catecholamine transport protein involved in the Uptake-one system (*27–29*). DMI has been observed to be a competitive inhibitor of NE transport in neuronal tissue (*27–29*). Since the described log dose-response graphs (Fig. 1) do not by themselves imply a specific mechanism of inhibition (*32*), a kinetic analysis of the DMI inhibition of the sodium-dependent uptake system was done. As shown in Fig. 3, 0.03 μM DMI competitively inhibited the sodium-dependent uptake of NE and 0.10 μM DMI competitively inhibited the sodium-dependent uptake of MIBG. These studies demonstrate that the sodium-dependent uptake system in cultured adrenomedullary cells is similar to the neuronally characterized Uptake-one and suggests that this uptake system is shared by NE and MIBG.

Inhibition kinetics of NE and MIBG upon the sodium-dependent uptake

MIBG, 1.6 μM, competitively inhibited the sodium-dependent uptake of NE (Fig. 4A), and NE, 2.5 μM, competitively inhibited the sodium-dependent uptake of MIBG (Fig. 4B). The endogenous NE concentrations in the media are small and constant during the uptake time and conditions and do not qualitatively affect these plots since all observed inhibitions were competitive. Therefore, both NE and MIBG are transported into cultured adrenomedullary cells by way of the same sodium-dependent (Uptake-one) system.

General comments on the in vitro studies

Using this in vitro model system, we have identified two shared uptake systems for NE and MIBG which operate independently and overlap at high and low concentrations. The sodium-dependent uptake system was characterized to be temperature-dependent, saturable, of high affinity and low capacity, ouabain-sensitive and energy-dependent (*19*). We have now shown that this system is inhibited by DMI and cocaine, selective Uptake-one inhibitors. Therefore, the sodium-dependent uptake system in cultured adrenomedullary cells fulfills all of the criteria of the neuronally characterized Uptake-one system (*27,28,31,33–37*). The sodium-independent uptake system was characterized as temperature-dependent, unsaturable, ouabain-insensitive, and energy-independent (*19*). We have now shown this system to be poorly inhibited by DMI and cocaine. Therefore, the characteristics of the sodium-independent uptake system in cultured adrenomedullary cells are consistent with a passive diffusion mechanism (*38*). As previously stated, the sodium-independent uptake system does not appear to represent nonspecific binding since uptake increased linearly as the substrate concentrations increased out to 5 mM (*19*). The ability of DMI and cocaine to block the sodium-independent uptake of NE and MIBG may be related to nonspecific effects of these very lipophilic agents (*29*); DMI and cocaine could, at the larger concentrations, interact with the membrane in such a way as to alter the permeability.

Based on these studies, the in vitro uptake mechanisms for NE and MIBG into cultured bovine adrenomedullary cells can be represented by the scheme in Fig. 5. Both NE and MIBG are transported across the plasma membrane by the sodium-dependent (Uptake-one) and sodium-independent (passive diffusion) mechanisms. However, even at the lowest concentrations tested, a greater percentage of MIBG, relative to NE, was taken up by the sodium-independent uptake system.

The predictive value of this model raises many issues. First, it demonstrates that MIBG uptake is competitive with NE; therefore, since MIBG is a cation at physiologic pH, NE must be transported across the plasma membrane in the cationic form. Second, it shows that NE can enter adrenomedullary cells through Uptake-one; the physiologic relevance of such an uptake system for the adrenal medullae is unknown. Third, it demonstrates the mechanism by which tricyclic antidepressants can interfere with [^{131}I]MIBG and [^{123}I]MIBG scintigraphy; this is consistent with the observed effects following the inadvertent administration of imipramine on [^{131}I]MIBG accumulation into the salivary glands in four patients (*39*). And fourth, it suggests that both

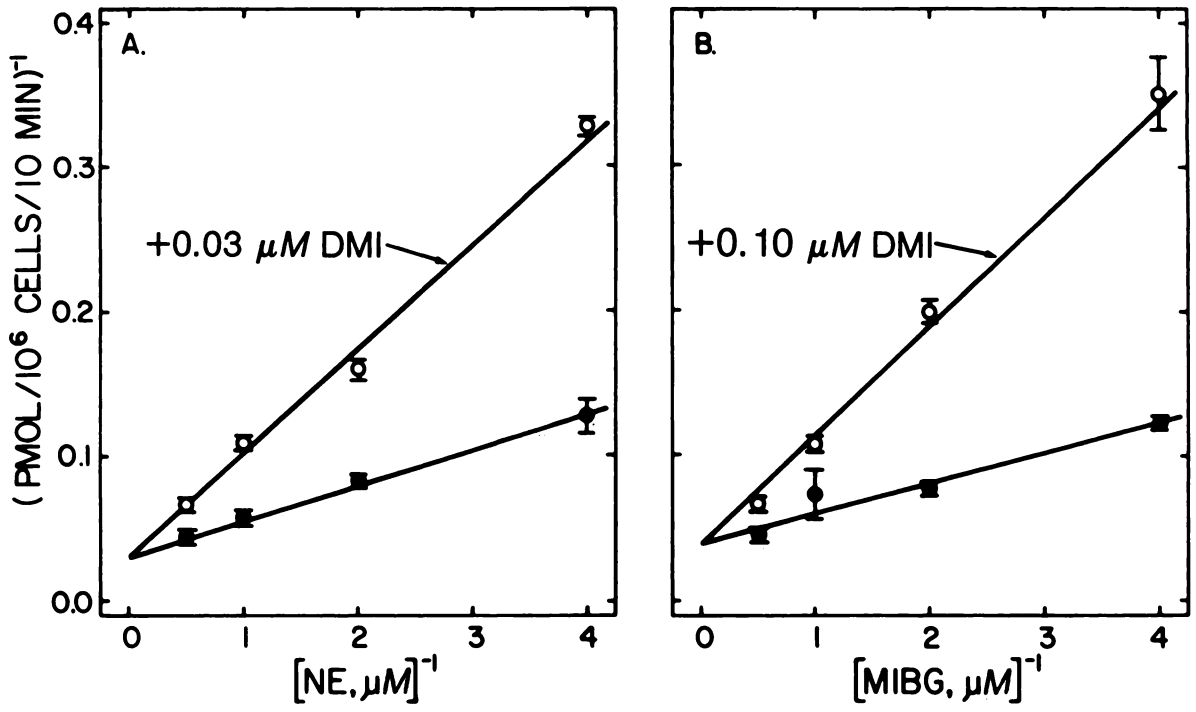

FIGURE 3
Kinetic analysis of competitive inhibition of sodium-dependent uptake of NE and MIBG by DMI, a tricyclic anti-depressant and selective Uptake-one blocker, using Lineweaver-Burk plots of: A. NE with 0.03 μM DMI and B. MIBG with 0.10 μM DMI

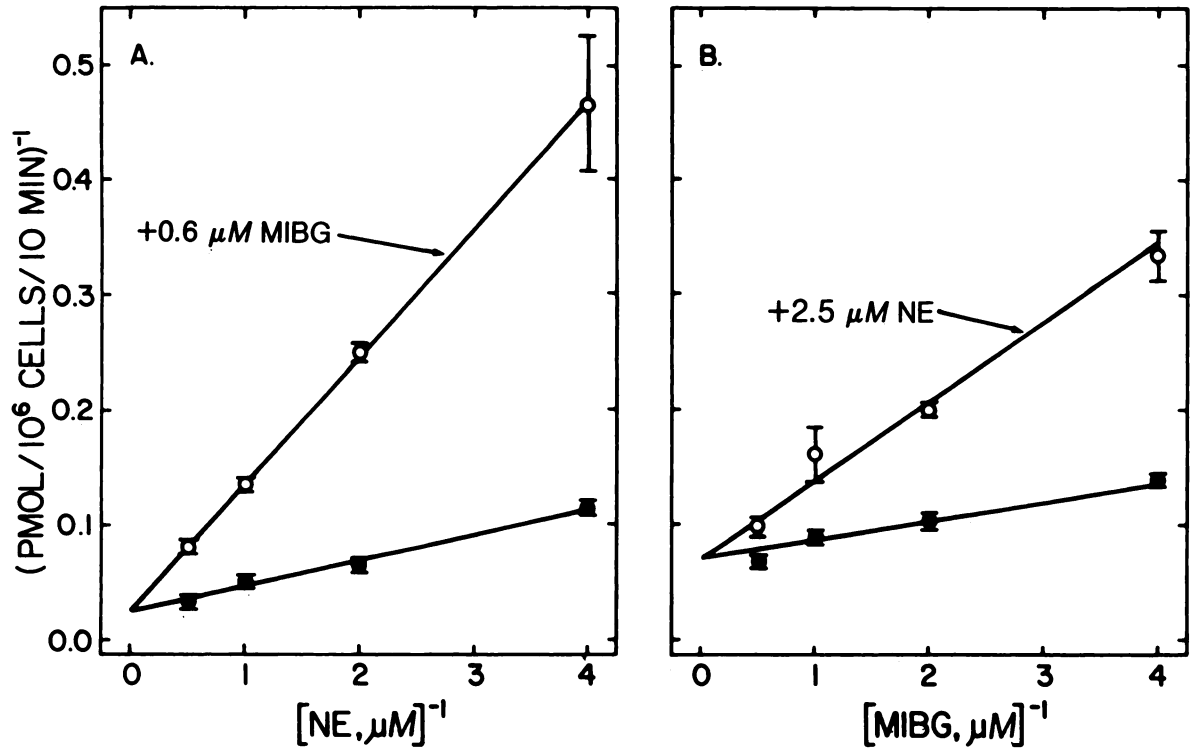

FIGURE 4
Kinetic analysis of the competitive inhibition of the sodium-dependent uptake of NE by MIBG and the sodium-dependent uptake of MIBG by NE, using Lineweaver-Burk plots of: A. NE with 1.6 μM MIBG and B. MIBG with 2.5 μM NE

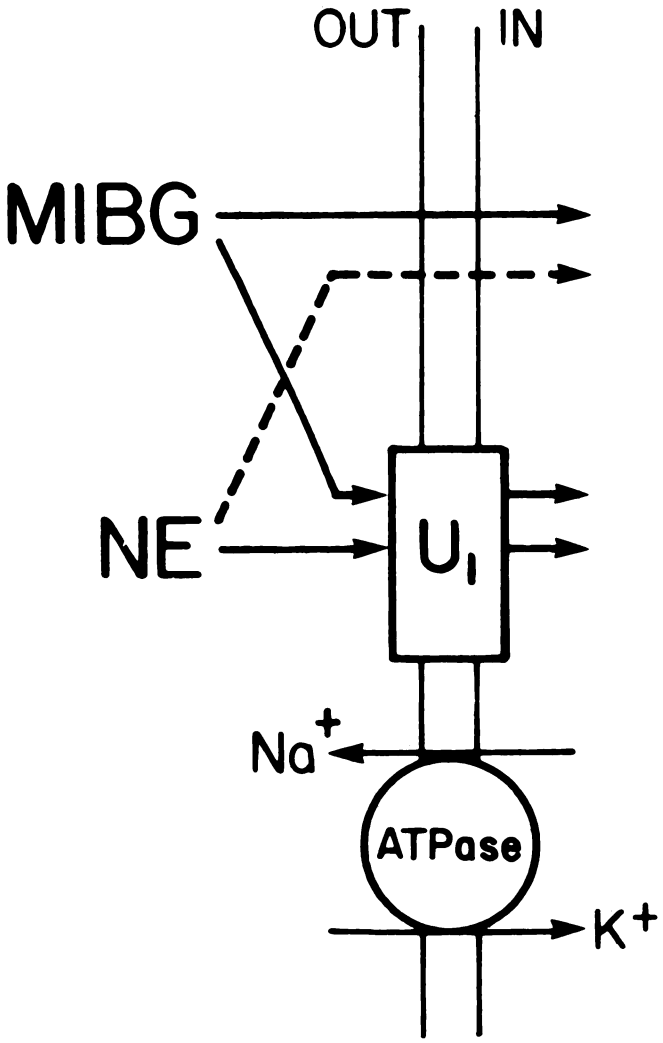

FIGURE 5
Scheme of the sodium-dependent and sodium-independent uptake systems for NE and MIBG into cultured bovine adrenomedullary cells

uptake systems may be important for [^{131}I]MIBG and [^{123}I]MIBG scintigraphy; the relative role of each uptake system will be dependent upon the blood levels of MIBG.

Arterial concentrations of MIBG to the adrenal

A key issue is whether or not both the sodium-dependent uptake system and the sodium-independent uptake system are equally important for [^{131}I]MIBG and [^{123}I]MIBG scintigraphy. The blood levels of [^{125}I]-MIBG reported for dog and monkey (*16,17*) and of [^{123}I]MIBG reported for man (*11*) suggest that arterial concentrations of MIBG will be less than 1 μM; however, it is difficult to predict the actual blood concentrations perfusing the adrenal medullae from these data. The sodium-dependent uptake system will play a greater role as the blood concentrations of MIBG get smaller. In an attempt to determine the arterial concentrations of MIBG perfusing the adrenal, we took samples every 30 sec before, during, and post-injection from three dogs catheterized in the abdominal aorta just above the branch of the phrenicoabdominal artery to the adrenal. The dogs were injected with a radioactive dose based on body weight similar to the clinically used diagnostic dose. The highest arterial concentration of MIBG observed was 0.023 μM which occurred 30 sec post-injection in one of the three dogs. By 5-min postinjection, the concentration of MIBG was less than or equal to 0.007 μM.

DMI and cocaine inhibition of the in vivo uptake of NE and MIBG

The in vitro studies and the very small arterial concentrations of MIBG suggest that the sodium-dependent (Uptake-one) system is the predominant uptake system. We, therefore, evaluated the effects of pretreatment with DMI and cocaine on the uptake and distribution of H-3-NE and [^{123}I]MIBG into the adrenal medullae and adrenergic rich tissues of the dog (Table 2). Pretreatment with DMI and cocaine markedly reduced the adrenomedullary uptake of both H-3-NE and [^{125}I]MIBG. Although pretreatment with DMI and cocaine reduced the heart uptake of H-3-NE by 41 to 42%, pretreatment with these agents increased heart uptake of [^{125}I]MIBG by 55 to 63%. Additionally, these pretreatments had variable effects on the uptake of H-3-NE and [^{125}I]MIBG into the lung, pancreas, spleen, and liver.

General comments on the in vivo studies

The in vivo studies demonstrate that the selective Uptake-one inhibitors, DMI and cocaine, inhibited the adrenomedullary uptake of both NE and MIBG. These results contrast to those of a recent report of 35% and 0% inhibition of MIBG uptake after pretreatment of rats with cocaine and DMI, respectively (*40*). The observed differences may reflect the chronic administration of cocaine and DMI that was used in their protocol. The arterial blood studies also demonstrate that the in vivo concentrations of MIBG perfusing the adrenal are so low (less than 0.03 μM) that the sodium-dependent (Uptake-one) system should be the primary uptake mechanism. Therefore, the in vivo data correlates well with the in vitro data and suggests that Uptake-one is the active uptake mechanism underlying scintigraphy.

Many factors may be involved in the observed differences in the effects of DMI and cocaine pretreatment on the uptake of H-3-NE and [^{125}I]MIBG into the heart, lung, pancreas, spleen, and liver. In particular, the ability of the cultured bovine adrenomedullary cells to predict the neuronal uptake of MIBG in the heart may be compromised by the large amount of nonneuronal tissue in the heart (*41,42*). There is evidence that,

TABLE 2

Effect of DMI and Cocaine Pretreatment* on Tissue Uptake and Distribution† of H-3-NE and [^{125}I]MIBG in Female Dogs

Tissue	NE Concentration§¶ Control (n = 3)	NE Concentration§¶ DMI (n = 2)	NE Concentration§¶ Cocaine (n = 3)	MIBG Concentration§¶ Control (n = 3)	MIBG Concentration§¶ DMI (n = 3)	MIBG Concentration§¶ Cocaine (n = 3)
Adrenal	14.022	0.475	1.756	6.914	0.673	1.266
Medullae	± 1.809	± 0.145	± 0.196	± 0.769	± 0.059	± 0.196
Heart	0.993	0.583	0.572	0.405	0.628	0.660
	± 0.041	± 0.031	± 0.029	± 0.017	± 0.038	± 0.072
Lung	0.450	0.188	0.187	1.127	0.233	0.247
	± 0.064	± 0.016	± 0.019	± 0.051	± 0.032	± 0.011
Pancreas	0.663	0.219	0.157	0.505	0.387	0.462
	± 0.045	± 0.003	± 0.042	± 0.087	± 0.026	± 0.053
Spleen	0.789	0.375	0.398	0.609	0.634	0.629
	± 0.106	± 0.037	± 0.080	± 0.032	± 0.027	± 0.015
Liver	0.488	0.501	0.451	0.657	0.533	0.925
	± 0.032	± 0.026	± 0.080	± 0.072	± 0.062	± 0.167

* DMI (10 mg/kg) was administered intravenously 60 min prior to receiving 100 μCi of [^{125}I]MIBG or H-3-NE. Cocaine (5 mg/kg) was administered 5 min prior to receiving 100 μCi of [^{125}I]MIBG or H-3-NE. Five min after receiving the 100 μCi dose of either radiotracer, the dogs were killed and tissue samples were taken.

† Tissue distribution data for all 11 tissues can be obtained upon request from the author.

§ Mean ± s.e.m. All tissue samples were taken in duplicate from each animal.

¶ Concentrations are expressed in % kg dose/g.

unlike H-3-NE, MIBG and similar radiopharmaceutical analogs bind significantly to nonneuronal tissue in the heart even at tracer levels (Wieland DM, unpublished results). Additionally, increased organ blood flow, especially to the heart, may increase non-neuronal uptake. Therefore, uptake mechanisms other than Uptake-one, and loci other than adrenergic nerves, may be involved in the net accumulation of MIBG in tissues other than the adrenal medullae, especially at short time intervals after injection.

CONCLUSIONS

Uptake, storage and secretion mechanisms are the cellular dynamics that allow for [^{131}I]MIBG and [^{123}I]MIBG scintigraphy. This paper focuses on the mechanism(s) of uptake. Results using the cultured bovine adrenomedullary cell model suggest that both the sodium-dependent (Uptake-one) and sodium-independent (passive diffusion) uptake systems are important for [^{131}I]MIBG and [^{123}I]MIBG scintigraphy of the adrenal medulla and its tumors. However, Uptake-one will be the predominant uptake mechanism if MIBG concentrations are low, as is the case with bolus injections of [^{131}I]MIBG presently used for clinical diagnosis. The ability of the in vitro bovine adrenomedullary cell system to serve as a model for in vivo uptake in human pheochromocytomas, adrenomedullary hyperplasia, neuroblastomas, and adrenergic-rich organs such as the heart and salivary glands has yet to be validated. However, the nearly complete in vivo blockade of MIBG uptake in the dog adrenal medullae by selective Uptake-one inhibitors and a similar blockade of MIBG accumulation in the human salivary glands by inadvertant administration of imipramine (*39*) strongly suggest that the model has predictive value. The in vitro model is currently being used to study the storage and secretion mechanisms of MIBG.

FOOTNOTES

† Sigma Chemical Co., St. Louis, MO.

‡ Du Pont NEN Medical Products, No. Billerica, MA.

§ Nunc Multidish plates, Vanguard International, Neptune, NJ.

¶ N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid.

** Biocount scintillation fluid, Research Products International, Mt. Prospect, IL.

†† Beckman LS 7500 microprocessor controlled liquid scintillation counter, Beckman Instruments, Fullerton, CA.

‡‡ Packard Model 5230 auto-gamma scintillation spectrometer, Packard Instruments, Downers Grove, IL.

§§ Packard Model 306 Tri-Carb sample oxidizer, Packard Instruments, Downers Grove, IL.

¶¶ Packard Model 3300 liquid scintillation counter, Packard Instruments, Downers Grove, IL.

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